Université de Montréal

Rôles des anomalies de récepteurs hormonaux dans la physiopathologie des tumeurs surrénaliennes

Par

Nina Mombo N'Diaye

Département de Sciences Biomédicales
Vice-Décanat Recherche et Études Supérieures

Thèse présentée à la Faculté des Études Supérieures
En vue de l'obtention du grade de
Philosophiae Doctor (Ph.D.)

Décembre 2001

SUMMARY

The hallmark of adrenal Cushing's syndrome (CS) is that the primary production of cortisol by adrenal tumors or hyperplasias leads to progressive feedback inhibition of CRH and POMC expression. The mechanisms by which cortisol is produced when ACTH is suppressed were previously unknown and believed to be "autonomous".

The first observations of food- and GIP (glucose-dependent insulinotropic polypeptide)-dependent CS suggested that abnormal hormone receptors could be coupled to steroidogenesis in adrenal tumors. We proposed that adrenal CS could result from the expression of ectopic or aberrant adrenal hormone receptors. This places adrenal cells under stimulation of a trophic factor, which is not under regulatory negative feedback exerted by glucocorticoids. This atypical hormonal regulation constitutes an unregulated new trophic stimulus, which leads to increased function and possibly to hyperplasia and proliferative advantage. The molecular mechanisms leading to aberrant hormone receptor expression (overexpression, ectopic expression or abnormal coupling to steroidogenesis) are still largely unknown.

The present work led to the molecular characterizarion of GIP and LH/hCG receptors present in GIP- and LH-dependent adrenal lesions. Yet, we demonstrated the ectopic expression of GIP receptor without any activating mutation. Moreover, the observation of the aberrant expression of the receptor at early stage of adrenal hyperplasia in a patient with GIP-dependent CS suggested that the GIP receptor expression could be partly responsible for the proliferative disease.

We then hypothesized the presence of a mutation in the promoter of the GIP receptor leading to ectopic expression. Sequencing of the putative promoter in a patient with GIP-dependent CS revealed 16 single nucleotide variations and 10 length polymorphisms. Further studies will help discriminate between actual polymorphisms and potential mutations.

For LH-dependent CS, our results suggest that the abnormal function of LH/hCG receptor may be secondary to its ectopic expression in adrenal cells originating from the fasciculata zone or to an increased activity and coupling of the eutopic receptor to steroidogenesis. The study of the zonal LH/hCG receptor expression will clarify this question.

The identification of abnormal adrenal receptors contribute to our understanding of the pathophysiology of ACTH-independent CS and leads to innovative pharmacological therapies.

Key words

Adrenal cortex, Cortisol, Cushing's syndrome, Ectopic expression, Hormone receptors

Université de Montréal

Vice-Décanat Recherche et Études Supérieures

Cette thèse intitulée :

Rôles des anomalies de récepteurs hormonaux dans la physiopathologie des tumeurs surrénaliennes

présentée par

Nina Mombo N'Diaye

a été évaluée par un jury composé des personnes suivantes :

Présidente du jury :	D[r] Jolanta Gutkowska
Membre du jury :	D[r] Malur Sairam
Examinatrice externe :	D[r] Nicole Gallo-Payet
Directeur de recherche :	D[r] André Lacroix
Co-directrice de recherche :	D[r] Johanne Tremblay

Thèse acceptée le : 17/05/02

SOMMAIRE

Le syndrome de Cushing (SC) surrénalien se caractérise par un hypercorticisme qui induit l'inhibition de l'expression de la proopiomélanocortine et de la corticolibérine, par un mécanisme de rétroaction négative; la sécrétion de cortisol provient de tumeurs (adénomes, carcinomes) ou d'hyperplasies surrénaliennes. Les mécanismes régissant la production de cortisol en absence d'ACTH, dite "autonome", étaient alors inconnus.

Suite aux premières observations d'hypercorticisme induit par le GIP (*glucose-dependent insulinotropic polypeptide*), notre équipe a émis l'hypothèse que le SC surrénalien pourrait résulter de l'expression surrénalienne de récepteurs hormonaux aberrants; cette régulation hormonale atypique échapperait à l'inhibition exercée par les glucocorticoïdes. Ce nouveau stimulus trophique non régulé entraîne une sécrétion anormale de cortisol (ou d'autres stéroïdes) et confère probablement un avantage prolifératif aux cellules atteintes. Les mécanismes moléculaires responsables de ces anomalies de récepteur (surexpression, anomalie de couplage ou expression ectopique) restent encore inconnus.

Les travaux présentés dans cette thèse ont abouti à la caractérisation moléculaire des récepteurs du GIP et de la LH/hCG exprimés dans les lésions surrénaliennes de patients atteints de SC GIP-dépendant et LH-dépendant. Ainsi, nous avons démontré l'expression ectopique du récepteur du GIP non muté dans les tissus pathologiques. L'observation de l'expression illicite du récepteur à un stade précoce du développement du SC GIP-dépendant suggère que l'expression de ce dernier pourrait en partie être responsable du syndrome prolifératif.

Nous avons ensuite émis l'hypothèse de la présence d'une mutation dans la région promotrice du récepteur du GIP, responsable de l'expression ectopique. Le séquençage du promoteur putatif chez une patiente atteinte du SC GIP-dépendant a permis d'identifier 16 variations nucléotidiques et 10 polymorphismes de longueur. Des études ultérieures permettront de discriminer les polymorphismes des mutations potentielles.

Pour le SC LH-dépendant, les résultats obtenus suggèrent que l'anomalie de fonction du récepteur LH/hCG serait due à l'expression ectopique du récepteur dans la zone fasciculée de la corticosurrénale ou à une anomalie de couplage du récepteur eutopiquement exprimé. L'étude de l'expression du récepteur LH/hCG dans les différentes zones de la corticosurrénale permettra d'éclaircir ce point.

La caractérisation des récepteurs hormonaux anormaux constitue une percée dans la compréhension des mécanismes physiopathologiques du SC ACTH-indépendant et débouche sur de nouvelles approches thérapeuthiques pharmacologiques.

Mots-clés

Corticosurrénale, Cortisol, Syndrome de Cushing, Expression ectopique, Récepteurs hormonaux

TABLE DES MATIÈRES

CHAPITRE 4 – ARTICLE 3

CHAPITRE 5

CHAPITRE - DISCUSSION

LISTE DES TABLEAUX

CHAPITRE 1 – INTRODUCTION

CHAPITRE 2 – ARTICLE 1

CHAPITRE 3 – ARTICLE 2

CHAPITRE 4 – ARTICLE 3

CHAPITRE 5

LISTE DES FIGURES

CHAPITRE 1 – INTRODUCTION

CHAPITRE 2 – ARTICLE 1

CHAPITRE 3 – ARTICLE 2

CHAPITRE 4 – ARTICLE 3

CHAPITRE 5

LISTE DES ABRÉVIATIONS

5-HT	Sérotonine
5-HT_4R	Récepteur de la sérotonine de type 4
AC	Adénylate cyclase
ACTH	Adrénocorticotropine
ADN	Acide désoxyribonucléique
ADNc	Acide désoxyribonucléique complémentaire
AIMAH	*ACTH-independent bilateral macronodular adrenal hyperplasia*
AMPc	Adénosine monophosphate cyclique
Ang-I	Angiotensine I
ANP	*Atrial natriuretic peptide*
AP_1	*Activating protein 1*
AR	Adrénorécepteur
ARNm	Acide ribonucléique messager
AT_1	Récepteur de l'angiotensine de type 1
AT_2	Récepteur de l'angiotensine de type 2
AVP, LVP	Arginine-vasopressine, Lysine-vasopressine
AVP-V1	Récepteur arginine-vasopressine de type 1
BLAST	*Basic local alignment search tool*
CBP	CREB *binding protein*
CCK	Cholécystokinine
cEBPβ	*CAAT enhancing binding protein beta*
CGRP	*Calcitonin-gene related peptide*
CNP	Peptide natriurétique de type C
CRE	*cAMP responsive element*
CREB	CRE *binding protein*
CRH	Corticolibérine
DHEA,DHEAS	Déhydroépiandrostérone, déhydroépiandrostérone sulfate

ET-1	Endothéline-1
FSH	Folicullostimuline
FT	Facteur de transcription
GC	Glucocorticoïdes
GHRH	*Growth hormone releasing hormone*
GIP	*glucose-dependent insulinotropic peptide*
GLP-1	*Glucagon-like polypeptide-1*
GnRH	*Gonadotropin releasing hormone*
GRE	*Glucocorticoid responsive element*
GRP	*Gastrin releasing peptide*
hCG	Hormone gonadotropine chorionique
HHS	Hypothalamo-hypophyso-surrénalien
i.v.	*intraveineux(se)*
IGF-I	*Insulin-like growth factor I*
IGF-II	*Insulin-like growth factor II*
K^+	Ion potassique
LH	Hormone lutéinisante
MAP-kinase	*Mitogen activated protein-kinase*
MC2R	*Melanocortin receptor type 2*, Récepteur de l'ACTH
Na^+	Ion sodique
NPY	Neuropeptide Y
Ob-R	Récepteur de la leptine
Oct-1	Octamère 1
p.o.	*Per os*
P450scc	P450 *side chain clivage*
PACAP	*Pituitary adenylate cyclase activation peptide*
PC2	Enzyme de conversion de POMC (*Protein convertase type 2*)
PCR	*Polymerase chain reaction*

PK_A	Protéine kinase A
PK_C	Protéine kinase C
PLA_2	Phospholipase A2
PLC	Phospholipase C
POMC	Proopiomélanocortine
PPNAD	*Primary pigmented nodular adrenal disease*
PTH	Hormone parathyroïdienne
RB	Rétinoblastome
RCPG	Récepteur couplé aux protéines G
RT-PCR	*Reverse transcription-polymerase chain reaction*
SC	Syndrome de Cushing
SF-1	Steroidogenic factor 1
SINE	*Short interspersed element*
SNP	*Single nucleotide polymorphism*
SP_1	*Stimulating protein 1*
SRA	Système rénine-angiotensine
StAR	*Steroidogenic acute regulatory* (peptide)
TGFβ1	*Transforming growth factor beta 1*
TNFα	*Tumor necrosis factor alpha*
TRH	Hormone thyréotrope
TSH	*Thyroid stimulating hormone*
VIP	*Vasoactive intestinal polypeptide*

A ma famille,

REMERCIEMENTS

Je tiens à remercier toutes les personnes qui ont participé, de près ou de loin, à ce travail de recherche et qui m'ont soutenue tout au long de mon cheminement ; ceci inclut les collaborateurs, les amis, ma famille et, plus particulièrement, mon mari.

Au Dr André Lacroix, j'exprime ma profonde reconnaissance pour m'avoir accueillie dans son laboratoire et pour m'avoir consacré son temps, son soutien académique, matériel et moral au cours de la réalisation de mon travail. Il a été pour moi une source de motivation et de stimulation intellectuelle dans la poursuite de mes études.

Je remercie également le Dr Johanne Tremblay pour son soutien académique et matériel, ses conseils judicieux sur l'orientation des divers projets de recherche sur lesquels nous avons travaillé.

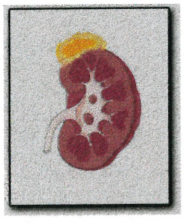
Je voudrais également remercier tous les membres du laboratoire et plus spécialement Mme Sylvie Oble pour son aide technique précieuse. La grande interaction avec les membres d'autres laboratoires a été particulièrement fructueuse et stimulante ; et je leur en sais gré.

Mes remerciements vont également au Dr Isabelle Bourdeau avec qui j'ai eu le plaisir de travailler sur plusieurs projets. J'ai notamment apprécié les discussions et échanges stimulants au cours de notre collaboration.

Enfin, je remercie les membres du jury d'avoir accepté d'évaluer cette thèse.

CHAPITRE 1

INTRODUCTION



1.1. Introduction : le syndrome de Cushing

1.1.1. Historique

C'est en 1912 qu'Harvey Cushing décrivit le premier cas de la maladie qui porte son nom, le syndrome de Cushing (SC) (Cushing 1912). En 1932, il émit l'hypothèse qu'une dysfonction hypophysaire primaire serait à l'origine du syndrome polyglandulaire qu'il avait décrit vingt ans auparavant (Cushing 1932). Un peu plus tard, le SC surrénalien fut découvert ainsi que le rôle thérapeutique de la surrénalectomie (Walters 1934).

Le syndrome de Cushing endogène se caractérise par les symptômes cliniques résultant de l'exposition chronique de l'organisme à des taux élevés de glucocorticoides ou autres stéroïdes sécrétés par la corticosurrénale (Nieman & Cutler 1995) (Orth & Kovacs 1998) (Yanovski & Cutler, Jr. 1994): obésité centrale progressive, myopathie proximale, hypertension, intolérance au glucose et hyperinsulinémie, manifestations dermatologiques diverses (atrophie de la peau, susceptibilité accrue aux ecchymoses, stries abdominales pourpres, hyperpigmentation), ostéoporose, hyperlipidémie et troubles neuropsychiatriques; l'excès d'androgènes surrénaliens entraîne hirsutisme, acné et oligoaménorrhée chez la femme, impotence et diminution de la libido chez l'homme suite à la suppression de l'axe hypophyso-gonadique.

1.1.2. Étiologies du Syndrome de Cushing

1.1.2.1. Syndrome de Cushing ACTH-dépendant

Dans la plupart des cas, le SC endogène est corticotropine (ACTH)-dépendant, résultant de la production excessive d'ACTH soit par un adénome hypophysaire (maladie de Cushing), soit par une tumeur extra-hypophysaire sécrétant de l'ACTH et son précurseur POMC, proopiomélanocortine (syndrome à ACTH ectopique) ; dans de rares cas, une tumeur surproduisant le facteur de relâche de l'ACTH, appelé corticolibérine (CRH), entraîne une production excessive d'ACTH par l'hypophyse (syndrome à CRH ectopique) (Tableau 1.I).

Tableau 1.I. Etiologies du syndrome de Cushing endogène

ACTH-dépendant	**Fréquence relative**
Adénomes hypophysaires	60-70%
Sécrétion ectopique d'ACTH	15%
Hyperplasie des cellules hypophysaires corticotrophes	rare
Sécrétion ectopique de CRH	rare
ACTH-indépendant	
Adénomes surrénaliens	8-10%
Carcinomes surrénaliens	5%
Dysplasie micronodulaire bilatérale (PPNAD)	rare
Hyperplasie macronodulaire bilatérale (AIMAH)	rare
Syndrome d'hypersensibilité au cortisol	très rare
Sécrétion ectopique de cortisol	très rare

Moins fréquemment, le SC est ACTH-indépendant, résultant de l'hypersécrétion de cortisol par des tumeurs corticosurrénaliennes bénignes (adénomes), malignes (carcinomes) ou des hyperplasies (Newell-Price *et al.* 1998) (Nieman & Cutler 1995) (Orth & Kovacs 1998) (Yanovski & Cutler, Jr. 1994) (Tableau 1.I). Cet hypercorticisme primaire d'origine surrénalienne induit, par un mécanisme de rétroaction négative, une inhibition de la synthèse et de la libération de CRH et d'ACTH. Les mécanismes physiopathologiques modulant la production de cortisol et la prolifération cellulaire dans ces lésions dites autonomes, puisqu'indépendantes de l'ACTH, restent encore mal définis et font l'objet des travaux de recherche présentés dans cette thèse.

De rares cas de sécrétion ectopique de cortisol par des tumeurs ovariennes entraînent un SC ACTH-indépendant (Marieb *et al.* 1983). Récemment, des symptômes cliniques de Cushing ont été décrits chez deux patients non exposés à des glucocorticoïdes exogènes qui présentaient des taux faibles ou anormalement régulés de cortisol et d'ACTH. L'hypothèse d'hypersensibilité au cortisol, appuyée par la démonstration d'une augmentation variable du nombre de récepteurs aux glucocorticoïdes (GR) chez ces patients, a été avancée pour expliquer cette symptomatologie (Iida *et al.* 1990) (Newfield *et al.* 2000).

1.2. Syndrome de Cushing surrénalien primaire

1.2.1. Données épidémiologiques

L'incidence du SC n'a jamais été déterminée avec grande précision. La fréquence croissante de lésions surrénaliennes responsables de SC dit sub-clinique, tel qu'identifié dans des cas d'incidentalomes surrénaliens, rend l'estimation de l'incidence de cette pathologie encore plus difficile. Pour le SC secondaire à un adénome surrénalien unilatéral, elle est approximativement de 2 cas sur 1 million par année (Ross 1994); elle est estimée à 1,7 cas par million par année pour les carcinomes dont 30 à 60% sont des

tumeurs sécrétrices cliniquement actives, responsables pour la moitié d'entre elles d'un hypercorticisme clinique (Bertagna & Orth 1981) (Bornstein *et al.* 1999) (Latronico & Chrousos 1997). La maladie de Cushing étant approximativement 3 fois plus fréquente que le SC surrénalien primaire, l'incidence serait estimée à 5 ou 6 cas par million par année. En tenant compte des cas de sécrétion ectopique d'ACTH cliniquement détectable, l'incidence totale du SC endogène atteindrait 10 cas par million par année.

L'hypercorticisme primaire d'origine surrénalienne, toutes étiologies confondues, rend compte de 15 à 20% des cas de SC endogène chez l'adulte (Tableau 1.I) ; dans 90 à 98% des cas, il est secondaire à des tumeurs unilatérales (Latronico & Chrousos 1997) (Nieman & Cutler 1995) (Orth & Kovacs 1998). En revanche, chez l'enfant, les lésions surrénaliennes primaires rendent compte de 65% des cas de SC; de plus, les carcinomes sécrétant du cortisol sont 3 à 4 fois plus fréquents que les adénomes. Pour des raisons encore inconnues, la présence de tumeurs surrénaliennes est plus fréquente chez la femme que chez l'homme avec un ratio de 4 pour 1 pour les adénomes et de 2 pour 1 pour les carcinomes (Bertagna & Orth 1981) (Bornstein *et al.* 1999) (Latronico & Chrousos 1997) (Ross 1994).

1.2.2. Physiopathologie du syndrome de Cushing surrénalien

1.2.2.1. Hyperplasies surrénaliennes bilatérales

Moins de 10% des cas de SC ACTH-indépendant sont secondaires à des lésions surrénaliennes bilatérales de physiopathologies diverses (Tableau 1.I). Ainsi, dans la dysplasie micronodulaire pigmentaire bilatérale surrénalienne ou PPNAD (*primary pigmented nodular adrenocortical disease*), de petits nodules noirs ou bruns sécréteurs sont répartis sur toute la glande, dont la taille reste habituellement normale, et sont entourés de cortex atrophique. Ces nodules expriment des taux élevés de synaptophysine

suggérant leur origine neuro-endocrine. Certains patients présentent une sécrétion paradoxale de cortisol lors du test de Liddle de suppression à la dexaméthasone (Stratakis *et al.* 1999). Des mutations de gènes encore non identifiés, situés au locus 2p16, et du gène codant pour la protéine kinase A de type 1-α, situé sur le chromosome 17, sont reliées au syndrome de Carney qui associe un PPNAD à des myxomes, des lésions pigmentées cutanées, des schwannomes et à plusieurs autres néoplasies endocriniennes (Carney *et al.* 1985) (Casey *et al.* 1998) (Kirschner *et al.* 2000) (Stratakis *et al.* 1996). Dans le syndrome de McCune-Albright, des mutations activatrices du gène codant pour la protéine Gsα (mutations gsp) entraînent une hyperactivité constitutive de la voie de signalisation de l'adénosine monophosphate cyclique (AMPc), responsable de l'augmentation de la stéroïdogénèse dans les nodules surrénaliens bilatéraux en l'absence d'ACTH (Weinstein *et al.* 1991). Ces mutations ne sont pas retrouvées dans le tissu cortical internodulaire qui devient alors atrophique.

Une cause encore plus rare de SC (moins de 1% des cas) est l'hyperplasie surrénalienne macronodulaire bilatérale ACTH-indépendante ou AIMAH (*ACTH-independent bilateral macronodular adrenal hyperplasia*) (Newell-Price *et al.* 1998) (Nieman & Cutler 1995) (Orth & Kovacs 1998) (Yanovski & Cutler, Jr. 1994). Cette pathologie est décrite sous divers noms : MMAD *(massive macronodular adrenal disease*), AMAH (*autonomous macronodular adrenal hyperplasia*), AIMBAD (*ACTH-independent massive bilateral adrenal disease*) ou encore maladie surrénalienne macronodulaire géante (Stratakis & Kirschner 1998). Les signes cliniques de la maladie apparaissent tardivement, vers 50-60 ans. On observe une égale distribution selon le sexe comparativement à la maladie de Cushing ou aux tumeurs surrénaliennes unilatérales qui ont une plus grande prévalence chez la femme. La plupart des cas sont sporadiques; cependant, quelques cas familiaux ont également été décrits (Cooper *et al.* 1998)

(Findlay *et al.* 1993) (Grunenberger *et al.* 1999) (Minami *et al.* 1996). Une mutation gsp (R201S) a récemment été décrite chez un patient atteint d'AIMAH ne présentant aucune autre caractéristique du syndrome de McCune-Albright (Fragoso *et al.* 1999).

1.2.2.2. Adénomes et carcinomes surrénaliens

Comme pour la plupart des tumeurs, la majorité des adénomes et carcinomes surrénaliens sont d'origine monoclonale, alors que les hyperplasies bilatérales sont d'origine polyclonale (Bornstein *et al.* 1999) (Reincke *et al.* 2000). Les hyperplasies et tumeurs corticosurrénaliennes sont plus fréquentes dans les syndromes pour lesquels des mutations de proto-oncogènes ou de gènes suppresseurs ont été identifiées (Latronico & Chrousos 1997). Une perte d'hétérozygotie du chromosome 17p et des mutations du gène suppresseur P53 ont été décrites dans des tumeurs corticosurrénaliennes isolées ou associées au syndrome de Li-Fraumeni, caractérisé par une grande prédisposition au développement de divers cancers (Gicquel *et al.* 2000). Des délétions du locus 11p15 ont été identifiées dans des lésions sporadiques ou associées au syndrome de Beckwith-Wiedemann, caractérisé par une série d'anomalies du développement (excédent de poids à la naissance, organomégalie, macroglosie, prédisposition aux tumeurs embryonnaires) due à la dérégulation de multiples gènes soumis à l'empreinte parentale. On observe fréquemment, associées à une expression biallélique d'IGF-II (*type 2 insulin-like growth factor*) dans des corticosurrénalomes malins, des isodisomies paternelles au locus 11p15.5 qui comprend, entre autres, les gènes de l'insuline, H-Ras1 (codant pour une GTPase de la famille ras, agissant comme une oncoprotéine), IGF-II, H-19 (impliqué dans la régulation d'IGF-II) et $P57^{KIP2}$ (Latronico & Chrousos 1997) (Gicquel *et al.* 2000).

Les adénomes et carcinomes corticosurrénaliens sont également très fréquents dans le syndrome d'endocrinopathies multiples qui est associé au gène MEN-1, situé au locus 11q13 (Chandrasekharappa *et al.* 1997), et code pour une protéine nucléaire dont la fonction reste encore inconnue (Guru *et al.* 1998). D'autres anomalies génétiques sont plus rarement retrouvées dans les corticosurrénalomes telles que des mutations du gène codant pour la protéine Giα, du proto-oncogène N-RAS et la surexpression du gène de susceptibilité au rétinoblastome (RB) (Latronico & Chrousos 1997) (Lyons *et al.* 1990). Une étude récente a rapporté la surexpression du gène anti-apoptotique *hDiminuto/Dwarf1* dans des adénomes corticosurrénaliens de patients atteints d'un SC ACTH-indépendant, suggérant une possible implication dans le processus de tumorigénèse (Sarkar *et al.* 2001).

1.3. Régulation hormonale des fonctions corticosurrénaliennes

1.3.1. L'axe hypothalamo-hypophyso-surrénalien (HSS)

1.3.1.1. Physiologie de l'axe HHS

La capacité de l'organisme de s'adapter à un environement variable est fonction de son aptitude à générer une réponse physiologique spécifique, transitoire et régulée face à un stress aïgu. Sont impliqués les systèmes HHS et sympatho-médullosurrénalien; ce dernier ne sera pas décrit ici.

L'activation de l'axe corticotrope implique principalement les neurones de la région parvocellulaire située dans le noyau paraventriculaire hypothalamique où la corticolibérine (CRH) et l'arginine-vasopressine (AVP) sont synthétisées puis libérées dans le système porte hypothalamo-hypophysaire via l'éminence médiane (Baylis 1991) (Grossman 1995). La liaison du CRH et de l'AVP à leur récepteur respectif, CRH-R1 (Chen *et al.* 1993) et AVP-V3R (ou V1bR) (de Keyzer *et al.* 1994), au niveau des cellules

corticotropes du lobe antérieur hypophysaire, stimule la synthèse et la maturation de POMC, conduisant à la sécrétion d'ACTH (Bertagna 1994). Celle-ci exerce son action sur la corticosurrénale en entraînant la sécrétion de glucocorticoïdes (GC) par la zone fasciculée/réticulée, de minéralocorticoïdes (aldostérone) par la zone glomérulée et, dans une moindre mesure, d'androgènes (déhydroépiandrostérone ou DHEA) par la zone réticulée (Figure 1.0). Une boucle de rétroaction négative s'instaure via les GC qui inhibent la synthèse et la sécrétion de CRH, d'AVP et d'ACTH aux niveaux hypophysaire et hypothalamique.

1.3.1.2. Régulation centrale de l'axe corticotrope

Le VIP (*vasoactive intestinal polypeptide*) et le PACAP (*pituitary adenylate cyclase activating peptide*), qui sont également synthétisés au niveau des neurones hypothalamiques, stimulent la sécrétion de CRH et d'ACTH (Nussdorfer & Malendowicz 1998) (Watanobe & Tamura 1994). La libération de CRH peut être régulée positivement, dans le noyau paraventriculaire, par les agonistes adrénergiques (αAR_1), sérotoninergiques (5-HTR_{1A}), muscariniques, nicotiniques et histaminiques. Elle est aussi stimulée par l'angiotensine II (Ang-II), le neuropeptide Y (NPY), la cholécystokinine (CCK) et le GRP (*gastrin-releasing peptide*) ou inhibée par le peptide natriurétique (ANP), la substance P, la somatostatine, monoxyde d'azote et les agonistes γ-aminobutyriques (Grossman 1995). Plusieurs cytokines dont l'interleukine-1, le TNF-α (tumor necrosis factor) et l'interleukine-6 augmentent la libération de CRH via la production de prostaglandines au niveau de l'endothélium vasculaire du cerveau (Lacroix & Rivest 1998).

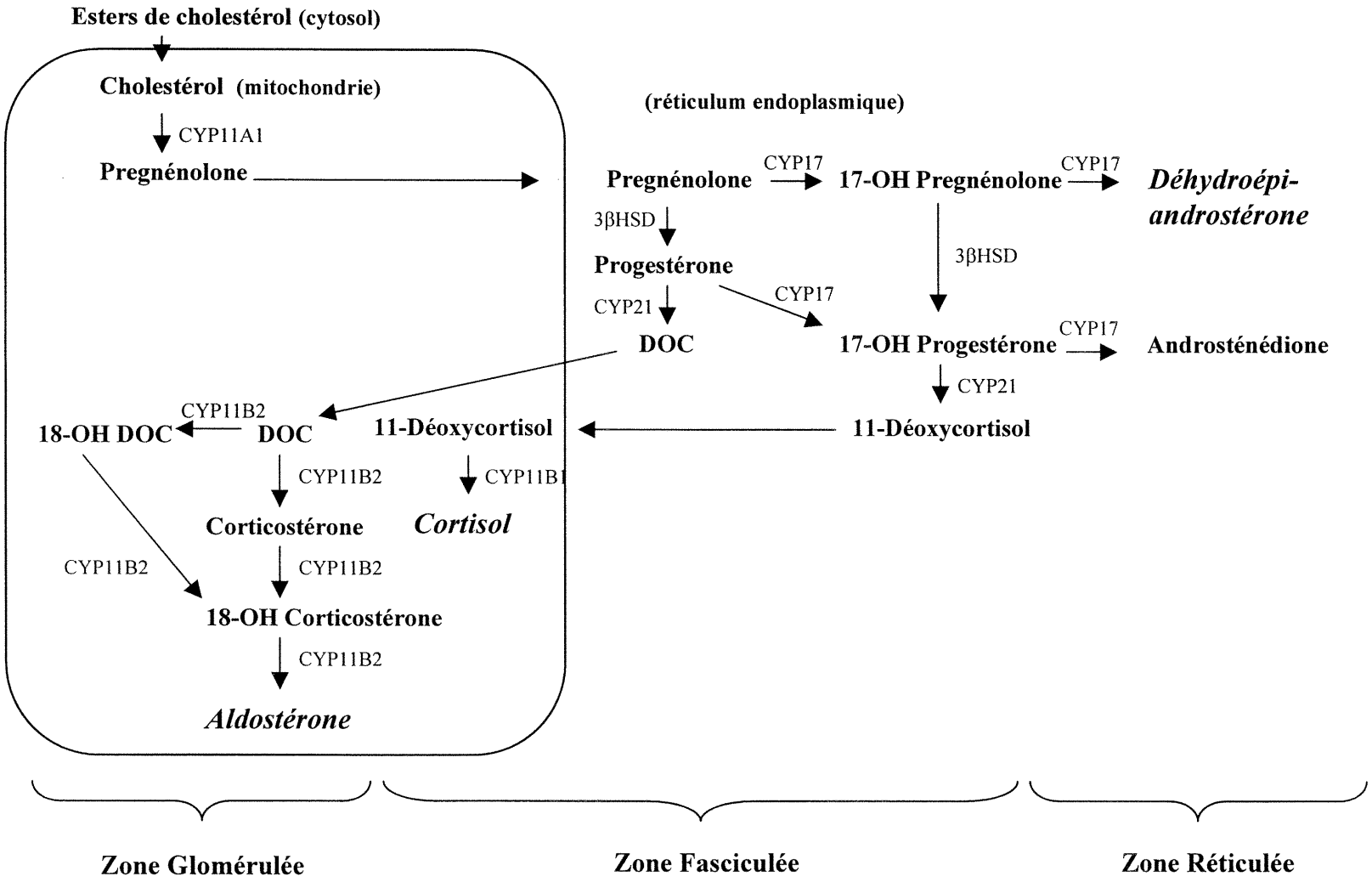


Figure 1.0. Stéroïdogénèse surrénalienne. La corticosurrénale comporte trois zones fonctionnellement distinctes: sous la capsule, se trouvent la zone glomérulée, la zone fasciculée et la zone réticulée qui touche la médullosurrénale. La zone glomérulée, qui n'exprime pas la CYP17, synthétise l'aldostérone (minéralocorticoïde); la zone fasciculée, qui possède les enzymes 3βHSD et CYP17, produit le cortisol (glucocorticoïde); la zone réticulée produit principalement la DHEA (androgène) via les enzymes CYP17 et DHEA sulfotransférase. 3βHSD: 3β-hydroxystéroide déshydrogénase; DOC: déoxycorticostérone.

La production d'ACTH peut être modulée par des interactions autocrines ou paracrines tant au niveau central que périphérique; en effet, il a été démontré que les cellules corticotropes de l'hypophyse expriment le CRH et qu'elles peuvent stimuler la sécrétion d'ACTH (Giraldi & Cavagnini 1998). Il existe également, dans la médullosurrénale, un système CRH/ACTH régulant de façon paracrine les fonctions de la corticosurrénale (Ehrhart-Bornstein *et al.* 1998) (Nussdorfer 1996). Chez le rat, il a été montré que le CRH et l'AVP d'origine surrénalienne peuvent augmenter la sécrétion d'ACTH *in vitro*.

1.3.2 Régulation de la stéroïdogénèse: rôle de l'ACTH

1.3.2.1. Phase précoce : mobilisation du cholestérol

L'ACTH, en se liant à son récepteur membranaire MC2R (*melanocortin receptor type 2*), génère une série de réponses spécifiques à court et long termes (effets aïgus et chroniques) tel qu'illustré dans la figure 1.1 (Lehoux *et al.* 1998) (Simpson & Waterman 1988). L'activation de la cascade de l'adénylate cyclase (AC)/AMPc/PKA (*AMPc-dependent protein kinase*) mène à la phosphorylation de protéines qui régulent les étapes précoces et tardives de la stéroïdogénèse (Miller 1988) (Stocco & Clark 1996). L'ACTH provoque rapidement (en quelques minutes) la mobilisation et le transfert du cholestérol vers la membrane mitochondriale interne (Stocco & Clark 1996). Le principal modulateur de cette étape est la protéine StAR (*steroidogenic acute regulatory*), inductible par l'ACTH, et dont le gène, lorsqu'invalidé (chez la souris) ou muté (chez l'homme), entraîne un déficit en hormones stéroïdiennes et une hyperplasie de la surrénale (hyperplasie surrénalienne congénitale lipoïdique) (Bose *et al.* 1996) (Lin *et al.* 1995). Une seconde protéine impliquée dans ce processus est le récepteur des benzodiazépines périphérique qui délivre le cholestérol à l'enzyme CYP11A1 (P450scc) pour être converti en prégnénolone.

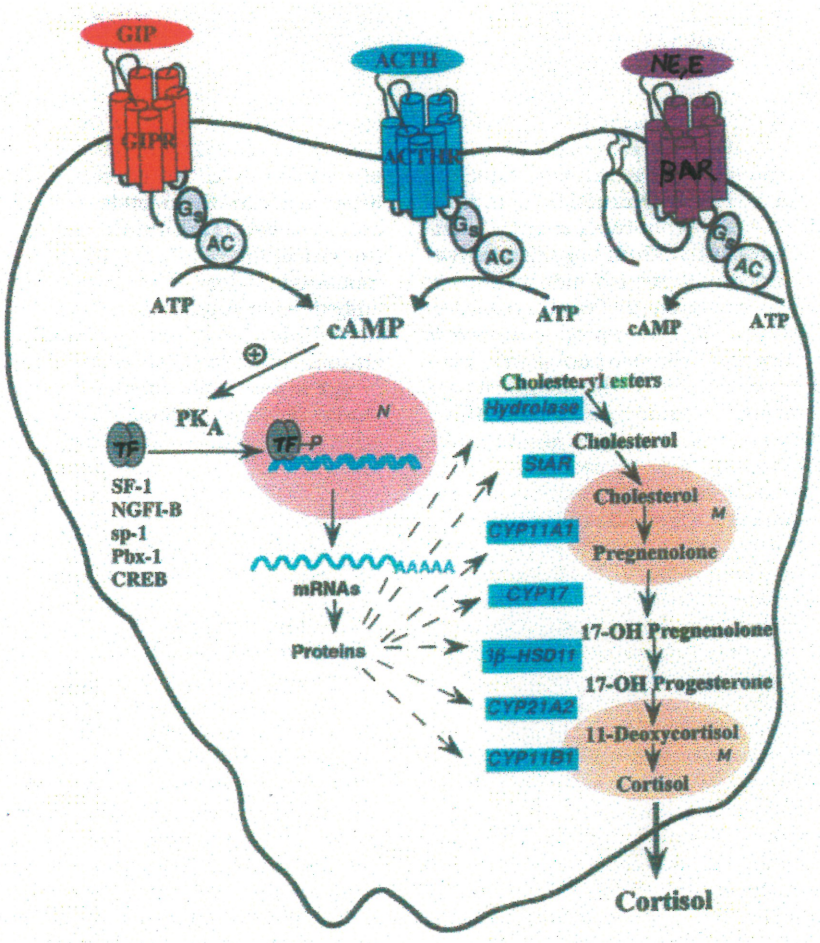


Figure 1.1. Régulation de la stéroïdogénèse par les récepteurs hormonaux anormaux ou ectopiques. Au niveau de la cellule corticosurrénalienne, l'ACTH est le modulateur physiologique principal de la stéroïdogénèse. Suite à la liaison de l'ACTH à son récepteur (ACTHR), la protéine de couplage Gs est activée, puis l'adénylate cyclase, qui active la production d'AMPc. Ce dernier active la PKA qui à son tour active les facteurs de transcription cibles (SF-1, NGFI-B, Sp1, Pbx-1, CREB). Ceux-ci régulent la disponibilité en cholestérol libre ainsi que l'expression des gènes de la stéroïdogénèse. Des effets directs, non illustrés ici, sont également relayés par l'ACTH, tels que l'activation des enzymes CYP et de la protéine StAR. L'expression ectopique de récepteurs hormonaux fonctionnellement couplés à la stéroïdogénèse confère une sensibilité inappropriée des cellules corticosurrénaliennes au GIP, aux cathécolamines (E,NE) ou à d'autres hormones. Ces récepteurs exerceraient leur action selon les mêmes mécanismes que le récepteur de l'ACTH. [Permission de A. Lacroix et *al.* : *Endocr Rev* 22 :75-110, 2001. © The Endocrine Society.]

L'ACTH induit l'augmentation de l'expression des gènes précoces c-Fos et c-Jun par la voie de signalisation PKA-dépendante, initiant ainsi une cascade d'événements intracellulaires menant à une réponse stéroïdogénique rapide (Hol *et al.* 1995) (Lehoux & Ducharme 1995) (Simpson & Waterman 1988). De plus, l'hormone exerce ses effets à long terme en établissant une boucle d'auto-régulation positive alimentée par l'ACTH qui stimule l'expression de son propre récepteur (Mountjoy *et al.* 1994).

1.3.2.2. Phase tardive: régulation transcriptionnelle

Les effets chroniques de l'ACTH durent plusieurs heures et impliquent la régulation transcriptionnelle et/ou post-transcriptionnelle de plusieurs gènes stéroïdogéniques, tels que la CYP11A1, la 3 β-hydroxystéroide déshydrogénase de type II (3β-HSD), la CYP17 ($P450c_{17}$), la CYP21A2 ($P450c_{21}$) et la CYP11B1 ($P450c_{11}$) (Di Blasio *et al.* 1987) (Miller 1988) (Simpson & Waterman 1988). Cette régulation à long terme semble complexe puisqu'aucune corrélation claire n'a pu être établie entre les taux de messager et de protéine de ces enzymes *in vivo* (Lehoux *et al.* 1998).

La plupart des effets de l'ACTH sont relayés par l'action de facteurs de transcription (FT) spécifiques dont les récepteurs nucléaires orphelins tels que nurr77 (aussi appelé NGFI–B) (Murphy & Conneely 1997) et SF-1 (*steroidogenic factor 1*) (Parker & Schimmer 1997) (Sugawara *et al.* 1997). En effet, un stimulus de stress active la transcription de ces deux facteurs dans les corticotropes et la corticosurrénale (Ingraham *et al.* 1994) (Murphy & Conneely 1997). Nurr77 et SF-1 sont activés respectivement par déphosphorylation et phosphorylation et modulent l'expression des gènes des enzymes de la stéroïdogénèse au niveau de la corticosurrénale (Li & Lau 1997) (Parker & Schimmer 1997) (Wilson *et al.* 1993).

Le facteur SF-1 module l'expression constitutive et régulée par l'AMPc des gènes CYP11A (Clemens *et al.* 1994) (Morohashi *et al.* 1993) (Takayama *et al.* 1994) (Watanabe *et al.* 1994)) et CYP17 (Zhang & Mellon 1996) (Zhang & Mellon 1997). L'analyse des promoteurs de ces gènes a mené à l'identification d'éléments de réponse à l'AMPc (CRE) et des FTs s'y liant ou favorisant la transcription stimulée par l'AMPc; des FTs généraux comme la protéine CREB (*cAMP responsive element binding*) et l'homéoprotéine Pbx1 se lient toutes deux à des motifs CRE et amplifient l'expression des gènes stéroïdogéniques stimulée par l'AMPc (Bischof *et al.* 1998a) (Inoue *et al.* 1991) (John *et al.* 1986) (Kagawa *et al.* 1994) (Ogo *et al.* 1997). Un autre facteur ubiquitaire, Sp1, régule l'expression basale et AMPc-dépendante du gène CYP11A (Venepally & Waterman 1995). Des données récentes suggèrent que le facteur SF-1 peut contrôler l'expression AMPc-dépendante du gène CYP17: l'élément CRE proximal situé de -80 à -40 pb a été identifié en tant que site de liaison du facteur SF-1 (Bakke & Lund 1995); de plus, il a été démontré, *in vitro*, qu'une mutation dominante négative empêchant la lisaison de SF-1 abolit également l'expression modulée par l'AMPc du gène rapporteur utilisé (Jacob & Lund 1998). L'intégration des signaux intracellulaires générés par ces FT (SF-1, Sp1, CREB et probablement Pbx1) serait effectuée par le co-activateur CBP (*CREB binding protein*) assurant ainsi une régulation coordonnée de l'expression des gènes CYP11A et CYP17 (Bischof *et al.* 1998b) (Monte *et al.* 1998).

Par ailleurs, SF-1 stimule l'expression et l'activité de la protéine StAR (Rust *et al.* 1998); nurr77 et nurr1 (*nur-related fator 1*) régulent positivement l'expression du précurseur POMC au niveau de l'hypophyse (Philips *et al.* 1997a) (Philips *et al.* 1997b). L'invalidation du gène nur77 n'entraîne aucun phénotype remarquable chez la souris; ce qui suggère que d'autres membres de la famille des facteurs nur peuvent suppléer aux fonctions de nur77 chez la souris et probablement chez l'homme. En revanche, SF-1 joue un rôle-clé dans le développement et la survie des organes stéroïdogéniques tel que le démontre le phénotype gravement altéré des souris SF-1$^{-/-}$ (agénésie des surrénales et des gonades, réversion du sexe de mâle à femelle) (Lee *et al.* 1995) (Parker 1998).

1.3.3. Régulation de la stéroïdogénèse: rôle des hormones peptidiques et neurotransmetteurs

1.3.3.1. L'arginine vasopressine

Bien que l'ACTH soit le principal activateur de la stéroïdogénèse, celle-ci est régulée par d'autres hormones peptidiques, neuropeptides, neurotransmetteurs, ions et cytokines (Clements & Funder 1986) (Ehrhart-Bornstein *et al.* 1998) (Gallo-Payet & Guillon 1998) (Jones & Gillham 1988;Turnbull & Rivier 1999). La vasopressine exerce son action stéroïdogénique au niveau central en tant que sécrétagogue de l'ACTH. Néanmoins, des études *in vivo* et *in vitro* ont clairement démontré que l'AVP stimule directement la sécrétion d'aldostérone et de cortisol à partir de la glande surrénale chez le bœuf (Bird *et al.* 1990b) (Bird *et al.* 1990a). Chez le rat, l'AVP stimule uniquement la sécrétion d'aldostérone. Chez l'homme, elle augmente la production d'aldostérone (250%) et de cortisol (60-260%) *in vitro* (Guillon *et al.* 1995) (Guillon *et al.* 1998) (Perraudin *et al.* 1993). Son action est relayée par l'activation des récepteurs vasopressinergiques de type VI (AVP V1R), principalement localisés dans la zone glomérulée et, dans une moindre mesure, dans la zone fasciculée (Arnaldi *et al.* 1998b) (Gallo-Payet *et al.* 1986) (Gallo-Payet & Guillon 1998) (Guillon & Gallo-Payet 1986). Bien que la présence de récepteur de type V2 ait été mise en évidence par RT-PCR dans la corticosurrénale humaine, ce dernier ne semble cependant pas jouer de rôle dans le contrôle de la stéroïdogénèse (Arnaldi *et al.* 1998b) (Lacroix *et al.* 1997b). Le récepteur de type V3 n'est pas exprimé dans la corticosurrénale humaine (Arnaldi *et al.* 1998b), mais dans les cellules chromaffines de la médullosurrénale d'où il peut stimuler la libération de cathécolamines tout comme chez le rat (Gallo-Payet & Guillon 1998) (Grazzini *et al.* 1996) (Guillon *et al.* 1998).

Etant exprimée dans l'hypophyse et dans la glande surrénale, l'AVP peut réguler les fonctions corticosurrénaliennes selon un mode endocrine ou paracrine. L'importance physiologique de ses effets reste toutefois incertaine puisqu'on n'observe aucune diminution significative de la sécrétion de cortisol chez les patients atteints de diabète insipide dû à un déficit vasopressinergique hypophysaire (Elias *et al.* 1997) (Mazza *et al.* 1994).

1.3.3.2. Cathécolamines et sérotonine (5-HT)

Il a été également démontré que les cathécolamines peuvent stimuler la sécrétion d'aldostérone et de cortisol, *in vitro*, via les adrénorécepteurs de type β1 (β1-AR) à partir de cellules corticosurrénalienne de boeuf, de porc et de volaille; elles n'ont cependant aucun effet sur la corticosurrénale humaine *in vitro* (Lefebvre *et al.* 1998).

La 5-HT est un autre neurotransmetteur qui joue un rôle important dans le contrôle de la stéroïdogénèse. Elle stimule, *in vitro*, la production d'aldostérone, de corticostérone et de cortisol directement à partir des cellules de corticosurrénale de rat, de grenouille et humaine (Delarue *et al.* 1988b) (Lefebvre *et al.* 1992) (Lefebvre *et al.* 1998), mais aussi indirectement, en augmentant le flux sanguin surrénalien (Hinson *et al.* 1989). Chez l'homme et la grenouille, ces effets sont relayés par le récepteur sérotoninergique de type 4 (5-HT_4R) qui active les voies de signalisation du calcium et de l'AMPc (Delarue *et al.* 1988b) (Lefebvre *et al.* 1992). Chez le rat, la sécrétion d'aldostérone est stimulée via le récepteur 5-HT_7 (Contesse *et al.* 1999). *In vivo*, les agonistes du récepteur 5-HT_4 tels que le cisapride et le zacopride induisent uniquement une augmentation de la sécrétion d'aldostérone chez l'homme (Lefebvre *et al.* 1993) (Lefebvre *et al.* 1995).

Tout comme pour l'AVP, un mode d'action paracrine a été proposé pour la 5-HT dont la présence a été démontrée dans les mastocytes périvasculaires humains ainsi que dans les cellules chromaffines de la médullosurrénale de grenouille, de rat et de souris (Delarue *et al.* 1988a) (Fernandez-Vivero *et al.* 1993) (Verhofstad & Jonsson 1983). La 5-HT est également un sécrétagogue connu de l'ACTH, agissant au niveau central. De plus, ce neurotransmetteur peut activer le sytème rénine-angiotensine (SRA) systémique, élevant ainsi la concentration sanguine d'aldostérone. L'hypothèse d'un mécanisme de sécrétion analogue, impliquant les sytèmes CRH/AVP, 5-HT et SRA surrénaliens, est plausible mais reste néanmoins à démontrer.

1.3.3.3. VIP et PACAP

Les peptides VIP et PACAP, synthétisés dans les cellules chromaffines de la médullosurrénale, régulent l'activité sécrétoire de la corticosurrénale de façon paracrine chez l'homme, le rat et le taureau (Nussdorfer & Malendowicz 1998). Le VIP stimule la sécrétion d'aldostérone en activant les récepteurs sélectifs de types 2 et 3 (VIPR2/VIPR3). Il stimule également la sécrétion de cortisol, bien que modérément, en activant de façon non spécifique le récepteur de l'ACTH, MC2R (Bodnar *et al.* 1997) (Bornstein *et al.* 1996) (Li *et al.* 1990).

Ces deux neuropeptides contrôlent également la stéroïdogénèse par des mécanismes de régulation indirects. En effet, tous deux stimulent, à partir des cellules chromaffines, la sécrétion de cathécolamines (Anderova *et al.* 1998) (Guo & Wakade 1994) entraînant ainsi la libération d'aldostérone via l'activation des récepteurs β-adrénergiques (Bernet *et al.* 1994) (Hinson *et al.* 1992). En outre, l'augmentation du flux sanguin surrénalien induite par le VIP et PACAP accroît la capacité stéroïdogénique de la corticosurrénale (Hinson *et al.* 1994) (Nussdorfer 1996).

1.3.3.4. L'angiotensine II

L'angiotensine II (Ang-II), peptide biologiquement actif du SRA, et l'ion potassium (K^+) sont les principaux modulateurs de la synthèse et de la sécrétion d'aldostérone (Orth & Kovacs 1998). Un surplus potassique, une baisse de la natrémie ou du volume sanguin activent le SRA conduisant séquentiellement à la libération d'Ang-II et d'aldostérone. L'Ang-II exerce son action via le récepteur AT_1 (AT_1R) qui se trouve couplé à la phospholipase C (PLC); elle provoque la diminution de l'excrétion de K^+ par le rein et la rétention de Na^+ à partir du colon réglant ainsi le bilan Na^+, K^+ de l'organisme.

Tout comme l'ACTH, l'Ang-II produit des effets à long terme en régulant l'activité transcriptionnelle des gènes stéroïdogéniques. Des études ont montré que l'Ang-II stimule la stéroïdogénèse en augmentant l'expression de la protéine StAR dans la lignée cellulaire de corticosurrénalome humain, H295R (Clark *et al.* 1995). De plus, elle favorise spécifiquement la synthèse d'aldostérone en stimulant la transcription de la CYP11B2 (P450 aldo synthase) et du récepteur AT1R chez le rat, et en inhibant la transcription de la CYP17 dans les cellules corticosurrénaliennes ovines (Bird *et al.* 1992). L'Ang-II inhibe également l'expression de son propre récepteur AT1R dans les cellules de fasciculée humaines et bovines (Naville *et al.* 1993) (Penhoat *et al.* 1991). L'existence d'un SRA local au niveau de la corticosurrénale suggère un mode de régulation paracrine de l'homéostasie de l'aldostérone (Gupta *et al.* 1995) (Mulrow 1998) (Vinson & Ho 1998).

L'aldostéronémie est aussi régulée par des signaux inhibiteurs: ainsi, la dopamine et la somatostatine abolissent la réponse sécrétoire induite par l'Ang-II (Kasprzak *et al.* 1991) (Missale *et al.* 1988). De plus, il a été démontré, *in vitro*, que les peptides natriurétiques ANP et CNP, présents aussi bien dans la circulation systémique

que dans la médullosurrénale, freinent la production d'aldostérone (Bodart *et al.* 1996) (Kawai *et al.* 1996). Par ailleurs, l'ANP contrecarre les effets de l'Ang-II et de l'ACTH sur la sécrétion d'aldostérone en réduisant le taux d'expression de la protéine StAR (Cherradi *et al.* 1998).

1.3.3.5. La leptine

D'autres neuropeptides modulent la fonction stéroïdogénique de la corticosurrénale tant au niveau central que périphérique. Par exemple, l'endothéline 1 (ET-1) (Hinson & Kapas 1998) (Nussdorfer *et al.* 1997) et le neuropeptide Y (Malendowicz *et al.* 1996) (Mazzocchi *et al.* 1996) stimulent la sécrétion de cortisol et d'aldostérone. Il est d'ailleurs reconnu que l'augmentation des taux hypothalamiques de NPY entraîne hypercorticisme, hyperinsulinémie et résistance à l'insuline, trois défauts endocriniens retrouvés dans les syndromes de Cushing et d'obésité (Vettor *et al.* 1994).

Récemment, un intérêt grandissant s'est porté sur la leptine. Cette cytokine, synthétisée et sécrétée par le tissu adipeux, a été initialement identifiée comme agent anorexigène. Agissant principalement au niveau hypothalamique, elle diminue les taux de NPY et donc la prise alimentaire. Plusieurs études montrent l'existence d'interactions entre l'axe corticotrope et la leptine. *In vivo*, les concentrations de leptine et d'ACTH sont négativement corrélés, dénotant leur action antagoniste (Licinio *et al.* 1997). Chez l'homme et la souris, l'injection de leptine exogène freine l'activation de l'axe HHS induite par le jeûne (Ahima *et al.* 1996) (Bornstein *et al.* 1997) (Heiman *et al.* 1997) (Huang *et al.* 1998) (Pralong *et al.* 1998) (Rohner-Jeanrenaud & Jeanrenaud 1996); cela se traduit par une baisse de l'induction des messagers de CRH et CYP17 dans l'hypophyse et la corticosurrénale. Le même effet inhibiteur est observé, en condition

basale (axe HHS non stimulé), chez des souris obèses ou non, traitées à la leptine: l'expression hypophysaire du précurseur POMC et de son enzyme de maturation PC2 est fortement diminuée (Renz *et al.* 2000). La leptine et son récepteur Ob-R sont exprimés aussi bien dans l'hypophyse que dans la surrénale (Cao *et al.* 1997) (Glasow *et al.* 1998) (Jin *et al.* 1999) (Pralong *et al.* 1998) (Shimon *et al.* 1998).

Certaines études montrent cependant un effet activateur de la leptine au niveau de l'hypophyse où les taux augmentés de CRH et d'ACTH entraînent la sécrétion de cortisol (Malendowicz *et al.* 1998) (Schwartz *et al.* 1996) (Raber *et al.* 1997). Il est possible que les effets chroniques et aigus de la leptine soient divergents, à l'exemple du CRH. Ce dernier agit comme anorexigène lorsque sécrété de façon aiguë, puis un effet orexigène s'instaure suite à l'activation chronique de l'axe corticotrope. Ces divergences peuvent également refléter des différences anatomiques et fonctionnelles des neurones à CRH sur lesquels la leptine exercerait une action inhibitrice ou stimulatrice selon le type de population neuronale ciblée.

Il est bien établi que les GC stimulent la sécrétion de leptine, contrecarrant ainsi leur effet orexigène (Slieker *et al.* 1996) (Spinedi & Gaillard 1998). On retrouve, en effet, des taux élevés de leptine chez les patients atteints de SC (Cizza *et al.* 1997) (Pralong *et al.* 1999). Cependant, l'ACTH régule négativement la production de leptine qui, elle-même, inhibe la sécrétion d'ACTH (Renz *et al.* 2000). Ainsi, dans un contexte physiologique normal, ces boucles de régulation multiples contrôlent finement les concentrations relatives des différentes hormones impliquées.

1.3.4. Régulation de la croissance cellulaire

Outre la fonction stéroïdogénique, la croissance cellulaire au sein de la glande surrénale est aussi finement régulée. La surrénale adulte est constituée de deux entités fonctionnelles d'origine embryonnaire distincte: les cellules de la crête neurale forment

la médullosurrénale; les cellules provenant du tractus urogénital donnent naissance à la corticosurrénale. Le cortex fœtal est constitué de cellules différenciées formant la zone fœtale et de cellules immatures formant la zone définitive. Celle-ci est le siège de prolifération et d'hyperplasie cellulaires et donne naissance aux trois zones corticales de la surrénale adulte. La zone foetale, qui constitue 80% de la surrénale fœtale, subit un remodelage cellulaire par apoptose menant à son involution peu après la naissance (Ramayya 2001). De récentes données indiquent que ce processus de mort cellulaire est régulé par l'Ang-II via son récepteur AT_2, majoritairement exprimé dans la zone foetale (Breault *et al.* 1996) (Chamoux *et al.* 1999). Le centre de la surrénale se retrouve alors colonisé par de petits amas de cellules chromaffines qui s'organisent pour former la médullosurrénale.

Selon la théorie dite de la "migration'', l'intégrité de la glande surrénale adulte est préservée grâce à un processus constant de division cellulaire dans la zone glomérulée (Wolkersdörfer & Bornstein 2001). Les cellules nouvellement produites migrent de façon centripète et se transforment en cellules fasciculées (Belloni *et al.* 1978). Une stimulation chronique par l'ACTH induit la différenciation des cellules glomérulées en cellules fasciculées (Kahri 1966). Les GC inhibent ce changement phénotypique en réduisant l'expression de l'enzyme CYP11A (Arola *et al.* 1994) (Salmenpera 1976) (Salmenpera *et al.* 1976). Ils semblent jouer un rôle important dans l'établissement et le maintien des zones anatomiques de la corticosurrénale. En effet, des taux élevés de GC (concentrations physiologiques observées au sein de la corticosurrénale du fait de la vascularisation centripète de l'organe) inhibent l'étape de 18-hydroxylation catalysée par la CYP21A2 dans des cellules de surrénale fœtale humaine traitées à l'ACTH, entraînant un déficit en 18-OH-déoxycorticostérone et en aldostérone (Ehrhart-Bornstein *et al.* 1998).

Contrairement aux GC, l'ACTH provoque une hypertrophie et une hyperplasie de la corticosurrénale, *in vivo*, selon un processus réversible (Payet & Lehoux 1980). Paradoxalement, l'hormone corticotrope inhibe la prolifération cellulaire *in vitro*: une courte exposition à l'ACTH (2 heures) entraîne un effet trophique corrélé à l'induction PKA-dépendante de c-Fos et c-Jun (Armelin *et al.* 1996) (Penhoat *et al.* 1996); après 24 heures d'exposition, le niveau d'expression de c-Myc s'effondre et un ralentissement de la croissance cellulaire est observé (Armelin *et al.* 1996) (Watanabe *et al.* 1997)). De récentes données suggèrent que l'action trophique de l'ACTH pourrait résulter de l'activation d'une voie de signalisation indépendante de l'AMPc (Kimura *et al.* 1993) (Lotfi *et al.* 1997). En effet, il a été démontré, *in vivo et in vitro,* que l'ACTH stimule l'expression des gènes précoces c-Fos, c-Jun et c-Myc en activant la voie des MAP (*mitogen activated protein*)-kinases (Karin 1995) (Watanabe *et al.* 1997).

D'autres hormones sont impliquées dans le contrôle de la prolifération cellulaire telles que l'Ang-II, ET-1, VIP et la somatostatine entre autre. Une stimulation chronique par l'Ang-II induit l'hypertrophie de la zone glomérulée, *in vivo,* en activant la voie des MAP-kinases par un mécanisme PKC-dépendant (Penhoat *et al.* 1996) (Watanabe *et al.* 1996). ET-1 stimule également la prolifération des cellules de la glomérulée *in vitro* et *in vivo*, en interagissant avec son récepteur ET_A (Hinson & Kapas 1998). Ce dernier est spécifiquement exprimé dans la zone glomérulée. Le VIP, quant à lui, provoque une hyperplasie modérée de la zone glomérulée *in vivo* (Malendowicz & Nussdorfer 1993) (Rebuffat *et al.* 1994). La somatostatine exerce un effet antiprolifératif direct sur la zone glomérulée *in vivo* et antagonise l'effet de l'Ang-II (Kasprzak *et al.* 1991).

La régulation de la fonction trophique de la corticosurrénale fait également intervenir des facteurs de croissance tels que l'IGF-I (*insulin-like growth factor I*), l'GIF-II et le TGFβ1 (transforming *growth factor beta 1).* Les IGF-I et II exercent une action mitogénique sur la corticosurrénale. L'IGF-II est plus exprimé dans la surrénale fœtale, siège de nombreux événements de remodelage cellulaire, comparativement à la

surrénale adulte (Voutilainen *et al.* 1994). Il semble jouer un rôle important dans l'acquisition et le développement du phénotype tumoral car il est fortement exprimé dans les corticosurrénalomes hormonalement actifs (Gicquel *et al.* 1995) (Ilvesmaki *et al.* 1993). IGF-I et TGFβ1 produisent des effets opposés chez le bœuf en stimulant ou inhibant la transcription de gènes spécifiquement exprimés dans la surrénale : l'IGF-I augmente l'expression de MC2R, StAR et quelques enzymes stéroidogéniques tandis que TGFβ1 l'inhibe (Le Roy *et al.* 2000). Ce dernier semble être impliqué dans le processus de remodelage de la surrénale fœtale humaine puisqu'il inhibe la prolifération des cellules de la zone fœtale et induit leur mort par apoptose *in vitro*. Cependant, ceci n'a jamais été montré *in vivo*.

1.4. Récepteurs hormonaux anormaux dans les tumeurs surrénaliennes : études *in vitro*

1.4.1. Carcinome surrénalien de rat

Le concept d'expression ectopique de récepteurs hormonaux surrénaliens a été proposé, pour la première fois, par Robert Ney et ses collaborateurs en 1971 (Hingshaw & Ney 1974) (Schorr *et al.* 1971) (Schorr & Ney 1971). L'étude de la modulation de la stéroïdogénèse chez le rat a permis de montrer que des hormones autres que l'ACTH telles que les cathécolamines et l'hormone thyréotrope (TSH) stimulent l'AC du carcinome surrénalien 494 produisant de la corticostérone, mais pas celle de la corticosurrénale normale (Schorr & Ney 1971). Les cathécolamines augmentent l'activité de l'AC en interagissant spécifiquement avec les β-adrénorécepteurs. La présence de β-ARs ectopiques fonctionnels a ensuite été confirmée par d'autres groupes (Brush *et al.* 1974) (Williams *et al.* 1977) ; cependant, aucun effet direct sur la stéroïdogénèse n'a pu être vérifié du fait de la faible efficacité de couplage de l'AC à la stéroïdogénèse dans ces cellules tumorales en culture (Brush *et al.* 1974). La présence d'α-adrénorécepteurs ectopiques stimulant la guanylate cyclase a également été démontrée (Perchellet & Sharma 1980) (Shanker & Sharma 1980). D'autres études sur cette même tumeur de rat ont révélé un plus large spectre de sensibilité hormonale. En effet, la folliculostimuline

(FSH), l'hormone lutéinisante (LH) et, dans une moindre mesure, la prostaglandine E_1 activent l'AC contrairement au glucagon, à l'insuline, l'AVP, la parthormone et la calcitonine. Ces hormones n'exercent aucun effet synergique indiquant que leurs récepteurs spécifiques stimulent une AC commune (Figure 1.1).

1.4.2. Tumeurs corticosurrénaliennes humaines

Hingshaw et Ney (Hingshaw & Ney 1974) ont étudié la modulation hormono-dépendante de l'activité AC dans 3 adénomes sécrétant du cortisol (SC) et 1 carcinome sécrétant des androgènes (syndrome virilisant). Dans le carcinome, la production d'AMPc est augmentée par la TSH et l'ACTH mais aucunement par l'adrénaline, la LH ou le glucagon ; la même observation a été rapportée pour 1 des 3 adénomes. Cependant l'AC est plus faiblement stimulée. Les auteurs en ont tiré la conclusion suivante : « *At present the physiological significance of these aberrant tumor responses is uncertain, and their relationship to tumor function has to remain speculative. However it is possible that, in certain cases, the autonomous behavior of endocrine tumors may be more apparent than real, and that this behavior is the result of stimulation of the tumor by hormones other than the appropriate ones for the parent gland.* » (Hingshaw & Ney 1974).

D'autres exemples de couplage fonctionnel de récepteurs membranaires hormonaux, liés aux protéines G pour la plupart, ont été décrits dans des tumeurs corticosurrénaliennes humaines bénignes ou malignes : tel est le cas des récepteurs de la prolactine, de l'hormone lactoplacentaire, de la LH, FSH, TSH, des agonistes β–adrénergiques, de l'angiotensine II, du glucagon et de l'interleukine-1 (Lacroix *et al.* 2001). Le couplage anormal du récepteur LH/hCG (LH/hCGR) à la stéroïdogénèse a aussi été rapporté *in vitro* dans 2 adénomes surrénaliens sécrétant des androgènes et dans un carcinome surrénalien sécrétant du cortisol (Leinonen *et al.* 1991) (Pittaway *et al.* 1973) (Why *et al.* 2000).

1.5. Diversité des récepteurs hormonaux anormaux dans le SC surrénalien

Le concept de récepteurs hormonaux aberrants démontré *in vitro* trouva une signification clinique, *in vivo*, avec la description originale d'un cas de SC lié à l'alimentation en 1987 (Hamet *et al.* 1987). Plus tard, les premières observations d'hypercorticisme induit par une hormone gastrointestinale appelée GIP (*glucose-dependent insulinotropic peptide*) effectuées par deux équipes vinrent supporter l'hypothèse étiologique de l'expression aberrante de récepteurs hormonaux dans le SC surrénalien (Lacroix *et al.* 1992) (Reznik *et al.* 1992).

1.5.1. SC lié à l'alimentation et GIP-dépendant

Hamet et collaborateurs (Hamet *et al.* 1987) ont été les premiers investigateurs à décrire une production de cortisol dépendante de l'alimentation chez un homme de 41 ans atteint d'un SC secondaire à un adénome surrénalien unilatéral et présentant une hormonogénèse périodique. Chez ce patient, la cortisolémie est inhabituellement basse le matin à jeun et très élevée après les repas; cette augmentation post-prandiale n'était pas inhibée par l'administration orale de fortes de doses de dexaméthasone. L'activité de l'AC mesurée sur des préparations membranaires issues de l'adénome est augmentée de 27% par l'ACTH et de 62% par la vasopressine alors que la FSH, le glucagon et l'Ang-II sont sans effet. Un autre cas de SC secondaire à un adénome surrénalien unilatéral est également rapporté chez un patient présentant un rythme de sécrétion du cortisol similaire au cas précédent avec des pics durant la journée aux heures présumées (mais non indiquées) des repas (Olsen *et al.* 1978).

Cinq ans plus tard, 2 études *in vivo* appronfondies sont effectuées sur 2 cas de SC liés à l'alimentation résultant d'AIMAH bilatérale (Lacroix *et al.* 1992) (Reznik *et al.* 1992). La première patiente est une canadienne-française de 48 ans présentant un SC cliniquement déclaré depuis environ 3 ans (Lacroix *et al.* 1992). La sécrétion de cortisol, basse le matin à jeun et élevée dans la journée après les repas, est indépendante de l'ACTH, puisqu'indécelable. L'hypothèse d'une production de cortisol modulée par une hormone gastro-intestinale est supportée par les observations suivantes: la cortisolémie est augmentée par le glucose per os (p.o.) et les repas riches en lipides ou en protéines et non par le glucose intraveineux (i.v.); de plus, la réponse induite par le glucose p.o. est abolie par la somatostatine. Le GIP et le GLP-1 (*glucagon-like peptide-1*), dont la sécrétion est stimulée par le glucose oral, les lipides et, plus faiblement, par les protéines, paraissent alors deux modulateurs potentiels de la stéroïdogénèse. Or chez cette patiente, la sécrétion de cortisol est étroitement corrélée à l'augmentation post-prandiale de GIP de même qu'elle est stimulée par l'infusion de GIP à doses physiologiques contrairement aux 4 sujets sains testés; elle est également induite par l'ACTH, mais ni par le CRH, le glucagon, l'insuline, la pentagastrine ou l'AVP. La présence de récepteurs du GIP (GIPR) a été démontrée par imagerie scintigraphique après injection de [^{123}I]-GIP *in vivo* et leur fonctionnalité testée *in vitro*. Seules les cellules tumorales de la patiente voient leur production de cortisol s'élever en réponse au GIP comparativement à des cellules de surrénale normale adulte ou foetale ou à d'autres issues d'adénomes sécrétant du cortisol ou de l'aldostérone. En outre, cette réponse est spécifique au GIP puisqu'elle n'est reproduite avec aucune des autres hormones testées (sécrétine, CCK, VIP, substance P, bombésine, CGRP, glucagon, AVP, ANP, CRH, TRH, GHRH, neurotensine, neurokinine A). Il apparaît donc clairement que l'hypercorticisme lié à l'alimentation résulte de la sensibilité anormale des cellules surrénaliennes à des doses physiologiques de GIP. L'expression illicite ou anormale des GIPR dans les surrénales hyperplasiques est l'hypothèse étiologique avancée pour ce nouveau syndrome (Figure 1.2) (Lacroix *et al.* 1992) (Lacroix *et al.* 2001) (N'Diaye *et al.* 1998b).

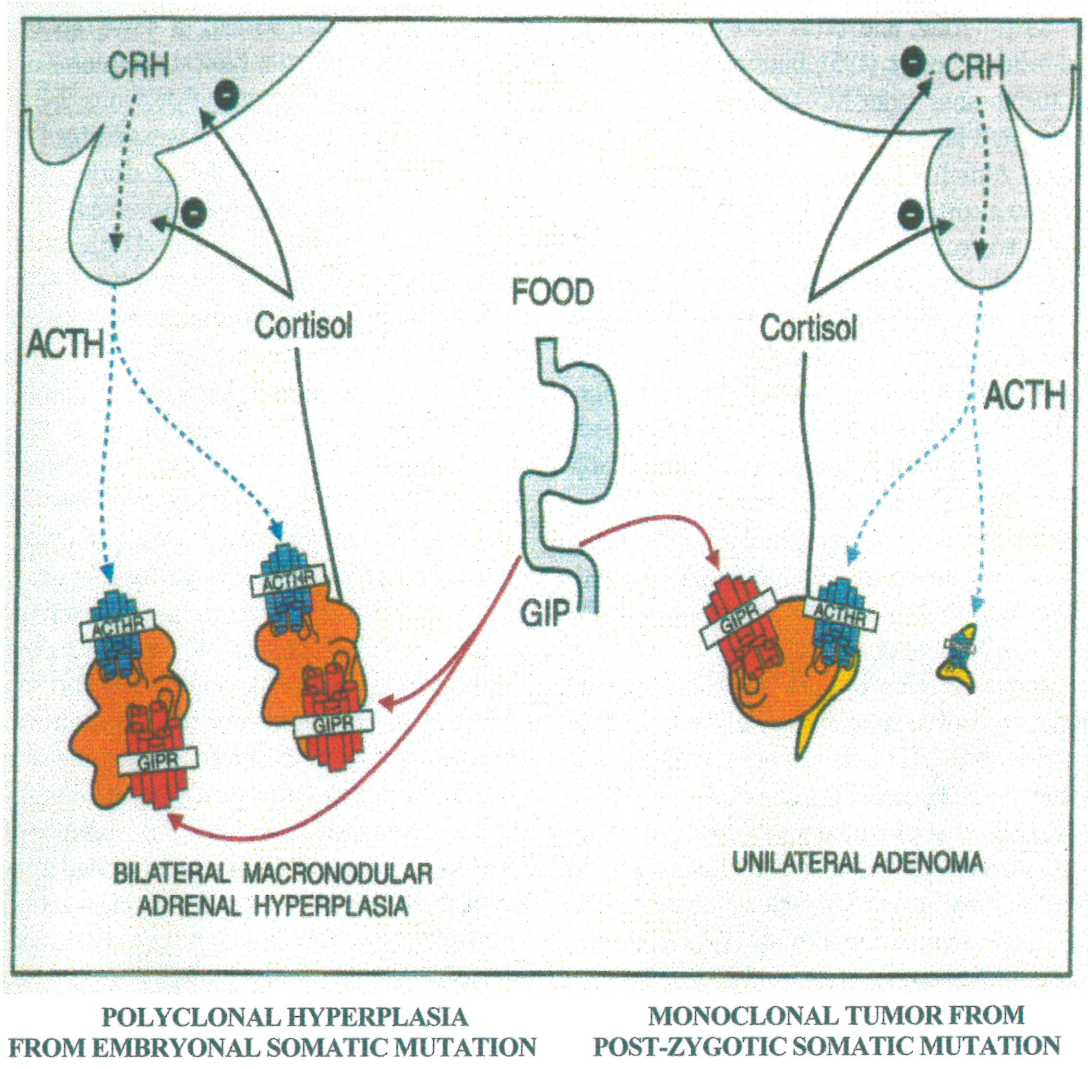


Figure 1.2. L'axe hypophyso-surrénalien dans le SC GIP-dépendant. L'expression ectopique de GIPR (en rouge) a été identifiée dans les hyperplasies surrénaliennes macronodulaires bilatérales (à gauche) et dans les adénomes surrénaliens unilatéraux (à droite). Le GIP est physiologiquement sécrété par les cellules K du duodénum et du petit intestin suite à l'ingestion de repas; il en résulte une augmentation post-prandiale du cortisol plasmatique, à des niveaux supra-physiologiques (traits pleins noirs); ce dernier exerce un rétro-contrôle négatif sur la synthèse de CRH et d'ACTH. Lorsque les niveaux plasmatiques de GIP sont bas (situation à jeun), la cortisolémie est basse, l'ACTH étant supprimée (traits bleux en pointillé). Un adénome unilatéral sensible au GIP résulterait de l'expansion clonale d'une cellule du cortex surrénalien ayant subi une mutation somatique post-zygotique. Les taux supprimés d'ACTH entraînent l'atrophie des surrénales adjacente et contra-latérale dans le cas d'atteintes unilatérales (à droite). Une mutation somatique apparaissant lors des premiers stades de la vie embryonnaire, responsable de l'expression ectopique de GIPR dans les cellules progénitrices du cortex surrénalien (expansion polyclonale) conduirait, à long terme, au développement d'un SC GIP-dépendant non familial avec une hyperplasie surrénalienne macronodulaire bilatérale (à gauche). [Permission de A. Lacroix et *al.* : *Endocr Rev* 22 :75-110, 2001. © The Endocrine Society.]

La seconde patiente est une française de 49 ans suivie depuis 5 ans pour un SC avec AIMAH traité par des inhibiteurs de la synthèse de cortisol (Reznik *et al.* 1992). Suite au rapport préliminaire paru sur le premier cas de SC GIP-dépendant (Lacroix *et al.* 1991), une investigation clinique poussée a été entreprise chez cette patiente qui présentait un profil de cortisolémie similaire au cas précédent: taux de cortisol bas le matin à jeun et augmentés par les repas mixtes, riches en lipides ou en protéines ainsi que par le glucose p.o. et non par le glucose i.v.; l'administration sous-cutanée d'octréotide bloque la réponse au glucose oral. La concentration plasmatique de GIP est parfaitement corrélée aux niveaux de cortisol, de même que l'infusion i.v. de GIP augmente la cortisolémie de la patiente et pas celle de 4 sujets sains prétraités à la dexaméthasone. Seule l'ACTH reproduit cette réponse *in vivo* contrairement à la lysine-vasopressine (LVP), l'insuline, la pentagastrine et au glucagon. L'administration chronique préprandiale d'octréotide a permis de corriger temporairement l'hypercorticisme (Lebrethon *et al.* 1998). Cependant, une surrénalectomie bilatérale s'est rapidement avérée nécessaire suite à la perte d'efficacité du traitement.

Le SC lié à l'alimentation ou GIP-dépendant a depuis été identifié chez 17 patients avec AIMAH (Archambeaud-Mouveroux *et al.* 1996) (Croughs *et al.* 2000) (Lacroix *et al.* 1992) (Lebrethon *et al.* 1998) (N'Diaye *et al.* 1999) (Pralong *et al.* 1999) (Reznik *et al.* 1992) (Lacroix *et al.* 2000) (Groussin *et al.* 2001) et 7 ayant un adénome unilatéral (Hamet *et al.* 1987) (Lebrethon *et al.* 1998) (Chabre *et al.* 1998) (Combes *et al.* 1998) (de Herder *et al.* 1996) (Luton *et al.* 1998) (Tsagarakis *et al.* 2001) (Lacroix *et al.* 2000) (Groussin *et al.* 2001). Sur ces 24 cas décrits, 85% sont des femmes. La plus grande prévalence de SC chez la femme est un phénomène connu qui reste néanmoins inexpliqué (Ross 1994). Il est à noter que l'âge moyen de dépistage de la maladie est plus élevé chez les patients atteints d'AIMAH comparativement aux cas d'adénomes unilatéraux parmi lesquels le plus jeune patient est âgé de 15 ans (Lieberman *et al.* 1994)

(Stratakis & Kirschner 1998). Ces lésions surrénaliennes ne montrent aucune caractéristique histopathologique distinctive par rapport à d'autres adénomes ou hyperplasies non GIP-dépendants. Un cas s'apparentant au syndrome de Carney a été rapporté chez un patient présentant des lésions cutanées pigmentées sur le visage, un naevus bleu sur une jambe et deux surrénales macronodulaires contenant de la lipofuchine, entourées d'un schwannome (Archambeaud-Mouveroux *et al.* 1996); toutefois, la sécrétion de cortisol à partir des macronodules est stimulée par le GIP *in vitro* (Lebrethon *et al.* 1998).

L'hypercorticisme chronique induit par le GIP entraîne, à long terme, la suppression des taux d'ACTH et de CRH. Ainsi, lorsque les niveaux de GIP sont faibles (périodes hors des repas), la production de cortisol est fortement diminuée provoquant un état d'insuffisance surrénalienne chez certains patients (de Herder *et al.* 1996) (Reznik *et al.* 1992). Chez d'autres, les taux plasmatiques de cortisol à jeun ne sont pas particulièrement bas. Il serait donc imprudent d'exclure la possibilité d'un SC GIP-dépendant sur la base des profils de cortisolémie sans effectuer de tests alimentaires au préalable (N'Diaye *et al.* 1999) (Pralong *et al.* 1999). Une telle situation clinique suggère deux hypothèses: une sous-population des cellules de l'hyperplasie ou de l'adénome surrénalien perdrait la sensibilité au GIP et serait alors contrôlée par d'autres mécanismes permettant une sécrétion de cortisol en absence d'ACTH et de GIP; les cellules pourraient également exprimer plusieurs récepteurs anormaux qui moduleraient la production de cortisol. La possibilité de récepteurs GLP-1 ectopiques a été éliminée jusqu'à présent car l'hormone, administrée *in vivo* ou *in vitro*, ne stimule aucunement la production de cortisol (Chabre *et al.* 1998) (Lebrethon *et al.* 1998) (Pralong *et al.* 1999). En revanche, chez un patient atteint d'AIMAH liée à l'alimentation et pour lequel les taux de cortisol à jeun sont relativement élevés, il a été montré que le sécrétion de cortisol est stimulée, *in vitro*, par le GIP et la leptine (Pralong *et al.* 1999). Un autre cas

d'AIMAH présentant deux récepteurs aberrants (GIPR et LH/hCGR) a été récemment rapporté (Bertherat *et al.* 2001). La présence potentielle de plus d'un récepteur illicite est donc susceptible de modifier l'expression phénotypique de ce syndrome. De plus, lorsque l'hypercorticisme est modéré, l'axe corticotrope peut n'être que partiellement supprimé. Ainsi, on peut observer une élévation des concentrations d'ACTH et de cortisol en réponse au CRH chez un patient atteint d'AIMAH GIP-dépendante d'évolution récente (Croughs *et al.* 2000). De même, une patiente ayant un adénome unilatéral sécrétant cortisol et androgènes en réponse à l'alimentation (*in vivo*) ou au GIP (*in vitro*) conserve une sécrétion résiduelle d'ACTH (Tsagarakis *et al.* 2001).

1.5.2. SC dépendant de l'hormone lutéinisante

Un autre exemple de récepteur aberrant nous est donné par le cas d'une canadienne-française de 63 ans présentant un SC avec AIMAH dont les symptômes cliniques sont clairement apparus 10 ans après la survenue de la ménopause (Lacroix *et al.* 1999a). Au cours des ses 4 grossesses, elle accusa une importante prise de poids (18-22kg), lui conférant un aspect cushinoïde malgré l'absence d'autres signes caractéristiques de la maladie (hypertension, stries abdominales ou hirsutisme). Après chaque accouchement, son poids revenait à la normale et durant 2 à 3 mois, elle souffrait de fatigue, de nausée et d'un manque d'appétit. L'administration *in vivo* de de gonadotrophine (GnRH), d'hCG ou de LH humaine (hLH) recombinante augmentait la cortisolémie de cette patiente. De même, la testostérone libre et l'oestradiol voyaient leur concentration s'élever suite à l'injection de hLH. Ni la folliculostimuline (FSH), ni le GnRH (après que la LH et la FSH aient été supprimées par l'acétate de leuprolide) n'avaient d'effet.

Dans les conditions physiologiques, la LH stimule la stéroïdogénèse à partir des gonades en activant l'AC et la PLC. Son récepteur, LH/hCGR, est principalement exprimé dans les tissus gonadiques mais aussi dans l'utérus, les trompes de Fallope, le placenta, le cerveau, l'hypothalamus et la prostate (Rao 1996). Sa présence a certes été identifiée dans la zone réticulée de la surrénale humaine adulte par immuno-histochimie et par hybridation *in situ* ; de même, il est connu que l'hCG stimule la sécrétion de DHEAS à partir des surrénales foetales humaines (Seron-Ferre *et al.* 1978). Cependant, aucun effet physiologique de l'hCG sur la surrénale n'a été démontré *in vivo* chez des sujets adultes sains. Les réponses hormonales observées chez cette patiente pourraient donc résulter de l'expression ectopique des récepteurs LH/hCG qui se trouveraient efficacement couplés à la stéroïdogénèse dans des cellules de type fasciculée (Figure 1.3). Un second modulateur semble être impliqué dans la physiopathologie de ce syndrome. En effet, la production de cortisol, de testostérone libre et de DHEAS est stimulée, *in vivo,* par deux agonistes sérotoninergiques spécifiques du 5HT4R, le cisapride et le métoclopramide alors que seule la sécrétion d'aldostérone est positivement régulée par la sérotonine chez l'adulte sain (voir section 1.3.3.2). L'hypercorticisme a néanmoins pu être contrôlé par l'administration d'un analogue à longue action du GnRH, l'acétate de leuprolide, qui abolit la sécrétion de LH et de FSH endogènes (Figure 1.3) (Lacroix *et al.* 1999a).

L'hypercorticisme est généralement cause d'infertilité, d'avortement, de naissance prématurée et de mortalité infantile. Les rares exemples de SC associé à une grossesse résultent plus fréquemment d'adénomes (44%) ou de carcinomes (17%) surrénaliens que d'adénomes hypophysaires (29%) ou d'une sécrétion ectopique d'ACTH (4%) (Sheeler 1994). Dans quelques cas, l'exacerbération de l'hypercorticisme durant une nouvelle grossesse (Kreines *et al.* 1964) ou la régression des symptomes en post-partum (Calodney *et al.* 1973) (Keegan *et al.* 1976) (Parra & Cruz-Krohn 1966) (Reschini *et al.* 1978) ont été rapportées.

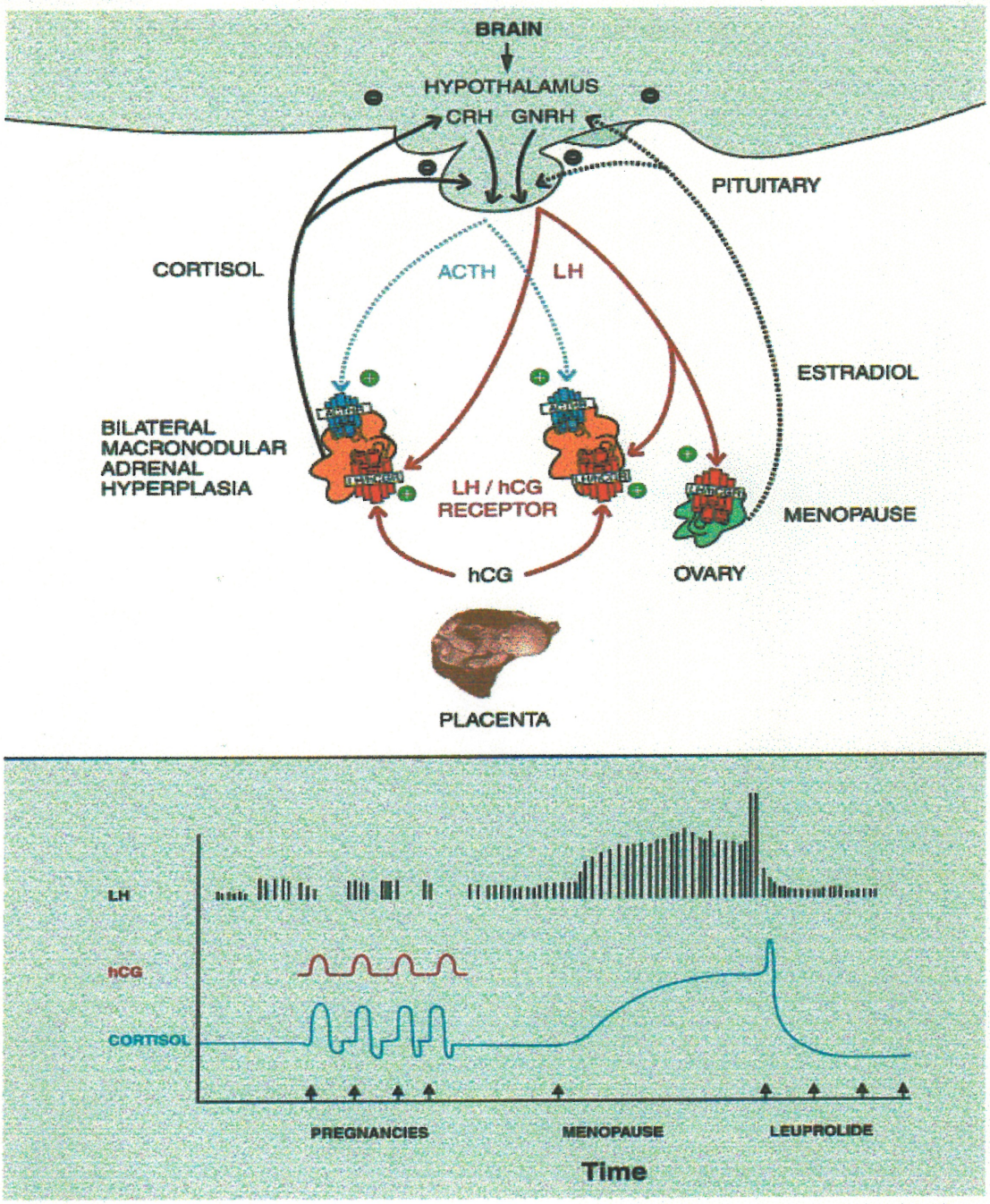


Figure 1.3. L'axe hypophyso-surrénalien dans le SC LH-dépendant. L'expression de LH/hCGR dans le cortex surrénalien est illustrée. Le développement de l'hyperplasie surrénalienne bilatérale et de l'hypercorticisme est dû à l'occupation du récepteur par l'hCG, d'origine placentaire, durant les grossesses ; l'accouchement est suivi d'une période d'hypocorticisme. A la ménopause, les taux chroniquement élevés de LH suite à l'absence d'oestrogènes conduisent à l'hyperplasie progressive des surrénales et à l'hypercorticisme. Le cortisol sécrété exerce un rétro-contrôle négatif sur la production de CRH et d'ACTH (panneau du haut). L'administration d'acétate de leuprolide induit initialement une stimulation transitoire de LH et de cortisol ; puis, s'ensuit une suppression à long terme des taux de LH et la production de cortisol se trouve normalisée (panneau du bas). [Permission de A. Lacroix et *al.* : *Endocr Rev* 22 :75-110, 2001. © The Endocrine Society.]

Une augmentation anormale de la cortisolémie induite par le GnRH et la LH a également été observée chez une femme ayant une hyperplasie surrénalienne macronodulaire bilatérale avec une cortisolurie normale et un axe corticotrope incomplètement supprimé par la dexaméthasone (SC sub-clinique) (Bourdeau *et al.* 2000). Les récepteurs hormonaux illicites peuvent donc s'exprimer dans des cas d'hyperplasie surrénalienne macronodulaire sub-cliniques.

Plusieurs équipes ont auparavant montré le rôle stimulateur joué par la LH ou le GnRH, *in vitro,* sur certaines tumeurs surrénaliennes virilisantes (Lacroix *et al.* 2001). Ceci n'est pas toujours confirmé *in vivo* puisque les taux plasmatiques de LH sont supprimés chez certaines patientes. De plus, pour d'autres cas, la suppression de la LH endogène par l'administration d'oestrogènes n'entraîne pas de diminution de la sécrétion d'androgènes (Blichert-Toft *et al.* 1975) (de Lange *et al.* 1980) (Smith *et al.* 1978). Bien que l'origine surrénalienne de certaines tumeurs ait été confirmée (Leinonen *et al.* 1991), la présence de cellules gonadiques ectopiques au sein des lésions surrénaliennes fut une des hypothèses avancées pour expliquer ce phénomène. La présence de récepteurs aberrants dans des tumeurs originant de cellules de la zone réticulée en fut une autre.

1.5.3. SC secondaire à l'expression d'autres récepteurs anormaux

1.5.3.1. SC vasopressine-dépendant

Une stimulation anormale de la sécrétion de cortisol en réponse à l'administration d'AVP ou de LVP a été rapportée chez plusieurs patients atteints de SC surrénalien ACTH-indépendant secondaire à un adénome unilatéral ou à une hyperplasie macronodulaire bilatérale (Arnaldi *et al.* 1998b) (Arnaldi *et al.* 1998a) (Horiba *et al.* 1995) (Lacroix *et al.* 1997b). Cette réponse exagérée est relayée par le récepteur vasopressinergique V1a qui est exprimé, dans ces lésions surrénaliennes, à un niveau

comparable à celui observé dans la corticosurrénale humaine normale (Arnaldi *et al.* 1998b) (Lacroix *et al.* 1997b) (Perraudin *et al.* 1995). Cependant, *in vivo*, l'AVP exogène n'augmente pas la cortisolémie de façon décelable lorsque la sécrétion d'ACTH est inhibée par la dexaméthasone, chez des sujets sains (Lacroix *et al.* 1997b). L'hypothèse étiologique de ce syndrome serait donc l'activation anormale d'un récepteur eutopique plutôt qu'une véritable expression ectopique.

L'administration de doses pharmacologiques d'AVP peut augmenter la sécrétion de cathécolamines (Gallo-Payet & Guillon 1998) et indirectement stimuler la sécrétion de cortisol comme cela a été démontré chez un patient atteint d'AIMAH et d'un SC cathécolamine-dépendant (Lacroix *et al.* 1997a). Il est donc important de montrer que la modulation de la production de cortisol est due à des variations physiologiques de l'AVP endogène; ceci est illustré par le cas de 2 patients ayant une hyperplasie surrénalienne macronodulaire bilatérale (Daidoh *et al.* 1998) (Mircescu *et al.* 2000).

1.5.3.2. SC cathécolamines-dépendant

La présence de récepteurs β-AR ectopiques fonctionnellement couplés à la stéroïdogénèse dans des tumeurs surrénaliennes a été clairement démontrée *in vitro* (Hirata *et al.* 1981) (Katz *et al.* 1985) (Matsukura *et al.* 1980). L'expression clinique de cette anomalie a récemment été rapportée chez 2 patients. Le premier cas rapporté est celui d'un homme de 56 ans atteint d'un SC avec AIMAH chez lequel la cortisolémie est corrélée aux variations physiologiques des cathécolamines endogènes (Lacroix *et al.* 1997a). Cette réponse est inhibée par un prétraitement avec un β-bloqueur, le propranolol. La perfusion d'isoprotérénol augmente rapidement la production de cortisol chez ce patient, mais pas chez des sujets sains dont l'ACTH est supprimé par la dexaméthasone. Ces données suggèrent la présence de récepteurs β_1 ou β_2 dans le tissu hyperplasique. Chez une seconde patiente de 50 ans présentant également une

hyperplasie macronodulaire des surrénales associée à un SC, cathécolamines et AVP sont les deux modulateurs endogènes de la stéroïdogénèse (Mircescu *et al.* 2000). Des études moléculaires permettront de caractériser le sous-type d'adrénorécepteur exprimé dans ces tissus et d'identifier le défaut moléculaire impliqué (mutation, anomalie d'expression ou de couplage).

1.5.3.3 SC sérotonine-dépendant

Les agonistes sérotoninergiques sont de puissants stimulateurs de la sécrétion d'aldostérone, mais restent sans effet sur la production de cortisol chez l'homme (cf section 1.3.3.2). Cette réponse est relayée par le 5-HT_4R exprimé principalement dans la zone glomérulée et plus faiblement dans la fasciculée (Lefebvre *et al.* 1992) (Lefebvre *et al.* 1993).

Chez la patiente canadienne-française atteinte d'un SC LH-dépendant (cf section 1.4.2.), les deux agonistes sérotoninergiques spécifiques de $5HT_4$R, le cisapride et le métoclopramide, stimulent la sécrétion de cortisol, l'ACTH étant supprimée (Lacroix *et al.* 1999a). Plusieurs autres observations de réponse exagérée au cisapride ont été rapportées chez des sujets ayant un SC déclaré avec AIMAH ou un SC sub-clinique (Bonnin *et al.* 2000) (Bourdeau *et al.* 2000). La présence du récepteur $5HT_4$ a été mise en évidence par RT-PCR dans les tissus surrénaliens des patients (Bonnin *et al.* 2000) (N'Diaye *et al.* 2001b). Cependant, ni le séquançage ni la distribution cellulaire de ce récepteur n'ont été effectuées.

1.5.3.4 SC angiotensine-dépendant

Un cas de SC associé à une AIMAH et pour lequel les taux de cortisol et d'aldostérone sont stimulés par le test de posture a récemment été décrit (Nakamura *et al.* 2001). Aucun effet de la vasopressine endogène sur la stéroïdogénèse n'a été observé. L'augmentation des concentrations de cortisol et d'aldostérone est totalement inhibée par l'administration à court terme de candesartan, un antagoniste du récepteur AT_1. Aucune infusion d'Ang-II n'a été effectuée chez ce patient, ni aucune thérapie pharmacologique avec un antagoniste spécifique des récepteurs AT_1. L'anomalie de récepteur (mutation, anomalie de couplage) n'a pas encore été identifiée.

1.5.4. Criblage systématique des récepteurs hormonaux aberrants

Une étude de criblage systématique pour des récepteurs aberrants a été effectuée sur une série de 20 patients atteints de SC surrénalien (Mircescu *et al.* 2000) selon un protocole consistant à moduler de façon transitoire la concentration des ligands spécifiques des récepteurs potentiellement anormaux. Les niveaux plasmatiques de stéroïdes (cortisol, aldostérone, testotérone libre, DHEAS et oestradiol) sont dosés sur différents intervalles de temps; toute variation supérieure à 50% de la valeur basale constitue une réponse positive (Lacroix *et al.* 1999b) (Lacroix *et al.* 2001). Ainsi, il a pu être établi que 2 des 6 patients avec AIMAH présentaient une réponse anormale au GIP, 1 au GnRH (récepteur LH/hCG) et au cisapride (récepteur 5-HT_4), les 3 derniers répondant à la posture et/ou à l'AVP (1 récepteur β-adrénergique, 1 récepteur AVP-V_1 et 1 récepteur β-adrénergique/ AVP-V_1 aberrants); 3 des 13 patients porteurs d'adénomes unilatéraux, avaient une réponse au test de posture, au repas mixte ou à l'AVP alors que le seul patient atteint d'un cancer surrénalien inclus dans l'étude ne présentait de réponse à aucun des tests effectués.

Ces résultats suggèrent que l'expression surrénalienne des récepteurs ectopiques ou anormaux serait plus fréquente dans les hyperplasies macronodulaires que dans les lésions unilatérales. Ceci a récemment été confirmé par d'autres études (Arnaldi *et al.* 1998b) (Bertherat *et al.* 2001) (Lacroix *et al.* 2001). Il faut cependant noter que seuls quelques récepteurs couplés aux protéines G ont été testés. D'autres études systématiques seront nécessaires afin de déterminer la prévalence des anormalies de récepteurs hormonaux dans le SC surrénalien.

1.6. Mécanismes moléculaires des anomalies de récepteurs surrénaliens

La découverte de nouvelles étiologies du SC surrénalien met en exergue la question de la régulation tissu-spécifique de l'expression génique. Les mécanismes moléculaires menant à l'expression ectopique de récepteurs hormonaux dans le SC surrénalien sont encore inconnus. Plusieurs hypothèses peuvent cependant être avancées: un réarrangement génique pourrait entraîner l'expression illicite d'un gène, spécifiquement au niveau de la surrénale (Figure 1.4). Des translocations chromosomiques ont été décrites dans le syndrome d'hyperaldostéronisme contrôlé par les glucocorticoïdes (Lifton *et al.* 1992) ainsi que dans des tumeurs endocrines tels que des adénomes de la parathyroïde (Rosenberg *et al.* 1991) et des carcinomes papillaires de la thyroïde (Gagel 1998) : en effet, il a été démontré que l'expression du gène chimère de l'aldostérone synthétase fusionné avec le promoteur du gène de la 11β–hydroxylase entraîne la production ectopique d'aldostérone dans la zone fasciculée (Lifton *et al.* 1992); de même, le promoteur du gène de la PTH, lorsque combiné avec le gène de la cycline D, dirige l'expression de l'oncogène Prad-1 dans les tumeurs parathyroïdiennes (Rosenberg *et al.* 1991); dans 25% des carcinomes papillaires (proportion allant jusqu'à

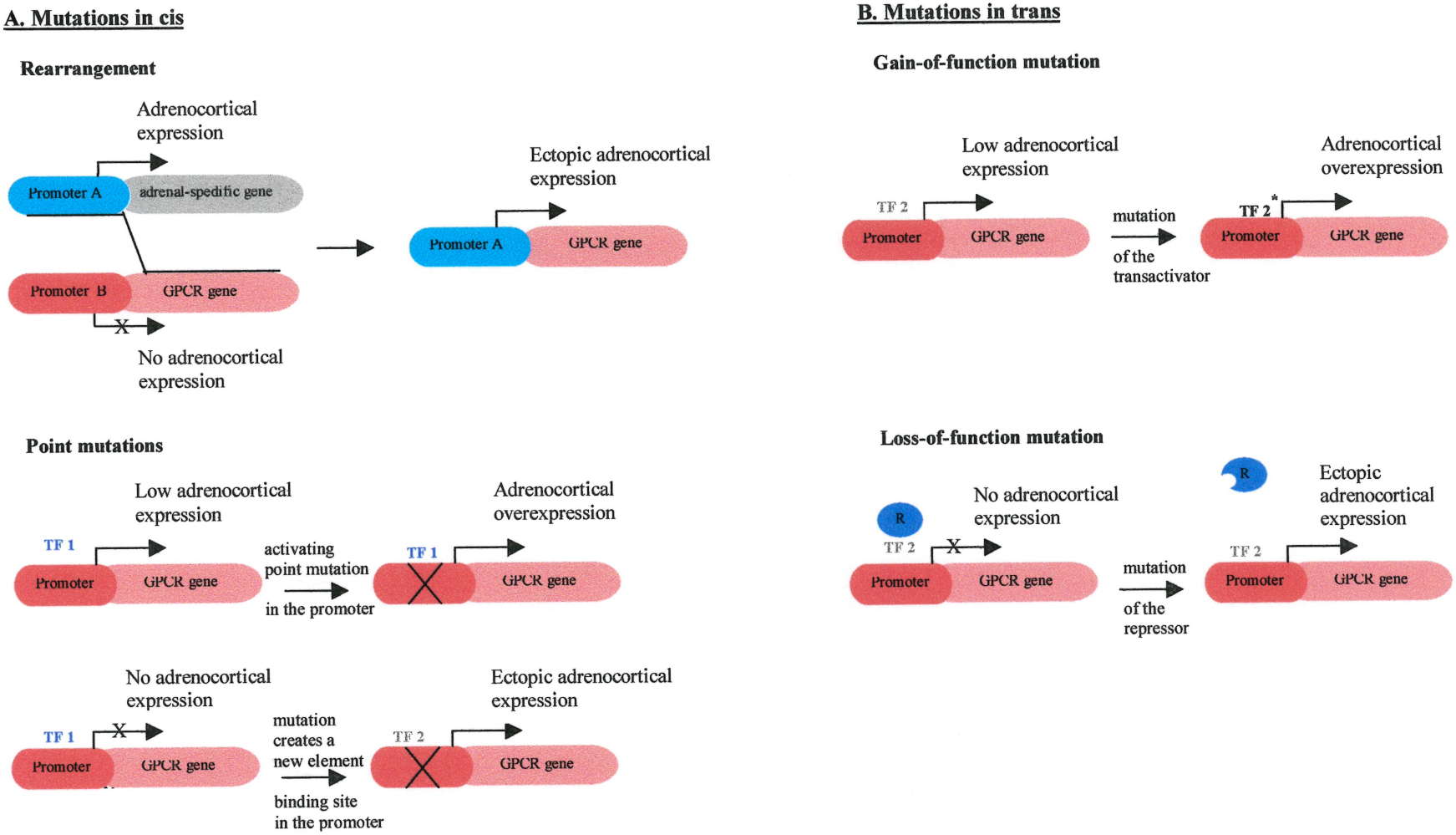


Figure 1.4. Mécanismes moléculaires potentiels d'ectopie. L'expression ectopique des RCPG dans le SC surrénalien pourraient résulter de mutations touchant les régions cis-régulatrices des gènes impliqués (A) ou les facteurs transcriptionnels (B). **A.** Un réarrangement génique pourrait fusionner un gène spécifique d'un RCPG non exprimé dans la surrénale avec un promoteur dirigeant spécifiquement l'expression dans la corticosurrénale; des mutations ponctuelles dans le promoteur d'un RCPG pourrait également mener à une expression anormale: soit en stimulant l'expression d'un récepteur qui est faiblement exprimé dans la surrénale et non couplé à la stéroïdogénèse (surexpression); soit en créant un nouveau site de liaison pour un FT spécifique de la surrénale (expression ectopique). **B.** Une mutation gain-de-fonction d'un FT surrénalien ou d'un co-activateur (non montré), de même qu'une mutation perte-de-fonction d'un répresseur ou d'un co-répresseur (non montré) empêchant l'expression d'un RCPG dans la surrénale pourraient conduire à une expression ectopique. [Permission de A. Lacroix et *al.* : *Endocr Rev* 22 :75-110, 2001. © The Endocrine Society.]

62% après l'irradiation de Chernobyl), une partie du gène RET codant pour le domaine tyrosine-kinase du récepteur Ret fusionne avec au moins 8 nouveaux promoteurs (Rabes *et al.* 2000) (Salassidis *et al.* 2000). Ceci entraîne l'activation constitutive du domaine tyrosine-kinase de Ret. Il est à noter qu'à ce jour, aucun réarrangement majeur n'a été décrit dans les adénomes ou hyperplasies surrénaliennes.

Des mutations discrètes des régions promotrices de gènes codant pour des récepteurs hormonaux pourraient également conduire à une expression ectopique en générant de nouveaux sites de liaison pour des complexes facteur de transcription/ co-activateur spécifiquement exprimés dans la surrénale ; une mutation ponctuelle pourrait aussi entraîner la surexpression d'un récepteur normalement exprimé dans la surrénale, ou si faiblement qu'il ne régulerait pas la stéroïdogénèse (Figure 1.4A).

Un autre mécanisme potentiel d'ectopie implique des anomalies de fonction de facteurs de transcription, co-activateurs ou co-répresseurs impliqués dans la régulation tissu-spécifique des gènes exprimés dans la surrénale (Figure 1.4B). Ainsi, une mutation activatrice ou entraînant la perte de fonction d'un facteur de transcription (ou co-régulateur) conduit à une surexpression ou à une perte d'expression du gène ciblé. Des mutations inactivatrices de Pit-1 et de Lhx3, deux facteurs de transcription impliqués dans le développement hypophysaire, sont associées au syndrome de déficience congénitale en hormones hypophysaires (Tatsumi *et al.* 1992) (Howard & Maurer 2001).

Un modèle de souris transgénique surexprimant une forme chimérique de la LH constitue un autre exemple de mécanisme conduisant à l'expression aberrante d'un récepteur. En effet, les taux chroniquement élevés de LH entraînent le développement d'un SC, d'ovaires polycystiques et de tumeurs ovariennes chez ces souris (Rilianawati *et al.* 1998). Il a été démontré que cela était dû à l'expression ectopique de LH/hCGR dans

al. 1998). Il a été démontré que cela était dû à l'expression ectopique de LH/hCGR dans la surrénale suite à l'augmentation des taux d'oestrogènes et de prolactine. Ainsi, le phénomène d'ectopie ne nécessite pas forcément la mutation d'un élément cis- ou trans-régulateur, mais peut être la conséquence de l'activation d'un gène normalement silencieux.

1.7. Récepteurs couplés aux protéines G

La plupart des récepteurs anormaux présents dans les hyperplasies et tumeurs corticosurrénaliennes appartiennent à la superfamille des récepteurs couplés aux protéines G. L'étude des voies de signalisation des récepteurs aberrants impliqués dans le SC surrénalien suggère que ces derniers miment les événements intracellulaires induits par l'activation de MC2R par son propre ligand ou de tout autre récepteur régulant la stéroïdogénèse surrénalienne. Ainsi, tout récepteur hormonal capable de se coupler efficacement aux systèmes de signalisation intracellulaires présents dans les cellules de la corticosurrénale peut virtuellement contrôler la production exagérée de stéroïdes et/ou la prolifération cellulaire anormale chez les patients atteints de SC surrénalien. Dans la prochaine section, nous présentons les deux récepteurs qui font l'objet des travaux exposés dans cette thèse, GIPR et LH/hCGR. La physiologie et la régulation de l'expression tissu-spécifique de ces récepteurs seront discutées.

1.7.1. Le GIP et son récepteur

1.7.1.1. Rôles physiologiques du GIP

Le GIP est une hormone peptidique de 42 acides aminés synthétisée par les cellules K du duodénum et du jejunum. Initialement décrit en tant qu'inhibiteur de la sécrétion d'acide gastrique (*gastric inhibitory polypeptide*) (Brown *et al.* 1970), le GIP est désormais considéré comme l'une des principales incrétines responsables de la

polypeptide) (Fehmann *et al.* 1995). Son importance physiologique est notamment soulignée dans des situations pathologiques telles que le diabète mellitus non insulino-dépendant (diabète de type 2) où l'action insulinotropique du GIP est sévèrement compromise, suggérant un défaut au niveau du récepteur ou de la transduction du signal (Holst *et al.* 1997) (Nauck *et al.* 1993). Cette hypothèse est appuyée par le modèle de souris invalidée pour le GIPR chez lequel se développe une intolérance au glucose (Miyawaki *et al.* 1999). D'autres actions du GIP ne sont cependant pas à exclure car il a été démontré, notamment chez le rat, que le récepteur du GIP est exprimé dans de nombreux tissus outre le pancréas: l'estomac, le cœur, la corticosurrénale, l'endothélium vasculaire, l'hypopyse, le tissu adipeux (Usdin *et al.* 1993) (Kaplan & Vigna 1994) (Yip *et al.* 1998). Ainsi, le GIP stimule la sécrétion de cortisol chez le rat (et non chez l'homme) (Mazzocchi *et al.* 1999), module le flux sanguin chez le chien et le rat (Kogire *et al.* 1988) (Svensson *et al.* 1997) et régule le métabolisme lipidique dans les adipocytes (Yip & Wolfe 2000).

1.7.1.2. Mécanismes d'action du GIP

Le récepteur du GIP (GIPR) est un membre de la famille des récepteurs du VIP et de la sécrétine appartenant à la superfamille des récepteurs couplés aux protéines G (RCPG) (Gremlich *et al.* 1995) (Volz *et al.* 1995) (Yamada *et al.* 1995). La liaison du GIP à son récepteur entraîne la stimulation de l'AC via la protéine Gs et l'augmentation des taux intracellulaires d'AMPc dans les îlots pancréatiques et les insulinomes de rat (Amiranoff *et al.* 1984) (Siegel & Creutzfeldt 1985), dans les cellules fasciculées de surrénale de rat (Mazzocchi *et al.* 1999) et dans les cellules endothéliales humaines de veine ombilicale (Zhong *et al.* 2000). De plus, il a été montré que l'activation de GIPR entraîne l'augmentation de la concentration du calcium intracellulaire aussi bien dans les insulinomes de rat et de hamster (Usdin *et al.* 1993) (Lu *et al.* 1993) que dans les cellules endothéliales humaines de veine ombilicale et d'artère pulmonaire (Zhong *et al.* 2000).

Ce récepteur est en effet couplé à différents types de canaux calciques (Wheeler *et al.* 1995), cependant le GIP ne semble pas stimuler la production d'IP_3 (Lu *et al.* 1993). D'autres études ont révélé la diversité des voies de signalisation activées par le GIPR et suggèrent une possible interaction entre ces divers signaux: ainsi, l'action insulinotropique du GIP peut être relayée par l'activation des MAP (*mitogen-activated protein*)-kinases via une voie de signalisation sensible à la wortmanine dont la séquence des événements reste à définir (Kubota *et al.* 1997) (Straub & Sharp 1996); cet effet physiologique est également induit par la production d'acide arachidonique via la PLA_2 Ca^{2+}-indépendante (Ehses *et al.* 2001). Une stimultion prolongée de GIPR par son ligand entraîne un phénomène de désensibilisation et l'internalisation des récepteurs activés (Tseng *et al.* 1996).

1.7.1.3 Région régulatrice du gène GIPR

Le gène codant pour GIPR a été cloné et séquencé dans le cadre du « projet génome humain » (HUGO). Localisé sur le chromosome 19q13.3, il est constitué de 14 exons et couvre une région de 13,8kb (Gremlich *et al.* 1995) (Stoffel *et al.* 1995). La séquence de la région 5'-non traduite est également connue; cependant, elle n'a pas encore été caractérisée fonctionnellement. Plusieurs éléments de cis-régulation potentiels ont été identifiés dans la région promotrice du gène de rat dont un élément CRE, un site de liaison octamère, trois sites Sp1 et un élément initiateur (Boylan *et al.* 1999). Les deux derniers éléments assurent le rôle des boîtes TATA et CAAT, absentes de ce promoteur, en participant à l'initiation de la transcription. La région plus distale du promoteur (en amont du site –181pb) contient des éléments cis-régulateurs négatifs qui inhibent la transcription dans les cellules n'exprimant pas GIPR (Boylan *et al.* 1999). Ces régions, encore non identifiées, semblent être impliquées dans la régulation de l'expression tissu-spécifique du récepteur.

1.7.2. L'hormone lutéinisante, la gonadotropine chorionique et leur récepteur

1.7.2.1. Rôles physiologiques

L'hormone lutéinisante (LH) et la gonadotropine chorionique sont deux hormones glycoprotéiques sécrétées l'une par l'hypophyse, l'autre par le placenta. Partageant 85% d'homologie, LH et hCG exercent leurs actions en se liant au même récepteur LH/hCGR (Segaloff & Ascoli 1993). L'hCG permet le maintien de la grossesse en stimulant la sécrétion d'hormones stéroïdiennes à partir de la surrénale fœtale et du corps lutéal de l'ovaire chez la mère. Au niveau de la zone fœtale de la surénale, elle stimule la sécrétion de DHEAS (Seron-Ferre *et al.* 1978), précurseur des oestrogènes impliqués dans le maintien de la grossesse et la maturation des tissus fœtaux (Pepe & Albrecht 1995). La LH, quant à elle, joue un rôle important dans la maturation sexuelle en régulant la stéroïdogénèse à partir des gonades chez l'adulte. En effet, dans l'ovaire, elle stimule la production d'androgènes à partir des cellules de la thèque, fournissant ainsi aux cellules de la granulosa les précurseurs nécessaires à la synthèse des oestrogènes; elle déclenche l'ovulation et maintient la production de progestérone par le corps lutéal. Dans les testicules, la LH stimule la production d'androgènes à partir des cellules de Leydig; ces derniers régulent la spermatogénèse.

1.7.2.2. Mécanismes d'action de la LH/hCG

Principalement exprimé dans les gonades, LH/hCGR est également présent dans de nombreux autres tissus tels que l'utérus, le placenta, l'hypothalamus, le cerveau, la surrénale, la prostate et la glande mammaire (Pabon *et al.* 1996) (Rao 1996) (Meduri *et al.* 1997) (Reiter *et al.* 1995). Les pathologies dues à des mutations de LH/hCGR (puberté précoce, pseudo-hermaphrodisme, hypoplasie des cellules de Leydig ou aménorhée) attestent l'importance physiologique des récepteurs gonadiques dans le contrôle de l'axe hypothalamo-hypophyso-gonadique (Themmen & Huhtaniemi 2000). Le rôle des récepteurs non gonadiques est encore peu connu.

La LH stimule la stéroïdogénèse en se liant au LH/hCGR qui active l'AC, via la protéine Gs, et la PLC (Segaloff & Ascoli 1993). Des études sur des modèles cellulaires gonadiques murins et porcins montrent que LH/hCGR peut se coupler à diverses isoformes des protéines G telles que Gi, Gq/11 et G13; il active également la voie des MAP-kinases et stimule la mobilisation du calcium intracellulaire (Themmen & Huhtaniemi 2000). L'activation de ces voies alternatives nécessite une forte concentration d'hormone ainsi qu'une grande densité de récepteurs, conditions rencontrées pendant la grossesse et l'ovulation (Zhu *et al.* 1994). Bien que LH/hCGR soit soumis à une désensibilisation par ses propres ligands, ce phénomène n'a lieu ni dans le corps lutéal de l'ovaire ni dans les cellules de Leydig fœtales pendant la grossesse (Themmen & Huhtaniemi 2000).

1.7.2.3. Région régulatrice du gène LH/hCGR

Le récepteur LH/hCG est l'un des récepteurs à 7 passages transmembranaires (683 acides aminés) possédant un large domaine extracellulaire. Il est encodé par deux gènes (gènes I et II) situés sur le chromosome 2p21-16 (Tsai-Morris *et al.* 1998). Les régions proximales des promoteurs des deux gènes ont été caractérisées (Atger *et al.* 1995): elles diffèrent de quelques bases et d'une délétion de 6pb dans la région codante (+55 à +60). Le site d'initiation de la transcription est identique dans les deux promoteurs (-176 pb); cependant, d'autres sites d'initiation de la transcription situés plus en amont ont été identifiés dans les cellules humaines de testicule et de choriocarcinome JAR. Le patron d'expression tissulaire du récepteur pourrait résulter de l'utilisation tissu-spécifique des sites d'initiation de la transcription et de la possibilité d'exprimer l'un des deux gènes (I ou II). Plusieurs éléments de cis-régulation potentiels ont été identifiés dans la région promotrice du gène humain (1 CRE, 7 sites AP-1 et un demi-site ERE) ainsi que trois régions inhibitrices qui abolissent l'activité du promoteur proximal dans les cellules de choriocarcinome JEG-3 (Hu *et al.* 1998). Ces régions doivent jouer un rôle important dans les tissus non gonadiques n'exprimant pas LH/hCGR.

1.8. Hypothèse et objectifs du présent travail de recherche

De nouvelles étiologies du SC surrénalien ont été identifiées grâce à l'investigation clinique qui a démontré l'importance physiopathologique du concept de récepteurs hormonaux aberrants. Les études *in vivo* et *in vitro* décrites précédemment soulignent la grande diversité de récepteurs membranaires anormaux impliqués dans le SC surrénalien. Ceci inclut les récepteurs dits ectopiques, car non exprimés dans la surrénale normale, tels que GIPR, βAR et tout autre récepteur capable de se coupler aux voies de signalisation de la stéroïdogénèse via les protéines G; sont également inclus les récepteurs aberrants dits eutopiques, car normalement exprimés dans la corticosurrénale, dont la fonction et/ou le niveau d'expression sont augmentés (AVP-V1aR, 5-HT_4R) (Figure 1.5). La présence de récepteurs ectopiques ou aberrants place les cellules surrénaliennes sous un stimulus qui échappe au rétrocontrôle négatif exercé par les glucocorticoïdes. Ce nouveau stimulus trophique non régulé entraîne une sécrétion anormale de cortisol (ou d'autres stéroïdes) et confère probablement un avantage prolifératif aux cellules atteintes. Les mécanismes moléculaires responsables de ces anomalies de récepteur (surexpression, anomalie de couplage ou expression ectopique) restent encore inconnus. Les travaux présentés dans cette thèse visent à identifier les mécanismes menant à de telles anomalies de fonction et d'expression et à caractériser les défauts moléculaires des récepteurs impliqués. Les objectifs spécifiques des études menées sont donc les suivants:

1- **caractériser la structure moléculaire et la fonction des récepteurs anormaux** exprimés dans les tumeurs et hyperplasies surrénaliennes de patients souffrant d'un SC surrénalien. **Les études porteront notamment sur les récepteurs du GIP et de la LH**;

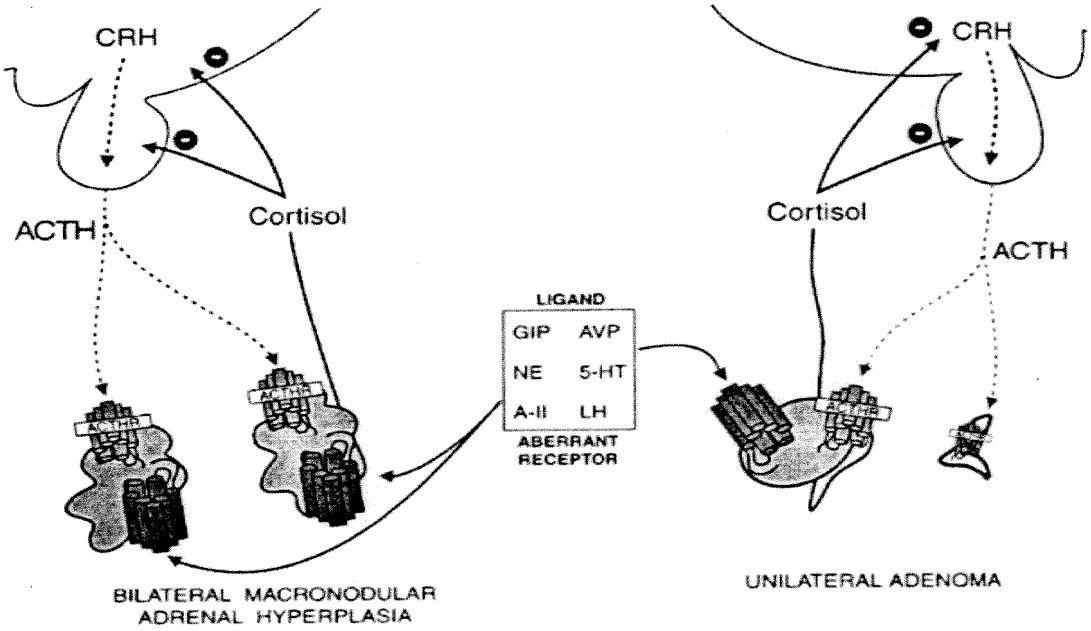
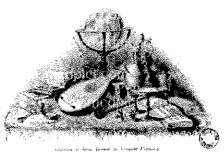


Figure 1.5. L'axe hypophyso-surrénalien dans le SC secondaire à l'expression ou la fonction anormale de récepteurs hormonaux. Divers récepteurs aberrants ont été identifiés dans les hyperplasies surrénaliennes macronodulaires bilatérales (à gauche) ou dans les adénomes surrénaliens unilatéraux (à droite). L'expression ectopique de récepteurs hormonaux fonctionnellement couplés à la stéroïdogénèse confère une sensibilité inappropriée des cellules du cortex surrénalien au GIP, au catécholamines (NE, E) ou à l'Ang-II. Dans certaines situations, des récepteurs eutopiques (AVP-V1R, LH/hCGR ou 5-HT_4R) se retrouvent très efficacement couplés à la stéroïdogénèse par des mécanismes encore inconnus. Ceci mène à une sécrétion exagérée de cortisol ainsi qu'à la suppression de l'axe hypohpyso-surrénalien. Les taux supprimés d'ACTH (ligne en pointillé) entraînent l'atrophie des surrénales adjacente et contra-latérale dans le cas d'atteintes unilatérales (à droite).

2- **aborder l'étude du rôle primaire des anomalies de récepteur dans l'établissement du processus de tumorigénèse**;

3- **rechercher, dans la région promotrice du gène GIPR, la présence d'une mutation** responsable de l'expression ectopique du récepteur dans les lésions surrénaliennes des sujets atteints du SC GIP-dépendant.

Les études ont été effectuées sur les tissus surrénaliens de patients atteints de SC ainsi que sur des surrénales normales adultes et fœtales, selon le code d'éthique en vigueur au sein de l'institution. Les techniques utilisées sont décrites dans la section "Matériel et Méthodes" des articles présentés dans cette thèse.



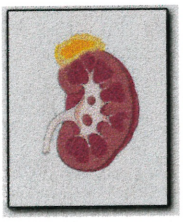
CHAPITRE 2

ARTICLE 1

Adrenocortical Overexpression of Gastric Inhibitory Polypeptide Receptor Underlies Food-dependent Cushing's Syndrome

Nina N'Diaye, Johanne Tremblay, Pavel Hamet, Wouter W. de Herder et André Lacroix

Journal of Clinical Endocrinology and Metabolism 83 : 2781-2785, 1998



0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 8
Printed in U.S.A.

Adrenocortical Overexpression of Gastric Inhibitory Polypeptide Receptor Underlies Food-Dependent Cushing's Syndrome*

NINA N'DIAYE, JOHANNE TREMBLAY, PAVEL HAMET, WOUTER W. DE HERDER, AND ANDRÉ LACROIX

Division of Endocrinology and Department of Medicine, Research Center, Campus Hôtel-Dieu, Centre Hospitalier de l'Université de Montréal, Montreal, Canada H2W 1T8; and the Department of Internal Medicine, University Hospital Rotterdam (W.W.D.H.), Rotterdam, The Netherlands

ABSTRACT

Abnormal responsiveness of adrenocortical cells to gastric inhibitory polypeptide (GIP) in food-dependent Cushing's syndrome suggested that adrenal expression of ectopic, overexpressed, or mutated GIP receptor (GIPR) underlies this syndrome. The expression of GIPR was studied by RT-PCR in human adrenal tissues from two patients with GIP-dependent Cushing's syndrome (adenoma, bilateral hyperplasia), five fetal or adult controls, one patient with Cushing's disease, and four patients with non-food-dependent cortisol-secreting adenomas or bilateral hyperplasias and compared to that in normal pancreas. Hybridization of the RT-PCR-amplified ribonucleic acids with the human GIPR complementary DNA showed an overexpression of GIPR in the adrenals of the two GIP-dependent Cushing's syndrome patients compared to that in normal adrenal tissues (2–3 orders of magnitude) or pancreas (10-fold); no signal could be seen in adrenal adenomas or macronodular hyperplasia from cases of non-food-dependent Cushing's syndrome. No mutation of the GIPR was identified by sequencing the full-length receptor in GIP-dependent adrenal tissue. New alternative spliced isoforms of the GIPR were found, but are identical in GIP-dependent and normal adrenal tissues. Incubation of adrenal cells with GIP stimulates cortisol secretion in GIP-dependent, but not in normal fetal, adult, or non-food-dependent Cushing's syndrome, adrenals. We conclude that the GIPR overexpression and its coupling to steroidogenesis underlie GIP-dependent Cushing's syndrome. (*J Clin Endocrinol Metab* **83:** 2781–2785, 1998)

CORTICOTROPIN-INDEPENDENT Cushing's syndrome is usually secondary to cortisol-secreting adrenal adenomas or carcinomas (1), which are essentially of unknown pathophysiology. Rare cases of corticotropin-independent bilateral adrenal hyperplasia have been reported (1, 2), and their pathophysiology is diverse. Primary pigmented nodular adrenocortical disease can be familial, associated with other tumors (Carney complex), and linked to an unknown gene on chromosome 2 (3). In McCune-Albright syndrome, activating mutations of $G_s\alpha$ in adrenal nodules induce constitutive steroidogenesis (4). Recently, we (5) and others (6, 7) identified food-dependent cortisol production and Cushing's syndrome in three women with corticotropin-independent bilateral adrenal hyperplasia and two patients with adrenal adenomas (8, 9). Abnormal adrenal regulation of cortisol production by gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic polypeptide) *in vivo* (5, 6, 9) or *in vitro* (5) suggested that this new etiology of Cushing's syndrome may be secondary to either ectopic expression or an activating mutation of GIP receptors (GIPR) not normally expressed or functional in adrenal cortical tissues; this hypothesis could not be studied directly at the time of the initial reports (5–7), as the GIPR was not yet well characterized. The GIPR complementary DNA (cDNA) has now been cloned from rat (10), hamster (11), and human (12–14) sources; the human GIPR is about 13.8 kb long and consists of 14 exons (13). GIPR was expressed predominantly in pancreatic β-cells in the hamster, as shown by Northern blot analysis (11); however, by using RT-PCR in rats, it was found to be distributed in several tissues, including the brain, pituitary, gut, fat, heart, vascular endothelium, and adrenals (10, 15). *In situ* hybridization studies indicated that the GIPR was localized in the inner layers of the rat adrenal cortex (10); it is unknown, however, whether GIP regulates steroidogenesis or adrenal growth in the rat. This study demonstrates that GIP-dependent Cushing's syndrome in humans is secondary to the adrenal overexpression of the GIPR that is able to be coupled efficiently to steroidogenesis.

Received January 22, 1998. Revision received May 1, 1998. Accepted May 11, 1998.

Address all correspondence and requests for reprints to: André Lacroix, M.D., Division of Endocrinology, Research Center, Pavillon Hôtel-Dieu, Centre Hospitalier de l'Université de Montréal, 3850 St. Urbain Street, Montreal, Quebec, Canada H2W 1T8. E-mail: lacroixa@ere.umontreal.ca.

* Presented in part at the 79th Annual Meeting of The Endocrine Society, Minneapolis, MN, June 12, 1997. This work was supported by a grant (MT-131–89) from the Medical Research Council of Canada.

Subjects and Methods

Patient's tissues

Adrenal tissues were obtained at the time of surgery from two women with previously reported GIP-dependent Cushing's syndrome (5, 9). One had bilateral macronodular adrenal hyperplasia (5), and another had an adrenal adenoma (9). Tissues were collected rapidly in liquid nitrogen and were stored at −80 C until analysis. Several other control adrenal cortical tissues were obtained from 1) three normal human fetuses and two normal adult multiple organ transplant donors; 2) one woman with pituitary Cushing's disease; and 3) three patients with ACTH-independent, non-food-dependent Cushing's syndrome: one with an adrenal adenoma (62-yr-old woman) and two others with ma-

cronodular adrenal hyperplasia secondary to ectopic adrenal β-adrenergic receptor (56-yr-old man) (16) and to increased V1-vasopressin receptor response (36-yr-old woman) (17), respectively. Normal pancreas was obtained from a woman undergoing a distal pancreatectomy for a benign pancreatic cyst. The study protocol was approved by the local institutional review committee, and informed consent was obtained from all subjects.

Ribonucleic acid (RNA) preparation and RT-PCR

Total RNA was extracted from adrenals by the guanidium-phenol chloroform method (18). First strand cDNA synthesis was carried out with 2 μg total RNA and random primers (hexamers) using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Burlington, Canada) as recommended by the manufacturer. In control reactions, reverse transcriptase was omitted to ensure that the PCR amplification did not result from contaminating genomic DNA. The PCR reaction contained 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM each of deoxy-NTP, 10 pmol each of sense and antisense primers specific for the human GIPR (GeneBank no. U39231), one fifth of the RT reaction, and 2.5 U *Taq* DNA polymerase. Two sets of primers were used to amplify the full-length GIPR cDNA: 5′-GGGACAGGCCTGATCGCCCCT-3′ (−50 to −30) and 5′-TGTAGCCGCCTGAACAAACTC-3′ (532–551); and 5′-TGCTAGCCCTGCTCATCTTGA-3′ (513–533) and 5′-ACACGGGGATCCCGCCCCCTA-3′(1453–1474). The amplification was achieved with 30 and 35 cycles (94 C for 30 s, 48 C for 30 s, and 72 C for 30 s). The PCR products were separated on agarose gel. The RNA samples were also amplified (94 C for 30 s, 51 C for 30 s, and 72 C for 30 s) with a pair of primers specific for the human β-actin cDNA (5′-GATTCCTATGTGGGCGA-3′ and 5′-GATTCCTATGTGGGCGA-3′).

DNA sequencing

The RT-PCR products were subcloned in Bluescript SK^+ (Stratagene, Aurora, Canada). Sequencing of the cDNA inserts was performed on double stranded DNA using the chain termination reaction technique (19) with Circumvent (New England Biolabs, Mississauga, Canada).

Hybridization on RT-PCR products and quantification

RT-PCR products from three independent PCR reactions were hybridized with the full-length GIPR cDNA under high stringency conditions. Filters were prehybridized at 42 C for 2–4 h in a solution containing 50% formamide, 5 × Denhardt's (1 × Denhardt's is 0.02% polyvinylpyrrolidone, Ficoll 400, and BSA), 6 × SSC (1 × SSC is 150 mmol/L NaCl and 15 mmol/L Na_3 citrate, pH 7.0), and 100 μg/mL salmon sperm DNA. Hybridization was performed for 16 h at 42 C with 2×10^5 cpm/mL cDNA probe labeled by random priming (Life Technologies). The filters were washed twice for 15 min each time at room temperature with a solution containing 2 × SSC and 0.1% SDS and then once for 30 min at 65 C with a solution containing 0.1 × SSC and 0. 5% SDS.

Quantification of RT-PCR products was performed with the ImageQuant program (1988–1992, Molecular Dynamics, Sunnyvale, CA). Each experiment (n = 3) was analyzed twice. Only the 30 cycle amplification products were quantified, as the most accurate results were obtained when the amplification rates of specific RNA were identical within exponential phase. The 546- and 453-bp bands were both analyzed.

In vitro stimulation of steroidogenesis by GIP in adrenal cells

Adrenal cells were dispersed from portions of freshly obtained adrenal tissues as reported previously (5, 9). The dispersed cells were incubated in DMEM (Life Technologies) without serum at a concentration of 1×10^6 cells/mL; 1-mL aliquots were incubated with human GIP (Bachem Fine Chemicals, Torrance, CA) in duplicate for 2 h at 37 C under 5% carbon dioxide-95% air. After the incubation, the medium was collected, stored at −20 C until measurement of cortisol concentrations using either a commercial RIA kit or an immunofluorometric assay (Technicon Immuno I System, Miles Diagnostics, Elkhart, IN). Each experiment was performed the day of the surgery. The results are expressed as a percentage of the response in the unstimulated condition.

Results

The expression of GIPR in adrenals was examined by RT-PCR using the first set of primers corresponding to nucleotides −50 to −30 and 532–551. Two fragments of 546 and 453 bp, corresponding to the extracellular domain of the GIPR, were amplified from the adrenals of the two patients with GIP-dependent Cushing's syndrome (Fig. 1A). The same two bands were revealed by ethidium bromide staining in normal adult pancreas, but not in normal fetal and adult adrenals or in adrenal adenoma or bilateral hyperplasia from patients with non-food-dependent Cushing's syndrome. Hybridization with the human GIPR cDNA showed weak expression in normal fetal and adult adrenals (2–3 orders of magnitude less than GIP-dependent Cushing's patient adrenals). No signal could be seen in adrenal adenomas or macronodular hyperplasias from patients with non-food-dependent Cushing's syndrome (Fig. 1B). β-Actin bands of the expected size were similar in all tissues examined (Fig. 1C).

Quantification of RT-PCR products (30-cycle amplification) showed a 10-fold increase in the expression of GIPR in the adrenals of the two GIP-dependent Cushing's patients

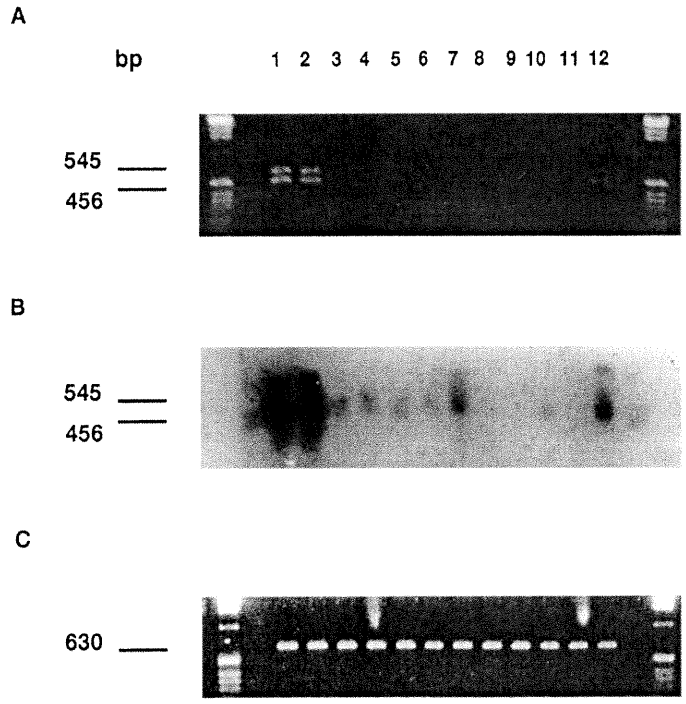


FIG. 1. Analysis of GIPR expression. GIPR was amplified by RT-PCR from 2 μg total RNA of human and pathologic adrenals as described in *Subjects and Methods* (A); the PCR products were run on a 1.5% agarose gel and stained by ethidium bromide. The analysis of the RT-PCR products was performed by hybridization of the full-length GIPR cDNA after 35-cycle amplification (B). The faint signal in the two border lanes corresponds to nonspecific hybridization of the probe with the DNA ladder. Procedures are described in *Subjects and Methods*. β-Actin was amplified as an internal control (C). Lane 1, GIP-dependent adrenal hyperplasia; lane 2, GIP-dependent adrenal adenoma; lanes 3–5, human fetal adrenals; lanes 6 and 7, human fasciculata cells and whole adult adrenal; lanes 8–11, non-food-dependent Cushing's adrenals; lane 8, adrenal hyperplasia secondary to increased activity of V1-vasopressin receptor; lane 9, ectopic β-adrenergic receptor; lane 10, pituitary Cushing's disease; lane 11, adrenal adenoma; lane 12, normal pancreas.

compared to that in normal pancreas (data not shown). RT-PCR products were confirmed by sequencing. The 546-bp fragment sequence was identical to the reported human cDNA (12–14). The 453-bp fragment corresponds to a form of the GIPR lacking exon 4 (Fig. 2). The relative level of expression was similar for both of the isoforms (Fig. 1).

The 3′-ends of the GIPR cDNAs from one GIP-dependent Cushing's patient (9) and from one normal adult adrenal were amplified using the nucleotide 513–533 and 1453–1474 primers and sequenced. Another isoform of the GIPR lacking exon 9 was identified in normal and GIP-dependent Cushing's adrenals. (Figs. 2) The sequence analysis of the full-length cDNAs revealed no mutations.

We have previously found that the incubation of adrenal cells with GIP resulted in a dose-dependent stimulation of steroidogenesis in cells from patients with GIP-dependent Cushing's syndrome (5, 9) (Table 1); GIP did not stimulate cortisol production in adrenal cells dispersed from normal adult or fetal adrenals, Cushing's disease, non-food-dependent cortisol-secreting adrenal adenomas, or hyperplasias.

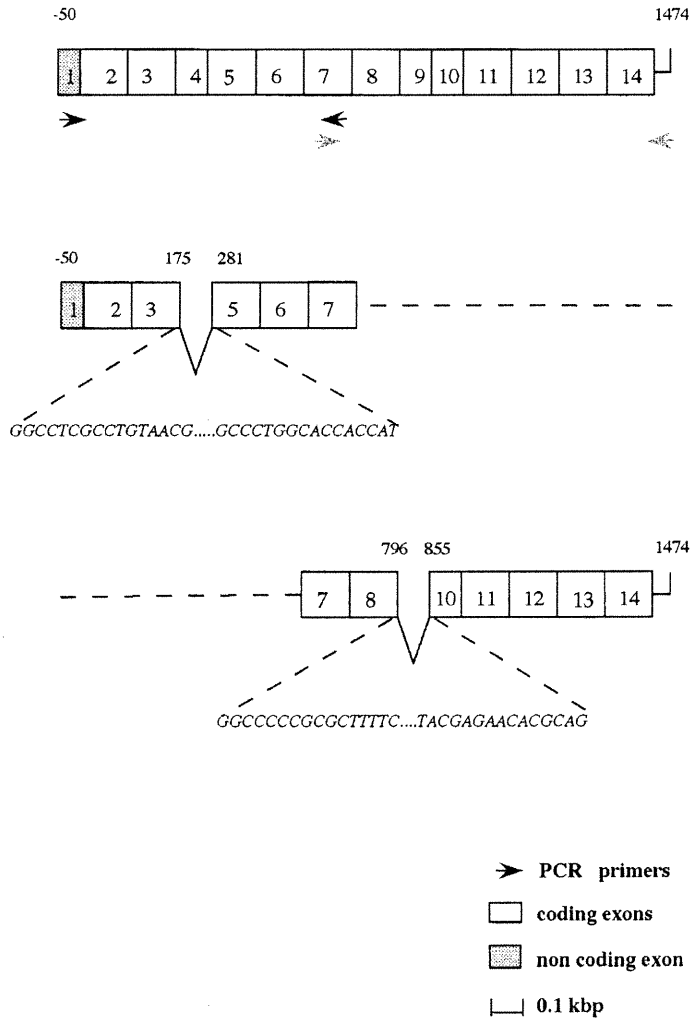


FIG. 2. Schematic representation of the three adrenal cDNAs; cDNAs were inserted in the *Sma*I site of Bluescript SK$^+$. *Open boxes* represent the coding exons, and the *dotted box* represents the noncoding first exon. *Arrows* represent the oligonucleotides used for the amplification of the full-length cDNA.

TABLE 1. Cortisol secretion by adrenal cells dispersed from the patients with Cushing's syndrome and from normal controls

Source of cells	GIP conc. (nmol/L)	Cortisol conc. in medium (% of unstimulated cells)
Normal adult adrenal	10.0	98
Normal fetal adrenal;	10.0	100
GIP-dependent bilateral hyperplasia[a]	0.01	102
	0.1	116
	1.0	350
	10.0	841
GIP-dependent adrenal adenoma[b]	100.0	745
Non food-dependent cortisol secreting adenoma	10.0	103
Non food-dependent cortisol secreting adenoma	10.0	96
Vasopressin-dependent bilateral hyperplasia	10.0	97
Cushing's disease	10.0	100

Cells were incubated for 2 h at a concentration of 10^6/mL in DMEM without serum; the cortisol concentration was determined in the incubation medium by RIA or by immunofluorometric assay.

[a] Ref. 5.

[b] Ref. 9.

Incubation with ACTH-(1–24) stimulated cortisol secretion in all adrenal cells studied (not shown).

Discussion

GIP is a 42-amino acid hormone produced in K cells of the duodenum and small intestine; it belongs to the vasoactive intestinal peptide/glucagon/secretin gastrointestinal hormone family (20, 21). Its main physiological role appears to be its insulinotropic effect on pancreatic islet β-cells; however, additional effects include metabolic regulation of adipose tissue, stimulation of glucagon and somatostatin release, and modulation of hepatic, portal, and mesenteric blood flow (20, 21). Initial radioligand studies in pancreatic β-cells indicated that the GIPR is a 56-kDa glycoprotein functionally coupled to adenylate cyclase (22–24). The recent cloning of rat (10), hamster (11), and human GIPR (12–14) confirmed that it was a member of the secretin-vasoactive intestinal peptide family of G protein-coupled receptors; the GIPR gene is localized on chromosome 19q13.3. In humans, two forms of GIPR resulting from alternate splicing have been cloned, one of 466 and one of 493 amino acids with a 27-amino acid insertion at the carboxyl-terminal of the cytoplasmic tail (12); they both bind GIP-(1–42) and are coupled to adenylate cyclase.

The presence of GIPR in the adrenals of these two patients with GIP-dependent Cushing's syndrome was suggested both *in vivo*, by the bilateral adrenal uptake of [^{123}I]GIP (5), and *in vitro*, where GIP stimulated cortisol secretion from dispersed adrenal cells (5, 7, 9). The present study confirmed, at the molecular level, the presence of GIPR messenger RNA (mRNA) in the adrenals of two patients with GIP-dependent Cushing's syndrome. GIPR expression was previously demonstrated by *in situ* hybridization in the adrenal adenoma of the patient with GIP-dependent Cushing's syndrome, whereas it was not present in a non-food-dependent cortisol-secreting adrenal adenoma (9); however, normal human

adrenal tissues were not studied in that initial report. The absence of functional GIPR in normal adult or fetal human adrenals was previously suggested by the lack of steroidogenic response to GIP *in vivo* (5, 6) and *in vitro* (5, 7), and is confirmed in this study. The low level of GIPR demonstrated by RT-PCR in normal fetal and adult adrenal tissues or in the hyperplastic adrenals of a pituitary Cushing's disease patient is not coupled to regulation of steroidogenesis by GIP *in vivo* (5, 6) or *in vitro* (5). Thus, this study indicates that an important overexpression of GIPR and its effective coupling to steroidogenesis are responsible for GIP-dependent Cushing's syndrome. We had initially suggested that this syndrome could result from the ectopic or aberrant expression of the GIPR (5); although a faint amount of GIPR mRNA is detectable by large RT-PCR amplification, we propose that the concept of an ectopic receptor, responsible for the physiopathology of this syndrome, remains valid because the small amount of mRNA does not appear to confer biological activity. It also remains to be determined whether the small amount of GIPR is expressed in steroidogenic cells or in others, such as endothelial cells in normal adrenals.

Three distinct forms of GIPR, the full-length cDNA, a cDNA without exon 4, and one without exon 9, have been identified in normal adrenals and in those of GIP-dependent Cushing's syndrome; it is not known whether the latter two are also functional receptors when overexpressed. Two GIPR cDNAs were described recently in human pancreas (12): one lacks exons 9 and 10 and encodes a nonfunctional receptor, whereas the other has an 81-nucleotide insertion at the 3'-end and encodes a functional receptor. In this study, these two forms of GIPR have not been seen in adrenals from either patients or normal subjects. Another potential pathophysiological mechanism of GIP-dependent Cushing's syndrome, an activating mutation of the GIPR, was excluded by direct sequencing of its full-length cDNA. A recent study reported the presence of a partially inactivating missense mutation of the hGIPR gene in close to 4% of the Japanese population (25); no association with noninsulin-dependent diabetes was found.

The molecular mechanisms regulating tissue-specific expression of GIPR are still unknown, as are those leading to increased adrenal expression; the promoter of the hGIPR has not yet been characterized. Transcriptional regulation is the most plausible mechanism, as the expressions of GIPR isoforms were similar to those of the full-length GIPR. A unilateral GIP-dependent cortisol-secreting adenoma may result from the clonal expansion of one cell in which a somatic mutation inducing the overexpression of GIPR had occurred. In the case of GIP-dependent bilateral macronodular adrenal hyperplasia, where all adrenal cells exhibit hyperplasia, the mutation must have occurred during embryogenesis; genetic transmission has not been demonstrated. The mechanisms by which GIPR expression is decreased in non-GIP-dependent adrenal Cushing's syndrome, as shown in this and a previous study (9), may also imply transcriptional regulation. It will be of interest to examine whether the GIPR expression is increased in tissues other than the adrenals in patients with bilateral diffuse hyperplasia and whether it results in other pathological conditions. Taking into account the high expression of GIPR in the rat brain (10, 15), it is noteworthy that the patient with bilateral adrenal disease (5) has persisted in presenting significant psychiatric symptoms several years after the correction of hypercortisolism.

The possibility that ectopic expression or abnormal activity of hormone receptors other than for GIP may be functionally coupled to adenylyl cyclase and steroidogenesis and underlies other cases of adrenal Cushing's syndrome is now supported by recent reports of vasopressin-dependent (17, 26, 27) and β-adrenergic-dependent Cushing's syndromes (16). The identification of the abnormal receptors can lead to novel pharmacological approaches to control hypercortisolism and potentially adrenal proliferation with inhibitors of the ligands (6) or antagonists of the ectopic receptors (16).

Acknowledgments

The authors thank Drs. Ted B. Usdin and Tom I. Bonner for providing the human GIPR cDNA; Drs. Nicole Gallo-Payet, Daniel Pharand, Jean-Marie Paquin, and Alfons Pomp for providing the adrenal tissues; and Marie-France Lepage for preparation of the manuscript.

References

1. **Nieman L, Cutler Jr GB.** 1995 Cushing's syndrome. In: De Groot LJ, eds. Endocrinology. Philadelphia: Saunders; 1741–1770.
2. **Lieberman SA, Eccleshall TR, Feldman D.** 1994 ACTH-independent massive bilateral adrenal disease (AIMBAD): a subtype of Cushing's syndrome with major diagnostic and therapeutic implications. Eur J Clin Endocrinol. 131:67–73.
3. **Stratakis CA, Carney JA, Lin J-P, et al.** 1996 Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. J Clin Invest. 97:699–705.
4. **Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM.** 1991 Activating mutations of the stimulatory G Protein in the McCune-Albright syndrome. N Engl J Med. 325:1688–1695.
5. **Lacroix A, Bolté E, Tremblay J, et al.** 1992 Gastric inhibitory polypeptide-dependent cortisol hypersecretion: a new cause of Cushing's syndrome. N Engl J Med. 327:974–980.
6. **Reznik Y, Allali-Zerah V, Chayvialle JA, et al.** 1992 Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. N Engl J Med. 327:981–986.
7. **Archambeaud-Mouveroux F, Comas JM, Tessier MP, et al.** Food-dependent Cushing's syndrome associated with the Carney complex. Proc of the 78th Annual Meeting of the Int Endocrine Soc. 1996; 899.
8. **Hamet P, Larochelle P, Franks DJ, Cartier P, Bolté E.** 1987 Cushing's syndrome with food-dependent periodic hormonogenesis. Clin Invest Med. 10:530–533.
9. **De Herder WW, Hofland LJ, Usdin TB, et al.** 1996 Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. J Clin Endocrinol Metab. 81:3168–3172.
10. **Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI.** 1993 Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology. 133:2861–2870.
11. **Yasuda K, Inagaki N, Yamada Y, Kubota A, Seino S, Seino Y.** 1994 Hamster gastric inhibitory polypeptide receptor expressed in pancreatic islets and clonal insulin-secreting cells; its structure and functional properties. Biochem Biophys Res Commun. 205:1556–1562.
12. **Gremlich S, Porret A, Hani EH, et al.** 1995 Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes. 44:1202–1208.
13. **Yamada Y, Hayami T, Nakamura K, et al.** 1995 Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. Genomics. 29:773–776.
14. **Voltz A, Goke R, Lankat-Buttgereit B, Fehmann HC, Bode HP, Goke B.** 1995 Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. FEBS Lett. 373:23–29.
15. **Kaplan AM, Vigna SR.** 1994 Gastric inhibitory polypeptide (GIP) binding sites in rat brain. Peptides. 15:297–302.
16. **Lacroix A, Tremblay J, Rousseau G, Bouvier M, Hamet P.** 1997 Propranolol therapy for ectopic β-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med. 337:1429–1434.
17. **Lacroix A, Tremblay J, Touyz R, et al.** 1997 Abnormal adrenal and vascular responses to vasopressin mediated by a V_1-vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia,

Cushing's syndrome and orthostatic hypotension. J Clin Endocrinol Metab. 82:2414–2422.

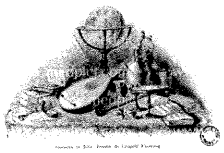
18. **Chomczynski P, Sacchi N.** 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156–159.
19. **Sanger F, Nicklen S, Coulson A.** 1977 DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA. 74:5463–5467.
20. **Fehmann HC, Goke R, Goke B.** 1995 Cell and molecular biology of the incretin hormones glucagon-like peptide-1 and glucose-dependent insulin releasing polypeptide. Endocr Rev. 16:390–410.
21. **Beck B.** 1989 Gastric inhibitory polypeptide: a gut hormone with anabolic functions. J Mol Endocrinol. 2:169–174.
22. **Amiranoff B, Couvineau A, Vauclin-Jacques N, Laburthe M.** 1986 Gastric inhibitory polypeptide receptor in pancreatic beta cells: direct cross-linking, solubilisation and characterization as a glycoprotein. Eur J Biochem. 159:353–358.
23. **Maletti M, Altman JJ, Hoa DH.** 1987 Evidence of functional gastric inhibitory polypeptide (GIP) receptors in human insulinoma. Binding of synthetic human GIP1–31 and activation of adenylate cyclase. Diabetes. 36:1336–1340.
24. **McIntosh CH, Wheeler MB, Gelling RW, Brown JC, Pederson RA.** 1996 GIP receptors and signal-transduction mechanisms. Acta Physiol Scand. 157:361–365.
25. **Kubota A, Yamada Y, Hayami T, et al.** 1996 Identification of two missense mutations in the GIP receptor gene: a functional study and association analysis with NIDDM. Diabetes. 45:1701–1705.
26. **Perraudin V, Delarue C, De Keyzer Y, et al.** 1995 Vasopressin-responsive adrenocortical tumor in a mild Cushing's syndrome: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab. 80:2661–2667.
27. **Horiba N, Suda T, Aioba M, et al.** 1995 Lysine vasopressin stimulation of cortisol secretion in patients with adreno-corticotropin-independent macronodular adrenal hyperplasia. J Clin Endocrinol Metab. 80:2336–2341.

Résumé

Suite à la description originale du SC GIP-dépendant en 1992 chez une patiente présentant une hyperplasie surrénalienne bilatérale (Lacroix *et al.* 1992), nous avions émis l'hypothèse d'une expression ou d'une fonction aberrante de GIPR au sein de la lésion surrénalienne sensible au GIP. Le clonage de l'ADNc du récepteur humain, en 1996, (Gremlich *et al.* 1995) (Volz *et al.* 1995) (Yamada *et al.* 1995) nous fournit les outils moléculaires afin de vérifier cette hypothèse. Une étude avait précédemment rapporté la forte expression de GIPR dans l'adénome surrénalien d'un patient atteint d'un SC GIP-dépendant comparativement au tissu surrénalien d'un patient ayant un SC non GIP-dépendant (de Herder *et al.* 1996). Cependant, ni l'étude de l'expression du récepteur dans la corticosurrénale humaine normale, ni la caractérisation moléculaire de ce dernier n'avaient été effectuées. Le premier article de cette thèse constitue la **première démonstration de l'expression ectopique de GIPR dans deux lésions surrénaliennes sensibles au GIP**.

Dans notre étude, l'expression de GIPR a été examinée, par RT-PCR, sur une série de tissus surrénaliens normaux et pathologiques : 1 hyperplasie (Lacroix *et al.* 1992) et 1 adénome (de Herder *et al.* 1996) surrénaliens GIP-dépendants, 3 surrénales issues de plusieurs cas de SC non GIP-dépendant ainsi que dans 5 surrénales normales adultes et fœtales. Un fort signal était observé uniquement dans les lésions surrénaliennes sensibles au GIP, tandis qu'une faible présence du messager était détectée dans les surrénales normales adultes et foetales. Les traces d'ARNm dans les surrénales normales ne sont décelées qu'après 35 cycles d'amplification et hybridation de type Southern ; elles proviennent probablement d'une contamination par l'endothélium vasculaire qui est un site connu d'expression de GIPR. Le concept d'expression ectopique de GIPR dans les tumeurs surrénaliennes sensibles au GIP fut donc validé.

L'ADNc entier de GIPR a été généré à partir de l'hyperplasie surrénalienne GIP-dépendante de notre patiente, sous-cloné et séquencé. Nous avons rapporté, pour la première fois, l'absence de mutation dans la partie codante du récepteur, ainsi que l'expression de plusieurs formes d'épissage dans les surrénales normales et pathologiques. Depuis cette démonstration initiale, l'expression ectopique de GIPR a été rapportée dans plusieurs autres hyperplasies et adénomes surrénaliens sensibles au GIP (voir revues présentées en annexe). L'ensemble de ces études suggèrent que l'expression illicite de GIPR dans les tissus surrénaliens pathologiques serait le défaut moléculaire responsable du SC GIP-dépendant. Cependant, les données présentées ici ne permettent pas d'exclure l'hypothèse d'une dédifférenciation des tumeurs surrénaliennes conduisant à l'expression ectopique de GIPR. Cette question sera abordée dans le deuxième article de cette thèse.



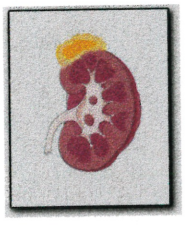
CHAPITRE 3

ARTICLE 2

Asynchronous Development of Bilateral Nodular Adrenal Hyperplasia in Gastric Inhibitory Polypeptide-dependent Cushing's Syndrome.

Nina N'Diaye, Pavel Hamet, Johanne Tremblay, Jean-Marie Boutin, Louis Gaboury et André Lacroix

Journal of Clinical Endocrinology and Metabolism 84 : 2616-2622, 1999



0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 8
Printed in U.S.A.

Asynchronous Development of Bilateral Nodular Adrenal Hyperplasia in Gastric Inhibitory Polypeptide-Dependent Cushing's Syndrome*

NINA N'DIAYE, PAVEL HAMET, JOHANNE TREMBLAY, JEAN-MARIE BOUTIN, LOUIS GABOURY, AND ANDRÉ LACROIX

Division of Endocrinology and Departments of Medicine (N.N., P.H., J.T., J.-M.B., A.L.) and Pathology (L.G.), Research Center, Hôtel-Dieu, Centre Hospitalier de l'Université de Montréal, 3850 Saint-Urbain, Montreal, Canada H2W 1T8

ABSTRACT

Gastric inhibitory polypeptide (GIP)-dependent Cushing's syndrome has been reported to occur either in unilateral adrenal adenoma or in bilateral macronodular adrenal hyperplasia. A 33-yr-old woman with Cushing's syndrome was found to have two 2.5- to 3-cm nodules in the right adrenal on computed tomography scan; the left adrenal appeared normal except for the presence of a small 0.8 × 0.6-cm nodule. Uptake of iodocholesterol was limited to the right adrenal. Plasma morning cortisol was 279 nmol/L fasting and 991 nmol/L postprandially, and ACTH remained suppressed. Plasma cortisol increased after oral glucose (202%) or a lipid-rich meal (183%), but not after a protein-rich meal (95%) or iv glucose (93%); the response to oral glucose was blunted by pretreatment with 100 μg octreotide, sc. Plasma cortisol and GIP levels were positively correlated ($r = 0.95$; $P = 0.0001$); cortisol was stimulated by the administration of human GIP iv (225%), but not by GLP-1, insulin, TRH, GnRH, glucagon, arginine vasopressin, upright posture, or cisapride orally. A right adrenalectomy was performed; GIP receptor messenger ribonucleic acid was overexpressed in both adrenal nodules and in the adjacent cortex. Histopathology revealed diffuse macronodular adrenal hyperplasia without internodular atrophy. Three months after surgery, fasting plasma ACTH and cortisol were suppressed, but cortisol increased 3.6-fold after oral glucose, whereas ACTH remained suppressed; this was inhibited by octreotide pretreatment, suggesting that cortisol secretion by the left adrenal is also GIP dependent. We conclude that GIP-dependent nodular hyperplasia can progress in an asynchronous manner and that GIPR overexpression is an early event in this syndrome. (*J Clin Endocrinol Metab* **84:** 2616–2622, 1999)

FOOD-DEPENDENT adrenal Cushing's syndrome has been reported in recent years in patients with either bilateral macronodular adrenal hyperplasia (1–3) or single unilateral adrenal adenoma (3–7); other cases of Cushing's syndrome and periodic hormonogenesis of unknown cause were also probably secondary to the same etiology (8). Abnormal adrenal regulation of cortisol production by gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic polypeptide) *in vivo* (1, 2, 5) or *in vitro* (1, 3, 5–7) suggested that this new etiology of Cushing's syndrome may be secondary to either an ectopic expression or an activating mutation of GIP receptors (GIPR) not normally expressed or functional in adrenal cortical tissues. The human GIPR complementary DNA (cDNA) and gene have now been cloned (9, 10); the gene is composed of 14 exons spanning approximately 13.8 kb of DNA and is localized on chromosome 19q13.3 (9). Recent studies indicate that GIP-dependent Cushing's syndrome results from the adrenal overexpression of the GIPR in the adrenal adenoma or hyperplasia tissues compared to that in normal adult (3, 6, 7, 11) or fetal adrenal cortex (3, 11) or that in non-GIP-dependent adrenal Cushing's syndrome tissues (3, 5, 6, 11); no mutation of the GIPR cDNA was identified in the affected adrenal tissues (6, 11). The small amount of GIPR messenger ribonucleic acid (mRNA) detected in normal adrenal tissues after at least 35 cycles of amplification was not efficiently coupled to steroidogenesis (1, 3, 6, 11), such that the concept of functional ectopic receptor remains valid.

The molecular mechanisms regulating tissue-specific expression of GIPR are still unknown, as are those leading to its increased adrenal expression. An acquired somatic mutation inducing the overexpression of GIPR may be responsible for the clonal expansion resulting in a single GIP-dependent cortisol-secreting adenoma; in the case of GIP-dependent bilateral macronodular adrenal hyperplasia, where all adrenal cells exhibit hyperplasia, the mutation must have occurred before the early stages of adrenal cortex embryogenesis. It is unclear, however, whether the ectopic expression of the GIPR precedes and is responsible for the adrenal overgrowth in addition to the regulation of cortisol secretion or whether the GIPR overexpression is secondary to dedifferentiation during a proliferative process caused by another pathophysiology. We now report a patient with GIP-dependent Cushing's syndrome with asynchronous development of bilateral nodular hyperplasia in whom there is evidence that the adrenal overexpression of the GIPR was present at the stage of hyperplasia as well as in larger nodules.

Received July 6, 1998. Revision received March 24, 1999. Accepted May 3, 1999.

Address all correspondence and requests for reprints to: André Lacroix, M.D., Division of Endocrinology, Research Center, Hôtel-Dieu, Centre Hospitalier de l'Université de Montréal, 3850 St. Urbain Street, Montreal, Quebec, Canada H2W 1T8. E-mail: lacroixa@ere.umontreal.ca.

* Presented in part at the 80th Annual Meeting of The Endocrine Society, New Orleans, LA, June 1998. This work was supported by a grant (MT-13189) from the Medical Research Council of Canada.

Case Report

A 33-yr-old woman was referred for evaluation of Cushing's syndrome, which had become symptomatic during the last 2–3 yr. She had experienced an 8-kg weight gain, headaches, high blood pressure up to 170/112 mm Hg, fatigue, sleep disturbances, lack of concentration, and emotional lability; she became amenorrheic during the last 4 months and noted muscle cramps and decreased muscle strength. There were no particular symptoms related to food intake and no gastrointestinal disturbances. There was no family history of endocrine diseases. Her oral daily medication included 4 mg perindopril (Coversyl, Servier Canada, Inc., Laval, Qc, Canada), 25 mg hydrochlorothiazide, 80 mg propranolol (Inderal, Wyeth-Ayerst, Labroatories, Inc., Saint-Laurent Qc, Canada) for control of high blood pressure, and 100 mg fluvoxamine (Luvox, Solvay Pharma, Inc., Scarborough, Ont, Canada) with 50 mg trazodone (Desyrel, Bristol-Myers Squibb Canada, Inc., Montreal, Qc, Canada) for symptoms of depression. On physical examination, height was 1.57 m, weight was 56.2 kg, and body mass index was 22.8. Blood pressure was 168/104 mm Hg, and heart rate was regular at 80 beats/min. There was central obesity, with rounded face and mild supraclavicular fat pads, but no abdominal striae. There was a mild facial down and normal skin pigmentation, but otherwise normal physical examination, including muscle strength.

Initial investigation had included elevated free urinary cortisol level of 1077 nmol/day (normal range, 90–330 nmol/day); morning fasting plasma cortisol was 279 nmol/L. Morning plasma ACTH was decreased at 0.3 pmol/L (normal range, 2–12 pmol/L). Serum levels were: dehydroepiandrosterone sulfate (DHEAS), 1.5 μmol/L (normal range, 0.9–11.6); testosterone, 0.4 nmol/L (normal range, <2.9); FSH, 3.6 U/L; LH, 0.4 U/L; and PRL, 10 μg/L. Blood electrolytes and fasting and postprandial glucose were normal, whereas low density lipoprotein cholesterol and triglycerides were elevated at 5.78 and 2.99 mmol/L, respectively. An abdominal computed tomography scan revealed that the right adrenal was the site of two 2.5- to 3-cm nodules; the left adrenal was of normal morphology, except for the presence of a 0.8 × 0.6-cm posterosuperior nodule. An iodocholesterol scan performed without dexamethasone suppression showed uptake of the tracer only in the right adrenal.

Materials and Methods

Clinical studies

The study protocols were approved by the institutional review committee, and written informed consent was obtained from the patient. Medications were discontinued for at least 1 week before conducting the evaluation. Studies were performed after an overnight fast in the supine position for 60 min before testing. An iv dexamethasone suppression test (1 mg/h from 1100–1500 h) was performed as described previously (1), where the patient remains fasting until the end of the infusion of dexamethasone. The protocol to screen for potential adrenal ectopic receptors included serial measurements at 30- to 60-min intervals during 2–3 h of plasma ACTH, cortisol, aldosterone, 17-hydroxyprogesterone, free testosterone, DHEAS, and estradiol during the course of the various tests, which were performed sequentially over the course of several days. Tests included the administration of 100 μg GnRH, iv (Factrel, Wyeth-Ayerst Laboratories, Inc., Saint-Laurent, Canada); 200 μg TRH, iv (Relefact, Hoechst-Roussel, Montreal, Canada); 10 IU arginine vasopressin, im (Pitressin, Parke-Davis, Scarborough, Canada); 1 mg glucagon, iv (Eli Lily Canada, Inc., Scarborough, Canada); 0.2 U/kg regular human insulin, iv (Humulin, Eli Lily Canada, Inc.); 10 mg cisapride, orally (Prepulsid, Janssen Pharmaceuticals, Mississauga, Canada); and 250 μg ACTH-(1–24), iv (Cortrosyn, Organon Canada, Scarborough, Canada). Other tests included a standard mixed meal and a posture test performed by a 2-h supine position, followed by a 2-h ambulation period.

To study the effects of carbohydrates, proteins, or lipids on cortisol secretion, the patient sequentially received orally at 3-h intervals 75 g oral glucose, an isocaloric protein-rich meal, or a lipid-rich meal as described previously (1). On a different day, 25 g glucose were administered iv, and 3 h later, 100 μg octreotide (Sandostatin, Novartis, Pointe Claire, Canada) were administered sc 60 min before repeating an oral 75-g glucose challenge; plasma levels of cortisol, ACTH, GIP, and insulin were determined at regular intervals during these tests. Human GIP (Bachem, Torrance, CA) was prepared and infused at a rate of 0.6 μg/kg·min during the administration of 150 cc/h 10% glucose as described previously (1); to maximize the response, endogenous levels of GIP were suppressed by the sc administration of 100 μg octreotide 90 min before starting the infusion of human (h) GIP. Glucagon-like peptide-1 (GLP-1; Bachem) was provided by Dr. John Dupre (London, Canada) as 50 μg/mL in 0.1% human serum albumin and was infused at a rate of 0.75 pmol/kg·min also under 10% glucose, as described previously (12); the GLP-1 infusion was not preceded by the administration of octreotide.

Assays

Plasma and urinary cortisol and plasma estradiol were measured by immunofluorometric assay (Bayer Immuno I System, Tarytown, NY), ACTH by immunoradiometric assay (Allegro, Nichols Institute Diagnostics , San Juan Capistrano, CA), and plasma GIP (Peninsula Laboratories, Inc. Belmont, CA) and other steroid hormones by commercial RIA kits.

RNA preparation and GIPR RT-PCR

Total RNA was extracted from adrenals by the guanidium-phenol chloroform method (13). First strand cDNA synthesis was carried out with 2 μg total RNA and random primers (hexamers) by using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Burlington, Canada) as recommended by the manufacturer. In control reactions, reverse transcriptase was omitted to ensure that the PCR amplification did not result from contaminating genomic DNA. The PCR reaction contained 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.2 mmol/L of each deoxy-NTP, 10 pmol each of sense and antisense primers specific for the human GIP receptor (GenBank U39231), one fifth of the RT reaction, 2.5 U *Taq* DNA polymerase, and 5% formamide. The amplification was achieved with 35 cycles (94 C, 30 s; 49 C, 30 s; 72 C, 30 s) with a pair of primers specific for hGIPR [5′-TGCTAGCCCTGCTCATCTTGA-3′ (513–533) and 5′-ACACGGGGATCCCGCCCCCTA-3′ (1453–1474)]. The PCR products were separated on agarose gel. The RNA samples were also amplified (94 C, 30 s; 51 C, 30 s; 72 C, 30 s) with a pair of primers specific for the human β-actin cDNA (5′-GATTCCTATGTGGGCGA-3′ and 5′-GATTCCTATGTGGGCGA- 3′).

Results

During an initial screening, morning fasting plasma cortisol increased from 279 to 455 nmol/L before and to 991 nmol/L 2 h after the noontime meal; plasma ACTH remained less than 0.4 pmol/L. An iv dexamethasone suppression test failed to decrease plasma cortisol levels and did not prevent postprandial elevations of cortisol (276%). A standard mixed meal was able to reproduce the increase in plasma cortisol from 376 to a peak value of 888 nmol/L at 90 min (Table 1); the iv administration of ACTH-(1–24) also resulted in a stimulation of plasma cortisol (180%), whereas the upright posture test, TRH, GnRH, glucagon, insulin-induced hypoglycemia, arginine vasopressin, and cisapride were without effect (Table 1). Plasma cortisol increased in response to 75 g oral glucose (202%) and a lipid-rich meal (183%), but not after a protein-rich meal (95%) or 25 g glucose, iv (93%); the response to oral glucose was decreased by pretreatment with 100 μg octreotide, sc (Fig. 1). Plasma cortisol elevations followed and were positively correlated with plasma GIP levels during these various tests ($r = 0.95$; $P = 0.0001$). Cortisol levels were stimulated by the infusion of 0.6 μg/kg·h hGIP (225%), but not by 0.75 pmol/kg·min GLP-1 (88%) (Fig. 2); plasma insulin levels increased from 138 to a peak value of 344 pmol/L after 60 min of GLP-1 infusion. The plasma levels of GIP reached during the hGIP infusion (1000 ng/L) were

TABLE 1. *In vivo* modulation of cortisol secretion by various tests in the patient with ACTH-independent adrenal Cushing's syndrome

Test	Basal plasma cortisol (nmol/L)	Peak plasma cortisol (nmol/L)	Change at peak (% of basal)
Mixed meal	376	888	236
ACTH-(1–24) (250 μg, iv)[a]	799	1440	180
Upright posture test	317	361	114
TRH (200 μg, iv)	447	469	105
GnRH (100 μg, iv)	480	524	109
Glucagon (1 mg, iv)	357	389	109
Insulin (0.2 U/kg, iv)	403	430	107
Arginine vasopressin (10 U, im)[a]	742	713	96
Cisapride (10 mg, orally)	314	320	102

[a] Tests were performed 2 h after a mixed meal.

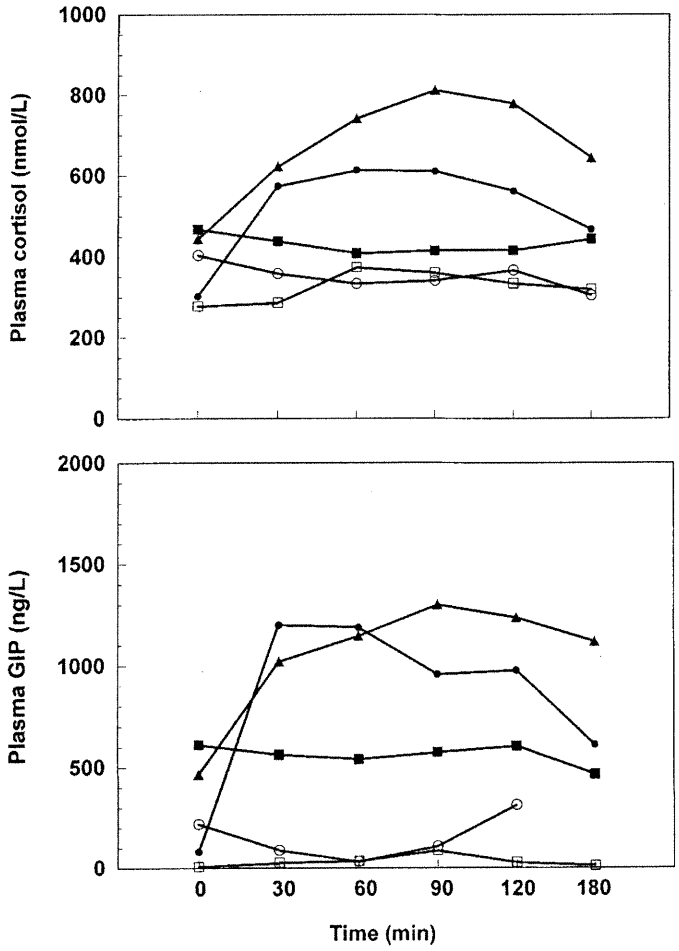


FIG. 1. Plasma cortisol and GIP responses to the oral administration of 75 g glucose (●), a protein-rich meal (■), or a lipid-rich meal (▲); iv administration of 25 g glucose (○); and oral administration of 75 g glucose 1 h after the sc injection of 100 μg octreotide (□) in the patient with food-dependent cortisol production. The first three tests were performed consecutively at 3-h intervals on the same day; the last two tests were also conducted sequentially at 3-h interval on the following day.

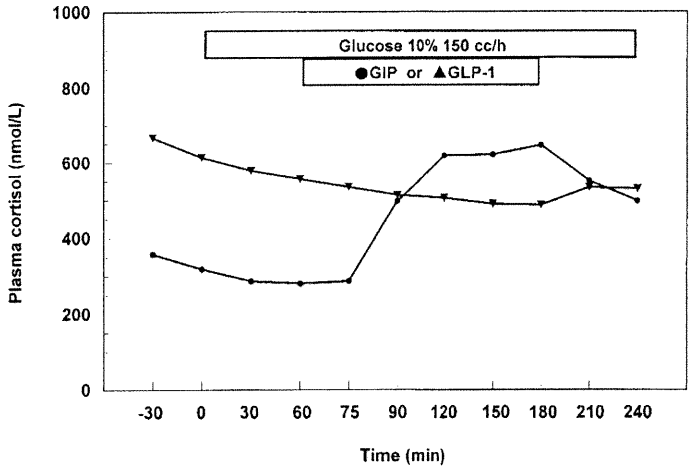


FIG. 2. Plasma cortisol concentrations during iv infusion of 10% glucose at 150 cc/h with the additional infusion of either GIP at a rate of 0.6 μg/kg·h (●) or GLP-1 at a rate of 0.75 pmol/kg·min (▲) during 120 min in the patient with food-dependent cortisol production. The GIP infusion was preceded 90 min earlier by the sc injection of 100 μg octreotide to suppress endogenous levels of GIP.

similar to those produced by the 75-g oral glucose test (1202 ng/L). The infusion of GIP *in vivo* induced an increase in plasma levels of 17-hydroxyprogesterone and free testosterone, but not of aldosterone, DHEAS, or estradiol (Table 2); plasma aldosterone increased after upright posture (46 to 234 pmol/L) or cisapride administration (53 to 524 pmol/L).

The right adrenal was removed by laparoscopy and was found to include two yellow-tan-colored macronodules, whereas the adjacent cortex appeared macroscopically normal (Fig. 3). However at histology, diffuse hyperplasia was present in the adrenal cortex, forming small micronodules outside of the two macronodules, which were composed of an alternance of clear and acidophilic cells (Fig. 3). The levels of GIPR mRNA in the adrenal and control tissues were detected using RT-PCR amplification and ethidium bromide staining. The expected size (980 bp) GIPR band was detected and overexpressed in both of this patient's right adrenal nodules (Fig. 4, lanes 2 and 3), whereas it was absent in the control normal adult adrenal cortex (lane 5). Interestingly, a GIPR band was also detectable in the patient's hyperplastic adrenal cortex adjacent to the macronodules (lane 4). The GIPR bands in this patient's macronodules were similar to those found in the previously reported positive control (5, 11) with a documented GIP-dependent adrenal adenoma (lane 1) or in normal pancreas (lane 7).

Suppression of the hypothalamic-pituitary-adrenal axis was present postoperatively, and oral replacement with hydrocortisone was adjusted progressively to 20 mg in the morning and 10 mg in the afternoon. Three months after surgery, fasting morning plasma ACTH and cortisol were still suppressed, but plasma cortisol increased reproducibly

TABLE 2. Plasma levels of various steroids in the patient with GIP-dependent Cushing's syndrome in response to the infusion of GIP *in vivo*

Time (min)	Cortisol (nmol/L)	Aldosterone (pmol/L)	DHAS (μmol/L)	17-Hydroxyprogesterone (nmol/L)	Free testosterone (pmol/L)	Estradiol (pmol/L)
0[a]	281	67	0.9	1.8	1.4	48
15	288	71	1.0	1.6	0.9	51
30	497	65	0.9	6.0	2.5	54
60	618	69	1.0	8.5	3.3	46
90	621	79	1.1	5.8	ND	57
120	646	74	1.1	8.7	2.0	43
150	551	56	1.1	4.8	1.9	39
180	498	53	1.1	4.7	1.5	41
Normal range basal levels	170–800	28–443	0.9–11.6	0.4–10.7	2.8–11.0	0–1336

[a] GIP was infused at a rate of 0.6 μg/kg · h starting at time zero until the 120 min point as described in *Materials and Methods.*

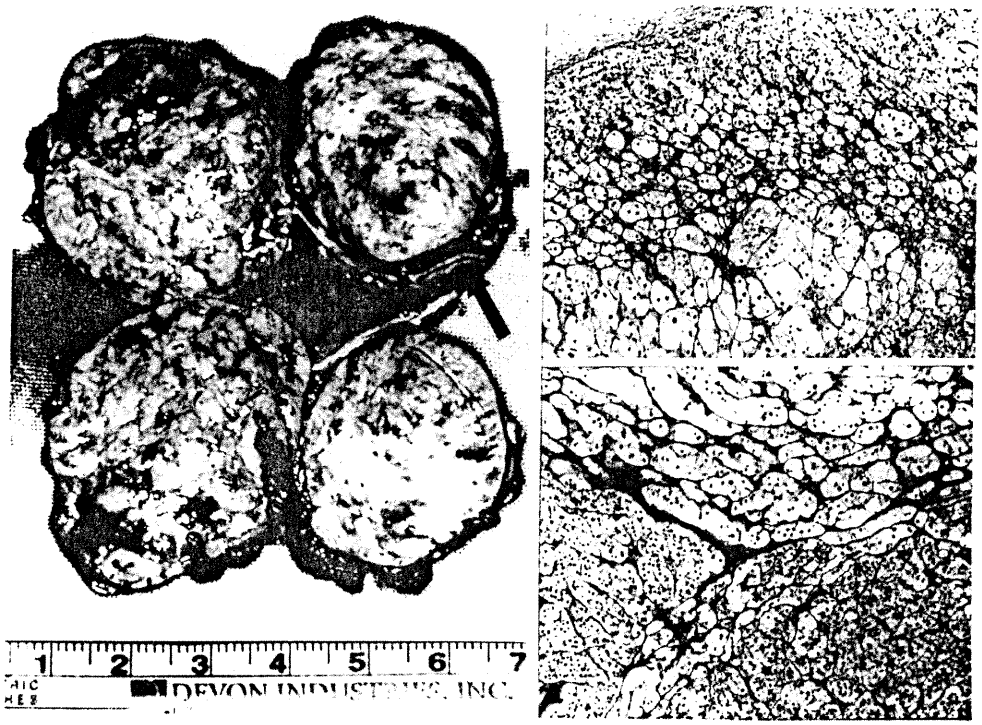


FIG. 3. Pathology of the right adrenal gland removed from the patient with GIP-dependent Cushing's syndrome. The macroscopic examination (*left panel*) shows the adrenal gland, which was sectioned in the middle of its horizontal plane; two large nodules were well demarcated, whereas the remaining extranodular portions of the adrenal cortex appeared of normal thickness (*arrow*). At histological examination (magnification, ×40) shown in the *right panel*, the cortex outside of the two main nodules was hyperplastic (*right upper panel*) and included several micronodules (*right lower panel*) composed of acidophilic cuboidal cells alternating with cells with clear cytoplasm. The adrenal capsule is seen in the *left top portion of the top panel.*

(362%) after 75 g glucose, orally. ACTH remained suppressed, but plasma GIP increased normally (Fig. 5). The response of plasma cortisol to 75 g oral glucose was abolished when the GIP stimulation was inhibited by pretreatment with 100 μg octreotide, sc. One year after the surgery, the patient still required replacement with 20 mg hydrocortisone daily because of the persistent suppression of the hypothalamic-pituitary-adrenal axis; 24-h urinary free cortisol levels are maintained in the normal range on this medication. Signs and symptoms of Cushing's syndrome have disappeared, weight has decreased to 50.8 kg, and the patient is normotensive without any other medication. On repeat abdominal computed tomography scan, the left adrenal nodule now measures 0.8 × 0.9 cm.

A 75-g oral glucose test was performed in the mother and two sisters of this patient; in each case, plasma levels of cortisol decreased, as expected, with the diurnal rhythm and were not stimulated by the increase in plasma levels of GIP (Fig. 5).

Discussion

Our investigation clearly indicated that this patient presented primary ACTH-independent Cushing's syndrome. Since the initial descriptions of GIP-dependent Cushing's syndrome (1, 2), we suggested that primary adrenal Cushing's syndrome could result from the ectopic or abnormal adrenal expression of a wide diversity of hormone receptors; this hypothesis is tested using a protocol that produces transient fluctuations of various hormones, which could be the ligands for potential ectopic adrenal receptors and thus induce ACTH-independent cortisol production. This protocol was recently successful in identifying abnormal responses to vasopressin (14) and ectopic β-adrenergic receptors (15) in patients with bilateral macronodular adrenal hyperplasia. The initial investigation of this patient clearly suggested the possibility of periodic hormonogenesis, as plasma cortisol levels showed erratic diurnal variations. The food dependence was suggested by an increase in cortisol 2 h postpran-

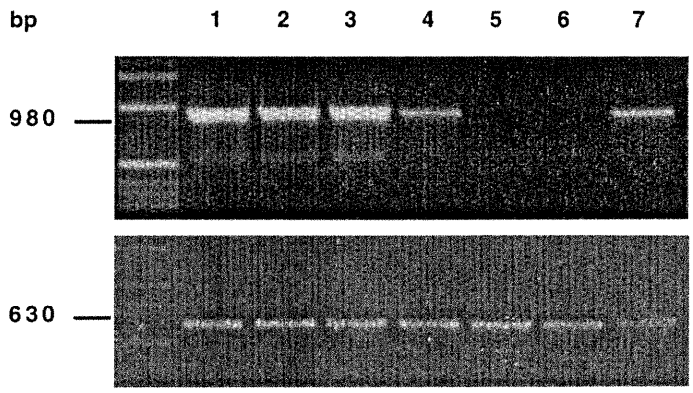


FIG. 4. Analysis of the expression of the GIPR (*upper panel*) and β-actin (*lower panel*) by RT-PCR. Two micrograms of total RNA from adrenal tissues from a previously studied patient (5, 11) (positive control) with a GIP-dependent adrenal adenoma (lane 1), from the two right adrenal macronodules (lanes 2 and 3) or from the adrenal cortex adjacent to the macronodules (lane 4) from this patient, from normal adult whole adrenal (lanes 5), from non-GIP-dependent macronodular adrenal hyperplasia (lane 6), and from normal adult pancreas (lane 7) were amplified by RT-PCR as described in *Materials and Methods*. The PCR products were run on a 1.5% agarose gel and stained by ethidium bromide. The *left part* of lane 1 contains base pair size markers, and the numbers of base pairs of the expected amplified bands are indicated.

dially, and this was confirmed by the tests with mixed meals with or without dexamethasone suppression. The pattern of cortisol response to various oral test meals, the absence of stimulation after iv glucose, and the inhibition of oral glucose response by octreotide all supported the idea that the mediator was a gastrointestinal hormone. The good correlation between plasma GIP and cortisol levels supported the idea that GIP could be the mediator; however, GLP-1, another important incretin that responds to the same secretagogues, could have been another candidate. The cortisol response to the infusion of physiological concentrations of GIP and the absence of response to GLP-1 clearly indicated that this patient had GIP-dependent adrenal Cushing's syndrome. The lack of response of GIP and cortisol to the protein-rich meal, a relatively weaker secretagogue of GIP, was probably secondary to the fact that the GIP levels had not completely returned to baseline after the more potent effects of the oral glucose test.

GIP-dependent Cushing's syndrome has been reported in a relatively small number of patients to date (1–7); however, this patient presented several features that render the case of particular interest. Previous patients with GIP-dependent Cushing's syndrome had fasting plasma cortisol levels ranging from as low as 4–47 nmol/L (5), 68–140 nmol/L (7), 102–119 nmol/L (2), 121–200 nmol/L (6), and 146 nmol/L (5) to 160–193 nmol/L (1); the current patient had fasting plasma cortisol ranging between 279–480 nmol/L, which indicates that GIP-dependent Cushing's syndrome should not be excluded without performing a test meal. It has been previously proposed that the suppression of ACTH coupled with the low levels of GIP in the fasting state were responsible for the decreased plasma cortisol levels, which can be accompanied by symptoms of relative cortisol insufficiency (1, 2). The various other tests performed were not able to identify another abnormal receptor that could have explained the relatively normal fasting levels of cortisol in this patient. We cannot rule out the existence of ectopic receptors for other hormones that our protocol would not have identified; alternatively, a proportion of cortisol production by the two large nodules may be autonomous and non-GIP dependent.

Food- or GIP-dependent Cushing's syndrome was previously identified in patients with either bilateral large macronodular adrenal hyperplasia (1–3) or single unilateral adrenal adenoma (3–7). We were initially unclear whether this patient had two distinct adenomas in the right adrenal and a nonfunctional incidentaloma in the left adrenal, as the iodocholesterol uptake was restricted to the right adrenal. The macroscopic appearance of the right adrenal tended to support the first hypothesis; however, the histological findings clearly indicate the presence of macronodular adrenal hyperplasia. There was one preliminary report of the coexistence of a schwannoma, pigmented skin lesions in a patient with GIP-dependent bilateral nodular hyperplasia that contained lipofuscin (3); there were no similar characteristics reminiscent of the Carney complex (16) in our or other patients.

This study confirmed the increased expression of GIPR mRNA in the two GIP-dependent macronodules, as reported previously in patients with large bilateral adrenal hyperplasia or unilateral adenomas and GIP-dependent Cushing's syndrome (3, 5–7, 11); however, GIPR overexpression was also detectable in this patient's adrenal cortex adjacent to the two larger nodules at a stage of relatively early hyperplasia. This finding supports the possibility that this patient has bilateral disease; the probable increased expression of GIPR in the small left adrenal cortex and nodule would explain the GIP-dependent cortisol production that was still present after right adrenalectomy. The previous sequencing of the GIPR cDNA indicated the existence of spliced isoforms lacking exons 4 and 9 in the GIP-dependent or normal adrenal tissues and the absence of receptor mutation in GIP-dependent adrenals (6, 11); the presence of an isoform lacking exon 9 is not detectable on the gel in Fig. 5 because the 61-bp difference is not resolved, and the two bands appear as a single 980-bp band.

The molecular mechanisms regulating tissue-specific expression of GIPR are still unknown, as are those leading to its increased adrenal expression. The cloning and characterization of the 5′-promoter and 3′-regulatory regions of the GIPR gene and of their specific transcription factors will be necessary to elucidate this question. It is unclear whether the ectopic expression of the GIPR precedes and is responsible for the adrenal overgrowth in addition to the regulation of cortisol secretion or whether the GIPR expression is a secondary phenomenon occurring during the course of the adrenal proliferation resulting from another primary pathophysiology. The presence of abnormal GIPR expression at the stage of early hyperplasia found in this patient argues in favor of a primary role and suggests that its overexpression precedes the nodular formation and may thus be at least partly responsible for the proliferative process. Chabre *et al.* (6) recently demonstrated a stimulation of thymidine incorporation by GIP in adrenal cells from GIP-dependent Cushing's syndrome, but not in normal cells. The steroidogenic

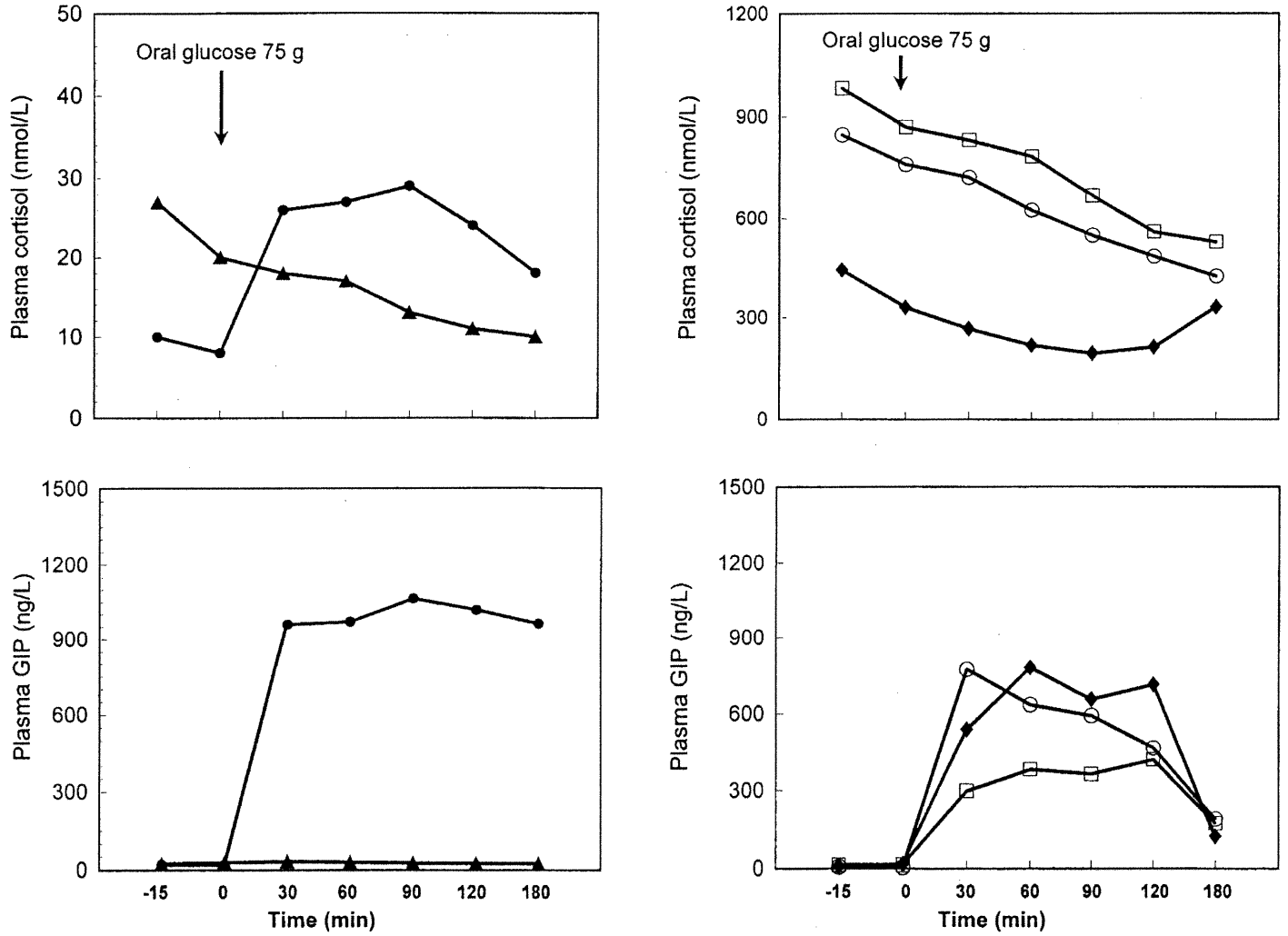


FIG. 5. Plasma cortisol and GIP responses to 75 g oral glucose administration given 3 months after right adrenalectomy in the patient with GIP-dependent Cushing's syndrome (*left panel*) and in her mother and two sisters (*right panel*). After the right adrenalectomy (*left panel*), tests were performed on two different mornings, after a 12-h overnight fast either without (●), or 60 min after the sc administration of 100 μg octreotide (▲); oral replacement with hydrocortisone was omitted until the end of the test. The oral administration of 75 g glucose was also performed in the mother (♦) and in two sisters (○ and □) in the morning after a 12-h overnight fast without octreotide (*right panel*).

secretory pattern suggests that in this case, the cells overexpressing the GIPR have a fasciculata phenotype, with a predominance of cortisol production without a significant stimulation of aldosterone or DHEAS production; different patterns were found previously in vasopressin- or catecholamine-dependent Cushing's syndrome (14, 15).

The concept of alterations in G protein coupled-receptors and/or postreceptor events leading to increased cAMP and proliferation is now well established (17) and was well studied in somatotroph and thyroid cells (18–20). Our hypothesis is that ectopic or abnormal expression of a hormone receptor capable of being coupled to adenylyl cyclase places the adrenal cells under the stimulation of a trophic factor that is not under a regulatory negative feedback by glucocorticoids; this constitutes an unregulated new trophic stimulus that leads to increased function and possibly to a proliferative advantage. A recent study indicates that the hormone-stimulated LH receptor can act as an adrenocortical tumor promoter when ectopically expressed in the adrenal cortex of mice transgenic for the inhibin α-subunit promoter/simian virus 40 T antigen fusion gene (21). It remains to be shown whether the adrenocortical expression of an ectopic receptor without another oncogenic event would be sufficient to induce adrenal overgrowth. The asynchronous nature of nodule formation observed in this patient suggests that the initial mutation is not uniformly distributed in all adrenocortical cells, or that other secondary events are necessary to generate within the hyperplastic cell population a clonal proliferation of selected cells. Bilateral macronodular adrenal hyperplasia is usually sporadic, but rare familial cases have been reported (22, 23); we have not found any evidence of GIP-dependent stimulation of cortisol production in three siblings of this patient.

The characterization of the pathophysiology of adrenal hyperplasias or tumors can eventually lead to diverse pharmacological therapies as alternatives to adrenalectomy. This has now been illustrated by the short term improvement of hypercortisolism with octreotide in GIP-dependent Cushing's syndrome (2, 5) and by the long term control of ectopic β-adrenergic receptors by propranolol (15). It would be ben-

eficial for this and other patients with GIP-dependent Cushing's syndrome to have access to an effective antagonist of the GIPR such as GIP-(7–30)-NH_2 (24) to correct the hypercortisolism and possibly prevent the progression of adrenal cell proliferation.

Acknowledgments

The authors thank Dr. Robert Wistaf for referring the patient, Ms. Marthe Ménard, R.N., and Ms. Manon Landry, R.N., for conducting the endocrine tests, Dr. Alfons Pomp and Wouter W. de Herder for providing us with the adrenal and pancreatic tissues, Drs. Jean-Louis Chiasson and John Dupre for helpful discussions, Ms. Sylvie Sauvé for illustrations, and Ms. Marie-France Lepage and Victoria Barranga for preparation of the manuscript.

References

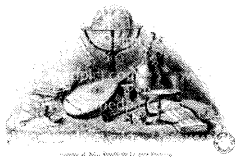
1. **Lacroix A, Bolté E, Tremblay J, et al.** 1992 Gastric inhibitory polypeptide-dependent cortisol hypersecretion: a new cause of Cushing's syndrome. N Engl J Med. 327:974–980.
2. **Reznik Y, Allali-Zerah V, Chayvialle JA, et al.** 1992 Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. N Engl J Med. 327:981–986.
3. **Lebrethon MC, Avallet O, Reznik Y, et al.** 1998 Food-dependent Cushing's syndrome: characterization and functional role of gastric inhibitory polypeptide receptor in the adrenals of three patients. J Clin Endocrinol Metab. 83:4514–4519.
4. **Hamet P, Larochelle P, Franks DJ, Cartier P, Bolté E.** 1987 Cushing's syndrome with food-dependent periodic hormonogenesis. Clin Invest Med. 10:530–533.
5. **De Herder WW, Hofland LJ, Usdin TB, et al.** 1996 Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. J Clin Endocrinol Metab. 81:3168–3172.
6. **Chabre O, Liakos P, Vivier J, et al.** 1998 Cushing's syndrome due to a gastric inhibitory polypeptide-dependent adrenal adenoma: insights into hormonal control of adrenocortical tumorigenesis. J Clin Endocrinol Metab. 83:3134–3143.
7. **Luton JP, Bertherat J, Kuhn JM, Bertagna X.** 1998 Aberrant expression of GIP (gastric inhibitory polypeptide) receptor in an adrenal cortical adenoma responsible for a case of food-dependent Cushing's syndrome. Bull Acad Natl Med. 182: 1839–1850.
8. **Olsen NJ, Fang VS, De Groot LJ.** 1978 Cushing's syndrome due to adrenal adenoma with persistent diurnal cortisol secretory rhythm. Metabolism 27:695–700.
9. **Yamada Y, Hayami T, Nakamura K, et al.** 1995 Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. Genomics. 29:773–776.
10. **Voltz A, Goke R, Lankatbuttgereit B, et al.** 1995 Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. FEBS Lett. 373:23–29.
11. **N'Diaye N, Tremblay J, De Herder WW, Hamet P, Lacroix A.** 1998 Adrenal overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. J Clin Endocrinol Metab. 83:2781–2785.
12. **Dupre J, Behme MT, Hramiak IM, McFarlane P, Williamson MP, Zabel P, McDonald TJ.** 1995 Glucagon-like peptide 1 reduces postprandial glycemic excursions in IDDM. Diabetes 44:626–630.
13. **Chomczynski P, Sacchi N.** 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156–159.
14. **Lacroix A, Tremblay J, Touyz R, et al.** 1997 Abnormal adrenal and vascular responses to vasopressin mediated by a V_1-vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia, Cushing's syndrome and orthostatic hypotension.: J Clin Endocrinol Metab. 82:2414–2422.
15. **Lacroix A, Tremblay J, Rousseau G, Bouvier M, Hamet P.** 1997 Propranolol therapy for ectopic β-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med. 337:1429–1434.
16. **Stratakis CA, Carney, JA, Lin J-P, et al.** 1996 Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. J Clin Invest. 97:699–705.
17. **Dhanasekaran N, Heasley LE, Johnson GL.** 1995 G Protein coupled-receptor systems involved in cell growth and oncogenesis. Endocr Rev. 16:259–270.
18. **Billestrup N, Swanson LW, Vale W.** 1986 Growth hormone releasing factor stimulates cell proliferation of somatotrophs *in vitro*. Proc Natl Acad Sci USA. 83:6854–6857.
19. **Dumont JE, Jaunaux JC, Roger PP.** 1989 The cyclic AMP mediated stimulation of cell proliferation. Trends Biochem Sci. 14:67–71.
20. **Ledent C, Dumont JE, Vassart G, Parmentier M.** 1992 Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J. 11:537–542.
21. **Rilianawati, Paukku T, Kero J, et al.** 1998 Direct Luteinizing hormone action triggers adrenocortical tumorigenensis in castrated mice for the murine inhibin α-subunit promoter/simian virus 40 T-antigen fusion gene. Mol Endocrinol 12:801–809.
22. **Findlay JA, Sheeler LR, Engeland WC, Aaron DC.** 1993 Familial adrenocorticotropin-independent Cushing's syndrome with bilateral macronodular adrenal hyperplasia. J Clin Endocrinol Metab. 76:189–191.
23. **Minami S, Sugihara H, Tatsukuchi A, et al.** 1996 ACTH independent Cushing's syndrome occuring in siblings. Clin Endocrinol. 44:483–488.
24. **Tseng CC, Kieffer TJ, Jarboe LA, Usdin TB, Wolfe MM.** 1996 Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. J Clin Invest. 98:2440–2445.

Résumé

Il a clairement été démontré que l'hypercorticisme GIP-dépendant est la conséquence de l'expression ectopique de GIPR dans les hyperplasies et adénomes surrénaliens. Cependant, l'implication potentielle de ce récepteur illicite dans le développement de tumeurs surrénaliennes n'a jamais été étudiée *in vivo*. L'expression ectopique de GIPR est-elle un événement précoce, responsable des capacités prolifératives et stéroïdogéniques accrues des cellules surrénaliennes affectées ou est-elle la conséquence d'un autre syndrome prolifératif entraînant la dédifférenciation des cellules surrénaliennes? Le présent article apporte un élément de réponse à cette question avec l'étude d'un cas atypique de SC GIP-dépendant pour lequel la **présence illicite de GIPR** fut **détectée à un stade précoce d'hyperplasie**.

Initialement diagnostiquée en tant que SC GIP-dépendant dû à la présence de deux nodules surrénaliens, une patiente, âgée de 33 ans, subit une surrénalectomie droite. L'étude de l'expression de GIPR par RT-PCR montra une forte présence du messager dans les deux nodules de la surrénale réséquée. La partie adjacente, d'apparence macroscopique normale, exprimait également le GIPR, bien que plus faiblement, contrairement aux surrénales normale et hyperplasique utilisées comme contrôles négatifs. Cependant, l'analyse histologique du spécimen révéla la présence de foyers d'hyperplasie précoce dans la région internodulaire, suggérant que l'expression ectopique de GIPR est décelable dès les premiers stades de l'hyperplasie. Par ailleurs, un test au repas effectué quelques mois après la chirurgie provoqua une stimulation anormale de la sécrétion de cortisol chez la patiente, indiquant la présence ectopique de GIPR dans la surrénale non réséquée. Ces résultats démontrent, pour la première fois, le **développement asynchrone d'une hyperplasie bilatérale GIP-dépendante**. L'observation de l'expression illicite du récepteur à une étape précoce du processus d'hyperplasie suggère que **la surexpression de GIPR précède la formation des nodules** et que ce dernier pourrait en partie être responsable du syndrome prolifératif.

Les mécanismes moléculaires conduisant à cette expression ectopique sont encore inconnus. L'hypothèse d'une mutation dans la région promotrice du gène GIPR a été avancée et éprouvée dans la dernière étude présentée dans cette thèse. Auparavant, nous caractériserons un autre type d'anomalie de récepteur, illustré par le SC LH-dépendant.



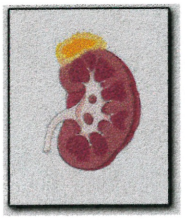
CHAPITRE 4

ARTICLE 3

Absence of Mutation of Aberrant LH/hCG Receptor in LH-dependent Adrenal Cushing's Syndrome

Nina N'Diaye, Isabelle Bourdeau, Johanne Tremblay, Pavel Hamet, et André Lacroix

Soumis à *Journal of Clinical Endocrinology and Metabolism,* 2001



Manuscript submitted to J. Clin. Endocrinol. Metab. On December 2001

Absence of Mutation of Aberrant LH/hCG Receptor in LH-dependent Adrenal Cushing's Syndrome

Nina N'Diaye, Isabelle Bourdeau, Johanne Tremblay, Pavel Hamet, and André Lacroix.

Division of Endocrinology, and Department of Medicine, Research Center, Hôtel-Dieu du Centre hospitalier de l'Université de Montréal (CHUM), 3850 Saint-Urbain street, Montréal, Canada H2W 1T8.

Running title: Aberrant adrenal LH/hCG Receptor

*: Presented in part at the 83rd Annual Meeting of the Endocrine Society, Denver, CO, June 2001.

Correspondence should be addressed to:
André Lacroix, M.D.
Division of Endocrinology
Research Center, Hôtel-Dieu du CHUM
3850 Saint-Urbain street
Montréal, Québec, Canada
H2W 1T8
Tel: (514) 980-8000 extension 12735
Fax: (514) 412-7204
e-mail: andre.lacroix@umontreal.ca

ABSTRACT

The aberrant expression or activity of various hormone receptors can regulate cortisol production in adrenal Cushing's syndrome. We have previously reported a woman with Cushing's syndrome secondary to bilateral adrenal hyperplasia in whom cortisol production was aberrantly regulated by LH/hCG and serotonin 5-HT_4 receptors agonists (N. Engl. J. Med. 341:1577-1581, 1999). We have now studied the expression and structure of the LH/hCG receptor in a biopsy of the adrenal tissue of this patient. The LH/hCGR, which is normally expressed in the zona reticularis of the human adrenal cortex, but not in zona glomerulosa or fasciculata, was identified by RT-PCR in a biopsy of this patient's macronodular adrenal hyperplasia. No mutation of the LH/hCG receptor was found by full sequencing of the cDNA and no overexpression of the receptor mRNA was identified by dot blot analysis when compared with normal or other cortisol-secreting (non LH/hCG dependent adenomas and hyperplasias) pathological adrenals. Several splicing variants of the LH/hCG receptor were detected in the patient's macronodular adrenal hyperplasia tissue as well as in normal adrenal cortex; they give rise to receptors with smaller extracellular domains.

This study confirms the presence of non-mutated LH/hCG receptor in the macronodular adrenal hyperplasia of this patient with LH/hCG-dependent Cushing's syndrome. It is still unclear whether the LH/hCGR is expressed ectopically in cells originating from the zona fasciculata or whether the aberrant response to LH/hCG results from an increased activity of the eutopic adrenal LH/hCG receptor.

INTRODUCTION

Recent work from our and other groups has led to the concept that some ACTH-independent cortisol-producing adrenal tumors and hyperplasias are not really autonomous, but can actually be regulated by various aberrantly expressed or overactive hormone receptors (1). As an example, we have recently described a patient with LH-dependent bilateral ACTH-independent macronodular adrenal hyperplasia (AIMAH) who developed transient clinical Cushing's syndrome during her four pregnancies, and which became persistent only after the long-term increase of LH levels at menopause (2). Cortisol secretion was stimulated by the administration of GnRH, hCG and LH, but not by FSH; in the same patient, the administration of cisapride and metoclopramide, two agonists of the 5-HT_4 receptor, also increased cortisol secretion. The pharmacological blockade of LH secretion with the long-acting GnRH analog leuprolide acetate led to normalization of urinary cortisol excretion, restoration of hypothalamic-pituitary-adrenal axis and to disappearance of clinical Cushing's syndrome without requirement for bilateral adrenalectomy.

The LH/hCGR is a large seven-transmembrane receptor (699 or 701 amino acids with or without LQ insertion at amino acid position 19-20) with a very large extracellular ligand binding domain (3,4); it normally stimulates gonadal steroidogenesis via adenylate cyclase (AC) and phospholipase C (PLC) pathways (5). Mainly expressed in gonadal tissues, but also in others such as uterus, fallopian tubes, placenta,

hypothalamus and prostate (6), the LH/hCGR was recently identified in the zona reticularis of the human adrenal cortex (7). However, although hCG enhances DHEAS secretion from human fetal adrenals, it normally does not induce cortisol or DHEAS secretion from adult adrenal glands, *in vivo* (2,8).

We proposed that LH-dependent cortisol secretion in this patient was secondary to the aberrant expression and/or function of LH/hCGR by a yet unknown mechanism (2). We have now determined the level of expression of the LH/hCGR in a biopsy of the AIMAH tissue of this patient, and sequenced its cDNA in order to verify the potential presence of an activating mutation.

MATERIAL AND METHODS

Patient's tissues

As the large bilateral adrenal lesions had not regressed radiologically after one year of experimental therapy with leuprolide acetate, we performed a biopsy of the left adrenal by laparoscopy. The pathological diagnosis was benign macronodular adrenal hyperplasia. A portion of this biopsy, and the adrenal specimens from other patients were rapidly collected at the time of surgery, frozen in liquid nitrogen, and stored at -80°C until analysis. The expression of LH/hCGR was also studied in adrenal cortex tissues obtained from 2 adult multiple organ donors (in one case, an adrenal fasciculata-reticularis enriched cell population was prepared and kindly provided by Dr Nicole Gallo-Payet, Université de Sherbrooke) and from patients with Cushing's syndrome from diverse etiologies: 2 women with persistent Cushing's disease following pituitary surgery; 2 patients (1 with AIMAH and 1 with unilateral adrenal adenoma) with GIP-dependent Cushing's syndrome (9); 1 patient with catecholamine-dependent AIMAH (10) and 1 with adrenal adenoma without aberrant hormone responsiveness (1). Ovarian and placental tissues (provided by Dr D. Provencher, Université de Montréal) were used as positive controls. The institutional ethics committee approved the study and written informed consent was obtained from the patients.

RNA preparation and LH/hCG receptor RT-PCR

Total RNA was extracted by the guanidium-phenol chloroform method (11). First strand cDNA synthesis was carried out with 2 μg total RNA and random primers using MMLV reverse transcriptase (InVitrogen, Burlington, ON, Canada) as recommended by the manufacturer. The amplification of LH/hCGR was mainly performed with two sets of primers, one encompassing the extracellular domain of the receptor (Forward: 5'-GGGGATCCGTCAAGACACTGGC-3'; Reverse: 5'-CTTTCTAGAGTGATGACGGTG-3'), and the other one, the transmembrane and cytoplasmic domains (Forward: 5'-CACTCTAGAAAGATGGCACAC-3';Reverse:5'-GGTCTAGACAGGGTTCCTACTC AC -3'). A third pair of primers spanning exons 5 to 10 was also used (Forward: 5'-GCATCTGTA ACACAGGCATC-3'; Reverse: 5'-CCCCGATGTGCTCCTGAACC-3'). PCR reactions were performed for 35 cycles (94°C for 30 sec; 50°C for 30 sec; 72°C for 30 sec) with one-fifth of RT reaction, 0.75mM $Mg(OAc)_2$, and 2.5 U Taq polymerase. In some experiments, 5% formamide was added to strengthen the PCR signal. Cycling was done at 94°C for 30 sec, 56°C for 30 sec, 72°C for 40 sec for the third set of primers.

DNA sequencing

RT-PCR products were subcloned in PUC19 (Roche Molecular Biochemicals, Laval, QC, Canada). Sequencing of cDNA inserts was performed on double stranded DNA using the chain termination reaction technique (12) with Circumvent (New England Biolabs, Mississauga, ON, Canada). Direct sequencing was also performed with automatic sequencer (ABI system, Perkin Elmer).

Dot blot analysis

Total RNA (2 μg) of each sample was spotted onto Hybond N+ membrane and hybridized with a 1.2 kb LH/hCGR cDNA, obtained by a HindIII-XbaI digestion and labeled with $dCT^{32}P$ using a random-prime labeling kit (Amersham-Pharmacia Biotech, Ste Anne-De-Bellevue, Qc, Canada) . Hybridization was performed with QuickHyb® as recommended by the manufacturer (Stratagene, La Jolla, CA, USA). Membranes were washed twice at room temperature for 15 min with 2X SSC and 0.1% SDS, then once for 30 min at 55°C with 0.5X SSC and 0.1% SDS. Quantification of LH/hCGR RNA was achieved with ImageQuant software (Molecular Dynamics Biotech, 1999, Piscataway, NJ, USA). Results were normalized with the house-keeping gene GAPDH. Each experiment (n=2) was performed in duplicate and analyzed twice.

RESULTS

The expression of LH/hCGR was examined by RT-PCR, using the set of primers spanning exons 5 to 11 (Fig.1), in the adrenal tissue of the patient with LH/hCG-dependent AIMAH, and normal adrenal fasciculata/reticularis enriched preparation and placenta as positive controls. The expected band of 655 bp was amplified in all samples (Fig.2). Using 2 other sets of primers (Fig.1), the whole LH/hCGR cDNA was generated from both normal and pathological adrenals. All RT-PCR fragments (of expected size or not) were subcloned and sequenced. This allowed us to identify several splicing variants that give rise to receptors with smaller extracellular domains (Table 1). The 469 bp- and 388 bp-bands detected in normal and pathological adrenals correspond to LH/hCGR$_{\Delta 9}$, LH/hCGR$_{\Delta 9\text{-}10}$ isoforms (Fig.2). For LH/hCGR$_{\Delta 9\text{-}10}$, there was one non conserved amino acid substitution (L316F) at the exon 8/11 boundary due to a transversion from TT (exon 8) G (exon 9) to TT (exon 8) T (exon 11). However, these variants are expressed in control adrenals as well. The isoforms LH/hCGR$_{\Delta 4\text{-}6}$, LH/hCGR$_{\Delta 3\text{-}6}$, and LH/hCGR$_{\Delta 3\text{-}6,\Delta 9}$ were also amplified from the pathological adrenals (data not shown); they lack critical regions involved in ligand binding (13-16). Furthermore, both allelic variants (with or without LQ insertion at amino acid position 19-20) of the LH/hCGR were expressed in the patient's LH-dependent adrenal tissue. No mutations were found in the coding region of LH/hCGR gene; however, three already reported single nucleotide polymorphisms (SNP) located in the 3'UTR of the cDNA were identified both in the patient's LH-dependent adrenal tissue and in the normal ovary: SNP1 at position 2528 T→C (17); SNP2 at position 2533 T→C (17); SNP3 at position 2742 G→C (Genbank cluster id: 1042551) (Fig.1).

Dot blot analysis revealed no overexpression of the LH/hCGR in the patient's LH-responsive adrenal tissue as compared to normal adrenals (Fig.3). Moreover, mRNA levels of the LH/hCGR were present, but highly variable in the adrenal tissues of patients with Cushing's syndrome from various etiologies; in none of those patients did we find any stimulation of cortisol secretion when endogenous LH levels were stimulated by administration of GnRH (not shown).

DISCUSSION

Recently, the presence of LH/hCGR was identified in the zona reticularis of the human adrenal by immunohistochemistry and *in situ* hybridization (7). However, hCG does not stimulate cortisol or DHEAS secretion in normal adult adrenal, whereas it induces DHEAS secretion in human fetal adrenal cells (2,8). The *in vivo* demonstration of LH/hCG-enhanced cortisol secretion has now been reported in 3 other patients with adrenal Cushing's syndrome in addition to this initial case (2,18,19,20). It was thus pertinent to characterize the LH/hCG receptor in a case of aberrant regulation of cortisol secretion induced by LH/hCG.

RT-PCR study performed on the adrenal tissue biopsy of our patient with AIMAH and LH-dependent Cushing's syndrome confirmed the presence of LH/hCGR; molecular analysis revealed no mutation of the LH/hCGR cDNA and no overexpression of the receptor mRNA as compared to normal and pathological (adenomas and hyperplasias) adrenals by dot blot analysis.

Interestingly, diverse splicing variants of the receptor were expressed in the patient's AIMAH tissue, most of them ($LH/hCGR_{\Delta 4\text{-}6}$, $LH/hCGR_{\Delta 3\text{-}6}$, $LH/hCGR_{\Delta 3\text{-}6,\Delta 9}$) being probably inactive due to loss of ligand binding capacity. Indeed, LH/hCGR extracellular domain harbors highly conserved leucine-rich repeats (LRR) encoded by exons 2-10 (21). Most of the functional studies were performed on the rat LHR and have identified LRR1-6 (encoded by exons 1-6) as essential regions for gonadotropin binding (13,15). Considering the 94% homology between rat and human LHR extracellular domains, it is reasonable to assume that $LH/hCGR_{\Delta 4\text{-}6}$, $LH/hCGR_{\Delta 3\text{-}6}$, and

LH/hCGR$_{\Delta 3\text{-}6,\Delta 9}$ are non-functional receptors. The two other variants LH/hCGR$_{\Delta 9}$ and LH/hCGR$_{\Delta 9\text{-}10}$ can be expressed in normal adrenal cortex indicating that the hCG-induced cortisol response *in vivo* is not due to abnormal splicing of LH/hCGR mRNA, nor to amino acid substitution (L316F). LH/hCGR$_{\Delta 9}$ isoform was already described in human and rat, but it is unknown whether it is a functional receptor (3,22). LH/hCGR lacking exon 10 was shown to be expressed and functional in marmoset monkey and ovine ovaries (23,24); yet, in human, homozygous deletion of 5kb encompassing exon 10 of LH/hCGR gene leads to type II Leydig cell hypoplasia (male hypogonadism) due to decreased LH/hCGR expression at the cell surface (14,25). LH/hCGR$_{\Delta 9\text{-}10}$ isoform has not been reported before and may have specific functional characteristics as proposed for LH/hCGR$_{\Delta 10}$. Indeed, this latter isoform displayed normal signaling properties to hCG but impaired ones to LH (25). The large number of variants occurring for LH/hCGR, as for many other hormone receptors, suggests a physiological relevance that remains to be determined (26).

The underlying mechanism responsible for LH/hCGR abnormal function in LH-dependent Cushing's syndrome is still elusive. The LH/hCGR was found in all studied adrenal tissues, but is coupled to steroidogenesis only in this patient's LH-responsive AIMAH; in other patients with adrenal Cushing's syndrome studied here, we found no stimulation of cortisol secretion following GnRH-induced increase of endogenous LH levels (27). It is thus unclear whether the LH/hCGR is expressed ectopically in adrenal cells originating from the fasciculata zone (rather than in reticularis cells in normal adrenal) of the patient's AIMAH tissues; alternatively, the aberrant response to hCG

may be secondary to an increased activity and coupling of the eutopic LH/hCGR to steroidogenesis, as hypothesized for vasopressin-responsive Cushing's syndrome (1,28). In this patient (2), the profile of steroids produced in response to LH (cortisol, free testosterone and estradiol, but not DHEAS or aldosterone) suggests that the LH/hCGR is expressed in cells with a fasciculata-reticularis phenotype.

In a woman with hCG-responsive Cushing's syndrome and unilateral adrenal adenoma, an activating gsp mutation (R201C) was found in the adrenal nodule (18). The effect of suppression of endogenous LH levels was not studied in that case, which does not allow to determine the contribution of the aberrant LH/hCG receptor to the secretion of cortisol. Hypercortisolism resulting from a gsp mutation should lead to constitutive activation of steroidogenesis independent from ligand activation of any receptor. However, multiple mutational events may occur in the same adrenal lesion, as a second receptor was aberrantly functional in this patient's AIMAH tissues (2); an exaggerated cortisol response to $5HT_4$-R agonists also revealed an aberrant expression or function of eutopic $5HT_4R$-effector system. The $5HT_4R$ subtype is normally expressed mainly in human adrenal zona glomerulosa, and to a lesser extent in zona fasciculata cells (29). $5HT_4R$ agonists are potent stimulators of aldosterone secretion in humans, but do not cause any increase in plasma cortisol levels in normal subjects. In a preliminary study, various isoforms of the $5HT_4R$ were identified in this patient's adrenal as well as in normal adrenal tissues (30); further quantitative studies and sequencing of the $5HT_4R$ are required.

Pharmacological blockade of endogenous LH secretion by leuprolide acetate was sufficient to completely normalize plasma cortisol levels of this patient in spite of the co-expression of an overactive $5HT_4R$ (2). Moreover, the patient did not suffer from hypocortisolism despite complete suppression of endogenous LH levels following treatment with leuprolide acetate suggesting that the function of $5HT_4R$ might contribute to maintain cortisol secretion. In another 45-y.o pre-menopausal woman with Cushing's syndrome and AIMAH, simultaneous expression of ectopic GIP receptor and increased activity of LH/hCGR appeared to be present (20).

Further molecular analysis dissecting the transduction pathways of aberrant adrenal LH/hCG receptor will be necessary to identify the molecular defect underlying LH-dependent Cushing's syndrome.

ACKNOWLEDGMENTS

We wish to acknowledge the contributions of Drs Alfons Pomp for performing the laparoscopic adrenal biopsy and of Dr Walter Schurch for the pathology study. The assistance of Mrs. Victoria Baranga in preparing the manuscript is also appreciated.

REFERENCES

1. **Lacroix A, N'Diaye N, Tremblay J, Hamet P.** 2001 Ectopic and abnormal hormonal receptors in adrenal Cushing's syndrome. Endocr Rev. 22:75-110.

2. **Lacroix A, Hamet P, Boutin JM.** 1999 Leuprolide acetate therapy in luteinizing hormone-dependent Cushing's syndrome. N Engl J Med. 341:1577-1581.

3. **Minegishi T, Nakamura K, Takakura Y, et al.** 1990 Cloning and sequencing of human LH/hCG receptor cDNA. Biochem Biophys Res Commun. 172: 1049-1054.

4. **Atger M, Misrahi M, Sar S, et al.** 1999 Structure of the luteinizing hormone-choriogonadotropin receptor gene: unusual promoter and 5' non-coding regions. Mol Cell Endocrinol. 111:113-123.

5. **Segaloff DL, Ascoli M.** 1993 The lutropin/ choriogonadotropin receptor. 4 years later. Endocr Rev. 14:324-347.

6. **Rao CV.** 1996 The beginning of a new era in reproductive biology and medicine: expression of low levels of functional luteinizing hormone/ human chorionic gonadotropin receptors in non-gonadal tissues. J Physiol Pharmacol. 47:41-53.

7. **Pabon JE, Li X, Lei ZM et al.** 1996 Novel presence of luteinizing hormone/ human chorionic gonadotropin receptors in human adrenal glands. J Clin Endocrinol Metab. 81:2397-2400.

8. **Seron-Ferre M, Lawrence CC, Jaffe RB**. 1978 Role of hCG in regulation of fetal zone of the human fetal adrenal gland. J Clin Endocrinol Metab. 46:834-837.

9. **Lacroix A, N'Diaye N, de Herder W, et al.** 2000 Adrenal GIP receptor overexpression in food-dependent Cushing's syndrome. Program and abstracts of the 11th International Congress of Endocrinology, Sydney, Australia, (Abstract P18), p99.

10. **Lacroix A, Tremblay J, Rousseau G et al.** 1997 Propranolol therapy for ectopic beta-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med. 337:1429-1434.

11. **Chomczynski P, Sacchi N.** 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156-159.

12. **Sanger F, Nicklen S, Coulson A.** 1977 DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA. 74:5463-5467.

13. **Thomas D, Rozell TG, Liu X, Segaloff DL.** 1996 Mutational analyses of the extracellular domain of the full length lutropin/ choriogonadotropin receptor suggest leucin-rich repeats 1-6 are involved in ligand binding. Mol Endocrinol. 10:760-768.

14. **Zhang R, Buczko E, Dufau ML.** 1996 Requirement of cysteine residues in exons 1-6 of the extracellular domain of the luteinizing hormone receptor for gonadotropin binding. J Biol Chem. 271:5755-5760.

15. **Hong S, Phang T, Ji I, Ji TH.** 1998 The amino-terminal region of the luteinizing hormone/choriogonadotropin receptor contacts both subunits of human chorio-gonadotropin. I. Mutational analysis. J Biol Chem. 273:13835-13840.

16. **Bhowmick N, Narayan P, Puett D.** 1999 Identification of ionizable amino acid residues of the extracellular domain of the lutropin receptor involved in ligand binding. Endocrinol. 140:4558-4563.

17. **Frazier AL, Robbins LS, Stork PJ, et al.** 1990 Isolation of TSH and LH/CG receptor cDNAs from human thyroid: regulation by tissue specific splicing. Mol Endocrinol. 4:1264-1276.

18. **Bugalho MJ, Li X, Rao CV, et al.** 2000 Presence of a Gs alpha mutation in an adrenal tumor expressing LH/hCG receptors and clinically associated with Cushing's syndrome. Gynecol Endocrinol. 14:50-54.

19. **Wieland RG, Schaffer MB, Glove RP.** 1971 Cushing's syndrome complicated pregnancy. A case report. Obstet Gynecol. 38:841-843.

20. **Bertherat J, Barrande G, Lefebvre H et al.** 2001 Systematic screening confirms that illicit membrane receptors are frequent and often multiple in bilateral ACTH-independent macronodular adrenal hyperplasia (AIMAH). The Endocrine Society's 83rd Annual Meeting, Denver, CO, (Abstract P1-397).

21. **Moyle WR, Campbell RK, Rao SN et al.** 1995 Model of human chorionic gonadotropin and lutropin receptor interaction that explains signal transduction of the glycoprotein hormones. J Biol Chem. 270:20020-20031.

22. **Aatsinki JT, Pietila EM, Lakkakorpi JT, Rajaniemi JH.** 1992 Expression of LH/CG receptor gene in rat ovarian tissue is regulated by an extensive alternative splicing of the primary transcript. Mol Cell Endocrinol. 84:127-135.

23. **Zhang FP, Rannikko AS, Pulak RM, et al.** 1997 Cloning and functional expression of the luteinizing hormone receptor complementary deoxyribonucleic acid from the marmoset monkey testis: absence of sequences encoding exon 10 in other species. Endocrinol. 138: 2481-2490.

24. **Bacich DJ, Rohan RM, Norman RJ, Rodgers RJ.** 1994 Characterization and relatively abundance of alternatively spliced luteinizing hormone receptor messenger ribonucleic acid in the ovine ovary. Endocrinol. 135:735-744.

25. **Gromoll J, Eiholzer U, Nieschlaq E, Simoni M.** 2000 Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. J Clin Endocrinol Metab. 85:2281-2286.

26. **Lou H, Gagel RF.** 2001 Alternative ribonucleic acid processing in endocrine systems. Endocr Rev. 22:205-225.

27. **Mircescu H, Jilwan J, N'Diaye N, et al.** 2000 Are ectopic or abnormal membrane hormone receptors frequently present in adrenal Cushing's syndrome ? J Clin Endocrinol Metab. 85:2281-2286.

28. **Lacroix A, Tremblay J, Touyz RM et al.** 1997 Abnormal adrenal and vascular responses to vasopressin mediated by V1-vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia, Cushing's syndrome, and orthostatic hypotension. J Clin Endocrinol Metab. 82:2414-2422.

29. **Lefebvre H, Comtesse V, Delarue C, et al.** 1998 Serotoninergic regulation of adrenocortical function. Horm Metab Res.30:398-403.

30. **N'Diaye N, Cartier D, Bourdeau I, et al.** 2001 Characterization of aberrant LH/hCG and serotonin 5-HT_4 receptors in adrenal Cushing's syndrome.The Endocrine Society's 83rd Annual Meeting, Denver, CO, (Abstract P1-407).

Table 1. Alternative splicing variants of LH/hCG receptor.

Receptor variants	Extracellular domain	Expression in LH-dependent AIMAH	Expression in normal adrenal
intact LH/hCGR	362 aa	+	+
LH/hCGR Δ9	300 aa	+	+
LH/hCGR Δ4-6	286 aa	+	+
LH/hCGR Δ9-10	273 aa	+	+
LH/hCGR Δ3-6	262 aa	+	N.D.
LH/hCGR Δ3-6, Δ9	200 aa	+	+

LH/hCGR was amplified from the adrenal tissue biopsy of the patient's LH-hCG-dependent AIMAH and normal control adrenals as defined in fig.3. Each splicing variant was identified by sequencing. N.D.: not detected.

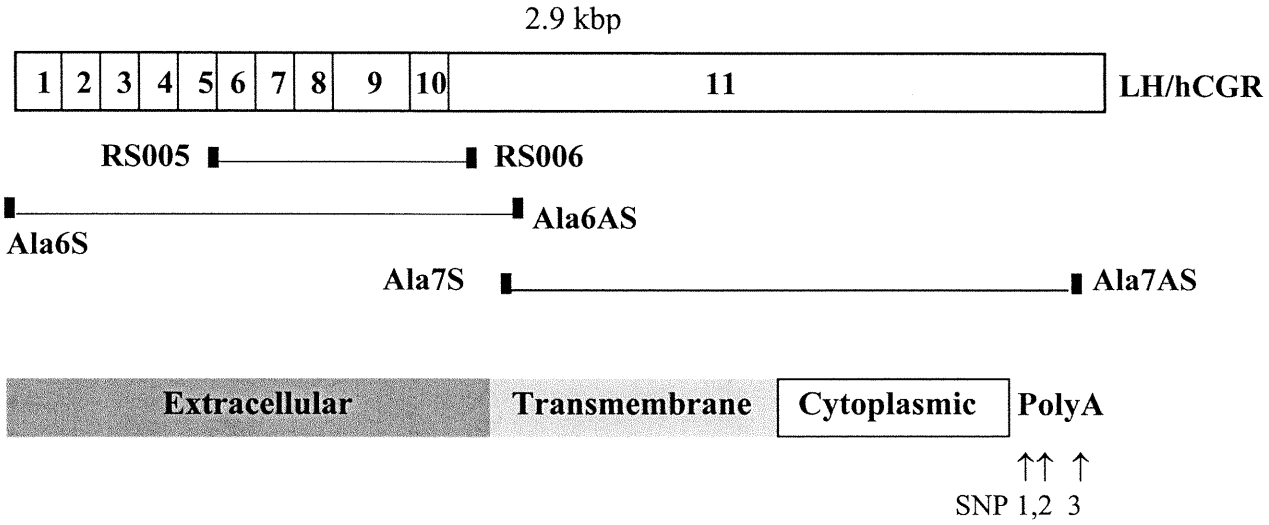


Figure 1. LH/hCG receptor sequencing strategy. The primers represented were used to amplify the hCG/LHR cDNA by PCR. The products were subcloned into Puc19 vector in SmaI site and sequenced. Single nucleotide polymorphisms (SNP) were identified in the 3'UTR of the LH/hCGR cDNA: SNP 1 at position 2528 (T→C), SNP 2 at position 2533 (T→C), and SNP 3 at position 2742 (G→C).

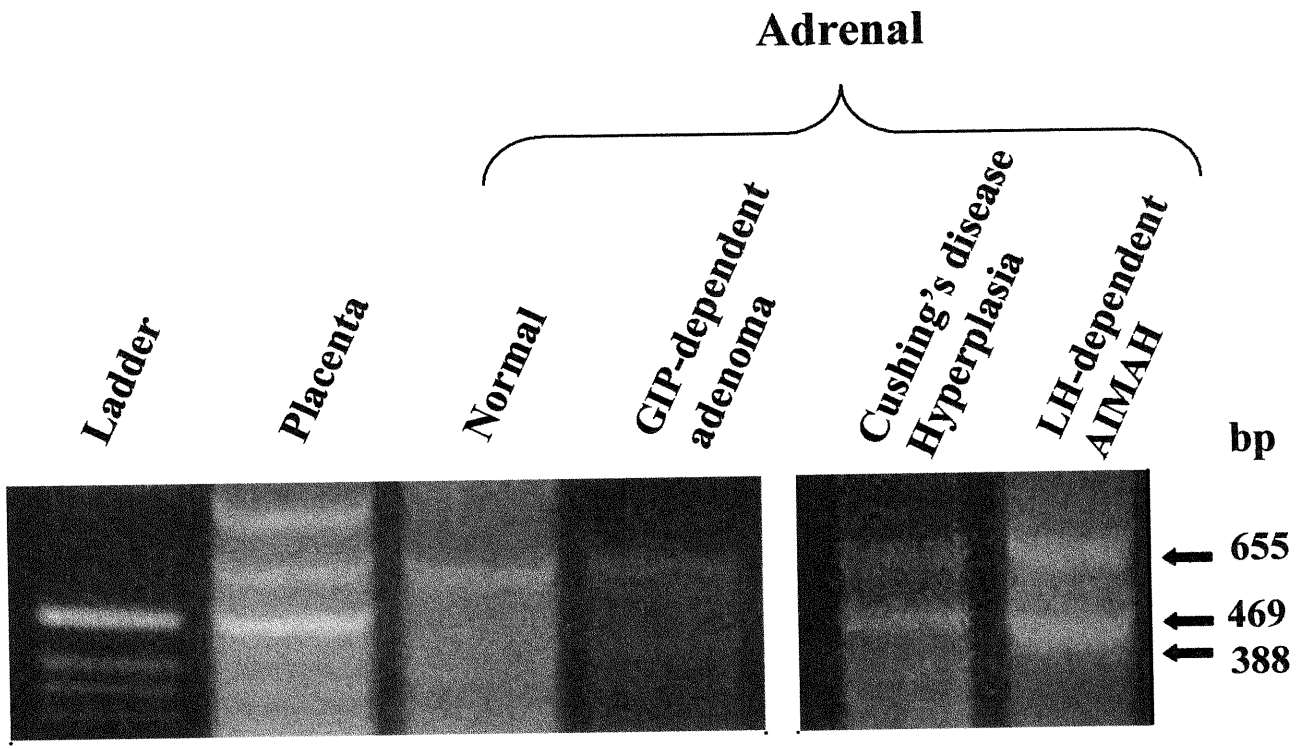


Figure 2. LH/hCG receptor expression in adrenal hyperplasias and tumors. LH/hCGR cDNA was amplified using RS005-RS006 primers. 2 µg total RNA from LH-dependent adrenal hyperplasia, normal and pathological human adrenals were used; human placenta was used as a positive control. PCR products were run on a 2% agarose gel and stained by ethidium bromide. The 655 bp-band corresponds to the intact LH/hCGR (no splicing), the 469bp-band to LH/hCGR$_{\Delta 9}$, and the 388bp-band to LH/hCGR$_{\Delta 9\text{-}10}$. Non specific bands can also be amplified from placental RNA.

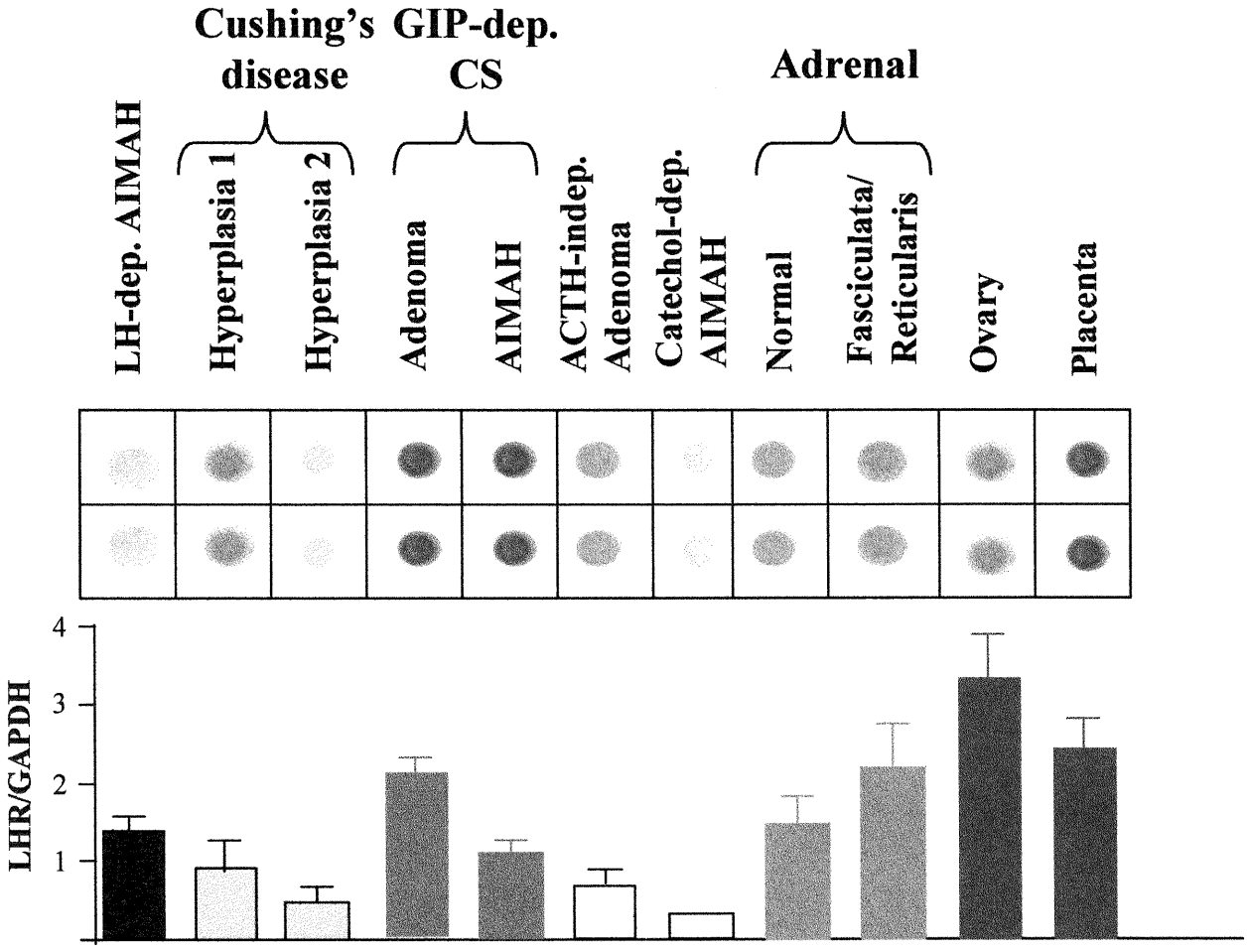


Figure 3. Relative levels of LH/hCGR RNA in adrenal hyperplasias and tumors. Hybridization was performed on 2 μg total RNA from LH-dependent adrenal hyperplasia, from adrenals of patients with Cushing's disease, non LH-dependent adrenal hyperplasia and adenoma, normal adrenal gland, ovaries, and placenta. The probe used corresponds to the extracellular domain of the LH/hCGR. Two independent experiments done in triplicate are shown.

Résumé

Le troisième article de cette thèse caractérise un autre type d'anomalie de récepteur avec le cas d'une femme atteinte d'un SC secondaire à une hyperplasie surrénalienne bilatérale et chez laquelle la sécrétion de cortisol est stimulée par la LH, l'hCG et des agonistes sérotoninergiques spécifiques de 5-HT_4R (Lacroix *et al.* 1999a). Or ni la LH, ni l'hCG ne stimulent la sécrétion de cortisol ou de DHEAS chez l'adulte sain. L'administration d'un analogue à longue action du GnRH (acétate de leuprolide) afin d'inhiber la sécrétion endogène de LH, chez cette patiente, a permis la disparition des signes cliniques du SC ainsi qu'une normalisation des taux de cortisol urinaire sans avoir recours à la surrénalectomie bilatérale (Lacroix *et al.* 1999a). Ces résultats suggérant l'implication clinique de LH/hCGR dans cette nouvelle étiologie du SC, **l'hypothèse physiopathologique avancée fut l'expression ectopique de LH/hCGR.**

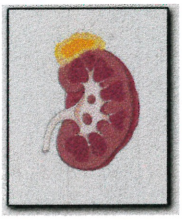
Nous avons donc étudié l'expression du LH/hCGR dans une biopsie surrénalienne provenant de la patiente. La présence de récepteur non muté dans le tissu hyperplasique fut démontrée par RT-PCR et séquençage. L'expression de LH/hCGR dans la surrénale humaine normale (zone réticulée) avait été documentée auparavant (Pabon *et al.* 1996) et fut confirmée dans notre étude; cependant, ce récepteur surrénalien n'est pas couplé à la sécrétion de cortisol ou de DHEAS chez l'adulte sain. Par ailleurs, aucune surexpression de l'ARNm de LH/hCGR ne fut détectée dans le tissu de la patiente comparativement aux surrénales normales. L'**anomalie de fonction de LH/hCGR** observée dans le SC LH-dépendant pourrait donc résulter :

- **de l'expression ectopique de LH/hCGR dans les cellules surrénaliennes provenant de la zone fasciculée**. Ceci est suggéré par le fait que ni la DHEAS, ni l'aldostérone ne sont sécrétées par l'AIMAH sensible à la LH/hCG ;
- d'une **anomalie de couplage d'un récepteur eutopique (LH/hCGR)** à la stéroïdogénèse, entraînant une hyperactivité de ce dernier.

Au terme de ces trois études rapportant la caractérisation moléculaire de deux récepteurs hormonaux aberrants, le SC GIP-dépendant est le seul syndrome associé à un défaut moléculaire clairement identifié. Nous avons donc poursuivi nos recherches sur un mécanisme physiopathologique potentiellement responsable de ce nouveau syndrome. Les résultats préliminaires de ce travail sont présentés le prochain chapitre.

CHAPITRE 5

Recherche de mutation dans le promoteur putatif de GIPR chez une patiente atteinte du Syndrome de Cushing GIP-dépendant



5.1. Introduction méthodologique

5.1.1. Promoteur putatif du récepteur du GIP humain

Le syndrome de Cushing GIP-dépendant se caractérise par une expression ectopique du récepteur du GIP dans les adénomes ou hyperplasies surrénaliennes des sujets atteints. Nous avons précédemment démontré que ce dernier n'est pas muté (N'Diaye *et al.* 1998a). L'objectif de cette étude est de vérifier l'hypothèse d'une mutation dans la région promotrice du gène conduisant à une telle anomalie. Bien que non défini fonctionnellement, le promoteur putatif du récepteur du GIP humain a été cloné et séquencé dans le cadre du « projet génome humain » (HUGO). Pour notre étude, nous avons considéré une région de 9kb en amont du site d'initiation de la traduction, bornée par la queue polyA du gène (non identifié) précédent. Cette région sera désignée pProm (promoteur putatif). L'analyse macroscopique de pProm a été réalisée afin d'identifier des éléments structuraux de base tels que des séquences répétées et des sites potentiels de régulation transcriptionnelle. La recherche de mutation a été effectuée en séquençant la totalité de la région pProm chez une patiente atteinte du SC GIP-dépendant.

5.1.2. Protocole expérimental

5.1.2.1. Patients

Le séquençage de la région pProm a été effectué à partir de l'ADN de surrénale provenant d'une patiente âgée de 33 ans (patient P1) qui présentait une hyperplasie bilatérale asynchrone associée à un SC GIP-dépendant (N'Diaye *et al.* 1999). Les segments d'ADN comportant des variations nucléotidiques ont été vérifiés chez 4 autres patients (au plus) pour lesquels le diagnostic de SC GIP-dépendant a été posé et l'expression aberrante du GIPR a été démontrée : l'un d'entre eux (patient), âgé de 32 ans, présentait un adénome unilatéral (Lacroix *et al.* 2000); les trois autres (patients P3,

P4 et P5) présentaient une hyperplasie surrénalienne bilatérale (Gerl *et al.* 2000) (Lacroix *et al.* 2000). De plus, l'ADN de rein provenant d'un donneur sain (normal) et l'ADN surrénalien de deux patients, l'un présentant un SC et une stimulation anormale de la sécrétion de cortisol en réponse à l'AVP (patient P6), l'autre souffrant de la maladie de Cushing (patient P7), ont été utilisés comme contrôles. Le protocole d'étude a été approuvé par le comité d'éthique de l'institution et un consentement écrit a été obtenu de tous les sujets.

5.1.2.2. Extraction d'ADN et amplification par PCR

L'ADN a été extrait des tissus selon la méthode classique du choc alcalin (Maniatis 1989). La région pProm a été découpée en 7 fragments de PCR de taille variable (0,72kb à 3,2kb) (Figure 5.1). Les fragments ont été amplifiés par PCR, à partir de 100 ng d'ADN génomique, selon les conditions indiquées ci-dessous (Tableau 5.I). Les produits de PCR ont ensuite été extraits du gel d'agarose en utilisant le papier filtre DAE81, élués puis purifiés au phénol-chloroforme afin d'éliminer sels, amorces et nucléotides libres.

5.1.2.3. Séquençage

Les produits de PCR provenant d'au moins 4 réactions indépendantes ont ensuite été séquencés directement selon une procédure automatique (système ABI, polymérase AmpliTaq). Lors du séquençage, des difficultés techniques ont été rencontrées avec les amplicons contenant des régions hautement répétitives (Tableau 5.I : Ala 11S-Ala9AS, Ala17S-Ala15AS, Ala25S-25AS) ont été sous-clonés intégralement ou partiellement avec la technologie « Gateway » selon les recommandations du fournisseur (InVitrogen-Gibco). Pour l'amplicon Ala25S-25AS, un fragment interne a été généré par PCR (amorces Ala36S, Ala36AS) pour fins de sous-clonage. Pour chaque construction, cinq clones ont été séquencés sur les deux brins.

Tableau 5.1. Protocole d'amplification par PCR

Amorces	Cycles de PCR	Polymérase	Taille (kpb)	Séquençage
Ala11S : 5'-ACCAGCCTGGGCAACATGGTG-3' Ala9AS : 5'-ATTGGGGAGCCCTCACCTGT-3'	30cycles(94°C/10″;61°C/20″;68°C/60″*)*: +10″/cycle	Taq (2,5U)	1,167	Direct + Sous-clonage
Ala14S : 5'-AGGGAAGGAGACAGGGT-3' Ala10AS : 5'-GCCATGGAAAGGCACAGTC-3'	30cycles (94°C/10″;56°C/20″;68°C/1'45″)	Taq (2,5U)	1,6	Direct
Ala17S : 5'-TGAGGGTGACGGTGTGAG-3' Ala15AS : 5'-ATGGCACCCAACGGGTAG-3'	10cycles (94°C/15″;58°C/20″;68°C/60″) 25cycles(94°C/15″;58°C/20″;68°C/60″*)*: +10″/cycle	Taq (2,5U)	1,4	Direct + Sous-clonage
Ala25S:5'-GCTACCGCACCAGGCCTTTTCTGG-3' Ala26A : 5'-CAGTCACCCACAGAGTCCTCAGTC-3'	10cycles (94°C/15″;58°C/20″;68°C/60″) 25cycles(94°C/15″;58°C/20″;68°C/60″*)*: +10″/cycle	Expand ( )	3,267	Direct
Ala25S : 5'-GCTACCGCACCAGGCCTTTTCTGG-3' Ala25AS : 5'-CTCGCTTCCTGGACTTAGGCTGCC-3'	10cycles (94°C/15″;58°C/20″;68°C/60″) 25cycles(94°C/15″;58°C/20″;68°C/60″*)*: +10″/cycle	Expand ( )	2,560	Direct + Sous-clonage
Ala27S : 5'-ACCAGGCATCTACCCACCCCTGAC-3' Ala22AS : 5'-TACTTGGGAGGCAGAGGCAG-3'	30cycles (94°C/10″;66°C/5″;72°C/60″)	Taq (2,5U)	0,923	Direct + Sous-clonage
Ala24S : 5'-AGCCTTCCCAACAGACAGG-3' Ala23AS : 5'-CTGGGCATCAGAGTGAGAC-3'	30cycles (94°C/10″;56°C/20″;68°C/1'45″)	Taq (2,5U)	1,377	Direct
Ala36S : 5'-TCTCTCCGGCTCAGTCCCTG -3' Ala36AS : 5'-ATGGTGGTGGTGCGCCTGTGG -3'	30cycles (94°C/15″;65°C/20″;68°C/50″)	Taq (2,5U)	0,72	Sous-clonage

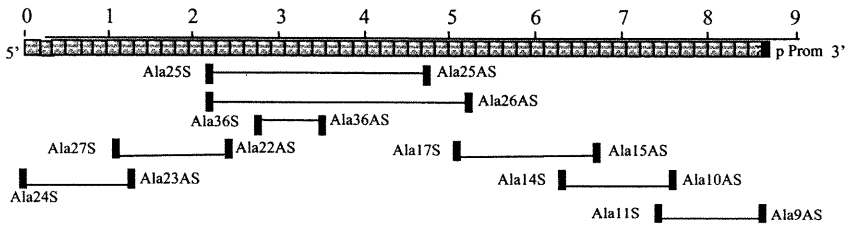


Figure 5.1 . Les paires d'amorces utilisées génèrent des fragments chevauchants qui couvrent la totalité de pProm. L'orientation est dans le sens 5' → 3'.

Les chromatogrammes issus de chaque amplicon séquencé ont été analysés et les séquences comparées à la séquence de référence disponible sur la base de données GenBank (cosmide R28204).

5.2. Résultats

5.2.1. Analyse structurale de pProm

En premier lieu, les caractérisitiques de pProm à l'échelle macroscopique ont été étudiées. Le profil nucléotidique, la distribution des séquences répétées et l'identification d'éléments de régulation potentiels ont été effectués en analysant la totalité ou certaines régions de pProm à l'aide d'alignements de séquences de type BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), du programme d'analyse d'ADN MacVector (version 6.5.1, 1999, Oxford Molecular Group plc), des logiciels de recherche de séquences répétées simples, d'îlots CpG (http://repeatmasker.genome. washington.edu/ ; http://www.cbi.ac.uk) et de sites de liaison potentiels pour facteurs de transcription , Tess (http://www.cbil.upenn.edu/tess/), Transfac (http://transfac.gbf.de/) et TFSearch (http://www.cbrc.jp/research/db/TFSEARCH. html).

Le profil nucléotidique de pProm est présenté en figure 5.2. Le contenu en GC varie grandement (20% à 65%) suggérant la présence de régions distinctes ayant un contenu en GC très élevé ; ces derniers constituent des domaines dont la présence est fortement corrélée à la densité en gènes. Seule la région proximale de pProm ([8500-8700 pb]) comporte une région riche en GC à plus de 70% (contenu GC variant de 60% à 80%), désignée domaine GC I, suggérant la présence d'éléments de réponse potentiels (boîte GC, élément Sp1) impliqués dans l'initiation de la transcription. Trois autres régions plus distales ([5550-5800 pb],[4500-5000 pb],[1980-2660 pb]), riches en GC à 60-70% constituent les domaines GC II, III et IV.

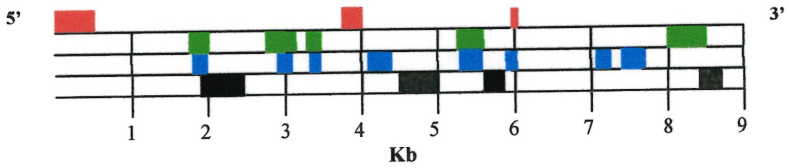
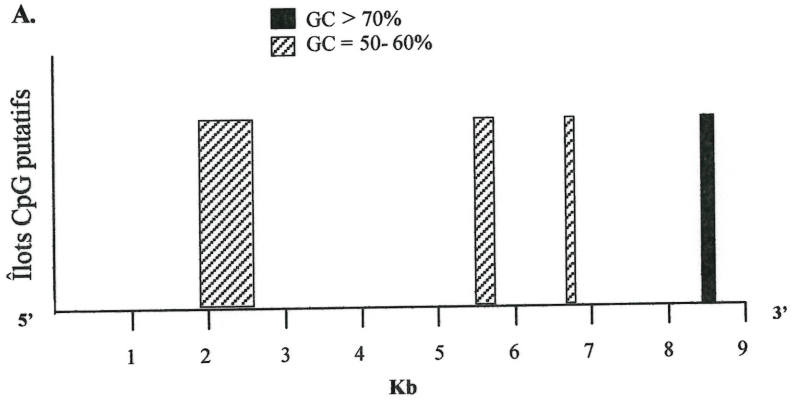


Figure 5.2 . Profil nucléotidique de pProm. Les régions riches à plus de 70% en A+C, G+T, A+T et C+G sont représentées en rouge, vert, bleu et noir, respectivement. L'analyse du profil nucléotidique a été effectuée selon un intervalle de 50 pb. L'orientation est dans le sens 5'→3'.

A.



B.

Caractéristiques des îlots CpG

Ilot CpG	Position	Taille	%GC	Obs/Att
I	8578-8641	64 pb	75,0	0,79
II	6776-6825	50 pb	54,0	0,66
III	5552-5728	177 pb	56,5	0,78
IV	1985-2271	287 pb	54,0	0,81
	2366-2533	168 pb	53,0	0,85
	2589-2697	109 pb	53,2	0,92

Figure 5.3. Ilots CpG putatifs. La recherche des îlots CpG a été effectuée sur un intervalle de 100 pb. Chaque segment a été analysé selon le contenu en GC (> 50%), la longueur du motif CpG (> 50 pb) et le rapport de la proportion observée sur la proportion attendue d'occurrence du motif CpG (> 0,6).

Une autre caractéristique reliée aux domaines GC est leur contenu en îlots CpG (Figure 5.3A). L'analyse de pProm a révélé la présence de 4 îlots CpG potentiels dont 1, l'îlot I situé dans la région proximale de pProm, a un contenu en GC supérieur à 70%. La majorité des îlots sont courts (<200 pb) avec un contenu en GC variant de 50 à 60% (Figure 5.3B).

5.2.2. Séquences simples répétées

Le promoteur putatif du gène GIPR contient essentiellement deux types de séquences répétées : l'ADN satellite constitué de séquences répétées simples (SSRs) représentées par un court motif tel que $(A)_n$ ou $(TG)_n$ répété en tandem (Figure 5.4) et les séquences SINEs (*short interspersed element*) de type *Alu* (Figure 5.5). Ainsi, pProm est particulièrement riche en séquences microsatellites de type $(A)_{3-16}$ qui couvrent 4 régions d'environ 1kb ([0-1000 pb]; [3500-4300 pb]; [5500-6300 pb]; [6900-8000 pb]). Moins fréquentes, mais néanmoins très denses, sont les séquences minisatellites $(T)_{5-30}$ regroupées entre 1700 et 4300 pb. Deux autres types de microsatellites $(TG)_{2-5}$ et $(T)_{5-10}$ sont également présents (Figure 5.4). Ces séquences, très sujettes au polymorphisme de longueur, constituent de précieux outils (marqueurs génétiques) pour les études de génétique des populations et l'identification de gènes impliqués dans des pathologies.

5.2.3. Motifs d'ADN

L'analyse par alignement de séquence de type BLAST (*basic local alignment search tool*) de courtes régions de pProm a permis la mise en évidence de deux motifs d'ADN de 249 et 618 pb (cassettes A et B), arrangées en répétitions directes et/ou inversées (Figure 5.5) : La cassette A ([3680-3920 pb]), constituée de 2 séquences *Alu* successives, *Alu*Sp et FLAM_C, est répétée 13 fois le long du promoteur ; la cassette B ([2172-2790 pb]), répétée 8 fois dans pProm, comporte également 2 séquences *Alu* successives, *Alu*Y et *Alu*Sx. La conservation des motifs varie de 70% à 90% et les profils de distribution des 2 cassettes sont superposables (Figure 5.5).

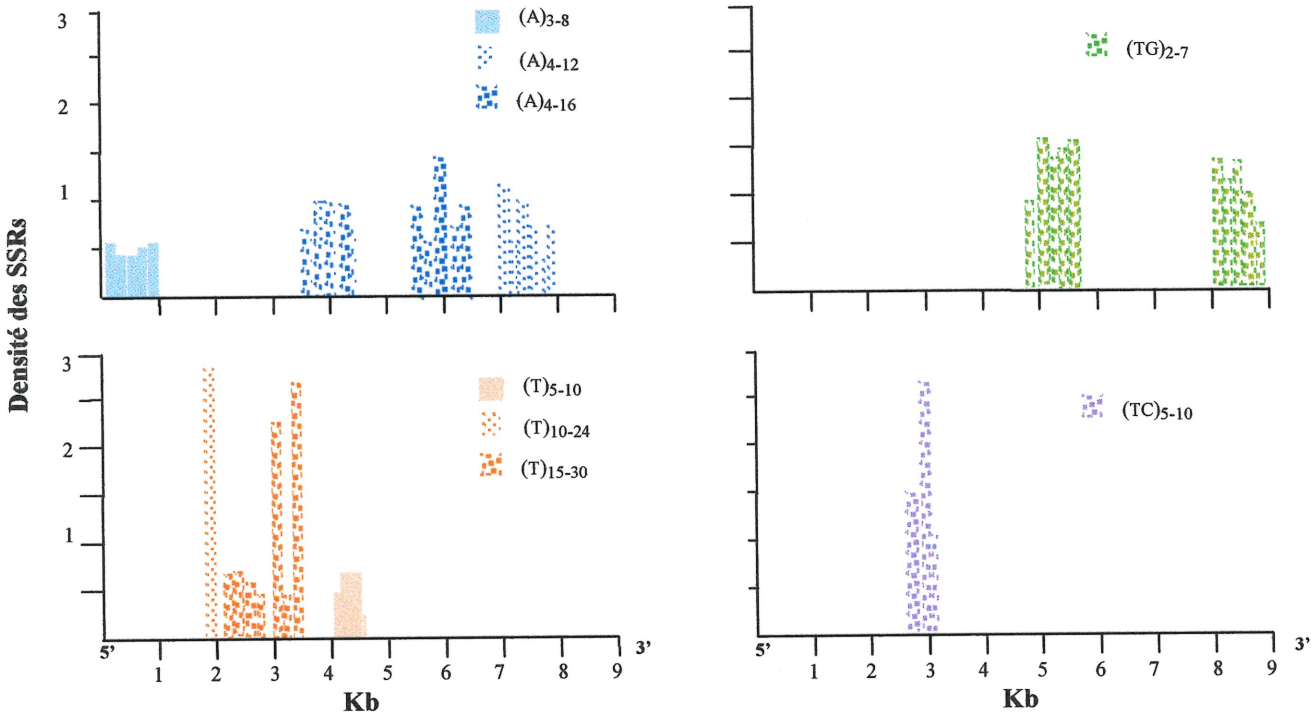


Figure 5.4. Distribution des séquences simples répétées (SSRs) de pProm. Ces séquences sont constituées de répétitions en tandem parfaites ou légèrement imparfaites de k-mères (n = 1-30). La densité est calculée sur un intervalle de 100 pb. L'orientation est dans le sens 5'→ 3'.

Les séquences *Alu* sont de courts rétrotransposons d'environ 300 pb. Elles dérivent du promoteur de l'ARN 7SL et possèdent toute la machinerie nécessaire à leur propre transcription. L'ADN de type *Alu* peut jouer un rôle dans la régulation de l'expression génique en intégrant des séquences porteuses d'éléments régulateurs en de nouveaux sites. Ainsi la cassette A contient-elle 3 éléments cis-régulateurs putatifs, tels qu'un site Sp1, une boîte E et un site cEBPα/β ; de même, la cassette B comporte un site Sp1, un site cEBPα/β et une boîte CAAT.

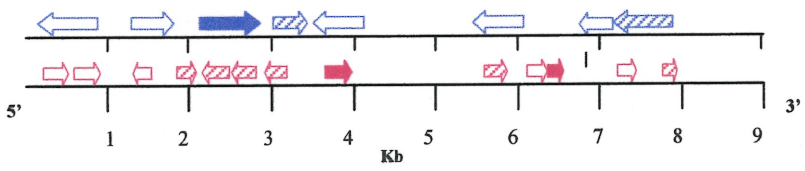


Figure 5.5. Distribution des séquences Alu le long de pProm. La flèche pleine rouge représente la cassette A, et la flèche pleine bleue, la cassette B. Les cassettes A et B sont répétées respectivement 13 et 8 fois, avec une plus ou moins grande conservation de séquence variant de 70-79% (⇨) et 80-90% (⇨). L'orientation est dans le sens 5'→ 3'.

On remarquera que la densité des séquences *Alu* est plus importante dans la partie distale de pProm. De plus, il y a une corrélation positive entre la densité en *Alu* et la densité en satellites $(A)_{3\text{-}16}$ et $(T)_{5\text{-}30}$, les profils de distribution des SSRs et des cassettes A et B étant superposables (Figures 5.4, 5.5). Il en est de même pour les domaines GC II et IV. En revanche, les domaines GC I et III ainsi que les régions riches en microsatellites $(TG)_{2\text{-}7}$ sont exempts d'éléments *Alu* ([4000-5500 pb], [8000-8700 pb]). Ces régions, notamment le domaine GC I qui comprend l'îlot CpG I, doivent probablement contenir des éléments de régulation en cis essentiels à l'activité du promoteur du gène GIPR puisqu'elles ne semblent pas tolérer la présence de SINEs. En effet, la région proximale de pProm ([8100-8700 pb]), qui inclut le domaine GC I, est

constituée d'un promoteur sans boîte TATA, comprenant 3 sites Sp1, 1 site Oct-1 et un élément initiateur potentiels (Figure 5.6), tout comme le promoteur de rat (Boylan *et al.* 1999). Ces éléments cis-régulateurs sont généralement retrouvés dans les promoteurs sans boîtes TATA et CAAT, deux éléments impliqués dans l'initiation de la transcription. Par ailleurs, plusieurs sites potentiels AP-1 (3), GRE (3) et CRE (2) sont retrouvés dans le promoteur humain, suggérant une possible régulation par les glucocorticoïdes et l'AMPc.

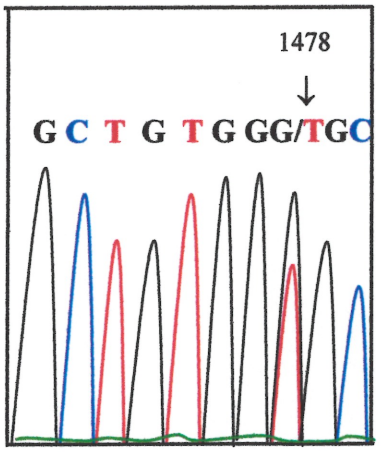
5.3. Analyse des résultats de séquençage

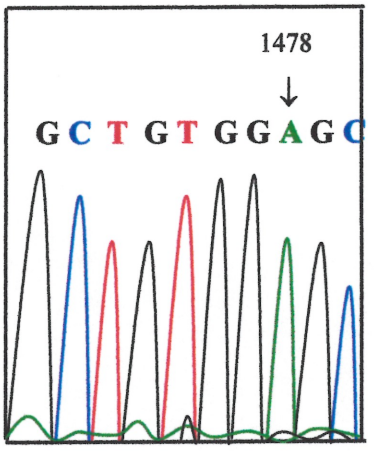
5.3.1. Variations mononucléotidiques

Le séquençage des 7 fragments de PCR amplifiés à partir de l'ADN surrénalien de la patiente 1 (Figure 5.7) a révélé la présence de 14 variations mononucléotidiques dont quelques-unes sont illustrées dans la figure 5.8 (chromatogrammes). Deux types de variations sont observés: les substitutions nucléotidiques uniques hétérozygotes (3566: AA→GA) ou homozygotes (4674: GG→AA) et les substitutions multiples rapportées à une même position (1478: TT→TG; 1478: TT→AA). Certains sites polymorphiques semblent donc soumis à une plus grande diversité nucléotidique.

La fréquences de ces variations a été examinée chez 6 autres sujets (Tableau 5.II): 4 cas de Cushing GIP-dépendant (patients P2 à P5), 1 cas de Cushing non-GIP-dépendant (patient P6), 1 cas de maladie de Cushing (patient P7) et un sujet normal (N). Bien que l'étude n'ait pu être complétée chez tous les sujets (amplification par PCR et/ou réaction de séquençage non optimales), l'analyse des résultats préliminaires nous apportent les informations suivantes: 7/14 variations sont retrouvées uniquement chez la patiente P1; les 7 autres sont présentes chez tous les patients Cushing GIP-dépendant étudiés. En outre, 12/14 sites polymorphiques rapportés sont localisés dans les séquences

Figure 5.6. Promoteur proximal du gène GIPR. Le schéma couvre la région [7647-8760] de pProm. La position +1 définit le site d'initiation de la transcription; la région [-1038- 0] constitue le promoteur proximal ([7647-8595]). Les éléments cis-régulateurs putatifs sont soulignés : InR (*initiator*), SP_1 (*stimulating protein 1*), Oct-1 (*octamer binding factor 1*), AP_1 (*activating protein 1*), GRE (*glucocorticoid responsive element*), CREB (*cAMP responsive element binding protein*); Ftz.2 (*fushi tarazu*), aussi nommé SF-1 (*steroidogenic factor 1*) chez l'homme, est impliqué dans le développement des organes sexuels et de la surrénale. Le promoteur minimal est représenté en gras.





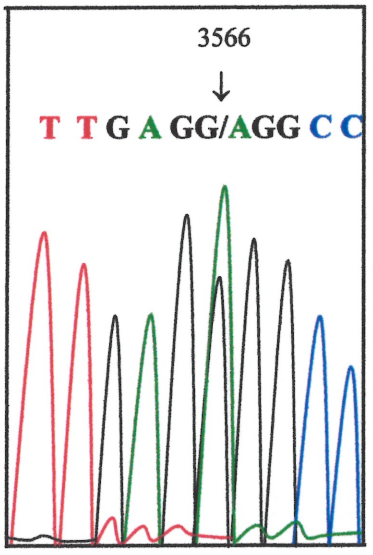


Figure 5.7. Variations mononucléotidiques dans la séquence de pProm. Sont illustrés trois exemples de variations, 3566: AA → GA, 1478: TT→ TG, 1478: TT→ AA. Les flèches indiquent la position des substitutions nucléotidiques qui sont soit hétérozygotes soit homozygotes.

Alu composant les cassettes A et B; 5 d'entre eux se situent dans les îlots CpG III (5584; 5686; 5736) et IV (2117; 2174). Les 2 autres variations (4614, 4674) sont situées dans le domaine GC III qui ne contient aucune séquence *Alu*. Ce profil de distribution suggère une forte corrélation entre la présence d'un polymorphisme et le contenu en GC. En effet, les îlots CpG et les séquences *Alu* sont reconnus pour leur caractère très polymorphique.

Ces variations (excepté cinq) comportent toutes un intérêt en tant que mutations ponctuelles potentiellement impliquées dans l'étiologie du syndrome de Cushing GIP-dépendant. Seules cinq variations (3566 ; 4674; 5584 ; 5686 ; 5736) peuvent, à priori, être exclues car retrouvées chez les patients P6 et P7, atteints, respectivement, d'un SC AVP-dépendant et de la maladie de Cushing. En outre, l'analyse des données présentées dans le tableau 5.II suggèrent l'existence d'un haplotype incluant les variations situées en [4674-5736]: en effet, les patients P1, P3, P4 et P5 partagent sensiblement le même groupe de variations nucléotidiques au sein de cette région qui se comporte donc comme une "unité génétique" (Figure 5.8).

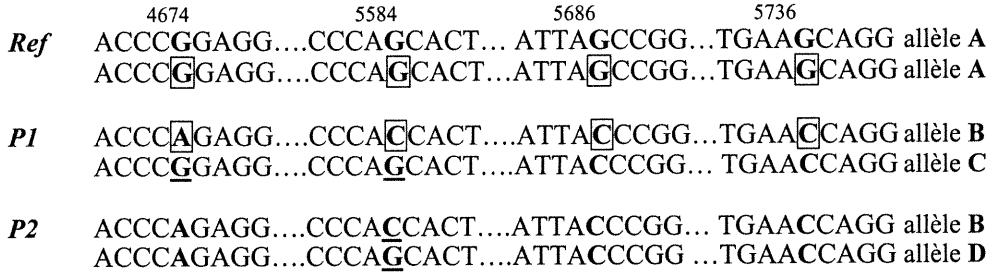


Figure 5.8. Haplotype de la [4674-5736]. Les séquences issues des patients P1 et P3 sont comparées à la séquence du cosmide R28204 (Ref). La combinaison des variations nucléotidiques (indiquées en gras en positions 4674, 5584, 5686, 5736) génère 4 allèles (A, B, C, D) répartis selon les génotypes suivants : $[4674\text{-}5736]^{AA}$, $[4674\text{-}5736]^{BC}$, $[4674\text{-}5736]^{BD}$. Les positions soulignées indiquent les sites hétérozygotes.

Tableau 5.II. Variations mononucléotidiques

	1455	1470	1478	2117	2174	3548	3560	3563	3566	4614	4674	5584	5686	5736
P1	**CC→AC**	**CC→TT**	**TT→TG**	**CC→AC**	**CC→TT**	**AA→AT**	**GG→TG**	**GG→GA**	**AA→GA**	**CC→GC**	**GG→GA**	**GG→GC**	**GG→CC**	**GG→CC**
P2	CC→CC	**CC→GG**	**TT→AA**	CC→CC	**CC→TT**		GG→GG	GG→GG	AA→AA			**GG→GC**	**GG→GC**	**GG→GC**
P3		**CC→TT**	**TT→AA**	CC→CC						CC→CC	**GG→AA**		**GG→CC**	**GG→CC**
P4						AA→AA	GG→GG	GG→GG	**AA→GA**	CC→CC	**GG→AA**		**GG→GC**	**GG→CC**
P5						AA→AA	GG→GG	GG→GG	**AA→GA**	CC→CC	**GG→AA**			**GG→GC**
P6										CC→CC	**GG→AA**			
P7							GG→GG	GG→GG	**AA→GA**	CC→CC	**GG→AA**	**GG→GC**	**GG→CC**	**GG→CC**
N										CC→CC	GG→GG			
Fréq.	1/2	3/3	3/3	1/3	2/2	1/3	1/5	1/5	4/5	1/7	6/7	3/3	5/5	6/6

Les sites polymorphiques sont indiqués en gras. Fréq. est la fréquence d'observation d'un polymorphisme dans l'échantillon de sujets étudiés. P1 à P5 : syndrome de Cushing GIP-dépendant ; P6 : syndrome de Cushing vasopressine-dépendant ; P7 : maladie de Cushing ; N : sujet sain.

L'impact possible de ces variations nucléotidiques sur la fonction régulatrice de pProm a été déterminé au moyen d'analyses informatiques. A cet égard, les polymorphismes situés dans les éléments cis-régulateurs putatifs sont d'un intérêt particulier. Les régions comprenant des sites polymorphiques ont donc été analysées, à la recherche de sites de liaison potentiels de facteurs transcriptionnels. Seuls les sites identifiés par les trois moteurs d'analyse matricielle utilisés, TESS, Transfac et TFSearch, ont été considérés (score > 0,7 n'autorisant qu'une divergence minimale par rapport à la séquence consensus). Les résultats sont résumés dans le tableau 5.III: 9/14 variations seraient localisées dans des éléments cis-régulateurs ubiquitaires, tels que AP_1, $cEBP_\beta$ (*CAAT enhancing binding protein beta)*, SP_1 et CRE, ou plus spécifiques tels que l'élément GRE. L'analyse de l'effet des polymorphismes rend compte du fait qu'une séquence peut abriter plus d'un élément de régulation potentiel et que l'introduction d'une ou plusieurs variations nucléotidiques peut avoir un impact différent selon le site cible considéré: ainsi, la séquence [5721-5750] incluant la variation 5736 (GG→CC) contient 3 éléments de réponse putatifs CRE, $cEBP_\beta$, et AP_1; la présence d'un polymorphisme entraîne la perte des sites $cEBP_\beta$ et CRE, mais n'a aucun effet sur l'élément AP_1. De plus, chaque substitution nucléotidique introduite au sein d'un élément de régulation a un impact spécifique sur la fonction de ce dernier : l'activité du facteur transcriptionnel impliqué peut être augmentée, abolie ou inchangée. Les données issues de l'analyse informatique devront être validées par des études fonctionnelles sur l'activité de pProm.

Tableau 5.III. Éléments cis-régulateurs putatifs de pProm

Séquences	Variations	Sites potentiels	Div_{cons}/ Div_{var}	Intégrité du site
[1441-1490]	1455	**AP_1** (-)	1/2	↘
	1478	cEBP$_\beta$ (+)	1/1	→
[2101-2140]	2117	CRE (+)	2/2	→
	2117	**½GRE** (+)	1/2	↘
[3541-3580]	3548,3560, 3563,3566	cEBP$_\beta$_1(+)	3/3	→
	3560,3563, 3566	**cEBP$_\beta$**_2(+)	5/6	↘
	3560,3563, 3566	**cEBP$_\beta$**_3(+)	1/3	↘
	3563,3566	***SP$_1$_1*** (+)	4/3	↗
	—	SP$_1$_2 (+)	3/3	→
[4601-4630]	4614	***cEBP$_\beta$*** (+)	1/0	↗
[5721-5750]	5736	**CRE** (+)	1/2	↘
	5736	**cEBP$_\beta$** (+)	1/2	↘
	5736	AP_1 (+)	2/2	→

Impact des variations mononucléotidiques sur l'intégrité des sites de liaison potentiels. Les séquences consensus des sites régulateurs sont issues de la base de données TFSearch. La valeur Div_{cons} indique le nombre de bases divergeant entre les séquences consensus et la séquence du cosmide R28204; la valeur Div_{var} indique le nombre de bases divergeant entre les séquences consensus et celle du patient P1 (incluant les variations). L'orientation des sites mentionnée par les sigles(+) et (-). L'intégrité des sites est représentée par les flèches en gras (→: intégrité conservée; ↘: divergence, ou ↗ : convergence par rapport à la séquence consensus).

5.3.2. *Polymorphismes de longueur*

Certaines régions de pProm contiennent des séquences répétées simples de type satellite (Figure 5.4). Le séquençage des SSRs et des régions avoisinantes a nécessité une étape de sous-clonage des fragments de PCR générés par les paires d'amorces (Ala11S; Ala9AS), (Ala17S; Ala15AS), (Ala36S; Ala36AS) et (Ala34S; Ala34AS). Cette stratégie a l'avantage de discriminer et d'identifier facilement des espèces moléculaires différant uniquement sur la longueur des séquences satellites. Les résultats proviennent du séquençage de l'ADN surrénalien de la patiente P1 uniquement (Tableau 5.IV).

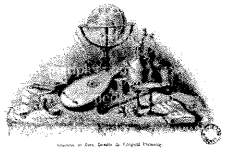
Tableau 5.IV. Polymorphismes de longueur

Amplicon	Position	Polymorphisme	Génotype*
27S-22AS	1589	$T_4 \rightarrow T_4$ $T_4 \rightarrow T_3$	$(\mathbf{T_3})_{1589}(\mathbf{T_3})_{1750}(T_2)_{1774}(\mathbf{T_{27}})_{1886}$
	1750	$T_4 \rightarrow T_3$	$(\mathbf{T_3})_{1589}(\mathbf{T_3})_{1750}(T_2)_{1774}(\mathbf{T_{21}})_{1886}$
	1774	$T_2 \rightarrow T_2$ $T_2 \rightarrow T_1$	
	1886	$T_{24} \rightarrow T_{20}$ $T_{24} \rightarrow T_{21}$	$(\mathbf{T_3})_{1589}(\mathbf{T_3})_{1750}(\mathbf{T_1})_{1774}(\mathbf{T_{23}})_{1886}$
		$T_{24} \rightarrow T_{22}$ $T_{24} \rightarrow T_{23}$	$(T_4)_{1589}(\mathbf{T_3})_{1750}(T_2)_{1774}(\mathbf{T_{23}})_{1886}$
36S-36AS	3003	$T_{26} \rightarrow T_{26}$	$(T_{26})_{3003}(\mathbf{T_{12}})_{3390}$
	1750	$T_{26} \rightarrow T_{12}$	
17S-15AS	5539	$A_{12} \rightarrow A_{12}$ $A_{12} \rightarrow A_{11}$	$(\mathbf{A_{11}})_{5550}(\mathbf{A_{11}})_{5843}(\mathbf{A_{12}})_{6290}$
		$A_{12} \rightarrow A_9$	$(A_{12})_{5550}(\mathbf{A_{11}})_{5843}(\mathbf{A_{14}})_{6290}$
	5842	$A_{12} \rightarrow A_{11}$	
	6270	$A_{16} \rightarrow A_{15}$ $A_{16} \rightarrow A_{14}$ $A_{16} \rightarrow A_{12}$	$(\mathbf{A_9})_{5550}(\mathbf{A_{11}})_{5843}(\mathbf{A_{15}})_{6290}$
11S-9AS	7939	$A_{12} \rightarrow A_{12}$	$(A_{12})_{7939}$
		$A_{12} \rightarrow A_{11}$	$(\mathbf{A_{11}})_{7939}$

* Les régions polymorphiques sont indiquées en gras.

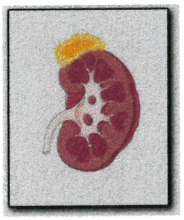
Pour une séquence satellite donnée, on peut donc distinguer, au plus, 2 allèles. Or, certaines séquences présentent 4 polymorphismes différents suggérant l'apparition d'erreurs au cours des étapes successives (PCR, sous-clonage et/ou séquençage). La signification biologique de ces polymorphismes reste à déterminer, notamment celui situé dans la région proximale de pProm (7939 : A_{12}/A_{11}). Le séquençage des segments d'ADN jouxtant les séquences satellites a permis l'identification de 2 autres SNPs potentiels (1860 :TT→TC ; 1954 : GG→GA).

En résumé, le séquençage de pProm chez un patient atteint du SC GIP-dépendant a permis d'identifier 16 variations mononucléotidiques et 10 polymorphismes de longueur. Le génotypage d'autres patients atteints de SC GIP-dépendant (ADN surrénalien et génomique) ainsi que de sujets contrôles (ADN génomique) permettra de discriminer les polymorphismes des mutations potentielles. De plus, les études fonctionnelles nous renseigneront sur l'effet de ces mutations sur l'activité promotrice de pProm.



CHAPITRE 6

DISCUSSION



6.1. Récepteurs hormonaux aberrants dans la physiopathologie du SC ACTH-indépendant

Le but du présent travail était d'identifier les défauts moléculaires responsables de la physiopathologie des hyperplasies et tumeurs corticosurrénaliennes sécrétant du cortisol. L'étude détaillée de deux types de récepteurs aberrants impliqués dans le SC surrénalien a apporté les premières démonstrations de l'expression ectopique de GIPR, non muté, dans un adénome et un cas d'AIMAH GIP-dépendants (N'Diaye *et al.* 1998a) et de la fonction aberrante de LH/hCGR (récepteur ectopique ou anomalie de couplage), non muté, dans un cas d'AIMAH sensible à la LH (N'Diaye *et al.* 2001a). Par ailleurs, la description originale de la progression asynchrone d'une hyperplasie surrénalienne bilatérale GIP-dépendante a fourni les premiers indices, observés *in vivo*, de l'implication potentielle de l'expression ectopique de GIPR dans le développement des tumeurs surrénaliennes (N'Diaye *et al.* 1999). L'ensemble de ces résultats appuie l'hypothèse selon laquelle l'expression ou la fonction aberrante d'un récepteur hormonal, dans des tumeurs surrénaliennes sécrétantes, constitue un stimulus trophique non régulé conférant des capacités stéroïdogénique et prolifération accrues aux cellules affectées.

Depuis lors, notre équipe a contribué à la caractérisation de 6 autres cas de SC GIP-dépendant secondaires à des atteintes unilatérales (1) ou bilatérales (5) (Lacroix *et al.* 2001) (Lacroix *et al.* 2000) (Gerl *et al.* 2000) (Noordam *et al.* 2001). De plus, le concept de récepteurs hormonaux aberrants a été illustré par plusieurs cas de SC surrénalien dont la physiopathologie implique des RCPG aussi divers que les récepteurs du GIP, de la LH/hCG, de l'Ang II (AT_1R), de l'AVP (V1aR), des cathécolamines (β-AR) et de la sérotonine ($5\text{-}HT_4R$) (cf section 1.5). Cette liste n'exclut pas la possibilité d'autres récepteurs anormaux, car seuls quelques RCPG ont été testés. Il est clair que des études de criblage systématique permettront d'évaluer la prévalence et la diversité des anomalies de récepteurs hormonaux dans le SC surrénalien (Lacroix *et al.* 2001) (Mircescu *et al.* 2000) (Arnaldi *et al.* 1998b).

Mis à part le SC GIP-dépendant, les défauts moléculaires sous-jacents à ces nouvelles étiologies du SC surrénalien n'ont pas toujours été étudiés ou clairement identifiés. Par exemple, notre étude sur le cas original d'AIMAH répondant à la LH/hCG montre que le SC LH-dépendant ne résulte ni d'une mutation dans la séquence codante de LH/hCGR, ni de la surexpression de ce dernier dans le tissu surrénalien affecté (N'Diaye *et al.* 2001a). La présence de LH/hCGR est observée dans des surrénales normales et pathologiques d'étiologies diverses, mais ce dernier n'est pas couplé à la production de cortiol ou de DHEAS. L'étude de la distribution zone-spécifique de LH/hCGR dans les tissus surrénaliens sensibles à la LH/hCG, comparativement au tissu normal, permettrait de déterminer précisément si le SC LH-dépendant résulte de l'expression ectopique de LH/hCGR dans la zone fasciculée du cortex surrénalien ou de l'hyperactivité du même récepteur, exprimé de façon eutopique. Le défaut moléculaire associé à ce syndrome reste donc inconnu. Il en va de même pour le SC vasopressine-dépendant. L'activation prolongée et soutenue du récepteur eutopique V1a, par son ligand, a été montrée chez une patiente présentant une hypotension orthostatique et un SC ACTH-indépendant associé à une hyperplasie surrénalienne macronodulaire bilatérale (Lacroix *et al.* 1997b). L'hypothèse d'une anomalie généralisée du système V1aR-effecteur causant un tel tableau clinique a été proposée. Les niveaux d'ARNm du récepteur ne sont pas plus élevés dans les lésions surrénaliennes que dans le tissu normal. L'ADNc de V1aR n'ayant pas été séquencé, on ne peut exclure la possibilité d'une mutation qui augmenterait l'efficacité de couplage du récepteur aux protéines G.

6.2. Clonalité des tumeurs surrénaliennes

De rares cas familiaux de SC ont été décrits dans la littérature (Findlay *et al.* 1993) (Minami *et al.* 1996) (Grunenberger *et al.* 1999) (Cooper *et al.* 1998); ces défauts d'expression et/ou de fonction de récepteur seraient donc fréquemment dûs à des mutations somatiques et, plus rarement, à des mutations de la lignée germinale. La présence de récepteurs hormonaux aberrants dans les adénomes unilatéraux sécrétants résulterait de l'expansion monoclonale d'une cellule corticosurrénalienne primaire ayant acquis une mutation somatique à un stade post-zygotique au cours du développement du cortex surrénalien ; le caractère monoclonal de certaines tumeurs corticosurrénaliennes est d'ailleurs confirmé par plusieurs études (Gicquel *et al.* 1995) (Latronico & Chrousos 1997).

Lors d'une atteinte surrénalienne bilatérale, l'événement doit avoir lieu très tôt au cours de l'embryogénèse de sorte que toutes les cellules corticales originant des cellules souches atteintes sont affectées. L'anomalie de récepteur pourrait également s'étendre à d'autres tissus selon la précocité de l'événement mutagène ; ce qui se traduirait par un spectre de manifestations aberrantes plus large (phénotype pléïotrope) tel qu'observé chez deux patientes : l'une, atteinte d'un SC vasopressine-dépendant, présentait une réponse vasculaire à l'AVP anormalement prolongée ainsi qu'un déficit vasopressinergique en situtation d'hypotension induite par le test de posture (Lacroix *et al.* 1997b); la seconde, atteinte d'un SC GIP-dépendant, souffrait de troubles psychiatriques qui ont persisté suite à la correction de l'hypercorticisme (Lacroix *et al.* 1992). Le récepteur du GIP étant exprimé dans le cerveau et la surrénale de rat (Usdin *et al.* 1993), il est possible d'envisager que l'expression ectopique de GIPR dans les lésions surrénaliennes de cette patiente s'étende également au cerveau.

Dans quelle cellule surrénalienne se produit l'événement conduisant à l'expression anormale du récepteur ? Selon le profil des hormones stéroïdiennes produites, l'événement peut avoir lieu dans les cellules de type fasciculée-réticulée (adénomes sécrétant uniquement du cortisol ou du cortisol et des androgènes) ainsi que dans les cellules de la réticulée (adénome sécrétant uniquement des androgènes) ; les hyperplasies macronodulaires sécrètent parfois les trois classes de stéroïdes surrénaliens suggérant que les trois zones corticosurrénaliennes sont affectées. On peut donc imaginer que certains cas d'hyperaldostéronisme primaire avec adénome unilatéral ou hyperplasie bilatérale peuvent aussi résulter d'anomalies de récepteur hormonaux dans la zone glomérulée.

6.3. Récepteurs hormonaux aberrants et tumorigénèse

6.3.1. Données in vivo

Quelle est l'implication des anomalies de récepteurs hormonaux dans le contrôle de la croissance cellulaire et la tumorigénèse ? On peut penser qu'un avantage prolifératif acquis par les cellules surrénaliennes, selon un mécanisme inconnu, entraîne leur dédifférenciation et l'activation de gènes codant pour des récepteurs hormonaux, normalement exprimés pendant la vie fœtale. Cette hypothèse pourrait s'appliquer au cas d'une patiente présentant un SC dû à une mutation gsp, associé à un adénome unilatéral sensible à la LH (Bugalho *et al.* 2000). En effet, il a été démontré que les cellules surrénaliennes de cette patiente étaient affectées par une mutation activatrice gsp; cette situation conduit à une stimulation constitutive de la stéroïdogénèse, indépendante de tout ligand. L'implication clinique de la fonction aberrante de LH/hCGR n'a pu être déterminée, l'effet de la suppression de la LH endogène n'ayant pas été étudié chez la patiente. Néanmoins, ces résultats suggèrent que l'hypercorticisme serait secondaire à l'apparition de la mutation gsp dans l'adénome surrénalien; la fonction aberrante de LH/hCGR (réactivation d'un récepteur fonctionnel pendant la vie foetale) serait un phénomène secondaire non impliqué dans la physiopathologie de ce syndrome.

On peut également émettre l'hypothèse d'une mutation primaire entraînant l'expression aberrante d'un récepteur hormonal dans la surrénale; ce dernier conférerait un avantage prolifératif accru aux cellules affectées et entraînerait éventuellement une augmentation de la production et de la sécrétion d'hormones. Le cas d'une patiente avec hyperplasie surrénalienne bilatérale GIP-dépendante, à développement asynchrone, constitue un modèle d'étude *in vivo* suceptible d'étayer cette hypothèse (N'Diaye *et al.* 1999). En effet, suite à une première investigation clinique, le diagnostic de SC GIP-dépendant secondaire à une atteinte unilatérale fut posé ; la surrénale droite de la patiente présentait deux nodules sensibles au GIP et fut réséquée. L'analyse histologique du spécimen révéla la présence de foyers d'hyperplasie précoce et diffuse dans la surrénale adjacente qui paraissait normale et ne présentait aucun signe d'atrophie malgré la suppression de l'ACTH. L'étude de l'expression de GIPR, dans les nodules et la partie adjacente, nous permit d'établir une chronologie dans la séquence des événements menant à la formation d'une tumeur sécrétante sensible au GIP. Ainsi, nous avons pu établir que l'expression ectopique de GIPR est un événement précoce, concomitant des premiers stades d'hyperplasie, et qui précède la formation des nodules. Nos données suggèrent l'implication de GIPR dans le processus d'hyperplasie. Cependant, la seule présence du récepteur illicite ne suffit pas au dévelopement des nodules. La survenue d'un second événement moléculaire serait nécessaire à la formation des nodules. La description d'un second cas d'AIMAH GIP-dépendante à développement asynchrone confirme nos premiers résultats (Lacroix *et al.* 2000).

Quelque soit le mécanisme impliqué, il est clair que l'expression phénotypique du défaut moléculaire nécessite une longue période de temps. En effet, dans les cas d'AIMAH, plusieurs années doivent s'écouler avant que l'hyperplasie et l'hyperactivité stéroïdogénique ne soient cliniquement apparentes. Ceci pourrait résulter de l'activation transitoire du récepteur par son ligand, comme c'est le cas pour les SC GIP- et LH/hCG-dépendants (cf Figure 1.5). Dans le SC GIP-dépendant, les surrénales ne sont stimulées

que brièvement, mais de façon répétée, après chaque repas. Le SC LH/hCG-dépendant, quant à lui, ne se développe qu'après une longue période d'exposition des surrénales à de fortes concentrations de ligands ; ces conditions sont rencontrées lors d'une grossesse, pour l'hCG, et après la ménopause, pour la LH. Les exemples de régression d'hyperplasies après accouchement dans les cas de SC LH/hCG-dépendant (Lacroix *et al.* 2001) confortent l'hypothèse selon laquelle l'anomalie de récepteur serait un événement primaire ; cependant, aucune régression de tumeur ou d'hyperplasie après inhibition complète de l'activité du récepteur aberrant n'a été rapportée à ce jour. Des données récentes sur des cas de SC sub-clinique associés une hyperplasie surrénalienne macronodulaire bilatérale montrent que la stéroïdogénèse est relativement peu efficace, à ce stade précoce de développement de la maladie, malgré une prolifération cellulaire accrue. Ceci peut traduire des cinétiques d'expression phénotypique différentes : les cellules portant l'anomalie moléculaire acquerraient, tout d'abord, un phénotype prolifératif et seraient peu sécrétantes; puis, elles évolueraient vers un phénotype sécréteur retrouvant ainsi les caractéristiques des cellules différenciées d'origine où l'événement primaire a eu lieu. Il est également possible que ces lésions surrénaliennes aient, intrinsèquement, de faibles capacités stéroïdogéniques ou que les récepteurs aberrants, exprimés à un faible niveau, soient plus efficacement couplés à la prolifération cellulaire qu'à la stéroïdogénèse.

6.3.2. Données in vitro

Nous avons émis l'hypothèse que la fonction aberrante de tout RCPG induit la stimulation de la glande surrénale par des facteurs trophiques échappant au rétro-contrôle négatif exercé par les glucocorticoïdes. Les cellules corticosurrénaliennes ainsi « sur-stimulées » gagneraient un avantage prolifératif. Les études suggèrent que les récepteurs aberrants impliqués dans la physiopathologie du SC surrénalien (β-AR, V1aR, GIPR) régulent la sécrétion hormonale en mimant les événements cellulaires générés par

l'activation des récepteurs normalement couplés à la stéroïdogénèse, notamment la voie de l'AMPc/PK_A (Chabre *et al.* 1998) (Lacroix *et al.* 1997b) (Lacroix *et al.* 1997a) (Lebrethon *et al.* 1998). Mais qu'en est-il des signaux mitogéniques ? L'augmentation de l'AMPc intracellulaire stimule-t-elle également la prolifération des cellules surrénaliennes affectées, comme cela a été démontré pour les cellules somatotrophes et thyroïdiennes (Dhanasekaran *et al.* 1995) (Farfel *et al.* 1999) (Spiegel 1996)?

Il est reconnu que des concentrations pharmacologiques ou physiologiques d'ACTH ne stimulent que peu ou pas la croissance des cellules surrénaliennes *in vitro* (Estivariz *et al.* 1982). Cependant, dans certaines conditions, l'ACTH stimule la voie mitogénique, dans la lignée de cellules surrénaliennes Y1, en activant les MAP-kinases P42-P44 et en induisant la protéine c-fos (Kimura *et al.* 1993) (Lotfi *et al.* 1997). Par ailleurs, une étude portant sur les effets prolifératifs du GIP sur des cultures primaires de cellules surrénaliennes humaines normales ou tumorales provenant de patients ayant un SC GIP-dépendant montre qu'une stimulation de la synthèse d'ADN est induite par le GIP, et ce, uniquement dans les cellules tumorales (Chabre *et al.* 1998). De plus, l'activation de la voie des MAP-kinases (P42-P44) est observée dans les cellules tumorales. Cependant, il existe des données contradictoires concernant l'effet du GIP sur la croissance cellulaire *in vitro* (Lebrethon *et al.* 1998). L'effet de la LH/hCG sur la prolifération des cellules surrénaliennes *in vitro* ainsi que la caractérisation des voies de signalisation impliquées n'ont pas été étudiées. L'utilisation de modèles animaux tels que des souris transgéniques exprimant spéciquement au niveau de la corticosurrénale des récepteurs hormonaux ectopiques permettra d'éprouver notre hypoythèse. Elle est d'ailleurs déjà confirmée par l'établissement d'un modèle de souris transgéniques pour bLHβ-CTP qui expriment LH/hCGR dans la surrénale (expression ectopique) et développent un syndrome de Cushing avec hyperplasie surrénalienne bilatérale (Kero *et al.* 2000).

6.3.3. Récepteur de l'ACTH: suppresseur de tumeurs

Il semblerait donc que, dans le cortex surrénalien, les voies stéroïdogéniques et mitogéniques soient distinctes. En effet, les données *in vitro* suggèrent que la voie de signalisation ACTH/Gs/PK_A est relativement peu impliquée dans le processus de croissance cellulaire; en revanche, elle régule principalement la sécrétion d'hormones stéroïdiennes, contribuant ainsi au maintien du caractère hautement différencié des cellules de la corticosurrénale. Aucune mutation activatrice du récepteur de l'ACTH n'a été décrite dans les hyperplasies et néoplasies surrénaliennes (Light *et al.* 1995). Il a été montré que la perte d'hétérozygocité du gène codant pour le récepteur de l'ACTH est associée au caractère malin ou à l'absence de phénotype sécrétoire de certaines tumeurs corticosurrénaliennes. De plus, l'expression du récepteur est plus faible dans les carcinomes que dans les adénomes corticosurrénaliens issus de patients atteints d'un SC (Arnaldi *et al.* 1998c) (Reincke *et al.* 1997a). Ces données suggèrent donc que le récepteur de l'ACTH pourrait agir comme suppresseur de tumeur (Reincke *et al.* 1997b), tout comme p53 qui est impliqué dans le développement de certaines tumeurs corticosurrénaliennes (Reincke *et al.* 1994). L'hypothèse qu'un défaut dans la voie de signalisation du récepteur de l'ACTH pourrait entraîner une dédifférenciation cellulaire et une capacité accrue de prolifération a été avancée (Reincke *et al.* 1997b).

6.4. Expression ectopique: Mutation dans le promoteur du gène GIPR ?

La démonstration de l'expression ectopique de GIPR dans les hyperplasies et tumeurs surrénaliennes sécrétantes GIP-dépendantes soulève la question de la régulation tissu-spécifique de ce récepteur. Les mécanismes moléculaires conduisant à l'expression illicite de GIPR sont encore inconnus. Cependant, le profil d'expression particulièrement tranché du récepteur dans divers tissus surrénaliens tumoraux (GIP et non GIP-dépendants) et sains suggère un mécanisme de régulation transcriptionnelle : l'hypothèse

proposée est la présence d'une mutation dans la région promotrice du gène GIPR (Lacroix *et al.* 2001) (N'Diaye *et al.* 1998a) (N'Diaye *et al.* 1999). L'obtention de la séquence du promoteur putatif de GIPR humain grâce au projet HUGO, a permis de vérifier cette hypothèse en séquençant une région de 9kb en amont du site d'initiation de la traduction, bornée par la queue polyA du gène précédent. Le séquençage de pProm chez une patiente atteinte du SC GIP-dépendant (N'Diaye *et al.* 1999) a conduit à l'identification de 16 variations mononucléotidiques et 10 polymorphismes de longueur.

Plusieurs caractérisitiques structurales de pProm sont à prendre en considération dans l'analyse des résultats de séquençage. Considérons tout d'abord la distribution des îlots CpG. Le dinucléotide CpG est largement sous-représenté dans le génome humain du fait de la transition spontanée du dinucléotide CpG méthylé (^{m}CpG) en dinucléotide TpG. En dépit des systèmes de réparation existants, le taux de mutation demeure élevé dans ces régions. Ces îlots CpG sont généralement retrouvés dans les régions promotrices des gènes et jouent un rôle important dans le contrôle de l'expression génique en fonction de leur état de méthylation : les régions hyperméthylées sont en général associées à des gènes inactifs (comme c'est le cas pour les pseudo-gènes). Sur les 14 variations identifiées dans l'ADN surrénalien de la patiente, 5 sont localisées dans des îlots CpG. Elles représentent probablement des polymorphismes puisque 4 de ces variations (5584; 5686 ; 5739 ; 2174) sont retrouvées chez des patients atteints de SC d'étiologies diverses (cf Tableau 5.II). En revanche, une seule (2117) pourrait être une mutation susceptible de modifier l'état de méthylation des îlots CpG potentiels et d'altérer l'expression du gène GIPR.

Une autre caractéristique intéressante de pProm est son contenu en séquences répétées. Bien qu'elles représentent au moins 50% du génome, ces séquences sont généralement considérées comme de l'ADN inutile (*junk DNA*), car on ne leur connaît pas de fonction (Lander *et al.* 2001b). Parmi elles, il y a les séquences satellites et *Alu*. Le

satellite (TG)n est d'un intérêt particulier en tant que vestige potentiel d'îlots CpG ; les îlots I et III sont tous deux placés dans un environnement $(TG)_{2-5}$ suggérant la perte de dinucléotides ^{m}CpG au cours de l'évolution (cf Figures 5.2, 5.3). Les séquences *Alu* ont une fonction inconnue. Toutefois, leur distribution dans le génome semble être régie par certaines règles. Notamment, un principe stipule que les séquences *Alu* s'accumulent dans les régions riches en GC (comme c'est le cas des domaines GC II et IV qui contiennent les cassettes A et B). Cependant, il est également rapporté dans la littérature que la densité des SINEs dans un environnement riche en AT est plus grande au voisinage des gènes (Smit 1999). Il semblerait que la densité des séquences *Alu* soit plutôt corrélée à celle des gènes activement transcrits, indépendemment de l'environnement nucléotidique (Lander *et al.* 2001). Les séquences satellites et *Alu*, présentes dans pProm, sont très polymorphiques. En effet, 12 des 14 variations mononucléotidiques rapportées sont localisées dans des séquences *Alu* ; 9 d'entre elles altèrent l'intégrité d'éléments cis-régulateurs putatifs et, potentiellement, leur fonction régulatrice. Quelques polymorphismes de longueur ont également été observés au sein des séquences satellites; leur signification biologique reste à déterminer, notamment celle du microsatellite situé dans la région proximale de pProm (7939 : A_{12}/A_{11}).

Afin de déterminer si ces changements mononucléotidiques sont de véritables polymorphismes de type SNP (*single nucleotide polymorphism*) ou de potentielles mutations et non des artéfacts dûs à un biais méthodologique, un échantillonnage d'ADNs surrénalien et génomique issus de patients Cushing GIP-dépendant et d'ADN génomique de sujets témoins devra être génotypé en ces 14 sites. Des données telles que la fréquence des allèles identifiés chez les patients et les sujets témoins, le patron de distribution des SNPs permettraient d'associer un ou plusieurs SNPs au SC GIP-dépendant. De plus, l'analyse fonctionnelle de pProm permettra d'identifier les régions essentielles à la régulation de l'expression du gène GIPR. L'étude de l'effet de mutations potentielles sur l'activité promotrice de pProm en sera facilitée.

6.5. Nouvelles approches thérapeutiques pharmacologiques

La caractérisation de la présence des récepteurs hormonaux anormaux constitue une percée dans la compréhension des mécanismes pathophysiologiques et débouche sur de nouvelles possibilités d'approches thérapeutiques. Le traitement conventionnel des tumeurs sécrétantes du cortex surrénalien repose essentiellement sur leur résection chirurgicale. La chirurgie des adénomes unilatéraux bénins pose peu de difficulté. De plus, l'approche laparoscopique rend l'intervention encore moins pénible pour le patient. Cependant, la suppression de l'axe hypophyso-surrénalien nécessite souvent 9 à 15 mois de remplacement par des glucocorticoïdes avant une récupération complète. Les hyperplasies bilatérales requièrent une surrénalectomie bilatérale, laissant le patient totalement dépendant d'une corticothérapie de remplacement permanente.

Certaines équipes ont réussi à améliorer l'hypercorticisme de patients atteint de SC GIP-dépendant en inhibant la relâche de GIP par l'administration sous-cutanée préprandiale d'octréotide (Croughs *et al.* 2000) (de Herder *et al.* 1996) (Reznik *et al.* 1992). Malheureusement, ce traitement relativement complexe et coûteux ne fut efficace que durant quelques mois. La perte d'efficacité de l'octréotide à long terme reflète probablement la désensibilisation du récepteur de la somatostatine au niveau des cellules intestinales sécrétant le GIP. La surrénalectomie restera donc le traitement de rigueur pour ce syndrome jusqu'à ce que des antagonistes spécifiques de GIPR soient disponibles.

Chez un patient présentant un SC catécholamine-dépendant avec AIMAH (Lacroix *et al.* 1997a), un traitement avec le propranolol a permis de réduire la sécrétion de cortisol de façon marquée; cependant, les niveaux de cortisol urinaires restant approximativement 2 fois plus élevés que la limite supérieure admise, le patient a dû subir une surrénalectomie unilatérale. Par la suite, il devint possible de normaliser

totatement la production de cortisol avec le propranolol. La dose de propranolol fut réduite progressivement afin d'éviter l'hypocorticisme. Ceci pourrait être secondaire à l'effet agoniste inverse du propranolol (Chidiac *et al.* 1994); il est également possible que le contrôle adéquat de l'hypercorticisme ait à son tour réduit l'expression de β-AR. Cependant, aucune régression morphologique de l'hyperplasie surrénalienne n'a été observée suite au traitement avec le propranolol.

Chez une patiente souffrant d'un SC LH-dépendant avec AIMAH, la suppression des taux endogènes de LH par l'administration d'un analogue à longue action du GnRH, l'acétate de leuprolide, a permis le contrôle de l'hypercorticisme et a évité la surrénalectomie bilatérale (Lacroix *et al.* 1999a). En dépit de la suppression complète des taux endogènes de LH, la patiente n'a pas souffert d'insuffisance surrénalienne. Cette dernière portant une deuxième anomalie de récepteur (5-HT_4R fonctionnel), il est possible que la production basale de cortisol soit maintenue par une stimulation sérotoninergique. L'absence de régression de l'hyperplasie bilatérale dans un contexte de supression des niveaux de LH suggère que la prolifération cellulaire serait régulée par le récepteur 5-HT_4 aberrant ou que les récepteurs anormaux contrôleraient la stéroïdogénèse uniquement, et non la croissance cellulaire. L'étude des effets d'un antagoniste spécifique de 5-HT_4R chez ce type de patients s'avérera riche d'enseignements.

6.6. Directions futures

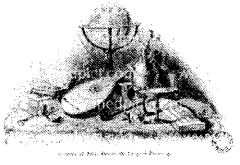
De nouvelles étiologies du SC surrénalien ont été identifiées grâce à l'investigation clinique qui a démontré l'importance pathophysiologique du concept de récepteurs hormonaux aberrants. Les travaux présentés dans cette thèse ont abouti à la caractérisation moléculaire des récepteurs du GIP et de la LH/hCG exprimés dans les lésions surrénaliennes de patients atteints de SC GIP-dépendant et LH-dépendant. Ainsi, nous avons démontré l'expression ectopique de GIPR non muté, dans les tissus surrénaliens pathologiques et avons émis l'hypothèse que l'expression ectopique de GIPR, dans la surrénale, serait le défaut moléculaire responsable du SC GIP-dépendant. L'observation de l'expression illicite du récepteur à un stade précoce du développement d'un SC GIP-dépendant suggère que l'expression de GIPR pourrait en partie être responsable du syndrome prolifératif.

Les mécanismes moléculaires conduisant à cette expression ectopique sont encore inconnus. Le séquençage du promoteur putatif du gène GIPR a révélé la présence de variations nucléotidiques. Ces résultats préliminaires n'ont pas permis l'identification formelle de mutation(s); cependant, une étude actuellement en cours dans notre laboratoire vise a identifier la présence de SNP potentiels chez 10 sujets normaux (travail effectué par le D^{r} Sonir Antonini). De plus, l'étude fonctionnelle du promoteur proximal de GIPR apportera une meilleure compréhension des mécanismes de régulation de l'expression de ce récepteur (travail effectué par Valérie Baldacchino).

Un autre type d'anomalie de récepteur, illustré par le SC LH-dépendant, a été étudié au cours de ce travail de recherche. LH/hCGR est exprimé dans la surrénale normale (zone réticulée), mais n'est pas couplé à la stéroïdogénèse. Les résultats obtenus suggèrent que l'anomalie de fonction de LH/hCGR observée dans le SC LH-dépendant pourrait résulter soit de l'expression ectopique de LH/hCGR dans les cellules

surrénaliennes provenant de la zone fasciculée, soit d'une anomalie de couplage d'un récepteur eutopique (LH/hCGR) à la stéroïdogénèse, entraînant une hyperactivité de ce dernier. La localisation zone-spécifique du récepteur, par immuno-histochimie ou hybridation *in situ,* éclaircira ce point.

Bien que le défaut moléculaire n'ait pas clairement été identifié pour le SC LH-dépendant, un modèle de souris transgénique surexprimant le récepteur LH/hCGR dans la surrénale a été élaboré dans notre laboratoire afin d'étudier les conséquences fonctionnelles d'une anomalie de récepteur hormonal sur la prolifération des cellules surrénaliennes (travail éffectué par Sylvie Oble). Ce modèle permettra notamment d'étudier les effets d'une modulation physiologique des taux de LH endogènes (castration, grossesse, suppression de la LH par l'acétate de leuprolide) et de déterminer si l'anomalie de récepteur est un événement primaire responsable du syndrome prolifératif.



BIBLIOGRAPHIE

Ahima R.S., Prabakaran D., Mantzoros C., Qu D., Lowell B., Maratos-Flier E., & Flier J.S. (1996) Role of leptin in the neuroendocrine response to fasting. *Nature* 382:250-252.

Amiranoff B., Vauclin J.N. , & Laburthe M. (1984) Functional GIP receptors in a hamster pancreatic beta cell line: specific binding and biological effects. *Biochem.Biophys.Res.Commun.* 123:671-676.

Anderova M., Duchene A.D., Barbara J.G., & Takeda K. (1998) Vasoactive intestinal peptide potentiates and directly stimulates catecholamine secretion from rat adrenal chromaffin cells. *Brain Res.* 809:97-106.

Archambeaud-Mouveroux F., Comas J.M. & Tessier M.P. (1996) Food-dependent Cushing's syndrome associated with the Carney complex. In: *Program and Abstracts of the 10th International Congress of Endocrinology*, San Francisco, CA. Abstract P3-158 p 899.

Armelin H.A., Lotfi C.F., & Lepique A.P. (1996) Regulation of growth by ACTH in the Y-1 line of mouse adrenocortical cells. *Endocr.Res.* 22:373-383.

Arnaldi G., de Keyzer Y., Gasc J.M., Clauser E., & Bertagna X. (1998a) Vasopressin receptors modulate the pharmacological phenotypes of Cushing's syndrome. *Endocr.Res.* 24:807-816.

Arnaldi G., Gasc J.M., de Keyzer Y., Raffin-Sanson M.L., Perraudin V., Kuhn J.M., Raux-Demay M.C., Luton J.P., Clauser E., & Bertagna X. (1998b) Variable expression of the V1 vasopressin receptor modulates the phenotypic response of steroid-secreting adrenocortical tumors. *J.Clin.Endocrinol.Metab.* 83:2029-2035.

Arnaldi G., Mancini V., Costantini C., Giovagnetti M., Petrelli M., Masini A., Bertagna X., & Mantero F. (1998c) ACTH receptor mRNA in human adrenocortical tumors: overexpression in aldosteronomas. *Endocr.Res.* 24:845-849.

Arola J., Heikkila P., Voutilainen R., & Kahri A.I. (1994) Corticosterone regulates cell proliferation and cytochrome P450 cholesterol side-chain cleavage enzyme messenger ribonucleic acid expression in primary cultures of fetal rat adrenals. *Endocrinology* 135:2064-2069.

Atger M., Misrahi M., Sar S., Le Flem L., Dessen P., & Milgrom E. (1995) Structure of the human luteinizing hormone-choriogonadotropin receptor gene: unusual promoter and 5' non-coding regions. *Mol.Cell Endocrinol.* 111:113-123.

Bakke M. & Lund J. (1995) Mutually exclusive interactions of two nuclear orphan receptors determine activity of a cyclic adenosine 3',5'-monophosphate-responsive sequence in the bovine CYP17 gene. *Mol.Endocrinol.* 9:327-339.

Baylis P.H. (1991) Vasopressin and its neurophysin. In: *Endocrinology* (eds DeGroot L.J., Besser M., Burger H.G., Jameson J.L., Loriaux D.L., Marshall J.C., Odell W.D., Potts J.T., Jr. & Rubenstein A.H.), 3rd edn, pp. 406-420. W.B. Saunders Company, Philadelphia.

Belloni A.S., Mazzocchi G. , Meneghelli V., & Nussdorfer G.G. (1978) Cytogenesis in the rat adrenal cortex: evidence for an ACTH-induced centripetal cell migration from the zona glomerulosa. *Arch.Anat.Histol.Embryol.* 61:195-205.

Bernet F., Bernard J., Laborie C., Montel V., Maubert E., & Dupouy J.P. (1994) Neuropeptide Y (NPY)- and vasoactive intestinal peptide (VIP)-induced aldosterone secretion by rat capsule/glomerular zone could be mediated by catecholamines via beta 1 adrenergic receptors. *Neurosci.Lett.* 166:109-112.

Bertagna C. & Orth D.N. (1981) Clinical and laboratory findings and results of therapy in 58 patients with adrenocortical tumors admitted to a single medical center (1951 to 1978). *Am.J.Med.* 71:855-875.

Bertagna X. (1994) Proopiomelanocortin-derived peptides. *Endocrinol.Metab Clin.North Am.* 23:467-485.

Bertherat J., Barrande G., Lefebvre H., Raffin-Sanson M.L., & Bertagna X. (2001) Systematic screening confirms that illicit membrane receptors are frequent and often multiple in bilateral ACTH-independent macronodular adrenal hyperplasia (AIMAH). *Program & Abstracts.The Endocrine Society's 83rd Annual Meeting,* Denver, Colorado. Abstract P1-397:p. 233.

Bird I.M., Magness R.R., Mason J.I., & Rainey W.E. (1992) Angiotensin-II acts via the type 1 receptor to inhibit 17 alpha-hydroxylase cytochrome P450 expression in ovine adrenocortical cells. *Endocrinology* 130:3113-3121.

Bird I.M., Nicol M., Williams B.C., & Walker S.W. (1990a) Vasopressin stimulates cortisol secretion and phosphoinositide catabolism in cultured bovine adrenal fasciculata/reticularis cells. *J.Mol.Endocrinol.* 5:109-116.

Bird I.M., Walker S.W., & Williams B.C. (1990b) Agonist-stimulated turnover of the phosphoinositides and the regulation of adrenocortical steroidogenesis. *J.Mol.Endocrinol.* 5:191-209.

Bischof L.J., Kagawa N., Moskow J.J., Takahashi Y. , Iwamatsu A., Buchberg A.M., & Waterman M.R. (1998a) Members of the meis1 and pbx homeodomain protein families cooperatively bind a cAMP-responsive sequence (CRS1) from bovine CYP17. *J Biol.Chem.* 273:7941-7948.

Bischof L.J., Kagawa N., & Waterman M.R. (1998b) The bovine CYP17 promoter contains a transcriptional regulatory element cooperatively bound by tale homeodomain proteins. *Endocr.Res.* 24:489-495.

Blichert-Toft M., Vejlsted H., Hehlet H., & Albrechtsen R. (1975) Virilizing adrenocortical adenoma responsive to gonadotrophin. *Acta Endocrinol.(Copenh)* 78:77-85.

Bodart V., Rainey W.E., Fournier A., Ong H., & De Lean A. (1996) The H295R human adrenocortical cell line contains functional atrial natriuretic peptide receptors that inhibit aldosterone biosynthesis. *Mol.Cell Endocrinol.* 118:137-144.

Bodnar M., Sarrieau A., Deschepper C.F., & Walker C.D. (1997) Adrenal vasoactive intestinal peptide participates in neonatal corticosteroid production in the rat. *Am.J.Physiol.* 273:R1163-R1172 .

Bonnin C., Monsaingeon M., Bex V., Duclos M., Tortigues D., Lefebvre H., Tabarin A. & Roger P. (2000) Hypercorticism by bilateral adrenal hyperplasia with several paradoxical responses. In: *Program & Abstracts. The 82nd Annual Meeting of The Endocrine Society.* Toronto, Ont., Abstract 1936, p 467.

Bornstein S.R., Haidan A., & Ehrhart-Bornstein M. (1996) Cellular communication in the neuro-adrenocortical axis: role of vasoactive intestinal polypeptide (VIP). *Endocr.Res.* 22:819-829.

Bornstein S.R., Stratakis C.A., & Chrousos G.P. (1999) Adrenocortical tumors: recent advances in basic concepts and clinical management. *Ann.Intern.Med.* 130:759-771.

Bornstein S.R., Uhlmann K. , Haidan A., Ehrhart-Bornstein M., & Scherbaum W.A. (1997) Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* 46:1235-1238.

Bose H.S., Sugawara T., Strauss J.F., III, & Miller W.L. (1996) The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. *N.Engl.J.Med.* 335:1870-1878.

Bourdeau I., D'Amour P., Hamet P., Boutin J.M. & Lacroix A. (2000) Abnormal membrane hormone receptors in subclinical bilateral macronodular adrenal hyperplasia. In: *In: Program & Abstracts. The 82nd Annual Meeting of The Endocrine Society,* Toronto, Ont., Abstract 1934, p 467.

Boylan M.O., Jepeal L.I., & Wolfe M.M. (1999) Structure of the rat glucose-dependent insulinotropic polypeptide receptor gene. *Peptides* 20:219-228.

Breault L., Lehoux J.G., & Gallo-Payet N. (1996) The angiotensin AT2 receptor is present in the human fetal adrenal gland throughout the second trimester of gestation. *J.Clin.Endocrinol. Metab.* 81:3914-3922.

Brown J.C., Mutt V., & Pederson R.A. (1970) Further purification of a polypeptide demonstrating enterogastrone activity. *J.Physiol. (Lond)* 209:57-64.

Brush J.S., Sutliff L.S., & Sharma R.K. (1974) Metabolic regulation and adenyl cyclase activity of adrenocortical carcinoma cultured cells. *Cancer Res.* 34:1495-1502.

Bugalho M.J., Li X., Rao C.V., Soares J., & Sobrinho L.G. (2000) Presence of a Gs alpha mutation in an adrenal tumor expressing LH/hCG receptors and clinically associated with Cushing's syndrome. *Gynecol.Endocrinol.* 14:50-54.

Calodney L., Eaton R.P., Black W., & Cohn F. (1973) Exacerbation of Cushing's syndrome during pregnancy: report of a case. *J.Clin.Endocrinol.Metab.* 36:81-86.

Cao G.Y., Considine R.V., & Lynn R.B. (1997) Leptin receptors in the adrenal medulla of the rat. *Am J Physiol* 273:E448-E452.

Carney J.A., Gordon H., Carpenter P.C., Shenoy B.V., & Go V.L. (1985) The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine (Baltimore)* 64:270-283.

Casey M., Mah C., Merliss A.D., Kirschner L.S., Taymans S.E., Denio A.E., Korf B., Irvine A.D., Hughes A., Carney J.A., Stratakis C.A., & Basson C.T. (1998) Identification of a novel genetic locus for familial cardiac myxomas and Carney complex. *Circulation* 98:2560-2566.

Chabre O., Liakos P., Vivier J., Chaffanjon P., Labat-Moleur F., Martinie M., Bottari S.P., Bachelot I., Chambaz E.M., Defaye G., & Feige J.J. (1998) Cushing's syndrome due to a gastric inhibitory polypeptide-dependent adrenal adenoma: insights into hormonal control of adrenocortical tumorigenesis. *J.Clin.Endocrinol.Metab.* 83:3134-3143.

Chamoux E., Breault L., Lehoux J.G., & Gallo-Payet N. (2002) Involvement of the angiotensin II type 2 receptor in apoptosis during human fetal adrenal gland development. *J.Clin. Endocrinol. Metab.* 84:4722-4730.

Chandrasekharappa S.C., Guru S.C., Manickam P., Olufemi S.E., Collins F.S., Debelenko L.V., Emmert_Buck M.R., Zhuang Z., Lubensky I.A., Liotta L.A., Crabtree J.S., Wang Y., Roe B.A., Weisemann J., Boguski M.S., Agarwal S.K., Kester M.B., Kim Y.S., Heppner C., Dong Q., Spiegel A.M., Burns A.L., & Marx S.J. (1997) Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 276:404-407.

Chen R., Lewis K.A., Perrin M.H., & Vale W.W. (1993) Expression cloning of a human corticotropin-releasing-factor receptor. *Proc.Natl. Acad. Sci. U. S. A.* 90:8967-8971.

Cherradi N., Brandenburger Y., Rossier M.F., Vallotton M.B., Stocco D.M., & Capponi A.M. (1998) Atrial natriuretic peptide inhibits calcium-induced steroidogenic acute regulatory protein gene transcription in adrenal glomerulosa cells. *Mol.Endocrinol.* 12:962-972.

Chidiac P., Hebert T.E., Valiquette M., Dennis M., & Bouvier M. (1994) Inverse agonist activity of beta-adrenergic antagonists. *Mol Pharmacol.* 45:490-499.

Cizza G., Lotsikas A.J., Licinio J., Gold P.W., & Chrousos G.P. (1997) Plasma leptin levels do not change in patients with Cushing's disease shortly after correction of hypercortisolism. *J.Clin.Endocrinol.Metab.* 82:2747-2750.

Clark B.J., Pezzi V., Stocco D.M., & Rainey W.E. (1995) The steroidogenic acute regulatory protein is induced by angiotensin II and K+ in H295R adrenocortical cells. *Mol.Cell.Endocrinol.* 115:215-219.

Clemens J.W., Lala D.S., Parker K.L., & Richards J.S. (1994) Steroidogenic factor-1 binding and transcriptional activity of the cholesterol side-chain cleavage promoter in rat granulosa cells. *Endocrinology* 134:1499-1508.

Clements J.A. & Funder J.W. (1986) Arginine vasopressin (AVP) and AVP-like immunoreactivity in peripheral tissues. *Endocr.Rev.* 7:449-460.

Combes J., Bourrinet-Pellet E., Narboni G., Saez J.M., Remy-Martin M.A., Mantion G., Cornette C., De Wazières B., & Penfornis A. (1998) Un nouveau cas de syndrome de Cushing dépendant de l'alimentation associé à un adénome surrénalien unique. *Ann.Endocrinol.(Paris)* 59:264.

Contesse V., Lenglet S., Grumolato L., Anouar Y., Lihrmann I., Lefebvre H., Delarue C., & Vaudry H. (1999) Pharnacological and molecular characterization of 5-hydroxytryptamine$_7$ receptors in the rat adrenal gland. *Mol.Pharmacol.* 56:552-561.

Cooper R.J., Udelsman R., Resta C. & Tollin S.R. (1998) Familial Cushing's syndrome secondary to bilateral macronodular hyperplasia. In: *Program and Abstracts The 80th Annual Meeting of the Endocrine Society*, New Orleans, LA, Bethesda, MD. Abstract P2-397, pp 333.

Croughs R.J., Zelissen P.M., Van Vroonhoven Th.J., Hofland L.J., N'Diaye N., Lacroix A., & de Herder W.W. (2000) GIP-dependent adrenal Cushing's syndrome with incomplete suppression of ACTH. *Clin.Endocrinol. (Oxf)* 52:235-240.

Cushing H.W. (1912) The pituitary body and its disorders. P.B. Lippincott, Philadelphia.

Cushing H.W. (1932) The basophilic adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull.Hohn Hopkins Hosp.* 50:137-195.

Daidoh H., Morita H., Hanafusa J., Mune T., Murase H., Sato M., Shibata T., Suwa T., Ishizuka T., & Yasuda K. (1998) In vivo and in vitro effects of AVP and V1a receptor antagonist on Cushing's syndrome due to ACTH-independent bilateral macronodular adrenocortical hyperplasia. *Clin.Endocrinol.(Oxf)* 49:403-409.

de Herder W.W., Hofland L.J., Usdin T.B., de Jong F.H., Uitterlinden P., van Koetsveld P., Mezey E., Bonner T.I., Bonjer H.J., & Lamberts S.W. (1996) Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. *J.Clin.Endocrinol.Metab.* 81:3168-3172.

de Lange W.E., Pratt J.J., & Doorenbos H. (1980) A gonadotrophin responsive testosterone producing adrenocortical adenoma and high gonadotrophin levels in an elderly woman. *Clin.Endocrinol.(Oxf)* 12:21-28.

Delarue C., Leboulenger F. , Morra M., Hery F., Verhofstad A.J., Berod A., Denoroy L., Pelletier G. , & Vaudry H. (1988a) Immunohistochemical and biochemical evidence for the presence of serotonin in amphibian adrenal chromaffin cells. *Brain Res.* 459:17-26.

Delarue C., Lefebvre H., Idres S., Leboulenger F., Homo-Delarche G., Lihrmann I., Feuilloley M., & Vaudry H. (1988b) Serotonin stimulates corticosteroid secretion by frog adrenocortical tissue in vitro. *J.Steroid.Biochem.* 29:519-525.

Dhanasekaran N., Heasley L.E., & Johnson G.L. (1995) G protein-coupled receptor systems involved in cell growth and oncogenesis. *Endocr.Rev.* 16:259-270.

Di Blasio A.M., Voutilainen R., Jaffe R.B., & Miller W.L. (1987) Hormonal regulation of messenger ribonucleic acids for P450scc (cholesterol side-chain cleavage enzyme) and P450c17 (17 alpha- hydroxylase/17,20-lyase) in cultured human fetal adrenal cells. *J.Clin.Endocrinol. Meta.b* 65:170-175.

Ehrhart-Bornstein M., Hinson J.P., Bornstein S.R., Scherbaum W.A., & Vinson G.P. (1998) Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr.Rev.* 19:101-143.

Ehses J.A., Lee S.S., Pederson R.A., & McIntosh C.H. (2001) A new pathway for glucose-dependent insulinotropic polypeptide (GIP) receptor signaling: evidence for the involvement of phospholipase A2 in GIP-stimulated insulin secretion. *J.Biol.Chem.* 276:23667-23673.

Elias L.L., Antunes-Rodrigues J., Elias P.C., & Moreira A.C. (1997) Effect of plasma osmolality on pituitary-adrenal responses to corticotropin-releasing hormone and atrial natriuretic peptide changes in central diabetes insipidus. *J.Clin.Endocrinol.Metab.* 82:1243-1247.

Estivariz F.E., Iturriza F., McLean C., Hope J., & Lowry P.J. (1982) Stimulation of adrenal mitogenesis by N-terminal proopiocortin peptides. *Nature* 297:419-422.

Farfel Z., Bourne H.R., & Iiri T. (1999) The expanding spectrum of G protein diseases. *N.Engl.J.Med.* 340:1012-1020.

Fehmann H.C., Goke R., & Goke B. (1995) Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr. Rev.* 16:390-410.

Fernandez-Vivero J., Rodriguez-Sanchez F., Verastegui C., Cordoba M.F., Romero A., & de Castro J.M. (1993) Immunocytochemical distribution of serotonin and neuropeptide Y (NPY) in mouse adrenal gland. *Histol.Histopathol.* 8:509-520.

Findlay J.C., Sheeler L.R. , Engeland W.C., & Aron D.C. (1993) Familial adrenocorticotropin-independent Cushing's syndrome with bilateral macronodular adrenal hyperplasia. *J.Clin.Endocrinol.Metab.* 76:189-191.

Fragoso M.C.B., Latronico A.C., Domenice S., Lando V.S. & Mendonca B.B. (1999) Activation mutation in the stimulatory guanine nucleotide-binding protein in a woman with Cushing's syndrome by ACTH-independent macronodular adrenal hyperplasia. In: *Program and Abstracts The 81st Annual Meeting of the Endocrine Society*, San Diego, CA, Bethesda, MD. Abstract P2-150, p 312.

Gagel R.F. (1998) Multiple Endocrine Neoplasia. In: *Williams Textbook of Endocrinology.* (eds Wilson J.D., Foster D.W., Kronenberg H.M. & Larsen P.R.), pp. 1627-1649. W.B. Saunders Company, Philadelphia.

Gallo-Payet N. & Guillon G. (1998) Regulation of adrenocortical function by vasopressin. *Horm.Metab.Res.* 30:360-367.

Gallo-Payet N., Guillon G. , Balestre M.N., & Jard S. (1986) Vasopressin induces breakdown of membrane phosphoinositides in adrenal glomerulosa and fasciculata cells. *Endocrinology* 119:1042-1047.

Gerl H., Rohde W., Biering H., Schulz N., & Lochs H. (2000) [Food-dependent Cushing syndrome of long standing with mild clinical features]. *Dtsch.Med.Wochenschr.* 125:1565-1568.

Gicquel C., Bertagna X., & Le Bouc Y. (1995) Recent advances in the pathogenesis of adrenocortical tumours. *Eur.J.Endocrinol.* 133:133-144.

Gicquel C., Bertherat J., Le Bouc Y., & Bertagna X. (2000) Pathogenesis of adrenocortical incidentalomas and genetic syndromes associated with adrenocortical neoplasms. *Endocrinol. Metab.Clin.North.Am.* 29:1-13.

Giraldi F.P. & Cavagnini F. (1998) Corticotropin-releasing hormone is produced by rat corticotropes and modulates ACTH secretion in a paracrine/autocrine fashion. *J.Clin.Invest.*101:2478-2484.

Glasow A., Haidan A., Hilbers U., Breidert M., Gillespie J., Scherbaum W.A., Chrousos G.P., & Bornstein S.R. (1998) Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. *J.Clin.Endocrinol.Metab.* 83:4459-4466.

Grazzini E., Lodboerer A.M., Perez-Martin A., Joubert D., & Guillon G. (1996) Molecular and functional characterization of V1b vasopressin receptor in rat adrenal medulla. *Endocrinology* 137 :3906-3914.

Gremlich S., Porret A., Hani E.H., Cherif D., Vionnet N., Froguel P., & Thorens B. (1995) Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes* 44:1202-1208.

Grossman A. (1995) Corticotropin-Releasing Hormone: Basic Physiology and Clinical Applications. In: *Endocrinology* (eds DeGroot L.J., Besser G.M., Burger H.G., Jameson J.L., Loriaux D.L., Marshall J.C., Odell W.D., Potts J.T., Jr. & Rubestein A.H.), 3 edn, pp. 341-354. W.B. Saunders Company, Philadelphia.

Groussin L., Perlemoine K. , Contesse V., Tabarin A., Thieblot P., Schlienger J.L., Bertagna X., & Bertherat J. (2001) Gastric inhibitory polypeptide receptor (GIP-R) gene expression in tumors of the adrenal cortex and bilateral ACTH-independent macronodular adrenal hyperplasia (AIMAH). *The 83rd Annual Meeting of the Endocrine Society*, Denver, Colorado.

Grunenberger F., Noal E., Bachellier P., Weber J.C., Jaeck D., & Schlienger J.L. (1999) Hypercorticisme familial non ACTH dépendant par hyperplasie macronodulaire bilatérale des surrénales. *Ann.Endocrinol. (Paris).Abstract A04* pp 60-290.

Guillon G. & Gallo-Payet N. (1986) Specific vasopressin binding to rat adrenal glomerulosa cells. Relationship to inositol lipid breakdown. *Biochem.J.* 235:209-214.

Guillon G., Grazzini E., Andrez M., Breton C., Trueba M., Serradeil-LeGal C., Boccara G., Derick S., Chouinard L., & Gallo-Payet N. (1998) Vasopressin: a potent autocrine/paracrine regulator of mammal adrenal functions. *Endocr.Res.* 24:703-710.

Guillon G., Trueba M., Joubert D., Grazzini E., Chouinard L., Cote M., Payet M.D., Manzoni O., Barberis C., & Robert M. (1995) Vasopressin stimulates steroid secretion in human adrenal glands: comparison with angiotensin-II effect. *Endocrinology* 136:1285-1295.

Guo X. & Wakade A.R. (1994) Differential secretion of catecholamines in response to peptidergic and cholinergic transmitters in rat adrenals. *J.Physiol. (Lond)* 475:539-545.

Gupta P., Franco-Saenz R., & Mulrow P.J. (1995) Locally generated angiotensin II in the adrenal gland regulates basal, corticotropin-, and potassium-stimulated aldosterone secretion. *Hypertension* 25:443-448.

Guru S.C., Goldsmith P.K., Burns A.L., Marx S.J., Spiegel A.M., Collins F.S., & Chandrasekharappa S.C. (1998) Menin, the product of the MEN1 gene, is a nuclear protein. *Proc.Natl.Acad.Sci.U.S.A.* 95:1630-1634.

Hamet P., Larochelle P., Franks D.J., Cartier P., & Bolte E. (1987) Cushing syndrome with food-dependent periodic hormonogenesis. *Clin.Invest Med.* 10:530-533.

Heiman M.L., Ahima R.S., Craft L.S., Schoner B., Stephens T.W., & Flier J.S. (1997) Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 138:3859-3863.

Hingshaw H.T. & Ney R.L. (1974) Abnormal Control in the Neoplastic Adrenal Cortex. In: *Hormones and Cancer* (ed McKerns K.W.), pp. pp 309-327. Academic Press, New York.

Hinson J.P., Cameron L.A., Purbrick A., & Kapas S. (1994) The role of neuropeptides in the regulation of adrenal vascular tone: effects of vasoactive intestinal polypeptide, substance P, neuropeptide Y, neurotensin, Met-enkephalin, and Leu-enkephalin on perfusion medium flow rate in the intact perfused rat adrenal. *Regul.Pept.* 51:55-61.

Hinson J.P. & Kapas S. (1998) The role of endothelial cell products in the regulation of adrenocortical function: actions of endothelin, nitric oxide, adrenomedullin and PAMP. *Horm.Metab.Res.* 30 :334-340.

Hinson J.P., Kapas S., Orford C.D., & Vinson G.P. (1992) Vasoactive intestinal peptide stimulation of aldosterone secretion by the rat adrenal cortex may be mediated by the local release of catecholamines. *J.Endocrinol.* 133:253-258.

Hinson J.P., Vinson G.P., Pudney J., & Whitehouse B.J. (1989) Adrenal mast cells modulate vascular and secretory responses in the intact adrenal gland of the rat. *J.Endocrinol.* 121:253-260.

Hirata Y., Uchihashi M., Sueoka S., Matsukura S., & Fujita T. (1981) Presence of ectopic beta-adrenergic receptors on human adrenocortical cortisol-producing adenomas. *J.Clin.Endocrinol.Metab.* 53:953-957.

Hol E.M., Gispen W.H., & Bar P.R. (1995) ACTH-related peptides: receptors and signal transduction systems involved in their neurotrophic and neuroprotective actions. *Peptides* 16:979-993.

Holst J.J., Gromada J., & Nauck M.A. (1997) The pathogenesis of NIDDM involves a defective expression of the GIP receptor. *Diabetologia* 40:984-986.

Horiba N., Suda T., Aiba M., Naruse M., Nomura K., Imamura M., & Demura H. (1995) Lysine vasopressin stimulation of cortisol secretion in patients with adrenocorticotropin-independent macronodular adrenal hyperplasia. *J.Clin.Endocrinol.Metab.* 80:2336-2341.

Howard P.W. & Maurer R.A. (2001) A point mutation in the LIM domain of Lhx3 reduces activation of the glycoprotein hormone alpha-subunit promoter. *J.Biol.Chem.* 276:19020-19026.

Hu Y.L., Lei Z.M., & Rao C.V. (1998) Analysis of the promoter of the luteinizing hormone/human chorionic gonadotropin receptor gene in neuroendocrine cells. *Life Sci.* 63:2157-2165.

Huang Q., Rivest R., & Richard D. (1998) Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. *Endocrinology* 139:1524-1532.

Iida S., Nakamura Y., Fujii H., Nishimura J., Tsugawa M., Gomi M., Fukata J., Tarui S., Moriwaki K., & Kitani T. (1990) A patient with hypocortisolism and Cushing's syndrome-like manifestations: cortisol hyperreactive syndrome. *J.Clin.Endocrinol.Metab.* 70:729-737.

Ilvesmaki V., Kahri A.I., Miettinen P.J., & Voutilainen R. (1993) Insulin-like growth factors (IGFs) and their receptors in adrenal tumors: high IGF-II expression in functional adrenocortical carcinomas. *J.Clin.Endocrinol.Metab.* 77:852-858.

Ingraham H.A., Lala D.S., Ikeda Y., Luo X., Shen W.H., Nachtigal M.W., Abbud R., Nilson J.H., & Parker K.L. (1994) The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. *Genes Dev.* 8:2302-2312.

Inoue H., Watanabe N., Higashi Y., & Fujii-Kuriyama Y. (1991) Structures of regulatory regions in the human cytochrome P-450scc (desmolase) gene. *Eur.J Biochem.* 195:563-569.

Jacob A.L. & Lund J. (1998) Mutations in the activation function-2 core domain of steroidogenic factor-1 dominantly suppresses PKA-dependent transactivation of the bovine CYP17 gene. *J Biol.Chem.* 273:13391-13394.

Jin L., Burguera B.G., Couce M.E., Scheithauer B.W., Lamsan J., Eberhardt N.L., Kulig E., & Lloyd R.V. (1999) Leptin and leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. *J.Clin.Endocrinol.Metab.* 84:2903-2911.

John M.E., John M.C., Boggaram V., Simpson E.R., & Waterman M.R. (1986) Transcriptional regulation of steroid hydroxylase genes by corticotropin. *Proc.Natl.Acad.Sci.U.S.A.* 83:4715-4719.

Jones M.T. & Gillham B. (1988) Factors involved in the regulation of adrenocorticotropic hormone/beta-lipotropic hormone. *Physiol.Rev.* 68:743-818.

Kagawa N., Ogo A., Takahashi Y., Iwamatsu A., & Waterman M.R. (1994) A cAMP-regulatory sequence (CRS1) of CYP17 is a cellular target for the homeodomain protein Pbx1. *J Biol.Chem.* 269:18716-18719.

Kahri A. (1966) Histochemical and electron microscopic studies on the cells of the rat adrenal cortex in tissue culture. *Acta Endocrinol. (Copenh)* 52:Suppl-96.

Kaplan A.M. & Vigna S.R. (1994) Gastric inhibitory polypeptide (GIP) binding sites in rat brain. *Peptides* 15:297-302.

Karin M. (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol.Chem.* 270:16483-16486.

Kasprzak A., Rebuffat P., Andreis P.G., Mazzocchi G., & Nussdorfer G.G. (1991) Effects of prolonged cysteamine administration on the rat adrenal cortex: evidence that endogenous somatostatin is involved in the control of the growth and steroidogenic capacity of zona glomerulosa. *J.Steroid Biochem.Mol.Biol.* 38:469-473.

Katz M.S., Kelly T.M., Dax E.M., Pineyro M.A., Partilla J.S., & Gregerman R.I. (1985) Ectopic beta-adrenergic receptors coupled to adenylate cyclase in human adrenocortical carcinomas. *J.Clin.Endocrinol.Metab.* 60:900-909.

Kawai M., Naruse M., Yoshimoto T., Naruse K., Shionoya K., Tanaka M., Morishita Y., Matsuda Y., Demura R., & Demura H. (1996) C-type natriuretic peptide as a possible local modulator of aldosterone secretion in bovine adrenal zona glomerulosa. *Endocrinology* 137:42-46.

Keegan G.T., Grabarits F., & Roland A.S. (1976) Pregnancy complicated by Cushing's syndrome. *South.Med.J.* 69:1207-1209.

Kero J., Poutanen M., Zhang F.P., Rahman N., McNicol A.M., Nilson J.H., Keri R.A., & Huhtaniemi I.T. (2000) Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex. *J.Clin.Invest.* 105:633-641.

Kimura E., Sonobe M.H., Armelin M.C., & Armelin H.A. (1993) Induction of FOS and JUN proteins by adrenocorticotropin and phorbol ester but not by 3',5'-cyclic adenosine monophosphate derivatives. *Mol.Endocrinol.* 7:1463-1471.

Kirschner L.S., Carney J.A., Pack S.D., Taymans S.E., Giatzakis C., Cho Y.S., Cho-Chung Y.S., & Stratakis C.A. (2000) Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat.Genet.* 26:89-92.

Kogire M., Inoue K., Sumi S., Doi R., Takaori K., Yun M., Fujii N., Yajima H., & Tobe T. (1988) Effects of synthetic human gastric inhibitory polypeptide on splanchnic circulation in dogs. *Gastroenterology* 95:1636-1640.

Kreines K., Perrin E., & Salzer R. (1964) Pregnancy in Cushing's syndrome. *J.Clin.Endocrinol.Metab.* 24:75-79.

Kubota A., Yamada Y., Yasuda K., Someya Y., Ihara Y., Kagimoto S., Watanabe R., Kuroe A., Ishida H., & Seino Y. (1997) Gastric inhibitory polypeptide activates MAP kinase through the wortmannin-sensitive and -insensitive pathways. *Biochem.Biophys.Res.Commun.* 235:171-175.

Lacroix A., Bolte E., Tremblay J., Dupre J., Poitras P., Fournier H., Garon J., Garrel D., Bayard F., Taillefer R., Flanagan R.J., & Hamet P. (1992) Gastric inhibitory polypeptide-dependent cortisol hypersecretion--a new cause of Cushing's syndrome. *N.Engl.J.Med.* 327:974-980.

Lacroix A., Bolté E., Poitras P., Fournier H., Garon J., Garrel D., Bayard F., Tremblay J., Taillefer R., Flanagan R. & Hamet P. (1991) Gastric Inhibitory Polypeptide-Dependent Macronodular Adrenal Hyperplasia: A New Etiology of Cushing's Syndrome. In: *Program and Abstracts The 73rd Annual Meeting of the Endocrine Society*, Washington, DC, Bethesda, MD. Abstract 241, p 91.

Lacroix A., Hamet P., & Boutin J.M. (1999a) Leuprolide acetate therapy in luteinizing hormone--dependent Cushing's syndrome. *N Engl.J.Med* 341:1577-1581.

Lacroix A., Mircescu H., & Hamet P. (1999b) Clinical Evaluation of the Presence of Abnormal Hormone Receptors in Adrenal Cushing's Syndrome. *The Endocrinologist* 9:9-15.

Lacroix A., N'Diaye N., de Herder W.W., Nieman L., Ezzat S., Hermus A.R., Noordam C., Gerl H., Lochs H., Pico A., Hamet P., & Tremblay J. (2000) Adrenal GIP receptor overexpression in food-dependent adrenal Cushing's syndrome. *Program & Abstracts of the 11th International Congress of Endocrinology,* Sydney, Australia.

Lacroix A., N'Diaye N., Tremblay J., & Hamet P. (2001) Ectopic and Abnormal Hormone Receptors in Adrenal Cushing's Syndrome. *Endocr.Rev.* 22:75-110.

Lacroix A., Tremblay J., Rousseau G., Bouvier M., & Hamet P. (1997a) Propranolol therapy for ectopic beta-adrenergic receptors in adrenal Cushing's syndrome. *N.Engl.J.Med.* 337:1429-1434.

Lacroix A., Tremblay J., Touyz R.M., Deng L.Y., Lariviere R., Cusson J.R., Schiffrin E.L., & Hamet P. (1997b) Abnormal adrenal and vascular responses to vasopressin mediated by a V1-vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia, Cushing's syndrome, and orthostatic hypotension. *J.Clin.Endocrinol.Metab.* 82:2414-2422.

Lacroix S. & Rivest S. (1998) Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J.Neurochem.* 70:452-466.

Lander E.S., Linton L.M., Birren B., Nusbaum C., Zody M.C., Baldwin J., Devon K., Dewar K., Doyle M., FitzHugh W., Funke R., Gage D., Harris K., Heaford A. & al. (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860-921.

Latronico A.C. & Chrousos G.P. (1997) Extensive personal experience: adrenocortical tumors. *J.Clin.Endocrinol.Metab.* 82:1317-1324.

Le Roy C., Li J.Y., Stocco D.M., Langlois D., & Saez J.M. (2000) Regulation by adrenocorticotropin (ACTH), angiotensin II, transforming growth factor-beta, and insulin-like growth factor I of bovine adrenal cell steroidogenic capacity and expression of ACTH receptor, steroidogenic acute regulatory protein, cytochrome P450c17, and 3beta- hydroxysteroid dehydrogenase. *Endocrinology* 141:1599-1607.

Lebrethon M.C., Avallet O. , Reznik Y., Archambeaud F., Combes J., Usdin T.B., Narboni G., Mahoudeau J., & Saez J.M. (1998) Food-dependent Cushing's syndrome: characterization and functional role of gastric inhibitory polypeptide receptor in the adrenals of three patients. *J.Clin.Endocrinol.Metab.* 83:4514-4519.

Lee S.L., Wesselschmidt R.L., Linette G.P., Kanagawa O., Russell J.H., & Milbrandt J. (1995) Unimpaired thymic and peripheral T cell death in mice lacking the nuclear receptor NGFI-B (Nur77). *Science* 269:532-535.

Lefebvre H., Contesse V., Delarue C., Feuilloley M., Hery F., Grise P., Raynaud G., Verhofstad A.A., Wolf L.M., & Vaudry H. (1992) Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin4 receptor subtype. *Neuroscience* 47:999-1007.

Lefebvre H., Contesse V., Delarue C., Legrand A., Kuhn J.M., Vaudry H., & Wolf L.M. (1995) The serotonin-4 receptor agonist cisapride and angiotensin-II exert additive effects on aldosterone secretion in normal man. *J.Clin.Endocrinol.Metab.* 80:504-507.

Lefebvre H., Contesse V., Delarue C., Soubrane C., Legrand A., Kuhn J.M., Wolf L.M., & Vaudry H. (1993) Effect of the serotonin-4 receptor agonist zacopride on aldosterone secretion from the human adrenal cortex: in vivo and in vitro studies. *J.Clin.Endocrinol.Metab.* 77:1662-1666.

Lefebvre H., Contesse V., Delarue C., Vaudry H., & Kuhn J.M. (1998) Serotonergic regulation of adrenocortical function. *Horm.Metab.Res.* 30:398-403.

Lehoux J.G. & Ducharme L. (1995) In vivo effects of adrenocorticotropin on c-jun, jun-B, c-fos and fos-B in rat adrenal. *Endocr.Res.* 21:267-274.

Lehoux J.G., Fleury A., & Ducharme L. (1998) The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonucleic acid and protein of steroidogenic enzymes in rat adrenal in vivo. *Endocrinology* 139:3913-3922.

Leinonen P., Ranta T., Siegberg R., Pelkonen R., Heikkila P., & Kahri A. (1991) Testosterone-secreting virilizing adrenal adenoma with human chorionic gonadotrophin receptors and 21-hydroxylase deficiency. *Clin.Endocrinol.(Oxf)* 34:31-35.

Li Y. & Lau L.F. (1997) Adrenocorticotropic hormone regulates the activities of the orphan nuclear receptor Nur77 through modulation of phosphorylation. *Endocrinology* 138:4138-4146.

Li Z.G., Queen G., & LaBella F.S. (1990) Adrenocorticotropin, vasoactive intestinal polypeptide, growth hormone- releasing factor, and dynorphin compete for common receptors in brain and adrenal. *Endocrinology* 126:1327-1333.

Licinio J., Mantzoros C., Negrao A.B., Cizza G, Wong M.L., Bongiorno P.B., Chrousos G.P., Karp B., Allen C., Flier J.S., & Gold P.W. (1997) Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat.Med.* 3:575-579.

Lieberman S.A., Eccleshall T.R., & Feldman D. (1994) ACTH-independent massive bilateral adrenal disease (AIMBAD): a subtype of Cushing's syndrome with major diagnostic and therapeutic implications. *Eur.J.Endocrinol.* 131:67-73.

Lifton R.P., Dluhy R.G., Powers M., Rich G.M., Cook S., Ulick S., & Lalouel J.M. (1992) A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 355:262-265.

Light K., Jenkins P.J., Weber A., Perrett C., Grossman A., Pistorello M., Asa S.L., Clayton R.N., & Clark A.J. (1995) Are activating mutations of the adrenocorticotropin receptor involved in adrenal cortical neoplasia? *Life Sci.* 56:1523-1527.

Lin D., Sugawara T., Strauss J.F., III, Clark B.J., Stocco D.M., Saenger P., Rogol A., & Miller W.L. (1995) Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 267:1828-1831.

Lotfi C.F., Todorovic Z., Armelin H.A., & Schimmer B.P. (1997) Unmasking a growth-promoting effect of the adrenocorticotropic hormone in Y1 mouse adrenocortical tumor cells. *J Biol.Chem.* 272:29886-29891.

Lu M., Wheeler M.B., Leng X.H., & Boyd A.E. (1993) The role of the free cytosolic calcium level in beta-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide I(7-37). *Endocrinology* 132:94-100.

Luton J.P., Bertherat J., Kuhn J.M., & Bertagna X. (1998) [Aberrant expression of the GIP (Gastric Inhibitory Polypeptide) receptor in an adrenal cortical adenoma responsible for a case of food-dependent Cushing's syndrome]. *Bull.Acad.Natl.Med.* 182:1839-1849.

Lyons J., Landis C.A., Harsh G., Vallar L., Grunewald K., Feichtinger H., Duh Q.Y., Clark O.H. , Kawasaki E., & Bourne H.R. (1990) Two G protein oncogenes in human endocrine tumors. *Science* 249:655-659.

Malendowicz L.K., Macchi C., Nussdorfer G.G., & Nowak K.W. (1998) Acute effects of recombinant murine leptin on rat pituitary-adrenocortical function. *Endocr.Res.* 24:235-246.

Malendowicz L.K., Markowska A., & Zabel M. (1996) Neuropeptide Y-related peptides and hypothalamo-pituitary-adrenal axis function. *Histol.Histopathol.* 11:485-494.

Malendowicz L.K. & Nussdorfer G.G. (1993) Unusual effect of prolonged vasoactive intestinal peptide (VIP) administration on the adrenal growth and corticosterone secretion in the rat. *Neuropeptides* 25:145-150.

Maniatis T. (1989) Analysis and cloning of eukaryotic genomic DNA. In: *Molecular cloning - A laboratory manual* (eds SambrookJ., Fritsch E.F. & Maniatis T.), 2nd edn, pp. 9.16-9.17. Cold Spring Harbor Laboratory Press.

Marieb N.J., Spangler S., Kashgarian M., Heimann A., Schwartz M.L., & Schwartz P.E. (1983) Cushing's syndrome secondary to ectopic cortisol production by an ovarian carcinoma. *J.Clin.Endocrinol.Metab.* 57:737-740.

Matsukura S., Kakita T., Sueoka S., Yoshimi H., Hirata Y., Yokota M., & Fujita T. (1980) Multiple hormone receptors in the adenylate cyclase of human adrenocortical tumors. *Cancer Res.* 40:3768-3771.

Mazza E., Goffi S., Barchi P., Arvat E., Bellone J., Limone P., Ghigo E., & Camanni F. (1994) Enhanced adrenocorticotrophic hormone and cortisol responses to corticotrophin-releasing hormone in central idiopathic diabetes insipidus. *Eur.J.Endocrinol.* 130:121-124.

Mazzocchi G., Malendowicz L.K., Macchi C., Gottardo G., & Nussdorfer G.G. (1996) Further investigations on the effects of neuropeptide Y on the secretion and growth of rat adrenal zona glomerulosa. *Neuropeptides* 30:19-27.

Mazzocchi G., Rebuffat P., Meneghelli V., Malendowicz L.K., Tortorella C., Gottardo G., & Nussdorfer G.G. (1999) Gastric inhibitory polypeptide stimulates glucocorticoid secretion in rats, acting through specific receptors coupled with the adenylate cyclase-dependent signaling pathway. *Peptides* 20:589-594.

Meduri G., Charnaux N., Loosfelt H., Jolivet A., Spyratos F., Brailly S., & Milgrom E. (1997) Luteinizing hormone/human chorionic gonadotropin receptors in breast cancer. *Cancer Res.* 57:857-864.

Miller W.L. (1988) Molecular biology of steroid hormone synthesis. *Endocr.Rev.* 9:295-318.

Minami S., Sugihara H., Sato J., Tatsukuchi A., Sugisaki Y., Sasano H., & Wakabayashi I. (1996) ACTH independent Cushing's syndrome occurring in siblings. *Clin.Endocrinol.(Oxf)* 44:483-488.

Mircescu H., Jilwan J., N'Diaye N., Bourdeau I., Tremblay J., Hamet P., & Lacroix A. (2000) Are ectopic or abnormal membrane hormone receptors frequently present in adrenal Cushing's syndrome? *J.Clin.Endocrinol.Metab.* 85:3531-3536.

Missale C., Memo M., Liberini P., & Spano P. (1988) Dopamine selectively inhibits angiotensin II-induced aldosterone secretion by interacting with D-2 receptors. *J.Pharmacol.Exp.Ther.* 246:1137-1143.

Miyawaki K., Yamada Y., Yano H., Niwa H., Ban N., Ihara Y., Kubota A., Fujimoto S., Kajikawa M., Kuroe A., Tsuda K., Hashimoto H., Yamashita T., Jomori T., Tashiro F., Miyazaki J., & Seino Y. (1999) Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc.Natl.Acad.Sci.U.S.A.* 96:14843-14847.

Monte D., DeWitte F., & Hum D.W. (1998) Regulation of the human P450scc gene by steroidogenic factor 1 is mediated by CBP/p300. *J Biol.Chem.* 273:4585-4591.

Morohashi K., Zanger U.M., Honda S., Hara M., Waterman M.R., & Omura T. (1993) Activation of CYP11A and CYP11B gene promoters by the steroidogenic cell-specific transcription factor, Ad4BP. *Mol.Endocrinol.* 7:1196-1204.

Mountjoy K.G., Bird I.M., Rainey W.E., & Cone R.D. (1994) ACTH induces up-regulation of ACTH receptor mRNA in mouse and human adrenocortical cell lines. *Mol.Cell.Endocrinol.* 99:R17-R20.

Mulrow P.J. (1998) Renin-angiotensin system in the adrenal. *Horm.Metab.Res.* 30:346-349.

Murphy E.P. & Conneely O.M. (1997) Neuroendocrine regulation of the hypothalamic pituitary adrenal axis by the nurr1/nur77 subfamily of nuclear receptors. *Mol.Endocrinol.* 11:39-47.

N'Diaye N., Bourdeau I., Tremblay J., Hamet P., & Lacroix A. (2001a) Absence of mutation of aberrant LH/hCG receptor in LH-dependent adrenal Cushing's syndrome. *J.Clin.Endocrinol. Metab.*- soumis-

N'Diaye N., Cartier D., Bourdeau I., Tremblay J., Hamet P., Lefebvre H., & Lacroix A. (2001b) Characterization of aberrant LH/hCG and serotonin 5-HT4 receptors in adrenal Cushing's syndrome. *The 83rd Annual Meeting of the Endocrine Society,* Denver, Colorado.Abstract P1-407p. 235.

N'Diaye N., Hamet P., Tremblay J., Boutin J.M., Gaboury L., & Lacroix A. (1999) Asynchronous development of bilateral nodular adrenal hyperplasia in gastric inhibitory polypeptide-dependent Cushing's syndrome. *J.Clin.Endocrinol.Metab.* 84:2616-2622.

N'Diaye N., Tremblay J., Hamet P., de Herder W.W., & Lacroix A. (1998a) Adrenocortical overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. *J.Clin.Endocrinol.Metab.* 83:2781-2785.

N'Diaye N., Tremblay J., Hamet P., & Lacroix A. (1998b) Hormone receptor abnormalities in adrenal Cushing's syndrome. *Horm.Metab.Res.* 30:440-446.

Nakamura Y., Son Y., Kohno Y., Shimono D., Kuwamura N., Koshiyama H., Sasano H., & Matsuda T. (2001) Case of adrenocorticotropic hormone-independent macronodular adrenal hyperplasia with possible adrenal hypersensitivity to angiotensin II. *Endocrine* 15:57-61.

Nauck M., Creutzfeldt W., Heimesaat M.M., Orskov C., Holst J.J., & Ebert R. (1993) Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J.Clin.Invest.* 91:301-307.

Naville D., Lebrethon M.C. , Kermabon A.Y., Rouer E., Benarous R., & Saez J.M. (1993) Characterization and regulation of the angiotensin II type-1 receptor (binding and mRNA) in human adrenal fasciculata-reticularis cells. *FEBS Lett.* 321:184-188.

Newell-Price J., Trainer P., Besser M., & Grossman A. (1998) The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr.Rev.* 19:647-672.

Newfield R.S., Kalaitzoglou G., Licholai T., Chilton D., Ashraf J., Thompson E.B., & New M.I. (2000) Normocortisolemic Cushing's syndrome initially presenting with increased glucocorticoid receptor numbers. *J.Clin.Endocrinol.Metab.* 85:14-21.

Nieman L.K. & Cutler G.B.J. (1995) Cushing's syndrome. In: (eds De Groot L.J., Besser M., Burger H.G. & et al.), pp. 1741-1769. Endocrinology. W.B. Saunders Company, Philadelphia.

Noordam K., Hermus A.R., Pesman G.J., N'Diaye N., Sweep F., Lacroix A., & Otten B. (2001) A child with food-dependent Cushing's syndrome due to ectopic expression of GIP-receptor in unilateral adenoma. *Clin.Endocrinol. (Oxf).*

Nussdorfer G.G. (1996) Paracrine control of adrenal cortical function by medullary chromaffin cells. *Pharmacol.Rev.* 48:495-530.

Nussdorfer G.G. & Malendowicz L.K. (1998) Role of VIP, PACAP, and related peptides in the regulation of the hypothalamo-pituitary-adrenal axis. *Peptides* 19:1443-1467.

Nussdorfer G.G., Rossi G.P., & Belloni A.S. (1997) The role of endothelins in the paracrine control of the secretion and growth of the adrenal cortex. *Int.Rev.Cytol.* 171:267-308.

Ogo A., Waterman M.R., & Kagawa N. (1997) cAMP-dependent transactivation involving the homeodomain protein Pbx1. *Arch.Biochem.Biophys.* 338:193-200.

Olsen N.J., Fang V.S., & DeGroot L.J. (1978) Cushing's syndrome due to adrenal adenoma with persistent diurnal cortisol secretory rhythm . *Metabolism* 27:695-700.

Orth D.N. & Kovacs W.J. (1998) The Adrenal Cortex. In: *Williams' Textbook of Endocrinology* (eds Wilson J.D., Foster D.W., Kronenberg H.M. & Larsen P.R.), 9 edn, pp. 517-664. W.B. Saunders Company, Philadelphia .

Pabon J.E., Li X., Lei Z.M., Sanfilippo J.S., Yussman M.A., & Rao C.V. (1996) Novel presence of luteinizing hormone/chorionic gonadotropin receptors in human adrenal glands. *J.Clin.Endocrinol.Metab.* 81:2397-2400.

Parker K.L. (1998) The roles of steroidogenic factor 1 in endocrine development and function. *Mol.Cell.Endocrinol.* 145:15-20.

Parker K.L. & Schimmer B.P. (1997) Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr.Rev.*18:361-377.

Parra A. & Cruz-Krohn J. (1966) Intercurrent Cushing's syndrome and pregnancy. *Am.J.Med.* 40:961-966.

Payet N. & Lehoux J.G. (1980) A comparative study of the role of vasopressin and ACTH in the regulation of growth and function of rat adrenal glands. *J.Steroid Biochem.* 12:461-467.

Penhoat A., Ouali R., Jaillard C., Langlois D., Begeot M., & Saez J.M. (1991) Characterization and regulation of angiotensin and corticotropin receptors on cultured bovine adrenal cells. *Endocr.Res.* 17:1-18.

Penhoat A., Ouali R., Viard I., Langlois D., & Saez J.M. (1996) Regulation of primary response and specific genes in adrenal cells by peptide hormones and growth factors. *Steroids* 61:176-183.

Pepe G.J. & Albrecht E.D. (1995) Actions of placental and fetal adrenal steroid hormones in primate pregnancy. *Endocr.Rev.* 16:608-648.

Perchellet J.P. & Sharma R.K. (1980) Ectopic alpha-adrenergic mediated accumulation of guanosine 3',5'- monophosphate in isolated adrenocortical carcinoma cells. *Endocrinology* 106:1589-1593.

Perraudin V., Delarue C., de Keyzer Y., Bertagna X., Kuhn J.M., Contesse V., Clauser E., & Vaudry H. (1995) Vasopressin-responsive adrenocortical tumor in a mild Cushing's syndrome: in vivo and in vitro studies. *J.Clin.Endocrinol.Metab.* 80:2661-2667.

Perraudin V., Delarue C., Lefebvre H., Contesse V. , Kuhn J.M., & Vaudry H. (1993) Vasopressin stimulates cortisol secretion from human adrenocortical tissue through activation of V1 receptors. *J.Clin.Endocrinol.Metab.* 76:1522-1528.

Philips A., Lesage S., Gingras R., Maira M.H., Gauthier Y., Hugo P., & Drouin J. (1997a) Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. *Mol.Cell.Biol.* 17:5946-5951.

Philips A., Maira M., Mullick A., Chamberland M., Lesage S., Hugo P., & Drouin J. (1997b) Antagonism between Nur77 and glucocorticoid receptor for control of transcription. *Mol.Cell Biol.* 17:5952-5959.

Pittaway D.E., Andersen R.N., & Givens J.R. (1973) In vitro on an HCG responsive, testosterone secreting adrenal cortical adenoma. *Steroids* 22:731-745.

Pralong F.P., Gomez F., Guillou L., Mosimann F., Franscella S., & Gaillard R.C. (1999) Food-dependent Cushing's syndrome: possible involvement of leptin in cortisol hypersecretion. *J.Clin.Endocrinol.Metab.* 84:3817-3822.

Pralong F.P., Roduit R., Waeber G., Castillo E., Mosimann F., Thorens B., & Gaillard R.C. (1998) Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland . *Endocrinology* 139:4264-4268.

Raber J., Chen S., Mucke L., & Feng L. (1997) Corticotropin-releasing factor and adrenocorticotrophic hormone as potential central mediators of OB effects. *J.Biol.Chem.* 272:15057-15060.

Rabes H.M., Demidchik E.P. , Sidorow J.D., Lengfelder E., Beimfohr C., Hoelzel D., & Klugbauer S. (2000) Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-

chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clin. Cancer Res.* 6:1093-1103.

Ramayya M.S. (2001) Adrenal organogenesis and steroidogenesis. In: *Adrenal disorders* (ed Humana Press), pp. 11-43.

Rao C.V. (1996) The Beginning of a New Era in Reproductive Biology and Medicine: Expression of Low Levels of Functional Luteinizing Hormone/Human Chorionic Gonadotropin Receptors in Nongondal Tissues. *J.Physiol.Pharmacol.* 47:41-53.

Rebuffat P., Nowak K.W., Tortorella C., Musajo F.G., Gottardo G., Mazzocchi G., & Nussdorfer G.G. (1994) Evidence that endogenous vasoactive intestinal peptide (VIP) plays a role in the maintenance of the growth and steroidogenic capacity of rat adrenal zona glomerulosa. *J.Steroid Biochem.Mol.Biol.* 51:81-88.

Reincke M., Beuschlein F., lawik M., & orm K. (2000) Molecular adrenocortical tumourigenesis. *Eur.J.Clin.Invest.* 30:63-68.

Reincke M., Beuschlein F., Latronico A.C., Arlt W. , Chrousos G.P., & Allolio B. (1997a) Expression of adrenocorticotrophic hormone receptor mRNA in human adrenocortical neoplasms: correlation with P450scc expression. *Clin.Endocrinol. (Oxf)* 46:619-626.

Reincke M., Karl M., Travis W.H., Mastorakos G., Allolio B., Linehan H.M., & Chrousos G.P. (1994) p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. *J.Clin.Endocrinol.Metab.* 78:790-794.

Reincke M., Mora P., Beuschlein F., Arlt W., Chrousos G.P., & Allolio B. (1997b) Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. *J.Clin.Endocrinol. Meta.* 82:3054-3058.

Reiter E., McNamara M., Closset J., & Hennen G. (1995) Expression and functionality of luteinizing hormone/chorionic gonadotropin receptor in the rat prostate. *Endocrinology* 136:917-923.

Renz M., Tomlinson E., Hultgren B., Gu Q., Shimkets R.A., Lewin D.A., & Stewart T.A. (2000) Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH. *J.Biol.Chem.* 275:10429-10436.

Reschini E., Giustina G., Crosignani P.G., & D'Alberton A. (1978) Spontaneous remission of Cushing syndrome after termination of pregnancy. *Obstet.Gynecol.* 51:598-602.

Reznik Y., Allali-Zerah V. , Chayvialle J.A., Leroyer R., Leymarie P., Travert G., Lebrethon M.C., Budi I., Balliere A.M., & Mahoudeau J. (1992) Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. *N.Engl.J.Med.* 327:981-986.

Rilianawati, Paukku T., Kero J., Zhang F.P., Rahman N., Kananen K., & Huhtaniemi I. (1998) Direct luteinizing hormone action triggers adrenocortical tumorigenesis in castrated mice

transgenic for the murine inhibin alpha-subunit promoter/simian virus 40 T-antigen fusion gene. *Mol.Endocrinol.* 12:801-809.

Rohner-Jeanrenaud F. & Jeanrenaud B. (1996) Obesity, leptin, and the brain. *N.Engl.J.Med.* 334:324-325.

Rosenberg C.L., Kim H.G., Shows T.B., Kronenberg H.M., & Arnold A. (1991) Rearrangement and overexpression of D11S287E, a candidate oncogene on chromosome 11q13 in benign parathyroid tumors. *Oncogene* 6:449-453.

Ross N.S. (1994) Epidemiology of Cushing's syndrome and subclinical disease. *Endocrinol.Metab Clin.North Am.* 23:539-546.

Rust W., Stedronsky K., Tillmann G., Morley S., Walther N., & Ivell R. (1998) The role of SF-1/Ad4BP in the control of the bovine gene for the steroidogenic acute regulatory (StAR) protein. *J.Mol.Endocrinol.* 21:189-200.

Salassidis K., Bruch J., Zitzelsberger H., Lengfelder E., Kellerer A.M., & Bauchinger M. (2000) Translocation t(10;14)(q11.2:q22.1) fusing the kinetin to the RET gene creates a novel rearranged form (PTC8) of the RET proto-oncogene in radiation-induced childhood papillary thyroid carcinoma. *Cancer Res.* 60:2786-2789.

Salmenpera M. (1976) Comparison of the ultrastructural and steroidogenic properties of mitochondria of fetal rat adrenals in tissue culture. A morphometric and a gas chromatographic analysis. *J.Ultrastruct.Res.* 56:277-286.

Salmenpera M., Kahri A.I., & Saure A. (1976) Effects of corticosterone on adrenocorticotrophin-induced mitochondrial differentiation with special reference to 11 beta- and 18-hydroxylation. *J.Endocrinol.* 70:215-222.

Sarkar D., Imai T., Kambe F., Shibata A., Sachiko O., Siddiq A., Hayasaka S., Funahashi H., & Seo H. (2001) The Human Homolog of Diminuto/Dwarf1 Gene (hDiminuto): A Novel ACTH-Responsive Gene Overexpressed in Benign Cortisol-Producing Adrenocortical Adenomas. *J.Clin.Endocrinol.Metab.* 86:5130-5137.

Schorr I. & Ney R.L. (1971) Abnormal hormone responses of an adrenocortical cancer adenyl cyclase. *J.Clin.Invest.* 50:1295-1300.

Schorr I., Rathnam P., Saxena B.B., & Ney R.L. (1971) Multiple specific hormone receptors in the adenylate cyclase of an adrenocortical carcinoma. *J.Biol.Chem.* 246:5806-5811.

Schwartz M.W., Seeley R.J. , Campfield L.A., Burn P., & Baskin D.G. (1996) Identification of targets of leptin action in rat hypothalamus. *J.Clin.Invest.* 98:1101-1106.

Segaloff D.L. & Ascoli M. (1993) The lutropin/choriogonadotropin receptor ... 4 years later. *Endocr.Rev.* 14:324-347.

Seron-Ferre M., Lawrence C.C., & Jaffe R.B. (1978) Role of hCG in regulation of the fetal zone of the human fetal adrenal gland. *J.Clin.Endocrinol.Metab.* 46:834-837.

Shanker G. & Sharma R.K. (1980) Characterization of ectopic alpha-adrenergic binding receptors of adrenocortical carcinoma cells. *Endocrinology* 106:1594-1598.

Sheeler L.R. (1994) Cushing's syndrome and pregnancy. *Endocrinol.Metab.Clin.North.Am.* 23:619-627.

Shimon I., Yan X., Magoffin D.A., Friedman T.C., & Melmed S. (1998) Intact leptin receptor is selectively expressed in human fetal pituitary and pituitary adenomas and signals human fetal pituitary growth hormone secretion. *J.Clin.Endocrinol.Metab.* 83:4059-4064.

Siegel E.G. & Creutzfeldt W. (1985) Stimulation of insulin release in isolated rat islets by GIP in physiological concentrations and its relation to islet cyclic AMP content. *Diabetologia* 28:857-861.

Simpson E.R. & Waterman M.R. (1988) Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. *Annu.Rev.Physiol.* 50:427-440.

Slieker L.J., Sloop K.W., Surface P.L., Kriauciunas A., LaQuier F., Manetta J., Bue-Valleskey J., & Stephens T.W. (1996) Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. *J.Biol.Chem.* 271:5301-5304.

Smit A.F. (1999) Interspersed repeats and other mementos of transposable elements in mammalian genomes. *Curr.Opin.Genet.Dev.* 9:657-663.

Smith H.C., Posen S., Clifton-Bligh P., & Casey J. (1978) A testosterone-secreting adrenal cortical adenoma . *Aust.N.Z.J.Med.* 8:171-175.

Spiegel A.M. (1996) Mutations in G proteins and G protein-coupled receptors in endocrine disease. *J.Clin.Endocrinol.Metab.* 81:2434-2442.

Spinedi E. & Gaillard R.C. (1998) A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH. *Endocrinology* 139:4016-4020.

Stocco D.M. & Clark B.J. (1996) Regulation of the acute production of steroids in steroidogenic cells. *Endocr.Rev.* 17:221-244.

Stoffel M., Fernald A.A., Le Beau M.M., & Bell G.I. (1995) Assignment of the gastric inhibitory polypeptide receptor gene (GIPR) to chromosome bands 19q13.2-q13.3 by fluorescence in situ hybridization. *Genomics* 28:607-609.

Stratakis C.A., Carney J.A., Lin J.P., Papanicolaou D.A., Karl M., Kastner D.L., Pras E., & Chrousos G.P. (1996) Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. *J.Clin.Invest.* 97:699-705.

Stratakis C.A. & Kirschner L.S. (1998) Clinical and genetic analysis of primary bilateral adrenal diseases (micro- and macronodular disease) leading to Cushing syndrome. *Horm.Metab.Res.* 30:456-463.

Stratakis C.A., Sarlis N., Kirschner L.S., Carney J.A., Doppman J.L., Nieman L.K., Chrousos G.P., & Papanicolaou D.A. (1999) Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. *Ann.Intern.Med.* 131:585-591.

Straub S.G. & Sharp G.W. (1996) A wortmannin-sensitive signal transduction pathway is involved in the stimulation of insulin release by vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide. *J.Biol.Chem.* 271:1660-1668.

Sugawara T., Kiriakidou M. , McAllister J.M., Holt J.A., Arakane F., & Strauss J.F., III (1997) Regulation of expression of the steroidogenic acute regulatory protein (StAR) gene: a central role for steroidogenic factor 1. *Steroids* 62:5-9.

Svensson A.M., Efendic S., Ostenson C.G., & Jansson L. (1997) Gastric inhibitory polypeptide and splanchnic blood perfusion: augmentation of the islet blood flow increase in hyperglycemic rats. *Peptides* 18:1055-1059.

Takayama K., Morohashi K., Honda S., Hara N., & Omura T. (1994) Contribution of Ad4BP, a steroidogenic cell-specific transcription factor, to regulation of the human CYP11A and bovine CYP11B genes through their distal promoters. *J.Biochem.(Tokyo)* 116:193-203.

Tatsumi K., Miyai K., Notomi T., Kaibe K., Amino N., Mizuno Y., & Kohno H. (1992) Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. *Nat.Genet.* 1:56-58.

Themmen A.P.N. & Huhtaniemi I.T. (2000) Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr.Rev.* 21:551-583.

Tsagarakis S., Tsigos C., Vassiliou V., Tsiotra P. , Pratsinis H., Kletsas D., Trivizas P., Nikou A., Mavromatis T., Sotsiou F., Raptis S., & Thalassinos N. (2001) Food-Dependent Androgen and Cortisol Secretion by a Gastric Inhibitory Polypeptide-Receptor Expressive Adrenocortical Adenoma Leading to Hirsutism and Subclinical Cushing's Syndrome: In Vivo and in Vitro Studies. *J.Clin.Endocrinol.Metab.* 86:583-589.

Tsai-Morris C.H., Geng Y., Buczko E., & Dufau M.L. (1998) A novel human luteinizing hormone receptor gene. *J.Clin.Endocrinol.Metab.* 83:288-291.

Tseng C.C., Boylan M.O., Jarboe L.A., Usdin T.B., & Wolfe M.M. (1996) Chronic desensitization of the glucose-dependent insulinotropic polypeptide receptor in diabetic rats. *Am J.Physiol.* 270:E661-E666.

Turnbull A.V. & Rivier C.L. (1999) Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol.Rev.* 79:1-71.

Usdin T.B., Mezey E., Button D.C., Brownstein M.J., & Bonner T.I. (1993) Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* 133:2861-2870.

Venepally P. & Waterman M.R. (1995) Two Sp1-binding sites mediate cAMP-induced transcription of the bovine CYP11A gene through the protein kinase A signaling pathway. *J.Biol.Chem.* 270:25402-25410.

Verhofstad A.A. & Jonsson G. (1983) Immunohistochemical and neurochemical evidence for the presence of serotonin in the adrenal medulla of the rat. *Neuroscience* 10:1443-1453.

Vettor R., Zarjevski N., Cusin I., & Rohner_Jeanrenaud F. (1994) Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. *Diabetologia* 37:1202-1208.

Vinson G.P. & Ho M.M. (1998) The adrenal renin/angiotensin system in the rat. *Horm.Metab. Res.* 30:355-359.

Volz A., Goke R., Lankat-Buttgereit B., Fehmann H.C., Bode H.P., & Goke B. (1995) Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. *FEBS Lett.* 373:23-29.

Voutilainen R., Ilvesmaki V., Ariel I., Rachmilewitz J., de Groot N., & Hochberg A. (1994) Parallel regulation of parentally imprinted H19 and insulin-like growth factor-II genes in cultured human fetal adrenal cells. *Endocrinology* 134:2051-2056.

Walters W. (1934) The suprarenal cortical syndrome with presentation of ten cases. *Ann.Surg.* 100:670-688.

Watanabe G., Lee R.J., Albanese C., Rainey W.E., Batlle D., & Pestell R.G. (1996) Angiotensin II activation of cyclin D1-dependent kinase activity. *J.Biol.Chem.* 271:22570-22577.

Watanabe G., Pena P., Albanese C., Wilsbacher L.D., Young J.B., & Pestell R.G. (1997) Adrenocorticotropin induction of stress-activated protein kinase in the adrenal cortex in vivo. *J. Biol.Chem.* 272:20063-20069.

Watanabe N., Inoue H., & Fujii-Kuriyama Y. (1994) Regulatory mechanisms of cAMP-dependent and cell-specific expression of human steroidogenic cytochrome P450scc (CYP11A1) gene. *Eur.J.Biochem.* 222:825-834.

Watanobe H. & Tamura T. (1994) Stimulation by peptide histidine methionine (PHM) of adrenocorticotropin secretion in patients with Cushing's disease: a comparison with the effect of vasoactive intestinal peptide (VIP) and a study on the effect of combined administration of corticotropin- releasing hormone with PHM or VIP. *J.Clin.Endocrinol.Metab.* 78:1372-1377.

Weinstein L.S., Shenker A. , Gejman P.V., Merino M.J., Friedman E., & Spiegel A.M. (1991) Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N.Engl.J. Med.* 325:1688-1695.

Wheeler M.B., Gelling R.W. , McIntosh C.H., Georgiou J., Brown J.C., & Pederson R.A. (1995) Functional expression of the rat pancreatic islet glucose-dependent insulinotropic

polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology* 136:4629-4639.

Why L., Carlson H.E., Kane P., Li X., Lei Z.M. & Rao C.V. (2000) Cushing's syndrome secondary to an hCG/LH receptor-positive adrenal carcinoma presenting in the postpartum period. In: *Program & Abstracts. The 82nd Annual Meeting of the Endocrine Society.* Toronto, Ont., Abstract 2040, p 493.

Williams L.T., Gore T.B., & Lefkowitz R.J. (1977) Ectopic beta-adrenergic receptor binding sites. Possible molecular basis of aberrant catecholamine responsiveness of an adrenocortical tumor adenylate cyclase. *J.Clin.Invest.* 59:319-324.

Wilson T.E., Mouw A.R., Weaver C.A., Milbrandt J., & Parker K.L. (1993) The orphan nuclear receptor NGFI-B regulates expression of the gene encoding steroid 21-hydroxylase. *Mol. Cell.Biol.* 13:861-868.

Wolkersdörfer G.W. & Bornstein S.R. (2001) Reappraisal of adrenal zonation theories based on the differential regulation of apoptosis. In: *Adrenal disorders* (ed Humana Press), pp. 45-57.

Yamada Y., Hayami T., Nakamura K., Kaisaki P.J., Someya Y., Wang C.Z., Seino S., & Seino Y. (1995) Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. *Genomics* 29:773-776.

Yanovski J.A. & Cutler G.B., Jr. (1994) Glucocorticoid action and the clinical features of Cushing's syndrome. *Endocrinol.Metab.Clin.North.Am.* 23:487-509.

Yip R.G., Boylan M.O., Kieffer T.J., & Wolfe M.M. (1998) Functional GIP receptors are present on adipocytes. *Endocrinology* 139:4004-4007.

Yip R.G. & Wolfe M.M. (2000) GIP biology and fat metabolism. *Life Sci.* 66:91-103.

Zhang P. & Mellon S.H. (1996) The orphan nuclear receptor steroidogenic factor-1 regulates the cyclic adenosine 3',5'-monophosphate-mediated transcriptional activation of rat cytochrome P450c17 (17 alpha-hydroxylase/c17-20 lyase). *Mol.Endocrinol.* 10:147-158.

Zhang P. & Mellon S.H. (1997) Multiple orphan nuclear receptors converge to regulate rat P450c17 gene transcription: novel mechanisms for orphan nuclear receptor action. *Mol.Endocrinol* 11:891-904.

Zhong Q., Bollag R.J., Dransfield D.T., Gasalla_Herraiz J., Ding K.H., & Isales C.M. (2000) Glucose-dependent insulinotropic peptide signaling pathways in endothelial cells. *Peptides* 21:1427-1432.

Zhu X., Gilbert S., Birnbaumer M., & Birnbaumer L. (1994) Dual signaling potential is common among Gs-coupled receptors and dependent on receptor density. *Mol.Pharmacol.* 46:460-469.

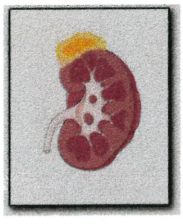
ANNEXE I

ARTICLE 4

Hormone Receptor Abnormalities in Adrenal Cushing's Syndrome

Nina N'Diaye, Johanne Tremblay, Pavel Hamet et André Lacroix

Hormone and Metabolic Research 30 : 440-446, 1998



Hormone Receptor Abnormalities in Adrenal Cushing's Syndrome

N. N'Diaye, J. Tremblay, P. Hamet, A. Lacroix

Division of Endocrinology, and Department of Medicine, Research Center, Campus Hôtel-Dieu, Centre Hospitalier de l'Université de Montréal (CHUM), Montreal, Canada

Cortisol secretion in ACTH-independent primary adrenal Cushing's syndromes was previously believed to be autonomous. In most cases, the pathophysiology of the disease was largely unknown. However, recent work by our group and others have shown that these cortisol-producing adrenocortical tumors may be under the control of inappropriate, illicit or ectopic hormone receptors. This review provides a rapid overview of the physiology of the normal adrenal cortex and outlines recent findings supporting the hypothesis that cortisol production may be regulated by a diversity of abnormal or ectopic hormone receptors in primary adrenal Cushing's syndrome.

Key words: Adrenal Cortex – Cortisol – Hormone Receptor – Ectopic – Cushing's Syndrome

Introduction

Cushing's syndrome is characterized by clinical symptoms and signs resulting from the chronic exposure to excess amounts of glucocorticoids. The pleïotropic effects of glucocorticoids are illustrated by the diversity of the symptoms: progressive central obesity, proximal myopathy, hypertension, glucose intolerance and hyperinsulinemia, dermatologic manifestations (skin atrophy, easy bruisability, abdominal striae, alterations in pigmentation), osteoporosis, hyperlipidemia and neuropsychiatric alterations; excess adrenal androgen production results in hirsutism, acne or oligomenorrhea in women, whereas, in men, adrenal androgens can suppress the pituitary-gonadal axis resulting in decreased libido and impotence (1).

Endogenous Cushing's syndrome is classically divided into two groups: hypercortisolism can be ACTH-dependent, resulting from an excess production of adrenocorticotropin (ACTH) by a pituitary corticotrope adenoma (Cushing's disease), or from an extra-pituitary tumor secreting either proopiomelanocortin and ACTH (ectopic ACTH syndrome) or, rarely, corticotropin releasing hormone (CRH) which stimulates excessive ACTH production from the pituitary (ectopic CRH syndrome). The second group consists of ACTH-independent Cushing's syndrome which results from excess secretion of cortisol by adrenocortical benign and malignant tumors or hyperplasias (1). The incidence of Cushing's syndrome is approximately two to four cases per million per year. ACTH-dependent forms represent approximately 85% of endogenous cases and 75 – 80% of those are secondary to pituitary Cushing's disease. ACTH-independent forms account for 15% of endogenous cases with approximately equal distribution between unilateral cortisol-secreting adenomas or carcinomas. Rare cases of ACTH-independent bilateral adrenal hyperplasia have also been reported and their pathophysiology is diverse. Primary pigmented nodular adrenocortical disease can be familial, associated with other tumors such as myxomas, schwannomas, pigmented cutaneous lesions and peripheral endocrine tumors (Carney's complex) and linked to an unknown gene on chromosome 2 (2). In McCune-Albright syndrome, activating mutations of $G_{s\alpha}$ in adrenal nodules induce constitutive steroidogenesis (3).

The mechanisms by which cortisol is produced in adrenal Cushing's syndrome in the absence of ACTH, its normal regulator, were previously unknown; recent work by our group and other investigators have shown that these previously so-called autonomously cortisol-producing adrenal tumors may actually be under the control of inappropriate, illicit or ectopic hormone receptors. Following a brief overview of the regulation of normal adrenocortical function by their main trophic hormones, the present review will focus on recent findings identifying defects of hormone receptor expression or function in primary adrenal Cushing's syndrome.

Control of cortisol production in the normal adrenal cortex

Under physiological circumstances, various hormones regulate adrenal function by interacting with their specific receptors which are expressed in the adrenal cortex and are functionally coupled to steroidogenesis. ACTH is the main regulator of glucocorticoid production in the zona fasciculata/reticularis of the adrenal cortex; it acts *via* its receptor which was shown to be a member of the G-protein-coupled seven-transmembrane family of cell surface hormone receptors (4). The binding of ACTH to its receptor activates adenylate cyclase (AC) and increases cAMP production. This leads to cAMP-dependent protein kinase (PKA) activation and phosphorylation of proteins

Horm. Metab. Res. 30 (1998) 440 – 446

Received: 8 Dec. 1997 Accepted after revision: 30 Dec. 1997

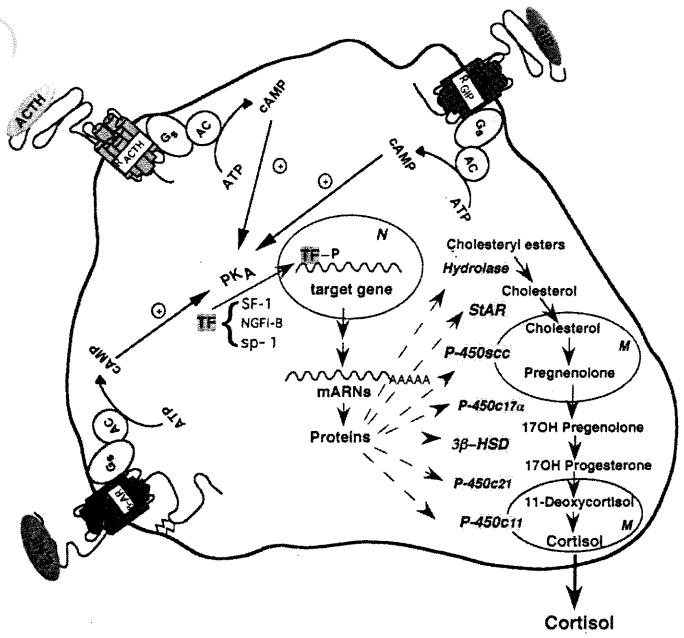


Fig. 1 Adrenal Cushing's syndrome: Regulation of stereogenesis by ectopic hormone receptors. ACTH is the physiological modulator of steroidogenesis in the adrenal cortex. The binding of ACTH to its receptor (R_{ACTH}) activates adenylate cyclase (AC) and leads to cAMP production. This leads to cAMP- dependent protein kinase (PKA) activation and to phosphorylation of specific transcription factors (SF-1; NGFI-B, Sp-1) which regulate free cholesterol availability and steroidogeneic ene expression. ACTH also regulates the early steps of steroid synthesis and the direct activation of P-450 enzymes. The ectopic expression of hormone receptors functionally coupled to steroidogenesis confers to adrenocortical cells inappropriate sensitivity either to GIP (gastric inhibitory polypetide) or to cathecolamines (E, NE). These ectopic or abnormal receptors could regulate steroidogenesis in adrenal Cushing's syndrome by mimicking the cellular events triggered by ACTH receptor activation. N: nucleus; M: mitochondria; TF: transcription factor; E: epinephrine; NE: norepinephrine.

which regulate early and late steps of steroid synthesis (5–6). Steroidogenesis results from the sequential specific enzymatic conversions of cholesterol substrate into steroid hormones (Fig. **1**). Cholesteryl esters are first hydrolyzed to liberate free cholesterol, which will become available for steroidogenesis. The transport of free cholesterol to the inner mitochondrial membrane through interactions with several proteins, including the sterol carrier protein (SCP2) and the steroidogenic acute regulatory (StAR) protein, is the initial rate-limiting step (5). This early step is positively regulated by ACTH, which increases the number of LDL receptors on the cell surface, and the activity of cholesterol esterase. The first steroidogenic enzymatic reaction consists of the removal of six carbons from the lateral chain of cholesterol by the integral inner mitochondrial membrane P450 side chain cleavage (P450 scc) enzyme to generate pregnenolone (6). Chronic effects of ACTH involve increases in gene transcription of most of the steroidogenic enzymes including P450 scc, P450 c17, P450 c21, and P450 c11 (Fig. **1**). The orphan nuclear receptor NGFI-B has been proposed to regulate steroidogenic gene expression (7). Steroidogenic factor 1 (SF-1) is also one of the important transcription factor that mediates the effects of ACTH on steroid enzyme expression (8); SF-1 is activated through its phosporylation by ACTH-stimulated PKA. Moreover, SF-1 was shown to regulate the expression and activity of StAR (9); other transcription factors, including sp-1, Pbx-1, have been implicated in the regulation of steroid hydroxylase genes. ACTH also increases the synthesis of other key proteins for steroidogenesis such as adrenodoxin, many growth factors and their receptors; it stimulates the autocrine production of insulin-like growth factor 1 (IGF-1), transforming growth factor beta (TGFβ) and others which regulate adrenocortical cell proliferation (10). ACTH up-regulates its own receptor leading to an increased sensitivity to the hormone (11).

A negative feed-back loop is established by glucocorticoids by inhibiting hypothalamic CRH, AVP and pituitary ACTH secretion. In primary adrenal disease, hypercortisolism induces a suppression of CRH and ACTH secretion and consequently lowers ACTH to undetectable levels. On the contrary, sustained ACTH overproduction, as it occurs in Cushing's disease or in chronic stress, leads to adrenocortical hypertrophy and high levels of glucocorticoids (1).

Although predominantly regulated by ACTH, adrenocortical steroidogenesis is also under the control of other hormones. Arginine vasopressin (AVP), an important ACTH secretagogue, also stimulates cortisol and aldosterone secretion directly in human adrenal tissues or dispersed cells *in vitro* (12). These actions are mediated by V_1-vasopressin subtype receptors which are normally expressed in the adrenal cortex, while V_3-vasopressin receptors are present in the rat adrenal medulla and elicit cathecolamine secretion; both receptors are coupled to the calcium pathway. AVP itself is also expressed in the normal adrenal gland, suggesting an autocrine/paracrine mechanism of action for the locally synthesized hormone. Cathecolamines have also been shown to stimulate cortisol secretion in bovine and fowl adrenocortical cells *via* β_1-adrenoreceptors (13,14). The physiological relevance of this finding remains to be determined *in vivo*; cathecolamine-driven cortisol production has never been described in normal human adrenals. Serotonin (5-HT) stimulates cortisol production in human adrenocortical tissues *in vitro* by binding to its 5-HT_4 subtype receptor (15). The presence of 5-HT was demonstrated in adrenocortical mast-like cells by immunochemistry suggesting the existence of a paracrine loop; 5-HT-induced steroidogenesis is mediated by calcium and cAMP pathways.

Ectopic adrenal hormone receptor expression: a pathopysiological mechanism in some ACTH-independent Cushing's syndromes

In most cases of primary adrenal Cushing's syndrome, the basic underlying pathophysiology has not been identified. The first indications for a potential role of abnormalities of hormone receptors came from work by *Robert Ney* et al. (16,17). They were studying the role of AC in mediating the effects of ACTH in adrenal steroidogenesis; in normal adrenal cortex, only ACTH was capable of stimulating AC. However, in rat or human adrenal adenomas or cancers, they demonstrated, *in vitro*, that AC was stimulated by hormones other than ACTH such as epinephrine, norepinephrine, TSH, LH and FSH. The hypothesis of ectopic or aberrant adrenal expression of hormone receptors was proposed as a potential explanation of ACTH-independent adrenal hyperfunction. Other *in vitro* studies (18–23) have supported further that several G-protein coupled receptors can be functionally coupled to steroidogenesis in some

Table 1 Ectopic hormone receptors in adrenocortical tumors.

Tissues	Ectopic receptors	References
Rat adrenal carcinoma 494	AC stimulation by adrenalin, TSH, LH, FSH. β-adrenergic receptors	Schoor et al., 1971 (16) Williams et al., 1977 (18)
Human adrenal adenomas and carcinomas	AC stimulation by TSH	Hingshaw and Ney, 1974 (17)
Human adrenal carcinomas	AC stimulation by GH, LH, and prolactin	Millington et al., 1976 (19)
Human cortisol producing adenomas	AC stimulation by NE, E, TSH, and LH	Matsukura et al., 1980 (20)
Human primary nodular hyperplasia	AC stimulation by glucagon	Matsukura et al., 1980 (20)
Human cortisol producing adenomas	β-adrenergic receptors	Hirata et al., 1981 (21)
Human carcinomas	β-adrenergic receptors	Katz et al., 1985 (22)
Human food-dependent cortisol producing adenoma	AC stimulation by vasopressin (probably GIP)	Hamet et al., 1987 (26)
Human virilizing adenoma	LH/hCG receptor *in vivo* and *in vitro* stimulation by hCG	Leionen et al., 1991 (23)
GIP-dependent bilateral macronodular adrenal hyperplasia	Steroidogenesis stimulated by GIP; GIPR present; Steroidogenesis stimulated by GIP	Lacroix et al., 1992 (24); N'Diaye et al., 1997 (32); Reznik et al., 1992 (25); Archambeaud-Mouveroux et al., 1996 (27)
GIP-dependent adrenal adenoma and Cushing's syndrome	Steroidogenesis stimulated by GIP	De Herder et al., 1996 (28)
Cathecolamine-dependent Cushing's syndrome	Steroidogenesis stimulated by β-adrenergic agonists and inhibited by propranolol	Lacroix et al., 1997 (36)

human adrenocortical benign and malignant tumors (Table **1**). The proposed concept of ectopic hormone receptors had been demonstrated *in vitro* only, until it found a clinical demonstration of its significance *in vivo* with the description of two cases of Cushing's syndrome modulated by a gastro-intestinal hormone called gastric inhibitory polypeptyde, or GIP (24,25).

Food-dependent Cushing's syndrome

The first case of food-dependent Cushing's syndrome was described by *Hamet* et al. (26) in a patient with a unilateral adrenal adenoma and low levels of ACTH. It was observed that, curiously, plasma cortisol was low in the morning and increased during the daytime; further observations revealed that the elevations of plasma cortisol followed food ingestion, and that fasting resulted in low cortisol levels. The endogenous modulator of steroidogenesis was not identified precisely in this first case, but the causal link between food ingestion and cyclic hormonogenesis had been established.

The second observation was reported by *Lacroix* et al., who studied a patient with bilateral macronodular adrenal hyperplasia and food-dependent cortisol production (24). The suggestion that the modulator of cortisol production was a gastro-intestinal hormone was supported by the observation that cortisol secretion was stimulated by oral administration of glucose or other nutrients, but not by intravenous glucose; in addition, administration of sandostatin inhibited the increase of plasma cortisol following oral glucose. GIP was a likely candidate modulator, as it is one of the rare gastrointestinal hormones which is stimulated by oral glucose as well as by oral lipids and to a lesser extent by oral proteins; a good correlation was found between plasma GIP and cortisol levels following the ingestion of various meals in this patient. Moreover, the *in vivo* infusion of physiological concentrations of GIP was shown to stimulate cortisol production in the patient, but not in normal controls. The presence of GIP receptors in the adrenal tissues was supported by the adrenal imaging following the injection of [^{123}I]-GIP to the patient *in vivo*. Incubation of the dispersed adrenal cells, *in vitro*, confirmed the GIP-mediated cortisol secretion in the patient's cells, whereas no cortisol response to GIP was found in adult or fetal normal adrenal cells nor in other cortisol or aldosterone secreting adenomas (24). GIP-dependent Cushing's syndrome has been reported in two other patients with bilateral macronodular hyperplasia (25,27) and in another patient with a unilateral adenoma (28); we are aware of four other unpublished cases (personal communications). It was suggested that this disease was secondary to the illicit or ectopic adrenal expression of the GIP receptor. The human GIP receptor cDNA has now been cloned (29–31). Recent molecular analyses indicated that the pathological adrenal tissues of the patients with GIP-dependent Cushing's syndrome highly overexpressed GIP receptors (28,32); a very weak expression was found in the normal human adrenals (32), but was not efficiently coupled to steroidogenesis. A pharmacological blockade of hypercorticism with octreotide which inhibits postprandial release of GIP was attempted as an alternative to surgery in two patients (25,28); during the first months, the therapy led to clinical and biological amelioration, but the long-term treatment proved to be ineffective.

Vasopressin-responsive Cushing's syndrome

Hyperresponsiveness to exogenous arginine- or lysine-vasopressin has recently been described in Cushing's syndrome secondary to unilateral adrenal adenomas or bilateral macronodular hyperplasias (33,34). Our own study also clearly established that exogenous AVP triggered an exagerated cortisol response (34) in a patient with Cushing's syndrome and bilateral macronodular adrenal hyperplasia with an unusual association of orthostatic hypotension. During upright posture and hypotension, cortisol and aldosterone secretion increased despite the suppression of ACTH and renin levels. AVP normally increases during upright posture and even further in orthostatic hypotension, but in our patient, AVP levels remained below the limit of detection until the correction of hypercortisolism. In addition, under dexamethasone suppression, plasma cortisol, aldosterone and androgens were increased by exogenous AVP in this patient, but not in controls. Cells freshly dis-

persed from the adrenal hyperplasia displayed a higher cortisol response to AVP than normal adrenal cells; the cortisol response was mediated by V1-AVP receptors which were shown to be expressed either at normal (35) or increased levels (33) in the pathological adrenal tissues. This new etiology of Cushing's syndrome constitutes an example of increased expression or response of a receptor which is normally expressed in the adrenal (eutopic receptor), but which does not normally mediate a large glucocorticoid synthesis response. It is still not known whether the receptor is mutated or whether the altered response is secondary to a defect of the V1 receptor-effector system. Interestingly, the alteration in the V1-receptor-effector system was not limited to the adrenal tissues in our patient, as it was shown that there was also an abnormal prolonged vascular vasoconstrictive response to AVP compared to arterioles of normal subjects. The persistence of a decreased stimulation of plasma vasopressin and endothelin levels during postural hypotension several months after the correction of hypercorticism also raises the possibility of an exagerated V_1-AVPR signal at the hypothalamic level in this patient.

Catecholamine-dependent Cushing's syndrome

The recent clinical demonstration by our group of the adrenal expression of β-adrenergic receptors (β-ARs) modulating cortisol and aldosterone secretion in a case of Cushing's syndrome (36) is another example of ectopic hormone receptor. The patient presented large bilateral macronodular adrenal hyperplasia and Cushing's syndrome; the ACTH-independent overproduction of cortisol was induced by various situations which increased physiological endogenous levels of catecholamines (upright posture, insulin-induced hypoglycemia, stress test). Plasma cortisol elevation during upright posture was blunted following pretreatment with the β-adrenergic antagonist propranolol. Infusion of isoproterenol, a β-adrenergic agonist, stimulated cortisol and aldosterone secretion in the patient; however, in normal subjects, in which ACTH had been suppressed by dexamethasone, the infusion of isoproterenol was without effect on cortisol secretion. High-affinity binding sites compatible with β_1-AR or β_2-AR were found in the adrenal tissue of the patient but not in controls. They were efficiently coupled to steroidogenesis as shown by the response of AC and cAMP to stimulation with isoproterenol *in vitro* and catecholamine-induced steroidogenesis *in vivo*. Further studies will be needed to properly characterize the receptor subtype which is expressed in the adrenal hyperplastic tissues and to determine whether it is mutated or not. Chronic treatment of the patient with the β-adrenergic antagonist propranolol was efficient in the long-term control of hypercortisolism; this constitutes the first example of a long-term pharmacological blockade of an ectopic adrenal hormone receptor. Interestingly, the control of hypercortisolism was followed by a decreasing requirement in the doses of antagonist; glucocorticoids are known to stimulate β_2-AR transcription (37) *via* glucocorticoid response elements (GRE) located in the promoters of the target genes (38). The normalization of cortisol level may have decreased β-AR density, which would explain the lower requirement for antagonist. The propranolol therapy did not decrease the size of the remaining adrenal; however, the doses of propranolol used were the minimal amounts which maintained normal cortisol production without blocking the receptors completely.

Possible mechanisms for ectopic receptor expression

These studies suggest that ectopic hormone receptors regulate steroidogenesis in adrenal Cushing's syndrome by mimicking the cellular events triggered normally by ACTH receptor activation (Fig. **1**). There has not been any description yet of familial transmission of adrenal ectopic hormone receptors; thus, the abnormalities of receptor expression in these new syndromes probably result from somatic mutations. In patients with bilateral adrenal hyperplasia, the mutational event may have occurred very early during embryogenesis, so that every cortical cell of both adrenals is concerned by the defect. A unilateral lesion may have arisen from clonal expansion of a primary adrenocortical cell abnormality which would have occurred later during life; most of the studies confirm the monoclonal composition of human adrenocortical tumors (39).

A gene rearrangement could potentially lead to adrenocortical-specific inappropriate expression of a hormone receptor gene. Precedents for this mechanism in endocrine tumors include rearrangements described in subsets of parathyroid adenomas (40) and in glucocorticoid-remediable aldosteronism (41). The parathyroid hormone promoter was found to be recombined with the cyclin D gene, giving rise to the prad-1 oncogene (40); the aldosterone synthase gene was shown to be fused with the 11β-hydroxylase promoter resulting in the ectopic production of aldosterone by zona fasciculata. Another mechanism by which altered expression of hormone receptors can be achieved involves transcription factor dysfunction. Indeed, excessive activity or mutations of transcription factors can induce overexpression or lack of gene expression (42). A gain-of-function mutation could affect an activator that specifically regulates gene expression in the adrenal cortex or the loss of a repressor would induce ectopic gene expression.

In case of a very early mutational event, the abnormal expression would affect diverse tissues, so that polymorph aberrant manifestations would be expected. This was the case for our patient with AVP-dependent Cushing's syndrome (35), who also displayed abnormal vascular response to AVP and decreased hypothalamic release of AVP and endothelin during postural hypotension. Our patient with GIP-dependent Cushing's syndrome (24) and bilateral macronodular adrenal hyperplasia suffered from psychiatric dysfunctions, which persisted after correction of hypercorticism; since the GIP receptor has been shown to be expressed in the rat brain (43), it would be of interest to determine whether altered function of the brain GIPR can also be present in such patients.

G protein coupled-receptors and tumorigenesis

What is the role of abnormal hormone receptors in the altered cell growth and tumorogenesis? Stimulation of G-protein-coupled receptors alone, or in association with tyrosine kinase receptors, is known to evoke powerful mitogenic signals *via* G-protein-mediated activation of RAS (44). Thus, altered activity at any step of the transduction signal cascade may predispose to tumor formation. The concept of alterations in G-protein-coupled receptors and/or post-receptor events leading to increased proliferation is now well established (45). Several lines of evidence indicate that cAMP exerts proliferative actions in a subset of cell types such as somatotroph and thyroid cells (46,47). Transgenic mice with thyroid-specific expression of

adenosine A2 receptor (which activates AC via Gs-protein) developed thyroid hyperplasia and severe hyperthyroidism (48). This experiment clearly demonstrated that the *in vivo* constitutive activation of cAMP cascade in thyroid cells is sufficient to stimulate autonomous hyperfunction and uncontrolled cell proliferation. There are many examples of hormone receptor mutations involved in endocrine pathologies: constitutive mutational activation of the TSH receptor results in hyperfunctioning thyroid adenomas and hyperplasias (49,50); familial male precocious puberty (which is characterized by Leydig cell hyperplasia and testosterone production) is due to constitutive activation of LH/hCG receptor (51). At the G-protein level, the mosaic activating mutation of Gsα leads to McCune-Albright syndrome (3); activating mutations of the inhibitory Gαi protein (Gip) have been identified in some, but not all adrenocortical and ovary tumors (52), and overexpression of Gsα was shown in insulinomas and other endocrine tumors (53). These mutated hormone receptors and G-proteins are most definitive examples of oncoproteins.

Our hypothesis is that ectopic or abnormal expression of hormone receptors could initiate or promote increased cell proliferation and tumorigenesis of the adrenal cortex. This mechanism could be valid for other types of endocrine organ proliferative diseases and may include ectopic receptors, hyperresponsiveness of an eutopic receptor-effector system or loss of receptor for inhibitory hormones. An elegant way to explore further this hypothesis would be to construct transgenic mice with adrenocortical-specific expression of eutopic or ectopic receptors. There are examples of transgenic mice with cardiac overexpression of β_2-AR or Gsα who display enhanced cardiac function and develop myocardial fibrosis (54). However, it must be stressed that cAMP is not mitogenic in all cell types. Counter-regulatory mechanisms are initiated in response to persistently elevated cAMP levels. This was the case for transgenic mice expressing gsp in pancreatic β-cells (55), where inhibitors of phosphodiesterases were required to obtain high levels of cAMP and enhanced insulin secretion. In the Y1 mouse adrenocortical cell line transfected with β_2-AR, the ectopic receptors were shown to be efficiently coupled to steroidogenesis, but cell growth was not studied (56).

A recent report suggested that ACTH receptor could act as a tumor suppressor gene in adrenal tumorigenesis (57) like p53, which is involved in many tumor types, including adrenocortical tumors (58). No activating mutations of the ACTH receptor were found in adrenocortical neoplasms (59). However, loss of heterozygosity of the ACTH receptor gene was shown to be associated with high malignancy or absence of secretion function in a subset of human adrenocortical tumors. Furthermore, lower expression of ACTH receptor was found in adrenocortical carcinomas compared to adrenocortical adenomas from patients with Cushing's syndrome (60). ACTH is known to be a differentiating factor with low potential for promotion of cell proliferation as demonstrated by *in vitro* experiments. It is thus reasonable to speculate that a defect in the ACTH receptor signal cascade would result in dedifferentiation and increased cell proliferation. Obviously, much work remains to be done to better understand the mechanisms underlying tumorigenesis of the adrenal cortex.

Conclusion

The recent studies by our group and other investigators now clearly support the hypothesis that corticotropin-independent adrenal hyperplasias or tumors may be secondary to diverse abnormalities of a broad variety of hormone receptors. This may include ectopic hormone receptors such as GIPR, β-AR or any other receptors capable of coupling to G-proteins, adenylate cyclase and steroidogenesis; a similar outcome may result from abnormalities of eutopic receptors such as those for corticotropin, AVP, angiotensin II, serotonin, ANP, or growth factors. The presence of the ectopic or abnormal receptor places the adrenal cells under the stimulation of a trophic factor not under a regulatory negative feed-back by glucocorticoids. This constitutes an unregulated new trophic stimulus, which leads to increased function and possibly to hyperplasia and proliferative advantage. The characterization of the pathophysiology of adrenal hyperplasias or tumors can eventually lead to diverse pharmacological therapies as alternatives to adrenalectomy; this has now been illustrated by the short-term improvement of hypercortisolism with triiodothyronine in a TSH-dependent adrenal cortisol-secreting adenoma (16), with octreotide in GIP-dependent Cushing's syndrome (25) and by the long-term control of ectopic β-AR by propranolol (36). Further studies will probably identify a larger diversity of hormone receptor abnormalities and should eventually elucidate their molecular pathophysiology, which will probably contribute to our understanding of the regulation of tissue-specific expression of genes.

References

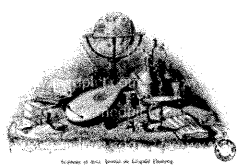
1. Nieman L, Cutler GB Jr. Cushing's syndrome. In: De Groot, editor. Endocrinology. Philadelphia: Saunders, 1995: 1741 – 70
2. Stratakis CA, Carney CA, Lin JP et al. Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. J Clin Invest 1996; 97: 699 – 705
3. Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM. Activating mutations of the stimulatory G protein in the McCune Albright syndrome. N Engl J Med 1991; 325: 1688 – 95
4. Mountjoy KG, Robbins LS, Mortrud MT, Cone RD. The cloning of a family of genes that encode the melancortin receptors. Science 1992; 257: 1248 – 51
5. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 1996; 17: 221 – 44
6. Miller WH. Molecular biology of steroid hormone synthesis. Endocr Rev 1988; 9: 295 – 318
7. Wilson TE, Mouw AR, Weaver CA, Milbrandt J, Parker KL. The orphan nuclear receptor NGFI-B regulates expression of the gene encoding steroid 21-hydroxylase. Mol Cell Biol 1993; 13: 861 – 8
8. Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 1997; 18: 361 – 77
9. Stocco DM, Clark BJ. The role of the steroidogenic acute regulatory protein in steroidogenesis. Steroids 1997; 62: 29 – 36
10. Mesiano S, Jaffe RB. Role of growth factors in the developmental regulation of the human fetal adrenal cortex. Steroids 1997; 62: 62 – 72
11. Penhoat A, Jaillard C, Saez JM. Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. Proc Natl Acad Sci USA 1989; 86: 4978 – 81

12. Perraudin V, Delarue C, Lefevre H, Comtesse V, Kuhn VM, Vaudry H. Vasopressin stimulates cortisol secretion from human adrenocortical tissues through activation of V1 receptors. J Clin Endocrinol Metab 1993; 76: 1522 – 8
13. Walker SW, Lightly ER, Clyne C, Williams BC, Bird IM. Adrenergic and cholinergic regulation of cortisol secretion from the zona fasciculata/reticularis of bovine adrenal cortex. Endocr Res 1991; 17: 237 – 65
14. Mazzocchi G, Gottardo G, Nussdorfer GG. Cathecolamines stimulate steroid secretion of dispersed fowl adrenocortical cells acting through the beta receptor subtype. Horm Metab Res 1997; 29: 190 – 2
15. Lefevre H, Contesse V, Delarue C, Feuilloley M, Hery F, Grise P, Raynaud G, Verhofstad AA, Wolf LM, Vaudry H. Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin 4 receptor subtype. Neuroscience 1992; 47: 999 – 1007
16. Schorr I, Rathman P, Saxena BB, Ney RL. Multiple specific hormone receptors in the adenylate cyclase of an adrenocortical carcinoma. J Biol Chem 1971; 246: 5806 – 11
17. Hingshaw HT, Ney RL. Abnormal control in the neoplastic adrenal cortex. In : MCKerns KW, editor. Hormones and cancer. New York: Academic Press, 1974: 309 – 21
18. Williams LT, Gore TB, Lefkowitz RJ. Ectopic β-adrenergic receptor binding sites. J Clin Invest 1997; 59: 319 – 24
19. Millington DS, Golder MP, Cowley T, London D, Roberts H, Butt WR, Griffiths K. *In vitro* synthesis of steroids by a feminising adrenocortical carcinoma: effect of prolactin and other protein hormones. Acta Endocrinol 1976; 82: 561 – 71
20. Matsukura S, Kakita T, Sueko S, et al. Multiple hormone receptors in the adenylate cyclase of human adrenocortical tumors. Cancer Res 1980; 40: 3768 – 71
21. Hirata Y, Uchihashi M, Sueko S, Matsukura S, Fujita T. Presence of ectopic β-adrenergic receptors on human adrenocortical cortisol producing adenomas. J Clin Endocrinol Metab 1981; 53: 953 – 7
22. Katz MS, Kelly TM, Dax EM, Pineyro MA, Partilla JS, Gregerman RI. Ectopic β-adrenergic receptors coupled to adenylate cyclase in human adrenocortical carcinomas. J Clin Endocrinol Metab 1985; 60: 900 – 9
23. Leinonen P, Ranta T, Siegberg R, Pelkonen R, Heikkila T, Kahri A. Testosterone-secreting virilizing adrenal adenoma with human chorionic gonadotrophin receptors and 21-hydroxylase deficiency. Clin Endocrinol 1991; 34: 31 – 5
24. Lacroix A, Bolté E, Tremblay J, Dupre J, Poitras P, Fournier H, Garon J, Garrel D, Bayard F, Taillefer R, et al. Gastric inhibitory polypeptide-dependent cortisol hypersecretion: a new cause of Cushing's syndrome. N Engl J Med 1992; 327: 974 – 80
25. Reznik Y, Allali-Zerah V, Chayvialle JA, Leroyer R, Leymarie P, Travert G, Lebrethon MC, Budi I, Baillere AM, Mahoudeau J. Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. N Engl J Med 1992; 327: 981 – 6
26. Hamet P, Larochelle P, Franks DJ, Cartier P, Bolté E. Cushings syndrome with food-dependent periodic hormogenesis. Clin Invest Med 1987; 10: 530 – 3
27. Archambeaud-Mouveroux F, Comas JM, Tessier MP, et al. Food-dependent Cushing's syndrome associated with the Carney complex. In: Proceedings of the 10 th Meeting of the international Endocrine Society, San Francisco. Bethesda, Md: Endocrine Society Press, 1996: Abstract, 899
28. De Herder WW, Hofland LJ, Usdin TB,De Jong FH, Uitterlinden P, Van Koetsveld P, Mezey E, Bonner TI, Bonjer HJ, Lamberts SW. Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. J Clin Endocrinol Metab 1996; 81: 3168 – 72
29. Gremlich S, Porret A, Hani EH, Cherif D, Vionnet N, Froguel P, Thorens B. Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes 1995; 44 : 1202 – 8
30. Yamada Y, Hayami T, Nakamura K, Kaisaki PJ, Someya Y, Wang CZ, Seino S, Seino Y. Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. Genomics 1995; 29: 773 – 6
31. Volz A, Goke R, Lankat-buttgereit B, Fehmann HC, Bode HP, Goke B. Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. FEBS Lett 1995; 373: 23 – 9
32. N'Diaye N, Tremblay J, de Herder WW, Hamet P, Lacroix A. Adrenal overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. In: Program and Abstract of the 79 th Annual Meeting of the Endocrine Society, Minneapolis. Bethesda, Md: Endocrine Society Press, 1997: Abstract, 304
33. Perraudin V, Delarue C, De Keyzer Y, Bertagna X, Kuhn JM, Contesse V, Clauser E, Vaudry H. Vasopressin-responsive adrenocortical tumor in a mild Cushing's syndrome: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab 1995; 80: 2661 – 7
34. Horiba N, Suda T, Aiba M, Naruse M, Nomura K, Imamura M, Demura H. Lysine vasopressine stimulation of cortisol secretion in patients with adrenocorticotropin-independent macronodular adrenal hyperplasia. J Clin Endocrinol Metab 1995; 80: 2336 – 41
35. Lacroix A, Tremblay J, Touyz RM, Deng LD, Lariviere R, Cusson JR, Schiffrin EL, Hamet P. Abnormal adrenal and vascular responses to vasopressin mediated by V1-vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia, Cushing's syndrome, and orthostatic hypotension. J Clin Endocrinol Metab 1997; 82: 2414 – 22
36. Lacroix A, Tremblay J, Rousseau G, Bouvier M, Hamet P. Propranolol therapy for ectopic β-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med 1997; 337: 1429 – 34
37. Fève B, Emorine LJ, Biend-Sutren MM, Lasnier F, Strosberg AD. Pairault J. Differential regulation of β1- and β2-adrenergic receptor protein and mRNA levels by glucocorticoids during 3 T3-F442 A adipose differentiation. J Biol Chem 1990; 265: 16343 – 9
38. Malbon CC, Hadock JR. Evidence that glucocorticoid response elements in the 5'-noncoding region of the hamster β2-adrenergic receptor gene are obligate for glucocorticoid regulation of receptor mRNA levels. Biochem Biophys Res Commun 1988; 154: 676 – 81
39. Gicquel C, Lelond-Francillard M, Bertagna X, Louvel A, Chapuis Y, Luton JP, Girard F, Le Bouc Y. Clonal analysis of human adrenocortical carcinomas and secreting adenomas. Clin Endocrinol 1994; 40: 465 – 77
40. Rosenberg CL, Kim HG, Shows TB, Kronenberg HM, Arnold A. Rearrangement and overexpression of D11 S287 E, a candidate oncogene on chromosome 11 q13 in benign parathyroidtumors. Oncogene 1991; 6: 449 – 53
41. Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM. A chimaeric 11 β-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature 1992; 355: 262 – 5
42. Lewin B. Oncogenic conversion by regulatory changes in transcription factors. Cell 1991; 64: 303 – 12
43. Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 1993; 133: 2861 – 70
44. Spiegel AM. The molecular basis of disorders caused by defects in G proteins. Horm Res 1997; 47: 89 – 96

45. Dhanasekaran N, Heasley LE, Johnson GL. G-protein-coupled receptor systems involved in cell growth and oncogenesis. Endocr Rev 1995; 16: 259–70
46. Billestrup N, Swanson LW, Vale W. Growth hormone releasing factor stimulates cell proliferation of somatotrophs *in vitro*. Proc Natl Acad Sci USA 1986; 83: 6854–7
47. Dumont JE, Jaunaux JC, Roger PP. The cyclic AMP mediated stimulation of cell proilferation. Trends Biochem Sci 1989; 14: 67–71
48. Ledent C, Dumont JE, Vassart G, Parmentier M. Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J 1992; 11: 537–42
49. Parma J, Duprey L, Van Sande J, Cochaux P, Gevry C, Mochel J, Dumont J, Vassart G. Somatic mutations in the thyrotropin receptor gene cause hyperfunctionning thyroid adenomas. Nature 1993; 365: 649–51
50. an Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G. Genetic basis of endocrine disease – Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab 1995; 80: 2577–85
51. Shenker A, Lane L, Kosugi S, Meredino Jr JJ, Minegiski T, Cutler Jr GB. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 1993; 369: 652–54
52. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR. Two G protein oncogenes in human endocrine tumors. Science 1990; 249: 655–9
53. Zeiger MA, Norton JA. GS alpha: identification of a gene highly expressed by insulinoma and other endocrine tumors. Surgery 1993; 114: 458–63
54. Drazner MH, Koch WJ, Lefkowitz RJ. Potential of beta-adrenergic signaling by gene transfer. Proc Ass Am Physic 1997; 109: 220–7
55. Ma YH, Tordis C, Tchao N, Wang J, Rodd G, Hanahan D, Bourne HR, Grodsky GM. Constitutively active stimulatory G-protein as in β-cells of transgenic mice causes counter-regulation of the increased 3'-5'-monophosphate and insulin secretion. Endocrinology 1994; 134: 42–7
56. Allen JM, Bartge EE, Abrass IB, Palmiter RD. Isoproterenol response following transfection of the mouse beta-2 adrenergic receptor gene into Y1 cells. EMBO J 1988; 7: 133–8
57. Reincke M, Mora P, Beuschlein F, Arlt W, Chrousos GP, Allolio B. Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis J Clin Endocrinol Metab 1997; 82: 3054–8
58. Reincke M, Karl M, Travis WH, Mastorakos G, Allolio B, Linehan HM, Chrousos GP. P53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. J Clin Endocrinol Metab 1994; 78: 790–4
59. Latronico AC, Reincke M, Mendonca BB, Arai K, Mora P, Allolio B, Wajchenberg BL, Chrousos GP, Tsigos C. No evidence for oncogenic mutations in the adrenocorticotropin receptor gene in human adrenocortical neoplasms. J Clin Endocrinol Metab 1995; 80: 875–7
60. Arnaldi G, Mancini V, De Keyzer Y, Bertagna X, Mantero F. Expression of ACTH receptor in cortisol secreting human adrenocortical tumors. In: Program and Abstract of the 79 th Annual Meeting of the Endocrine Society, Minneapolis. Bethesda, Md: Endocrine Society Press, 1997: Abstract, 312

Requests for reprints should be addressed to:

A. Lacroix, M.D.
Division of Endocrinology
Research Center, Campus Hôtel-Dieu, CHUM
3850 St-Urbain
Montreal, Quebec
Canada
H2W 1T8
E-mail: lacroixa@ere.umontreal.ca



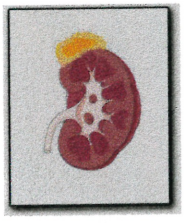
ANNEXE II

ARTICLE 5

Ectopic and Abnormal Hormone Receptor in Adrenal Cushing's Syndrome

André Lacroix, Nina N'Diaye, Johanne Tremblay et Pavel Hamet

Endocrine Review 22 : 75-110, 2001



0163-769X/01/$03.00/0
Endocrine Reviews 22(1): 75–110

Ectopic and Abnormal Hormone Receptors in Adrenal Cushing's Syndrome*

ANDRÉ LACROIX, NINA N'DIAYE, JOHANNE TREMBLAY, AND PAVEL HAMET

Division of Endocrinology, Department of Medicine, Research Center, Hôtel-Dieu du Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada H2W 1T8

ABSTRACT

The mechanism by which cortisol is produced in adrenal Cushing's syndrome, when ACTH is suppressed, was previously unknown and was referred to as being "autonomous." More recently, several investigators have shown that some cortisol and other steroid-producing adrenal tumors or hyperplasias are under the control of ectopic (or aberrant, illicit, inappropriate) membrane hormone receptors. These include ectopic receptors for gastric inhibitory polypeptide (GIP), β-adrenergic agonists, or LH/hCG; a similar outcome can result from altered activity of eutopic receptors, such as those for vasopressin (V1-AVPR), serotonin (5-HT_4), or possibly leptin. The presence of aberrant receptors places adrenal cells under stimulation by a trophic factor not negatively regulated by glucocorticoids, leading to increased steroidogenesis and possibly to the proliferative phenotype. The molecular mechanisms responsible for the abnormal expression and function of membrane hormone receptors are still largely unknown. Identification of the presence of these illicit receptors can eventually lead to new pharmacological therapies as alternatives to adrenalectomy, now demonstrated by the long-term control of ectopic β-AR- and LH/hCGR-dependent Cushing's syndrome by propanolol and leuprolide acetate. Further studies will potentially identify a larger diversity of hormone receptors capable of coupling to G proteins, adenylyl cyclase, and steroidogenesis in functional adrenal tumors and probably in other endocrine and nonendocrine tumors. (*Endocrine Reviews* **22:** 75–110, 2001)

I. Introduction

ENDOGENOUS Cushing's syndrome (CS) is characterized by clinical symptoms and signs resulting from chronic exposure to increased secretion of glucocorticoids (GCs) and other steroids by the adrenal cortex (1–3). Most frequently, endogenous CS is ACTH dependent, arising from excess ACTH production by pituitary corticotrope adenoma (Cushing's disease) or from an extrapituitary tumor secreting POMC and ACTH (ectopic ACTH syndrome); rarely, a CRH-secreting tumor causes excessive ACTH production from the pituitary (ectopic CRH syndrome). Less frequently, CS is ACTH independent, as it results from excess secretion of cortisol by benign and malignant adrenocortical tumors or hyperplasias (1–4). Rare cases of ectopic cortisol production from ovarian tumors that led to ACTH-independent CS have been described (5). Lastly, cortisol hypersensitivity with variable increases in GC receptor numbers has been proposed to explain the clinical features of CS in two patients with low or dysregulated cortisol and ACTH levels and no exposure to exogenous GC (6, 7).

The mechanisms by which cortisol is produced in adrenal CS, when ACTH is suppressed, were previously unknown and referred to as being "autonomous." Studies by several groups have now shown that some of the cortisol-producing

Address reprint requests to: André Lacroix, M.D., Division of Endocrinology, Research Center, Hôtel-Dieu du CHUM, 3840 St. Urbain Street, Montréal, Québec, Canada H2W 1T8. E-mail: andre.lacroix@umontreal.ca

* Supported by a grant from the Medical Research Council of Canada (MA-10339).

adrenal tumors or hyperplasias may actually be under the control of ectopic (or aberrant, illicit, inappropriate) hormone membrane receptors (8–10). After a brief overview of the regulation of normal adrenocortical function by its main trophic hormones and of the etiologies of adrenal CS, the present review will focus on *in vitro* and *in vivo* findings, identifying abnormalities of expression or function of receptors for various hormones in primary adrenal CS. The mechanisms regulating tissue-specific expression of eutopic membrane receptors in the normal adrenal cortex and the potential molecular alterations leading to the ectopic expression of hormone receptors in adrenocortical tumors and hyperplasias will also be discussed. The identification of abnormal membrane hormone receptors in adrenal CS has now opened the field of new therapeutic strategies to control hypercortisolism by interfering with ligand binding to these receptors and will also be presented.

II. Hormonal Regulation of the Normal Adrenal Cortex

The normal regulation of adrenocortical function has been the subject of recent reviews (11, 12) and, hence, will be discussed only briefly here. An important site of regulation of the hypothalamic-pituitary-adrenal axis (HPA) is located in neurons of the medial parvocellular part of the hypothalamic paraventricular nucleus (PVN) where CRH and arginine vasopressin (AVP) are produced and travel along their axons to the median eminence to be released in the hypophyseal portal blood system (13, 14). The binding of CRH or AVP to its respective specific receptors CRH-R1 (15) and AVP V3R (16) on corticotrophs of the anterior lobe stimulates the synthesis and maturation of POMC, leading to ACTH secretion (17). Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP), which are also produced in hypothalamic neurons, enhance CRH and ACTH release (18, 19). CRH secretion can be stimulated in the PVN by α_1-adrenoreceptor agonists, serotonin ($5\text{-}HT_{1A}$) receptor agonists, muscarinic and nicotinic receptor agonists of acetylcholine, histamine, and γ-aminobutyric acid ($GABA_A$), whereas it is inhibited by $GABA_B$ agonists (14). CRH release is also stimulated by angiotensin II (Ang-II), neuropeptide Y (NPY), cholecystokinin (CCK), and gastrin-releasing peptide, or suppressed by atrial natriuretic peptide (ANP), substance P, somatostatin, and nitric oxide (NO) (14). Several cytokines, including interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and IL-6, stimulate CRH, possibly through the production of prostaglandins in brain vascular endothelium (20). ACTH secretion can also be modulated by paracrine/autocrine interactions, as corticotroph cells have been shown to express CRH, which can effectively stimulate ACTH release (21).

ACTH binds to its G protein-coupled membrane melanocortin type 2 receptor (22, 23) to elicit short-term (acute) and long-term (chronic) specific responses, as illustrated in Fig. 1 (24, 25). Activation of the adenylyl cyclase (AC)/cAMP/cAMP-dependent protein kinase (PKA) pathway leads to the phosphorylation of proteins that regulate the early and late steps of steroidogenesis (26, 27). ACTH rapidly (within a few minutes) promotes the mobilization and transfer of free cholesterol to the inner mitochondrial membrane (27). Cloning of the steroidogenic acute regulatory (StAR) protein (28), the subsequent finding of mutations in the StAR gene responsible for the steroid deficiency disease, lipoid adrenal congenital hyperplasia (29, 30), as well as the knockout of this gene in the mouse (31) have identified this ACTH-inducible protein as a key modulator of cholesterol transport into mitochondria. A second protein involved in this process is the peripheral-type benzodiazepine receptor (PBR), which completes the final step of cholesterol delivery to CYP11A1 ($P450_{scc}$) for transformation into pregnenolone (32, 33). ACTH also up-regulates the immediate early genes c-*fos* and c-*jun* via the PKA pathway (25, 34, 35). A positive feedback loop for the long-term effects of ACTH is established by the hormone up-regulating its own receptor (36, 37).

The chronic effects of ACTH require several hours and involve transcriptional and/or posttranscriptional regulation of most genes coding for steroidogenic enzymes, such as CYP11A1, 3β-hydroxysteroid dehydrogenase II (3β-HSD), CYP 17 ($P450_{c17}$), CYP21A2 ($P450_{c21}$), and CYP11B1 ($P450_{c11}$) (24, 26, 38). This long-term regulation is complex, as no clear correlation exists between mRNA and protein levels of steroidogenic enzymes *in vivo* (25).

Many ACTH effects are mediated by specific transcription factors (TFs), including orphan nuclear receptors such as nur77 (also called NGFI-B) (39) or steroidogenic factor 1 (SF-1) (40, 41). Indeed, stressful stimuli induce SF-1 and nur77 transcription in corticotrophs and in the adrenal cortex (39, 42). Nur77 and SF-1 both modulate the expression of steroidogenic enzyme genes in the adrenal cortex, nur77 being activated by dephosphorylation and SF-1 by putative PKA-dependent phosphorylation (41, 43, 44).

As an example, SF-1 is involved in the regulation of CYP YP11A (45–48) and CYP17 (49, 50), where it has been postulated to play a role in constitutive and cAMP-regulated expression. The analysis of the promoter regions of these genes has led to the identification of cAMP-responsive sequences (CRS) and TFs that bind them or synergize cAMP-dependent transcription; general TFs, as cAMP response element (CRE)-binding (CREB) protein and the homeodomain protein Pbx1, both bind CRS and drive cAMP-dependent expression of steroidogenic genes (51–55). Another ubiquitous TF, Sp1, was shown to regulate basal and cAMP-dependent expression of the CYP11A gene (56). Recent data have suggested that SF-1 is able to mediate cAMP-induced transcription of the CYP17 gene: the proximal CRS (CRS2: −80 to −40) has been identified as a SF-1 binding site (57); moreover, a dominant negative mutation preventing SF-1 binding suppresses cAMP-regulated expression of a reporter gene (58). The coactivator CREB-binding protein (CBP/p300) has been proposed to integrate the effects of TFs such as SF-1, Sp1, CREB, and probably Pbx1 for the regulation of CYP11A and CYP17 genes (59, 60). Moreover, nur77 and nurr1 (nur-related factor 1) positively regulate POMC expression in the pituitary (61, 62). SF-1 up-regulates StAR expression and activity (63). Knockout nur77−/− mice demonstrate no remarkable phenotype (64), suggesting that other members of the nur family play redundant roles, perhaps in humans as well. In contrast, SF-1 appears to be essential for the devel-

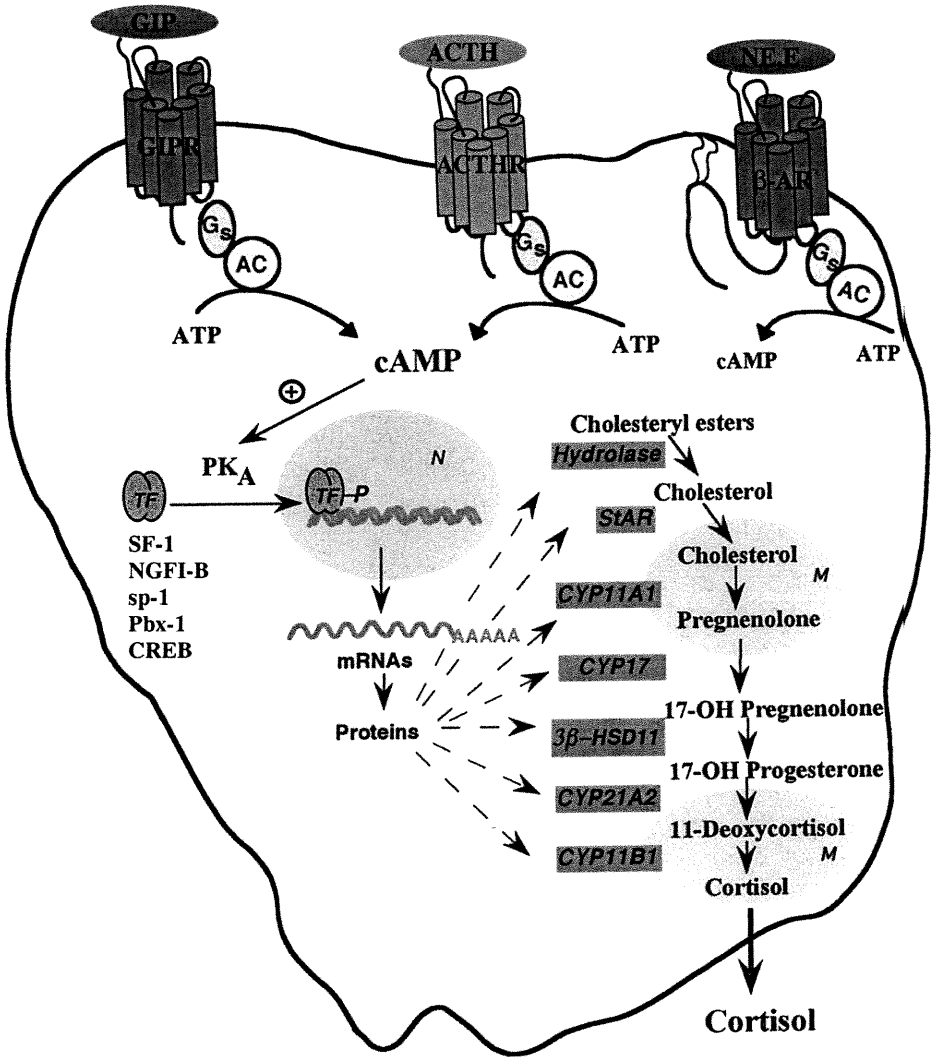


FIG. 1. Regulation of steroidogenesis by ectopic hormone receptors in fasciculata cells of adrenal CS. ACTH is the physiological modulator of steroidogenesis in the adrenal cortex. Binding to its receptor (ACTHR) activates AC and leads to cAMP production with cAMP-dependent protein kinase (PKA) activation and phosphorylation of specific TFs (SF-1, NGFI-B, Sp-1, Pbx-1, CREB) that regulate free cholesterol availability and steroidogenic enzymes expression. ACTH also regulates the early steps of steroid synthesis by the direct activation of CYP enzymes. The ectopic expression of membrane hormone receptors functionally coupled to steroidogenesis confers inappropriate sensitivity to adrenocortical cells either to GIP, to catecholamines (E, NE), or to other hormones (LH/hCG, TSH, etc. . .). These ectopic or abnormal receptors probably regulate steroidogenesis in adrenal CS by mimicking the cellular events triggered by ACTHR activation. N, Nucleus; M, mitochondria; E, epinephrine; NE, norepinephrine. [Modified with permission from N. N'Diaye *et al.*: *Horm Metab Res* 30: 440–446, 1998 (10). © Georg Thieme Verlag.]

opment and survival of steroidogenic organs, as SF-1−/− mice lack adrenal glands and gonads and exhibit male-to-female sex reversal of their genitalia (65, 66).

Increasing evidence indicates that adrenocortical steroidogenesis is modulated not solely by ACTH but also by multiple circulating and local peptide hormones, neuropeptides, neurotransmitters, ions, and cytokines (11, 67–70). Both *in vivo* and *in vitro* studies have clearly demonstrated that AVP stimulates aldosterone and cortisol secretion in bovine adrenals (71, 72); in rat cells, AVP stimulates aldosterone but not corticosterone secretion (73, 74). However, it stimulates aldosterone (250%) and cortisol (60–260%) secretion from normal human adrenals *in vitro* (75–77) via activation of V1-AVP receptors (V1-AVPR) localized mainly in compact cells of the zona reticularis and, to a lesser extent, in the zona glomerulosa (ZG) and fasciculata (68, 74, 78, 79). V2-AVPR were not detected initially in human adrenal cortex tissues (68), but were identified recently by RT-PCR studies (79); their stimulation by DDAVP does not modulate steroidogenesis (79, 80). V3-AVPR (or V1bR) are not detected in the normal human adrenal cortex (79), but are expressed in rat and human chromaffin cells (68, 77, 81), where AVP can stimulate catecholamine release from the adrenal medulla. Thus, AVP could exert significant direct effects on adrenal cortex function, both in endocrine and paracrine modes, but its physiological role has not yet been clearly established. However, in patients with congenital central diabetes insipidus, there is no evidence for clinically significant decreased cortisol secretion (82, 83).

Catecholamines have also been shown to stimulate cortisol and aldosterone secretion *in vitro* in bovine, pig, and fowl via β1-adrenoreceptors (11, 84, 85), but this does not appear to

occur in human adrenocortical cells (86). Serotonin (5-HT) is another neurotransmitter that may play a role in the control of steroidogenesis (87). 5-HT is able to directly trigger cortisol and aldosterone release, as demonstrated *in vitro*, in rat, frog, and human adrenal cells (87–89) but also, indirectly, by stimulating adrenal blood flow (90). The receptor subtype involved in these adrenal effects is still controversial in the rat, but was determined to be 5-HT4 receptor (5-HT_4R) in frogs and humans (88, 89). The 5-HT_4R is positively coupled to the cAMP and calcium pathways. *In vivo*, 5-HT4 agonists such as cisapride or zacopride induce an increase in aldosterone but not in cortisol secretion in humans (91, 92). Possible paracrine control of steroidogenesis by 5-HT can be proposed since its presence has been demonstrated in human perivascular mast cells and in chromaffin cells of the frog, rat, and mouse adrenals (93–95). Central 5-HT is known to enhance ACTH release from the pituitary and to activate the systemic renin-angiotensin system (RAS) to stimulate aldosterone secretion. However, no study has established whether these secretory responses can occur within the adrenal gland *in vivo*.

VIP and PACAP have been shown to play a paracrine role in the secretory activity of the adrenal cortex in the rat, human, and cow, as they are synthesized by adrenomedullary chromaffin cells (18). VIP stimulates aldosterone release from ZG through the activation of selective VIP receptors (VIPR2/VIPR3), whereas it stimulates cortisol secretion moderately through the nonspecific activation of ACTH receptor (ACTHR) (96–98). VIP/PACAP-induced adrenal steroidogenesis can also be enhanced by an indirect mechanism: indeed, both stimulate catecholamine secretion from adrenal chromaffin cells (99, 100), which in turn elicit a β-adrenoreceptor-mediated aldosterone release (101, 102). Moreover, cortisol secretion can be raised by increasing the intraadrenal blood flow as it is stimulated by VIP and PACAP (103, 104).

Ang-II, the biologically active peptide of the RAS, and potassium ion are the major regulators of aldosterone synthesis and secretion (2). A decrease in potassium balance activates the RAS, leading to Ang-II, and then to aldosterone release. Ang-II mediates its effect on steroidogenesis via AT1 receptors (AT1R), which are coupled to phospholipases C and A_2 (PLC, PLA_2). It has been demonstrated that Ang-II inhibits the expression of P450c17 at the transcriptional level in ovine adrenocortical cells (105). Moreover, it augments the expression of StAR protein (106). In the rat, Ang-II enhances the transcription of AT1R and P450 aldo synthase (CYP 11B2) *in vivo* and *in vitro* (107, 108). However, Ang-II seems to inhibit AT1R expression in bovine and human fasciculata cells (109, 110). The presence of a local RAS in the adrenal cortex suggests that Ang-II can regulate aldosterone production in a paracrine fashion (111) (for review see Refs. 112 and 113). Inhibitory signals contribute to maintain aldosterone homeostasis. Dopamine and somatostatin blunt Ang-II-induced aldosterone production (114, 115). The natriuretic peptides ANP and C-type natriuretic peptide (CNP), which are present in the circulation but are also expressed in the adrenal medulla, have been demonstrated to exert an inhibitory action on aldosterone release *in vitro* (116, 117). ANP also inhibits ACTH and Ang-II-induced cortisol production by decreasing the level of StAR expression (118). Other neuropeptides regulate the steroidogenic function of the adrenal cortex by acting both at the central and adrenal levels, as endothelin 1 (ET-1) (119, 120) and NPY (121, 122) enhance cortisol and aldosterone release.

Recent attention has been drawn to leptin as a negative regulator of the HPA axis. Acute injection of leptin in humans (123) and mice (124) counteracts fasting-induced activation of the HPA axis. This effect is proposed to be driven by a direct action of the peptide, both at the hypothalamic and adrenal levels (125). Leptin and its receptor, Ob-R, are expressed in the pituitary (126, 127) and in human, rat, and mouse adrenal glands (128–130). Moreover, the adrenal is embedded in adipose tissue, the physiological source of leptin, which acts at the transcriptional level to prevent the stress-induced stimulation of CRH and CYP17 mRNAs in the hypothalamus and adrenal, respectively (131–133). Other studies have shown opposite effects of leptin on the pituitary where CRH (known to suppress appetite and food intake) and ACTH levels are stimulated, leading to cortisol secretion (134, 135). These discrepancies may arise from anatomic and functional differences in CRH neurons in the PVN where leptin might have inhibitory effects on some and stimulatory effects on other populations of cells. Leptin is induced by GCs (136, 137), resulting in higher plasma levels in CS patients (138, 139).

The integrity of adult adrenal size is maintained by a continuous process of cell division in the ZG and centripetal migration and differentiation into fasciculata cells (140). Chronic stimulation by ACTH induces a phenotypic change of glomerulosa cells into fasciculata cells (141) whereas GCs inhibit this differentiation process namely by reducing P450scc expression (142–144); it was proposed that GCs may play a role in the functional zonation of the adrenal cortex (11). Indeed, high levels of GC (as high as in the inner adrenal cortex owing to centripetal blood flow) were shown to inhibit the 18-hydroxylation step in ACTH-treated cultures of human fetal adrenals, thus decreasing 18-OH-deoxycorticosterone (DOC) and aldosterone levels (11). In contrast to GC, ACTH can lead *in vivo* to hypertrophy and hyperplasia of the adrenal cortex, a process that is reversible. Paradoxically, it seems to harbor inhibitory effects on cell proliferation *in vitro*. A trophic effect is observed after a 2-h exposure to ACTH. This is correlated with a PKA-dependent increase of c-Jun and c-Fos expression (145, 146). After 24 h of stimulation, c-Myc expression is decreased, and inhibition of cell growth is observed (145, 147). Recent data suggest a cAMP-independent proliferation-promoting effect of ACTH (148, 149). Indeed, ACTH was shown to stimulate the mitogen-activated protein (MAP)-kinase pathway *in vivo* and *in vitro*, leading to the accumulation of c-Fos, c-Jun, and c-Myc (147, 150). Ang-II is another peptidic hormone that can also activate the MAP-kinase cascade in adrenal cells in a PKC-dependent mechanism (146, 151). *In vivo*, a chronic stimulation with Ang-II induces ZG hypertrophy. ET-1 also augments cell proliferation in the ZG *in vitro* and *in vivo* by interacting with its ET_A receptor, which is specifically expressed in the ZG (119). Chronic treatment with VIP exerts a moderate hyperplasia of ZG *in vivo* (152, 153). Somatostatin exerts direct antiproliferative effects on the ZG *in vivo* (115). It can also antagonize the mitogenic action of Ang-II. ACTH stimulates the autocrine production of growth factors (GFs)

such as insulin-like growth factor I (IGF-I), IGF-II, and transforming growth factor-β1 (TGF-β1), which regulate the trophic and steroidogenic functions of the adrenal cortex *in vivo* (11, 154). IGF-I and IGF-II have mitogenic effects. IGF-II is more highly expressed in fetal than in adult adrenals (155). In addition, it is highly expressed in hormonally active adrenocortical carcinomas but not in benign tumors, which suggests an important role in tumor acquisition or progression (156, 157). In bovine cells, IGF-I and TGF-β1 exert opposite effects on adrenocortical function by inhibiting the expression of specific adrenal genes; IGF-I enhances the transcription level of ACTH-R, StAR, and specific steroidogenic enzymes, whereas TGF-β1 inhibits it (158). TGF-β1 is thought to play a role in human fetal adrenal remodeling, as it inhibits fetal zone cell proliferation and promotes apoptosis *in vitro* (159, 160). However, this has not been demonstrated *in vivo*.

III. Primary Adrenal Cushing's Syndrome (CS)

The incidence of CS has not been determined with great precision. The increasing frequency of subclinical cortisol-secreting adrenal lesions, identified during the evaluation of adrenal incidentalomas, renders precise estimation of the true incidence even more difficult. The incidence of clinical CS secondary to unilateral adrenal adenoma is approximately two cases per million per year (161); this estimate is close to that of 1.7 per million per year for adrenocortical carcinoma, where clinically significant hormonal secretion occurs in 30–60% of cases, including clinical hypercortisolism, in approximately half of the hormonally active cases (162–164). Since pituitary Cushing's disease is approximately 3-fold more frequent than primary adrenal disease, its incidence would be close to five to six cases per million per year. When clinically detectable ectopic ACTH secretion is also taken into account, the overall incidence of endogenous CS would reach approximately 10 cases per million per year.

Primary adrenal etiologies account for 15–20% of endogenous CS in adults and are secondary to unilateral tumors in 90–98% of cases (1, 2, 163); in contrast, in prepubertal children, primary adrenal causes are responsible for almost 65% of CS. In adults, some case series have suggested that adenomas and carcinomas are equally responsible for adrenal CS, whereas in other series, adenomas were responsible for up to 80% of cases (165, 166). Cortisol-secreting adrenal carcinomas are 3–4 times more frequent than adrenal adenomas in children. For unclear reasons, adrenal tumors are more frequent in females than in males with a ratio of 4:1 for adenomas and 2:1 for carcinomas (161–164).

Less than 10% of ACTH-independent CS can be secondary to bilateral adrenal lesions, and their pathophysiology is diverse. Primary pigmented nodular adrenocortical disease (PPNAD) or micronodular adrenal dysplasia can be familial, associated with other tumors such as myxomas, schwannomas, pigmented cutaneous lesions, and peripheral endocrine tumors (Carney's complex), and linked to unknown genes on chromosome 2 or to mutations of protein kinase A Type 1-α located on chromosome 17 (167–169, 169a). In PPNAD, the overall size of the adrenal gland is usually not enlarged, but is occupied by several small black or brown nodules spread in an otherwise atrophic cortex. High synaptophysin expression in PPNAD nodules suggests a neuroendocrine phenotype of these cells (170). A paradoxical increase in cortisol production is often found in these patients during Liddle's dexamethasone suppression test (171). In McCune-Albright syndrome, activating mutations of $G_{s\alpha}$ occur in some adrenal cells in a mosaic pattern during early embryogenesis and lead to the formation of adrenal nodules, in which constitutive activation of AC and the steroidogenic cascade produce increased cortisol secretion with ACTH suppression; the internodular adrenal cortex, where the $G_{s\alpha}$ mutation is not present, becomes atrophic (172, 173).

ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) is a rare cause of CS, as it is estimated to represent less than 1% of all endogenous cases of this syndrome (1–4). In a review by Lieberman *et al.* (174) in 1994, only 24 published cases had been identified, but several other cases and series have been reported since then (175–178). AIMAH has been described by various terms, including massive macronodular adrenocortical disease (MMAD), autonomous macronodular adrenal hyperplasia (AMAH), ACTH-independent massive bilateral adrenal disease (AIMBAD), and "giant" or "huge" macronodular adrenal disease (175). The clinical syndrome becomes evident during the patient's fifth or sixth decade and has a relatively even gender distribution when compared with Cushing's disease or unilateral adrenal tumors, which are more prevalent in women. Most cases have been sporadic, but a few familial cases have been reported as well (179–182). An activating R201S mutation of $G_{s\alpha}$ was found in the AIMAH tissues of a patient without any other features of McCune-Albright syndrome (183).

IV. Initial *in Vitro* Evidence of Ectopic Adrenal Membrane Hormone Receptors

The concept of ectopic adrenal membrane receptor expression was proposed initially by Robert Ney and his collaborators in 1971 (8, 9, 184). In studying the role of AC in mediating the effects of ACTH in rat adrenal steroidogenesis, only ACTH was capable of stimulating AC in normal cortex membrane preparations; however, in corticosterone-producing rat adrenocortical carcinoma 494, they demonstrated that AC was stimulated by hormones other than ACTH, such as epinephrine, norepinephrine, and TSH (8). Catecholamine effects on AC were induced by β-, but not by α-, adrenergic agonists. Further studies (Table 1) indicated that AC from this tumor was also stimulated by FSH, LH, and slightly by PGE_1) (184), but not by glucagon, insulin, vasopressin, PTH, or calcitonin. Propranolol was able to block the effects of catecholamines but not of other hormones on AC. The illicit hormones exerted no additive or synergistic actions, suggesting that the tumor possessed multiple specific receptors which activated a common AC (Fig. 1). The presence of ectopic and functional β-adrenergic receptors was also confirmed by other groups (185, 186); high-affinity β-adrenergic binding sites and AC stimulation were observed in rat adrenocortical carcinoma 494 membranes, but not in normal

TABLE 1. Initial *in vitro* studies of abnormal hormone receptors in adrenocortical tumors

Tissues	Abnormal receptors	References
Rat adrenal carcinoma 494	AC stimulation by epinephrine (E), norepinephrine (NE), TSH, LH, FSH, PGE_1	(8,184)
	β-AR binding	(185)
	cGMP stimulation by α-adrenergic agonists	(188,189)
Y1 mouse tumor cell line	AC stimulated only by ACTH	(187)
Human cortisol-secreting adrenal adenomas and carcinomas	AC stimulated by TSH	(9)
Human steroid-secreting adrenal carcinoma	AC and steroid stimulation by FSH, LH, GH, human placental lactogen, and PRL; inhibition by insulin	(190)
Human cortisol-producing adenomas	AC stimulation by NE, E, TSH, LH, and Ang-II	(191)
Human primary nodular hyperplasia	AC stimulation by glucagon	(191)
Human adrenal carcinoma	AC not stimulated by any hormone	(191)
Human cortisol-producing adenomas	β-AR binding and stimulation of cortisol secretion	(192)
Human cortisol-producing carcinomas	β-AR binding and AC stimulation; AC stimulation by TSH in one tumor	(193)
Human adenomas and carcinomas	AC not stimulated by any hormone	(194)
Human androgen-secreting adenomas	LH/hCGR binding and stimulation of androgen secretion	(195,196)
Human cortisol-producing adenoma	Type I, IL-1R expression and stimulation of cortisol secretion by IL-1	(198)
Human cortisol-secreting adrenal carcinoma	LH/hCGR by immunohistochemistry and *in situ* hybridization	(197)

adrenal membranes (185). A direct effect on steroidogenesis could not be verified in these initial studies, as AC was not efficiently coupled to steroidogenesis in rat adrenal carcinoma 494 (186). The aberrant response of AC to various hormones is not a universal phenomenon, as the AC of the Y1 mouse adrenocortical tumor cell line was found to be stimulated by ACTH, but not by epinephrine, PTH, insulin, glucagon, TSH, or PGE_1 (187). The presence of ectopic α-adrenergic receptors stimulating guanylate cyclase and cGMP production was also demonstrated in rat adrenal carcinoma 494 (188, 189).

Hingshaw and Ney (9) studied AC activity in three cortisol-secreting adenomas and one androgen-secreting carcinoma removed from patients with CS or virilization. AC stimulation was induced by TSH and ACTH, but not by epinephrine, LH, or glucagon in the androgen-secreting carcinoma; in only one of three adenomas, AC was stimulated slightly only by TSH and ACTH. They concluded that "at present the physiological significance of these aberrant tumor responses is uncertain, and their relationship to tumor function has to remain speculative. However it is possible that, in certain cases, the autonomous behavior of endocrine tumors may be more apparent than real, and that this behavior is the result of stimulation of the tumor by hormones other than the appropriate ones for the parent gland." (9).

Other *in vitro* studies have further supported the functional coupling of several, most frequently G protein-linked, membrane hormone receptors to steroidogenesis in some human adrenocortical benign and malignant tumors (Table 1). Millington *et al.* (190) investigated the effects of various hormones on the secretion of steroids in a human feminizing adenocarcinoma secreting mostly estrogens and androgens, but also some GC. AC activity was stimulated more by PRL, human placental lactogen, LH, and FSH preparations than by ACTH; insulin inhibited AC slightly, while TSH was without effect. In tumor explant culture, estrone and estradiol secretion was stimulated by PRL, insulin, and ACTH, but little by LH or GH. Androstenedione secretion was augmented by LH, GH, PRL, and ACTH. The synthesis of 11-hydroxycorticosteroids was stimulated by LH, GH, and PRL, but very little by ACTH. It must be stressed that hormone preparations available at that time were not pure and that contamination was quite possible. Matsukura *et al.* (191) studied AC activity in human cortisol-secreting adrenal tissues from adenomas, adenocarcinoma, and primary nodular hyperplasia (AIMAH), compared with normal adrenals and bilateral hyperplasias from pituitary Cushing's disease. In normal tissues, only ACTH and PGE_1 stimulated AC activity; in most adenomas, AC activity was increased by norepinephrine, in some by epinephrine, and in a few by TSH, LH, or Ang-II. In a case of AIMAH, AC was stimulated by glucagon and ACTH only. No stimulation of AC was found in adrenal carcinoma tissue. Hirata *et al.* (192) demonstrated the presence of high-affinity β-adrenergic binding sites in two of three cortisol-secreting adenomas, but not in the normal adrenal cortex or in one case of aldosterone-producing adenoma; furthermore, epinephrine stimulated cortisol secretion in cultured tumor cells from one of the patients with an adenoma, and Katz *et al.* (193) studied six human adrenal carcinomas with diversified steroidogenic activities and compared them with the normal adrenal cortex from three individuals; AC was stimulated by β-adrenergic agonists in four of six tumors but not in normal tissues. In one tumor examined for other hormone responses, AC was also stimulated by TSH, but not by glucagon or hCG. In two cases, membranes from metastatic adrenocortical cancer were compared with the primary tumor and had lost stimulation of AC by epinephrine or ACTH. Specific high-affinity β-adrenergic binding sites were detected only in tumors in which AC was stimulated by β-adrenergic agonists. In contrast, Saez *et al.* (194) did not find any AC responsiveness to norepinephrine, glucagon, and TSH in crude adrenal membranes from 11 patients with adenomas and carcinomas.

The aberrant expression of LH/hCG receptors was also previously reported *in vitro* in androgen-secreting adrenal adenomas (195, 196). Testosterone production was stimulated by hCG and ACTH in adrenal adenoma cells in culture, while only ACTH but not hCG was able to stimulate secre-

tion of cortisol, testosterone, and other steroids from the adjacent normal adrenal cortex (195); binding studies performed on cell membranes from hCG-responsive adrenal adenoma demonstrated high-affinity (0.14 nM) binding capacity (198 fmol/g). A preliminary report of the presence of LH/hCG receptor in a cortisol-secreting adrenocortical carcinoma was presented recently (197).

Willenberg *et al.* (198) investigated the adrenal adenoma of a 62-yr-old woman who presented CS with no particular clinical characteristics; striking lymphocytic infiltration of the adenoma was identified at histology. In contrast to normal control human adrenals or other cortisol-secreting adenomas or carcinomas, immunostaining revealed CD45 and CD68-positive macrophage-like cells in this patient's adenoma, and these cells are a major source of IL-1. Type I IL-1 receptor, which is not a seven-transmembrane G-coupled-receptor, was also found to be aberrantly expressed in the adenoma, by *in situ* hybridization and RT-PCR, but not in the normal adrenal cortex or other tumors. In cells dispersed from the adenoma, cortisol secretion was stimulated 2.6-fold by IL-1β, but poorly by ACTH (198); in normal adrenocortical cells or other cortisol-secreting adenomas, cortisol secretion was increased by approximately 1.5-fold during incubation with IL-1β. Since infiltration of mononuclear cells occurs in 15% of adrenal tumors, it will be of interest to further explore the prevalence of abnormal cytokine receptor expression in adrenal hyperplasias and tumors.

V. *In Vivo* Demonstration of the Functionality of Ectopic or Abnormal Membrane Hormone Receptors

The proposed concept of ectopic hormone receptors had been demonstrated *in vitro* only, until it found a clinical manifestation of its significance, *in vivo*, with the description of food-dependent CS (199); this resulted from ectopic adrenal expression of the receptor for a gastrointestinal hormone called gastric inhibitory polypeptide or GIP (200, 201).

A. Food- and GIP-dependent CS

Hamet *et al.* (199) were the first to identify "food-dependent" cortisol production in a 41-yr-old male patient presenting with CS secondary to a unilateral adrenal adenoma and periodic hormonogenesis. Plasma cortisol was consistently low in the morning or during fasting, but increased to abnormal levels after meals; food-induced elevations of plasma cortisol were not suppressed by high oral doses of dexamethasone. AC activity in the resected adrenal adenoma membrane preparation was stimulated 27% by ACTH and 62% by vasopressin, but not by FSH, glucagon, or Ang-II; the effects of various gastrointestinal hormones were not examined in this case. Another female patient with CS secondary to an adrenal adenoma had been previously reported to have "persistent diurnal cortisol secretory rhythm" (202); the low fasting plasma cortisol levels in the morning increased during the day at the presumed, but not indicated, meal times, suggesting that this patient also had food-dependent CS.

Two patients with bilateral AIMAH and food-dependent cortisol production were studied in detail a few years later and allowed to clarify the pathophysiology of this syndrome (200, 201). The first patient, a 48-yr-old French-Canadian woman, presented with typical symptoms of CS, which had become manifest during the previous 2–3 yr (200). Initial investigation revealed low plasma cortisol levels, fasting in the morning, and higher levels during the day, whereas plasma ACTH was always suppressed. The suspicion that cortisol production was regulated by a gastrointestinal hormone came from the observation that plasma cortisol was stimulated by oral administration of glucose or by lipid-rich or protein-rich meals, but not by intravenous glucose. In addition, somatostatin pretreatment inhibited the cortisol-stimulatory effect of oral glucose. A review of the various secretagogues of gastrointestinal hormones indicated that only GIP and the glucagon-like peptides (GLPs) were stimulated significantly by oral glucose and lipids, and to a lesser extent by proteins. Plasma cortisol levels were correlated with plasma GIP concentrations during the various test meals. *In vivo* GIP infusion, to reproduce physiological postprandial concentrations, augmented cortisol production in the patient, but not in four normal controls. In the patient, plasma cortisol was stimulated by the administration of ACTH but not by CRH, glucagon, insulin-induced hypoglycemia, pentagastrin, or AVP. The presence of GIP receptors (GIPRs) in adrenal tissues was supported by adrenal imaging after the injection of [^{123}I]-GIP *in vivo* (200). The incubation of dispersed adrenal cells *in vitro* confirmed GIP-mediated cortisol secretion in the patient's cells, whereas no cortisol response to GIP was found in normal adult or fetal adrenal cells or in other cortisol- or aldosterone-secreting adenomas (200); there was no stimulation of cortisol production in the patient's adrenal cells after *in vitro* incubation with secretin, CCK, VIP, substance P, bombesin, calcitonin gene-related peptide, glucagon, vasopressin, ANP, CRH, TRH, GHRH, neurotensin, or neurokinin A. It was thus concluded that food-dependent cortisol secretion resulted from the abnormal responsiveness of adrenal cells to the physiological secretion of GIP; "illicit" or ectopic GIPR expression on adrenal cells (Figs. 1 and 2) presumably were the basis for this new etiology of CS (200).

The second patient, a 49 yr-old French woman, had been followed for approximately 5 yr for CS and AIMAH (201). Unusual fluctuations of plasma cortisol were noted, and the patient was treated with cortisol biosynthesis inhibitors. After the preliminary report on the first case of GIP-dependent CS (203), the potential food-dependent nature of plasma cortisol secretion was also explored in this patient. Fasting plasma cortisol was low in the morning and increased after mixed meals, oral glucose, lipid-rich meals, and protein-rich meals, but not after intravenous glucose (201). Subcutaneous octreotide administration blocked the oral glucose effect on plasma cortisol. Plasma GIP levels were closely correlated with plasma cortisol levels during these various tests. Intravenous infusion of GIP produced an elevation of plasma cortisol levels in this patient, but not in four normal subjects pretreated with dexamethasone. Here again, plasma cortisol did not rise after *in vivo* administration of lysine-vasopressin (LVP), glucagon, insulin, or pentagastrin, but was stimulated by ACTH. Chronic octreotide administration, up to 100 μg three times daily resulted in a temporary improvement of the clinical syndrome and a return of urinary free cortisol levels

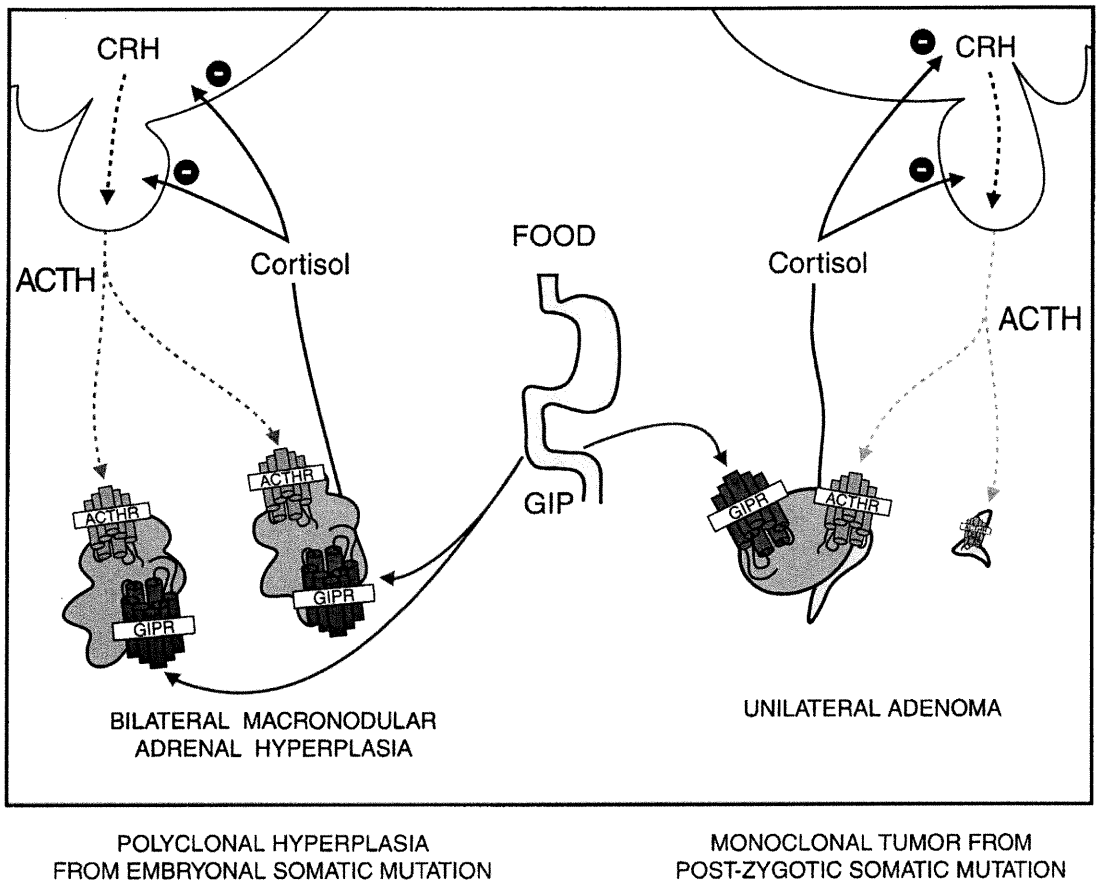


FIG. 2. HPA axis in GIP-dependent CS. The ectopic adrenal expression of the GIPR (in *red*) has been identified both in bilateral macronodular adrenal hyperplasia (*left* of figure) or in unilateral adrenal adenomas (*right side* of figure). After food ingestion, GIP is released in physiological concentrations by K cells from the duodenum and small intestine and binds to the ectopic adrenal GIPR; this results in postprandial supraphysiological increases of plasma cortisol (*full black lines*) which exerts its negative feedback on CRH and ACTH synthesis. In the absence of food ingestion (low plasma GIP levels), the suppressed levels of plasma ACTH (*dashed blue lines*) leads to decreased occupation of the ACTHR (decreased expression) and low fasting levels of plasma cortisol. A somatic postzygotic mutation occurring in a single cell leading to GIPR expression would eventually result in the growth of a GIP-dependent monoclonal unilateral cortisol-secreting adenoma with adjacent and contralateral adrenal cortex atrophy (*right side* of figure). A somatic mutation occurring during early embryonal life and responsible for ectopic GIPR expression in the progenitor cells of the adrenal cortex (polyclonal) would be responsible for the long-term development of nonfamilial GIP-dependent bilateral macronodular adrenal hyperplasia and CS (*left hand side* of figure).

to the upper limit of normal. However, there was an eventual escape from octreotide after 5 months of therapy, requiring bilateral adrenalectomy (204, 205).

Food- or GIP-dependent CS has now been identified in 13 patients with AIMAH (139, 200, 201, 205–208a) and in seven with unilateral adenoma (199, 205, 208a–213), as summarized in Table 2. At pathological examination, no distinctive features were reported, compared with non-GIP-dependent cortisol-secreting adenomas or bilateral macronodular hyperplasia, except in one case (207). This patient was described in a preliminary report to have facial pigmented spots, a blue nevus on one leg, lipofuscin pigments in bilateral adrenal macronodules, and a periadrenal schwannoma suggestive of Carney's complex without any family history; a full description has not yet been published, but *in vitro* studies clearly confirmed GIP-induced stimulation of cortisol secretion by adrenal cells (205). In two cases of AIMAH, the patient initially presented with a unilateral lesion and developed contralateral enlargement only later in time (206, 208a). Except for three patients [the first patient described with food-dependent CS but not proven to be GIP-dependent (199) and two recent ones with AIMAH (GIPR overexpression not yet confirmed)], all other patients are females; adrenal CS is more frequent in females (161), but it remains to be seen whether an even higher female frequency will be found in GIP-dependent CS and what molecular mechanism underlies this sex distribution. Average age at the time of diagnosis may be somewhat greater in patients with AIMAH than in patients with unilateral adrenal adenoma (Table 2) (174, 175); the youngest patient with a unilateral adenoma was only 15 yr old. In GIP-dependent CS, chronic GIP-induced hypercortisolism eventually leads to suppression of CRH and ACTH; this suppression, coupled with low GIP levels in the fasting state, is responsible for the decreased plasma cortisol levels, which can be accompanied by symptoms of relative cortisol insufficiency (201, 209). However, in certain patients (Table 2), fasting plasma cortisol levels were not particularly low, indicating that GIP-dependent CS should not be excluded without performing a test meal (139, 206); this finding could indicate that subpopulations of adrenal cells in the

TABLE 2. Summary of cases of food- and GIP-dependent adrenal Cushing's syndrome[a]

Sex	Age (yr)	Lowest fasting plasma cortisol (nmol/liter)	Cortisol stimulation by GIP		GIP receptor overexpression	Treatment with octreotide	References
			In vivo	*In vitro*			
Bilateral macronodular adrenal hyperplasia							
F	48	138	6.0-fold	8.4-fold	Yes	ND	(200,219)
F	49	124	3.7-fold	1.5-fold	Yes	Partial improvement for 5 months	(201,205)
F	45	149	ND	1.4-fold	Yes	ND	(205,207)
F	33	279	2.5-fold	ND	Yes	ND	(206)
F	60	140	ND	2.1-fold	Yes	Partial improvement for 5 months	(208)
F	43	414	ND	ND	Yes	ND	(208a)
F	40	122	ND	ND	Yes	ND	(208a)
F	57	338	2.4-fold	ND	Yes	ND	(208a)
F	36	556	ND	1.6-fold[b]	ND	ND	(139)
F	35	190	1.6-fold	1.7-fold	Yes	ND	(208a)
F	54	198	ND	ND	Yes	ND	(208a)
M	49	420	ND	ND	ND	ND	(208a)
M	34	140	ND	ND	ND	ND	C. Siame-Mourot and J. P. Cappoen[c]
Unilateral adrenal adenoma							
M	41	132	ND	ND	ND	ND	(199)
F	47	4	7.8-fold	7.5-fold	Yes	Partial improvement during 3.5 months	(209)
F	32	121	ND	10-fold	Yes	ND	(210)
F	43	140	4.5-fold	7.7-fold	Yes	Very transient improvement	(205,212)
F	33	66	ND	2.1-fold	Yes	ND	(211)
F	15	20	ND	15-fold	Yes	ND	(208a)
F	41	114	ND	5.5-fold	Yes	ND	(213)

ND, not done.
[a] In all cases, the food-dependent stimulation of cortisol secretion was clearly demonstrated.
[b] Also stimulated by leptin.
[c] Unpublished observations.

tumor or hyperplasia have lost their GIP dependency and are secreting cortisol under different mechanisms, or that more than one abnormal receptor regulating cortisol production are expressed in these cells. In one patient with food-dependent AIMAH but in whom fasting plasma cortisol was relatively elevated, Pralong *et al.* (139) reported that, in addition to GIP, leptin also aberrantly stimulated cortisol secretion in dispersed adrenal cells; thus, the potential presence of more than one abnormal receptor may modify the phenotypic appearance. The potential presence of ectopic GLP-1 receptors has been excluded to date by the lack of stimulation of cortisol production after GLP-1 administration, either *in vivo* or *in vitro* (139, 206, 210). In one patient with GIP-dependent AIMAH, plasma ACTH and cortisol responses to CRH were still preserved, presumably because the intermittent food-dependent stimulation of cortisol had not yet completely suppressed the HPA axis (208). In a female patient with hirsutism and a unilateral adenoma, both adrenal androgens and cortisol were found to be stimulated by food intake *in vivo* and GIP *in vitro* (213); hypercortisolism was modest and ACTH was not fully suppressed.

The abnormal adrenal regulation of cortisol production by GIP suggested that this aberrant adrenal sensitivity to GIP was secondary either to ectopic expression or activating mutation of GIPR, not normally expressed or functional in adrenal cortical tissues. Cloning of GIPR cDNA from rat (214), hamster (215), and later human (216–218) sources allowed these hypotheses to be investigated.

De Herder *et al.* (209) used *in situ* hybridization to demonstrate abundant GIPR mRNA in adrenal adenoma cells from their patient with GIP-dependent CS; this signal was not present in the adenoma from a patient with non-food-dependent CS, but was not examined in the normal adrenal cortex in this initial study. Using RT-PCR amplification, N'Diaye *et al.* (219) demonstrated pronounced adrenal GIPR overexpression in adrenal adenoma or hyperplastic tissues from GIP-dependent CS compared with the normal human pancreas, normal adult or fetal adrenal cortex, or non-GIP-dependent adrenal CS tissues. A small amount of GIPR mRNA was detected in normal fetal and adult adrenal tissues after at least 35 cycles of amplification and hybridization with the labeled cDNA but was not coupled efficiently to steroidogenesis. Sequence analysis of the full-length cDNA of normal and GIP-dependent adrenal tissues revealed no mutation of GIPR in the affected adrenal tissues (219); similar proportions of isoforms lacking exons 4 and 9 were identified in normal and GIP-dependent adrenals. Chabre *et al.* (210) confirmed the presence of the same overexpressed GIPR isoforms in a GIP-dependent adenoma by RT-PCR and sequencing; no GIPR bands could be detected in the atrophic adrenal cortex adjacent to the tumor or in normal adult adrenals, but only ethidium bromide staining was used. The ACTHR was found to be expressed at a lower level in GIP-dependent adenoma compared with normal tissues (210); this may be secondary to the chronic suppression of endogenous ACTH, which is known to up-regulate ACTHR expression (36, 37). If the relative suppression of ACTHR in GIP-dependent adrenal tissues is confirmed in further stud-

ies, this would indicate that GIP cannot substitute for ACTH in inducing the expression of ACTHR; it must be noted, however, that plasma GIP levels are only elevated transiently postprandially, which is different from conditions where ACTH is elevated chronically. GIPR overexpression was confirmed in other cases (Table 2) of GIP-dependent adrenal macronodular hyperplasias (205, 206, 208, 208a) and adenomas (205, 208a–210, 213) and was not demonstrated in non-GIP-dependent CS adrenal tissues (205, 210, 213, 219) or the human adrenocortical carcinoma cell line H295 (211). GIPR overexpression was detected, even in the early stages of adrenal hyperplasia (206). The small amount of GIPR mRNA sometimes found in normal fetal or adult adrenal tissues after amplification was not efficiently coupled to steroidogenesis (219) and may reflect a low number of GIPR in endothelial cells (214) rather than in adrenocortical cells. Thus, the concept of functional ectopic receptors remains valid in explaining the pathophysiology of GIP-dependent CS (Figs. 1 and 2).

It has been reported that the *in vitro* cortisol-stimulating effects of GIP are coupled to an increase of cAMP, but not of IP_3 production (205, 210). In studying GIP-dependent adrenal cells in primary culture, GIPR down-regulation by its own ligand has been demonstrated, as assessed by the induction of steroidogenic enzyme expression, cortisol secretion, or GIPR mRNA levels by *in situ* hybridization and RT-PCR studies (205, 220). By stimulating steroidogenic enzyme activity, ACTH pretreatment of cells increased the GIP-induced cortisol response but did not appear to modify GIPR expression directly (205).

Stimulation of thymidine incorporation into newly synthesized DNA by GIP was observed in primary cultures of adrenal cells from GIP-dependent CS, but not in normal cells (210). Activation of p42-p44 MAP kinases was observed after treatment of pathological cells with GIP (210). Depending on the cell culture conditions used, ACTH can be shown to inhibit or stimulate markers of cell proliferation in adrenal cells. In the studies by Lebrethon *et al.* (205), under conditions where ACTH inhibited thymidine incorporation in normal and GIP-dependent adrenal cells, GIP was also found to suppress DNA synthesis only in GIP-dependent, and not in normal adrenal cells. Such results suggest that GIP is possibly capable of regulating cell proliferation, in addition to steroidogenesis, in these tissues; however, cell growth stimulation by GIP has not yet been clearly demonstrated.

It should be stressed that food-induced cortisol secretion has been reported in some non-GIP-dependent CS. Bercovici *et al.* (221) described a patient with pituitary Cushing's disease in whom ACTH and cortisol were increased strikingly after mixed meals. ACTH secretion was stimulated by protein-rich meals, but not by oral glucose or lipid-rich meals. Intravenous infusion of amino acids was capable of inducing this response, while octreotide administration did not modify urinary cortisol levels. It was concluded that the pituitary corticotroph adenoma of this patient retained the capacity that normal corticotroph cells have to enhance their release of ACTH after protein ingestion. It has been shown very clearly that, in normal individuals, mixed meals produce an increase in ACTH release and in plasma cortisol levels; this is more evident at lunchtime than after breakfast, when the diurnal peak of ACTH and cortisol may mask the response (222–224). This stimulation is of hypothalamic-pituitary origin and is abolished by dexamethasone administration (225). It is believed that the effect may be secondary to the heightened serotonin production and related to tryptophan content in the meal (224). α-Adrenergic agonists can also increase postprandial stimulation of ACTH (226).

B. Vasopressin-responsive CS

A large proportion of pituitary corticotroph adenomas have been shown to augment their ACTH release after LVP administration, resulting in increased plasma cortisol levels (227, 228). In contrast, in adrenal CS, where ACTH is suppressed, it is expected that plasma cortisol should not increase after LVP administration (229). However, abnormal adrenal stimulation of cortisol secretion in response to exogenous AVP or LVP administration has been described in canine (230) and human ACTH-independent CS, secondary to unilateral adrenal adenomas, carcinomas, or AIMAH (Table 3).

In comparing the response of plasma ACTH and 11-hydroxycorticosteroids to insulin-induced hypoglycemia and LVP infusion in 10 patients with CS of various etiologies, Demura *et al.* (231) noted an unexpected increase in plasma cortisol after LVP in two of two patients with an adrenal adenoma, while ACTH remained suppressed. Makino *et al.* (232) described a 51-yr-old male with AIMAH in whom a combined LVP-CRH test elevated plasma cortisol levels, without any detectable rises in plasma ACTH. Itagaki *et al.* (233) studied a 53-yr-old woman with CS and AIMAH in whom plasma ACTH was undetectable basally and remained so after a metyrapone test or after intramuscular injection of 10 IU LVP; surprisingly, plasma cortisol increased 2.2-fold, and aldosterone increased 3.1-fold, after LVP administration. After bilateral adrenalectomy, dispersed adrenal cells from this patient augmented cortisol production 2-fold when incubated with LVP, while there was no stimulation in cells from another cortisol-secreting adenoma. Since plasma cortisol was not suppressed by the administration of a 1.2-liter water load, the role of endogenous vasopressin in regulating cortisol secretion by the tumor was considered to be uncertain by the authors.

Horiba *et al.* (234) reported two male Japanese patients with bilateral macronodular adrenal hyperplasia and clinical CS in whom im injection of 10 IU LVP increased plasma cortisol 2.3- to 2.6-fold, while plasma ACTH remained undetectable; there were no ACTH or cortisol responses to CRH or dexamethasone. Upon pathological examination, the glands were replaced by macronodules composed of compact and clear cells, but there were some regions of cortical internodular atrophy. In dispersed adrenal cells from both patients, LVP stimulated cortisol secretion (2.8- to 3.2-fold) more efficiently than ACTH. In seven other patients with CS and unilateral adenoma, LVP injection resulted in small increases of plasma cortisol, varying between 9.8 and 25.3%. In four normal subjects pretreated with 2 mg dexamethasone at bedtime and 0.5 mg on the morning of the test, LVP injection elevated plasma cortisol 1.6- to 1.8-fold (up to 45 nmol/liter from basal levels of 20.9 nmol/liter). An exaggerated 2.6-fold

TABLE 3. *In vivo* and *in vitro* studies of abnormal hormone receptors other than GIPR in adrenal tumors or hyperplasia

Tissues	Abnormal receptor	References
Vasopressin-responsive, cortisol-secreting adrenal tumors and AIMAH	Steroidogenesis overstimulated by LVP or AVP; variable eutopic expression of V1 vasopressin receptor	(80,182,229,231,232,234, 235,237–240)
	One familial case of AIMAH responsive to vasopressin	(182)
Vasopressin-responsive preclinical AIMAH	Increased secretion of cortisol after vasopressin administration	(242)
Catecholamine-dependent AIMAH and CS	Steroidogenesis stimulated by β-adrenergic agonists and inhibited by propranolol	(86,240)
LH-dependent CS and AIMAH	Cortisol secretion stimulated by hCG, LH, and GnRH; hypercortisolism normalized by leuprolide acetate	(251)
	Stimulation of cortisol secretion by LH in preclinical AIMAH	(242)
Transient CS during pregnancies; no permanent adrenal lesions	Stimulation of 17-OH-corticosteroids by hCG	(262)
Human virilizing adenoma	LH/hCG receptor binding; *in vivo* and *in vitro* stimulation of androgens by hCG	(195,264,266–268)
Serotonin-responsive CS with AIMAH	Cortisol secretion stimulated by 5-HT_4R agonists	(251,272)
	Cortisol secretion increased by 5-HT_4R agonists in preclinical AIMAH	(242)
Estrogen-stimulated bilateral nodular adrenal hyperplasia	Transient hypercortisolism during three pregnancies; *in vitro* stimulation of cortisol secretion by estrogens	(273)
PPNAD nodules	Paradoxical stimulation of cortisol by dexamethasone; increased GC receptors in nodules	(171,274)

rise in plasma cortisol after 10 IU of LVP was also reported in a patient with a unilateral cortisol-secreting adenoma and mild ACTH-independent CS (235). Intracellular calcium flux in dispersed tumor cells was stimulated by AVP and inhibited by a V1-AVPR antagonist. Using RT-PCR amplification, the V1-AVPR signal was stronger in the cortisol-secreting tumor than in the normal gland; there was a faint V2-AVPR signal in normal and tumoral adrenal tissues, and no V3-AVPR in either.

A 36-yr-old female American patient with CS and AIMAH presented an unusual association with orthostatic hypotension (80). Exogenous AVP, but not desmopressin, triggered large elevations of plasma cortisol (3.4-fold) and aldosterone (67-fold) levels. During upright posture and hypotension, cortisol and aldosterone secretion increased, despite the suppression of ACTH and renin levels. AVP, which normally rises during upright posture and even further in orthostatic hypotension, remained below the limit of assay detection, until the correction of hypercortisolism. Under dexamethasone suppression, plasma cortisol, aldosterone, and androgens were elevated by exogenous AVP in the patient, but not in the controls. Cells freshly dispersed from the diffuse adrenal hyperplasia displayed higher cortisol stimulation (4.2-fold) during incubation with AVP than normal adrenal cells (1.3-fold); the cortisol response was mediated by V1-AVPR, as shown by the effects of V1 antagonists and the lack of effect of V2 agonists. The presence of V1-AVPR was supported by binding studies, intracellular Ca^{2+} flux studies, and RT-PCR amplification of mRNA for all three AVPR. The binding studies revealed a similar V1-AVPR affinity (2.63 nM) in AIMAH adrenal cells, compared with membranes from human glomerulosa-rich normal adrenal cells or myometrium (236). The ED_{50} of AVP on $[Ca^{2+}]_i$ was similar in the adrenal cells of the patient (0.9 nM) compared with glomerulosa-rich cells (1.4 nM) from normal adrenals (76). Interestingly, CRH administration stimulated cortisol *in vivo* but not *in vitro* without any stimulation of ACTH; it is possible that CRH increased the adrenal production of vasopressin (68) and cortisol in a paracrine manner. Alteration of the V1-receptor-effector system was not limited to the adrenal tissues of this patient, as there was also an abnormal, prolonged vascular vasoconstrictive response to AVP, compared with the arterioles of normal or hypertensive subjects. The persistence of decreased stimulation of plasma vasopressin and endothelin levels during postural hypotension, several months after correction of the hypercortisolism, also raised the possibility of an exaggerated V1-AVPR signal at the hypothalamic level in this patient. The causal relationship between abnormal V1-AVPR-mediated-responses and postural hypotension remains uncertain (80). Another male Japanese patient with AIMAH and CS was found to have a 1.8-fold increase in plasma cortisol after LVP injection (237); food intake, GIP infusion, octreotide, and CRH were without effects. Removal of the large bilateral macronodular adrenals showed no areas of internodular atrophy; LVP stimulated cortisol production in cells freshly dispersed from a macronodule. Stimulation of plasma cortisol by administration of 0.2 IU AVP was noted in a Japanese man with AIMAH and coincident multiple adenomatous polyps and colon cancer (238); a point mutation of the *APC* gene was revealed in the colon cancer but not in the adrenal nodules.

In a retrospective study of 26 patients with CS secondary to unilateral cortisol-secreting tumors, Arnaldi *et al.* (79) observed an increase of plasma cortisol greater than 30 ng/ml after LVP testing in 27% of cases (five adenomas and two carcinomas). Quantitative RT-PCR assay of V1-AVPR showed that the levels of message were similar in 20 cortisol-secreting adenomas, compared with three normal adult adrenals; the levels were lower in 19 adrenocortical carcinomas, but there was a large overlap with adrenal adenomas. The normal adrenal glands and the majority of tumors also expressed low amounts of V2-AVPR, but no V3-AVPR. Only six of the patients for whom adrenal tumor material was available had undergone LVP testing; responders had somewhat higher V1-AVPR concentrations in their tumors than nonresponders, but the levels were not higher than in normal

adrenal tissues. In one patient with an *in vivo* cortisol response (~1.6-fold) to LVP, the AVP-induced cortisol secretion (2-fold) of perifused adrenal cells was inhibited by V1-AVPR antagonists.

The demonstration of an exaggerated cortisol response to pharmacological levels of exogenous vasopressin does not constitute direct evidence that fluctuations of endogenous AVP levels are the main regulator of steroidogenesis in these patients. This was illustrated in a male patient with AIMAH who was shown to have increased plasma cortisol in response to upright posture and administration of 10 IU AVP (86); however, the modulation of endogenous AVP levels by water dilution or hypertonic saline infusion did not modify plasma cortisol levels. In addition, *in vivo* administration of a V1-AVPR antagonist inhibited the response of cortisol to exogenous AVP, but not to upright posture. In fact, this patient was found to have ectopic β-adrenergic receptors (see *Section V.C.*) in his adrenal tissues; it is believed that pharmacological AVP levels stimulated catecholamine release, including from the adrenal medulla (68), and then mediated cortisol release in this case. Further support comes from the fact that there was no evidence of V1-AVPR in his adrenal tissues (N. N'Diaye and A. Lacroix, unpublished observation).

Daidoh *et al.* (239) studied a 49-yr-old man with very large bilateral AIMAH and severe CS; intravenous injection of small amounts of AVP (0.3 IU) increased plasma cortisol 3.7-fold without any detectable rise in ACTH. Similarly, insulin-induced hypoglycemia elevated plasma AVP and cortisol without any increase in plasma ACTH; catecholamine effects were not studied however. Upright posture augmented plasma AVP and cortisol. Oral administration of the V1-AVPR antagonist OPC-21268 for 8 days decreased urinary free cortisol levels, but potential spontaneous fluctuations of cortisol secretion were not evaluated for long periods. It was further shown, in dispersed adrenal cells, that AVP stimulated cortisol secretion in AIMAH cells but not in normal control cells, and that this effect was inhibited by OPC-21268; GIP was without effects on AIMAH cells, but catecholamine and insulin were not tested directly. We recently studied a 50-yr-old American woman with CS and AIMAH in whom plasma cortisol was stimulated by upright posture (1.7-fold) and exogenous AVP (3.4-fold), but not by dDVAP (240). In this patient, we were able to demonstrate that plasma cortisol was inhibited by water loading (24% decrease), and elevated during hypertonic saline infusion (1.7-fold). This patient was also found to have abnormal responses to β-adrenergic receptor agonists (see *Section V.C.*), in addition to the abnormal V1-AVPR response in her adrenals. These last two cases represent the first demonstrations of fluctuations in plasma cortisol levels in parallel with small physiological changes in endogenous vasopressin levels. All the previously reported cases of cortisol stimulation by lysine- (231–235, 237) or arginine-vasopressin (80) were related to exogenous pharmacological amounts. In these last two patients, as in another patient (80), plasma vasopressin was found to be suppressed to undetectable levels basally and showed only a very modest increase upon potent physiological stimulation. This may be due to the suppressive effects of hypercortisolism on vasopressin gene expression (241). It has also been postulated that abnormal V1-AVPR may modify vasopressin production via a short loop regulation mechanism in hypothalamic nuclei (80).

An abnormal increase of plasma cortisol in response to vasopressin administration was also noted in patients with preclinical bilateral macronodular adrenal hyperplasia (242). Recently, an exaggerated plasma cortisol response to LVP was seen in a 67-yr-old woman with CS and bilateral macronodular adrenal hyperplasia, whose brother had died after bilateral adrenalectomy for CS and AIMAH (182); the precise nature of the abnormal hormone receptor implicated is unknown, but this constitutes the first demonstration of abnormal hormone responsiveness in familial AIMAH.

Since V1-AVPR are present in the normal adrenal cortex and modulate modest effects of vasopressin on steroidogenesis, the exaggerated steroidogenic responses to vasopressin in these patients would be secondary to the abnormal function of an "eutopic" receptor-effector system, rather than to the presence of an ectopic receptor. V1-AVPR mRNA levels were found to be expressed either at higher (235) or similar (79, 80) levels, compared with normal control adrenal tissues. The binding affinity and dose response of intracellular calcium flux for V1-AVPR noted in the adrenal tissues of a patient with AIMAH (80) were not different from those reported in other normal tissues. Thus, no evidence of ectopic receptor or gross overexpression of the eutopic V1-AVPR has been presented to date; the molecular mechanisms leading to the abnormal response of V1-AVPR or its effector system, which would increase the response to AVP, remain to be elucidated.

Recently, V3-AVPR were shown to be expressed ectopically in a series of bronchial carcinoids secreting ACTH (243). A large proportion of patients with Cushing's disease, but not normal individuals, secrete ACTH in response to DDAVP (244, 245). V3-AVPR were found to be overexpressed in corticotroph adenomas (229); as DDAVP can also bind in part to V3-AVPR, this may explain the effects of DDAVP on ACTH release in Cushing's disease. Thus, stimulation of cortisol levels after vasopressin administration in CS cannot directly distinguish between pituitary corticotroph adenoma, ACTH-independent primary adrenal tumor or hyperplasia, or relatively well differentiated carcinoid tumors producing ACTH.

C. Catecholamine-dependent CS

Catecholamines are known to modulate HPA activity. Activation of α_1-adrenoreceptors in the PVN leads to CRH release with increased plasma levels of ACTH and cortisol (14). Administration of β_1- or β_2-adrenergic agonists or antagonists has no effect on ACTH or cortisol secretion (246). Peripherally administered α_1-adrenoreceptor agonists fail to activate the HPA, as the blood-brain barrier prevents their access to the PVN. Direct adrenal stimulatory or inhibitory effects of catecholamines on GC or mineralocorticoid secretion have been noted in several species, but are limited to aldosterone secretion in humans, where cortisol secretion is unaffected (11).

As discussed in *Section IV*, the abnormal presence of β-adrenergic receptors or the activation of AC activity by cat-

echolamines has been reported *in vitro* in several cases of human adrenal tumors associated with CS (191–193); no evidence of such receptors has been found in the normal adrenal cortex. However, the clinical expression of this abnormality was appreciated only recently in two patients. A 56-yr-old French-Canadian man with AIMAH and CS (86) was shown to have ACTH-independent overproduction of cortisol and aldosterone during elevations of endogenous catecholamines level (upright posture, insulin-induced hypoglycemia, and EKG stress test). Augmented plasma cortisol during upright posture was decreased after pretreatment with the β-adrenergic antagonist, propranolol; in contrast, this did not occur after inhibition of the RAS system with captopril or losartan, or of AVP with a V1-AVPR antagonist. Isoproterenol infusion stimulated cortisol (2.1-fold) and aldosterone (2.2-fold) secretion in the patient, but not in normal subjects, in whom ACTH had been suppressed by dexamethasone. Plasma cortisol was not influenced by mixed meals, or administration of TRH, GnRH, glucagon, or cisapride; as discussed previously, a late increase of cortisol after AVP administration was believed to result from stimulation of release of adrenomedullary catecholamines. High-affinity binding sites compatible with β_1-adrenergic receptor (β_1-AR) or β_2-AR were found in the adrenal tissues of the patient, but not in the controls. They were efficiently coupled to steroidogenesis (Fig. 1), as shown by AC stimulation with isoproterenol *in vitro* and catecholamine-induced steroidogenesis *in vivo* (86). Further molecular studies are needed to properly characterize the β-adrenergic receptor subtype expressed in hyperplastic adrenal tissues and to determine whether or not it is mutated.

Another 50-yr-old American woman with CS and AIMAH (240) was found to have abnormal responses to catecholamines in addition to an exaggerated response to AVP (described previously in *Section V.B.*). In this patient, plasma cortisol had risen after upright posture (1.7-fold) and exogenous AVP (3.4-fold), but also after insulin-induced hypoglycemia (2.7-fold), while ACTH remained suppressed. Infusion of isoproterenol for 30 min increased plasma cortisol from 323 to 630 nmol/liter, which returned rapidly to baseline when the infusion was discontinued. Pretreatment of the patient with the angiotensin receptor type-1 antagonist losartan did not prevent the elevation of plasma cortisol during upright posture. There were no increases of plasma cortisol after mixed meals, GnRH, TRH, glucagon, or cisapride. It was concluded that cortisol secretion was mediated by the abnormal presence and function of β-adrenergic and V1-AVPR, and medical therapy with the β-blocker propranolol was proposed to the patient; she did not tolerate this medication well and elected to undergo surgery in her home city (tissues not available).

D. LH-dependent CS

The LH/hCG receptor (LH/hCGR) normally activates AC and PLC to stimulate gonadal steroidogenesis (247). The receptor is mainly expressed in gonadal tissues, but also in other tissues, including the uterus, fallopian tubes, placenta, brain, hypothalamus, and prostate (248); recently, the presence of LH/hCGR was identified in the zona reticularis of the human adrenal (249) by immunohistochemistry and *in situ* hybridization. hCG stimulates DHEAS secretion in human fetal adrenal cells (250).

A 63-yr-old French-Canadian woman was studied for CS and AIMAH (251). Retrospectively, she described having gained between 18–22 kg during each of four full-term pregnancies, with Cushingoid fat distribution, but without high blood pressure, purple skin striae, or hirsutism. Her weight returned rapidly to baseline after delivery with symptoms of lack of appetite, nausea, and fatigue, which subsided within 2–3 months. Chronic hypercortisolism became clinically manifest only 10 yr after menopause (Fig. 3). Cortisol production was increased by the *in vivo* administration of GnRH, hCG, and recombinant human LH (hLH). Plasma free testosterone and estradiol were also augmented by hLH administration. Abnormal stimulation of cortisol, free testosterone, and DHEAS production was also evoked in this patient by oral intake of cisapride and metoclopramide, two 5-HT_4R agonists (251). Administration of the long-acting GnRH analog leuprolide acetate initially increased LH and FSH secretion, which was paralleled by a rise in cortisol secretion; however, this was followed within 10 days by suppression of endogenous LH and FSH levels and normalization of cortisol production. Stimulation of cortisol by hCG and recombinant hLH, but not by FSH, suggests that a functional adrenocortical LH/hCGR was coupled to steroidogenesis (Fig. 3); the lack of stimulation by GnRH, when LH levels were suppressed by chronic administration of leuprolide acetate, excludes an adrenal GnRH receptor. Studies of normal adult controls did not indicate any coupling of LH/hCGR to adrenal synthesis of cortisol or DHEAS. Abnormal stimulation of plasma cortisol after GnRH and LH administration was also found in one woman with bilateral macronodular adrenal hyperplasia and normal urinary cortisol levels, which did not suppress normally with dexamethasone (242). This suggests that diverse ectopic hormone receptors can be present in preclinical bilateral macronodular adrenal hyperplasia.

Pregnancy is relatively rare in women with CS, as only about 100 cases have been summarized in recent reviews (252–254). GC and androgen excess induce suppression of the pituitary-gonadal axis, causing oligomenorrhea or amenorrhea in 75% of woman of reproductive age affected by CS (1–3). In women in whom CS was associated with pregnancy (252), the etiology was more often secondary to an adrenal adenoma (44%) or carcinoma (17%) than to pituitary corticotroph adenoma (29%) or ectopic ACTH secretion (4%). Hypercortisolism is often responsible for high rates of abortion, premature labor, stillbirths, and perinatal deaths (252). In some cases of pregnancy and CS secondary to adrenal adenomas (255–257) or large bilateral macronodular adrenal hyperplasia (258), the clinical syndrome regressed after abortion or delivery. The syndrome was identified in a few cases only after exacerbation of the hypercortisolism during a subsequent pregnancy (259). When these patients were tested after delivery, biochemical evidence of residual abnormal cortisol secretion was still present, despite substantial improvement, and was fully corrected only after surgical removal of the adenoma. In the case of a woman with a large AIMAH reported by Calodney *et al.* (258), clinical CS oc-

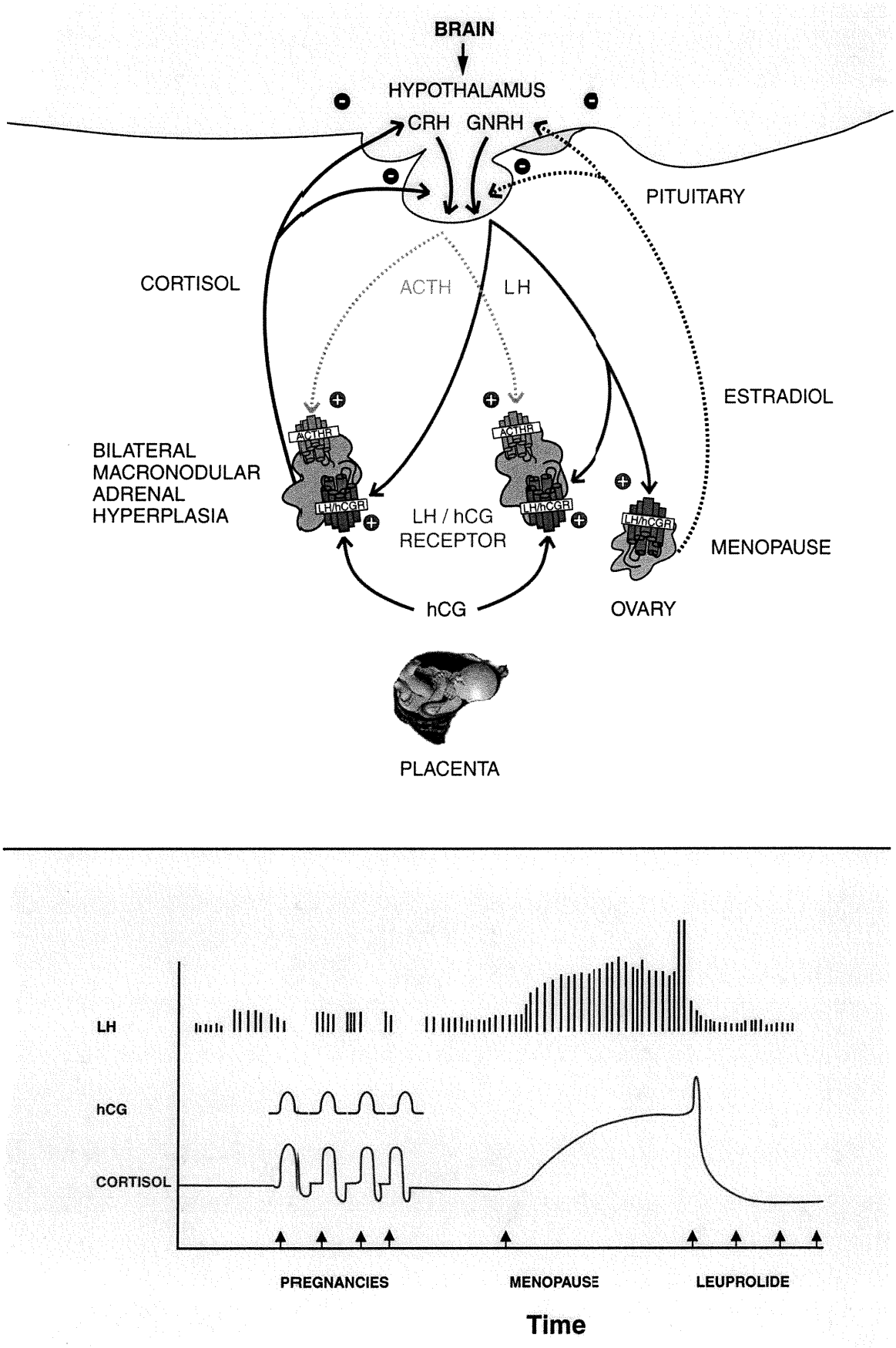


FIG. 3. HPA axis in CS secondary to the ectopic adrenal expression of LH/hCGR. Adrenal expression of the LH/hCGR in the adrenal cortex is illustrated. Occupation of this receptor either by hCG of placental origin or by LH of pituitary origin induces cortisol secretion, which exerts negative feedback inhibition on CRH and ACTH production (*upper panel*). The development of bilateral adrenal hyperplasia and hypercor-

curred only during the sixth pregnancy; the hypercortisolism was not suppressible with dexamethasone, but rather displayed paradoxical increases in urinary steroid secretion. Basal urinary 17-ketogenic and 17-ketosteroids returned to normal basal levels during the next 2 yr with the disappearance of clinical CS; however, the diurnal rhythm of plasma cortisol remained abnormal, as was dexamethasone suppressibility, leading to bilateral adrenalectomy. There was no evidence of pituitary CS in this patient, but ACTH assays were not available, and no metyrapone test was performed.

Transient corticotropin-independent CS during pregnancy with complete resolution after spontaneous abortion or delivery was described in two patients with mild bilateral adrenal hyperplasia (260, 261) or unknown adrenal pathology (262). A paradoxical increase in cortisol excretion during dexamethasone administration in pregnancy completely returned to normal after delivery (261). One patient developed severe biochemical and clinical evidence of CS during each of three pregnancies, and ACTH or cortisol levels were not stimulated by vasopressin administration (260); there was a transient period of hypocortisolism after each delivery, followed by complete clinical regression. In one case, short-term hCG administration elevated urinary 17-hydroxycorticosteroid levels, while sequential estrogen and progestogen administration had no effect (262).

It is thus possible that some of these patients with transient CS during pregnancy, or in whom hypercortisolism increased during pregnancy, also expressed ectopic LH receptors in their adrenal adenomas or in their adrenal cortex. Specific testing of the regulation of steroidogenesis with LH or estrogens in future cases of transient CS during pregnancy will help in elucidating the pathophysiology. It must be pointed out that spontaneous remission of CS after delivery has also been reported in a patient with ACTH-dependent CS of probable pituitary origin (263); the mechanisms involved in this regression have not been elucidated.

E. LH-dependent adrenal androgen-secreting tumors

Although the regulation of cortisol secretion by LH in adrenal CS was demonstrated only recently *in vivo*, there have been several reports indicating that the regulation of steroidogenesis in some rare, pure, androgen-secreting tumors was stimulated by hCG or GnRH (195, 264, 265). As plasma LH was found to be relatively suppressed in some of these patients, the role of endogenous LH in maintaining androgen production may be uncertain. In some cases, suppression of endogenous LH levels by administration of estrogens (266, 267) or by ACTH stimulation of GC (268) inhibited androgen production. In other cases, estrogens were unsuccessful in depressing androgen production (264, 269, 270). It has been suggested that gonadal cells localized in the adrenals could explain this phenomenon; however, clear evidence of adrenal origin of the tumors was identified in certain cases (195).

F. Serotonin-responsive CS

5-HT is produced by intraadrenal mast cells in humans and can regulate corticosteroid production via a paracrine mechanism (87, 271); these effects are mediated by the 5-HT_4R subtype, which is expressed mainly in adrenal ZG but also in zona fasciculata cells (89, 91). 5-HT_4R agonists are potent stimulators of aldosterone secretion in humans; they are weak stimulators of cortisol secretion by human adrenocortical cells *in vitro*, but not of plasma cortisol in normal subjects (87).

In the patient with LH-dependent CS (251), cisapride and metoclopramide, two 5-HT_4R agonists, produced 4.8- and 2.6-fold peak elevations, respectively, in plasma cortisol 120 min after their oral administration. Plasma corticotropin levels remained undetectable during cisapride and metoclopramide testing. Stimulation of plasma cortisol in this patient after treatment with cisapride and metoclopramide was proportional to their respective affinity for 5-HT_4R (87); no such response to cisapride was found in five other patients with bilateral adrenal hyperplasia, 11 with unilateral adenoma, and one with carcinoma and CS (240). A patient with CS and AIMAH was found to increase plasma cortisol in response to cisapride as well as to LVP and CRH, despite suppression of ACTH (272). Recent observations in patients with bilateral macronodular adrenal hyperplasia and preclinical hypercortisolism also documented marked stimulation of cortisol secretion upon cisapride administration (242).

The exaggerated cortisol responses to cisapride in these patients could be secondary to the increased zona fasciculata expression or abnormal function of an "eutopic" 5-HT_4R-effector system, rather than to the presence of an ectopic receptor. The presence of a 5-HT_4R has been detected by RT-PCR in the adrenal tissues of one of these patients and was similar to that found in normal adrenal cortex; however, full receptor sequencing and adrenal zone distribution have not been performed (272).

G. Steroid-responsive CS

Caticha *et al.* (273) described a 33-yr-old woman who developed transient and reversible clinical and biochemical signs of ACTH-independent CS during three pregnancies and during intake of oral contraceptives. Her adrenal histology was described as being compatible with primary nodular dysplasia, but there were no comments on pigmentation of her adrenal nodules; there was also no family history of adrenal disease and no other features of Carney's complex. Paradoxical increases in cortisol production were noted during oral dexamethasone suppression tests. Dose-responsive stimulation of cortisol secretion occurred after bilateral adrenalectomy when the cells were exposed to estradiol; the *in vitro* addition of dexamethasone was not reported, nor were the effects of antiestrogens.

Paradoxical increases in plasma cortisol and urinary free

tisolism is produced transiently and reversibly due to occupation of the receptor by hCG during pregnancies; delivery is followed by a transient period of hypocortisolism (*upper* and *lower panels*). At the time of menopause, a sustained elevation of LH levels follows a decrease in ovarian estrogen production and results in a progressive increase of bilateral adrenal hyperplasia and hypercortisolism. Administration of long-acting leuprolide acetate initially induced transient stimulation of LH and cortisol, followed by long-term suppression of LH and restoration of normal cortisol production.

cortisol were observed during the last 2 days of classical Liddle's 4-day low- and high-dose oral dexamethasone tests in patients with PPNAD with or without Carney's complex (171). We found no evidence of ectopic membrane hormone receptors in two patients with PPNAD, who showed an increase in cortisol secretion during prolonged dexamethasone administration; GC receptors appeared to be highly expressed by immunohistochemistry in PPNAD micronodules, compared with the adjacent internodular atrophic adrenal or to the normal control adrenal cortex (274). Similar paradoxical elevations of cortisol production during dexamethasone have been reported in several cases of CS during pregnancy (258, 261).

H. Other abnormal hormone responses in adrenal CS

Hashimoto *et al.* (275) described a 51-yr-old male with large bilateral AIMAH in whom plasma cortisol increased during insulin-induced hypoglycemia, while ACTH, measured by RIA, remained at undetectable levels (<10 pg/ml); *in vitro*, dispersed adrenal cells stimulated cortisol secretion with ACTH, but not with insulin, catecholamines, vasopressin, or Ang-II. A very similar patient with AIMAH studied by the same group (232) also displayed elevated plasma cortisol during insulin-induced hypoglycemia and combined LVP-CRH tests while plasma ACTH remained undetectable; *in vitro* studies were not performed in this case. It remained unclear whether insulin itself, a factor increased during hypoglycemia, or subdetectable rises in plasma ACTH were responsible for the regulation of cortisol secretion in these cases.

Leptin synthesis is stimulated by GC (136), and leptin receptors are expressed in normal adrenals as well as in adrenocortical adenomas and carcinomas (128, 276). Plasma leptin has been found to be elevated in patients with CS. The leptin receptor is expressed in the adrenal cortex, where leptin normally inhibits cortisol secretion. Leptin negatively regulates the HPA, both at the pituitary level, where it suppresses CRH secretion, and the adrenal level, where it depresses steroidogenesis (124, 130, 276). Pralong *et al.* (139) recently reported a 36-yr-old woman with AIMAH and CS in whom a mixed meal heightened plasma cortisol levels, and this effect was decreased by octreotide pretreatment. GIP was not infused, but GIP stimulated cortisol secretion *in vitro*. Leptin (single dose of 100 nM) increased cortisol secretion *in vitro*, whereas in normal adrenal tissues, it normally suppresses this parameter. Plasma leptin levels were elevated in this patient with CS but did not increase after meals. GIPR or leptin receptor were not measured directly. Thus, this case raises the possibility of paradoxical stimulation of steroidogenesis by leptin in some cases of AIMAH, but more detailed studies are required in other similar cases to confirm this possibility.

VI. Investigation Strategy

A. Initial clinical screening protocol

A protocol has been developed to screen patients with adrenal CS for the presence of ectopic/abnormal adrenal hormone receptors (277); the strategy is based on monitoring plasma levels of steroids during various tests that transiently modulate the levels of ligands for potentially abnormal receptors. The protocol includes serial measurements of plasma ACTH, cortisol, and other steroids or hormones as indicated (aldosterone, free testosterone, DHAS, and estradiol) at 30- to 60-min intervals for 2–3 h during the course of various tests performed after an overnight fast and in a supine posture for at least 1 h. Initial screening includes a posture test performed in a 2-h supine position, followed by a 2-h ambulatory period (to evaluate potential modulation by Ang-II, vasopressin, catecholamines, ANP, etc.); this is followed by a standard mixed meal (to evaluate the response of gastrointestinal hormones) and then by the administration of 250 μg ACTH 1–24 iv, which serves as a reference test. On another day, the administration of 100 μg GnRH iv (modulation by FSH, LH, GnRH) is followed by 200 μg TRH iv (modulation by TSH, PRL, TRH). Responses to 1 mg glucagon iv, 10 IU AVP im, and 10 mg cisapride orally (a serotonin 5-HT_4R agonist; this is now replaced by 10 mg metoclopramide as cisapride was withdrawn from the market) are tested sequentially on the third day. A change of less than 25% plasma cortisol is arbitrarily defined as no response, a 25–49% change is defined as a partial response, and a change of 50% or greater is considered a positive response. If a partial or positive cortisol response is found, the test is repeated to verify its consistency and to determine whether other steroids, such as aldosterone, DHAS, testosterone, and estradiol, are also modified. At the same time, fluctuations of potentially interesting ligand hormones (*i.e.*, catecholamines, vasopressin, renin/Ang-II, and ANP during a posture test) are measured. If a prolonged response to a test masks the evaluation of the following test, it is repeated separately.

B. Further characterization of abnormal hormone receptors

After initial screening, other tests can be performed to confirm the responses or to elucidate which hormone is implicated (Fig. 4). For example, if cortisol stimulation by upright posture is found, the inverse effect, *i.e.*, suppression by assuming a supine posture after ambulation, is verified. The respective contributions of vasopressin, catecholamines, and Ang-II or ANP modifications need to be distinguished. An exaggerated cortisol response to pharmacological levels of exogenous vasopressin is followed by evaluation of whether physiological fluctuations of endogenous vasopressin would modify plasma cortisol levels. An increase of plasma vasopressin during an upright posture test should parallel the elevation of plasma cortisol levels. Endogenous plasma AVP levels can be modulated by a 20 cc/kg water load, followed by infusion of NaCl 3% at 0.1 cc/kg/min for 120 min. The expected result would be an initial suppression of AVP and cortisol during water loading, followed by an increase of AVP and cortisol levels. To determine whether the vasopressin receptor involved in this response is a V1, V2, or V3 receptor type, 2.5 μg desmopressin, a preferential V2 receptor agonist, is administered subcutaneously (80, 86); the absence of a response to desmopressin would suggest a V1 or V1b/V3 receptor-mediated response. Pretreatment with a specific oral V1 receptor antagonist (SR 49049) has been used

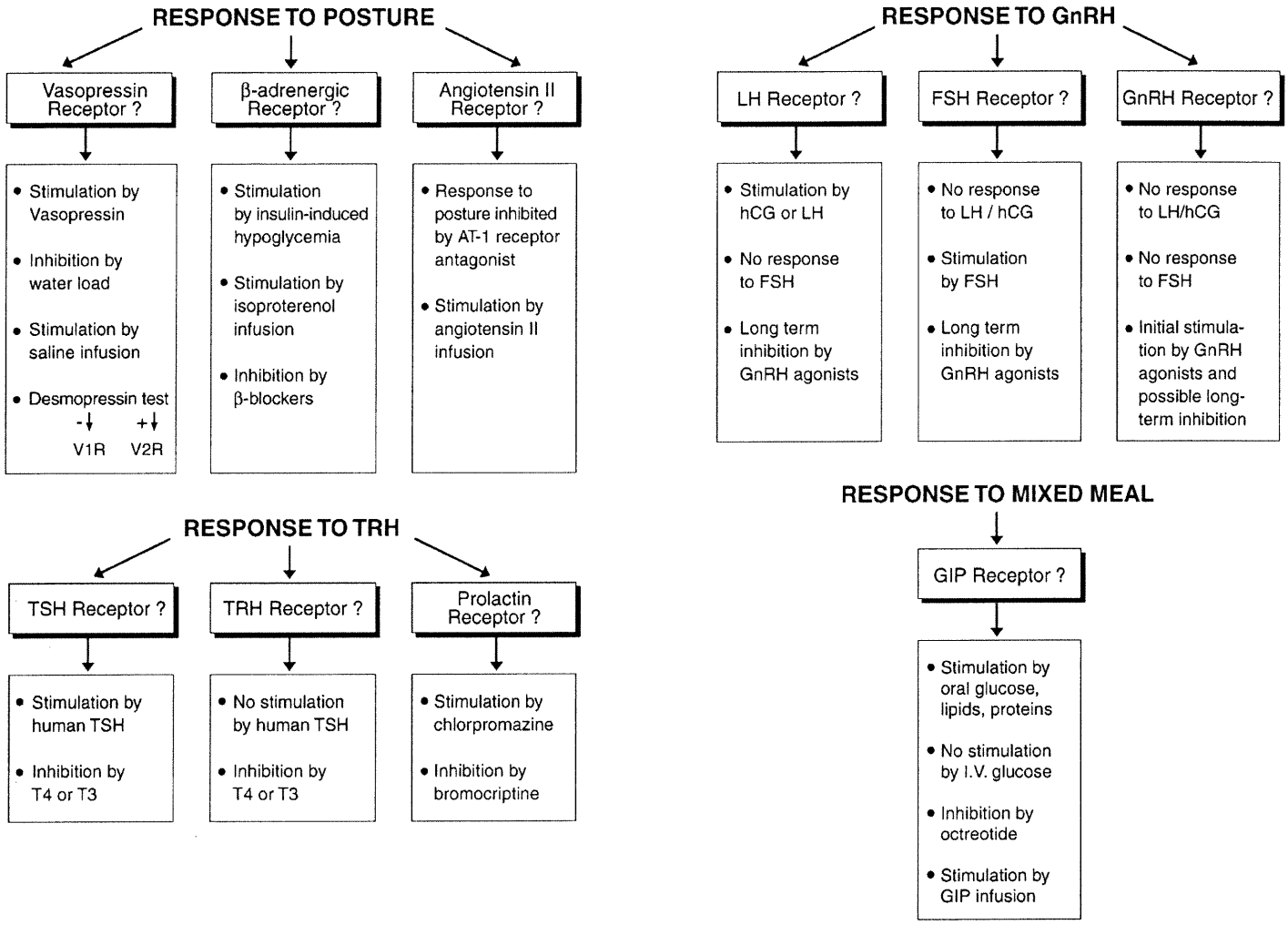


FIG. 4. Outline of the investigation protocol to further characterize abnormal adrenal hormone receptors when a positive response is identified during initial screening. Plasma cortisol levels are monitored during the various tests. [Reproduced with permission from A. Lacroix *et al.*: *The Endocrinologist* 9:9–15, 1999 (277). © Lippincott Williams & Wilkins.]

to demonstrate *in vivo* the involvement of the V1 receptor in this response (86). In case of no response to exogenous AVP, the role of Ang-II is assessed by repeating the posture test after administration of an AT1R antagonist or by direct infusion of Ang-II. If a catecholamine response is suspected, endogenous catecholamine stimulation is produced by insulin-induced hypoglycemia, and, if positive, by isoproterenol infusion (86). An attempt to block the response and to treat the patient with a β-blocker would be conducted if the stimulation of cortisol production is reproduced.

In the case of a response to a mixed meal, confirmation and identification of a specific gastrointestinal hormone involvement are based on evaluation of the effects of carbohydrates, proteins, or lipids on cortisol secretion. Patients ingest, at 3-h intervals, 75 g oral glucose, an isocaloric protein-rich meal, or a lipid-rich meal (200, 206). Plasma cortisol, ACTH, GIP, and insulin levels are measured at regular intervals during these tests. The absence of a cortisol response to the administration of 25 g glucose iv, or when 100 μg octreotide is administered sc 60 min before repeating the oral 75 g glucose challenge, confirms the role of a gastrointestinal hormone (200, 206). As only GIP and GLP-1 respond well to oral glucose, lipids, and partially to proteins, human GIP is infused at a rate of 0.6 μg/kg/min during the administration of 150 cc/h of 10% glucose and compared with the response to GLP-1 infused at a rate of 0.75 pmol/kg/min, also under 10% glucose (200, 206). The pattern of response to the various secretagogues would be different in the presence of abnormal receptors for gastrointestinal hormones other than GIP. Various candidate hormones would then be infused to confirm the identity of the steroidogenesis modulator.

Stimulation of cortisol production after GnRH administration could result from the abnormal adrenocortical presence of receptors for LH/hCG, FSH, or GnRH itself. The cortisol response after the administration on different days, of hCG 10,000 U im, purified human FSH 150–300 U im, and recombinant LH 300 U iv can be compared (251). A response to GnRH coupled to an absence of response to FSH, LH, and hCG would suggest an ectopic GnRH receptor; various analogs and antagonists of this receptor are available for testing the hypothesis. In addition, the response to an acute dose of GnRH should persist despite the suppression of endogenous gonadotropins by the administration of supraphysiological doses of gonadal steroids or the use of long-acting GnRH

analogs. In the presence of an ectopic LH/hCGR, a response to exogenous hCG or LH, but not to exogenous FSH, should be evident; the response to acute GnRH administration should disappear when the LH response is abolished by exogenous gonadal steroids or after the chronic administration of long-acting GnRH analogs. Therapy with long-acting GnRH analogs should produce eventual suppression of the endogenous LH ligand and normalize cortisol production, as demonstrated recently by our group in one such patient (251). In the presence of an ectopic FSH receptor, there should be no response to hCG or LH, but cortisol production should be increased after the administration of purified FSH. Here again, long-acting GnRH analogs should suppress the biologically active ligand and correct the hypercortisolism.

Stimulation of cortisol synthesis after TRH administration has not yet been reported. However, AC stimulation by TSH has been demonstrated in adrenocortical adenomas *in vitro* (9). Thus, a response to TRH would suggest the possibility of an ectopic receptor either for TSH, TRH, or PRL. The PRL receptor does not belong to the family of G-coupled seven-transmembrane receptors, which could mimic the ACTHR and activate AC. However, adrenocortical stimulation by PRL has been described *in vitro* (190), and the presence of this receptor in adrenal tumors has been confirmed (278). Elevation of endogenous PRL levels after a chlorpromazine test and its inhibition by a bromocriptine test would easily clarify the role of endogenous PRL. The potential presence of an ectopic TSH receptor would be assessed directly by the administration of purified human TSH and by inhibiting endogenous TSH production with exogenous T_4. The lack of an ectopic TRH receptor would be confirmed by disappearance of the cortisol response when the response of TSH to TRH has been suppressed by T_4 administration.

The *in vitro* response of AC to glucagon has been demonstrated previously in a cortisol-secreting adenoma (190), but a clinical case has not yet been reported. If a response to 1 mg of exogenous glucagon is found, it would be necessary to show that fluctuations of endogenous glucagon levels during insulin-induced hypoglycemia, fasting, or oral administration of glucose correlate well with fluctuations of cortisol levels.

The oral administration of 10 mg cisapride, a 5-HT_4R agonist, is expected to induce a large increase in aldosterone, but not in cortisol levels in normal individuals (91). If a cortisol response to cisapride is found, a response to other 5-HT_4R agonists such as zacopride or metoclopramide should be seen, but not to specific 5-HT-1,2,3 agonists. Although some specific 5-HT_4R antagonists are currently under investigation, their availability is limited, but they should become very valuable in confirming the role of this abnormally expressed receptor.

C. Systematic clinical screening for ectopic/abnormal hormone receptors

There has been only one report to date of the systematic clinical screening of patients with adrenal CS for the presence of diverse abnormal hormone receptors (240). In that study, 20 consecutive patients with adrenal CS secondary to either bilateral macronodular adrenal hyperplasia (n = 6), unilateral adenoma (n = 13), or carcinoma (n = 1) were tested for evidence of an abnormal hormone receptor. All six patients with AIMAH had a positive response to at least one test, in addition to ACTH 1–24: two patients, to the mixed meal (GIP-dependent); one patient, to GnRH (LH/hCGR) and cisapride (5-HT_4R); and three patients, to the upright posture and vasopressin (1 β-AR, 1 V1-AVPR, 1 β-AR, and V1-AVPR). In patients with unilateral adenoma, only one patient had a positive response to upright posture, while three partial responses to either mixed meals, vasopressin, or posture were also noted but were not further characterized. In the patient with adrenocortical carcinoma or in two patients with micronodular adrenal dysplasia (274), plasma cortisol was not modified by any of the tests. Initial experience suggests that the adrenal expression of various ectopic or abnormal hormone receptors is frequently implicated in the pathophysiology of bilateral macronodular adrenal hyperplasia (240), but less frequently in unilateral adenoma (79). It must be noted that the initial protocol used to date did not screen for many other G protein-coupled membrane receptors, such as those for PTH, calcitonin, acetylcholine, dopamine, opiates, prostaglandins, etc; it may thus become pertinent to investigate these other potential abnormal receptors in the future.

VII. Molecular Mechanisms of Ectopic/Abnormal Hormone Receptors

A. Tissue-specific expression and regulation of membrane hormone receptors

The hormonal regulation of adrenal cortex development and function requires the tissue-specific expression of hormone receptors. This implies the existence of fine-tuned mechanisms of regulation that involve *cis*-acting regulatory elements (promoters) and *trans*-acting factors (TFs) for these receptors. It is thus pertinent to briefly review which factors regulate the appropriate tissue-specific expression of the membrane hormone receptors of interest in adrenal CS before considering which molecular mechanisms could be responsible for their abnormal adrenal expression and function.

The ACTH MC-2 receptor gene, localized on human chromosome 18 (18p11.2), is highly expressed in the adrenal cortex and, at lower levels, in fat tissue and skin (22, 279, 280). The proximal promoter region (~1,000 bp) of the human ACTHR (hACTHR) gene is responsible for the basal transcriptional rate and contains several potential regulatory elements: one SP1 element, four AP1 elements, seven CRE (cAMP-responsive element)-like regulatory elements, and three SF-1-like elements (SF-35, SF-209, and SF-98) (23, 281, 282). Both SF-35 and SF-98 sites were shown to be essential for the cAMP regulation of ACTHR transcription. Although absolutely required, SF-1 is not sufficient for ACTHR expression in the adrenals, since it is not expressed in gonads, whereas both Leydig and ovarian cells express SF-1 (41). The well known up-regulation of the receptor by its own ligand (36, 37, 283–285) is probably mediated by one of the CREs. The same regulatory elements are present in the proximal promoter of the mouse ACTHR, except for CREs, which have

been changed for GRE (GC-responsive element) sites (286). A negative regulatory region (silencer), located between –1,236 and –908 from the transcription start site, prevents expression of the receptor in heterologous systems or in non-SF-1-containing cell sites (286). This suggests that other factors are needed for the receptor to be expressed properly.

β-Adrenergic receptors (β-AR) are subject to extremely tight regulation. In addition to short-term regulatory phosphorylation of receptor proteins, their gene expression is also regulated. Cloning of the 5′-flanking region of human β_1-AR (chromosome 10q24–26) revealed several potential thyroid response elements (TRE), GREs, and CREs (287). These putative response elements support the pathophysiological evidence that thyroid and GC hormones regulate β_1-AR by affecting receptor expression (287, 288). Hyper- and hypothyroidism have been associated with increases or decreases in β_1-AR number and activity. Thus, the presence of TRE in the 5′-flanking region of β_1-AR is consistent with these clinical conditions (289, 290). β_2-AR (chromosome 5q32) expression is up-regulated by GC in various tissues and is due to a direct increase in the rate of its transcription (291, 292). This is probably mediated by GRE, as demonstrated for hamster β_2-AR (293). In contrast, β_1-AR is down-regulated by GC. The stability of β_1-AR mRNA is not influenced by GC, but nuclear run-on assays have revealed that down-regulation is due to a decline in the relative transcription rate of the receptor (294). Homologous desensitization of β-AR has been observed for the three receptor types, β_1-, β_2-, and β_3-AR (291, 295, 296). This is compatible with the presence of CRE in the promoters of both β_1- and β_2-AR (287). Moreover, β-adrenergic stimulation causes not only down-regulation of β-AR but also loss of coupling to G_s/AC effectors (297). *In vivo* investigations of GC effects on β-agonist-induced down-regulation of β_1- and β_2-AR have shown that GC can prevent down-regulation of β_2-AR number and mRNA at the transcriptional level; the TF CREB may be involved (294).

LH/hCGR have also been reported in the human adrenal zona reticularis (249), although they are more highly expressed in gonadal tissues. The LH/CGR is one of the largest seven-transmembrane receptors (683 amino acids) as it harbors an unusually long extracellular ligand-binding domain (247). This receptor is encoded by two genes: gene I isolated from a lymphocyte library, and gene II isolated from a placental library (298). The four copies of hLHR genes are localized on chromosome 2p16–21 loci. The two proximal 5′-untranslated regions have been well characterized (299–301) and differ by several base changes and a 6-bp deletion in the coding region (+55 to +60). The transcription initiation site is localized at position –176 bp for both promoter regions. Additional upstream transcription start sites have been identified in human testicular and choriocarcinoma JAR cells. These data suggest that tissue-selective LHR promoter utilization and gene (I or II) expression may underlie the specific pattern of LHR expression. TATA and CAAT-like boxes have been identified in human, but not in mouse and rat, promoters; the human promoter contains one CRE, seven AP1 sites, and one half-ERE site. Three negative control regions (NCRs), when complexed with proteins of JEG-3 cell nuclear extracts, disable the proximal promoter activity (300); these regions might be very important in nongonadal tissues.

The actions of vasopressin are mediated by three G protein-coupled membrane receptor subtypes. V2 receptors are expressed almost exclusively in renal collecting ducts to promote water permeability via activation of G_s and AC (302, 303). VIa (or VI) receptors are expressed in blood vessels, where they promote vasoconstriction (304), and in the liver, where they promote glycogenolysis (305), while VIb (or V3) receptors are located mainly in the anterior pituitary, but also in the adrenal medulla. VIa and Vlb receptors are coupled to various pertussis toxin-sensitive G proteins and activate PLA_2, PLC, and PLD through activation of ligand-gated calcium channels (306). VIa receptors are also present in the adrenal cortex where they are involved in steroid secretion (see *Section II*). Dexamethasone increases the expression of VIa receptors in the rat liver and forebrain (307, 308). The elevation of mRNA levels precedes the rise in binding activity, suggesting a transcriptional effect. Isolation and analysis of the 5′-regulatory region of the rat VIa receptor have demonstrated that *trans*-acting factors such as CREB, AP-2, and GR are involved in the expression of the receptor gene (309, 310). At the protein level, GC have been shown to produce an early decrease in binding site density, followed later by an increase, which becomes more prevalent with time. Perhaps GC initially affects the stability of receptor protein or that of mRNA levels (307, 311). GC can also negatively regulate the stimulated expression of V1a receptor by a mechanism not involving GR-binding to DNA (310). Furthermore, it has been reported that GC amplify the vasopressin-induced transduction signal (increased IP accumulation in the presence of dexamethasone) (312, 313). This mechanism of regulation was demonstrated for the V1b receptor in the anterior pituitary where prolonged exposure to dexamethasone decreased the number of receptors, while increasing their coupling efficiency. Potentiation was found to be due, in part, to an increase in the guanylyl nucleotide-binding protein, Gq (314). The effect of GC on adrenal VIa receptors has not been studied.

The recent cloning of rat (214), hamster (215), and human GIPR (216–218) has revealed that it is a member of the secretin-VIP family of receptors. This gene is proposed to be involved in the pathogenesis of diabetes as GIPR knockout mice displayed glucose intolerance with impaired insulin secretion (218a). The human GIPR gene is localized on the chromosome 19q13.3 locus and consists of 14 exons; it is expressed in several tissues, including the rat brain, fat, gut, vascular endothelium, and adrenals (214, 315, 316). *In situ* hybridization studies indicate that the GIPR is localized in the inner layers of the rat adrenal cortex (214); GIP is also able to stimulate AC and corticosterone synthesis in the rat adrenal cortex (317). In humans, the tissue distribution of GIPR mRNA has not yet been examined extensively, but has been discovered in the pancreas and brain, but not in spleen (210). Several splice variants of the receptor have been described in the human pancreas, of which one with a 27-amino acid insertion in the cytoplasmic tail is functional (216, 219). The rat GIPR has been shown to be desensitized by its own ligand *in vivo* and *in vitro* (318). The rat 5′-flanking region of the receptor gene has recently been cloned (319); it is a TATA-less promoter harboring one CRE, an octamer-binding site, three Sp1 sites, and an initiator element (319). Distal negative con-

trol sequences, not yet clearly identified, seem to confer cell-specific regulation of GIPR expression (319). The human GIPR promoter, however, has not yet been characterized.

5-HT_4R-mediated stimulation of corticosteroid secretion is the only known endocrine effect mediated by this receptor. Activation of 5-HT_4R augments AC activity and elevates cAMP. Homologous desensitization has been postulated to occur via a specific receptor kinase. Splice variants of 5-HT_4R have been detected in several human tissues, and their tissue distribution suggests some degree of tissue specificity (320). These splice variants differ in their capacity to trigger the signal transduction cascade after receptor activation.

B. Potential mechanisms of ectopic or abnormal hormone receptors

The molecular mechanisms responsible for the ectopic or abnormal expression and function of membrane receptors in adrenal CS have not yet been identified. In fact, the important question of regulation of the tissue-specific expression of genes is raised by this new pathophysiology of adrenal CS. Several hypothesis can be proposed, however (Fig. 5). A gene rearrangement could potentially lead to adrenocortical-specific, inappropriate expression of a hormone receptor gene. Examples of this mechanism in endocrine tumors include rearrangements described in subsets of parathyroid adenomas (321), in GC-remediable aldosteronism (322), and in papillary carcinoma of the thyroid (323). The PTH promoter has been found to be recombined with the cyclin D gene, giving rise to the prad-1 oncogene (321). The aldosterone synthase gene has been shown to be fused with the 11β-hydroxylase promoter, resulting in ectopic production of aldosterone in zona fasciculata (322). In 25% of human papillary carcinomas (up to 62% after exposure to Chernobyl irradiation), a chromosomal break fuses the intracellular tyrosine kinase domain of the growth factors receptor RET to one of at least eight new promoters including H4, ELE1, R1α, NTRK1, RFG, and other genes (324, 325); this results in constitutive dimerization and activation of the tyrosine kinase of RET, bypassing the requirement for ligand binding. None of the ectopic hormone receptors identified to date in adrenal CS is located on the same chromosome as the ACTHR promoter; gross gene rearrangements have not been reported to date. More discrete mutations in the promoter regions of the membrane hormone receptor could also greatly increase the expression of a receptor normally expressed at such a low level that it would not play a significant role in steroidogenesis. A point mutation in the promoter region of the hormone receptor could generate an appropriate binding site for an adrenocortical-specific TF/co-activator complex, leading to ectopic expression (Fig. 5A).

Another mechanism by which altered expression of hormone receptors could be achieved would involve abnormalities in TFs, their coactivators, or corepressors (Fig. 5B). Indeed, excessive activity or mutations of TFs can induce

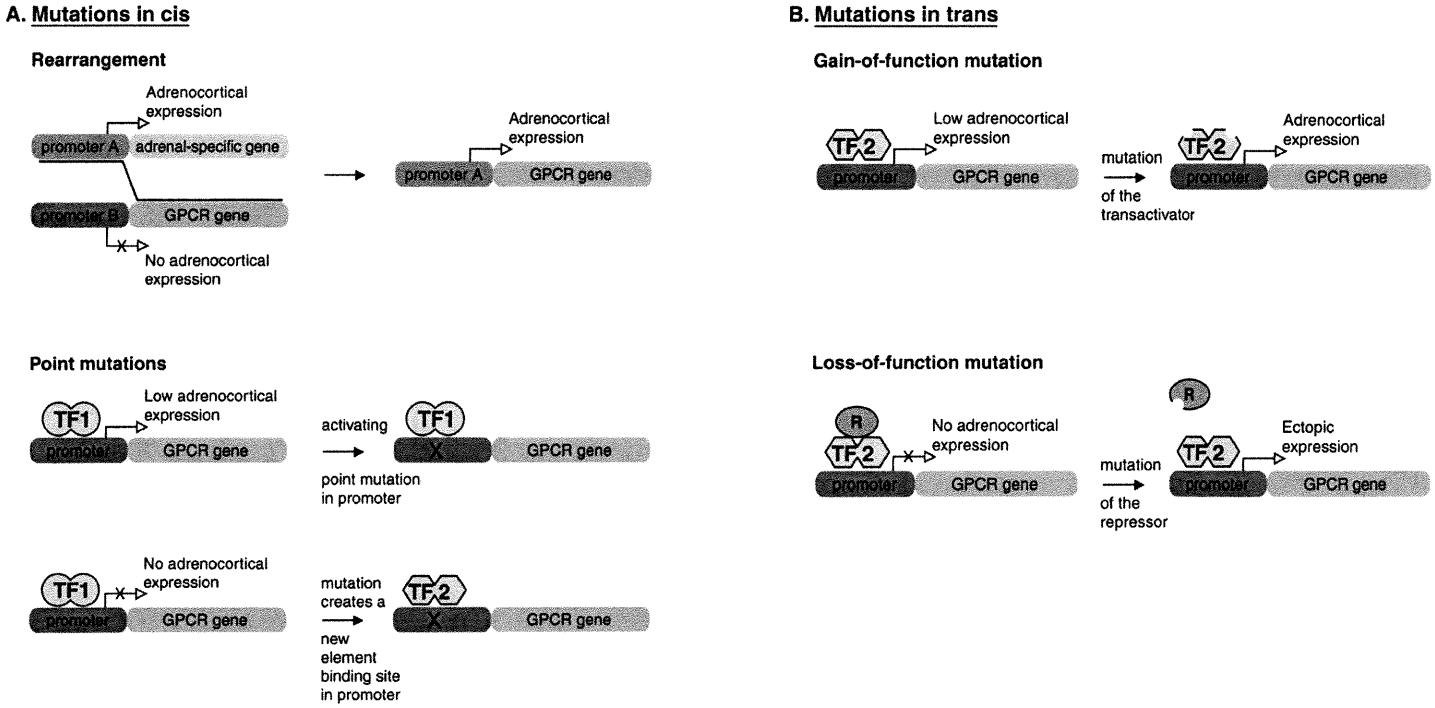


FIG. 5. The potential molecular mechanisms leading to ectopic expression of G protein-coupled receptor (GPCR) in adrenal CS may include mutations in *cis*-acting regulatory regions (A) or in *trans*-acting factors (B). A, Gene rearrangement could fuse a specific GPCR gene that is not expressed in adrenals with an adrenocortical-specific promoter, resulting in ectopic expression of the receptor in the adrenal cortex. More discrete mutations in the GPCR promoter could also lead to abnormal expression. Thus, a point mutation could enhance the transcription of a receptor that is normally weakly expressed in the adrenals and not coupled to steroidogenesis; a point mutation in the promoter of a GPCR gene that is not expressed in the adrenals could generate a new binding site for an adrenocortical-specific TF leading to ectopic expression. B, A gain-of-function mutation of an adrenocortical-specific activator could induce overexpression of an adrenal receptor; the ectopic adrenal expression of a transcription factor (TF) or of its coactivator (not shown) could also result in the ectopic expression of a GPCR. A loss-of-function mutation of a repressor (R) or corepressor (not shown) that prevents the GPCR adrenal expression could result in ectopic expression.

overexpression or a lack of gene expression (61, 326). A gain-of-function mutation could affect an activator that specifically regulates gene expression in the adrenal cortex, or the loss of a repressor could induce ectopic gene expression. In the case of overexpression of a single receptor, any of these hypotheses is plausible. In contrast, when more than one ectopic adrenal receptor is present, mutation in a factor regulating cell differentiation or in a TF common for the different receptors involved may be more likely. It is improbable that acquired mutations of several unrelated promoters would occur simultaneously.

Another interesting hypothesis emerges from a recent study by Kero *et al.* (327), who observed that transgenic mice expressing bLHβ-CTP (a chimeric protein of β-subunit fragments of bovine LH and hCG) in their pituitary develop adrenal CS in addition to polycystic ovaries and ovarian tumors after chronically elevated serum LH levels. It was shown that this resulted from ectopic expression of LH/CG receptors in the adrenal cortex, not detectable or functional in control mice. Since this induction is abolished by gonadectomy, it was proposed that elevated estrogens and PRL levels were responsible for inducing the illicit expression of the LH/CG receptor in the adrenal cortex. This observation would thus raise the possibility that the "ectopic expression" of a receptor may not require a mutation of *cis*- or *trans*-acting regulators, but may result from exaggerated stimulation of a gene that is normally silent.

The presence of abnormal membrane hormone receptors in unilateral cortisol-secreting adenomas may arise from the monoclonal expansion of a primary adrenocortical cell that acquired a somatic mutation, leading to the abnormal expression and function of that receptor at the postzygotic stage of adrenal cortex development (Fig. 2); most studies confirm the monoclonal composition of human adrenocortical tumors (157, 163). In patients with ectopic membrane hormone receptors in bilateral macronodular adrenal hyperplasia, the mutational event must have occurred very early during embryogenesis so that every cortical cell of both adrenals would be affected by the defect, which is polyclonal. There have been rare reports of familial AIMAH (179–182), and in only one case, an abnormal response to LVP was demonstrated in one sibling (182); thus, abnormalities of receptor expression in these syndromes may frequently be the consequence of somatic mutations but, in some cases, could also be germline mutations. In the case of a very early mutational event resulting in AIMAH, the abnormal expression could affect diverse tissues so that polymorph aberrant manifestations would be expected. This was the case in the patient with vasopressin-dependent CS (80) who also displayed an abnormal vascular response to AVP and decreased hypothalamic AVP release during postural hypotension. One patient with GIP-dependent CS and AIMAH suffered from psychiatric dysfunctions that persisted even after correction of the hypercortisolism (200); since GIPR have been shown to be expressed in the brain (214), it is possible that the brain GIPR is also altered. The McCune-Albright syndrome is an example of a somatic mutation occurring during embryogenesis and leading to defects in the adrenal cortex as well as in several other tissues (172); it is still unclear how a somatic mutation could affect all cells in a polyclonal mode in one case (ectopic hormone receptors in AIMAH and CS) and result in a mosaic or oligoclonal pattern of distribution in another case (McCune-Albright syndrome).

The majority of ectopic or abnormal hormone receptors in adrenocortical tumors or hyperplasias (8–10) belong to the G protein-coupled receptor superfamily. Studies of the second messengers implicated in ectopic/abnormal hormone receptors in adrenal CS suggest that they regulate steroidogenesis by mimicking the cellular events triggered normally by ACTHR activation (Fig. 1). It is thus expected that only ectopic hormone receptors capable of coupling efficiently to the intracellular signaling systems present in adrenocortical cells (*i.e.*, those for ACTH, V1-AVP, etc.) will be able to regulate steroidogenesis aberrantly. Certain receptors may be involved more frequently than others, however, if they share more characteristics of the promoters or TFs essential for adrenal cell type-specific tissue expression. It is thus noteworthy that GIPR and the β-AR are expressed normally and are functional in the adrenal fasciculata cells of rodents; it will be interesting to compare the structures of promoters and TFs between species. The LH/hCGR is expressed in the human adrenocortical reticularis during embryonic life; it remains to be seen which events render its expression possible in the fasciculata in adrenal CS (251). Similarly, the 5-HT_4R is usually very efficiently coupled to aldosterone synthesis in the human glomerulosa and already possesses some tropism for fasciculata cells; increased functional coupling to cortisol secretion may require only the inactivation of a relative silencer in fasciculata cells.

C. Role of ectopic hormone receptors in adrenocortical cell proliferation

What is the role of abnormal hormone receptors in altered cell growth and tumorigenesis? One could postulate that the primary event is a mutation resulting in aberrant adrenal expression of the receptor, leading to increased proliferation and eventually to increased hormone production. Alternatively, it can be proposed that the primary event is an unknown proliferative one resulting in cell dedifferentiation with resultant expression of "embryonal" type genes, including one or several hormone receptors. In either hypothesis, it is clear that a relatively long time period is necessary before phenotypic expression of the abnormal hormone receptor becomes evident. This is particularly true for AIMAH, as several decades are necessary before the hyperplasia and hyperfunction become clinically manifest. This may be secondary to the transient occupation of the receptor by the ligand, as illustrated by the cases of GIP (Fig. 2) and LH-dependent (Fig. 3) CS. In GIP-dependent CS, the adrenal tissues are stimulated only briefly but repeatedly after each food ingestion; in LH/hCG-dependent CS, the hyperplasia and hyperfunction occurred only after intense and prolonged exposure to the ligands, *i.e.*, during pregnancy for hCG, or after menopause for LH. Reversal of the hyperplasia between pregnancies would favor the hypothesis that the ectopic receptor is a primary event rather than one that is secondary to another proliferative event; however, this awaits clear demonstration of adenoma or AIMAH regression after complete blockade of the ectopic receptor. There is

also indication, based on cases of preclinical cortisol production in bilateral macronodular disease, that steroidogenesis can be relatively inefficient, despite significant proliferation. This suggests poor steroidogenic enzyme activities in the adrenal lesions or that the low expression of abnormal receptors is better coupled to proliferative signals than to hormone synthesis.

The elucidation of this question requires better understanding of the factors regulating normal adrenal gland development. Knowledge of the ontogeny of steroidogenic tissues (adrenals and gonads) was provided by the identification of tissue-specific TFs (328, 329). Indeed, by using SF-1 as a marker, it became possible to trace steroidogenic cells back to the earliest stage of differentiation (330). Investigation of the spatiotemporal expression of SF-1 revealed the existence of the adreno-genital primordium (AGP) which is composed of a SF-1-immunoreactive single cell population (for review see Ref. 331). This structure lies between the coelomic epithelia of the urogenital bridge and the dorsal aorta. The AGP then gives rise to adrenocortical and gonadal primordia, which both express SF-1. Studies in SF-1 knockout mice have shown that the earliest stages of urogenital ridge development occur normally; however, regression of the adrenals and gonads is observed as soon as gonadal sexual differentiation takes place (66). These results suggest a complex cascade of transcriptional events for establishment of the endocrine axis. The adrenocortical primordium gives rise to the adrenal cortex that differentiates into three zonae (glomerulosa, fasciculata, and reticularis). The adrenal medulla is composed of neural cells (SF-1-negative cells) that have migrated from a dorsal root ganglion to the adrenal primordium. In adult mice, SF-1 is expressed in adrenocortical, testicular Leydig, ovarian theca, and granulosa cells, and, at a lower level, in spleen and pituitary gonadotropes (reviewed in Ref. 41).

DAX-1 (dosage-sensitive sex reversal, AHC critical region on the X chromosome, gene 1) is another steroidogenic-specific TF involved in the adrenal cortex and gonads, as demonstrated by disorders due to DAX-1 mutations (332–334). DAX-1 belongs to the orphan nuclear receptor superfamily as does NGFI-B and SF-1 and it acts as a transcriptional repressor. However, the protein is atypical since it possesses no DNA-binding domain, suggesting possible interactions with other TFs. Indeed, DAX-1 has an expression profile similar to that of SF-1, suggesting a functional correlation between these two proteins (335–337). Moreover, SF-1 was shown to be a critical regulator of DAX-1 expression, as functional SF-1-binding sites have been identified in the promoter region of DAX-1 gene (338, 339). Another transcriptional repressor has been shown to play a role in adrenal development. Initially designated as an essential actor throughout nephrogenesis (340, 341), the Wilm's tumor suppressor gene (WT1) has recently been implicated in adrenogenesis (342). Unlike SF-1, WT1 expression is not detectable during adrenal cortex formation (343, 344) but is in the developing kidney and urogenital system. Taken together, these results suggest, first, that the WT1 gene may be expressed in a very transitory manner in adrenocortical precursor cells, and second, that WT1 activity may be required at early steps of adrenal development, probably in the AGP stage. Functional interactions between SF-1, DAX-1, and WT1 have been demonstrated for transcriptional regulation of the Mullerian inhibiting substance (MIS) sex-specific gene *in vitro* (344). Such combinational regulation may occur for the expression of genes determining the fate of the AGP. It should be interesting to determine whether any alterations in SF-1, DAX-1, or WT1 could be present, particularly in cases of AIMAH with ectopic membrane hormone receptors.

The concept of abnormal G protein-coupled receptors and/or postreceptor events leading to increased cAMP and proliferation is now well established (Table 4, reviewed in Refs. 345–347), especially in somatotroph and thyroid cells (348, 349). Stimulation of G-protein-coupled receptors, alone or in association with tyrosine kinase receptors, is known to evoke powerful mitogenic signals via G protein-mediated activation of ras (346). Thus, altered activity at any step of the transduction signal cascade may predispose to tumor formation. Transgenic mice with thyroid-specific expression of adenosine A2 receptor (which activates AC via G_s protein) develop thyroid hyperplasia and severe hyperthyroidism (350), clearly demonstrating that *in vivo* constitutive activation of the cAMP cascade in thyroid cells is sufficient to stimulate autonomous hyperfunction and uncontrolled cell proliferation. There are many examples of hormone receptor mutations involved in endocrine pathologies (Table 4). Some include somatic or germline constitutive mutational activation of the TSH receptor, resulting in hyperfunctionning thyroid adenomas and hyperplasias (351, 352); familial male precocious puberty (characterized by Leydig cell hyperplasia and testosterone production) is due to constitutive activation of LH/hCGR (353). At the G protein level, the mosaic-activating mutation of $G_{s\alpha}$ leads to McCune-Albright syndrome (172); activating mutations of inhibitory $G_{\alpha i}$ protein (Gip) have been identified in some, but not all, adrenocortical and ovarian tumors (355), and $G_{s\alpha}$ overexpression has been shown in insulinomas and other endocrine tumors (356). There are examples of transgenic mice with cardiac overexpression of β_2-AR or $G_{s\alpha}$ that display enhanced cardiac function and develop myocardial fibrosis (357). However, it must be stressed that cAMP is not mitogenic in all cell types. Counterregulatory mechanisms are initiated in response to persistently elevated cAMP levels. This was the case for transgenic mice expressing gsp in pancreatic β-cells (358) where inhibitors of phosphodiesterases were required to obtain high cAMP levels and enhanced insulin secretion. In the Y1 mouse adrenocortical cell line transfected with β_2-AR, ectopic receptors have been found to be efficiently coupled to steroidogenesis, but cell growth has not been studied (359).

We hypothesize that the ectopic expression of any G protein-coupled receptor could induce the stimulation of adrenal cells by trophic factors lacking regulatory negative feedback by cortisol. This stimulus may lead to increased function and confer a proliferative advantage. The event provides a gain of function to adrenocortical cells; thus, this category of mechanism, *i.e.*, the abnormal tissue-specific expression of membrane hormone receptors should be added to the list of abnormalities of hormone receptors implicated in certain human diseases (Table 4). GIP has been shown to stimulate the cAMP production and DNA synthesis in GIP-dependent, cortisol-secreting adenoma cells in a manner similar to

TABLE 4. Examples of human diseases resulting from gain-of-function mutations of membrane hormone receptor-effector systems

Receptor	Disease
I. Membrane Hormone Receptors	
a. Activating mutations of G protein-coupled hormone receptors	
TSHR: somatic	Toxic thyroid adenomas (351)
TSHR: germline	Familial neonatal hyperthyroidism (352)
LHR: germline	Testotoxicosis (353)
CaR: germline	Familial hypoparathyroidism (394)
PTH/PTHrPR: germline	Jansen metaphysical chondrodysplasia (395)
TSHR mutation with hCG affinity	Transient hyperthyroidism during pregnancy (396)
b. Illicit activation of a normal receptor	
TSHR activated by thyroid stimulating immunoglobulins	Grave's disease (397)
TSHR activated by high concentrations of hCG	Hyperthyroidism in choriocarcinoma (397)
Insulin receptor activation by IGF-I or IGF-II	Hypoglycemia of malignancy (398)
c. Ectopic G-protein coupled hormone receptors	
GIPR in adrenal adenomas or AIMAH	Food-dependent Cushing's syndrome (see Table 2)
LH/hCGR in adrenal AIMAH or adenomas	Postmenopausal and transient Cushing's syndrome during pregnancy; virilization (see Table 3)
β-AR in adrenal AIMAH or adenomas	Cushing's syndrome (see Table 3)
Glucagon receptor in pheochromocytomas	Pheochromocytomas (367,368)
TRH receptor in GH-secreting pituitary adenomas	Acromegaly (370–375)
d. Increased activity of eutopic G-protein coupled receptor-effector systems	
V1-AVP receptor in adrenal AIMAH and adenomas	Cushing's syndrome (see Table 3)
5-HT_4R in adrenal AIMAH	Cushing's syndrome (see Table 3)
e. Other membrane receptors	
Mutations of RET oncongene: germline	MEN 2A, MEN 2B, FMTC (323)
Mutations of RET oncogene: somatic	Medullary carcinoma of thyroid; rarely pheochromocytomas (323)
RET rearrangements	Papillary thyroid carcinomas (323)
EGFR, CSF-1 receptor	Various malignancies (399)
IL-1R	Adrenal Cushing's syndrome adenoma (198)
II. G Proteins	
$G_s\alpha$: somatic; mosaic in embryo	McCune-Albright syndrome (172,173)
$G_s\alpha$: somatic mutation	Acromegaly (355,400)
$G_s\alpha$: somatic mutation	Toxic thyroid nodules (355,401)
$G_s\alpha$: somatic overexpression	Insulinomas (356)
$G_{i2}\alpha$: somatic mutation	Ovarian and adrenal tumors (355)
$G_s\alpha$: germline	Mixed testotoxicosis with pseudohypoparathyroidism type Ia (402)
Gβ3: germline	Essential hypertension (354)

ACTH (210). Hormone-stimulated LH/hCGR can act as an adrenocortical tumor promoter when ectopically expressed in the adrenal cortex of gonadectomized mice transgenic for the inhibin α-subunit promoter/simian virus 40 T-antigen fusion gene (360); it remains to be seen whether the expression of ectopic adrenocortical receptors, in the absence of other oncogenic events, is sufficient for adrenal overgrowth. Future animal models such as transgenic mice expressing ectopic membrane hormone receptors in the adrenal cortex will be informative in this regard. This is already supported by the demonstration of bilateral adrenal hyperplasia and CS in the mice transgenic for bLHβ-CTP with ectopic adrenal expression of LH/CGR (327).

What is the cell of origin in which the receptor is expressed abnormally? Based on the profile of steroids produced, it appears that it can occur in well differentiated cells of the fasciculata/reticularis (pure cortisol- or mixed cortisol-/androgen-secreting adenoma), and in cells from the reticularis (pure androgen-secreting adenoma); the three classes of adrenal steroids are sometimes secreted in macronodular hyperplasia, suggesting that all zonae are affected. It remains to be seen whether some cases of unilateral adenomas or bilateral hyperplasia in primary hyperaldosteronism can also be secondary to ectopic hormone receptors.

No constitutive activating mutations of the ACTHR have yet been found in adrenocortical neoplasms or hyperplasias (361). Recent studies suggested that ACTHR could act as a tumor suppressor gene in adrenal tumorigenesis (362) in a way similar to p53, which is involved in many tumor types, including adrenocortical tumors (363). Loss of heterozygosity of the ACTHR gene was shown to be associated with high malignancy or the absence of secretion in a subset of human adrenocortical tumors. Furthermore, lower expression of ACTHR was found in adrenocortical carcinomas compared with adrenocortical adenomas from patients with CS (364, 365). ACTH is known to be a differentiating factor with low potential for promotion of cell proliferation, as demonstrated by *in vitro* experiments. It has thus been speculated that a defect in the ACTHR signal cascade could result in dedifferentiation and increased cell proliferation (362). Obviously, much work remains to be done to better understand the mechanisms underlying tumorigenesis of the adrenal cortex.

VIII. Ectopic/Abnormal Hormone Membrane Receptors in Nonadrenocortical Tumors

The ectopic or abnormal expression of membrane hormone receptors is not limited to endocrine tumors of the adrenal cortex; an extensive review of their abnormal expression in nonadrenal cortex tissues is beyond the objective of this article, but a few examples will be cited. The aberrant stimulation of AC in other human endocrine tumors has been explored initially by Robert Ney and colleagues (366). AC stimulation was induced by glucagon in three of nine pheochromocytomas and in two of three parathyroid adenomas, by LH and TSH in a thyroid follicular carcinoma, and by ACTH in a pituitary chromophobe adenoma (9, 366). The AC of normal adrenal medulla was not stimulated by glucagon, suggesting the ectopic expression of glucagon receptor in pheochromocytoma (9, 367). This finding served, for a prolonged period of time, as a diagnostic provocative test, particularly in periodically secreting pheochromocytomas. With the advent of more sensitive catecholamine determinations, the glucagon provocative test was rarely used, and this may have contributed to the paucity of molecular characterization of the receptor in pheochromocytomas (368). We do not know whether the glucagon receptor structure in pheochromocytomas is normal, or what regulates its expression. The presence of glucagon receptor in pheochromocytomas remains of clinical relevance as glucagon is commonly used in premedication for endoscopic and radiological investigation of the digestive system, and inadvertent crisis still occurs after its administration in unsuspected pheochromocytomas (369).

Matsukura *et al.* (370) found aberrant AC stimulation in four GH-secreting pituitary adenomas by TRH (two of four), GnRH (two of four), norepinephrine (three of four), dopamine (one of four), glucagon (one of three), or PGE_1 (four of four); in one ACTH-secreting pituitary adenoma, AC was stimulated by GnRH, norepinephrine, and glucagon, but not by TRH. The paradoxical stimulation of GH or ACTH after the GnRH or TRH tests *in vivo* in patients before surgery correlated well with the AC stimulation *in vitro*. The AC of two ectopic ACTH secreting tumors (gastric carcinoid and malignant thymoma) was also stimulated by TRH, GnRH, norepinephrine, epinephrine, serotonin, and PGE_1 (370).

The frequently observed paradoxical increase in GH in acromegalic patients after administration of TRH, or in a lesser proportion, of GnRH (371–373) and the AC stimulation found *in vitro* (370) suggested the presence of ectopic TRH or GnRH receptors in GH-secreting pituitary tumors. The expression of TRH receptors type 1 has been confirmed in GH-secreting adenomas (374), where the structure of the receptor does not appear to be mutated (375); the TRHR-1 is normally expressed in rat somatotroph cells (376), and it is unknown whether the abnormal response of GH in acromegaly results from ectopic expression of one of the TRH receptors, or rather from abnormal coupling of this receptor to GH secretion in adenoma cells. In a preliminary report, the paradoxical increase in GH following oral glucose in acromegaly was found to result from aberrant GH-tumor response to GIP (376a); this would suggest that ectopic GIPR could also occur in acromegaly. Epidermal growth factor (EGF) receptor is overexpressed in several types of human cancers including aggressive GH-secreting tumors (377).

As a corollary to the ectopic expression of LH/hCGR in the adrenal cortex, the stimulation of androgen secretion in patients with ovarian arrhenoblastomas, after administration of ACTH, and their suppression by dexamethasone indicate the ectopic expression of ACTHR in some of those tumors (378, 379).

In a sporadic human medullary thyroid carcinoma (MTC), Matsakura *et al.* (380) found that the AC was activated by TRH, glucagon, epinephrine, norepinephrine, and serotonin, but not by TSH, ACTH, or PRL. A large number of studies have now evaluated the expression and function of hormone and growth factor receptors in MTC (381, 382). It is problematic to distinguish which of the receptors identified are indeed ectopic, as frequently, the search for their expression in normal C cells has not been performed. Mutations of the normally C cell-expressed RET protooncogene (eutopic receptor) are present in almost all cases of genetic forms of familial MTC and MEN-2 (multiple endocrine neoplasia, type 2), and in a proportion of sporadic MTC cases (somatic), and play a crucial role in initiation of C cell proliferation (323). Clearly, other receptors contribute to the development and progression of MTC, *e.g.*, the trk family, neurotrophin receptors, where the type trkB is reduced, while trkC expression is increased during the progression of the disease (381). Some of these proliferative-related receptors are expressed also in normal thyroid; this appears to be the case for transforming growth factor-α (TGF-α and EGF), as well as for their common EGF receptor (382). However, EGF binding protein, particularly EGFBP-2 and -3, are detected only in MTC (382). Rat MTC cell line 6/23 also expresses GLP-1 receptor, VIP receptor, and PACAP receptor (383); in addition, several splice variants of PACAP were expressed in 6/23 cell line. The GLP-1 receptor expression is responsible for glucagon effect on calcitonin secretion via cAMP stimulation (384). Additional receptors in which ectopic or increased expression may be related to the progression of the disease include progesterone receptors, which are focally detected in all studied cases of MTC without the concurrent presence of estrogen receptors (385). Expression of gastrointestinal hormones and their receptors, particularly those of CCK-B/gastrin, also received attention in MTC. Thus, CCK-B/gastrin receptors were detected in all biopsy specimens, while they were not found in normal thyroid tissues or in other thyroid tumors such as follicular adenoma, papillary carcinoma, or anaplastic carcinoma (386). Therefore, the presence of CCK-B/gastrin receptor in MTC may have clinical implications. Much attention has been paid, over the last decade, to somatostatin receptor expression in MTC and many other tumor types. The genomic structure and transcription regulation of the various types of somatostatin receptors are now better understood in MTC (387).

Many other receptors have been described, during the last decade, as being expressed in the adrenal medulla tumors without apparent clinical evidence of their ectopic activities. Such is the case for the ANP receptor and its effect on catecholamine release in human pheochromocytoma (388). Most of the receptors studied more recently have, at least, a potential relevance for control of proliferation. Thus, IGF-II

itself is produced and released by the adrenal and is accompanied by the presence of IGF-II R in pheochromocytomas (389). As the ectopic expression of Src homology 2 (SH2) and SH3-containing oncogenic adaptor protein v-Crk in PC12 cells results in EGF-inducible neuronal differentiation, v-Crk was studied and demonstrated able to regulate the strength of a tyrosine kinase signal that leads to prolonged activation of Ras and MAP kinase, respectively (390). Pheochromocytoma shares the expression of several genes with MTC; one example is TGFα gene and its receptor EGFR (391). Both of these tumors express these receptors *in vivo* and *in vitro*, and it has been suggested that TGFα is involved in the regulation of tumor cell growth. Since the signaling pathway from the TrkA receptor via the MAP kinase is not altered in PC12 cells, it has been proposed that p300 could play a pivotal role in triggering the antimitogenic effect of NGF and neuronal differentiation (392).

Since all cells are regulated in their function and proliferation by a series of hormone and growth factors that signal the cells via membrane receptors, it appears quite plausible that several other examples of ectopic or abnormal membrane receptors will be identified in various hyperplasias and tumors in diverse endocrine and nonendocrine human tissues.

IX. An Opportunity for New Pharmacological Therapeutic Strategies

The identification of ectopic or abnormal adrenal hormone receptors in cortisol-secreting hyperplasias or tumors provides new opportunities to use specific pharmacological therapies as alternatives to adrenalectomy. This was initially suggested by the short-term improvement of hypercortisolism when T_3 was administered briefly to a patient before resection of an adrenal adenoma in which AC was stimulated by TSH (9); however, these data were very preliminary and could not clearly distinguish whether the changes in cortisol levels reflected spontaneous fluctuations of cortisol secretion or were truly the result of endogenous TSH suppression.

Pharmacological blockade of postprandial GIP release with octreotide was attempted in a few patients with GIP-dependent CS as an alternative to surgery (Table 2; Refs. 201, 208, and 209). During the first months of subcutaneous octreotide administration before each meal, clinical and biological improvements were documented, but long-term treatment proved to be ineffective. It is presumed that the escape of octreotide efficacy was secondary to down-regulation of somatostatin receptors in GIP-secreting intestinal cells. Thus, adrenalectomy remains the long-term treatment of choice for this syndrome until specific GIPR antagonists become available. Short-term use of the oral V1-AVPR antagonist OPC-21268 for 8 days decreased urinary free cortisol levels in a patient with vasopressin-responsive AIMAH and CS (239).

In the patient with catecholamine-dependent CS and bilateral AIMAH (86), initial treatment with propranolol up to 320 mg daily was able to considerably reduce cortisol secretion; however, urinary cortisol levels remained approximately twice the upper limit of normal, and it was decided to remove one of the two very large adrenals surgically. It then became possible, upon restoration of propranolol administration, to completely normalize cortisol production. Interestingly, the control of hypercortisolism was followed by a decreased requirement in the dosage of the β-blocker from 320 mg to 20 mg of propranolol daily, as higher doses were causing adrenal insufficiency. GC are known to stimulate β_2-AR transcription (292) via GRE located in promoters of the target genes (293). The normalization of cortisol levels may have decreased β-AR density, which would explain the lower requirement for the antagonist. Propranolol therapy did not reduce the size of the remaining adrenal even after 3 yr of follow-up; however, the minimal dose of propranolol necessary to maintain normal cortisol production was administered, without blocking the receptors completely. This constituted the first example of long-term pharmacological blockade of an ectopic adrenal membrane hormone receptor.

TABLE 5. Potential pharmacological therapy for abnormal hormone receptors in adrenocortical tumors

Abnormal receptor	Therapy
GIPR	Somatostatin or GIPR antagonist
β-AR	β-blockers
TSHR	L-T_4
V1-AVPR	V1-AVPR antagonist
Angiotensin-II R	AT-1 R antagonist
LH/hCGR	GnRH analogs
5-HT_4R	5-HT_4R antagonists

In the patient with LH/hCG-dependent AIMAH and CS, the suppression of endogenous LH levels with chronic, long-acting leuprolide acetate controlled the hypercortisolism (Fig. 3) and avoided bilateral adrenalectomy (251). Leuprolide acetate, a long-acting GnRH agonist, initially stimulated gonadotropin release, which increased cortisol production for 1 week; this was followed by suppression of endogenous LH levels and normalization of cortisol production. Despite complete suppression of endogenous LH levels, the patient did not present cortisol insufficiency. It is possible that basal cortisol production was maintained by serotonin stimulation, since there was also evidence of abnormal 5-HT_4R function in the same adrenals. The absence of regression of bilateral adrenal hyperplasia, despite chronic suppression of endogenous LH, indicates that its size was maintained by abnormal function of 5-HT_4R, or that aberrant receptors regulate steroidogenesis but not cell proliferation. It will be interesting to study the effects of a specific 5-HT_4R antagonist in this patient when it becomes available. A GnRH analog has previously been used successfully in long-term suppression of testosterone-secreting ovarian tumor (393).

Further studies will probably identify a larger diversity of hormone receptor abnormalities and should eventually allow the use of new pharmacological tools to inhibit either the production of endogenous ligands or block the receptors with appropriate specific antagonists (Table 5). Since it is also possible to detect the presence of ectopic/abnormal hormone receptors at the stage of preclinical steroid hormone production (242), it will be of great interest to investigate whether the progression of adrenal tumors or hyperplasias can be prevented by these new pharmacological approaches.

X. Summary and Conclusions

Taken together, the results of *in vitro* and *in vivo* studies indicate that a wide diversity of abnormal adrenocortical membrane hormone receptors can be present in adrenal CS. These may include ectopic hormone receptors, such as those for GIP, β-adrenergic agonists, LH/hCG, or other receptors capable of coupling to G proteins, AC, and steroidogenesis. There is evidence that the IL-1R, which do not belong to the seven-transmembrane receptor family and do not use the same signaling pathway as the ACTHR, may also become coupled to steroidogenesis. A similar outcome may result from increased or altered activity of eutopic receptors, such as those for vasopressin (V1-AVPR), or 5-HT. The presence of ectopic or abnormal receptors places adrenal cells under stimulation of a trophic factor that is not under the main regulatory negative feedback exerted by GC. This constitutes an unregulated new trophic stimulus, which leads to increased function and possibly to hyperplasia and proliferative advantage. The molecular mechanisms responsible for the ectopic expression of hormone receptors or to increased activation of the signaling cascade and steroidogenesis are still largely unknown. Characterization of the pathophysiology of adrenal hyperplasias or tumors can eventually lead to diverse pharmacological therapies as alternatives to adrenalectomy; this has now been illustrated by the short-term improvement of hypercortisolism with T_3 in TSH-dependent adrenal cortisol-secreting adenoma (9), with octreotide in GIP-dependent CS (201, 208, 209), and by the long-term control of ectopic β-AR and LH/hCGR by propranolol (86) and leuprolide acetate, respectively (251). Further studies will probably identify a larger diversity of hormone receptor abnormalities in adrenal and other endocrine and nonendocrine tissues. Elucidation of the molecular mechanisms leading to abnormal hormone receptor expression will probably contribute to our understanding of the regulation of tissue-specific expression of genes.

Acknowledgments

We wish to acknowledge the late Dr. E. Bolté for his initial contributions in elucidating food- and GIP-dependent Cushing's syndrome. In addition, we thank Drs. J.-M. Boutin, H. Mircescu, W. W. de Herder, I. Bourdeau, R. M. Touyz, L. Y. Deng, R. Larivière, J. R. Cusson, E. L. Schiffrin, G. Rousseau, M. Bouvier, the patients and their referring physicians, CHUM Endocrine Division members, nurses, and laboratory technicians for their contributions to this work. The assistance of Mrs. Victoria Baranga in preparing the manuscript, of Mr. Bruno St-Pierre for medical illustrations, and of Mr. Ovid Da Silva in editing the text is also appreciated.

References

1. **Nieman L, Cutler Jr GB** 1995 Cushing's syndrome. In: De Groot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology. W.B. Saunders Co, Philadelphia, pp 1741–1769
2. **Orth DN, Kovacs WJ** 1998 The adrenal cortex. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (eds) Williams' Textbook of Endocrinology. W.B. Saunders Co, Philadelphia, pp 517–664
3. **Yanovski JA, Cutler Jr GB** 1994 Glucocorticoid action and the clinical features of Cushing's syndrome. Endocrinol Metab Clin North Am 23:487–509
4. **Newell-Price J, Trainer P, Besser M, Grossman A** 1998 The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev 19:647–672
5. **Marieb NJ, Spangler S, Kashgarian M, Heimann A, Schwartz ML, Schwartz PE** 1983 Cushing's syndrome secondary to ectopic cortisol production by an ovarian carcinoma. J Clin Endocrinol Metab 57:737–740
6. **Iida S, Nakamura Y, Fujii H, Nishimura J, Tsugawa M, Gomi M, Fukata J, Tarui S, Moriwaki K, Kitani T** 1990 A patient with hypocortisolism and Cushing's syndrome-like manifestations: cortisol hyperreactive syndrome. J Clin Endocrinol Metab 70:729–737
7. **Newfield RS, Kalaitzoglou G, Licholai T, Chilton D, Ashraf J, Thompson EB, New MI** 2000 Normocortisolemic Cushing's syndrome initially presenting with increased glucocorticoid receptor numbers. J Clin Endocrinol Metab 85:14–21
8. **Schorr I, Ney RL** 1971 Abnormal hormone responses of an adrenocortical cancer adenyl cyclase. J Clin Invest 50:1295–1300
9. **Hingshaw HT, Ney RL** 1974 Abnormal control in the neoplastic adrenal cortex. In: McKerns KW (ed) Hormones and Cancer. Academic Press, New York, pp 309–327
10. **N'Diaye N, Tremblay J, Hamet P, Lacroix A** 1998 Hormone receptor abnormalities in adrenal Cushing's syndrome. Horm Metab Res 30:440–446
11. **Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP** 1998 Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocr Rev 19:101–143
12. **Nussdorfer GG, Mazzocchi G** 1998 Immune-endocrine interactions in the mammalian adrenal gland: facts and hypotheses. Int Rev Cytol 183:143–184
13. **Baylis PH** 1991 Vasopressin and its neurophysin. In: DeGroot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology. W.B. Saunders Co, Philadelphia, pp 406–420
14. **Grossman A** 1999 Corticotropin-releasing hormone: basic physiology and clinical applications. In: DeGroot LJ, Besser GM, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology. W.B. Saunders Co, Philadelphia, pp 341–354
15. **Chen R, Lewis KA, Perrin MH, Vale WW** 1993 Expression cloning of a human corticotropin-releasing-factor receptor. Proc Natl Acad Sci USA 90:8967–8971
16. **de Keyzer Y, Auzan C, Lenne F, Beldjord C, Thibonnier M, Bertagna X, Clauser E** 1994 Cloning and characterization of the human V3 pituitary vasopressin receptor. FEBS Lett 356:215–220
17. **Bertagna X** 1994 Proopiomelanocortin-derived peptides. Endocrinol Metab Clin North Am 23:467–485
18. **Nussdorfer GG, Malendowicz LK** 1998 Role of VIP, PACAP, and related peptides in the regulation of the hypothalamo-pituitary-adrenal axis. Peptides 19:1443–1467
19. **Watanobe H, Tamura T** 1994 Stimulation by peptide histidine methionine (PHM) of adrenocorticotropin secretion in patients with Cushing's disease: a comparison with the effect of vasoactive intestinal peptide (VIP) and a study on the effect of combined administration of corticotropin- releasing hormone with PHM or VIP. J Clin Endocrinol Metab 78:1372–1377
20. **Lacroix S, Rivest S** 1998 Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. J Neurochem 70:452–466
21. **Giraldi FP, Cavagnini F** 1998 Corticotropin-releasing hormone is produced by rat corticotropes and modulates ACTH secretion in a paracrine/autocrine fashion. J Clin Invest 101:2478–2484
22. **Mountjoy KG, Robbins LS, Mortrud MT, Cone RD** 1992 The cloning of a family of genes that encode the melanocortin receptors. Science 257:1248–1251
23. **Naville D, Jaillard C, Barjhoux L, Durand P, Begeot M** 1997 Genomic structure and promoter characterization of the human ACTH receptor gene. Biochem Biophys Res Commun 230:7–12
24. **Simpson ER, Waterman MR** 1988 Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu Rev Physiol 50:427–440
25. **Lehoux JG, Fleury A, Ducharme L** 1998 The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonu-

cleic acid and protein of steroidogenic enzymes in rat adrenal *in vivo*. Endocrinology 139:3913–3922
26. **Miller WL** 1988 Molecular biology of steroid hormone synthesis. Endocr Rev 9:295–318
27. **Stocco DM, Clark BJ** 1996 Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 17:221–244
28. **Clark BJ, Wells J, King SR, Stocco DM** 1994 The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 269:28314–28322
29. **Lin D, Sugawara T, Strauss III JF, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL** 1995 Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267:1828–1831
30. **Bose HS, Sugawara T, Strauss III JF, Miller WL** 1996 The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. N Engl J Med 335:1870–1878
31. **Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL** 1997 Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. Proc Natl Acad Sci USA 94:11540–11545
32. **Papadopoulos V** 1998 Structure and function of the peripheral-type benzodiazepine receptor in steroidogenic cells. Proc Soc Exp Biol Med 217:130–142
33. **Amri H, Ogwuegbu SO, Boujrad N, Drieu K, Papadopoulos V** 1996 *In vivo* regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by Ginkgo biloba extract EGb 761 and isolated ginkgolides. Endocrinology 137:5707–5718
34. **Lehoux JG, Ducharme L** 1995 *In vivo* effects of adrenocorticotropin on c-jun, jun-B, c-fos and fos-B in rat adrenal. Endocr Res 21:267–274
35. **Hol EM, Gispen WH, Bar PR** 1995 ACTH-related peptides: receptors and signal transduction systems involved in their neurotrophic and neuroprotective actions. Peptides 16:979–993
36. **Penhoat A, Jaillard C, Saez JM** 1989 Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. Proc Natl Acad Sci USA 86:4978–4981
37. **Mountjoy KG, Bird IM, Rainey WE, Cone RD** 1994 ACTH induces up-regulation of ACTH receptor mRNA in mouse and human adrenocortical cell lines. Mol Cell Endocrinol 99:R17–R20
38. **Di Blasio AM, Voutilainen R, Jaffe RB, Miller WL** 1987 Hormonal regulation of messenger ribonucleic acids for P450 scc (cholesterol side-chain cleavage enzyme) and P450c17 (17α-hydroxylase/17,20-lyase) in cultured human fetal adrenal cells. J Clin Endocrinol Metab 65:170–175
39. **Murphy EP, Conneely OM** 1997 Neuroendocrine regulation of the hypothalamic pituitary adrenal axis by the nurr1/nur77 subfamily of nuclear receptors. Mol Endocrinol 11:39–47
40. **Sugawara T, Kiriakidou M, McAllister JM, Holt JA, Arakane F, Strauss III JF** 1997 Regulation of expression of the steroidogenic acute regulatory protein (StAR) gene: a central role for steroidogenic factor 1. Steroids 62:5–9
41. **Parker KL, Schimmer BP** 1997 Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 18:361–377
42. **Ingraham HA, Lala DS, Ikeda Y, Luo X, Shen WH, Nachtigal MW, Abbud R, Nilson JH, Parker KL** 1994 The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. Genes Dev 8:2302–2312
43. **Li Y, Lau LF** 1997 Adrenocorticotropic hormone regulates the activities of the orphan nuclear receptor Nur77 through modulation of phosphorylation. Endocrinology 138:4138–4146
44. **Wilson TE, Mouw AR, Weaver CA, Milbrandt J, Parker KL** 1993 The orphan nuclear receptor NGFI-B regulates expression of the gene encoding steroid 21-hydroxylase. Mol Cell Biol 13:861–868
45. **Morohashi K, Zanger UM, Honda S, Hara M, Waterman MR, Omura T** 1993 Activation of CYP11A and CYP11B gene promoters by the steroidogenic cell-specific transcription factor, Ad4BP. Mol Endocrinol 7:1196–1204
46. **Takayama K, Morohashi K, Honda S, Hara N, Omura T** 1994 Contribution of Ad4BP, a steroidogenic cell-specific transcription factor, to regulation of the human CYP11A and bovine CYP11B genes through their distal promoters. J Biochem (Tokyo) 116: 193–203
47. **Watanabe N, Inoue H, Fujii-Kuriyama Y** 1994 Regulatory mechanisms of cAMP-dependent and cell-specific expression of human steroidogenic cytochrome P450 scc (CYP11A1) gene. Eur J Biochem 222:825–834
48. **Clemens JW, Lala DS, Parker KL, Richards JS** 1994 Steroidogenic factor-1 binding and transcriptional activity of the cholesterol side-chain cleavage promoter in rat granulosa cells. Endocrinology 134: 1499–1508
49. **Zhang P, Mellon SH** 1996 The orphan nuclear receptor steroidogenic factor-1 regulates the cyclic adenosine 3′,5′-monophosphate-mediated transcriptional activation of rat cytochrome P450c17 (17α-hydroxylase/c17–20 lyase). Mol Endocrinol 10:147–158
50. **Zhang P, Mellon SH** 1997 Multiple orphan nuclear receptors converge to regulate rat P450c17 gene transcription: novel mechanisms for orphan nuclear receptor action. Mol Endocrinol 11:891–904
51. **Inoue H, Watanabe N, Higashi Y, Fujii-Kuriyama Y** 1991 Structures of regulatory regions in the human cytochrome P-450 scc (desmolase) gene. Eur J Biochem 195:563–569
52. **John ME, John MC, Boggaram V, Simpson ER, Waterman MR** 1986 Transcriptional regulation of steroid hydroxylase genes by corticotropin. Proc Natl Acad Sci USA 83:4715–4719
53. **Bischof LJ, Kagawa N, Moskow JJ, Takahashi Y, Iwamatsu A, Buchberg AM, Waterman MR** 1998 Members of the meis1 and pbx homeodomain protein families cooperatively bind a cAMP-responsive sequence (CRS1) from bovine CYP17. J Biol Chem 273:7941–7948
54. **Kagawa N, Ogo A, Takahashi Y, Iwamatsu A, Waterman MR** 1994 A cAMP-regulatory sequence (CRS1) of CYP17 is a cellular target for the homeodomain protein Pbx1. J Biol Chem 269:18716–18719
55. **Ogo A, Waterman MR, Kagawa N** 1997 cAMP-dependent transactivation involving the homeodomain protein Pbx1. Arch Biochem Biophys 338:193–200
56. **Venepally P, Waterman MR** 1995 Two Sp1-binding sites mediate cAMP-induced transcription of the bovine CYP11A gene through the protein kinase A signaling pathway. J Biol Chem 270:25402–25410
57. **Bakke M, Lund J** 1995 Mutually exclusive interactions of two nuclear orphan receptors determine activity of a cyclic adenosine 3′,5′-monophosphate-responsive sequence in the bovine CYP17 gene. Mol Endocrinol 9:327–339
58. **Jacob AL, Lund J** 1998 Mutations in the activation function-2 core domain of steroidogenic factor-1 dominantly suppresses PKA-dependent transactivation of the bovine CYP17 gene. J Biol Chem 273:13391–13394
59. **Monte D, DeWitte F, Hum DW** 1998 Regulation of the human P450 scc gene by steroidogenic factor 1 is mediated by CBP/p300. J Biol Chem 273:4585–4591
60. **Bischof LJ, Kagawa N, Waterman MR** 1998 The bovine CYP17 promoter contains a transcriptional regulatory element cooperatively bound by tale homeodomain proteins. Endocr Res 24: 489–495
61. **Philips A, Lesage S, Gingras R, Maira MH, Gauthier Y, Hugo P, Drouin J** 1997 Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. Mol Cell Biol 17:5946–5951
62. **Philips A, Maira M, Mullick A, Chamberland M, Lesage S, Hugo P, Drouin J** 1997 Antagonism between Nur77 and glucocorticoid receptor for control of transcription. Mol Cell Biol 17:5952–5959
63. **Rust W, Stedronsky K, Tillmann G, Morley S, Walther N, Ivell R** 1998 The role of SF-1/Ad4BP in the control of the bovine gene for the steroidogenic acute regulatory (StAR) protein. J Mol Endocrinol 21:189–200
64. **Lee SL, Wesselschmidt RL, Linette GP, Kanagawa O, Russell JH, Milbrandt J** 1995 Unimpaired thymic and peripheral T cell death in mice lacking the nuclear receptor NGFI-B (Nur77). Science 269: 532–535
65. **Luo X, Ikeda Y, Parker KL** 1994 A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 77:481–490
66. **Parker KL** 1998 The roles of steroidogenic factor 1 in endocrine development and function. Mol Cell Endocrinol 145:15–20

67. **Jones MT, Gillham B** 1988 Factors involved in the regulation of adrenocorticotropic hormone/β-lipotropic hormone. Physiol Rev 68:743–818
68. **Gallo-Payet N, Guillon G** 1998 Regulation of adrenocortical function by vasopressin. Horm Metab Res 30:360–367
69. **Clements JA, Funder JW** 1986 Arginine vasopressin (AVP) and AVP-like immunoreactivity in peripheral tissues. Endocr Rev 7:449–460
70. **Turnbull AV, Rivier CL** 1999 Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev 79:1–71
71. **Bird IM, Walker SW, Williams BC** 1990 Agonist-stimulated turnover of the phosphoinositides and the regulation of adrenocortical steroidogenesis. J Mol Endocrinol 5:191–209
72. **Bird IM, Nicol M, Williams BC, Walker SW** 1990 Vasopressin stimulates cortisol secretion and phosphoinositide catabolism in cultured bovine adrenal fasciculata/reticularis cells. J Mol Endocrinol 5:109–116
73. **Hinson JP, Vinson GP, Porter ID, Whitehouse BJ** 1987 Oxytocin and arginine vasopressin stimulate steroid secretion by the isolated perfused rat adrenal gland. Neuropeptides 10:1–7
74. **Gallo-Payet N, Guillon G, Balestre MN, Jard S** 1986 Vasopressin induces breakdown of membrane phosphoinositides in adrenal glomerulosa and fasciculata cells. Endocrinology 119:1042–1047
75. **Perraudin V, Delarue C, Lefebvre H, Contesse V, Kuhn JM, Vaudry H** 1993 Vasopressin stimulates cortisol secretion from human adrenocortical tissue through activation of V1 receptors. J Clin Endocrinol Metab 76:1522–1528
76. **Guillon G, Trueba M, Joubert D, Grazzini E, Chouinard L, Cote M, Payet MD, Manzoni O, Barberis C, Robert M** 1995 Vasopressin stimulates steroid secretion in human adrenal glands: comparison with angiotensin-II effect. Endocrinology 136:1285–1295
77. **Guillon G, Grazzini E, Andrez M, Breton C, Trueba M, Serradeil-LeGal C, Boccara G, Derick S, Chouinard L, Gallo-Payet N** 1998 Vasopressin: a potent autocrine/paracrine regulator of mammal adrenal functions. Endocr Res 24:703–710
78. **Guillon G, Gallo-Payet N** 1986 Specific vasopressin binding to rat adrenal glomerulosa cells. Relationship to inositol lipid breakdown. Biochem J 235:209–214
79. **Arnaldi G, Gasc JM, de Keyzer Y, Raffin-Sanson ML, Perraudin V, Kuhn JM, Raux-Demay MC, Luton JP, Clauser E, Bertagna X** 1998 Variable expression of the V1 vasopressin receptor modulates the phenotypic response of steroid-secreting adrenocortical tumors. J Clin Endocrinol Metab 83:2029–2035
80. **Lacroix A, Tremblay J, Touyz RM, Deng LY, Lariviere R, Cusson JR, Schiffrin EL, Hamet P** 1997 Abnormal adrenal and vascular responses to vasopressin mediated by a V1- vasopressin receptor in a patient with adrenocorticotropin-independent macronodular adrenal hyperplasia, Cushing's syndrome, and orthostatic hypotension. J Clin Endocrinol Metab 82:2414–2422
81. **Grazzini E, Lodboerer AM, Perez-Martin A, Joubert D, Guillon G** 1996 Molecular and functional characterization of V1b vasopressin receptor in rat adrenal medulla. Endocrinology 137:3906–3914
82. **Mazza E, Goffi S, Barchi P, Arvat E, Bellone J, Limone P, Ghigo E, Camanni F** 1994 Enhanced adrenocorticotrophic hormone and cortisol responses to corticotrophin-releasing hormone in central idiopathic diabetes insipidus. Eur J Endocrinol 130:121–124
83. **Elias LL, Antunes-Rodrigues J, Elias PC, Moreira AC** 1997 Effect of plasma osmolality on pituitary-adrenal responses to corticotropin-releasing hormone and atrial natriuretic peptide changes in central diabetes insipidus. J Clin Endocrinol Metab 82:1243–1247
84. **Walker SW, Lightly ER, Clyne C, Williams BC, Bird IM** 1991 Adrenergic and cholinergic regulation of cortisol secretion from the zona fasciculata/reticularis of bovine adrenal cortex. Endocr Res 17:237–265
85. **Mazzocchi G, Gottardo G, Nussdorfer GG** 1997 Catecholamines stimulate steroid secretion of dispersed fowl adrenocortical cells, acting through the β-receptor subtype. Horm Metab Res 29:190–192
86. **Lacroix A, Tremblay J, Rousseau G, Bouvier M, Hamet P** 1997 Propranolol therapy for ectopic beta-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med 337:1429–1434
87. **Lefebvre H, Contesse V, Delarue C, Vaudry H, Kuhn JM** 1998 Serotonergic regulation of adrenocortical function. Horm Metab Res 30:398–403
88. **Delarue C, Lefebvre H, Idres S, Leboulenger F, Homo-Delarche G, Lihrmann I, Feuilloley M, Vaudry H** 1988 Serotonin stimulates corticosteroid secretion by frog adrenocortical tissue *in vitro*. J Steroid Biochem 29:519–525
89. **Lefebvre H, Contesse V, Delarue C, Feuilloley M, Hery F, Grise P, Raynaud G, Verhofstad AA, Wolf LM, Vaudry H** 1992 Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin4 receptor subtype. Neuroscience 47:999–1007
90. **Hinson JP, Vinson GP, Pudney J, Whitehouse BJ** 1989 Adrenal mast cells modulate vascular and secretory responses in the intact adrenal gland of the rat. J Endocrinol 121:253–260
91. **Lefebvre H, Contesse V, Delarue C, Soubrane C, Legrand A, Kuhn JM, Wolf LM, Vaudry H** 1993 Effect of the serotonin-4 receptor agonist zacopride on aldosterone secretion from the human adrenal cortex: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab 77:1662–1666
92. **Lefebvre H, Contesse V, Delarue C, Legrand A, Kuhn JM, Vaudry H, Wolf LM** 1995 The serotonin-4 receptor agonist cisapride and angiotensin-II exert additive effects on aldosterone secretion in normal man. J Clin Endocrinol Metab 80:504–507
93. **Delarue C, Leboulenger F, Morra M, Hery F, Verhofstad AJ, Berod A, Denoroy L, Pelletier G, Vaudry H** 1988 Immunohistochemical and biochemical evidence for the presence of serotonin in amphibian adrenal chromaffin cells. Brain Res 459:17–26
94. **Verhofstad AA, Jonsson G** 1983 Immunohistochemical and neurochemical evidence for the presence of serotonin in the adrenal medulla of the rat. Neuroscience 10:1443–1453
95. **Fernandez-Vivero J, Rodriguez-Sanchez F, Verastegui C, Cordoba MF, Romero A, de Castro JM** 1993 Immunocytochemical distribution of serotonin and neuropeptide Y (NPY) in mouse adrenal gland. Histol Histopathol 8:509–520
96. **Bornstein SR, Haidan A, Ehrhart-Bornstein M** 1996 Cellular communication in the neuro-adrenocortical axis: role of vasoactive intestinal polypeptide (VIP). Endocr Res 22:819–829
97. **Bodnar M, Sarrieau A, Deschepper CF, Walker CD** 1997 Adrenal vasoactive intestinal peptide participates in neonatal corticosteroid production in the rat. Am J Physiol 273:R1163–R1172
98. **Li ZG, Queen G, LaBella FS** 1990 Adrenocorticotropin, vasoactive intestinal polypeptide, growth hormone-releasing factor, and dynorphin compete for common receptors in brain and adrenal. Endocrinology 126:1327–1333
99. **Anderova M, Duchene AD, Barbara JG, Takeda K** 1998 Vasoactive intestinal peptide potentiates and directly stimulates catecholamine secretion from rat adrenal chromaffin cells. Brain Res 809: 97–106
100. **Guo X, Wakade AR** 1994 Differential secretion of catecholamines in response to peptidergic and cholinergic transmitters in rat adrenals. J Physiol (Lond) 475:539–545
101. **Bernet F, Bernard J, Laborie C, Montel V, Maubert E, Dupouy JP** 1994 Neuropeptide Y (NPY)- and vasoactive intestinal peptide (VIP)-induced aldosterone secretion by rat capsule/glomerular zone could be mediated by catecholamines via β 1 adrenergic receptors. Neurosci Lett 166:109–112
102. **Hinson JP, Kapas S, Orford CD, Vinson GP** 1992 Vasoactive intestinal peptide stimulation of aldosterone secretion by the rat adrenal cortex may be mediated by the local release of catecholamines. J Endocrinol 133:253–258
103. **Hinson JP, Cameron LA, Purbrick A, Kapas S** 1994 The role of neuropeptides in the regulation of adrenal vascular tone: effects of vasoactive intestinal polypeptide, substance P, neuropeptide Y, neurotensin, Met-enkephalin, and Leu-enkephalin on perfusion medium flow rate in the intact perfused rat adrenal. Regul Pept 51:55–61
104. **Nussdorfer GG** 1996 Paracrine control of adrenal cortical function by medullary chromaffin cells. Pharmacol Rev 48:495–530
105. **Bird IM, Magness RR, Mason JI, Rainey WE** 1992 Angiotensin-II acts via the type 1 receptor to inhibit 17α-hydroxylase cytochrome P450 expression in ovine adrenocortical cells. Endocrinology 130: 3113–3121

106. **Clark BJ, Pezzi V, Stocco DM, Rainey WE** 1995 The steroidogenic acute regulatory protein is induced by angiotensin II and K^+ in H295R adrenocortical cells. Mol Cell Endocrinol 115:215–219
107. **Harrison-Bernard LM, El Dahr SS, O'Leary DF, Navar LG** 1999 Regulation of angiotensin II type 1 receptor mRNA and protein in angiotensin II-induced hypertension. Hypertension 33:340–346
108. **Giacchetti G, Opocher G, Sarzani R, Rappelli A, Mantero F** 1996 Angiotensin II and the adrenal. Clin Exp Pharmacol Physiol Suppl 3:S119–S124
109. **Penhoat A, Ouali R, Jaillard C, Langlois D, Begeot M, Saez JM** 1991 Characterization and regulation of angiotensin and corticotropin receptors on cultured bovine adrenal cells. Endocr Res 17: 1–18
110. **Naville D, Lebrethon MC, Kermabon AY, Rouer E, Benarous R, Saez JM** 1993 Characterization and regulation of the angiotensin II type-1 receptor (binding and mRNA) in human adrenal fasciculata-reticularis cells. FEBS Lett 321:184–188
111. **Gupta P, Franco-Saenz R, Mulrow PJ** 1995 Locally generated angiotensin II in the adrenal gland regulates basal, corticotropin-, and potassium-stimulated aldosterone secretion. Hypertension 25: 443–448
112. **Mulrow PJ** 1998 Renin-angiotensin system in the adrenal. Horm Metab Res 30:346–349
113. **Vinson GP, Ho MM** 1998 The adrenal renin/angiotensin system in the rat. Horm Metab Res 30:355–359
114. **Missale C, Memo M, Liberini P, Spano P** 1988 Dopamine selectively inhibits angiotensin II-induced aldosterone secretion by interacting with D-2 receptors. J Pharmacol Exp Ther 246:1137–1143
115. **Kasprzak A, Rebuffat P, Andreis PG, Mazzocchi G, Nussdorfer GG** 1991 Effects of prolonged cysteamine administration on the rat adrenal cortex: evidence that endogenous somatostatin is involved in the control of the growth and steroidogenic capacity of zona glomerulosa. J Steroid Biochem Mol Biol 38:469–473
116. **Bodart V, Rainey WE, Fournier A, Ong H, De Lean A** 1996 The H295R human adrenocortical cell line contains functional atrial natriuretic peptide receptors that inhibit aldosterone biosynthesis. Mol Cell Endocrinol 118:137–144
117. **Kawai M, Naruse M, Yoshimoto T, Naruse K, Shionoya K, Tanaka M, Morishita Y, Matsuda Y, Demura R, Demura H** 1996 C-type natriuretic peptide as a possible local modulator of aldosterone secretion in bovine adrenal zona glomerulosa. Endocrinology 137:42–46
118. **Cherradi N, Brandenburger Y, Rossier MF, Vallotton MB, Stocco DM, Capponi AM** 1998 Atrial natriuretic peptide inhibits calcium-induced steroidogenic acute regulatory protein gene transcription in adrenal glomerulosa cells. Mol Endocrinol 12:962–972
119. **Hinson JP, Kapas S** 1998 The role of endothelial cell products in the regulation of adrenocortical function: actions of endothelin, nitric oxide, adrenomedullin and PAMP. Horm Metab Res 30: 334–340
120. **Nussdorfer GG, Rossi GP, Belloni AS** 1997 The role of endothelins in the paracrine control of the secretion and growth of the adrenal cortex. Int Rev Cytol 171:267–308
121. **Mazzocchi G, Malendowicz LK, Macchi C, Gottardo G, Nussdorfer GG** 1996 Further investigations on the effects of neuropeptide Y on the secretion and growth of rat adrenal zona glomerulosa. Neuropeptides 30:19–27
122. **Malendowicz LK, Markowska A, Zabel M** 1996 Neuropeptide Y-related peptides and hypothalamo-pituitary-adrenal axis function. Histol Histopathol 11:485–494
123. **Rohner-Jeanrenaud F, Jeanrenaud B** 1996 Obesity, leptin, and the brain. N Engl J Med 334:324–325
124. **Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS** 1996 Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252
125. **Flier JS** 1998 Clinical review 94: What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab 83:1407–1413
126. **Jin L, Burguera BG, Couce ME, Scheithauer BW, Lamsan J, Eberhardt NL, Kulig E, Lloyd RV** 1999 Leptin and leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. J Clin Endocrinol Metab 84:2903–2911
127. **Shimon I, Yan X, Magoffin DA, Friedman TC, Melmed S** 1998 Intact leptin receptor is selectively expressed in human fetal pituitary and pituitary adenomas and signals human fetal pituitary growth hormone secretion. J Clin Endocrinol Metab 83:4059–4064
128. **Glasow A, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP, Bornstein SR** 1998 Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. J Clin Endocrinol Metab 83:4459–4466
129. **Cao GY, Considine RV, Lynn RB** 1997 Leptin receptors in the adrenal medulla of the rat. Am J Physiol 273:E448–E452
130. **Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, Gaillard RC** 1998 Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. Endocrinology 139:4264–4268
131. **Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS** 1997 Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 138:3859–3863
132. **Huang Q, Rivest R, Richard D** 1998 Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. Endocrinology 139:1524–1532
133. **Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA** 1997 Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. Diabetes 46:1235–1238
134. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG** 1996 Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101–1106
135. **Malendowicz LK, Macchi C, Nussdorfer GG, Nowak KW** 1998 Acute effects of recombinant murine leptin on rat pituitary-adrenocortical function. Endocr Res 24:235–246
136. **Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J, Stephens TW** 1996 Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J Biol Chem 271:5301–5304
137. **Spinedi E, Gaillard RC** 1998 A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH. Endocrinology 139:4016–4020
138. **Cizza G, Lotsikas AJ, Licinio J, Gold PW, Chrousos GP** 1997 Plasma leptin levels do not change in patients with Cushing's disease shortly after correction of hypercortisolism. J Clin Endocrinol Metab 82:2747–2750
139. **Pralong FP, Gomez F, Guillou L, Mosimann F, Franscella S, Gaillard RC** 1999 Food-dependent Cushing's syndrome: possible involvement of leptin in cortisol hypersecretion. J Clin Endocrinol Metab 84:3817–3822
140. **Belloni AS, Mazzocchi G, Meneghelli V, Nussdorfer GG** 1978 Cytogenesis in the rat adrenal cortex: evidence for an ACTH-induced centripetal cell migration from the zona glomerulosa. Arch Anat Histol Embryol 61:195–205
141. **Kahri A** 1966 Histochemical and electron microscopic studies on the cells of the rat adrenal cortex in tissue culture. Acta Endocrinol (Copenh) 52:[Suppl 108]:1–96
142. **Arola J, Heikkila P, Voutilainen R, Kahri AI** 1994 Corticosterone regulates cell proliferation and cytochrome P450 cholesterol side-chain cleavage enzyme messenger ribonucleic acid expression in primary cultures of fetal rat adrenals. Endocrinology 135:2064–2069
143. **Salmenpera M, Kahri AI, Saure A** 1976 Effects of corticosterone on adrenocorticotrophin-induced mitochondrial differentiation with special reference to 11 β- and 18-hydroxylation. J Endocrinol 70: 215–222
144. **Salmenpera M** 1976 Comparison of the ultrastructural and steroidogenic properties of mitochondria of fetal rat adrenals in tissue culture. A morphometric and a gas chromatographic analysis. J Ultrastruct Res 56:277–286
145. **Armelin HA, Lotfi CF, Lepique AP** 1996 Regulation of growth by ACTH in the Y-1 line of mouse adrenocortical cells. Endocr Res 22:373–383
146. **Penhoat A, Ouali R, Viard I, Langlois D, Saez JM** 1996 Regulation of primary response and specific genes in adrenal cells by peptide hormones and growth factors. Steroids 61:176–183
147. **Watanabe G, Pena P, Albanese C, Wilsbacher LD, Young JB,**

Pestell RG 1997 Adrenocorticotropin induction of stress-activated protein kinase in the adrenal cortex *in vivo*. J Biol Chem 272:20063–20069

148. **Lotfi CF, Todorovic Z, Armelin HA, Schimmer BP** 1997 Unmasking a growth-promoting effect of the adrenocorticotropic hormone in Y1 mouse adrenocortical tumor cells. J Biol Chem 272:29886–29891
149. **Kimura E, Sonobe MH, Armelin MC, Armelin HA** 1993 Induction of FOS and JUN proteins by adrenocorticotropin and phorbol ester but not by 3′,5′-cyclic adenosine monophosphate derivatives. Mol Endocrinol 7:1463–1471
150. **Karin M** 1995 The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 270:16483–16486
151. **Watanabe G, Lee RJ, Albanese C, Rainey WE, Batlle D, Pestell RG** 1996 Angiotensin II activation of cyclin D1-dependent kinase activity. J Biol Chem 271:22570–22577
152. **Malendowicz LK, Nussdorfer GG** 1993 Unusual effect of prolonged vasoactive intestinal peptide (VIP) administration on the adrenal growth and corticosterone secretion in the rat. Neuropeptides 25:145–150
153. **Rebuffat P, Nowak KW, Tortorella C, Musajo FG, Gottardo G, Mazzocchi G, Nussdorfer GG** 1994 Evidence that endogenous vasoactive intestinal peptide (VIP) plays a role in the maintenance of the growth and steroidogenic capacity of rat adrenal zona glomerulosa. J Steroid Biochem Mol Biol 51:81–88
154. **Mesiano S, Katz SL, Lee JY, Jaffe RB** 1997 Insulin-like growth factors augment steroid production and expression of steroidogenic enzymes in human fetal adrenal cortical cells: implications for adrenal androgen regulation. J Clin Endocrinol Metab 82:1390–1396
155. **Voutilainen R, Ilvesmaki V, Ariel I, Rachmilewitz J, de Groot N, Hochberg A** 1994 Parallel regulation of parentally imprinted H19 and insulin-like growth factor-II genes in cultured human fetal adrenal cells. Endocrinology 134:2051–2056
156. **Ilvesmaki V, Kahri AI, Miettinen PJ, Voutilainen R** 1993 Insulin-like growth factors (IGFs) and their receptors in adrenal tumors: high IGF-II expression in functional adrenocortical carcinomas. J Clin Endocrinol Metab 77:852–858
157. **Gicquel C, Bertagna X, Le Bouc Y** 1995 Recent advances in the pathogenesis of adrenocortical tumours. Eur J Endocrinol 133: 133–144
158. **Le Roy C, Li JY, Stocco DM, Langlois D, Saez JM** 2000 Regulation by adrenocorticotropin (ACTH), angiotensin II, transforming growth factor-β and insulin-like growth factor I of bovine adrenal cell steroidogenic capacity and expression of ACTH receptor steroidogenic acute regulatory protein cytochrome P450c17, and 3β-hydroxysteroid dehydrogenase. Endocrinology 141:1599–1607
159. **Riopel L, Branchaud CL, Goodyer CG, Adkar V, Lefebvre Y** 1989 Growth-inhibitory effect of TGF-B on human fetal adrenal cells in primary monolayer culture. J Cell Physiol 140:233–238
160. **Spencer SJ, Mesiano S, Lee JY, Jaffe RB** 1999 Proliferation and apoptosis in the human adrenal cortex during the fetal and perinatal periods: implications for growth and remodeling. J Clin Endocrinol Metab 84:1110–1115
161. **Ross NS** 1994 Epidemiology of Cushing's syndrome and subclinical disease. Endocrinol Metab Clin North Am 23:539–546
162. **Bertagna C, Orth DN** 1981 Clinical and laboratory findings and results of therapy in 58 patients with adrenocortical tumors admitted to a single medical center (1951 to 1978). Am J Med 71: 855–875
163. **Latronico AC, Chrousos GP** 1997 Extensive personal experience: adrenocortical tumors. J Clin Endocrinol Metab 82:1317–1324
164. **Bornstein SR, Stratakis CA, Chrousos GP** 1999 Adrenocortical tumors: recent advances in basic concepts and clinical management. Ann Intern Med 130:759–771
165. **Samuels MH, Loriaux DL** 1994 Cushing's syndrome and the nodular adrenal gland. Endocrinol Metab Clin North Am 23:555–569
166. **Trainer PJ, Grossman A** 1991 The diagnosis and differential diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf) 34:317–330
167. **Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL** 1985 The complex of myxomas, spotty pigmentation, and endocrine overactivity. Medicine (Baltimore) 64:270–283
168. **Stratakis CA, Carney JA, Lin JP, Papanicolaou DA, Karl M, Kastner DL, Pras E, Chrousos GP** 1996 Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. J Clin Invest 97:699–705
169. **Casey M, Mah C, Merliss AD, Kirschner LS, Taymans SE, Denio AE, Korf B, Irvine AD, Hughes A, Carney JA, Stratakis CA, Basson CT** 1998 Identification of a novel genetic locus for familial cardiac myxomas and Carney complex. Circulation 98:2560–2566

169a. **Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA** 2000 Mutations of the gene encoding the protein kinase A Type 1-α regulatory subunit in patients with the Carney complex. Nat Genet 26:89–92

170. **Stratakis CA, Carney JA, Kirschner LS, Willenberg HS, Brauer S, Ehrhart-Bornstein M, Bornstein SR** 1999 Synaptophysin immunoreactivity in primary pigmented nodular adrenocortical disease: neuroendocrine properties of tumors associated with Carney complex. J Clin Endocrinol Metab 84:1122–1128
171. **Stratakis CA, Sarlis N, Kirschner LS, Carney JA, Doppman JL, Nieman LK, Chrousos GP, Papanicolaou DA** 1999 Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. Ann Intern Med 131:585–591
172. **Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM** 1991 Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med 325:1688–1695
173. **Boston BA, Mandel S, LaFranchi S, Bliziotes M** 1994 Activating mutation in the stimulatory guanine nucleotide-binding protein in an infant with Cushing's syndrome and nodular adrenal hyperplasia. J Clin Endocrinol Metab 79:890–893
174. **Lieberman SA, Eccleshall TR, Feldman D** 1994 ACTH-independent massive bilateral adrenal disease (AIMBAD): a subtype of Cushing's syndrome with major diagnostic and therapeutic implications. Eur J Endocrinol 131:67–73
175. **Stratakis CA, Kirschner LS** 1998 Clinical and genetic analysis of primary bilateral adrenal diseases (micro- and macronodular disease) leading to Cushing syndrome. Horm Metab Res 30:456–463
176. **Malchoff CD, Rosa J, DeBold CR, Kozol RA, Ramsby GR, Page DL, Malchoff DM, Orth DN** 1989 Adrenocorticotropin-independent bilateral macronodular adrenal hyperplasia: an unusual cause of Cushing's syndrome. J Clin Endocrinol Metab 68:855–860
177. **Doppman JL, Miller DL, Dwyer AJ, Loughlin T, Nieman L, Cutler GB, Chrousos GP, Oldfield E, Loriaux DL** 1988 Macronodular adrenal hyperplasia in Cushing disease. Radiology 166:347–352
178. **Doppman JL, Nieman LK, Travis WD, Miller DL, Cutler Jr GB, Chrousos GP, Norton JA** 1991 CT and MR imaging of massive macronodular adrenocortical disease: a rare cause of autonomous primary adrenal hypercortisolism. J Comput Assist Tomogr 15: 773–779
179. **Findlay JC, Sheeler LR, Engeland WC, Aron DC** 1993 Familial adrenocorticotropin-independent Cushing's syndrome with bilateral macronodular adrenal hyperplasia. J Clin Endocrinol Metab 76:189–191
180. **Minami S, Sugihara H, Sato J, Tatsukuchi A, Sugisaki Y, Sasano H, Wakabayashi I** 1996 ACTH independent Cushing's syndrome occurring in siblings. Clin Endocrinol (Oxf) 44:483–488
181. **Cooper RJ, Udelsman R, Resta C, Tollin SR**, Familial Cushing's syndrome secondary to bilateral macronodular hyperplasia. Program and Abstracts of The 80th Annual Meeting of The Endocrine Society, New Orleans, LA, 1998 (Abstract P2–397), p 333
182. **Grunenberger F, Noal E, Bachellier P, Weber JC, Jaeck D, Schlienger JL** 1999 Hypercorticisme familial non ACTH dépendant par hyperplasie macronodulaire bilatérale des surrénales. Ann Endocrinol (Paris) 60:290 (Abstract A04)
183. **Fragoso MCB, Latronico AC, Domenice S, Lando VS, Mendonca BB,** Activation mutation in the stimulatory guanine nucleotide-binding protein in a woman with Cushing's syndrome by ACTH-independent macronodular adrenal hyperplasia. Program and Abstracts of The 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999 (Abstract P2–150), p 312
184. **Schorr I, Rathnam P, Saxena BB, Ney RL** 1971 Multiple specific hormone receptors in the adenylate cyclase of an adrenocortical carcinoma. J Biol Chem 246:5806–5811
185. **Williams LT, Gore TB, Lefkowitz RJ** 1977 Ectopic β-adrenergic receptor binding sites. Possible molecular basis of aberrant cate-

cholamine responsiveness of an adrenocortical tumor adenylate cyclase. J Clin Invest 59:319–324
186. **Brush JS, Sutliff LS, Sharma RK** 1974 Metabolic regulation and adenyl cyclase activity of adrenocortical carcinoma cultured cells. Cancer Res 34:1495–1502
187. **Taunton OD, Roth J, Pastan I** 1969 Studies on the adrenocorticotropic hormone-activated adenyl cyclase of a functional adrenal tumor. J Biol Chem 244:247–253
188. **Perchellet JP, Sharma RK** 1980 Ectopic alpha-adrenergic mediated accumulation of guanosine 3′,5′- monophosphate in isolated adrenocortical carcinoma cells. Endocrinology 106:1589–1593
189. **Shanker G, Sharma RK** 1980 Characterization of ectopic α-adrenergic binding receptors of adrenocortical carcinoma cells. Endocrinology 106:1594–1598
190. **Millington DS, Golder MP, Cowley T, London D, Roberts H, Butt WR, Griffiths K** 1976 *In vitro* synthesis of steroids by a feminising adrenocortical carcinoma: effect of prolactin and other protein hormones. Acta Endocrinol (Copenh) 82:561–571
191. **Matsukura S, Kakita T, Sueoka S, Yoshimi H, Hirata Y, Yokota M, Fujita T** 1980 Multiple hormone receptors in the adenylate cyclase of human adrenocortical tumors. Cancer Res 40:3768–3771
192. **Hirata Y, Uchihashi M, Sueoka S, Matsukura S, Fujita T** 1981 Presence of ectopic β-adrenergic receptors on human adrenocortical cortisol-producing adenomas. J Clin Endocrinol Metab 53: 953–957
193. **Katz MS, Kelly TM, Dax EM, Pineyro MA, Partilla JS, Gregerman RI** 1985 Ectopic β-adrenergic receptors coupled to adenylate cyclase in human adrenocortical carcinomas. J Clin Endocrinol Metab 60:900–909
194. **Saez JM, Tell GP, Dazord A** 1978 Human adrenocortical tumors: alterations in membrane-bound hormone receptors and cAMP protein kinases. In: Sharma RK, Criss WE (eds) Endocrine Control in Neoplasia. Raven Press, New York, pp 53–69
195. **Leinonen P, Ranta T, Siegberg R, Pelkonen R, Heikkila P, Kahri A** 1991 Testosterone-secreting virilizing adrenal adenoma with human chorionic gonadotrophin receptors and 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 34:31–35
196. **Pittaway DE, Andersen RN, Givens JR** 1973 *In vitro* on an HCG responsive, testosterone secreting adrenal cortical adenoma. Steroids 22:731–745
197. **Why L, Carlson HE, Kane P, Li X, Lei ZM, Rao CV,** Cushing's syndrome secondary to an hCG/LH receptor-positive adrenal carcinoma presenting in the postpartum period. Program and abstracts of The 82nd Annual Meeting of The Endocrine Society, Toronto Ontario, Canada, 2000 (Abstract 2040), p 493
198. **Willenberg HS, Stratakis CA, Marx C, Ehrhart-Bornstein M, Chrousos GP, Bornstein SR** 1998 Aberrant interleukin-1 receptors in a cortisol-secreting adrenal adenoma causing Cushing's syndrome. N Engl J Med 339:27–31
199. **Hamet P, Larochelle P, Franks DJ, Cartier P, Bolte E** 1987 Cushing syndrome with food-dependent periodic hormonogenesis. Clin Invest Med 10:530–533
200. **Lacroix A, Bolte E, Tremblay J, Dupre J, Poitras P, Fournier H, Garon J, Garrel D, Bayard F, Taillefer R, Flanagan RJ, Hamet P** 1992 Gastric inhibitory polypeptide-dependent cortisol hypersecretion—a new cause of Cushing's syndrome. N Engl J Med 327: 974–980
201. **Reznik Y, Allali-Zerah V, Chayvialle JA, Leroyer R, Leymarie P, Travert G, Lebrethon MC, Budi I, Balliere AM, Mahoudeau J** 1992 Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. N Engl J Med 327:981–986
202. **Olsen NJ, Fang VS, DeGroot LJ** 1978 Cushing's syndrome due to adrenal adenoma with persistent diurnal cortisol secretory rhythm. Metabolism 27:695–700
203. **Lacroix A, Bolté E, Poitras P, Fournier H, Garon J, Garrel D, Bayard F, Tremblay J, Taillefer R, Flanagan R, Hamet P** 1991 Gastric inhibitory polypeptide-dependent macro-nodular adrenal hyperplasia: a new etiology of Cushing's syndrome. Program and Abstracts of the 73rd Annual Meeting of The Endocrine Society, Washington, DC, 1991 (Abstract 241), p 91
204. **Mahoudeau J, Reznik Y** 1993 Appetite comes with eating, cortisol too. A new cause of Cushing's syndrome (editorial). Presse Méd 22:407–408
205. **Lebrethon MC, Avallet O, Reznik Y, Archambeaud F, Combes J, Usdin TB, Narboni G, Mahoudeau J, Saez JM** 1998 Food-dependent Cushing's syndrome: characterization and functional role of gastric inhibitory polypeptide receptor in the adrenals of three patients. J Clin Endocrinol Metab 83:4514–4519
206. **N'Diaye N, Hamet P, Tremblay J, Boutin JM, Gaboury L, Lacroix A** 1999 Asynchronous development of bilateral nodular adrenal hyperplasia in gastric inhibitory polypeptide-dependent Cushing's syndrome. J Clin Endocrinol Metab 84:2616–2622
207. **Archambeaud-Mouveroux F, Comas JM, Tessier MP,** Food-dependent Cushing's syndrome associated with the Carney complex. Program and abstracts of the 10th International Congress of Endocrinology, San Francisco, CA, 1996 (Abstract P3–158), p 899
208. **Croughs RJ, Zelissen PM, Van Vroonhoven ThJ Hofland LJ, N'Diaye N, Lacroix A, de Herder WW** 2000 GIP-dependent adrenal Cushing's syndrome with incomplete suppression of ACTH. Clin Endocrinol (Oxf) 52:235–240
208a. **Lacroix A, N'Diaye N, de Herder W, Nieman L, Ezzat S, Hermus A, Noordam C, Gerl H, Lochs H, Pico A, Hamet P, Tremblay J,** Adrenal GIP receptor overexpression in food-dependent Cushing's Syndrome. Program and Abstracts of the 11th International Congress of Endocrinology, Sydney, Australia, 2000 (Abstract P18), p 99
209. **de Herder W, Hofland LJ, Usdin TB, de Jong FH, Uitterlinden P, van Koetsveld P, Mezey E, Bonner TI, Bonjer HJ, Lamberts SW** 1996 Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. J Clin Endocrinol Metab 81:3168–3172
210. **Chabre O, Liakos P, Vivier J, Chaffanjon P, Labat-Moleur F, Martinie M, Bottari SP, Bachelot I, Chambaz EM, Defaye G, Feige JJ** 1998 Cushing's syndrome due to a gastric inhibitory polypeptide-dependent adrenal adenoma: insights into hormonal control of adrenocortical tumorigenesis. J Clin Endocrinol Metab 83:3134–3143
211. **Luton JP, Bertherat J, Kuhn JM, Bertagna X** 1998 Aberrant expression of the GIP (gastric inhibitory polypeptide) receptor in an adrenal cortical adenoma responsible for a case of food-dependent Cushing's syndrome. Bull Acad Natl Med 182:1839–1849
212. **Combes J, Bourrinet-Pellet E, Narboni G, Saez JM, Remy-Martin MA, Mantion G, Cornette C, De Wazières B, Penfornis A** 1998 Un nouveau cas de syndrome de Cushing dépendant de l'alimentation associé à un adénome surrénalien unique. Ann Endocrinol (Paris) 59:264 (Abstract A171)
213. **Tsagarakis S, Tsigos C, Vassiliou V, Tsiotra P, Pratsinis H, Kletsas D, Trivizas P, Nikou A, Mavromatis T, Sotsiou F, Raptis S, Thalassinos N** 2001 Food-dependent androgen and cortisol secretion by a GIP-receptor expressive adrenocortical adenoma leading to hirsutism and subclinical Cushing's syndrome: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab 86:583–589
214. **Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI** 1993 Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 133:2861–2870
215. **Yasuda K, Inagaki N, Yamada Y, Kubota A, Seino S, Seino Y** 1994 Hamster gastric inhibitory polypeptide receptor expressed in pancreatic islets and clonal insulin-secreting cells: its structure and functional properties. Biochem Biophys Res Commun 205:1556–1562
216. **Gremlich S, Porret A, Hani EH, Cherif D, Vionnet N, Froguel P, Thorens B** 1995 Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes 44:1202–1208
217. **Volz A, Goke R, Lankat-Buttgereit B, Fehmann HC, Bode HP, Goke B** 1995 Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. FEBS Lett 373:23–29
218. **Yamada Y, Hayami T, Nakamura K, Kaisaki PJ, Someya Y, Wang CZ, Seino S, Seino Y** 1995 Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. Genomics 29: 773–776
218a. **Miyawaki K, Yamada Y, Yano H, Niwa H, Ban H, Ihara Y, Kubota**

A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y 1999 Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci USA 96:14843–14847

219. **N'Diaye N, Tremblay J, Hamet P, de Herder WW, Lacroix A** 1998 Adrenocortical overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. J Clin Endocrinol Metab 83:2781–2785
220. **Lacroix A, Bolté É, Tremblay J, Hamet P** 1993 Syndrome de Cushing induit par le GIP: expression clinique d'un récepteur ectopique. Med Sci 9:706–715
221. **Bercovici JP, Monguillon P, Merceur C, Codet JP, Roger P, Floch HH** 1996 Cushing's disease: role of feeding. Presse Méd 25:1326–1330
222. **Follenius M, Brandenberger G, Hietter B** 1982 Diurnal cortisol peaks and their relationships to meals. J Clin Endocrinol Metab 55:757–761
223. **Quigley ME, Yen SS** 1979 A mid-day surge in cortisol levels. J Clin Endocrinol Metab 49:945–947
224. **Ishizuka B, Quigley ME, Yen SS** 1983 Pituitary hormone release in response to food ingestion: evidence for neuroendocrine signals from gut to brain. J Clin Endocrinol Metab 57:1111–1116
225. **Larochelle P, Du SP, Bolte E, Lelorier J, Goyer R** 1983 Tixocortol pivalate, a corticosteroid with no systemic glucocorticoid effect after oral, intrarectal, and intranasal application. Clin Pharmacol Ther 33:343–350
226. **Al Damluji S, Iveson T, Thomas JM, Pendlebury DJ, Rees LH, Besser GM** 1987 Food-induced cortisol secretion is mediated by central α-1 adrenoceptor modulation of pituitary ACTH secretion. Clin Endocrinol (Oxf) 26:629–636
227. **Coslovsky R, Wajchenberg BL, Nogueira O** 1974 Hyperresponsiveness to lysine-vasopressin in Cushing's disease. Acta Endocrinol (Copenh) 75:125–132
228. **Raux MC, Binoux M, Luton JP, Gourmelen M, Girard F** 1975 Studies of ACTH secretion control in 116 cases of Cushing's syndrome. J Clin Endocrinol Metab 40:186–197
229. **Arnaldi G, de Keyzer Y, Gasc JM, Clauser E, Bertagna X** 1998 Vasopressin receptors modulate the pharmacological phenotypes of Cushing's syndrome. Endocr Res 24:807–816
230. **van Wijk PA, Rijnberk A, Croughs RJ, Wolfswinkel J, Selman PJ, Mol JA** 1994 Responsiveness to corticotropin-releasing hormone and vasopressin in canine Cushing's syndrome. Eur J Endocrinol 130:410–416
231. **Demura R, Demura H, Nunokawa T, Baba H, Miura K** 1972 Responses of plasma ACTH, GH, LH and 11-hydroxycorticosteroids to various stimuli in patients with Cushing's syndrome. J Clin Endocrinol Metab 34:852–859
232. **Makino S, Hashimoto K, Sugiyama M, Hirasawa R, Takao T, Ota Z, Saegusa M, Ohashi T, Omori H** 1989 Cushing's syndrome due to huge nodular adrenocortical hyperplasia with fluctuation of urinary 17-OHCS excretion. Endocrinol Jpn 36:655–663
233. **Itagaki E, Nozaki M, Abe Y, Takizawa M, Furukawa H, Murakawa S** 1989 Direct effect of lysine-vasopressin on cortisol production in isolated adrenal cells from nodular adrenocortical hyperplasia. In: Mantero F (ed) Serono Symposia, Raven Press, New York, vol 57:403–408
234. **Horiba N, Suda T, Aiba M, Naruse M, Nomura K, Imamura M, Demura H** 1995 Lysine vasopressin stimulation of cortisol secretion in patients with adrenocorticotropin-independent macronodular adrenal hyperplasia. J Clin Endocrinol Metab 80:2336–2341
235. **Perraudin V, Delarue C, de Keyzer Y, Bertagna X, Kuhn JM, Contesse V, Clauser E, Vaudry H** 1995 Vasopressin-responsive adrenocortical tumor in a mild Cushing's syndrome: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab 80:2661–2667
236. **Guillon G, Balestre MN, Roberts JM, Bottari SP** 1987 Oxytocin and vasopressin: distinct receptors in myometrium. J Clin Endocrinol Metab 64:1129–1135
237. **Iida K, Kaji H, Matsumoto H, Okimura Y, Abe H, Fujisawa M, Kamidono S, Chihara K** 1997 Adrenocorticotrophin-independent macronodular adrenal hyperplasia in a patient with lysine vasopressin responsiveness but insensitivity to gastric inhibitory polypeptide. Clin Endocrinol (Oxf) 47:739–745
238. **Yamakita N, Murai T, Ito Y, Miura K, Ikeda T, Miyamoto K, Onami S, Yoshida T** 1997 Adrenocorticotropin-independent macronodular adrenocortical hyperplasia associated with multiple colon adenomas/carcinomas which showed a point mutation in the APC gene. Intern Med 36:536–542
239. **Daidoh H, Morita H, Hanafusa J, Mune T, Murase H, Sato M, Shibata T, Suwa T, Ishizuka T, Yasuda K** 1998 *In vivo* and *in vitro* effects of AVP and V1a receptor antagonist on Cushing's syndrome due to ACTH-independent bilateral macronodular adrenocortical hyperplasia. Clin Endocrinol (Oxf) 49:403–409
240. **Mircescu H, Jilwan J, N'Diaye N, Bourdeau I, Tremblay J, Hamet P, Lacroix A** 2000 Are ectopic or abnormal membrane hormone receptors frequently present in adrenal Cushing's Syndrome? J Clin Endocrinol Metab 85:3531–3536
241. **Eckland DJ, Todd K, Lightman SL** 1988 Immunoreactive vasopressin and oxytocin in hypothalamo-hypophysial portal blood of the Brattleboro and Long-Evans rat: effect of adrenalectomy and dexamethasone. J Endocrinol 117:27–34
242. **Bourdeau I, D'Amour P, Hamet P, Boutin JM, Lacroix A** 2000 Abnormal membrane hormone receptors in subclinical bilateral macronodular adrenal hyperplasia. Program and abstracts of the 82nd Annual Meeting of The Endocrine Society, Toronto, Ontario, Canada, 2000 (Abstract 1934), p 467
243. **de Keyzer Y, Lenne F, Auzan C, Jegou S, Rene P, Vaudry H, Kuhn JM, Luton JP, Clauser E, Bertagna X** 1996 The pituitary V3 vasopressin receptor and the corticotroph phenotype in ectopic ACTH syndrome. J Clin Invest 97:1311–1318
244. **Sakai Y, Horiba N, Tozawa F, Sakai K, Kuwayama A, Demura H, Suda T** 1997 Desmopressin stimulation test for diagnosis of ACTH-dependent Cushing's syndrome. Endocr J 44:687–695
245. **Malerbi DA, Mendonca BB, Liberman B, Toledo SP, Corradini MC, Cunha-Neto MB, Fragoso MC, Wajchenberg BL** 1993 The desmopressin stimulation test in the differential diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf) 38:463–472
246. **Al Damluji S, Perry L, Tomlin S, Bouloux P, Grossman A, Rees LH, Besser GM** 1987 Alpha-adrenergic stimulation of corticotropin secretion by a specific central mechanism in man. Neuroendocrinology 45:68–76
247. **Segaloff DL, Ascoli M** 1993 The lutropin/choriogonadotropin receptor .. 4 years later. Endocr Rev 14:324–347
248. **Rao CV** 1996 The beginning of a new era in reproductive biology and medicine: expression of low levels of functional luteinizing hormone/human chorionic gonadotropin receptors in nongondal tissues. J Physiol Pharmacol 47:41–53
249. **Pabon JE, Li X, Lei ZM, Sanfilippo JS, Yussman MA, Rao CV** 1996 Novel presence of luteinizing hormone/chorionic gonadotropin receptors in human adrenal glands. J Clin Endocrinol Metab 81: 2397–2400
250. **Seron-Ferre M, Lawrence CC, Jaffe RB** 1978 Role of hCG in regulation of the fetal zone of the human fetal adrenal gland. J Clin Endocrinol Metab 46:834–837
251. **Lacroix A, Hamet P, Boutin JM** 1999 Leuprolide acetate therapy in luteinizing hormone-dependent Cushing's syndrome. N Engl J Med 341:1577–1581
252. **Sheeler LR** 1994 Cushing's syndrome and pregnancy. Endocrinol Metab Clin North Am 23:619–627
253. **Aron DC, Schnall AM, Sheeler LR** 1990 Cushing's syndrome and pregnancy. Am J Obstet Gynecol 162:244–252
254. **Buescher MA, McClamrock HD, Adashi EY** 1992 Cushing syndrome in pregnancy. Obstet Gynecol 79:130–137
255. **Keegan GT, Grabarits F, Roland AS** 1976 Pregnancy complicated by Cushing's syndrome. South Med J 69:1207–1209
256. **Parra A, Cruz-Krohn J** 1966 Intercurrent Cushing's syndrome and pregnancy. Am J Med 40:961–966
257. **Reschini E, Giustina G, Crosignani PG, D'Alberton A** 1978 Spontaneous remission of Cushing syndrome after termination of pregnancy. Obstet Gynecol 51:598–602
258. **Calodney L, Eaton RP, Black W, Cohn F** 1973 Exacerbation of Cushing's syndrome during pregnancy: report of a case. J Clin Endocrinol Metab 36:81–86
259. **Kreines K, Perrin E, Salzer R** 1964 Pregnancy in Cushing's syndrome. J Clin Endocrinol Metab 24:75–79
260. **Wallace C, Toth EL, Lewanczuk RZ, Siminoski K** 1996 Pregnancy-

induced Cushing's syndrome in multiple pregnancies. J Clin Endocrinol Metab 81:15–21
261. **Close CF, Mann MC, Watts JF, Taylor KG** 1993 ACTH-independent Cushing's syndrome in pregnancy with spontaneous resolution after delivery: control of the hypercortisolism with metyrapone. Clin Endocrinol (Oxf) 39:375–379
262. **Wieland RG, Shaffer MBJ, Glove RP** 1971 Cushing's syndrome complicating pregnancy. A case report. Obstet Gynecol 38:841–843
263. **Aron DC, Schnall AM, Sheeler LR** 1990 Spontaneous resolution of Cushing's syndrome after pregnancy. Am J Obstet Gynecol 162: 472–474
264. **de Lange WE, Pratt JJ, Doorenbos H** 1980 A gonadotrophin responsive testosterone producing adrenocortical adenoma and high gonadotrophin levels in an elderly woman. Clin Endocrinol (Oxf) 12:21–28
265. **Takahashi H, Yoshizaki K, Kato H, Masuda T, Matsuka G, Mimura T, Inui Y, Takeuchi S, Adachi H, Matsumoto K** 1978 A gonadotrophin-responsive virilizing adrenal tumour identified as a mixed ganglioneuroma and adreno-cortical adenoma. Acta Endocrinol (Copenh) 89:701–709
266. **Werk EEJ, Sholiton LE, Kalejs L** 1973 Testosterone-secreting adrenal adenoma under gonadotropin control. N Engl J Med 289: 767–770
267. **Larson BA, Vanderlaan WP, Judd HL, McCullough DL** 1976 A testosterone-producing adrenal cortical adenoma in an elderly woman. J Clin Endocrinol Metab 42:882–887
268. **Givens JR, Andersen RN, Wiser WL, Coleman SA, Fish SA** 1974 A gonadotropin-responsive adrenocortical adenoma. J Clin Endocrinol Metab 38:126–133
269. **Blichert-Toft M, Vejlsted H, Hehlet H, Albrechtsen R** 1975 Virilizing adrenocortical adenoma responsive to gonadotrophin. Acta Endocrinol (Copenh) 78:77–85
270. **Smith HC, Posen S, Clifton-Bligh P, Casey J** 1978 A testosterone-secreting adrenal cortical adenoma. Aust NZ J Med 8:171–175
271. **Lefebvre H, Gonzalez KN, Contesse V, Delarue C, Vaudry H, Kuhn JM** 1998 Effect of prolonged administration of the serotonin4 (5-HT4) receptor agonist cisapride on aldosterone secretion in healthy volunteers. Endocr Res 24:749–752
272. **Bonnin C, Monsaingeon M, Bex V, Duclos M, Tortigues D, Lefebvre H, Tabarin A, Roger P** 2000 Hypercorticism by bilateral adrenal hyperplasia with several paradoxical responses. Program and abstracts of the 82nd Annual Meeting of The Endocrine Society, Toronto, Ontario, Canada, 2000 (Abstract 1936), p 467
273. **Caticha O, Odell WD, Wilson DE, Dowdell LA, Noth RH, Swislocki AL, Lamothe JJ, Barrow R** 1993 Estradiol stimulates cortisol production by adrenal cells in estrogen-dependent primary adrenocortical nodular dysplasia. J Clin Endocrinol Metab 77:494–497
274. **Bourdeau I, Caron P, Schürch W, N'Diaye N, Antakly T, Lacroix A** 2000 Paradoxical response to dexamethasone correlates with high expression of glucocorticoid receptors in primary pigmented nodular adrenocortical disease. Program and Abstracts of the 82nd Annual Meeting of The Endocrine Society,Toronto, Ontario, Canada, 2000 (Abstract 1935), p 467
275. **Hashimoto K, Kawada Y, Murakami K, Hattori T, Suemaru S, Kageyama J, Ota Z, Hayata S, Ohashi T, Omori H** 1986 Cortisol responsiveness to insulin-induced hypoglycemia in Cushing's syndrome with huge nodular adrenocortical hyperplasia. Endocrinol Jpn 33:479–487
276. **Glasow A, Bornstein SR, Chrousos GP, Brown JW, Scherbaum WA** 1999 Detection of Ob-receptor in human adrenal neoplasms and effect of leptin on adrenal cell proliferation. Horm Metab Res 31:247–251
277. **Lacroix A, Mircescu H, Hamet P** 1999 Clinical evaluation of the presence of abnormal hormone receptors in adrenal Cushing's syndrome. The Endocrinologist 9:9–15
278. **Glasow A, Haidan A, Gillespie J, Kelly PA, Chrousos GP, Bornstein SR** 1998 Differential expression of prolactin receptor (PRLR) in normal and tumorous adrenal tissues: separation of cellular endocrine compartments by laser capture microdissection (LCM). Endocr Res 24:857–862
279. **Cammas FM, Kapas S, Barker S, Clark AJ** 1995 Cloning, characterization and expression of a functional mouse ACTH receptor. Biochem Biophys Res Commun 212:912–918
280. **Boston BA, Cone RD** 1996 Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3–L1 cell line. Endocrinology 137:2043–2050
281. **Marchal R, Naville D, Durand P, Begeot M, Penhoat A** 1998 A steroidogenic factor-1 binding element is essential for basal human ACTH receptor gene transcription. Biochem Biophys Res Commun 247:28–32
282. **Naville D, Penhoat A, Durand P, Begeot M** 1999 Three steroidogenic factor-1 binding elements are required for constitutive and cAMP-regulated expression of the human adrenocorticotropin receptor gene. Biochem Biophys Res Commun 255:28–33
283. **Lebrethon MC, Naville D, Begeot M, Saez JM** 1994 Regulation of corticotropin receptor number and messenger RNA in cultured human adrenocortical cells by corticotropin and angiotensin II. J Clin Invest 93:1828–1833
284. **Penhoat A, Jaillard C, Saez JM** 1994 Regulation of bovine adrenal cell corticotropin receptor mRNA levels by corticotropin (ACTH) and angiotensin-II (A-II). Mol Cell Endocrinol 103:R7–10
285. **Picard-Hagen N, Penhoat A, Hue D, Jaillard C, Durand P** 1997 Glucocorticoids enhance corticotropin receptor mRNA levels in ovine adrenocortical cells. J Mol Endocrinol 19:29–36
286. **Cammas FM, Pullinger GD, Barker S, Clark AJ** 1997 The mouse adrenocorticotropin receptor gene: cloning and characterization of its promoter and evidence for a role for the orphan nuclear receptor steroidogenic factor 1. Mol Endocrinol 11:867–876
287. **Collins S, Ostrowski J, Lefkowitz RJ** 1993 Cloning and sequence analysis of the human β1-adrenergic receptor 5′-flanking promoter region. Biochim Biophys Acta 1172:171–174
288. **Tseng YT, Waschek JA, Padbury JF** 1995 Functional analysis of the 5′ flanking sequence in the ovine β1-adrenergic receptor gene. Biochem Biophys Res Commun 215:606–612
289. **Williams LT, Lefkowitz RJ, Watanabe AM, Hathaway DR, Besch HRJ** 1977 Thyroid hormone regulation of β-adrenergic receptor number. J Biol Chem 252:2787–2789
290. **Malbon CC, Moreno FJ, Cabelli RJ, Fain JN** 1978 Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. J Biol Chem 253:671–678
291. **Hadcock JR, Malbon CC** 1988 Down-regulation of β-adrenergic receptors: agonist-induced reduction in receptor mRNA levels. Proc Natl Acad Sci USA 85:5021–5025
292. **Mak JC, Nishikawa M, Barnes PJ** 1995 Glucocorticosteroids increase β 2-adrenergic receptor transcription in human lung. Am J Physiol 268:L41–L46
293. **Malbon CC, Hadcock JR** 1988 Evidence that glucocorticoid response elements in the 5′-noncoding region of the hamster beta 2-adrenergic receptor gene are obligate for glucocorticoid regulation of receptor mRNA levels. Biochem Biophys Res Commun 154:676–681
294. **Mak JC, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ** 1995 Protective effects of a glucocorticoid on downregulation of pulmonary β 2-adrenergic receptors *in vivo*. J Clin Invest 96:99–106
295. **Zhou XM, Fishman PH** 1991 Desensitization of the human β1-adrenergic receptor. Involvement of the cyclic AMP-dependent but not a receptor-specific protein kinase. J Biol Chem 266:7462–7468
296. **Nantel F, Marullo S, Krief S, Strosberg AD, Bouvier M** 1994 Cell-specific down-regulation of the β3-adrenergic receptor. J Biol Chem 269:13148–13155
297. **Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ** 1990 β-Arrestin: a protein that regulates β-adrenergic receptor function. Science 248:1547–1550
298. **Tsai-Morris CH, Geng Y, Buczko E, Dufau ML** 1998 A novel human luteinizing hormone receptor gene. J Clin Endocrinol Metab 83:288–291
299. **El Hefnawy T, Krawczyk Z, Nikula H, Vihera I, Huhtaniemi I** 1996 Regulation of function of the murine luteinizing hormone receptor promoter by cis- and trans-acting elements in mouse Leydig tumor cells. Mol Cell Endocrinol 119:207–217
300. **Hu YL, Lei ZM, Rao CV** 1996 cis-Acting elements and trans-acting proteins in the transcription of chorionic gonadotropin/luteinizing hormone receptor gene in human choriocarcinoma cells and placenta. Endocrinology 137:3897–3905
301. **Hu YL, Lei ZM, Rao CV** 1998 Analysis of the promoter of the

luteinizing hormone/human chorionic gonadotropin receptor gene in neuroendocrine cells. Life Sci 63:2157–2165
302. **Elalouf JM, Di Stefano A, de Rouffignac C** 1986 Sensitivities of rat kidney thick ascending limbs and collecting ducts to vasopressin *in vivo*. Proc Natl Acad Sci USA 83:2276–2280
303. **Skorecki KL, Ausiello DA** 1998 Vasopressin receptor-adenylate cyclase interactions: a model for cAMP metabolism in the kidney. In: Cowley, AWJ, Liard J-F, Ausiello DA (eds) Vasopressin Cellular and Integrative Functions. Raven Press, New York, vol 7:55–73
304. **Guillon G** 1989 Vasopressin, oxytocin and angiotensin receptors in mammals. Ann Endocrinol (Paris) 50:425–433
305. **Keppens S, de Wulf H** 1975 The activation of liver glycogen phosphorylase by vasopressin. FEBS Lett 51:29–32
306. **Briley EM, Lolait SJ, Axelrod J, Felder CC** 1994 The cloned vasopressin V1a receptor stimulates phospholipase A2, phospholipase C, and phospholipase D through activation of receptor-operated calcium channels. Neuropeptides 27:63–74
307. **Watters JJ, Swank MW, Wilkinson CW, Dorsa DM** 1996 Evidence for glucocorticoid regulation of the rat vasopressin V1a receptor gene. Peptides 17:67–73
308. **Watters JJ, Wilkinson CW, Dorsa DM** 1996 Glucocorticoid regulation of vasopressin V1a receptors in rat forebrain. Brain Res Mol Brain Res 38:276–284
309. **Morel A, O'Carroll AM, Brownstein MJ, Lolait SJ** 1992 Molecular cloning and expression of a rat V1a arginine vasopressin receptor. Nature 356:523–526
310. **Iwasaki Y, Oiso Y, Saito H, Majzoub JA** 1997 Positive and negative regulation of the rat vasopressin gene promoter. Endocrinology 138:5266–5274
311. **Murasawa S, Matsubara H, Kizima K, Maruyama K, Mori Y, Inada M** 1995 Glucocorticoids regulate V1a vasopressin receptor expression by increasing mRNA stability in vascular smooth muscle cells. Hypertension 26:665–669
312. **Colson P, Ibarondo J, Devilliers G, Balestre MN, Duvoid A, Guillon G** 1992 Upregulation of V1a vasopressin receptors by glucocorticoids. Am J Physiol 263:E1054–E1062
313. **Watanabe Y, Tokuda H, Suzuki A, Shinoda J, Kotoyori J, Ito Y, Oiso Y, Kozawa O** 1995 Glucocorticoid amplifies vasopressin-induced phosphoinositide hydrolysis in aortic smooth muscle cells. J Cell Biochem 57:522–529
314. **Rabadan-Diehl C, Aguilera G** 1998 Glucocorticoids increase vasopressin V1b receptor coupling to phospholipase C. Endocrinology 139:3220–3226
315. **Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B, Schuit F** 1996 Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 45:257–261
316. **Yip RG, Boylan MO, Kieffer TJ, Wolfe MM** 1998 Functional GIP receptors are present on adipocytes. Endocrinology 139:4004–4007
317. **Mazzocchi G, Rebuffat P, Meneghelli V, Malendowicz LK, Tortorella C, Gottardo G, Nussdorfer GG** 1999 Gastric inhibitory polypeptide stimulates glucocorticoid secretion in rats, acting through specific receptors coupled with the adenylate cyclase-dependent signaling pathway. Peptides 20:589–594
318. **Tseng CC, Boylan MO, Jarboe LA, Usdin TB, Wolfe MM** 1996 Chronic desensitization of the glucose-dependent insulinotropic polypeptide receptor in diabetic rats. Am J Physiol 270:E661–E666
319. **Boylan MO, Jepeal LI, Wolfe MM** 1999 Structure of the rat glucose-dependent insulinotropic polypeptide receptor gene. Peptides 20: 219–228
320. **Blondel O, Gastineau M, Dahmoune Y, Langlois M, Fischmeister R** 1998 Cloning, expression, and pharmacology of four human 5-hydroxytryptamine 4 receptor isoforms produced by alternative splicing in the carboxyl terminus. J Neurochem 70:2252–2261
321. **Rosenberg CL, Kim HG, Shows TB, Kronenberg HM, Arnold A** 1991 Rearrangement and overexpression of D11S287E, a candidate oncogene on chromosome 11q13 in benign parathyroid tumors. Oncogene 6:449–453
322. **Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM** 1992 A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature 355:262–265
323. **Gagel RF** 1998 Multiple endocrine neoplasia. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (eds) Williams Textbook of Endocrinology. W.B. Saunders Co, Philadelphia, pp 1627–1649
324. **Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D, Klugbauer S** 2000 Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. Clin Cancer Res 6:1093–1103
325. **Salassidis K, Bruch J, Zitzelsberger H, Lengfelder E, Kellerer AM, Bauchinger M** 2000 Translocation t(10;14)(q11.2:q22.1) fusing the kinetin to the RET gene creates a novel rearranged form (PTC8) of the RET proto-oncogene in radiation-induced childhood papillary thyroid carcinoma. Cancer Res 60:2786–2789
326. **Tatsumi K, Miyai K, Notomi T, Kaibe K, Amino N, Mizuno Y, Kohno H** 1992 Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. Nat Genet 1:56–58
327. **Kero J, Poutanen M, Zhang FP, Rahman N, McNicol AM, Nilson JH, Keri RA, Huhtaniemi IT** 2000 Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex. J Clin Invest 105:633–641
328. **Morohashi KI, Omura T** 1996 Ad4BP/SF-1, a transcription factor essential for the transcription of steroidogenic cytochrome P450 genes and for the establishment of the reproductive function. FASEB J 10:1569–1577
329. **Waterman MR** 1994 Biochemical diversity of cAMP-dependent transcription of steroid hydroxylase genes in the adrenal cortex. J Biol Chem 269:27783–27786
330. **Hatano O, Takakusu A, Nomura M, Morohashi K** 1996 Identical origin of adrenal cortex and gonad revealed by expression profiles of Ad4BP/SF-1. Genes Cells 1:663–671
331. **Morohashi K** 1997 The ontogenesis of the steroidogenic tissues. Genes Cells 2:95–106
332. **Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER** 1994 An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372:635–641
333. **Muscatelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A, Bardoni B, Guioli S, Zehetner G, Rabl W** 1994 Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature 372:672–676
334. **Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, Sassone-Corsi P** 1997 A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. Mol Endocrinol 11:1950–1960
335. **Ikeda Y, Swain A, Weber TJ, Hentges KE, Zanaria E, Lalli E, Tamai KT, Sassone-Corsi P, Lovell-Badge R, Camerino G, Parker KL** 1996 Steroidogenic factor 1 and Dax-1 colocalize in multiple cell lineages: potential links in endocrine development. Mol Endocrinol 10:1261–1272
336. **Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G** 1996 Mouse Dax1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nat Genet 12:404–409
337. **Giguere V** 1999 Orphan nuclear receptors: from gene to function. Endocr Rev 20:689–725
338. **Yu RN, Ito M, Jameson JL** 1998 The murine Dax-1 promoter is stimulated by SF-1 (steroidogenic factor-1) and inhibited by COUP-TF (chicken ovalbumin upstream promoter-transcription factor) via a composite nuclear receptor-regulatory element. Mol Endocrinol 12:1010–1022
339. **Kawabe K, Shikayama T, Tsuboi H, Oka S, Oba K, Yanase T, Nawata H, Morohashi K** 1999 Dax-1 as one of the target genes of Ad4BP/SF-1. Mol Endocrinol 13:1267–1284
340. **Hastie ND** 1994 The genetics of Wilms' tumor-a case of disrupted development. Annu Rev Genet 28:523–558
341. **Semenza GL** 1994 Transcriptional regulation of gene expression: mechanisms and pathophysiology. Hum Mutat 3:180–199
342. **Moore AW, McInnes L, Kreidberg J, Hastie ND, Schedl A** 1999 YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. Development 126:1845–1857
343. **Moore AW, Schedl A, McInnes L, Doyle M, Hecksher-Sorensen J, Hastie ND** 1998 YAC transgenic analysis reveals Wilms' tumour

1 gene activity in the proliferating coelomic epithelium, developing diaphragm and limb. Mech Dev 79:169–184
344. **Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA** 1998 Wilms' tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell 93:445–454
345. **Dhanasekaran N, Heasley LE, Johnson GL** 1995 G protein-coupled receptor systems involved in cell growth and oncogenesis. Endocr Rev 16:259–270
346. **Spiegel AM** 1996 Mutations in G proteins and G protein-coupled receptors in endocrine disease. J Clin Endocrinol Metab 81:2434–2442
347. **Farfel Z, Bourne HR, Iiri T** 1999 The expanding spectrum of G protein diseases. N Engl J Med 340:1012–1020
348. **Billestrup N, Swanson LW, Vale W** 1986 Growth hormone-releasing factor stimulates proliferation of somatotrophs *in vitro*. Proc Natl Acad Sci USA 83:6854–6857
349. **Dumont JE, Jauniaux JC, Roger PP** 1989 The cyclic AMP-mediated stimulation of cell proliferation. Trends Biochem Sci 14:67–71
350. **Ledent C, Dumont JE, Vassart G, Parmentier M** 1992 Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J 11:537–542
351. **Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G** 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature 365:649–651
352. **Kopp P, Van Sande J, Parma J, Duprez L, Gerber H, Joss E, Jameson JL, Dumont JE, Vassart G** 1995 Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N Engl J Med 332:150–154
353. **Shenker A, Laue L, Kosugi S, Merendino Jr JJ, Minegishi T, Cutler Jr GB** 1993 A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 365:652–654
354. **Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann HE, Jakobs KH, Horsthemke B** 1998 Association of a human G-protein $\beta 3$ subunit variant with hypertension. Nat Genet 18:45–48
355. **Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR** 1990 Two G protein oncogenes in human endocrine tumors. Science 249: 655–659
356. **Zeiger MA, Norton JA** 1993 Gsα—identification of a gene highly expressed by insulinoma and other endocrine tumors. Surgery 114:458–462
357. **Drazner MH, Koch WJ, Lefkowitz RJ** 1997 Potentiation of β-adrenergic signaling by gene transfer. Proc Assoc Am Physicians 109:220–227
358. **Ma YH, Landis C, Tchao N, Wang J, Rodd G, Hanahan D, Bourne HR, Grodsky GM** 1994 Constitutively active stimulatory G-protein αs in β-cells of transgenic mice causes counterregulation of the increased adenosine 3′,5′-monophosphate and insulin secretion. Endocrinology 134:42–47
359. **Allen JM, Baetge EE, Abrass IB, Palmiter RD** 1988 Isoproterenol response following transfection of the mouse $\beta 2$-adrenergic receptor gene into Y1 cells. EMBO J 7:133–138
360. **Rilianawati, Paukku T, Kero J, Zhang FP, Rahman N, Kananen K, Huhtaniemi I** 1998 Direct luteinizing hormone action triggers adrenocortical tumorigenesis in castrated mice transgenic for the murine inhibin α-subunit promoter/simian virus 40 T-antigen fusion gene. Mol Endocrinol 12:801–809
361. **Light K, Jenkins PJ, Weber A, Perrett C, Grossman A, Pistorello M, Asa SL, Clayton RN, Clark AJ** 1995 Are activating mutations of the adrenocorticotropin receptor involved in adrenal cortical neoplasia? Life Sci 56:1523–1527
362. **Reincke M, Mora P, Beuschlein F, Arlt W, Chrousos GP, Allolio B** 1997 Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. J Clin Endocrinol Metab 82:3054–3058
363. **Reincke M, Karl M, Travis WH, Mastorakos G, Allolio B, Linehan HM, Chrousos GP** 1994 p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. J Clin Endocrinol Metab 78:790–794
364. **Reincke M, Beuschlein F, Latronico AC, Arlt W, Chrousos GP, Allolio B** 1997 Expression of adrenocorticotrophic hormone receptor mRNA in human adrenocortical neoplasms: correlation with P450 scc expression. Clin Endocrinol (Oxf) 46:619–626
365. **Arnaldi G, Mancini V, Costantini C, Giovagnetti M, Petrelli M, Masini A, Bertagna X, Mantero F** 1998 ACTH receptor mRNA in human adrenocortical tumors: overexpression in aldosteronomas. Endocr Res 24:845–849
366. **Schorr I, Hinshaw HT, Cooper MA, Mahaffee D, Ney RL** 1972 Adenyl cyclase hormone responses of certain human endocrine tumors. J Clin Endocrinol Metab 34:447–451
367. **Dexter RN, Allen DO** 1970 A glucagon sensitive adenyl cyclase system in pheochromocytoma. Clin Res 18:601 (Abstract)
368. **Levey GS, Weiss SR, Ruiz E** 1975 Characterization of the glucagon receptor in a pheochromocytoma. J Clin Endocrinol Metab 40: 720–723
369. **Barancik M** 1989 Inadvertent diagnosis of pheochromocytoma after endoscopic premedication. Dig Dis Sci 34:136–138
370. **Matsukura S, Kakita T, Hirata Y, Yoshimi H, Fukase M** 1977 Adenylate cyclase of GH and ACTH producing tumors of human: activation by non-specific hormones and other bioactive substances. J Clin Endocrinol Metab 44:392–397
371. **Lawrence AM, Goldfine ID, Kirsteins L** 1970 Growth hormone dynamics in acromegaly. J Clin Endocrinol Metab 31:239–247
372. **Irie M, Tsushima T** 1972 Increase of serum growth hormone concentration following thyrotropin-releasing hormone injection in patients with acromegaly or gigantism. J Clin Endocrinol Metab 35:97–100
373. **Hanew K, Kokubun M, Sasaki A, Mouri T, Yoshinaga K** 1980 The spectrum of pituitary growth hormone responses to pharmacological stimuli in acromegaly. J Clin Endocrinol Metab 51:292–297
374. **Yamada M, Hashimoto K, Satoh T, Shibusawa N, Kohga H, Ozawa Y, Yamada S, Mori M** 1997 A novel transcript for the thyrotropin-releasing hormone receptor in human pituitary and pituitary tumors. J Clin Endocrinol Metab 82:4224–4228
375. **Faccenda E, Melmed S, Bevan JS, Eidne KA** 1996 Structure of the thyrotrophin-releasing hormone receptor in human pituitary adenomas. Clin Endocrinol (Oxf) 44:341–347
376. **Konaka S, Yamada M, Satoh T, Ozawa H, Watanabe E, Takata K, Mori M** 1997 Expression of thyrotropin-releasing hormone (TRH) receptor mRNA in somatotrophs in the rat anterior pituitary. Endocrinology 138:827–830
376a. **Ohshima K, Okada S, Onai T, Sato M, Umahara M, Nakamura Y, Kato M, Kakegawa T, Tatemoto K, Mori M,** Program of the 76th Annual Meeting of The Endocrine Society, Anaheim, CA, 1994 (Abstract 51C), p 213
377. **LeRiche VK, Asa SL, Ezzat S** 1996 Epidermal growth factor and its receptor (EGF-R) in human pituitary adenomas: EGF-R correlates with tumor aggressiveness. J Clin Endocrinol Metab 81:656–662
378. **Tucci JR, Zah W, Kalderon AE** 1973 Endocrine studies in an arrhenoblastoma responsive to dexamethasone, ACTH and human chorionic gonadotropin. Am J Med 55:687–694
379. **Gallagher TF, Spencer H, Bradlow HL, Allen L, Hellman L** 1962 Steroid production and metabolism in metastatic arrhenoblastoma. J Clin Endocrinol Metab 22:970–977
380. **Matsukura S, Kakita T, Fukase M, Fujita T** 1981 Adenylate cyclase of a human medullary thyroid carcinoma. Experientia 37:523–524
381. **McGregor LM, McCune BK, Graff JR, McDowell PR, Romans KE, Yancopoulos GD, Ball DW, Baylin SB, Nelkin BD** 1999 Roles of trk family neurotrophin receptors in medullary thyroid carcinoma development and progression. Proc Natl Acad Sci U S A 96:4540–4545
382. **van der Laan BF, Freeman JL, Asa SL** 1995 Expression of growth factors and growth factor receptors in normal and tumorous human thyroid tissues. Thyroid 5:67–73
383. **Vertongen P, Ciccarelli E, Woussen-Colle MC, De Neef P, Robberecht P, Cauvin A** 1994 Pituitary adenylate cyclase-activating polypeptide receptors of types I and II and glucagon-like peptide-I receptors are expressed in the rat medullary carcinoma of the thyroid cell line 6/23. Endocrinology 135:1537–1542
384. **Crespel A, De Boisvilliers F, Gros L, Kervran A** 1996 Effects of glucagon and glucagon-like peptide-1-(7–36) amide on C cells from

rat thyroid and medullary thyroid carcinoma CA-77 cell line. Endocrinology 137:3674–3680
385. **Colomer A, Martinez-Mas JV, Matias-Guiu X, Llorens A, Cabezas R, Prat J, Garcia-Ameijeiras A** 1996 Sex-steroid hormone receptors in human medullary thyroid carcinoma. Mod Pathol 9:68–72
386. **Amiri-Mosavi A, Ahlman H, Tisell LE, Wangberg B, Kolby L, Forssell-Aronsson E, Lundberg PA, Lindstedt G, Nilsson O** 1999 Expression of cholecystokinin-B/gastrin receptors in medullary thyroid cancer. Eur J Surg 165:628–631
387. **Mato E, Matias-Guiu X, Chico A, Webb SM, Cabezas R, Berna L, De Leiva A** 1998 Somatostatin and somatostatin receptor subtype gene expression in medullary thyroid carcinoma. J Clin Endocrinol Metab 83:2417–2420
388. **Nakamaru M, Ogihara T, Saito H, Rakugi H, Hashizume K, Yamatodani A, Wada H, Kumahara Y** 1989 Effect of atrial natriuretic peptide on catecholamine release from human pheochromocytoma. Acta Endocrinol (Copenh) 120:107–112
389. **Gelato MC, Vassalotti J** 1990 Insulin-like growth factor-II: possible local growth factor in pheochromocytoma. J Clin Endocrinol Metab 71:1168–1174
390. **Teng KK, Lander H, Fajardo JE, Hanafusa H, Hempstead BL, Birge RB** 1995 v-Crk modulation of growth factor-induced PC12 cell differentiation involves the Src homology 2 domain of v-Crk and sustained activation of the Ras/mitogen-activated protein kinase pathway. J Biol Chem 270:20677–20685
391. **Nilsson O, Wangberg B, Kolby L, Schultz GS, Ahlman H** 1995 Expression of transforming growth factor α and its receptor in human neuroendocrine tumours. Int J Cancer 60:645–651
392. **Billon N, van Grunsven LA, Rudkin BB** 1996 The CDK inhibitor p21WAF1/Cip1 is induced through a p300-dependent mechanism during NGF-mediated neuronal differentiation of PC12 cells. Oncogene 13:2047–2054
393. **Barnes RB, Ehrmann DA** 1997 Long-term suppression of testosterone after treatment with a gonadotropin-releasing hormone agonist in a woman with a presumed testosterone secreting ovarian tumor. J Clin Endocrinol Metab 82:1746–1748
394. **Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, Seidman JG** 1994 Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. Nat Genet 8:303–307
395. **Schipani E, Kruse K, Juppner H** 1995 A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. Science 268:98–100
396. **Rodien P, Bremont C, Sanson ML, Parma J, Van Sande J, Costagliola S, Luton JP, Vassart G, Duprez L** 1998 Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. N Engl J Med 339: 1823–1826
397. **McKenzie JM, Zakarija M** 1995 Hyperthyroidism. In: De Groot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology. W.B. Saunders Co, Philadelphia, pp 676–711
398. **Service FJ** 1995 Hypoglycemia including hypoglycemia in neonates and children. In: De Groot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology.W.B. Saunders Co, Philadelphia, pp 1605–1623
399. **Gammaltoft S, Kahn CR** 1995 Hormone signaling via membrane receptors. In: De Groot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD, Potts Jr JT, Rubenstein AH (eds) Endocrinology. W.B. Saunders Co, Philadelphia, pp 17–65
400. **Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L** 1989 GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature 340:692–696
401. **Russo D, Arturi F, Wicker R, Chazenbalk GD, Schlumberger M, DuVillard JA, Caillou B, Monier R, Rapoport B, Filetti S** 1995 Genetic alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab 80:1347–1351
402. **Iiri T, Herzmark P, Nakamoto JM, van Dop C, Bourne HR** 1994 Rapid GDP release from Gsα in patients with gain and loss of endocrine function. Nature 371:164–168

4th Biennial Congress of the European Society for Sexual and Impotence Research
Rome, Italy—Cavalieri Hilton Hotel
September 30th–October 3rd, 2001

For information, contact the organizing secretariat at the following address:

Studio Congressi
Via Francesco Ferrara, 40
00191 Rome, Italy
Telephone: 39-06-329-0250, 39-06-329-0239
Fax: 39-06-3630-6897
E-Mail: sc.congressi@agora.stm.it
Website: http://www.essir2001.it