University of Montreal

Modulation of Oxytocin Receptors in Right Ventricular Hypertrophy

By

Yang Wang

Department of Biomedical Sciences

Faculty of Medicine

This thesis was submitted to the Faculty of Graduated Studies and Research in partial fulfilment of the requirements of the degree of Master in Biomedical Sciences.

April 2009

©Yang Wang, 2009

University of Montreal

Faculty of graduate studies

This thesis entitled

‘‘Modulation of Oxytocin Receptors in Right Ventricular Hypertrophy’’

Presented by

Yang Wang

Has been evaluated by a jury made up of the following people

President of jury:	Dr. Pangala Bhat
Director of research:	Dr. Marek Jankowski
Co-director of research:	Dr. Jolanta Gutkowska
Member of Jury:	Dr. Jean-Marc Lavoie

April 2009

Thesis accepted on:

RÉSUMÉ

L'hypertension pulmonaire (HP) est une maladie dont l'étiologie est inconnue et qui entraîne ultimement une défaillance du ventricule droit (VD) et le décès. L'HP peut être induite chez le rat par la la monocrotaline (MCT), un alcaloïde pyrrolizidique extrait de la plante Crotalaria Spectabilis, causant des lésions à l'endothélium des artères pulmonaires, menant à un épaississement de ces dernières et à une augmentation de la résistance vasculaire. Ceci à pour conséquence de causer une hypertrophie du VD, de l'inflammation, une dysfonction endothéliale NO-dépendante des artères coronariennes et une augmentation des peptides natriurétiques circulants.

Objectif: Nous avons testé l'hypothèse selon laquelle l'étiopathologie de l'HP impliquerait le récepteur à ocytocine (OTR) dû à son implication fonctionnelle avec les cytokines inflammatoires et la libération du peptide natriurétique atrial (ANP) et du NO.

Méthodes: Des rats mâles Sprague-Dawley pesant 220-250g reçurent une seule injection sous-cutanée de MCT (60 mg/kg). 6 à 7 semaines (46±1 jours) suivant l'injection, les rats furent sacrifiés et l'expression génique et protéique fut déterminée par PCR en temps réel et par western blot, respectivement, dans le VD et le ventricule gauche (VG)

Résultats: Les rats traités au MCT démontrèrent une augmentation significative du VD. Une hypertrophie du VD était évidente puisque le ratio du VD sur le VG ainsi que le poids du septum étaient près de 77% plus élevés chez les rats traités au MCT que chez les rats contrôles. Le traitement au MCT augmenta l'expression génique d'ANP (3.7-fois dans le VG et 8-fois dans le VD) ainisi que le NP du cerveau (2.7-fois dans le VG et 10-fois dans le VD). Les transcrits de trois récepteurs de NP augmentèrent significativement (0.3-2 fois) seulement dans le VD. L'expression protéique de la NO synthase (iNOS) fut également augmentée de façon sélective dans le VD. Par contre, les transcripts de NOS endothéliale et de NOS neuronale étaient plus élevés (0.5-2 fold) dans le VG. L'ARNm et l'expression protéique d'OTR furent diminués de 50% dans le VD, tandis qu'une augmentation de l'expression des cytokines IL-1β and IL-6 fut

observée. L'ARNm de Nab1, un marqueur d'hypertrophie pathologique, fut augmentée de deux-fois dans le VD.

Conclusion: L'augmentation d'expression génique de NP dans le VD des rats traités au MCT est associée à une augmentation des transcripts du récepteur NP, suggérant une action locale de NP dans le VD durant l'HP. L'expression d'OTR est atténuée dans le VD, possiblement par des cytokines inflammatoires puisque le promoteur du gène de l'OTR contient de multiples éléments de réponse aux interleukines. Diminuer l'expression d'OTR dans le VD durant l'hypertension pulmonaire pourrait influencer de manière positive la fonction cardiaque car l'OTR régule la contractilité et le rythme cardiaque.

Mots clés: hypertension pulmonaire, hypertrophie du ventricule droit monocrotaline, récepteur à ocytocine, inflammation, peptides natriurétiques.

SUMMARY

Pulmonary hypertension (PH) is a disease of unknown etiology that ultimately causes failure of right ventricle (RV) with a lethal outcome. PH can be induced in the rat with monocrotaline (MCT), a pyrrolizidine alkaloid from the plant *Crotolaria spectabilis* that damages the pulmonary artery endothelium leading to thickening of the pulmonary arteries and increased vascular resistance. This subsequently results in RV hypertrophy, inflammation, nitric oxide (NO)-associated coronary endothelial dysfunction and increment of natriuretic peptides (NP) in the circulation.

Objective: We verified hypothesis that the etiopathogenesis of PH involves the oxytocin receptor (OTR) because of its functional association with inflammatory cytokines and release of atrial natriuretic peptide (ANP) and NO.

Methods: Male Sprague-Dawley rats weighing 220-250g received a single subcutaneous injection of 60 mg/kg of MCT. Six to 7 weeks (46±1 days) following the injection, rats were sacrificed and gene and protein expression were detected by real-time PCR and western-blot analysis, respectively, in the RV and LV (left ventricle).

Results: MCT-treated rats displayed significant increases in RV weight. RV hypertrophy was evident as the ratio of the RV to LV plus septum weight was nearly 77% higher in MCT-treated rats compared to control rats. MCT treatment increased transcripts of ANP (3.7-fold in the LV and 8-fold in RV) and brain NP (2.7-fold in the LV and 10-fold in RV). Transcripts for three NP receptors significantly increased (0.3-2 fold) only in the RV. iNOS (inducible NO synthase) protein expression also increased selectively in the RV. In contrast, the endothelial NOS and neural NOS transcripts heightened (0.5-2 fold) in the LV. Both OTR mRNA and protein were decreased by 50% in the RV, whereas an up-regulation of cytokines IL-1β and IL-6 was observed. Nab1 mRNA, a marker of pathological hypertrophy, increased two-fold in the RV.

Conclusion: Increased gene expression of NP in the RV of the MCT-treated rat correlates with upregulation of NP receptor transcripts indicating local NP action in the RV during PH. OTR expression is decreased in the RV possibly by inflammatory cytokines, IL-1β and IL-6 because OTR promoter region contains

multiple putative interleukin-response elements. Lowering OTR in RV during pulmonary hypertension can influence cardiac function since OT regulates heart rate and cardiac contractility and is linked with cardioprotective system ANP and NO.

Keywords: pulmonary hypertension, right ventricular hypertrophy, monocrotaline, oxytocin receptor, inflammation, natriuretic peptides.

CONTENTS

Summary…………………………………………………………………….....3

List of figures………………………………………………………………..13

List of tables……………………………………………………………….…..14

List of abbreviations…………………………………………………………..15

Acknowledgements…………………………………………………….......19

Dedication ……………………………………………………………………..20

I. Introduction……………………………………………………………….21

I.I. Myocardial hypertrophy……………………………………………..…21

I.I.1. General……………………………………………………………….…21

I.I.2. Pathogenesis……………………………………………………...22

I.I.2.1. Role of genetic factors…………………………………...…23

I.I.2.2. Role of oxidative stress………………………………….....23

I.I.2.3. Role of inflammation…………………………………………24

I.I.2.4. Role of energy metabolism…………………………………..26

I.I.3. Molecular mechanisms involved in cardiac hypertrophy.…………27

I.I.3.1. Vasoactive peptides………………………………………....27

I.I.3.1.1 ANP and BNP as markers of cardiac hypertrophy...27

I.I.3.1.2 Cardioprotective role of GC-A, GC-B,

and NPR-C in the hypertrophic heart……………….29

I.I.3.1.3 Role of other vasoactive peptides in
cardiac hypertrophy……………………………………30
I.I.3.2. Peptide growth factors…………………………………………30
I.I.3.3. Hormones…………………………………………………….32
I.I.4. Molecular pathways………………………………………………….32
I.I.4.1. PI3K/Akt pathway…………………………………………………33
I.I.4.2. MAPKs pathway…………………………………………………..33
I.I.4.3. PKC pathway………………………………………………...34
I.I.4.4. NF-κB pathway…………………………………………………34
I.I.5. Apoptosis……………………………………………………………35
I.II. Pulmonary hypertension and right ventricular hypertrophy…………….36
I.II.1. Modulation of cardiac makers in this pathology……….…………36
I.II.2. Investigations of right and left ventricular function following
induction of pulmonary hypertension with the monocrotaline…….37
I.III. Oxytocin (OT) and Oxytocin receptor (OTR) in the heart…………....38
I.III.1. General features of OT and OTR……………………………………38
I.III.1.1. Cardiovascular functions of OT……………..…………..…39
I.III.1.2. OT-ANP interaction in central nervous system…………..…41
II. Hypothesis and specific aims……………………………………………..…42
II.1. Hypothesis……………………………………………………………42

II.2. Specific aims..……………………………………………………………42

II.2.1. Aim #1……..…………………………………………………….….42

II.2.2. Aim #2………………….……………………………………………...42

II.2.3. Aim #3………………….………………………………………….…..42

III. Experimental design…………………………………………………………..43

IV. Material and methods…………………………………………………………44

IV.1 Animal treatment and collection of specimens……………………….44

IV.2. Measurement of gene expression……………………………………44

IV. 2.1. Table 1. Materials for Real-time PCR…………………………44

IV.2.2. Preparation of total RNA and quantification of RNA

Concentration…………………………………………………...45

IV.2.2.1. Preparation of total RNA……………………………...45

IV.2.2.2. Determination of RNA concentration……………….…..45

IV.2.3. Reverse transcription of mRNA to cDNA………………………46

IV.2.4. Sequences of the primers used for PCR amplification of

cDNA samples………………………………………......……46

IV.2.5. Programs for amplification of gene sequences by

Real-time PCR and control of amplification conditions……. 48

IV.2.6 Selection of housekeeping gene for measurement of gene

expression semi quantitatively…………………………………48

IV.2.7 Methodology used for calculation of the gene expression

on the basis results provided by Real-time cycler…….………49

IV.2.8 Statistical evaluation of the results obtained by Real-time PCR……………………………………………………………50

IV.3. Cardiac protein extraction and Western blotting…………………..50

IV.3.1. Processing of cardiac samples and measurement of protein concentration……………………………………….....50

IV.3.1.1. Processing of cardiac samples……………………50

IV.3.1.2. Measurement of protein concentration…………....50

IV.3.2. Table 2. Reagents for Western-blot…………………………51

IV.3.3. Electrophoresis conditions, electro-transfer proteins to the nylon membrane……………………………………..52

IV.3.4. Membrane processing, incubations time, reaction with specific primary antibodies, washing conditions, incubation with secondary antibodies………………………..52

IV.3.5. Development of Western blotting reaction by chemiluminescence method……………………………….…..53

IV.3.6. Molecular weight of the bands obtained for the studied protein of interest and expected molecular size as recommended by antibody provider and published in the literature……………………………………………………...54

IV.3.7. Quantification of the bands intensity using computer Software……………………………………………………….54

IV.3.8. Methodology used for calculation of protein expression in Western-blot……………………………………..55

IV.3.9. Statistical evaluation of the results obtained by Western blotting…………………………………………………..55

V. Results…………………………………………………………………..56

Ⅴ.1. Effect of MCT treatment on body weights and weights
of selected organs…………………………………………………..56
Ⅴ.2. Effect of MCT treatment on NPs gene expressions in
the right and the left ventricles…………………………………..……57
Ⅴ.3. Effect of MCT treatment on NP receptors gene
expressions in the right and the left ventricles…………………………57
Ⅴ.4. Effect of MCT treatment on NOS gene and protein
expressions in the right and the left ventricles…………………………58
Ⅴ.5. Effect of MCT treatment on OTR gene and
protein expressions in the right and the left ventricles……………..….59
Ⅴ.6. Effect of MCT treatment on expressions of
proinflammatory cytokines in the right and the left ventricles………..60
Ⅴ.7. Effect of MCT treatment on a marker of pathological
hypertrophy gene and protein expressions
in the right and the left ventricles…………………………………..……60
Ⅴ.8. Effect of MCT treatment on ventricular expression of
factors involved in metabolic functions………………………………...61
Ⅵ. Discussion………………………………………………………………70
Ⅵ.1. Rat model of RV hypertrophy…………………………………………70
Ⅵ.2. Possible role of inflammatory cytokines
in the regulation of OTR…………………………………….…………72
Ⅵ.3. Involvement of NO in RV hypertrophy………………………………..74

VI.4. Role of Natriuretic Peptides……………………………………………...75

VI.5. Role of OT-Associated Metabolic Factors…………………………...77

VII. Conclusions and perspectives……………………………………………...79

VII.1. Conclusions……………………………………….………………79

VII.2. Perspectives………………………………………………………79

VII.2.1. OTR and inflammation……………………………………..79

VII.2.2. OTR and metabolism ………………………………………80

VIII. References…………………………………………………………………82

LIST OF FIGURES

Results:

Figure 1. Effect of MCT treatment on NPs gene expressions in the right and the left ventricles………………..…………….63

Figure 2. Effect of MCT treatment on NP receptors gene expressions in the right and the left ventricles……………..64

Figure 3. Effect of MCT treatment on NOS gene and protein expressions in the right and the left ventricles……………..65

Figure 4. Effect of MCT treatment on OTR gene and protein expressions in the right and the left ventricles……..66

Figure 5. Effect of MCT treatment on expressions of proinflammatory cytokines in the right and the left ventricles……………………………………………………67

Figure 6. Effect of MCT treatment on a marker of pathological hypertrophy gene and protein expressions in the right and the left ventricles…………………………….68

Figure 7. Effect of MCT treatment on ventricular expression of factors involved in metabolic functions……………………..69

LIST OF TABLES

Material and methods:

Table 1. Materials for Real-time PCR………………………………….44

Table 2. Reagents for Western-blot……………………………………51

Table 3. Primary and secondary antibodies used in Westen-Blot….53

Table 4. The size of Protein Bands……………………………………54

Results:

Table 1. The effect of monocrotaline treatment on selected organ weights……………………………………………………………..62

Table 2. The effect of monocrotaline treatment on heart weights……62

LIST OF ABBREVIATIONS

AIF	: apoptosis-inducing factor
ANF	: atrial natriuretic factor
Ang II	: angiotensin II
Akt	: protein kinase B
ANP	: atrial natriuretic peptides
AP-1	: activator protein 1
ATP	: adenosine triphosphate
AT-1	: angiotensin II type 1 receptor
Bcl2	: B-cell CLL/lymphoma 2
BH4	: tetrahydrobiopterin
BNP	: brain natriuretic peptides
BSA	: bovine serum albumin
CNP	: C-type natriuretic peptide
CT-1	: cardiotrophin-1
C_T	: threshold cycle
Cyt c	: cytochrome c
DEPC	: diethylpyrocarbonate
DNA	: deoxyribonucleic acid
dNTP	: deoxyribonucleotide triphosphate
DTT	: dithiothreitol
EDTA	: ethylenediaminetetraacetic acid
eNOS	: endothelial nitric oxide synthase
erg	: early growth reponse
ET-1	: endothelin-1

FGF-2	: fibroblast growth factors-2
FLICE	: fas-associated death domain-like interleukin-1β-converting enzyme
GAPDH	: glyceraldehyde-3-phosphate dehydrogenase
GC-A	: guanylyl cyclase A
GC-A-/-	: GC-A knockout
GC-B	: guanylyl cyclase B
GH	: growth hormone
cGMP	: cyclic guanosine monophosphate
HCM	: hypertrophic cardiomyopathy
H_2O_2	: hydrogen peroxide
HPRT	: hypoxanthine guanine phosphoribosyl transferase
IGF-1	: insulin-like growth factor-1
JNK	: c-Jun N-terminal kinase
iNOS	: inducible nitric oxide synthase
IRAP	: insulin regulated aminopeptidase
IL-1β	: interteukin-1 β
IL-6	: interleukin-6
LIF	: leukaemia-inhibitory factor
LV	: left ventricle
MAPK	: mitogen-activated protein kinase
MCT	: monocrotaline
MMP	: matrix metalloproteinase
mRNA	: messanger RNA
Nab1	: NGF1A-binding protein

NF-κB : nuclear factor kappa-light-chain-enhancer of activated B cells

nNOS : neuronal nitric nxide synthase

NO : nitric oxide

NOS : nitric oxide synthases

NP : natriuretic peptides

NPR-A : natriuretic peptide receptor A

NF-IL6 : nuclear factor IL-6

NT-proBNP : N-terminal proBNP

OD : optical density

OT : oxytocin

OTR : oxytocin receptor

PCR : polymerase chain reaction

PE : phenylephrine

PH : Pulmonary hypertension

PI-3/Akt : phosphatidylinositol 3-kinase/Akt

PKB : protein kinase B

PKC : protein kinase C

PMSF : phenylmethanesulphonylfluoride

RIPA : radio immunoprecipitation assay

RNA : ribonucleic acid

rRNA : ribosomal RNA

ROS : reactive oxygen species

RT : room temperature

RV : right ventricle

RVH : right ventricle hypertrophy

SDHA : succinate dehydrogenase complex, subunit A

SDS : sodium dodecyl sulfate

TBS : Tris Buffered Saline

TEMED : tetramethylethylenediamine

TNFα : tumor necrosis factor α

TGF-β1 : transforming growth factor β-1

UBC : ubiquitin C

UV : ultraviolet

YWHAQ : tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide

ACKNOWLEDGEMENTS

First and foremost, I would like to express my most sincere appreciation to my supervisors Dr. Marek Jankowski and Dr. Jolanta Gutkowska, for their solid and skillful guidance, as well as their encouragement throughout this dissertation. Furthermore, I acknowledge Dr. Tom Broderick for kindly providing us experimental samples and my colleague, Dr. Donghao Wang, enthusiastic supporter of my efforts.

DEDICATION

I dedicate my work to my loving and caring wife and my lovely daughter and son, whose love and strength have sustained my energy to complete this work.

I. INTRODUCTION

I.I. Myocardial hypertrophy

I.I.1. General

Myocardial hypertrophy and its subsequent heart failure are one of the leading causes of morbidity and mortality in Western world. This disease is characterized in an increase in heart muscle mass, which reflects a remodeling of the myocardium in response to mechanical stress and various stimuli. Cardiac hypertrophy is classified into two types: physiological and pathological hypertrophies. Physiological hypertrophy is reversible, not associated with cardiac damage, and occurs in healthy individuals following exercise and in pregnancy. In contrast, pathological hypertrophy is caused by the ventricle adapting to increased stress, such as chronically increased volume load or increased pressure load. Hypertrophy can also result from disease of the heart (valve disease, cardiomyopathies), genetic abnormalities (e.g., hypertrophic cardiomyopathy), and as a consequence of coronary artery disease. Sustained pathological hypertrophy can eventually result in a decline in left and right ventricular sizes and functions, which lead to heart failure. The major causes of pathological hypertrophy are hypertension, genetic polymorphisms, and loss of myocytes following ischemic damage. Altered cardiac metabolism can also be an important component leading to hypertrophy [Rajabi et al. 2007].

I.I.2. Pathogenesis

Many factors have been implicated in cardiac remodeling including alterations in gene expression in myocytes, cardiomyocytes apoptosis, cytokines, and growth factors. These factors influence cardiac dynamics and deficits in energy metabolism as well as alterations in cardiac extracellular matrix composition. Cardiac hypertrophy develops in two ways. The first one is a concentric hypertrophy caused by chronic pressure overload leading to reduced left ventricular volume and increased wall thickness. The second one is the eccentric hypertrophy due to volume overload and causing dilation and thinning of the heart wall. The mechanism of eccentric expansion is induced by the addition of the contractile sarcomere in series, which causes cell elongation. In contrast, the mechanism of concentric enlargement is caused by addition of sarcomeres in parallel, which results in increased cell thickness [Barry et al 2008]. The primary molecular cause of enlargement of the heart is hypertrophy of myocytes without an increase in the cell number. The increase in myocyte size accompanies an increase in the number of cardiac fibroblasts releasing fibrotic deposits and increased myocardial stiffness [Wakatsuki et al. 2004]. This in turn leads to overload and promotes further hypertrophy and cell death, resulting in a detrimental cycles of cardiac enlargement and myocyte loss. In experimental animal model, monocrotaline (MCT), a toxic pyrrolizidine alkaloid of plant origin has been used extensively to produce pulmonary hypertension (PH) in rats [Schultze et al 1998]. MCT can selectively injure the vascular endothelium of the lung and induces pulmonary vasculitis. As a result develops muscularization and hypertrophy of media in pulmonary arteries thus increasing vascular resistance

and stimulating local hypertension. Then, the PH leads to compensated right ventricle hypertrophy (RVH), which can progress to failure within weeks depending on the dose of MCT and the age of the animals.

I.I.2.1. Role of genetic factors

Genetic factors are important determinants of phenotypic expression of cardiac hypertrophy, both in single- or in complex-gene disorders. Hypertrophic cardiomyopathy (HCM) defined clinically by left ventricular hypertrophy is the most common inherited cardiac disorder and this condition is the major cause of sudden death in the young under 35 years and in athletes [Keren et al. 2008]. HCM is usually inherited as an autosomal dominant mutation in genes that encode protein constituents of the sarcomere.

I.I.2.2. Role of oxidative stress

Oxidative stress is characterized by the excessive formation of reactive oxygen species (ROS) from the respiratory chain that cannot be adequately countered by intrinsic antioxidant systems. There are two sources of free radicals [Gupta et al. 2007]. One is oxidative phosphorylation of mitochondria-generated oxygen radicals, such as superoxide radicals, H_2O_2, and hydroxyl radicals. Another source is lipid oxidation. It has been shown that excessive ROS generation triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death [Murdoch et al 2006, Sawyer et al 2002, Giordano et al 2005]. In the myocardium, oxygen radicals have been shown to alter Na+/Ca2+ exchange, Na+-K+ ATPase, and Ca2+ ATPase activities. Mutant

mice that lack manganese superoxide dismutase (a scavenging enzyme) show a severe reduction in complex II of the respiratory chain and rapidly develop dilated cardiomyopathy [Li et al 1995]. The involvement of eNOS and eNOS uncoupling in cardiac hypertrophy has been documented. The essential cofactor tetrahydrobiopterin (BH4) has been shown to be a key factor in eNOS catalysis. eNOS coupled with BH4 generates NO and L-citrulline. When BH4 availability is limiting, eNOS no longer produces NO but instead generates superoxide [Wever et al 1997; Alp et al 2004]. Studies have demonstrated that the normal (coupled) eNOS pathway inhibits hypertrophy and hypertrophic signaling, matrix metalloproteinase (MMP) activation, and cardiac dysfunction. In the presence of oxidant stress, depletion of substrate (arginine) and cofactors (BH4), NOS can become uncoupled and generate more ROS. This enhanced ROS diverts (forming peroxynitrite) and stimulates pathological cardiac hypertrophy and remodeling [Takimoto et al 2007].

I.I.2.3. Role of inflammation

Cytokines are soluble peptides that mediate cell-to-cell interactions via specific cell surface receptors and regulate the activation, differentiation, growth, death or acquisition of effector functions of immune cells. Most cytokines act as local autocrine or paracrine mediators and do not act in endocrine fashion. Therefore, systemic elevation of cytokines produces a series of local pathologic reactions. Accumulating evidence indicates that inflammatory cytokines play a pathogenic role in cardiac diseases by influencing heart contractility, inducing hypertrophy and promoting apoptosis or fibrosis. In the pressure overloaded ventricle, the

myocardium first develops adaptive hypertrophy. In the later stage this pattern of hypertrophy underwent a transition to chronic heart failure. Cytokines appear to play a significant role in this process by accelerating myocyte growth and down-regulating cardiac function [Sasayama et al. 1999]. It has been found that, in the patients, sustained increases in inflammatory cytokines, such as TNFα, IL-1 and IL-6, are the major pathogenic factors involved in cardiac remodeling and in the progression of chronic heart failure [Deswal et al 2001, Mann 2002, Malave et al 2003]. Animal studies have indicated that inflammatory cytokines induce cardiac hypertrophy [Li et al 2000], inhibit heart contractility [Yu et al 2003], and promote cardiomyocyte apoptosis [Nian et al 2004]. The main hypertrophic cytokines are the members of the IL-6 family including IL-6 itself, leukaemia-inhibitory factor (LIF) and cardiotrophin-1 (CT-1). Study demonstrated that transgenic mice overexpressing IL-6 and IL-6 receptor developed hypertrophy of ventricular myocardium [Hirota et al 1995]. In another model of cardiac hypertrophy due to pressure overload in rats, IL-6 was significantly increased [Pan et al 1998]. CT-1 was shown to promote cardiac myocyte survival and induce an eccentric form of hypertrophy both in vitro and in vivo [Jin et al 1996, Sheng et al 1996, Wollert et al 1996]. BNP is one of the most widely used marker of heart failure. Study has recently determined that combining CT-1 and BNP measurements may be a greater predictor of mortality in patients suffering from congestive heart failure than either marker alone [Tsutamoto et al 2007]. Study has indicated that IL-1β induces unique cardiac hypertrophy and the marked secretion of ANP and BNP [Harada et al 1999]. In this study, they examined the effects of IL-1β on the morphological changes of myocyte (MC) in comparison with the effects

of ET-1. They found that IL-1β induced the star-shaped MC hypertrophy characterized by elongation and pointed edges, while ET-1 induced the MC hypertrophy characterized by shapes of squares, triangles or circles. Therefore, they concluded that IL-1β induces unique form of cardiac hypertrophy.

I.I.2.4. Role of energy metabolism

Circulating fatty acids, glucose, lactate, amino acids, and ketone bodies as well as intracellular glycogen and triglycerides can be used by the heart to produce energy [Lehman et al 2002, Saddik et al 1991, Needly et al 1974]. Rates of glycolysis are accelerated in heart hypertrophy induced by pathological stimuli. In contrast, long-chain fatty acid oxidation is reduced in pathologically hypertrophied hearts [Bowles et al 1992, El Alaoui-Talibi et al 1997, Allard et al 1994]. Evidence from experimental and clinical studies suggest that the catabolic fate of glucose is an important determinant of post-ischemic myocardial function [Depre et al 1998, Taegtmeyer et al 2000]. Dichloroacetate [Wambolt et al 2000], an activator of pyruvate dehydrogenase and trimetazidine, an anti-ischemic drug [Lopaschuk et al 2002] are two agents that modulate energy metabolism. In the ischemic heart these two agents have been shown to normalize cardiac function by stimulation of glucose oxidation and reduction of glycolysis. In addition to enlargement of cardiac myocytes, the hypertrophic response to pathological and physiological stimuli is accompanied by qualitative changes within the cardiac myocytes. Energy metabolism is one aspect of cardiac myocyte biology that is well recognized as being altered during the heart hypertrophy [Richey et al 1998, Bowles et al 1992, El Alaoui-Talibi et al 1997, Allard et al 1994]. Of importance is

the fact that changes in energy metabolism are recognized as key determinants in both adaptive and maladaptive cardiac hypertrophy.

I.I.3. Molecular mechanisms involved in cardiac hypertrophy

I.I.3.1. Vasoactive peptides

I.I.3.1.1 ANP and BNP as markers of cardiac hypertrophy

Cardiac natriuretic peptides contribute importantly to the maintenance of sodium and volume homeostasis in health and disease. ANP was originally identified in atrial myocardial extracts as a substance promoting natriuresis [de Bold et al 1981]. It is principally produced in the atria of normal adults, but is also accumulated in the ventricles during development [Cameron et al 1996, Mercadier et al 1989] and in hypertrophied ventricles [Gu et al 1989]. BNP was first purified from porcine brain [Porter et al 1989]. Although expressed in brain, BNP is most abundant in the cardiac ventricles, where its expression is induced in decompensated heart failure [Langenickel et al 2000]. CNP was discovered in the central nervous system, although later studies indicated that expression of CNP in the endothelium [Suga et al 1992], macrophages [Ishizaka et al 1992], and cardiac fibroblasts [Horio et al 2003] is higher than that in the brain. As shown in figure 1, three natriuretic peptides bind to 3 natriuretic peptide receptors. Calderone et al [1998] investigated the effect of exogenously administered ANP on the cardiac cell hypertrophy induced by norepinephrine. They reported that ANP inhibited the cardiac myocyte enlargement, stimulated by norepinephrine, by mechanism involving cGMP-and Ca^{2+} influx. This finding demonstrated that endogenous ANP suppress the development of cardiac

myocyte hypertrophy. Furthermore, Horio et al [2000] have shown that inhibition of natriuretic peptide receptors by HS-142-1, antagonist increased the size of cardiac myocytes. The same research group [Horio et al 2000] has found that zaprinast, a specific cGMP phosphodiesterase inhibitor, suppressed the basal and phenylephrine (PE)-stimulated protein syntheses. These observations indicate that endogenous ANP inhibits cardiac myocyte hypertrophy under basal as well as phenylephrine-stimulated conditions, probably through a cGMP-dependent process. Thus, ANP may play a role as autocrine factor in the regulation of cardiac myocyte growth. BNP exerts many similar actions as ANP on target organs. Accordingly, the BNP is viewed as a cardiomyocyte-derived antifibrotic factor and plays a role in ventricular remodeling [Tamura et al 2000]. Prolonged cardiac hypertrophy and heart failure are characterized by the increase in the expression of the ANP and BNP [Chien et al 1991]. Previous studies implicated the expression of ANP and BNP genes as markers for ventricular dysfunction [Yoshimura et al 1993]. Clarkson et al [1996] investigated therapeutic effects of BNP administration in six patients with isolated diastolic heart failure with moderately severe exertional symptoms (exercise intolerance), five of whom had hypertension and four of whom had LV hypertrophy. Overall, their findings suggest that ANP, BNP/NPR-A/cGMP system plays an important role in counteracting of cardiac hypertrophy.

I.I.3.1.2 Cardioprotective role of GC-A, GC-B, and NPR-C in the hypertrophic heart

The biological actions of natriuretic peptides are mediated by specific guanylyl cyclase receptors (GC-A, also called natriuretic peptide receptor (NPR)-A and GC-B) through generation of cGMP [Hamet et al 1984, Chinkers et al 1989, Lowe et al 1989]. The ligand selectivity of GC-A is ANP≥ BNP>CNP [Suga et al 1992]. The preferred ligand for the GC-B receptor is CNP whereas ANP and BNP are bound with relatively less affinity [Samson 2000]. A third receptor, namely NPR-C does not show any ligand selectivity and thus may promote clearance of the peptides from the circulation. GC-A mediates the endocrine effects of ANP and BNP on regulating arterial blood pressure and volume homeostasis, and also the local antihypertrophic actions in the heart. It has paracrine function in vasodilatation and endochondral ossification. The important cardiovascular role of the ANP/GC-A system has been emphasized in gene knockout mouse models. For example, NPR-A deficiency in mice leads to marked cardiac hypertrophy [Oliver et al 1997], suggesting that the endogenous ANP-NPR-A system may have a role in the regulation of cardiac cell growth. Targeted deletion of the peptide (ANP-/-) or its receptor (GC-A-/-), leads to severe, chronic arterial hypertension, cardiac hypertrophy, and sudden death [Kuhn et al 2002]. Holtwick et al [2003] studied mice with conditional, selective deletion of GC-A in cardiomyocytes (GC-A-/-) and demonstrated that the ANP/GC-A system play an autocrine/paracrine roles in the heart, which include cardiomyocyte growth inhibition and the stimulation of diastolic relaxation.

I.I.3.1.3 Role of other vasoactive peptides in cardiac hypertrophy

Vasoactive peptides such as endothelin-1 (ET-1) and angiotensin II (Ang II) have been reported to induce cardiac hypertrophy. ET-1 activates several intracellular signaling pathways in cardiac myocytes, notably the protein kinase C (PKC) pathway, the mitogen-activated protein kinase (MAPK) cascades, and phosphatidylinositol 3-kinase/Akt (PI-3/Akt) pathway [Ito et al 1993]. It is likely that activation of these pathways contributes to the hypertrophic response. Ang II causes cardiomyocyte hypertrophy and cardiac fibroblast proliferation concomitant with deposition of extracellular matrix, both actions being mediated by the Ang II type 1 (AT-1) receptors [Sadoshima et al 1993]. Ang II has also been reported to stimulate autocrine/paracrine release of other growth factors such as platelet-derived growth factor, transforming growth factor β-1 (TGF-β1), insulin-like growth factor-1 (IGF-1), and ET-1 in various cell types [Gibbons et al 1992, Delafontaine et al 1993, Ito et al 1993] These growth factors may, in part, indirectly mediate the physiological effects of Ang II as well. Interestingly, the receptor for Ang IV known as insulin regulated aminopeptidase (IRAP), functioning as oxytocinase, has pleotropic actions and plays a role in degradation of oxytocin, arginine-vasopressin and other peptide hormones.

I.I.3.2. Peptide growth factors

TGF-β1 has activities important for the regulation of development, cell differentiation, tissue maintenance, and repair in a variety of cells and tissues [Massague et al 1990, Brand et al 1995]. TGF-β1 is upregulated in myocardium by increased work load and stimulates the hypertrophic program of cardiac gene

expression [Parker et al 1990]. As mentioned before, it has been reported that TGF- β1 is an important mediator of cardiac hypertrophy stimulated by Ang II [Schultz Jel et al 2002]. Fibroblast growth factor-2 (FGF-2) is also naturally produced by cardiac myocytes and non-myocytes in the heart, where it plays an autocrine and paracrine roles in cardiomyocyte hypertrophy [Takahashi et al 1994]. IGF-1, a 70 amino-acid basic peptide, is an essential growth factor for somatic-cell proliferation and differentiation, and during development mediates biological effects of growth hormone (GH) [Rinderknecht et al 1986, Sacca et al 1994]. IGF-1 has been shown to activate several pathways of the hypertrophic response, where it enhances the expression of contractile proteins such as actin, myosin and troponin. Rats undergoing left ventricular hypertrophy have increased IGF-1 mRNA and protein levels [Wahlander et al 1992, Donahuc et al 1994]. The signal is transduced by intracellular events. IGF-1 is one of the most potent natural activators of the protective AKT signaling pathway, a stimulator of cell growth and a potent inhibitor of programmed cell death. Therefore, IGF-1-dependent signaling pathways may be important in the initiation of beneficial cardiac hypertrophy *in vivo*, possibly *via* autocrine or paracrine effects. Several clinical trials tested IGF-1 for a variety of indications, including growth failure, type 1 diabetes, type 2 diabetes. Epidemiological observations suggested that high IGF-I plasma levels are associated with decreased risks for heart failure. [Vasan et al 2003].

I.I.3.3. Hormones

Thyroid hormone is generally considered as classic hormonal mediator of cardiac hypertrophy. Excess of thyroid hormone in experimental small animals and primates increases heart weight [Khoury et al 1996]. GH, the major stimulus for IGF-1 production and release, induces cardiac enlargement in experimental animals [Lei et al 1988]. Animal studies also indicate an anti-hypertrophic action of estrogen in the heart. In ovariectomized mice, estrogen supplementation activates ANP-mediated mechanism causing a 30% reduction of pressure overload and reduction cardiac hypertrophy [van Eickels et al 2001]. Estrogen prevents development of cardiac hypertrophy through the inhibition of molecules important in hypertrophic pathways such as calcium-sensitive protein phosphatase, calcineurin, Ang II or ET-1.

I.I.4. Molecular pathways

In response to hemodynamic overload, several parallel and interconnected intracellular signal transduction cascades are activated and mediate biological effects including cardiac hypertrophy [Chien et al 1993]. These intracellular pathways can activate transcription factors such as c-*fos*, c-*jun*, c-*myc*, and *egr*-1, which in turn regulate many genes involved in cellular metabolism, myocardial growth and apoptosis [Sadoshima et al 1997]. The intracellular pathways are discussed below.

I.I.4.1. PI3K/Akt pathway

PI3K/Akt plays an important role in the heart through regulation of cardiomyocyte growth, survival, function, and metabolism [Matsui et al 2003]. PI3K is activated by several receptor tyrosine kinases, such as the IGF-1 receptor and G protein-coupled receptors. Study showed that PI3K is activated in pressure overload hypertrophy in a Gβγ-dependent fashion [Naga et al 2000]. Over-expression of a constitutively active PI3K mutant resulted in cardiac hypertrophy in transgenic mice [Shioi et al 2000]. Conversely, a dominant-negative form of PI3K led to significantly reduced heart weight/body weight ratios in transgenic mice. These changes indicate that PI3K in fact regulates the hypertrophic response. One of the principal targets of PI3K signaling is the serine/threonine kinase Akt, also known as protein kinase B (PKB). Akt is activated via binding of PI3K phosphorylated phosphoinositides, which in turn results in its translocation to the membrane. It has been shown that transgenic overexpression of Akt/PKB is sufficient to induce significant cardiac hypertrophy in mice [Shioi et al 2002, Matsui et al 2002]. Constitutive activation of Akt in skeletal muscle also causes hypertrophy [Bodine et al 2001].

I.I.4.2. MAPKs pathway

MAPKs play a significant role in hypertrophic signaling [Frey et al 2003]. This pathway provides an important link between external stimuli and the nucleus via phosphorylation and regulation of multiple transcription factors. MAPK superfamily is grouped into three categories including ERKs, JNKs, and p38 MAPKs. ERKs have been reported to be responsible for the development of

hypertrophy [Glennon et al 1996]. Being a potent activator of AP-1 [Karin et al 1996], the JNK/AP-1 cascade is considered to be involved in the increased expression of ANF, skeletal α-actin, TGF-β1, and collagen type I in cardiac hypertrophy [Kim et al 1999]. P38 MAPK has a potential role in cardiomyocyte hypertrophy in response to stress stimuli [Glennon et al 1996, Nemoto et al 1998, Wang et al 1998]. Among the p38 isoforms, the p38α and p38β are most important for hypertrophic responses [Molkentin et al 2001].

I.I.4.3. PKC pathway

PKC family consists of several serine/threonine kinases that are ubiquitously expressed and act downstream of almost all the membrane-associated signal transduction pathways [Nishizuka et al 1986]. Multiple studies implicate various PKC isoforms in the pathogenesis of cardiac hypertrophy. Transgenic overexpression of PKCβ in heart is sufficient to elicit cardiac hypertrophy and sudden death [Bowman et al 1997]. However, these findings were not confirmed in other studies [Huang et al 2001, Tian et al 1999]. *In vivo*, PKCε involvement has been shown in compensated cardiac hypertrophy [Takeishi et al 2000]. Study of Braz et al. [2002] showed that only PKCα is required to stimulate cardiomyocyte hypertrophy *in vitro*, further complicating the issue of the relative importance of PKC isoforms in this process.

I.I.4.4. NF-κB pathway

NF-κB is a redox-sensitive transcription factor and its signaling is critical in the development of atherosclerosis, myocarditis, ischemia, cardiac hypertrophy and

dilated cardiomyopathy [Jones et al 2003, Purcell et al 2003, Valen et al 2001]. Recent studies have suggested that the NF-κB activation plays an important role in the pathogenesis of cardiac remodeling and heart failure [Kalra et al 2000, Ritchie et al 1998, Wong et al 1998]. A major focus is also directed toward NF-κB contribution in cardiac hypertrophy, which is investigated *in vitro* [Gupta et al 2002, Purcell et al 2001] and *in vivo* [Li et al 2004].

I.I.5. Apoptosis

Apoptotic nuclei are often observed in hypotrophic myofibers from patients with heart diseases. Apoptosis which contributes to the suppression of cardiac hypertrophy is the gene-regulated process of physiological cell self-destruction [Thompson 1995]. However, apoptosis has been shown to worsen cardiac structure and plays a role in the transition of hypertrophic heart to heart failure [Nadal-Ginard et al 2003, Van Empel et al 2004]. Diez et al [1997] and Fortuno et al [1998] reported that cardiomyocyte apoptosis is reduced after prolonged inhibition of the angiotensin pathway during the established phase of hypertension in the spontaneously hypertensive rats. Evidence suggests that two independent pathways may lead to cardiomyocyte apoptosis [Bishopric et al 2001, Foo et al 2005]. The extrinsic (type I) pathway is initiated by ligands that bind to death receptors such as TNF receptor 1, whereas the intrinsic (type II) pathway is governed by the release of various proapoptotic proteins such as Cytochrome c (Cyt c), procaspases 2, 3, 9, and apoptosis-inducing factor (AIF) from the mitochondria. The inhibitors of apoptosis and cellular FLICE-like inhibitory protein prevent activation of the extrinsic pathway. Bcl-2 is a major

regulator of mitochondrial membrane permeability and prevents activation of the intrinsic pathway.

I.II. Pulmonary hypertension (PH) and right ventricular hypertrophy

I.II.1. Modulation of cardiac makers in this pathology

The most common causes of right ventricle hypertrophy (RVH) are RV outflow obstruction and PH. These changes damage the blood vessels in the lung, causing increased pressure in the remaining vessels. Natriuretic peptides are important markers of heart hypertrophy including RVH. However there are some differences in the biology of natriuretic peptides produced in the heart. ANP is mostly stored in granules in the atria, whereas BNP is released to blood as a result of ongoing synthesis in ventricular myocardium. ANP has shorter plasma half-time than BNP [Mukoyama et al 1991]. Nagaya et al [1998] found similar rate of elevation of both ANP and BNP in patients with volume overload, however, changes of ANP plasma levels were less evident than plasma BNP levels in response to RV pressure overload. Two studies [Nagaya et al 1998, Tulevski et al 2001] showed similar significant correlations of both plasma ANP and BNP with RV ejection fraction and similar correlations for both ANP and BNP in the plasma with haemodynamic variables. However, a head-to-head comparison of the two natriuretic peptides showed that changes of ANP plasma level were much less related to the effects of treatment and survival in patients with primary PH [Nagaya et al 2000], then BNP is why the interest regarding clinical application of natriuretic peptides in monitoring RV failure due to chronic PH focused on BNP. BNP synthesis and secretion are regulated at both gene and

protein levels. At the protein level, proBNP, a high-molecular-weight precursor is synthesis at the final step. It is then cleaved into biologically inactive N-terminal segment (NT-proBNP) and the proper low-molecular-weight BNP [Wang et al 2003]. Both BNP and NT-proBNP levels was useful in the diagnosis of left ventricular (LV) heart failure [Wensel et al 2002, McDonagh et al 2004, Doust et al 2005, Steg et al 2005, Jankowski, 2008]. NT-proBNP has longer half-life and better stability than BNP, both in the circulation and during storage. Studies [Fijalkowska et al 2006, Gan et al 2006] also found that the both BNP and NT-proBNP plasma levels are related to the severity of right ventricular (RV) dysfunction, therefore, they could be considered as potential prognostic markers in this disease.

I.II.2. Investigations of RV and LV function following induction of PH with the monocrotaline

Monocrotaline (MCT) is a toxic pyrrolizidine alkaloid isolated from the plant Crotalaria spectabilis and has been used extensively to produce PH in rats [Schultze et al 1998]. There have been numerous animal investigations of RV and LV function following induction of PH with the ~~toxin~~ MCT [Brunner et al 2002, Chen et al 1997, Chen et al 1997, Henriques-Coelho et al 2004, Wolkart et al 2000]. A single dose of MCT in the rat results 7 weeks later in progressive and sustained PH [Meyrick et al 1980]. The pathological features such as pulmonary vasculitis, PH, and RVH seen following MCT administration are similar to those evident in human primary and secondary PH, which features are the thickening of the pulmonary arteries and narrowing of these blood vessels, as well as the

subsequent RVH. RV dysfunction in the setting of chronic PH has been well described both clinically and experimentally [Bonnet et al 2004, Kerbaul et al 2004, Rich et al 2005]. In MCT-treated animals, there is evidence of elevated pulmonary vascular resistance, increased pulmonary artery pressure, and subsequent RVH [Meyrick et al 1980, Reindel et al 1990, Rosenberg et al 1988]. These changes are accompanied by increases in RV systolic and diastolic pressures and ultimately by RV failure [Brown et al 1998, Farahmand et al 2004, Honda et al 1992, Seyfarth et al 2000]. However, Akhavein et al [2007] showed that the impairment of cardiac function was associated with myocarditis and coronary arteriolar medial thickening and unrelated to the degree of PH, indicating a direct cardiotoxic effect of MCT.

I.III. Oxytocin (OT) and Oxytocin receptor (OTR) in the heart

I.III.1. General features of OT and OTR

OT is a nine amino acid peptide structurally similar to closely related peptide arginine vasopressin, an antidiuretic hormone. OT is produced predominantly in magnocellular neurosecretory cells of the hypothalamus, transported to the posterior pituitary and secreted into the circulation. Although OT has an established role as a circulating hormone, it can also act as a “neurotransmitter and as a neuromodulator” by interacting with its central OTR within the brain [Gould et al 2003]. Circulating OT may originate from the brain or from peripheral tissues, because OT can also be synthesized in peripheral tissues such as corpus luteum, uterus, placenta, amnion, testis, pineal, thymus glands, mammary glands, kidney, heart, aorta and vena cava. OT is involved in female reproduction

where it is involved in the maintenance of parturition and the initiation of lactation [Blanks et al 2003]. OT is involved in male reproduction too. Metabolic activity of OT involves the triggering the insulin and glucagons secretions from the pancreas [Kolesnyk et al 2000]. Recent studies associated OT with increased glucose uptake by cardiac rat myocytes [Florian et al. 2009, Abstract QHA, Quebec City]. In adipocytes OT is also involved in insulin-like action [Hanif et al 1982, Egan et al 1990]. The multiple hormonal and neurotransmitter functions of OT are mediated by the specific OTRs. There is only one OTR gene and therefore, the same receptor protein is expressed in brain and peripheral organs. The OTR is a typical class I G protein-coupled receptor that is primarily coupled via G(q) proteins to phospholipase C-beta. The high-affinity receptor state requires both Mg(2+) and cholesterol, which probably function as allosteric modulators [Gimpl et al 2001]. OTRs have also been demonstrated in many peripheral tissues, including the kidney, heart, thymus, pancreas, and adipocytes. Kimura et al [1992] first isolated and identified a cDNA encoding the human OTR using an expression cloning strategy. To date, the OTR encoding sequences from pig [Gorbulev et al 1993], rat [Rozen et al 1995], sheep [Riley et al 1995], bovine [Bathgate et al 1995], mouse [Kubuta et al 1996], and rhesus monkey [Salvatore et al 1998] have also been identified.

I.III.1.1. Cardiovascular functions of OT

In rat heart, OT is expressed in atria at higher level than in ventricles. *In vitro* studies demonstrated that atrial myocytes secrete OT [Jankowski et al. 2000]. OT is reported to be a regulator of cell proliferation and a mediator for myoepithelial

cells differentiation in a mouse model [Reversi et al 2005]. OT exerts functions in the heart and vasculature by binding to OTR. The presence of OTR in the rat heart was demonstrated by autoradiography performed on frozen sections and the number of OT binding sites was similar in the atria and ventricles [Gutkowska et al 1997]. In the brain, the ANP and OT are co-localized in the same hypothalamic magnocellular neurons [Jirikowski et al 1986] with the highest percentage (90%) of oxytocinergic neurons containing ANP immunoreactivity found in the intersupraoptico-paraventricular island. The co-localization of both these peptides provides a morphological basis for their interaction in the modulation of physiological actions. Interaction between these hormonal system is also proposed in the heart. Experiments demonstrated physiologic function of OT in the mediation of ANP release from the heart [Gutkowska et al. 1997; Favaretto et al 1997]. An OT antagonist inhibited the stimulated and basal ANP release from perfused rat heart suggesting that the OT action in the heart is mediated by specific OTR [Gutkowska et al 1997]. OT is also able to modulate electrical and mechanical activities of atria, given that it promotes a reduction in both heart rate and force of atrial contraction, in concentrations that also stimulate ANP release [Gutkowska et al 1997, Mukaddam-Daher et al 2001, Favaretto et al 1997]. In addition, OT knockout mice show higher intrinsic heart rate, suggesting the existence of an intracardiac oxytocinergic system that modulates electrical activity of the heart [Michelini et al 2003]. Recent study has reported that the negative chronotropic action of oxytocin participates in its protective effects on ischemia-reperfusion-induced myocardial injury in a rat model [Ondrejcakova et al 2009].

I.III.1.2. OT-ANP interaction in central nervous system

OT released from the neural lobe may reach the heart by circulation to induce ANP release, but the intracardiac OT may also play a paracrine role in stimulating ANP release as mentioned above. On the other hand, endogenous hypothalamic ANP seems necessary to stimulate OT release in the hyperosmolality condition [Chirguer et al 2001]. The mature magnocellular neurons respond to perturbations in water balance by releasing large amounts of stored OT into the general circulation. This is accompanied by functional remodeling of the activity of the magnocellular neurosecretory neurons, the molecular basis of which is still unclear.

II. HYPOTHESIS AND SPECIFIC AIMS

II.1. Hypothesis

The hypothesis proposed in this thesis is that right ventricular hypertrophy induced in rat by pulmonary hypertension is related to the inflammation and changes of heart metabolism and these alterations inhibit local OTR expression. Therefore, impairment of OTR expression in right heart can be linked with local hypertrophy, and possibly, increased expression of cardioprotective hormones.

This hypothesis is consistent with the recent findings suggesting OT role in heart metabolism (stimulation of glucose uptake in cardiomyocytes), cardiac cell growth (promotion of endothelial cell multiplication; differentiation of cardiac stem cells from the functional cardiomyocytes) and activation of genes involved in heart protection (natriuretic peptides and nitric oxide synthases).

II.2. Specific aims

II.2.1. Aim #1: To investigate gene and protein expressions of cardioprotective factors including OTR, NPs and NPRs and marker of pathological hypertrophy, Nab1 in RV and LV of MCT-treated and control rats.

II.2.2. Aim #2: To investigate gene and protein expressions of inflammatory markers including TNFα, IL-1β, and IL-6 and NOS in RV and LV of MCT-treated and control rats.

II.2.3. Aim #3: To investigate gene and protein expressions of metabolic factors including GLUT4 and IRAP in RV and LV of MCT-treated and control rats.

III. EXPERIMENTAL DESIGN

Male Sprague-Dawley rats with an initial body weight ranging from 220–250 grams were used in this study. These rats were randomly divided into two groups: monocrotaline (MCT) - treated (n=7) or control (n=7). Since MCT was known to cause severe pulmonary hypertension in the rats, six to 7 weeks following the injection of MCT, rats were sacrificed. The hearts were extracted. The ratio of right ventricle to left ventricle plus septum weight (RV/LV + S) was used as an index of right ventricular hypertrophy. The tissues of right and left ventricles from MCT-treated and control rats were kindly provided by Dr. Tom Broderick and his colleague, Dr.Tiffany King. Total RNA and proteins were extracted from the tissues of right and left ventricles in the MCT treated and control rats. The gene expressions of oxytocin receptor (OTR), natriuretic peptides (NP) and its receptors (NPRs), nitric oxide synthases (NOS) including eNOS, iNOS, and nNOS, inflammatory cytokines including TNFalpha, IL-1beta, and IL-6, insulin regulated aminopeptidase (IRAP, oxytocinase), glucose transporter GLUT4, and marker of pathological hypertrophy, Nab1 were measured by real-time PCR. The protein expressions of OTR, TNFalpha, eNOS and iNOS, IRAP, GLUT4, and Nab1 were measured by Western-blot. Statistical evaluation of the results was done using Prism 3 software.

IV. MATERIAL AND METHODS

IV.1. Animal treatment and collection of specimens

Rats treated with MCT received a single injection at the concentration of 60 mg/kg subcutaneously in the nape. MCT was dissolved in 0.5 N HCl and the pH was adjusted to 7.4 using 0.5 N of NaOH. Control rats received an equivalent amount of isotonic saline. Rats were placed two per cage and fed with standard rat food and water until sacrifice. The rats were kept in a temperature-controlled room with 12:12-h light-dark cycle. Six to seven weeks before animals were sacrificed and the heart was extracted, weighed and dissected into the left ventricle, right ventricle, and septum and then frozen with tongs cooled to the temperature of liquid nitrogen.

IV.2. Measurement of gene expression

IV.2.1. Table 1. Materials for Real-time PCR

Reagents	Company	Cat No.
Trizol	Invitrogen	15596-026
Chloroform	Sigma	34854
Isopropanol	Sigma	19516
RNase-free Water	QIAGEN	129112
DNase 1	Invitrogen	18047019
RNaseOUT	Invitrogen	10777-019
Random Primer	Invitrogen	48190-011
M-MLV reverse transcriptase	Invitrogen	28025-013
dNTP set (100mM each A,C,G,T)	Amersham	28-4065-51
iQ™ SYBR® Green Supermix	BIO-RAD	170-8882

IV.2.2. Preparation of total RNA and quantification of RNA concentration

IV.2.2.1. Preparation of total RNA

Homogenize tissue samples in 1 ml of TRIZOL reagent per 50 to 100 mg of tissue. Add 0.2ml chloroforme per 1 ml of TRIZOL reagent. Shake samples vigorously for 15 seconds and incubate them at room temperature (RT) for 3 minutes. Centrifuge samples for 15 minutes at 12000 x g at 4^oC. Transfer the upper aqueous phase carefully without disturbing the interphase into a fresh tube. Add 0.5 (1 volume) isopropanol to precipitate RNA. Incubate samples for 10 minutes at RT. Centrifuge samples for 10 minutes at 12000 x g at 4^oC. Remove the supernatant completely. Wash the pellet with 1ml 75% ethanol and vortex samples for 1minutes. Centrifuge samples for 5 minutes at 7500 x g at 4^oC. Remove all ethanol solution. Air-dry RNA pellet for 5 to 10minutes. Dissolve RNA in 19µl DEPC-treated water by passing solution a few times through a pipette tip. Incubate RNA solution for 5 minutes at 65^oC.

IV.2.2.2. Determination of RNA concentration

Take 0.5ml DEPC water into new sterile test tubes. Vortex the RNA solutions 1 to 2 seconds and spin them for 10 seconds. Pick up 2µl RNA and put into 0.5ml DEPC water. Determine the sample concentration and purity using UV/visible spectrophotometer to take OD at 260nm and 280 nm. The A_{260}/A_{280} ratio should be above 1.6. Apply the convertion that 1 OD at 260 equals 40 µg /ml RNA.

IV.2.3. Reverse transcription of mRNA to cDNA

Take 4ug RNA solution from each sample and complete them with DEPC H_2O to the final volume 17 μl. Add 4ul Random Primer, vortex for 2 to 3 seconds and incubate these samples at 70°C for 10 minutes and then keep them on ice. Spin samples for 10 seconds and add 19ul master mix (8ul 5x buffer, 4ul DTT 0.1M, 4ul dNTP 2.5mM, 1μl RNAseOut, 2ul MLV) to reach total volume of 40ul. Vortex above mixture of samples and master mix for 2 to 3 seconds and incubate them at 42°C for 60 minutes, and then followed incubation at 70°C for 5 minutes. Obtain cDNA and store them at -20°C.

IV.2.4. Sequences of the primers used for PCR amplification of cDNA samples

1) OTR:

Forward primer: 5' CGTCAATGCGCCCAAGGAGA 3'
Reverse primer: 5' ATGCAAACGAATAGACACCT 3'

2) ANP:

Forward primer: 5' CAGCATGGGCTCCTTCTCCA 3'
Reverse primer: 5' GTCAATCCTACCCCCGAAGCAGCT 3'

3) BNP:

Forward primer: 5' CCATCGCAGCTGCCTGGCCCATCACTTCTG 3'
Reverse primer: 5' GACTGCGCCGATCCGGTC 3'

4) GC-A:

Forward primer : 5' ATCACAGTGAATCACCAGGAGTTC 3'
Reverse primer : 5'AGATGTAGATAACTCTGCCCTTTCG 3'

5) GC-B:

Forward primer: 5' GCTACATGGTACCACCATATTTGGACAACCTC 3'

Reverse primer: 5’ CAGGAGTCCAGGAGGTCCTTTTCG 3’

6) NPR-C:

Forward primer: 5’ ATCGTGCGCCACATCCCAGGCCAGT 3’
Reverse primer: 5’ TCCAAAGTAATCACCAATAACCTCCTGGGTACCCGC3’

7) TNFalpha:

Forward primer: 5’ CCCAGGGACCTCTCTCTAATCA 3’
Reverse primer: 5’ GCTACAGGCTTGTCACTCGG 3’

8) IL-1beta:

Forward primer: 5’ TCCCCAGCCCTTTTGTTGA 3’
Reverse primer: 5’ TTAGAACCAAATGTGGCCGTG 3’

9) IL-6:

Forward primer: 5’ TCTCCACAAGCGCCTTCG 3’
Reverse primer: 5’ CTCAGGGCTGAGATGCCG 3’

10) eNOS:

Forward primer: 5’ TACAGAGCAGCAAATCCAC 3’
Reverse primer: 5’ CAGGCTGCAGTCCTTTGATC 3’

11) iNOS:

Forward primer: 5’ GATCAATAACCTGAAGCCCG 3’
Reverse primer: 5’ GCCCTTTTTTGCTCCATAGG 3’

12) nNOS:

Forward primer: 5’ GGGCCATGTACCTGTCGTCCTC 3’
Reverse primer: 5’ TCCCTTTGTTGGTGGCATACTTGA 3’

13) GLUT4:

Forward primer: 5’ ACCCTGGGCTCTGTATCCC 3’
Reverse primer: 5’ CCCTGACCACTGAGTGCAAA 3’

14) IRAP:

Forward primer: 5’ GTCTTGGTGAGCATGAGATGG 3’
Reverse primer: 5’ CTAAGGTCCTGGCAGAGGGTA 3’

15) Nab1:

Forward primer: 5' TGCTGACAAGAAGAGATGAG 3' ’
Reverse primer: 5’ TCCTGGTTTCCACAGACTAC 3'
16) GAPDH:

primer: 5’ TTCAATGGCACAGTCAAGGC 3’
Reverse primer: 5’ TCACCCCATTTGATGTTAGCC 3’

IV.2.5. Programs for amplification of gene sequences by Real-time PCR and control of amplification conditions

Cycle 1:	95°C, 01’ 30’’
Cycle 2 (40x):	95°C, 00’ 25’’
	60°C, 00’ 25’’’
	72°C, 00’ 40’’
Cycle 3:	95°C, 01’ 00’’
Cycle 4:	55°C, 01’ 00’’
Cylce 5 (40x):	55°C, 00’ 10’’

IV.2.6. Selection of housekeeping gene for measurement of gene expression semi quantitatively

Housekeeping genes are used to normalize mRNA levels between samples for sensitive comparisons of mRNA transcription. A suitable housekeeping gene should be adequately expressed in the tissue of interest and shows minimal variability in expression between samples and under different experimental conditions. Commonly used housekeeping genes in real-time PCR include beta actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosome small subunit (18S) ribsosomal RNA (rRNA), Ubiquitin C (UBC), hypoxanthine guanine phosphoribosyl transferase (HPRT), succinate dehydrogenase complex, subunit A (SDHA) and Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide (YWHAQ). We choose GAPDH as housekeeping gene in our study for normalization, because this gene showed consistent expression

relative to other housekeeping genes among the treatment groups in our experiments.

IV.2.7. Methodology used for calculation of the gene expression on the basis results provided by Real-time cycler

Calculation of gene expression used the $\Delta\Delta C_T$ method, which calculates relative expression with the equation: fold induction = $2^{-[\Delta\Delta CT]}$. The $\Delta\Delta C_T$ method assumes that the amplification efficiencies of the target gene and the house-keeping gene are very similar. Data from the real-time cycler are displayed as amplification plots with fluorescence plotted against the number of cycles. The threshold as a broken line is either set manually by the user or automatically by the real-time cycler. It is the value where the fluorescence is above background, significantly below the plateau, and within the log-linear phase of the PCR. The threshold cycle (C_T) is the cycle at which the amplification plot crosses the threshold. This is the cycle at which there is a significant detectable increase in fluorescence. A low C_T value indicates high expression, as the template was detected after a low number of cycles. A high C_T value indicates low expression, as the template was only detected after a high number of cycles. $\Delta\Delta C_T$= Ct gene of interest (unknown sample) – Ct GAPDH (unknown sample) – Ct gene of interest (calibrator sample) – Ct GAPDH (calibrator sample). One of the control samples, chosen as the calibrator sample, was employed in each PCR.

IV.2.8. Statistical evaluation of the results obtained by Real-time PCR

Values are means ± SE of a minimum of five different animals. Statistical comparisons were made using Prism 3 software. Data were analyzed by two-way ANOVA. Comparisons between two groups were conducted by unpaired Student's *t*-test.

IV.3. Cardiac protein extraction and Western blotting

IV.3.1. Processing of cardiac samples and measurement of protein concentration

IV.3.1.1. Processing of cardiac samples

The heart was dissected into the left ventricle and right ventricle and then frozen with liquid nitrogen. Tissues were diced into small pieces, then transferred part of them into a big cylinder with 1 ml RIPA buffer containing the inhibitor of proteases (PMSF, EDTA and pepstatin; 10µl/ml). Samples were homogenized and incubated on a rotor at 4°C for 2h. Samples were then centrifuged at 12000 rpm for 20 minutes and supernatants were picked up into new tubes.

IV.3.1.2. Measurement of protein concentration

Prepare dye reagent by diluting 1 part Dye Reagent Concentrate with 4 parts distilled, deionized water. Prepare six dilutions of BSA standard. Prepare test solution by mixing well 2ul samples with 98ul water in clean and dry glass test tubes. Protein solutions were assayed in duplicate. Add 2 ml of diluted dye reagent to each sample and standard solution tube and vortex. Incubate at room

temperature for at least 5 minutes. Transfer these solutions into cuvettes and measure absorbance at 595 nm.

IV.3.2. Table 2. Reagents for Western-blotting

Reagents	Company	Cat No.
PMSF	Sigma	P7626
EDTA	Sigma	E6758
Pepstatin	Sigma	P5318
Glycine	Sigma	E6758
Tris	Bio-Rad	161-0715
SDS	Invitrogen	15525-017
Beta-mercaptoethanol	Sigma	M7154
Acrylamide	Invitrogen	15512-023
N,N'-methylene-bis-acrylamide	Sigma	M7279
NaCl	Sigma	S3014
Methanol	Sigma	179337
Acetic acid	Sigma	242853
TEMED	Invitrogen	15524-010
Ammonium persulfate	Sigma	A3678
Tween 20	Sigma	P9416
Protein standard for blotting	Bio-Rad	161-0374
BSA	Sigma	B4287
Skimmed milk	Milfresh	500g
ECL detection reagents	Amersham	RPN2105
Anti-OTR antibody	Santa Cruz	sc-8102
Anti-TNFalpha antibody	Santa Cruz	sc-8301
Anti-eNOS antibody	Santa Cruz	sc-654
Anti-iNOS antibody	Santa Cruz	sc-7271
Anti-Nab1 antibody	Santa Cruz	sc-12147
Anti-IRAP antibody	Alpha Diagnostic International	IRAP11-S
Anti-GLUT4 antibody	Santa Cruz	sc-1608
Anti-GAPDH antibody	Santa Cruz	sc-25778
Anti-goat IgG-HRP antibody	Santa Cruz	sc-2033
Anti-rabbit IgG-HRP antibody	Santa Cruz	sc-2317

IV.3.3. Electrophoresis conditions, electro-transfer proteins to the nylon membrane

10% acrylamide electrophoresis mini-vertical gel was prepared. This gel was set up in the tank which was filled up with 1X electrophoresis buffer. Samples were prepared with 5X SDS loading buffer, boiled for 3 minutes, and loaded into the wells of gel. The gel was run with 110-120V at 4°C until the line of blue dye of samples reached the top of separating gel and then run with 170-180V until the line of blue dye of samples run out of the gel. The separating gel was cut out and put into the transfer solution for 15 minutes. Filter paper and a piece of nylon membrane were cut wider than the gel. The gel, filter paper, and nylon membrane were immersed in transfer solution and then protein transfer sandwich was made. Proteins in SDS are negatively-charged, so place sandwich form white side of transfer cassette as follows (flatten with each layer except for gel layer because gel is fragile): 1 piece pad, 3 pieces filter paper, nylon membrane, gel, 3 pieces filter paper, 1 piece pad. The transfer conditions were set at 20 to 30V for overnight at 4°C.

IV.3.4. Membrane processing, incubations time, reaction with specific primary antibodies, washing conditions, incubation with secondary antibodies

After transfer of the protein to nylon membrane, the membrane was put in a box with Ponceau red for 3 minutes. Membrane was then rinsed with distilled water for 3 times and scanned. The membrane was washed 3 times with the buffer (1X TBS, 0.1% Tween 20, 10% milk) for 5, 10, and 15 minutes respectively which

was the washing conditions in our western-blot protocol. After washing, the membrane was incubated in the buffer (1X TBS, 0.1% Tween 20, 5% skimmed milk) for overnight at 4°C. After the membrane was blocked by milk, it was washed 3 times under the washing conditions. The membrane then probed with specific primary antibodies for 1 to 3 days at 4°C. After washing again, the membrane was incubated with an appropriate peroxidase-conjugated secondary antibody according to the manufacturer's protocol. Detailed information of primary and secondary antibodies used in our study is listed at follows:

Table 3. Primary and secondary antibodies used in Westen-Blot

Primary antibodies	Concentrations	Secondary antibodies	Concentrations
Anti-OTR	1:10000	Anti-goat IgG-HRP*	1:10000
Anti-TNFalpha	1:2000	Anti-rabbit IgG-HRP	1:5000
Anti-eNOS	1:4000	Anti-rabbit IgG-HRP	1:10000
Anti-iNOS	1:400	Anti-rabbit IgG-HRP	1:5000
Anti-Nab1	1:1000	Anti-goat IgG-HRP	1:10000
Anti-IRAP	1:2000	Anti-rabbit IgG-HRP	1:5000
Anti-GLUT4	1:5000	Anti-goat IgG-HRP	1:10000
Anti-GAPDH	1:20000	Anti-rabbit IgG-HRP	1:20000

* HRP: Horseradish peroxidase, an enzyme commonly used as an indicator for chemical reactions produces peroxide which after reacting with appropriate substrates provides color or photolumininiscent effects.

IV.3.5. Development of Western blotting reaction by chemiluminescence method

Chemiluminescence method is that membrane-bound proteins are detected using specific protein-enzyme conjugates. The enzyme catalyzes chemiluminescence detection substrates to emit light, which produces an image on photosensitive film. Detailed procedures are as follows. Take the detection reagents substrates that are supplied from ECL kit and mix 2ml solution A and 50ul solution B to obtain sufficient to cover the blots. Take out the blots from

washing buffer and remove the excessive washing buffer by placing the blots on paper towels. Transfer blots to a fresh container. Add mixed detection solution directly to the blots on the surface carrying the proteins. Incubate for precisely 5 minutes at room temperature. Drain off excessive detection buffer and wrap blots in Saran Wrap. Place the blots in a film cassette. Switch off the light and place a sheet of Kodak X-Omat film on top of the blots, close the cassette, and expose for various times according to the sensitivity of target protein molecules. Develop films using developing equipment.

IV.3.6. Molecular weight of the bands obtained for the studied protein of interest and expected molecular size as recommended by antibody provider and published in the literature

Table 4. The size of Protein Bands

Studied protein	Obtained MW kDa	Expected molecular size kDa
OTR	66	66
TNFalpha	26	26
eNOS	140	140
iNOS	130	130
Nab1	65	54
IRAP	150	150
GLUT4	45	40-45
GAPDH	37	35.8

IV.3.7. Quantification of the bands intensity using computer software

The Western blots were scanned and saved as “tif’’ files. The intensities of the bands were evaluated using the Adobe Photoshop 3.0 software. The procedures are as follows: Open the scanned image in Photoshop. Invert the image to let the dark parts of the film become light and the light parts become dark. On the first

band, use the lasso tool to draw a line all the way around the edges of the first band. This is where the judgement comes in to play, determining where the edges of the band are, and what is simply background. Check the values for the selection and record them to Excel.

IV.3.8. Methodology used for calculation of protein expression in Western-blot

Calculation of protein expression in Western-blot was done using Microsoft Excel software. We used target protein values divided its GAPDH protein values to get ratios. The average value of these ratios of LVC (AV-R-LVC) was then calculated. Each ratio of target/GAPDH protein value in LVC, LVM, RVC, and RVM groups was divided by AV-R-LVC to have final percentage of protein expression in these groups.

IV.3.9. Statistical evaluation of the results obtained by Western blotting

Values are means ± SE of a minimum of five different animals. Statistical comparisons were made using Prism 3 software. Data were analyzed by one-way or two-way ANOVA. Comparisons between two groups were conducted by unpaired Student's *t*-test.

V. RESULTS

V.1. Effect of MCT treatment on body weights and weights of selected organs

Table 1 shows the effect of MCT treatment on selected organ weights. Rats injected with MCT had no significant differences in body weight, lung weight, and liver weight compared with the age-matched controls. Therefore, the ratios of the lung weight to body weight and the liver weight to body weight similar to the control rats was not affected by MCT-treatment. Table 2 shows the effect of MCT treatment on heart weight. As illustrated, the treatment of rats with MCT showed similar values in LV weight and septum weight compared with the controls. Consequently, in MCT-treated and control rats, the calculated ratio of LV weight to body weight displayed no change. In contrast, RV weight and total heart weight in MCT-treated rats markedly increased and were significantly different compared to the control groups. As a result, the RV weight to LV + septum weight, the RV weight to body weight, and the total heart weight to body weight ratios were all significantly increased in the MCT-treated rats as compared to control rats. These results provided evidence of RV hypertrophy, because the ratio of the RV weight to the weight of LV plus septum and the ratio of the RV weight to body weight were nearly 77% and 131% higher in MCT-treated rats than that in control rats, respectively.

V.2. Effect of MCT treatment on NPs gene expressions in the right and the left ventricles

The effect of MCT treatment on myocardial ANP and BNP gene expression in the LV and RV is illustrated in Figure 1. MCT treatment increased transcripts of ANP mRNA by 8-fold and BNP mRNA by 10-fold in RV (ANP gene expression in MCT-RV *vs* control: p=0.0004, BNP gene expression in MCT-RV *vs.* control: p=0.0003). At the lower extend, the MCT treatment increased transcripts of ANP mRNA (3.7-fold) and BNP mRNA (2.7-fold) in LV (ANP gene expression MCT-LV *vs.* control: p=0.03, BNP gene expression in MCT-LV *vs* control: p=0.0005). It has to be noted that LV expresses ANP and BNP mRNA at the higher level than RV both in control and MCT-treated groups (Statistical significance: ANP gene expression in control-LV *vs.* control-RV: p=0.0001, BNP gene expression in control-LV *vs*. control-RV: p=0.0001; ANP gene expression in MCT-LV *vs*. MCT-RV: p=0.02, BNP gene expression in MCT-LV *vs*. MCT-RV: p=0.0001). The significant ANP and BNP mRNA increases in RV is consistent with elevation these transcripts in heart hypertrophy. However, in the MCT-LV, the ANP and BNP transcripts were elevated in the absence of the weight increase. Elevated ANP and BNP level in the LV can protect this chamber against hypertrophy.

V.3. Effect of MCT treatment on NP receptors gene expressions in the right and the left ventricles

As shown in Figure 2, MCT-induced specific hypertrophy in the RV was associated with increased gene expression of myocardial NP receptors. In the hypertrophied RV significantly increased the transcripts of three NP receptors

and about 2 fold for GC-A, 1.5 fold for GC-B, and 2 fold for NPR-C (statistical significance were: GC-A gene expression in MCT-RV *vs* control: p=0.002; GC-B gene expression in MCT-RV *vs* control: p=0.02; NPR-C gene expression in MCT-RV *vs* control: p=0.03). The changes in NP receptors mRNA were absent in the MCT-LV. This is specific effect in the right side of the heart or right ventricle.

Ⅴ.4. Effect of MCT treatment on NOS gene and protein expressions in the right and the left ventricles

Figure 3 shows that in the LV of MCT-treated group, the eNOS was elevated 1.5-fold both at the mRNA and protein levels. These results were significantly different compared with control group (eNOS gene expression in MCT-LV *vs*. control: p=0.03; eNOS protein expression in MCT-LV *vs*. control: p=0.0001). No changes of eNOS mRNA or proteins were found in the RV. In MCT-treated group, the level of eNOS protein in the LV was higher than that in RV (eNOS protein expression in MCT-LV *vs* MCT-RV: p=0.005) and in the control group, the level of eNOS protein in the LV was similar to that in RV. eNOS elevation in the LV can contribute in protection against hypertrophy.

The iNOS protein expression in MCT-treated rats was significantly higher in enlarged RV compared with RV of control group (iNOS protein expression in MCT-RV *vs*. control: p=0.0001). The effect of MCT treatment was not found at the level of iNOS mRNA expression. In addition, the iNOS protein levels were lower in the LV of MCT-treated than in the LV of control rats (iNOS protein expression in MCT-LV *vs*. control: p=0.02). However, in MCT-treated group, iNOS gene expression in LV was significantly higher than that in RV (iNOS gene

expression in MCT-LV *vs* MCT-RV: p=0.008). In contrast, iNOS protein expression in RV was greater than that in LV (iNOS protein expression in MCT-RV *vs* MCT-LV: p=0.0001). These results show disagreement between transcript and protein levels of iNOS regarding MCT treatment as well as in relation to these measures in the ventricular chambers. Assuming that functional role is attributed to the iNOS protein, its elevation in the MCT-RV suggests local inflammation, as confirmed by pro-inflammatory cytokines.

The nNOS transcripts were heightened 2 fold in the LV compared with control group (nNOS gene expression in MCT-LV *vs* control: p=0.007), but no change were observed in RV.

V.5. Effect of MCT treatment on OTR gene and protein expressions in the right and the left ventricles

As shown in Figure 4, in RVH, both OTR mRNA and protein were decreased by ~2-fold compared with corresponding OTR expressions in control groups (OTR gene expression in MCT-RV *vs* control: p=0.006, OTR protein expression in MCT-RV *vs* control: p=0.0005). In LV, OTR protein was about ~25% lower compared with control group (OTR protein in MCT-LV *vs* control: p=0.05), but no changes of OTR mRNA were detected. Interestingly, in control group, the OTR transcript in RV was significantly higher than that in LV (OTR gene expression in control-RV *vs* control-LV: p= 0.0001). This finding was in disagreement with the OTR protein levels observed in these heart chambers. As demonstrated in Figure 4, the level of OTR protein in LV was significantly higher than that in RV in MCT-treated rats (OTR protein expression in MCT-LV *vs* MCT-RV: p= 0.0009). These

results suggest that post-transcriptional regulatory mechanisms of OTR processing can be different in RV and LV.

V.6. Effect of MCT treatment on expressions of proinflammatory cytokines in the right and the left ventricles.

Figure 5 shows that proinflammtory cytokines IL-1β and IL-6, but not TNFα were modulated by MCT treatment. Both IL-1β and IL-6 mRNAs increased 4-fold in hypertrophied RV compared to control RV (IL-1β gene expression in MCT-RV *vs* control: p=0.002, IL-6 gene expression in MCT-RV *vs* control: p=0.008), but no changes were found in LV. In MCT-treated rats, the levels of IL-1β and IL-6 mRNA in the RV were markedly elevated comparing to the LV (IL-1β gene expression in MCT-RV *vs* MCT-LV: p=0.0003, IL-6 gene expression in MCT-RV *vs* MCT-LV: p=0.04). These findings suggest that MCT-induced inflammatory changes occur in RVH. We did not find any alterations in TNFα gene and protein in RV and LV in MCT-treated rats. More experiments for TNFα expression need to be conducted in order to reach a considerable conclusion.

V.7. Effect of MCT treatment on a marker of pathological hypertrophy gene and protein expressions in the right and the left ventricles

To further verify markers of pathological myocardial hypertrophy in MCT treated rats, the Nab1 gene expressions were tested in RV and LV and results were shown in Figure 6. In MCT-treated rats, the level of Nab 1 mRNA displayed two-fold increase in RV hypertrophy compared to that of control group (Nab1 gene

expression in MCT-RV *vs* control: p=0.002), but not in LV. These results indicate the development of pathological right ventricle hypertrophy.

V.8. Effect of MCT treatment on ventricular expression of factors involved in metabolic functions

Figure 7 illustrates the gene and protein expressions of metabolic factors associated with cardiac OT system: the IRAP and GLUT4. IRAP gene expression significantly increased in RV hypertrophy compared to control RV (IRAP gene expression in MCT-RV *vs* control: p=0.004). In MCT-treated rats, the level of IRAP protein in RV was also significantly higher than that in LV (IRAP protein expression in MCT-RV *vs* MCT-LV: p=0.002). There were no significant differences between IRAP protein levels in MCT-treated group and control group both in the respect to RV and LV. GLUT4 protein content in LV was significantly lower in MCT-treated rats as compared to LV of control group (GLUT4 protein expression in MCT-LV *vs* control: p=0.001), but no changes were observed in RV. There were no differences in the GLUT4 gene expression both in RV and LV between MCT-treated group and controls. However, in MCT-treated rats, the GLUT4 gene expression in the RV was significantly lower than in the LV (GLUT4 gene expression in MCT-RV *vs* MCT-LV: p=0.02). In contrast, the GLUT4 protein in the RV was significantly higher than in LV (GLUT4 protein expression in MCT-RV *vs* MCT-LV: p=0.03). Additional experiments should be performed to find correct conclusion for GLUT4 expression both at gene and protein level in MCT-treated and control groups.

Table 1. Effect of monocrotaline treatment on selected organ weights.

Parameter	*Control*	*Monocrotaline*
Body weight (g)	452 ± 10	436 ± 31
Lung weight (g)	2.04 ± 0.21	2.05 ± 0.08
Liver weight (g)	13.28 ± 0.06	13.27 ± 1.20
Lung weight/body weight (x 10^{-3})	4.51 ± 0.47	4.91 ± 0.55
Liver weight/body weight (x 10^{-3})	29.35 ± 1.25	30.20 ± 1.00

Values are means ± SEM for 7 control and 7 monocrotaline-treated rats.
Values were determined 46 ± 1 day following a single dose injection
of monocrotaline.

Table 2. Effect of monocrotaline treatment on heart weight.

Parameter	*Control*	*Monocrotaline*
Heart weight (g)	1.13 ± 0.04	1.35 ± 0.07 *
LV weight (g)	0.56 ± 0.04	0.51 ± 0.04
RV weight (g)	0.20 ± 0.01	IRAP
Septum weight (g)	0.37 ± 0.02	0.40 ± 0.04 17 kDa
RV/LV + septum weight (g)	0.74 ± 0.03	1.31 ± 0.15*
RV/BW ratio (x10^{-}	0.45 ± 0.01	1.04 ± 0.18 *
LV/BW ratio (x10^{-3})	1.23 ± 0.07	1.20 ± 0.12
HW/BW ratio (x10^{-3})	2.49 ± 0.08	3.15 ± 0.19 *

Values are means ± SEM for 7 control and 7 monocrotaline-treated rats. Values
were determined 46 ± 1 day following a single dose injection of monocrotaline.
* $P < 0.05$ compared to control.

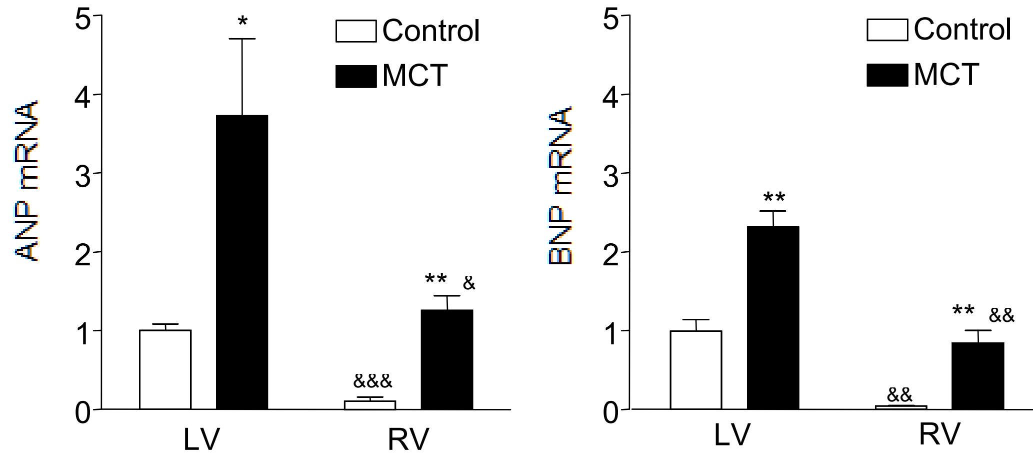

Figure 1. Effect of MCT on ANP and BNP gene expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean±SEM for 7 hearts in each group. MCT *vs* control: *, $P<0.05$; **, $P<0.001$; RV *vs* LV: [&,] $P<0.05$; [&&], $P<0.001$; [&&&], $P<0.0001$.

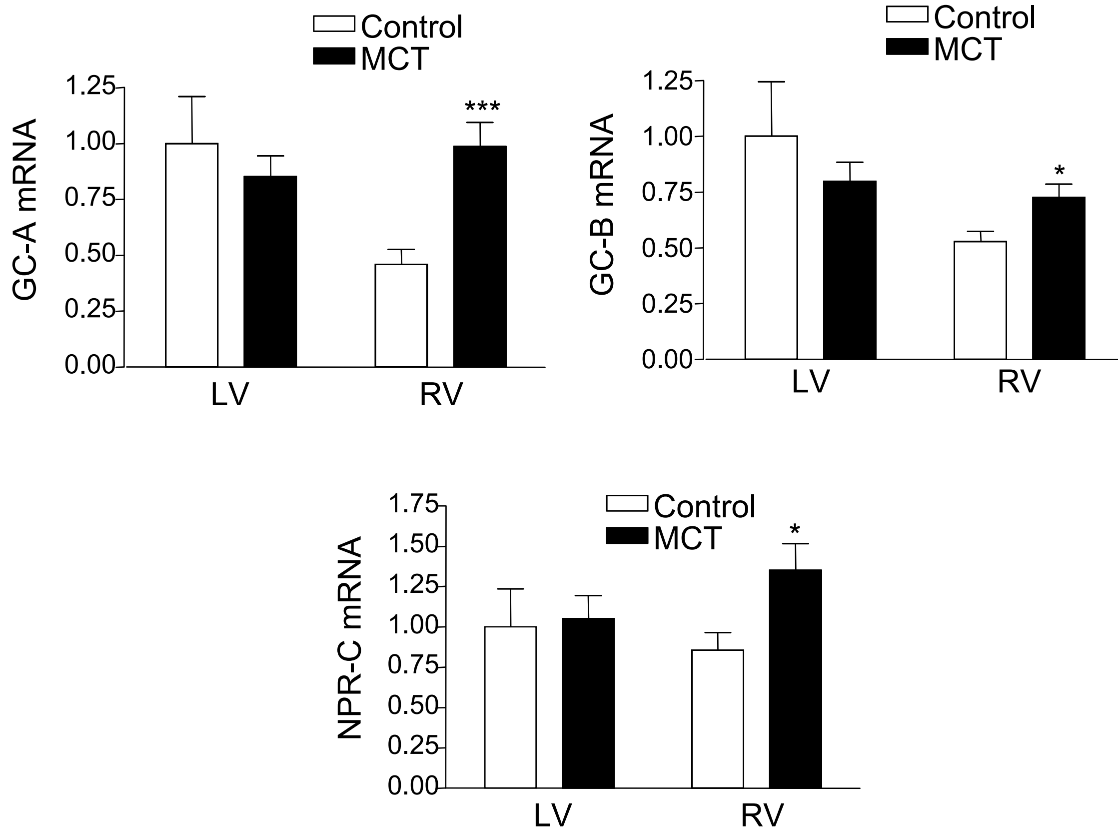

Figure 2. Effect of MCT on NPR-A, NPR-B, and NPR-C gene expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean±SEM for 7 hearts in each group. MCT *vs* control: *, $P<0.05$; ***, $P<0.0001$.

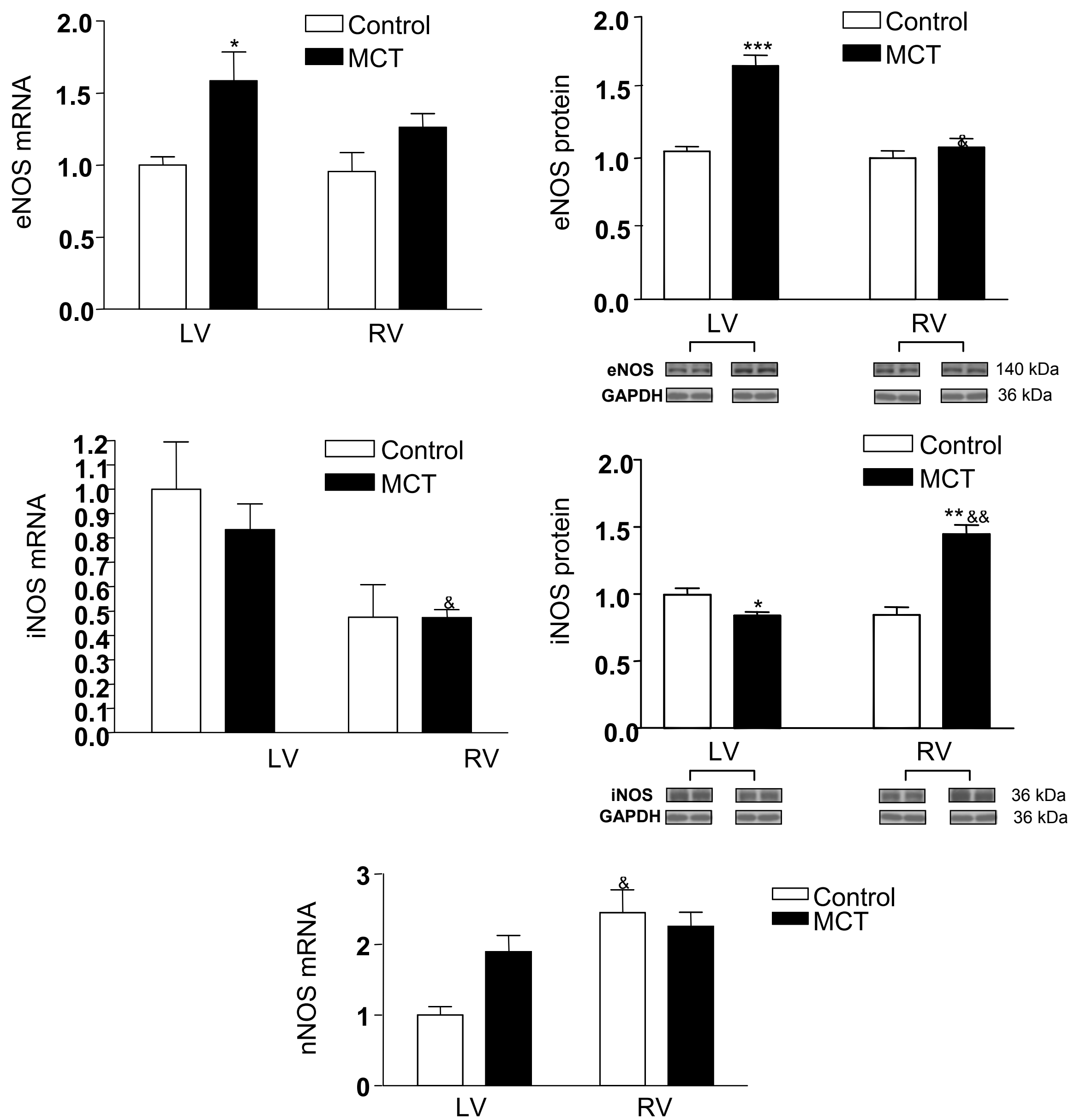

Figure 3. Effect of MCT on eNOS and iNOS gene and protein expressions, and nNOS gene expression in the right and the left ventricle. Data illustrated on the graph bar represent the mean±SEM for 7 hearts in each group. MCT *vs* control: *, P<0.05; **, P<0.001; ***, P<0.0001. RV *vs* LV: $^{\&}$, P<0.05; $^{\&\&}$, P<0.001.

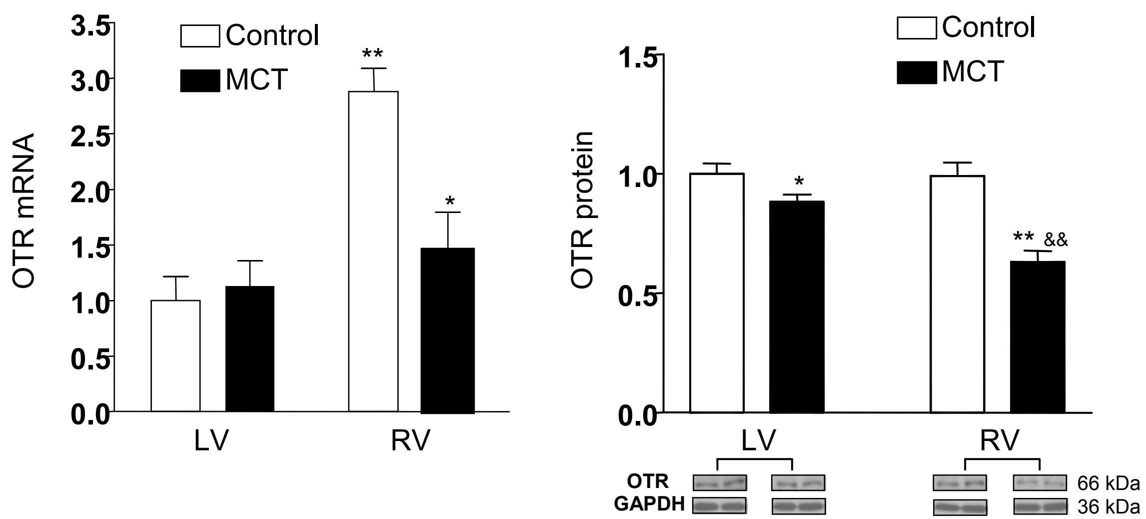

Figure 4. Effect of MCT on OTR gene and protein expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean$\pm$SEM for 7 hearts in each group. MCT *vs* control: *, $P<0.05$; **, $P<0.001$. RV *vs* LV: $^{\&\&}$, $P<0.001$.

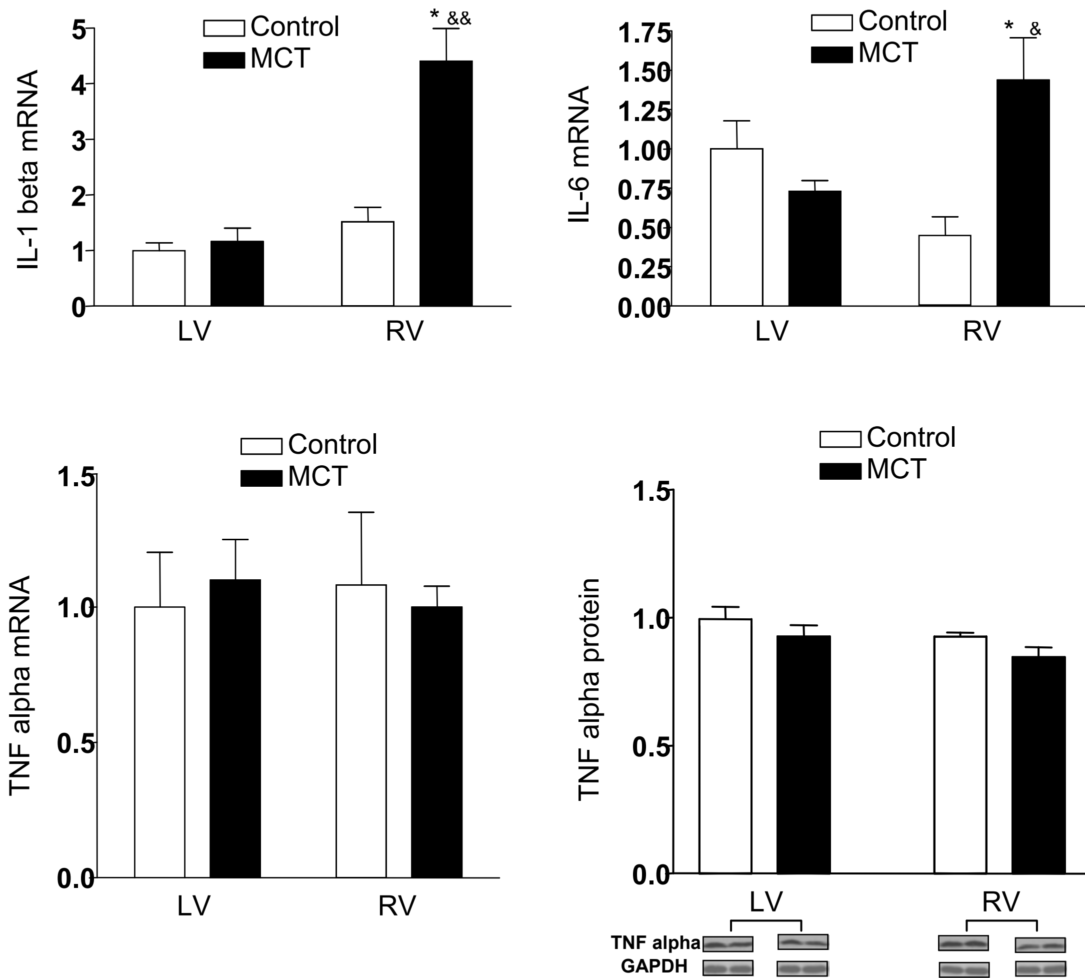

Figure 5. Effect of MCT on IL-1beta and IL-6 gene expressions, and TNFa gene and protein expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean+SEM for 7 hearts in each group. MCT *vs* control: *, *P*<0.05. LV *vs* RV: [&], *P*<0.05; [&&], *P*<0.001.

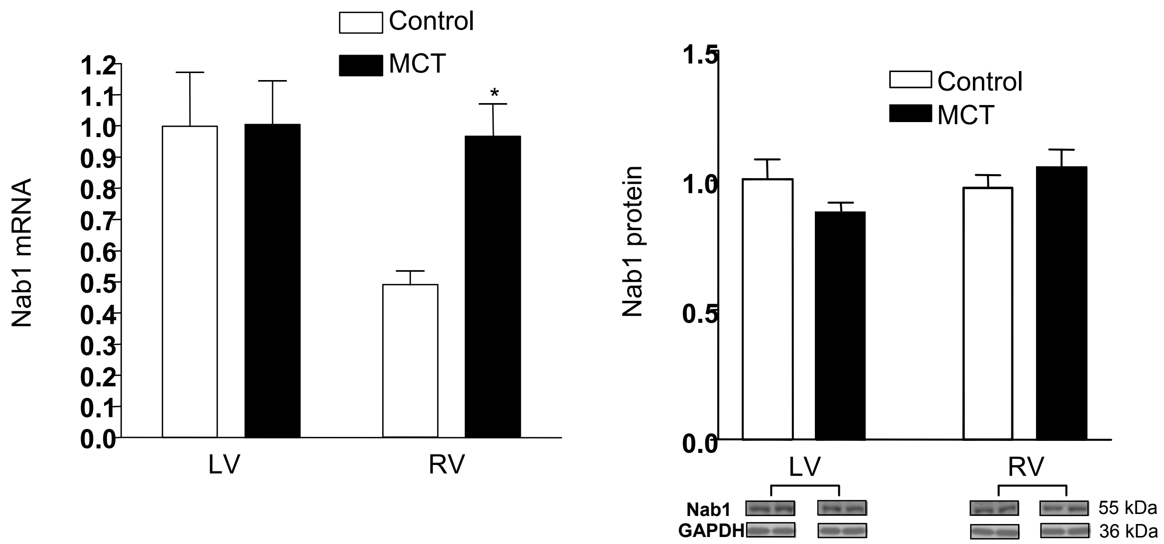

Figure 6. Effect of MCT on Nab1 gene and protein expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean±SEM for 7 hearts in each group. MCT *vs* control: *, $P<0.05$.

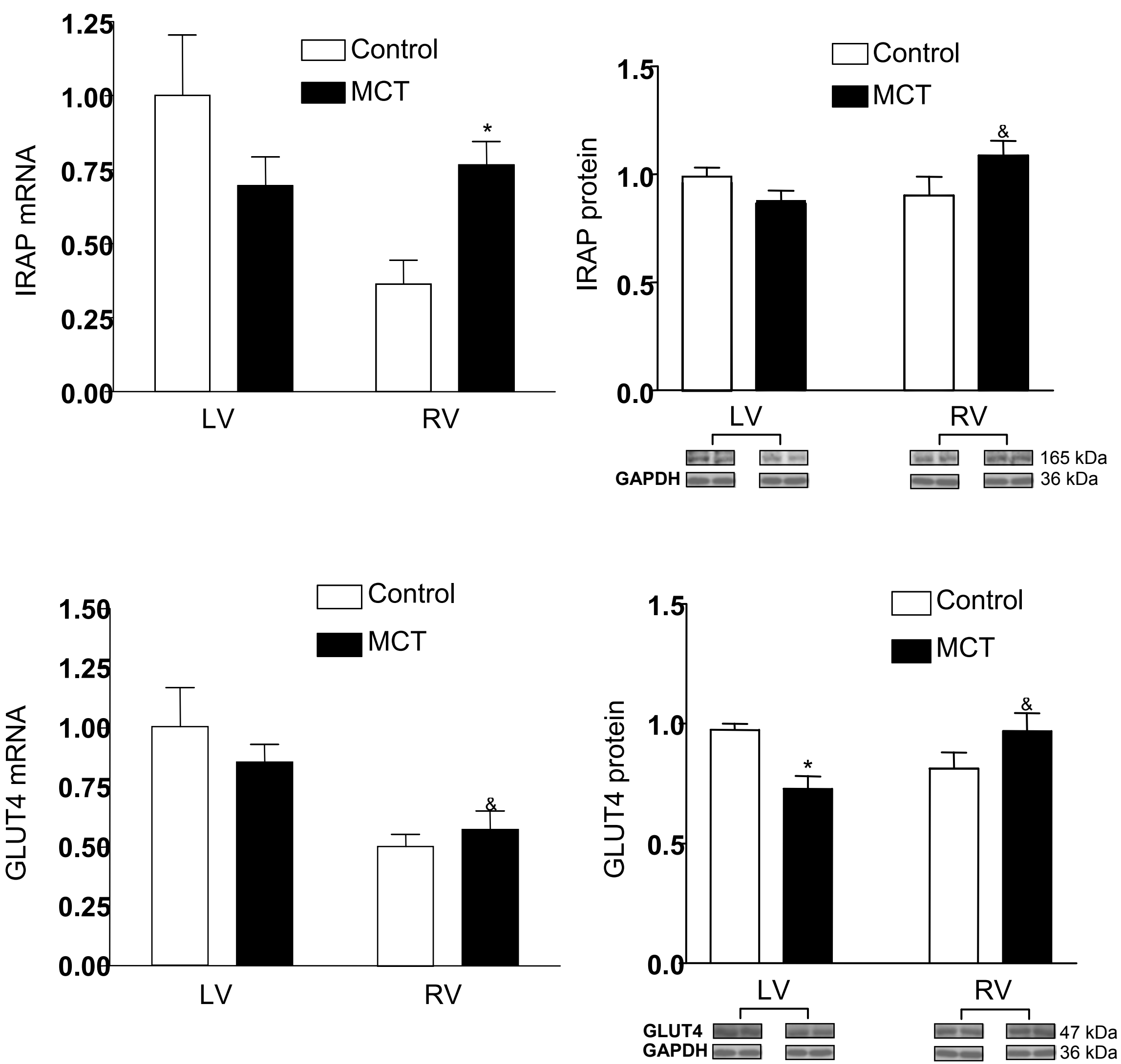

Figure 7. Effect of MCT on IRAP and GLUT4 gene and protein expressions in the right and the left ventricle. Data illustrated on the graph bar represent the mean±SEM for 7 hearts in each group. MCT *vs* control: *, $P<0.05$. RV *vs* LV: &, $P<0.05$.

VI. DISCUSSION

VI.1. Rat model of RV hypertrophy

Myocardial hypertrophy is a compensatory mechanism whereby cardiac tissue adapts to increased workload. Depending on the degree and duration of increased workload, ventricular hypertrophy may progress from a compensatory state to impaired systolic and/or diastolic function and heart failure. The model frequently used for studies of functional, structural and molecular changes due to RV compensated hypertrophy and RV failure, is animal treated with MCT. MCT selectively injures the endothelium of the lung vessels and induces pulmonary vasculitis. As a result develops muscularization and hypertrophy of media in pulmonary arteries thus increasing vascular resistance and stimulating local hypertension. Next, the PH leads to compensated RVH, which can progress to failure within weeks depending on the dose of MCT and the age of the animals. In the present study rats received a single dose (60 mg/kg) of MCT and the heart hypertrophy was investigated after 7 weeks. This protocol has been used extensively in experimental rats to cause PH and RVH [Broderick et al. 2008]. Our data demonstrated that MCT-treated rats displayed significant increases in heart weight and RV weight. The presence of RVH induced by MCT was evidenced by an increase in the heart weight/body, RV/body weight, and RV/LV+S ratios. Indeed, the ratio of the RV weight to the weight of LV plus septum and the ratio of the RV weight to body weight were nearly 77% and 131% higher in MCT-treated rats than that in control rats, respectively. In contrast, MCT

treatment did not induce LV hypertrophy. These results are in accord with previous investigations [Miyauchi et al 1993, Honda et al. 1992, Brunner et al 1999]. At molecular level, the RV hypertrophy was confirmed by elevated expression of hypertrophic markers such as ANP, BNP and Nab1 (NGF1A-binding protein). Nab1, a member of a family of corepressors for early growth response (Egr) transcription factors, was found to interact with the inhibitory R1 repression domain of Egr1, inhibiting its ability to activate transcription. Buitrago et al [2005] have described that Nab1 was an endogenous regulator of cardiac growth and increased in both mouse and human heart failure. They found that Nab1 is highly expressed in mammalian cardiac myocytes and inhibits cardiac myocyte hypertrophy through repression of Egr gene. Next, *in vivo* study of transgenic mice with selective overexpression Nab1 gene in the heart identified that Nab1 is a potent inhibitor of heart hypertrophy stimulated by pathological stimuli. Indeed, they found that Nab1 overexpression suppressed hypertrophy induced by adrenergic activation and pressure overload, whereas excess of Nab1 not affected physiological heart growth during development and in response to exercise (Buitrago et al. 2005). These findings indicate the Nab1-Egr1 axis acts as a selective regulator of heart growth in pathology. In the present study, the RVH induced by MCT was associated with the increased Nab1 transcript in enlarged RV but not in the LV displaying the normal weight. This result was not confirmed by Western-blot. Because, the Nab1 protein is not available on the market, the verification of Nab1 antibody specificity by immune tests was not possible. Therefore, our results are only consistent in part with the suggestion that Nab1 can be used as a marker of pathological hypertrophy in the heart.

VI.2. Possible role of inflammatory cytokines in the regulation of OTR

OT exerts the functions in the heart and vasculature by binding to its receptor. OTR expression is regulated in multiple organs in a tissue- and development-specific manner which may account for the distinct roles of OT in these tissues. The previous study demonstrated the presence of OTR in the mature rat heart by autoradiography performed on frozen sections [Gutkowska et al 1997]. The high OTR expression has observed during the rat early heart development [Jankowski et al. 2004]. Therefore, it is possible that high expression OTR in the RV can be associated with predominant circulatory function of RV in fetal life. OT administration reduces heart rate and force of contraction, and induces ANP release from heart which has been found both *in vivo* and *in vitro* studies [Haanwinckel et al 1995, Gutkowska et al 1997, Jankowski et al 1998]. Because most of these functions of OT were blocked by specific antagonist, it suggests that OTR play a key role in mediating OT cardiac functions. In the present study, we have demonstrated for the first time that OTR is down-regulated in RVH of MCT-treated rats. This was observed both on OTR mRNA and protein levels and by ~2-fold OTR lowering in RV of treated rats as compared to the RV of control rats. We found that in LV, only OTR protein expression, but not mRNA, was only slightly lower in MCT-treated rats compared to control rats (20%). The decrease of OTR in RVH may have functional significance in the hypertrophic heart and heart failure. The mechanisms involved in these changes are not clear. We hypothesize that decrease of OTR in RVH can cause decrease both of ANP and NO productions, which in turn induce cardiovascular diseases. Indeed, Schmid

et al [2001] demonstrated that the 5'-flanking region of the human OTR gene contains several putative binding sites for nuclear factor-IL6 (NF-IL6), also known as CAAT/enhancer binding protein-beta. This trans-acting factor modulates the expression of genes involved in acute inflammatory responses. In the present study have been shown that both IL-1β and IL-6 gene expression increased about 4-fold in RVH. Relevant to this finding is observation of Schmid et al [2001] who using deletion analysis and functional transfection studies in HeLa cells observed that both IL-1β and IL-6 treatment resulted in a significant decrease of OTR mRNA measured by ribonuclease protection assay. They reported that OTR gene promoter displays constitutive basal activity and is negatively regulated by both IL-1β and IL-6. This suppressive ability of IL-1β and IL-6 depends on the -1203/-722 region of the OTR promoter, which contains binding sites for NF-IL6, acute phase response element, and NF-κB. Therefore, it is likely that the OTR expression is attenuated in the RV by inflammatory cytokines.

Besides above investigations, accumulating evidence indicates that inflammatory cytokines play a pathogenic role in cardiac hypertrophy. In the patients, sustained increase in inflammatory cytokines, such as TNFα, IL-1 and IL-6, contribute to cardiac remodeling and in the development of chronic heart failure [Deswal et al 2001, Mann 2002, Malave et al 2003]. Animal studies have indicated that inflammatory cytokines induce cardiac hypertrophy [Li et al 2000]. IL-6 significantly increased in rat model of cardiac hypertrophy due to pressure overload [Pan et al 1998]. Transgenic mice overexpressing IL-6 and IL-6 receptor developed hypertrophy of ventricular myocardium [Hirota et al 1995]. In MCT-induced experimental models of PH, increased IL-6 mRNA expression was

associated with RVH [Bhargava et al 1999]. It has also been found that IL-1 was excessively produced in animals treated with MCT and the administration of an IL-1 receptor antagonist reduced PH and RVH [Voelkel et al 1994]. The observation from the present study showing increased IL-1β and IL-6 mRNA in the RV of MCT-treated rats is consistent with the above findings. It has been also reported that overexpression of TNF α in mice resulted in severe PH [Fujita et al 2002]. This finding suggested that MCT-induced PH and its subsequent heart hypertrophy may also involve increased expression of TNFα. However, in present study, we did not find any changes in TNFα gene and protein expression. It is possible that TNFα contributes in the development cardiac hypertrophy in MCT model but its level is normalized when RVH is established. The time course studies are required to verify this hypothesis.

VI.3. Involvement of NO in RV hypertrophy

As mentioned above, the evidence suggests the capability of IL-1β, IL-6, and TNFα to modulate myocardial functions. The literature indicates that pro-inflammatory cytokines modulate myocardial functions through at least two different pathways. One is an immediate pathway that is manifested within minutes and involves activation of the neutral sphingomyelinase signaling [Oral et al 1997]. Second pathway, termed a delayed pathway, requires hours to days to develop and is mediated by NO [Gulick et al 1989, Baligand et al 1993]. Several studies have shown that treatment with IL1β leads to a depression in cardiac myocyte contractility, which is mediated by NOS [Combes et al 2002, Hosenpud 1993, Schulz et al 1995]. NOS are NAPDH-requiring heme containing oxido-

reductases that convert one of the chemically equivalent guanidino nitrogens of L-arginine to NO and the by product L-citrulline. In the heart, eNOS is expressed within the endothelium of the epicardial and microvascular coronary vessels and the endocardium, but also in cardiac myocytes and in cardiac pacemaker and conduction tissue, such as sinoatrial and atrioventricular nodal cells. In addition, the iNOS and nNOS are also present in cardiac myocytes. In the cultures cells, IL-1β was shown to induce myocyte growth and modulate cell morphology [Thaik et al 1995, Palmer et al 1995]. In their studies, they postulated that the signaling pathways for the effects of IL-1β may involve induction of iNOS gene in cardiac myocytes [Tsujino et al 1994] and NO independent mechanisms [Thaik et al 1995, Palmer et al 1995]. In the study of Sasayama [1990], IL-6 enhanced de novo synthesis of iNOS protein, increased NO production and decreased rat cardiac myocyte contractility after 2h incubation. Activation iNOS in the heart is recognized as an inflammatory response (Anker and von Haehling. Inflammatory mediators in chronic heart failure: an overview. *Heart.*2004; 90: 464-470). We have found that iNOS heightened in the RV of MCT-treated rats in parallel to IL-1β and IL-6 mRNA increase. Based on these findings, it is possible that proinflammatory cytokines such as IL-1β and IL-6 modulate cardiac functions through iNOS-associated mechanism.

VI.4. Role of Natriuretic Peptides

As mentioned in the introduction section, the natriuretic peptides contribute importantly to the maintenance of sodium and volume homeostasis in health and disease. Both ANP and BNP are synthesized predominantly by cardiac myocytes

and their production is increased by cardiac pressure and volume overload. In the present study, MCT-stimulated cardiac pressure or volume overload not changed LV mass. However the upregulation of the local ANP and BNP expression suggested that PH had an effect also on the gene expression in the LV. Correspondingly, we found that MCT treatment increased transcripts of ANP 3.7-fold and BNP 2.7-fold in the LV compared to the control groups. Our findings not confirmed previous report of Usui et al. that after 8 weeks a single dose (60 mg/kg) of MCT caused hypertrophy both in the RV and in the LV [2006]. On the other hand, they found that MCT treatment increased transcripts of ANP by 4-fold and BNP by 2-fold in the LV which is in agreement to our findings. In our study, the expression of natriuretic peptides mRNA in the LV was higher than in the RV. This difference was observed in the control rats and in MCT-treated rats. It can be speculated that the mechanisms by which MCT-treatment caused changes of both ANP and BNP mRNAs in non-hypertrophic LV may involve neurohumoral factors such as glucocorticoids, catecholamines, arginine vasopressin, angiotensin II, and endothelin [de Bold et al 2001]. This regulation may involve OT system, because several lines of evidence indicate a possible physiologic link of OT in ANP release from the heart [Gutkowska et al. 1997; Favaretto et al 1997]. We found that MCT treatment increased transcripts of ANP with 8-fold and BNP with 10-fold in RV compared to the control groups. These results are consistent with the findings reported in the literature. Usui et al [2006] reported that MCT treatment increased transcripts of ANP by 3-fold and BNP by 5-fold in the RVH compared to the control groups. Chen et al [2001] has found that ANP gene expression increased about 4-fold in RVH of MCT-treated rats. The reason of increased expression of natriuretic peptides in the LV of rat with PH

hypertension is unknown. We can speculate that the increased synthesis of these peptides in LV is associated with stretch of ventricles and increased plasma ANP and BNP levels observed in MCT-treated rats [Usui et al 2006].

The biological actions of natriuretic peptides are mediated by their specific receptors. Three NPR genes are expressed in ventricular tissues [Brown et al 1993, Nunez et al 1992, Wilcox et al 1991] and transcripts for all three NPR subtypes have been found in ventricular cardiomyocytes [Lin et al 1995]. Deletion of GC-A in mice (GC-A –/–) prevents the vasorelaxant effects of ANP and BNP and leads to chronic hypertension and cardiac hypertrophy [Holtwick et al 2003]. In the present study, we found that transcripts for three NP receptors significantly increased about 0.3-2 fold only in the RV of MCT-treated rats compared to that of control groups. These findings suggest that increased gene expression of NP in the RV of the MCT-treated rat correlates with upregulation of NP receptor transcripts indicating local NP action in the RV during PH.

VI.5. Role of OT-Associated Metabolic Factors

OT has metabolic activity to trigger the insulin and glucagons secretions from the pancreas [Kolesnyk et al 2000] and is associated with increased glucose uptake by cardiac rat myocytes [Florian et al. 2009, Abstract QHA, Quebec City]. OT also acts on adipocytes and displays a role in metabolic regulation related to feeding [Egan et al 1990]. Oxytocin mimics a number of insulin effects on adipocytes [Rodbell 1966, Rodbell 1964, Kanif et al 1982, Honeyman et al 1983]. However, in contrast to insulin, oxytocin stimulates phospholipid metabolism and diacylglycerol formation in adipocytes [Pennington et al 1985, Augert et al 1988].

Moreover, exposure of isolated rat adipocytes to oxytocin increases PKC activity in adipocyte plasma membranes [Egan et al 1990]. The insulin-regulated membrane aminopeptidase (IRAP, oxytocinase) was originally identified in fat and muscle cells as a major protein in intracellular vesicles. IRAP colocalizes with the insulin-responsive glucose transporter GLUT4 in intracellular membrane vesicles. In cardiomyocytes, a fraction of intracellular GLUT4 has been found in large secretory granules that contain atrial natriuretic factor. IRAP has wide tissue distribution and is expressed by the heart. In IRAP-knockout mice and GLUT4-deficient mice develop cardiac hypertrophy. A recent study from Broderick et al [2008] has reported that MCT-induced RVH resulted in an increase in GLUT4 content in the right ventricle of the heart. This GLUT4 level increase in RV may potentially alter substrate energy metabolism. In present study, we found the IRAP gene expression significantly increased in RV hypertrophy compared to control RV and the level of IRAP protein in RV was significantly higher than that in LV in MCT-treated groups. However, the GLUT4 gene and protein expression obtained by RT-PCT and Western-blot analysis were not consistent with the recent findings from Broderick et al [2008]. This discrepancy requires explanation and additional experiments should be performed to find correct conclusion.

VII. CONCLUSIONS AND PERSPECTIVES

VII.1. Conclusions

Increased gene expression of NPs in the RV of the MCT-treated rat correlates with upregulation of NP receptor transcripts indicating local NP action in the RV during PH. OTR expression decreases in the RV possibly by inflammatory cytokines because OTR promoter region contains multiple putative interleukin-response elements. This alteration possibly involves inflammatory NO-dependent mechanisms. Lowering OTR in RV during pulmonary hypertension can influence cardiac function since OT regulates heart rate and cardiac contractility.

VII.2. Perspectives

VII.2.1. OTR and inflammation

Some of the molecular, and cellular events implicated in the development of cardiac hypertrophy include cardiac myocyte-autonomous and endocrine/paracrine pathways [Molkentin et al 2001]. It has been suggested that the interaction between cardiac myocytes and surrounding non-myocytes, which consist mainly fibroblasts, is likely to be an important component of the hypertrophic process [Weber et al 1991]. This interaction might be mediated via a set of growth factors and cytokines, acting in an autocrine and/or paracrine fashions. Cytokines of the IL-6 family are of particular interest given recent reports of their ability to promote cross-talk between cardiomyocytes and fibroblasts [Mann 2003]. Some studies have demonstrated that oxytocin displayed antioxidant and anti-inflammatory effects [Detillion et al 2004, Iseri et al

2005a, Iseri et al 2005b, Petersson et al 2001]. It has been found that OTRs were expressed by several cells involved in inflammation, such as cultured human vascular endothelial cells [Thibonnier et al 1999], aortic smooth muscle cells [Yazawa et al 1996], and monocytes and macrophages [Szeto et al 2008]. The recruitment of macrophages into the myocardium has been identified in a model of cardiac hypertrophy [Behr et al 2004]. In the present study, we have demonstrated that OTR expression decreased both at the gene and protein levels in RVH and this alteration likely correlates with the increased inflammatory cytokines such as IL-1β and IL-6. However, the functional relevance of the downregulation of OTR in RVH remains unclear. To address this question, we propose to investigate the molecular mechanisms involved in OTR expression in RVH. The experiments should be performed in cultured cardiac myocytes, cultured cardiac fibroblasts, and cultured macrophages, and their co-cultures. In cardiac myocytes the changes of OTR expression, cell morphology and ANP accumulation should be investigated. These parameters will be examined in response to added cytokines or to conditioned media collected from fibroblast and macrophages growing in the presence and the absence of OT. The addition of OT and cytokines receptor antagonists will confirm specificity of results obtained in the experiments. These studies will provide information whether cross-talks between cardiac myocytes and cells activated during heart inflammation involves OT mediation.

VII.2.2. OTR and metabolism

In the present study, we found that IRAP expression significantly increased in RV hypertrophy induced in rats by MCT treatment. The interaction between OTR and IRAP in RVH has not been studied yet. IRAP is recently found as a signal transducing receptor of angiotensin IV and as a member of the family of zinc-dependent membrane aminopeptidases. The members of this family have been shown to process regulatory peptides, thereby changing their activities [Barrett et al 1998]. It is important to note that the physiological roles of aminopeptidases A and N in the cleavage and regulation of the actions of angiotensin II and angiotensin III, respectively, have been established [Reaux et al 2000]. It has been documented in *ex vivo* studies that IRAP cleaves several peptide hormones including OT and AVP [Tsujimoto et al 1992, Herbst et al 1997, Matsumoto et al 2001]. IRAP cleaves OT and thus prevents OTR activation. OT is known to mimic many of the effects of insulin in adipocytes, stimulating glucose oxidation, lipogenesis, and glycogen synthesis. These insulin-like activities are due to OT binding to the OTR and not to the insulin receptor itself [Hanif et al 1982]. We have presently found that OTR receptor was down-regulated in RVH of MCT-treated rats. On the other hand, it is possible that increased IRAP in RVH degrades OT and further attenuates OTR signal transduction. Therefore, we propose to investigate functional interaction between IRAP and OT/OTR in cultured cardiac myocytes and also to study the molecular mechanisms involved.

VIII. REFERENCES

Akhavein F, St-Michel EJ, Seifert E, Rohlicek CV. Decreased left ventricular function, myocarditis, and coronary arteriolar medial thickening following monocrotaline administration in adult rats. *J Appl Physiol* 2007,103:287-295.

Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *Am J Physiol* 1994,267:H742–H750.

Alp NJ, Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler Thromb Vasc Biol.* 2004, 24:413–420.

Augert G, Exton JH. Insulin and oxytocin effects on phosphoinositide metabolism in adipocytes. *J Biol Chem* 1988,263:3600-3609.

Balligand JL, Ungureanu D, Kelly RA, Kobzik L, Pimental D, Michel T, Smith TW. Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium. *J Clin Invest* 199391:2314-2319

Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997,336:1066-1071.

Barrett AJ, Rawlings ND, Woessner JF. Handbook of Proteolytic Enzymes, eds. *Academic Press* 1988, pp. 996-1008.

Barry SP, Davidson SM, Townsend PA. Molecular regulation of cardiac hypertrophy. *Int J Biochem Cell Biol* 2008,40:2023-2039.

Bathgate R, Rust W, Balvers M, Hartung S, Morley S, and Ivell R. Structure and expression of the bovine oxytocin receptor gene. *DNA Cell Biol* 1995,14: 1037-1048.

Behr TM, Willette RN, Coatney RW, Berova M, Angermann CE, Anderson K, Sackner-Bernstein JD, Barone FC. Eprosartan improves cardiac performance, reduces cardiac hypertrophy and mortality and downregulates myocardial monocyte chemoattractant protein-1 and inflammation in hypertensive heart disease. *J Hypertens* 2004,22:583-592.

Bhargava A, Kumar A, Yuan N, Gewitz MH, Mathew R. Monocrotaline induces interleukin-6 mRNA expression in rat lungs. *Heart Dis* 1999,1:126-132.

Bishopric, N.H., Andreka, P., Slepak, T., Webster, K.A. Molecular mechanisms of apoptosis in the cardiac myocyte. *Curr Opin Pharmaco.* 2001,1:141-150.

Blanks AM, Thornton S: The role of oxytocin in parturition. *BJOG* 2003,110:46-51.

Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 2001,3:1014–1019

Bonnet P, Bonnet S, Boissiere J, Le Net JL, Gautier M, Dumas de la Roque E, Eder V. Chronic hypoxia induces nonreversible right ventricular dysfunction and dysplasia in rats. *Am J Physiol Heart Circ Physiol* 2004,287:H1023–H1028.

Bowles DK, Farrar RP, Starnes JW. Exercise training improves cardiac function after ischemia in the isolated, working rat heart. *Am J Physiol* 1992,263:H804–H809.

Bowman JC, Steinberg SF, Jiang TR, Geenen DL, Fishman GI, Buttrick PM. Expression of protein kinase C beta in the heart causes hypertrophy in adult mice and sudden death in neonates. *J Clin Invest* 1997,100:2189–2195

Brand, T; Schneider, MD. The TGF beta superfamily in myocardium: ligands, receptors, transduction, and function. *J Mol Cell Cardiol* 1995,27:5–18.

Braz JC, Bueno OF, De Windt LJ, Molkentin JD. PKC alpha regulates The hypertrophic growth of cardiomyocytes through extracellular signalregulated kinase1/2 (ERK1/2). *J Cell Biol* 2002,156:905–919

Brown LA, Nunez DJR, Wilkins MR. Differential regulation of natriuretic peptide receptor messenger RNAs during the development of cardiac hypertrophy in the rat. *J Clin Invest* 1993,92:2702-2712.

Brown L, Miller J, Dagger A, Sernia C. Cardiac and vascular responses after monocrotaline-induced hypertrophy in rats. *J Cardiovasc Pharmacol* 1998,31: 108–115.

Brunner F. Cardiac endothelin and big endothelin in right-heart hypertrophy due to monocrotaline-induced pulmonary hypertension in rat. *Cardiovasc Res* 1999,44:197-206.

Brunner F, Wolkart G, Haleen S. Defective intracellular calcium handling in monocrotaline-induced right ventricular hypertrophy: protective effect of long-term endothelin A receptor blockade with 2-benzo[1,3]dioxol-5-yl-3-benzyl-4-(4-methoxy-phenyl-)-4-oxobut-2-enoate-sodium. *J Pharmacol Exp Ther* 2002,300: 442–449.

Buitrago, M., Lorenz, K., Maass, A.H., Oberdorf-Maass, S., Keller, U., Schmitteckert, E.M., Ivashchenko, Y., Lohse, M.J., Engelhardt, S. The transcriptional repressor Nab1 is a specific regulator of pathological cardiac hypertrophy. *Nat Med* 2005,11:837-844

Calderone A, Thaik CM, Takahashi N, Chang DLF, Colucci WS. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. *J Clin Invest* 1998,101:812–818.

Cameron VA, Aitken GD, Ellmers LJ, et al. The sites of gene expression of atrial, brain, and C-type natriuretic peptides in mouse fetal development: temporal changes in embryos and placenta. *Endocrinology* 1996,137:817–824.

Chen EP, Bittner HB, Davis RD, Van Trigt P. Right ventricular failure—insights provided by a new model of chronic pulmonary hypertension. *Transplantation* 1997,63: 209–216.

Chen EP, Bittner HB, Tull F, Craig D, Davis RD, Van Trigt P. Nitric oxide improves pulmonary vascular impedence, transpulmonary efficiency, and left ventricular filling in chronic pulmonary hypertension. *J Thorac Cardiovasc Surg* 1997,113: 849–857.

Chen L, Gan XT, Haist JV, Feng Q, Lu X, Chakrabarti S, Karmazyn M. Attenuation of compensatory right ventricular hypertrophy and heart failure following monocrotaline-induced pulmonary vascular injury by the Na+-H+ exchange inhibitor cariporide. *J Pharmacol Exp Ther* 2001,298:469-476.

Chien KR, Knowlton KU, Zhu H, Chien S. Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. *FASEB J* 1991,5:3037–3046.

Chien KR, Zhu H, Knowlton KU, Miller HW, van-Bilsen M, O'Brien TX, Evans SM. Transcriptional regulation during cardiac growth and development. *Annu Rev Physiol* 1993,55:77–95.

Chinkers M, Garbers DL, Chang MS, Lowe DG, Chin H, Goeddel DV & Schulz S. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* 1989,338:78-83.

Chariguer RS, Rocha MJ, Antunes-rodrigues J, Franci CR. Hypothalamic atrial natriuretic peptide and secretion of oxytocin. *Brain Res* 2001,889:239-242.

Cicutti NJ; Smyth CE; Rosaeg OP; Wilkinson M. Oxytocin receptor binding in rat and human heart. *The Canadian journal of cardiology* 1999,15:1267-1273.

Clarkson PBM, Wheeldon NM, MacFadyen RJ, Pringle SD, MacDonald TM. Effects of brain natriuretic peptide on exercise hemodynamics and neurohormones in isolated diastolic heart failure. *Circulation* 1996,93:2037-2042.

Combes A, Frye CS, Lemster BH, Brooks SS, Watkins SC, Feldman AM, McTiernan CF. Chronic exposure to interleukin 1beta induces a delayed and

reversible alteration in excitation-contraction coupling of cultured cardiomyocytes. *Pflugers Arch* 2002,445:246-256.

de Bold A.J., Borenstein B., Veress A.T., et al. A rapid and potent natruiretic response to intravenous injection of atrial myocardial extracts in rats. *Life Sci* 1981,28:89–94.

de Bold AJ, MaKK, Zhang Y. The physiological and pathophysiological modulation of the endocrine function of the heart. *Can J Physiol Pharmacol* 2001,79:705-714.

Delafontaine, P., and Lou, H. Angiotensin II regulates insulin-like growth factor I gene expression in vascular smooth muscle cells. *J Biol Chem* 1993,268:16866–16870.

Depre C, Rider MH, Hue L. Mechanisms of control of heart glycolysis. *Eur J Biochem* 1998,258:277–290.

Deswal A, Petersen NJ, Feldman AM, Young JB, White BG & Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation* 2001,103: 2055–2059.

Detillion CE, Craft TKS, Glasper ER, Prendergast BJ and DeVries AC. Social facilitation of wound healing. *Psychoneuroendocrinology* 2004,29:1004-1011.

Diez J, Panizo A, Hernandez M, Vega F, Sola I, Fortuno MA, Pardo J. Cardiomyocyte apoptosis and cardiac angiotensin-converting enzyme in spontaneously hypertensive rats. *Hypertension.* 1997;30:1029–1034.

Donohue TJ, Dworkin LD, Lango MN, Fliegner K, Lango RP, Benstein JA, Slater WR, Catanese VM. Induction of myocardial insulin-like growth factor-I gene expression in left ventricular hypertrophy. *Circulation* 1994,89:799-809.

Doust JA, Pietrzak E, Dobson A, Glasziou P. How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review. *BMJ* 2005,330:625.

Dupraz P, Cottet S, Hamburger F, Dolci W, Felley-Bosco E, and Thorens B. Dominant negative MyD88 proteins inhibit interleukin-1β/interferon-γ-mediated induction of nuclear factor κB-dependent nitrite production and apoptosis in β cells. *J Biol Chem* 2000,275:37672–37678.

Egan JJ, Saltis J, Wek SA, Simpson IA, Londos C. Insulin, oxytocin, and vasopressin stimulate protein kinase C activity in adipocyte plasma membranes. *Proc Natl Acad Sci USA* 1990,87:1052-1056.

El Alaoui-Talibi Z, Guendouz A, Moravec M, Moravec J. Control of oxidative metabolism in volume-overloaded rat hearts: effect of propionyl-L-carnitine. *Am J Physiol* 1997,272:H1615–H1624.

Farahmand F, Hill MF, Singal PK. Antioxidant and oxidative stress changes in experimental cor pulmonale. *Mol Cell Biochem* 2004,260: 21–29.

Favareto AL, Ballejo GO, Albuquerque-araujo WI et al: Oxytocin releases atrial natriuretic peptide from rat atria in vitro that exerts negative inotropic and chronotropic action. *Peptides* 1997,18:1377-1381

Fijalkowska A, Kurzyna M, Torbicki A, Szewczyk G, Florczyk M, Pruszczyk P, Szturmowicz M. Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. *Chest* 2006,129:1313–1321.

Fijalkowska A. Torbickia Role of cardiac biomarkers in assessment of RV function and prognosis in chronic pulmonary hypertension. *Eur Heart J Suppl* 2007,9: H41 - H47.

Foo, R.S., Mani, K., Kitsis, R.N. Death begets failure in the heart. *J. Clin. Invest* 2005,115:565-571.

Fortuno MA, Ravassa S, Etayo JC, Diez J. Overexpression of Bax protein and enhanced apoptosis in the left ventricle of spontaneously hypertensive rats: effects of AT_1 blockade with losartan. *Hypertension* 1998,32:280–286.

Frey N and Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 2003,65:45–79.

Fujita M, Mason RJ, Cool C, Shannon JM, Hara N, Fagan KA. Pulmonary hypertension in TNF-alpha-overexpressing mice is associated with decreased VEGF gene expression. *J Appl Physiol* 2002,93:2162-2170.

Gan CT, McCann GP, Marcus JT, van Wolferen SA, Twisk JW, Boonstra A, Postmus PE, Vonk-Noordegraaf A. NT-proBNP reflects right ventricular structure and function in pulmonary hypertension. *Eur Respir J* 2006,28:1190–1194.

Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. *J Clin Invest* 1992,90:456–461.

Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* 2001,81:629-683.

Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 2005,115:500–508.

Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ, and Sugden PH. Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. *Circ Res* 1996,78:954–961.

Gorbulev V, Buchner H, Akhundova A, and Fahrenholz F. Molecular cloning and functional characterization of V2 [8-lysine] vasopressin and oxytocin receptors from a pig kidney cell line. *Eur J Biochem* 1993,215:1-7.

Gould BR, Zingg HH. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor-LacZ reporter mouse. *Neuroscience* 2003,122:155-167.

Gu J, D'Andrea M, Seethapathy M. Atrial natriuretic peptide and its messenger ribonucleic acid in overloaded and overload-released ventricles of rat. *Endocrinology* 1989,125:2066–2074.

Gulick T, Chung MK, Pieper SJ, Lange LG, Schreiner GF. Interleukin 1 and tumor necrosis factor inhibit cardiac myocyte beta-adrenergic responsiveness. *Proc Natl Acad Sci U S A* 1989,86:6753-6757.

Gupta S, Das B, Sen S. Cardiac hypertrophy: mechanisms and therapeutic opportunities. *Antioxid Redox Signal* 2007,9:623-652.

Gupta S, Purcell NH, Lin A, and Sen S. Activation of nuclear factor-kappaB is necessary for myotrophin-induced cardiac hypertrophy. *J Cell Biol* 2002,159: 1019–1028.

Gutkowska J, Jankowski M, Lambert C, Mukaddam-Daher S, Zingg HH & McCann SM. Oxytocin releases atrial natriuretic peptide by combining with oxytocin receptors in the heart. *Proceedings of the National Academy of Sciences USA* 1997,94:11704-11709.

Hamet P, Tremblay J, Pang SC, Garcia R, Thibault G, Gutkowska J, Cantin M & Genest J. Effect of native and synthetic atrial natriuretic factor on cyclic GMP. *Biochemical and Biophysical Research Communications 1984*,123: 515-527.

Haanwinckel MA, Elias LK, Favaretto AL, Gutkowska J, McCann SM, Antunes-Rodrigues J. Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. *Proc Natl Acad Sci U S A* 1995,92:7902-7906.

Hanif K, Goren HJ, Hollenberg D, Lederis K. Oxytocin action. Mechanisms for insulin-like activity in isolated rat adipocytes. *Mol Pharmacol* 1982,22:381–388.

Harada E, Nakagawa O, Yoshimura M, Harada M, Nakagawa M, Mizuno Y, Shimasaki Y, Nakayama M, Yasue H, Kuwahara K, Saito Y, Nakao K. Effect of interleukin-1 beta on cardiac hypertrophy and production of natriuretic peptides in rat cardiocyte culture. *J Mol Cell Cardiol* 1999,31:1997-2006.

Henriques-Coelho T, Correia-Pinto J, Roncon-Albuquerque R, Baptista MJ, Lourenco AP, Oliveira SM, Brandao-Nogueira A, Teles A, Fortunato JM, Leite-

Moreira AF. Endogenous production of ghrelin and beneficial effects of its exogenous administration in monocrotaline-induced pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2004,287: H2885–H2890.

Herbst JJ, Ross SA, Scott HM, Bobin SA, Morris NJ, Lienhard GE, Keller SR. Insulin stimulates cell surface aminopeptidase activity toward vasopressin in adipocytes. *Am J Physiol* 1997,272:E600-E606.

Holtwick R, van Eickels M, Skryabin BV, Baba HA, Bubikat A, Begrow F, Schneider MD, Garbers DL, Kuhn M. Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. *J Clin Invest* 2003, 111:1399-407.

Honda M, Yamada S, Goto Y, Ishikawa S, Yoshikane H, Ishinaga Y, Kuzuo H, Morioka S, Moriyama K. Biochemical and structural remodeling of collagen in the right ventricular hypertrophy induced by monocrotaline. *Jpn Circ J* 1992,56:392–403.

Hirota, H, Yoshida K, Kishimoto T, and Taga T. Continuous activation of gp 130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice. *Proc Natl Acad Sci USA* 1995,92:4862-4866.

Honda M Yamada S, Goto Y, Ishikawa S, Yoshikane H, Ishinaga Y, Kuzuo H, Morioka S, Moriyama K. Biochemical and structural remodelling of collagen in the right ventricular hypertrophy induced by monocrotaline. *Jpn Circ J* 1992,56:392-403.

Honeyman T W, Strohsnitter W, Scheid CR, Schimmel RJ. Phosphatidic acid and phosphatidylinositol labelling in adipose tissue. Relationship to the metabolic effects of insulin and insulin-like agents. *Biochem J* 1983,212:489-498.

Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. *Hypertension* 2000,35:19–24.

Horio T, Tokudome T, Maki T, Yoshihara F, Suga S, Nishikimi T, et al. Gene expression, secretion, and autocrine action of C-type natriuretic peptide in cultured adult rat cardiac fibroblasts. *Endocrinology* 2003,144:2279–2284.

Hosenpud JD. The effects of interleukin-1 on myocardial function and metabolism. *Clin Immunol Immunopathol* 1993,68:175-180.

Huang L, Wolska BM, Montgomery DE, Burkart EM, Buttrick PM, Solaro RJ. Increased contractility and altered Ca(2C) transients of mouse heart myocytes conditionally expressing PKCbeta. *Am J Physiol Cell Physiol* 2001,280:C1114–C1120.

Iseri SO, Sener G, Saglam B, Gedik N, Ercan F and Yegen BC. Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. *Peptides* 2005a,26:483-491.

Iseri SO, Sener G, Saglam B, Gedik N, Ercan F and Yegen BC. Oxytocin Protects Against Sepsis-Induced Multiple Organ Damage: Role of Neutrophils. *J Surg Res* 2005b,126:73-81.

Ishizaka Y, Kangawa K, Minamino N, Ishii K, Takano S, Eto T, et al. Isolation and identification of C-type natriuretic peptide in human monocytic cell line, THP-1. *Biochem Biophys Res Commun* 1992,189:697–704.

Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Murumo F, Hiroe M. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin II-induced hypertrophy in cultured rat cardiomyocytes. *J Clin Invest* 1993.92:398-403.

Jankowski M. B-type natriuretic peptide for diagnosis and therapy. *Recent Pat Cardiovasc Drug Discov* 2008,3:77-83.

Jankowski M, Danalache B, Wang D, Bhat P, Hajjar F, Marcinkiewicz M, Paquin J, McCann SM, Gutkowska J. Oxytocin in cardiac ontogeny. *Proc Natl Acad Sci U S A* 2004,101:13074-1309.

Jankowski M, Hajjar F, Kawas SA, Mukaddam-Daher S, Hoffman G, McCann SM, Gutkowska J. Rat heart: a site of oxytocin production and action. *Proc Natl Acad Sci U S A* 1998,95:14558-14563.

Jankowski M, Wang D, Hajjar F, Mukaddam-Daher S, McCann SM, Gutkowska J. Oxytocin and its receptors are synthesized in the rat vasculature. *Proc Natl Acad Sci U S A* 2000,97:6207-6211.

Jin H, Yang R, Keller GA, Ryan A, Ko A, Finkle D, et al. In vivo effects of cardiotrophin-1. *Cytokine* 1996,8:920–926.

Jirikowski GF, Back H, Forssmann WG & Stumpf WE. Coexistence of atrial natriuretic factor (ANF) and oxytocin in neurons of the rat hypothalamus. *Neuropeptides* 1986,8:243-249.

Jones WK, Brown M, Ren X, He S, and McGuinness M. NFkappaB as an integrator of diverse signaling pathways: the heart of myocardial signaling? *Cardiovasc Toxicol* 2003,3:229–254.

Kalra D, Baumgarten G, Dibbs Z, Seta Y, Sivasubramanian N, and Mann DL. Nitric oxide provokes tumor necrosis factor-alpha expression in adult feline myocardium through a cGMP-dependent pathway. *Circulation* 2000,102: 1302–1307.

Hanif K, Goren HJ, Hollenberg MD, Lederis K. Oxytocin action. Mechanisms for insulin-like activity in isolated rat adipocytes. *Mol Pharmacol* 1982,22:381-388.

Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *Philos Trans R Soc Lond B Biol Sci* 1996,351:127–134.

Kerbaul F, Rondelet B, Motte S, Fesler P, Hubloue I, Ewalenko P, Naeije R, Brimioulle S. Effects of norepinephrine and dobutamine on pressure load-induced right ventricular failure. *Crit Care Med* 2004,32:1035–1040.

Keren A, Syrris P, McKenna WJ. Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nat Clin Pract Cardiovasc Med* 2008,5:158-168.

Khoury SF, Hoit BD, Dave V, Pawloski-Dahm CM, Shao Y, Gabel M, Periasamy M, Walsh RA. Effects of thyroid hormone on left ventricular performance and regulation of contractile and Ca(2+)-cycling proteins in the baboon. Implications for the force-frequency and relaxation-frequency relationships. *Circ Res* 1996,79:727-735.

Kim S and Iwao H. Activation of mitogen-activated protein kinases in cardiovascular hypertrophy and remodeling. *Jpn J Pharmacol* 1999,80:97–102.

Kimura T, Tanizawa O, Mori K, Brownstein MJ, and Okayama H. Structure and expression of a human oxytocin receptor. *Nature* 1992,356:526-529.

Kolesnyk IUM, Trzhetsyns'kyi SD, Abramov AV. The effect of oxytocin on Langerhans beta-islet cell function and on carbohydrate metabolic indices in intact rats and in rats with diabetes. *Fiziol Zh* 2000,46:37-43.

Kubota Y, Kimura T, Hashimoto K, Tokugawa Y, Nobunaga K, Azuma C, Saji F, and Murata Y. Structure and expression of the mouse oxytocin receptor gene. *Mol Cell Endocrinol* 1996,124: 25-32.

Kuhn M, Holtwick R, Baba HA, Perriard JC, Schmitz W, Ehler E. Progressive cardiac hypertrophy and dysfunction in atrial natriuretic peptide receptor (GC-A) deficient mice. *Heart* 2002,87:368-374.

Langenickel T, Pagel I, Hohnel K, et al. Differential regulation of cardiac ANP and BNP mRNA in different stages of experimental heart failure. *Am J Physiol Heart Circ Physiol* 2000,278:H1500–1506.

Lehman JJ, Kelly DP. Gene regulatory mechanisms governing energy metabolism during cardiac hypertrophic growth. *Heart Fail Rev* 2002,7:175–185.

Lei LQ, Rubin SA, Fishbein MC. Cardiac architectural changes with hypertrophy induced by excess growth hormone in rats. *Lab Invest* 1988,59:357-362.

Lin X, Hanze F, Heese R, Sodmann, Lang RE. Gene expression of natriuretic peptide receptors in myocardial cells. *Circ Res* 1995,77:750-758.

Li YY, Feng YQ, Kadokami T, McTiernan CF, Draviam R, Watkins SC & Feldman AM. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor α can be modulated by anti-tumor necrosis factor α therapy. *Proc Natl Acad Sci U S A* 2000,97:12746–12751.

Li Y, Ha T, Gao X, Kelley J, Williams DL, Browder IW, Kao RL, and Li C. NF-kB activation is required for the development of cardiac hypertrophy in vivo. *Am J Physiol Heart Circ Physiol* 2004,287: H1712–H1720.

Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, and Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995,11:376–381.

Lopaschuk GD, Rebeyka IM, Allard MF. Metabolic modulation: a means to mend a broken heart. *Circulation* 2002,105:140–142.

Lowe DG, Chang MS, Hellmiss R, Chen E, Singh S, Garbers DL & Goeddel DV. Human atrial natriuretic peptide receptor defines a new paradigm for second messenger signal transduction. *EMBO Journal* 1989,8:1377-1384.

Malave HA, Taylor AA, Nattama J, Deswal A & Mann DL. Circulating levels of tumor necrosis factor correlate with indexes of depressed heart rate variability: a study in patients with mild-to-moderate heart failure. *Chest* 2003,123,716–724.

Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res* 2002,91:988–998.

Mann DL. Stress-activated cytokines and the heart: From adaptation to maladaptation. *Annu Rev Physiol* 2003,65:81–101.

Massague, J. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990,6:597–641.

Matsui T, Li L, Wu JC, Cook SA, Nagoshi T, et al. Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. *J Biol Chem* 2002,277:22896–22901.

Matsui T, Nagoshi T, and Rosenzweig A. Akt and PI 3-kinase signaling in cardiomyocyte hypertrophy and survival. *Cell Cycle* 2003,2:220–223.

Matsumoto H, Nagasaka T, Hattori A, Rogi T, Tsuruoka N, Mizutani S, Tsujimoto M. Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides. *Eur J Biochem* 2001,268:3259-3266.

McDonagh TA, Holmer S, Raymond I, Luchner A, Hildebrant P, Dargie HJ. NT-ProBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. *Eur J Heart Fail* 2004,6:269–273.

Mercadier JJ, Zongazo MA, Wisnewsky C et al. Atrial natriuretic factor messenger ribonucleic acid and peptide in the human heart during ontogenic development. *Biochem Biophys Res Commun* 1989,159:777–782.

Meyrick B, Gamble W, Reid L. Development of *Crotalaria* pulmonary hypertension: hemodynamic and structural study. *Am J Physiol Heart Circ Physiol* 1980,239:H692–H702.

Michelini LC, Marcelo MC, Amico J, Morris M. Oxytocinergic regulation of cardiovascular function: studies in oxytocin-deficient mice. *Am J Physiol Heart Circ Physiol* 2003,284:H2269-2276.

Miyauchi T, Yorikane R, Sakai S, Sakurai T, Okada M, Nishikibe M, Yano M, Yamaguchi I, Sugishita Y, Goto K. Contribution of endogenous endothelin-1 to the progression of cardiopulmonary alterations in rats with monocrotaline-induced pulmonary hypertension. *Circ Res* 1993,73:997-897.

Molkentin JD, Dorn GW II. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu Rev Physiol* 2001,63:391–426.

Molkentin JD and Dorn II GW. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu Rev Physiol* 2001,63: 391–426.

Mukaddam-Daher S, Yin YL, Roy J, Gutkowska J, Cardinal R. Negative inotropic and chronotropic effects of oxytocin. *Hypertension* 2001,38:292-296.

Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki M, Obata K, Yasue H. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* 1991,87:1402–1412.

Murdoch CE, Zhang M, Cave AC, Shah AM. NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. *Cardiovasc Res* 2006,71:208–215.

Nadal-Ginard B, Kajstura J, Leri A, and Anversa P. Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. *Circ Res* 2003,92:139–150.

Naga PrasadSV, Esposito G,MaoL,Koch WJ, Rockman HA. G-dependent phosphoinositide 3-kinase activation in hearts with in vivo pressure overload hypertrophy. *J Biol Chem* 2000,2757:4693–4698.

Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S, Kuribayashi S, Hamada S, Kakishita M, Nakanishi N, Takamiya M, Kunieda T, Matsuo H,

Kangawa K. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. *J Am Coll Cardiol* 1998,31:202–208.

Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Kakishita M, Fukushima K, Okano Y, Nakanishi N, Miyatake K, Kangawa K. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* 2000,102:865–870.

Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol* 1974,36:413–457.

Nemoto S, Sheng Z, and Lin A. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. *Mol Cell Biol* 1998,18:3518–3526.

Nian M, Lee P, Khaper N & Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res* 2004,94:1543–1553.

Nishikimi T, Maeda N, Matsuoka H. The role of natriuretic peptides in cardioprotection. *Cardiovasc Res* 2006,69:318-328.

Nishizuka Y. Studies and perspectives of protein kinase C. *Science* 1986,233: 305–312.

Nunez DJR, Dickson MC, Brown MJ. Natriuretic peptide receptor mrnas in the rat and human heart. *J Clin Invest* 1992,90:1966-1971.

Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL et al. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci U S A* 1997,94:14730–14735.

Ondrejcakova M, Ravingerova T, Bakos J, Pancza D, Jezova D. Oxytocin exerts protective effects on in vitro myocardial injury induced by ischemia and reperfusion. *Can J Physiol Pharmacol* 2009,87:137-142.

Oral H, Dorn GW 2nd, Mann DL. Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian cardiac myocyte. *J Biol Chem* 1997,272:4836-4842.

Palmer JN, Hartogensis WE, Patten M, Fortuin FD, Long CS. Interleukin-1 β induces cardiac myocyte growth but inhibits cardiac fibroblast proliferation in culture. *J Clin Invest* 1995,95:2555–2564.

Pan, J, Fukuda K, Kodama H, Sano M, Takahashi T, Makino S, Kato T, Manabe T, Hori S, and Ogawa S. Involvement of gp130-mediated signaling in pressure overload-induced activation of the JAK/STAT pathway in rodent heart. *Heart Vessels* 1998,13:199-208.

Parker, TG, Packer, SE, Schneider, MD. Peptide growth factors can provoke "fetal" contractile protein gene expression in rat cardiac myocytes. *J Clin Invest* 1990,85:507-514.

Pennington SR, Martin BR. Insulin-stimulated phosphoinositide metabolism in isolated fat cells. *J Biol Chem* 1985,260:11039-11045.

Petersson M, Wiberg U, Lundeberg T and Uvnas-Moberg K. Oxytocin decreases carrageenan induced inflammation in rats. *Peptides* 2001,22: 1479-1484.

Porter JG, Arfsten A, Palisi T, et al. Cloning of a cDNA encoding porcine brain natriuretic peptide. *J Biol Chem* 1989,264:6689–6692.

Purcell NH and Molkentin JD. Is nuclear factor kappaB an attractive therapeutic target for treating cardiac hypertrophy? *Circulation* 2003,108:638–640.

Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, and Lin A. Activation of NF-kappa B is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. *Proc Natl Acad Sci U S A* 2001,98:6668–6673.

Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H. Return to the fetal gene program protects the stressed heart: A strong hypothesis. *Heart Fail Rev* 2007,12:331–343.

Reaux A, Iturrioz X, Vazeux G, Fournie-Zaluski MC, David C, Roques BP, Corvol P, Llorens-Cortes C. Aminopeptidase A, which generates one of the main effector peptides of the brain renin-angiotensin system, angiotensin III, has a key role in central control of arterial blood pressure. *Biochem Soc Trans* 2000,28:435-440.

Reindel JF, Ganey PE, Wagner JG, Slocombe RF, Roth RA. Development of morphologic, hemodynamic, and biochemical changes in lungs of rats given monocrotaline pyrrole. *Toxicol Appl Pharmacol* 1990,106:179–200.

Reversi A, Cassoni P, Chini B. Oxytocin receptor signaling in myoepithelial and cancer cells. *J Mammary Gland Biol Neoplasia* 2005,10:221-229.

Richey PA, Brown SP. Pathological versus physiological left ventricular hypertrophy: a review. *J Sports Sci* 1998,16:129–141.

Rich S, McLaughlin VV. Pulmonary hypertension. In: *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine* (7th ed.), edited by Zipes DP, Libby P, Bonow RO, Braunwald E. Philadelphia, PA: Elsevier Saunders, 2005.

Riley PR, Flint AP, Abayasekara DR, and Stewart HJ. Structure and expression of an ovine endometrial oxytocin receptor cDNA. *J Mol Endocrinol* 1995,15:195-202.

Rinderknecht E, Humbel RE. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem* 1986,253:2769 –2776.

Ritchie ME. Nuclear factor-kappaB is selectively and markedly activated in humans with unstable angina pectoris. *Circulation* 1998,98:1707–1713.

Rodbell, M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 1964,239:375-380.

Rodbell M. Metabolism of isolated fat cells. II. The similar effects of phospholipase C (Clostridium perfringens alpha toxin) and of insulin on glucose and amino acid metabolism. *J Biol Chem* 1966,241:130-139.

Rosenberg HC, Rabinovitch M. Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 1988,255:H1484–H1491.

Rozen F, Russo C, Banville D, and Zingg HH. Structure, characterization, and expression of the rat oxytocin receptor gene. *Proc Natl Acad Sci USA* 1995,92: 200-204.

Sacca L, Cittadini A, Fazio S. Growth hormone and the heart. *Endocrin Rev* 1994,15:555–573.

Saddik M, Lopaschuk GD. Myocardial triglyceride turnover and contribution to energy substrate utilization in isolated working rat hearts. *J Biol Chem* 1991,266:8162–8170.

Sadoshima, J, Izumo, S. Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. *Circ Res* 1993,73:413–423.

Sadoshima J, Izumo S. The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu Rev Physiol* 1997,59:551–571.

Salvatore CA, Woyden CJ, Guidotti MT, Pettibone DJ, and Jacobson MA. Cloning and expression of the rhesus monkey oxytocin receptor. *J Recept Signal Transduct Res* 1998,18: 5-24.

Samson WK. The power of two--molecular differentiation of the vascular and bone actions of the natriuretic peptides. *Endocrinology* 2000,141:3525-3526.

Sasayama S. For OPC-8212 multicenter Research Group: A placebo-controlled, randomized, double-blind study of OPC-8212 in patients with mild chronic heart failure. *Cardiovasc Drugs Ther* 19904:419–426.

Sasayama S, Matsumori A, Kihara Y. New insights into the pathophysiological role for cytokines in heart failure. *Cardiovasc Res* 1999,42:557-564.

Sawyer DB, Siwik DA, Xiao L, Pimentel DR, Singh K, Colucci WS. Role of oxidative stress in myocardial hypertrophy and failure. *J Mol Cell Cardiol* 2002,34:379–388.

Schmid B, Wong S, Mitchell BF. Transcriptional regulation of oxytocin receptor by interleukin-1beta and interleukin-6. *Endocrinology* 2001,142:1380-1385.

Schulz R, Panas DL, Catena R, Moncada S, Olley PM, Lopaschuk GD. The role of nitric oxide in cardiac depression induced by interleukin-1 beta and tumour necrosis factor-alpha. *Br J Pharmacol* 1995,114:27-34.

Schultz Jel J, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, Doetschman T. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest* 2002,109:787-796.

Schultze AE, Roth RA. Chronic pulmonary hypertension—the monocrotaline model and involvement of the hemostatic system. *J Toxicol Environ Health* 1998,1:271–346.

Seyfarth T, Gerbershagen HP, Giessler C, Leineweber K, Heinroth-Hoffman I, Ponicke K, Brodde OE. The cardiac β-adrenoceptor-G-protein(s)-adenylyl cyclase system in monocrotaline-treated rats. *J Mol Cell Cardiol* 2000,32: 2315–2326.

Sheng Z, Pennica D, Wood WI, Chien KR. Cardiotrophin-1 displays early expression in the murine heart tube and promotes cardiac myocyte survival. *Development* 1996,122:419–428.

Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, et al. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J* 2000,19:2537–2548.

Shioi T, McMullen JR, Kang PM, Douglas PS, Obata T, et al. Akt/protein kinase B promotes organ growth in transgenic mice. *Mol Cell Biol* 2002,22:2799–2809.

Steg PG, Joubin L, McCord J, Abraham WT, Hollander JE, Omland T, Mentre F, McCullough PA, Maisel AS. B-type natriuretic peptide and echocardiographic determination of ejection fraction in the diagnosis of congestive heart failure in patients with acute Dyspnea. *Chest* 2005,128:21–29.

Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K & Imura H. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* 1992,130:229-239.

Suga S, Nakao K, Itoh H, Komatsu Y, Ogawa Y, Hama N et al. Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-beta. Possible existence of "vascular natriuretic peptide system". *J Clin Invest* 1992,90:1145–1149.

Szeto A, Nation DA, Mendez AJ, Dominguez-Bendala J, Brooks LG, Schneiderman N, McCabe PM. Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *Am J Physiol Endocrinol Metab* 2008,295:E1495-1501.

Taegtmeyer H. Metabolism-The Lost Child of Cardiology. *J Am Coll Cardiol* 2000,36:1386–1388.

Takahashi, N, et al. Hypertrophic stimuli induce transforming growth factor-beta 1 expression in rat ventricular myocytes. *J Clin Invest* 1994,94:1470–1476.

Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, Walsh RA. Transgenic overexpression of constitutively active protein kinase C epsilon causes concentric cardiac hypertrophy. *Circ Res* 2000,86:1218–1223.

Takimoto E, Kass DA. Role of Oxidative Stress in Cardiac Hypertrophy and Remodeling. *Hypertension* 2007,49:241.

Tamura N, Ogawa Y, Chusho H et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci USA* 2000,97:4239–4244.

Thaik CM, Calderone A, Takahashi N, Colucci WS. Interleukin-1 β modulates the growth and phenotype of neonatal rat cardiac myocytes. *J Clin Invest* 1995,96:1093–1099.

Thibonnier M, Conarty DM, Preston JA, Plesnicher CL, Dweik RA and Erzurum SC. Human Vascular Endothelial Cells Express Oxytocin Receptors. *Endocrinology* 1999,140:1301-1309.

Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995,267:1456–1462.

Tian R, Miao W, Spindler M, Javadpour MM, McKinney R, et al. Long-term expression of protein kinase C in adult mouse hearts improves postischemic recovery. *Proc Natl Acad Sci USA* 1999,96:13536–13541.

Tsujimoto M, Mizutani S, Adachi H, Kimura M, Nakazato H, Tomoda Y. Identification of human placental leucine aminopeptidase as oxytocinase. *Arch Biochem Biophys* 1992,292:388-392.

Tsujino M, Hirata Y, Imai T, Kanno K, Eguchi S, Ito H, Marumo F. Induction of nitric oxide synthase gene by interleukin-1 β in cultured rat cardiocytes. *Circulation* 1994,90:375–383.

Tsutamoto T, Asai S, Tanaka T, Sakai H, Nishiyama K, Fujii M, et al. Plasma level of cardiotrophin-1 as a prognostic predictor in patients with chronic heart failure. *Eur J Heart Fail* 2007,9:1032–1037.

Tulevski II, Groenink M, van der Wall EE, van Veldhuisen DJ, Boomsma F, Stoker J, Hirsch A, Lemkes JS, Mulder BJ. Increased brain and atrial natriuretic peptides in patients with chronic right ventricular pressure overload: correlation between plasma neurohormones and right ventricular dysfunction. *Heart* 2001,86:27–30.

Usui S, Yao A, Hatano M, Kohmoto O, Takahashi T, Nagai R, Kinugawa K. Upregulated neurohumoral factors are associated with left ventricular remodeling and poor prognosis in rats with monocrotaline-induced pulmonary arterial hypertension. *Circ J* 2006,70:1208-1215.

Valen G, Yan ZQ, and Hansson GK. Nuclear factor kappa-B and the heart. *J Am Coll Cardiol* 2001,38:307–314.

van Eickels M, Grohe C, Cleutjens JP, Janssen BJ, Wellens HJ. Doevendans, PA. *Circulation* 2001,104:1419–1423.

Van Empel VPM and De Windt LJ. Myocyte hypertrophy and apoptosis: a balancing act. *Cardiovasc Res* 2004,63:487–499.

Vasan RS, Sullivan LM, D'Agostino RB, Roubenoff R, Harris T, Sawyer DB, Levy D, and Wilson PWF. Insulin-like Growth Factor I Levels and Heart Failure Risk in Older People. *Issue of Annals of Internal Medicine* 2003 (Oct. 21),139:642-648.

Voelkel NF, Tuder RM, Bridges J, Arend WP. Interleukin-1 receptor antagonist treatment reduces pulmonary hypertension generated in rats by monocrotaline. *Am J Respir Cell Mol Biol* 1994,11:664-675.

Wåhlander H, Isgaard J, Jennische E, Friberg P. Left ventricular insulin-like growth factor I increases in early renal hypertension. *Hypertension* 1992,19:25-32.

Wakatsuki T, Schlessinger J, Elson EL. The biochemical response of the heart to hypertension and exercise. *Trends Biochem Sci* 2004,29:609–617.

Wambolt RB, Lopaschuk GD, Brownsey RW, Allard MF. Dichloroacetate improves postischemic function of hypertrophied rat hearts. *J Am Coll of Cardiol* 2000,36:1378-1385.

Wang TJ, Larson MG, Levy D, Benjamin EJ, Corey D, Leip EP, Vasan RS. Heritability and genetic linkage of plasma natriuretic peptide levels. *Circulation* 2003,108:13–16.

Wang Y, Huang S, Sah VP, Ross JJ, Brown JH, Han J, and Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 1998,273: 2161–2168.

Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and rennin–angiotensin–aldosterone system. *Circulation* 1991,83:1849–1865.

Wensel R, Opitz CF, Anker SD, Winkler J, Hoffken G, Kleber FX, Sharma R, Hummel M, Hetzer R, Ewert R. Assessment of survival in patients with primary pulmonary hypertension: importance of cardiopulmonary exercise testing. *Circulation* 2002,106:319–324.

Wever RMF, van Dam T, van Rijn HJM, et al. Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem Biophys Res Commun* 1997,237:340–344.

Wilcox JN, Augustine A, Goeddel DV, Lowe DG. Differential regional expression of three natriuretic peptide receptor genes within primate tissues. *Mol Cell Biol* 1991,11:3454-3462.

Wolkart G, Stromer H, Brunner F. Calcium handling and role of endothelin-1 in monocrotaline right ventricular hypertrophy of the rat. *J Mol Cell Cardiol* 2000,32:1995–2005.

Wollert KC, Taga T, Saito M, Narazaki M, Kishimoto T, Glembotski CC, et al. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways. *J Biol Chem* 1996,271:9535–9545.

Wong SC, Fukuchi M, Melnyk P, Rodger I, and Giaid A. Induction of cyclooxygenase-2 and activation of nuclear factor-kappaB in myocardium of patients with congestive heart failure. *Circulation* 1998,98:100–103.

Yazawa H, Hirasawa A, Horie K, Saita Y, Iida E, Honda K and Tsujimoto G. Oxytocin receptors expressed and coupled to Ca2+ signalling in a human vascular s mooth muscle cell line. *Brit J Pharmacol* 1996,117:799-804.

Yoshimura M, Yasue H, Okumura K, Ogawa H, Jougasaki M, Mukoyama M, Nakao K, Imura H. Different secretion patterns of atrial natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. *Circulation* 1993,87:464–469.

Yu X, Kennedy RH, Liu SJ. JAK2/STAT3, not ERK1/2, mediates interleukin-6-induced activation of inducible nitric-oxide synthase and decrease in contractility of adult ventricular myocytes. *J Biol Chem* 2003,278:16304–16309.