

MILD HYPOTHERMIA PREVENTS BRAIN EDEMA AND ATTENUATES UP-REGULATION OF THE ASTROCYTIC BENZODIAZEPINE RECEPTOR IN EXPERIMENTAL ACUTE LIVER FAILURE

Mireille Bélanger, Paul Desjardins, Nicolas Chatauret, Christopher Rose, Roger F. Butterworth

Neuroscience Research Unit, C.H.U.M. (Hôpital Saint-Luc), 1058 St-Denis, Montreal, Que., Canada H2X 3J4

ABSTRACT

Background/Aims Mild hypothermia has proven useful in the clinical management of patients with acute liver failure. Acute liver failure in experimental animals results in alterations in the expression of genes coding for astrocytic proteins including the “peripheral-type” (astrocytic) benzodiazepine receptor (PTBR), a mitochondrial complex associated with neurosteroid synthesis. To gain further insight into the mechanisms whereby hypothermia attenuates the neurological complications of acute liver failure, we investigated PTBR expression in the brains of hepatic devascularized rats under normothermic (37°C) and hypothermic (35°C) conditions.

Methods PTBR mRNA was measured using semi-quantitative RT-PCR in cerebral cortical extracts and densities of PTBR sites were measured by quantitative receptor autoradiography. Brain pregnenolone content was measured by radioimmunoassay.

Results At coma stages of encephalopathy, animals with acute liver failure manifested a significant increase of PTBR mRNA levels. Brain pregnenolone content and [3H]PK 11195 binding site densities were concomitantly increased. Mild hypothermia prevented brain edema and significantly attenuated the increased receptor expression and pregnenolone content.

Conclusions These findings suggest that an attenuation of PTBR up-regulation resulting in the prevention of increased brain neurosteroid content represents one of the mechanisms by which mild hypothermia exerts its protective effects in ALF.

Keywords Peripheral-type benzodiazepine receptor; Astrocyte; Acute liver failure; Hepatic encephalopathy; Brain edema; Pregnenolone

INTRODUCTION

Up-regulation of the peripheral-type (astrocytic) benzodiazepine receptor (PTBR) in the brain is a consistent finding in various hyperammonemic syndromes including acute and chronic liver failure (see [1] for review). Unlike the central benzodiazepine receptor which is expressed solely in the central nervous system (CNS) in association with GABAA receptors, PTBR is found in the CNS as well as in various peripheral tissues and is not allosterically coupled to GABAA receptors [2]. In the CNS, PTBR is localized on the outer mitochondrial membrane [3] of glial cells where it forms a heteromeric complex of three subunit proteins namely the isoquinoline carboxamide binding protein (IBP; 18 kDa), a voltage-dependent anion channel (VDAC; 32 kDa) and an adenine nucleotide carrier (ANC; 30 kDa) [4].

PTBR has been implicated in a number of cellular functions including the regulation of cellular proliferation [5], heme biosynthesis [6], immunomodulation [2], regulation of the mitochondrial permeability transition pore and

apoptosis [7] and [8]. However, its role in steroidogenesis is probably the best characterized function of PTBR [9]. PTBR mediates the transport of cholesterol to the inner mitochondrial membrane where it is metabolized to pregnenolone by the cytochrome P450 side-chain cleavage enzyme. Pregnenolone is the precursor molecule for the synthesis of steroids, some of which are synthesized in the brain [10]. It has been suggested that increased production of neurosteroids with potent modulatory effects on GABAergic and glutamatergic neurotransmitter systems may lead to disturbances in excitatory and inhibitory neurotransmission and contribute to hepatic encephalopathy (HE) [11] and brain edema [12] in acute liver failure (ALF).

Previous studies conducted in animal models as well as in patients with ALF have demonstrated protective effects of mild hypothermia in this condition which significantly delays the onset of encephalopathy and prevents brain edema in rats with ALF due to hepatic devascularization [13]. The present study was undertaken to determine the effects of mild hypothermia on PTBR expression in the brains of rats with ALF in order to provide a better understanding of the role of the PTBR in the pathogenesis of hepatic encephalopathy and brain edema, two major complications of ALF.

MATERIALS AND METHODS

Animal surgery

Male Sprague-Dawley rats (175–200 g) were anesthetized with halothane, and an end-to-side portacaval anastomosis was performed according to the guidelines of Lee and Fisher [14]. Rats underwent a laparotomy, the inferior vena cava and portal vein were isolated and clamped using an anastomosis clamp (Roboz Instruments Inc, Washington, DC) and an elliptical portion of the vein 1.5 times the diameter of the portal vein was removed. The portal vein was ligated and cut, and an end-to-side anastomosis was performed under a dissecting microscope. Total surgery time was <15 min. Sham-operated control rats, matched for weight, were similarly anesthetized and the inferior vena cava was clamped for 15 min. Following surgery all animals were individually housed with free access to food and water under constant conditions of temperature, humidity, and light cycles. Twenty-four hours after portacaval anastomosis, rats were reanesthetized and subjected to hepatic artery ligation (HAL). Following HAL, body temperature was monitored and maintained at 37 °C by means of heating pads (ALF-37). Hypothermia occurred spontaneously in the absence of external heating and body temperature was maintained at 35 °C using heating pads when necessary (ALF-35). When coma stage of encephalopathy was reached (defined as the loss of righting and corneal reflexes), normothermic animals were sacrificed by decapitation and the brain was rapidly removed, dissected on ice and immediately frozen in isopentane. All tissues were stored at –70 °C until use. Hypothermic animals were sacrificed in parallel with comatose normothermic animals. All the above surgical methods were conducted in accordance with the Guidelines of Canadian Council of Animal care and were approved by Animal Research Committee at Saint-Luc Hospital (C.H.U.M.).

Brain water measurement

Brains were kept at 4 °C and cut into 2-mm slices. 1-mm punch biopsy specimens were obtained from the gray matter of the cerebral cortex. Water content of each specimen was measured gravimetrically using a density gradient of bromobenzene-kerosene precalibrated with K₂SO₄ as previously described [15]. The cortical samples were placed onto the fluid column, and the equilibration point was measured within 2 min. The specific gravity of the tissue was calculated and results were expressed as percentage of water content. Eight measurements were made per animal, and values were averaged.

RNA extraction

Total RNA was extracted using TRI Reagent (MRC Inc., Ohio) according to the manufacturer's protocol. Putative contaminating DNA was eliminated by adding 100 U of RNase-free DNase I per 50 µg of total RNA at 37 °C for 1 h.

Purified RNA was then extracted with phenol, precipitated with ethanol and resuspended in diethylpyrocarbonate-treated water. RNA samples were kept at -70°C until use.

RT-PCR analysis

β -Actin was used as an internal standard to monitor loading variations. Total RNA (1 μg) was mixed with 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 0.01%(w/v) bovine serum albumin, 100 μM dNTPs, primers at 1 μM each, AMV reverse transcriptase (80 U/ml), Taq DNA polymerase (20 U/ml) and 50 $\mu\text{Ci/ml}$ [α 32P]dCTP (3000 Ci/mmol), for a total reaction volume of 50 μl . The reactions were initially heated at 50°C for 15 min followed by PCR at 95°C for 30 s, 62°C for 45 s and 72°C for 1 min. Amplification efficiency conditions were determined after a kinetic study, to ensure all experiments were performed within the exponential phase of amplification where PCR product remains proportional to initial template concentration (data not shown). β -Actin and PTBR were amplified for 18 and 30 cycles, respectively. After amplification, the samples were electrophoresed onto 9% polyacrylamide gels, dried, autoradiographed at -70°C with an intensifying screen. Each band was excised and Cerenkov radiation was quantitated using a β -counter. Oligonucleotide primers were designed using the PRIME program (Genetic Computer Group, Wisconsin) and synthesized by the Sheldon Biotechnology Center (McGill University, Quebec) based on the following GeneBank accession numbers: V01217 (β -Actin: [16]) and J05122 (PTBR: [17]). The forward and reverse primer sequences were as follows: 5'-CATCCCCAAAGTTCTAC-3' and 5'-CCAAAGCCTTCATACATC-3' (β -Actin, 347 bp); 5'-CCATGCTCAACTACTATGTATGGC-3' and 5'-GTACAAGTGTCCCGCATG-3' (PTBR, 234 bp). The specificity of the oligonucleotide primers was verified using the program BLASTN (National Center for Biotechnology Information, Bethesda, MD).

[3H]PK 11195 autoradiography

PTBR binding sites were determined by autoradiography using the specific PTBR/IBP ligand [3H]PK 11195 as described before [18]. 20 μM thick sagittal brain sections were cut using a cryostat and thaw-mounted on gelatin-coated microscope slides. Sections were allowed to dry and stored at -20°C until use. Sections were then warmed up to room temperature and incubated for 60 min at in 170 mM Tris-HCl buffer (pH=7.4, 25°C) containing 1 nM [3H]PK 11195 (specific activity 85.5 Ci/mmol; PerkinElmer, Boston, MA). Non-specific binding was determined on adjacent sections incubated in the same buffer in the presence of 1 mM unlabelled PK 11195. Following the incubation period, sections were rinsed twice for 5 min in ice-cold buffer, dipped in ice-cold distilled water and dried rapidly under a stream of cold air. Dried sections and calibrated [3H] microscale standards (Amersham, NJ) were apposed to tritium sensitive Hyperfilm (Amersham) for 4 weeks at 4°C . Films were developed and tissue concentrations of [3H]PK 11195 were determined by quantitative densitometry analysis using an MCID computer-based densitometer and image-analysis system (Imaging Research Inc., Canada). The amount of ligand bound to various brain regions was calculated from the specific activity of the ligand. Specific binding was calculated by subtracting the non-specific binding from the total binding of corresponding brain regions on adjacent tissue sections.

Brain pregnenolone content measurement

Cerebral cortex was dissected and homogenized in 2.5 mL hexane (Mallinckrodt Baker, NJ) together with an internal standard solution containing trace amounts of [3H]pregnenolone for recovery determination. Samples were vortexed for 1 min and supernatant was removed after centrifugation (3000 rpm, 5 min). Extraction was then repeated with an additional 2.5 mL of hexane and the supernatants from both extractions were pooled. Samples were evaporated to dryness and resuspended in steroid diluent (ICN Biomedicals, CA). Pregnenolone content was measured using a radioimmunoassay kit according to the instructions supplied by the manufacturer (ICN Biomedicals).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA). Differences between groups were evaluated by the post-hoc Tukey Test. A P value <0.05 was considered to indicate a significant difference.

RESULTS

Following hepatic devascularization, normothermic animals developed symptoms of encephalopathy progressing from lethargy to loss of righting ability (precoma stage) and loss of corneal reflex (coma stage). Hypothermia significantly slowed the onset of encephalopathy with the result that at the time normothermic rats were comatose, hypothermic animals had not started to show significant neurological deterioration. Rats sacrificed at coma stage of encephalopathy had significantly higher brain water content ($P < 0.01$) while paired rats kept mildly hypothermic had brain water content similar to that of sham-operated animals (Fig. 1).

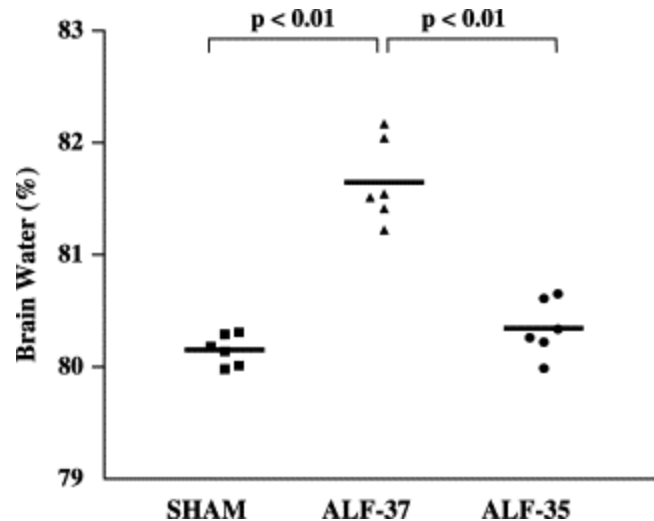


Fig. 1. Brain water content in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic rats (ALF-37; \blacktriangle) had significantly higher brain water content compared to sham-operated controls (SHAM; \blacksquare) or hypothermic rats (ALF-35; \bullet). Data points represent individual animals and horizontal bars indicate mean values of $n=6$ animals per experimental group. Significant differences between groups are indicated by respective P values.

RT-PCR analysis revealed a significant increase in the steady state level of PTBR mRNA following hepatic devascularization both in normothermic (113%, $P < 0.001$) and hypothermic (80%, $P < 0.001$) animals. However, mRNA levels were significantly lower ($P < 0.05$) in hypothermic compared to normothermic animals (Fig. 2). This increase of PTBR gene expression was selective since mRNA level of glutamine synthetase, an astrocytic marker protein, was unchanged (data not shown). Hepatic devascularization also resulted in a significant (42%; $P < 0.05$) increase in [3H]PK 11195 binding sites in the cerebral cortex of rats with ALF and maintained mildly hypothermic (Fig. 3). [3H]PK 11195 binding sites were further increased in ALF rats maintained normothermic (157% compared to sham, $P < 0.001$; 81% compared to ALF-35, $P < 0.001$) (Fig. 3). Brain pregnenolone levels were significantly elevated (2.1-fold compared to sham; $P < 0.05$) in the cerebral cortex of ALF rats maintained normothermic (Fig. 4); mild hypothermia prevented the increase of brain pregnenolone (Fig. 4).

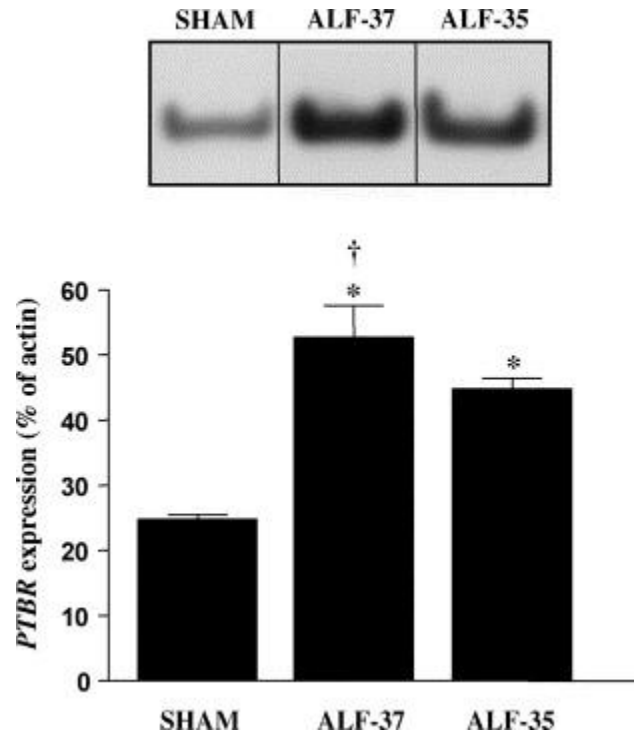


Fig. 2. Increased expression of IBP in the cerebral cortex of rats with ALF due to hepatic devascularization. Total RNA was extracted from the frontal cortices of sham-operated rats and ALF rats maintained normothermic (ALF-37) or mildly hypothermic (ALF-35). β -actin (347 bp) and IBP (234 bp) were reverse-transcribed and amplified by PCR for 18 and 30 cycles, respectively. Representative samples are shown. Values represent mean \pm SEM (n=4). *P<0.001 compared to sham-operated group; †P<0.05 compared to ALF-35 group determined by ANOVA and post-hoc Newman-Keuls test.

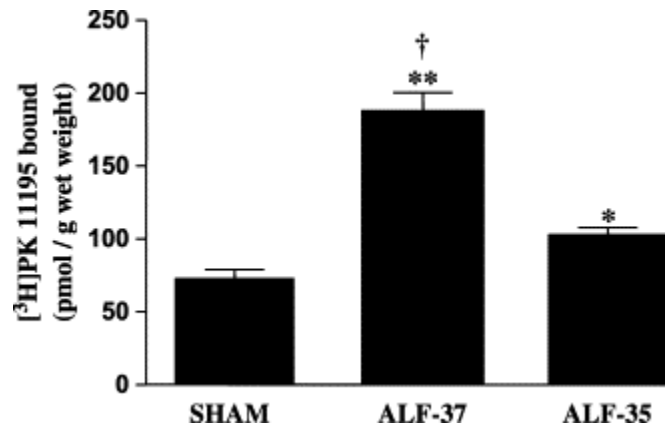


Fig. 3. [³H]PK 11195 binding site densities in the cerebral cortices of rats with ALF due to hepatic devascularization. ALF rats maintained normothermic (ALF-37; n=5) or mildly hypothermic (ALF-35; n=6) had higher [³H]PK 11195 binding site densities compared to sham-operated controls (SHAM; n=6). Values represent mean \pm SEM. *P<0.05 compared to shams; **P<0.001 compared to shams; †P<0.001 compared to ALF-35.

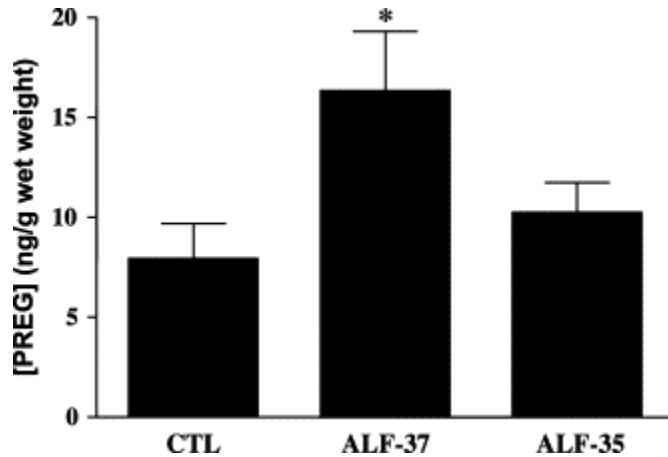


Fig. 4. Pregnenolone concentration in the cerebral cortices of rats with ALF due to hepatic devascularization. ALF rats maintained normothermic (ALF-37; n=5) had significantly higher pregnenolone levels compared to ALF rats maintained mildly hypothermic (ALF-35; n=6) or sham-operated controls (SHAM; n=6). Values represent mean±SEM. *P<0.05 compared to shams.

DISCUSSION

Induction of PTBR expression in the brain is a consistent finding associated with both acute and chronic liver failure. Increased densities of binding sites for the PTBR ligand [3H] PK 11195 were reported in the brains of rats following portacaval anastomosis, an experimental model of chronic liver failure [19] and [20] as well as in the frontal cortex and caudate nuclei of cirrhotic patients who died in hepatic coma [21]. The use of Positron Emission Tomography (PET) with [11C]PK 11195 as a ligand revealed that the increase in brain PTBR binding sites was positively correlated with the degree of cognitive impairment in cirrhotic patients as assessed by psychometric testing [22]. Furthermore, the increase of PTBR expression in the cerebral cortex of cirrhotic patients is correlated with the presence of Alzheimer type II astrocytosis, a characteristic neuropathological finding in patients with chronic liver disease, suggesting a possible role of PTBR in the occurrence of astrocytic changes associated in chronic HE [1]. Experimental ALF induced by the administration of the hepatotoxin thioacetamide (TAA) to mice also resulted in increased brain PTBR binding sites [23] and [24] as a result of increased levels of PTBR/isoquinoline binding protein mRNA [24].

The precise mechanisms responsible for the induction of PTBR in liver failure are not completely understood. However, a number of studies suggest that hyperammonemia, which is a common feature of chronic and acute liver failure, may be implicated. In support of this hypothesis, brains of mice administered ammonium acetate manifested an increased number of binding sites for [3H] PK 11195 and [3H] Ro5-4864, two specific PTBR ligands [23]. In addition, mice with chronic hyperammonemia due to congenital ornithine transcarbamylase deficiency (sparse fur (spf/Y) mice) also display increased brain [3H] PK 11195 binding site densities [25]. Furthermore, exposure of cultured astrocytes to pathophysiological concentrations of ammonia for 24 h results in an increased number of [3H] PK 11195 binding sites [26].

Considering the various cellular functions attributed to PTBR, unravelling the precise consequences of an increased number of these receptors has proven challenging. However, the role of PTBR in steroidogenesis is arguably its most extensively studied function. PTBR mediates the transport of cholesterol from the outer to the inner mitochondrial membrane where it is converted to pregnenolone by the action of the mitochondrial side chain cleavage enzyme

[9] and [27]. Pregnenolone is the precursor molecule for the synthesis of neurosteroids, some of which have potent modulatory effects on excitatory and/or inhibitory neurotransmission (see Ref. [10] for review). Interestingly, the administration of the neurosteroids tetrahydrodeoxycorticosterone (THDOC) and, more effectively, tetrahydroprogesterone (THP) to mice recapitulates many of the symptoms observed in HE (i.e. ataxia, loss of righting reflex, coma) [28]. Results from the present study indicate that the induction of PTBR was accompanied by increased brain levels of the neurosteroid precursor pregnenolone in the cerebral cortex of rats at coma stage of encephalopathy (Fig. 4). Similar increases of brain levels of pregnenolone have been reported in mice with ALF resulting from administration of TAA [23].

In vitro studies using cultured astrocytes demonstrate that the PTBR and neurosteroids influence ammonia-induced astrocytic swelling; it was demonstrated that the PTBR antagonist PK 11195 attenuates ammonia-induced swelling whereas the PTBR agonist Ro5-4864 increases ammonia-induced swelling [12]. In addition, various neurosteroids can either potentiate or inhibit ammonia-induced swelling, depending on the concentration used [12]. Whether or not the reported increase of PTBR expression and the increased levels of neurosteroid precursor pregnenolone contributes to astrocytic swelling and brain edema in vivo during ALF awaits further studies. However, this view is supported by the results of the present study demonstrating that mild hypothermia prevented both the increase of PTBR expression, pregnenolone content and the rise of brain water content observed in paired ALF animals kept normothermic. Studies in rats with hepatic devascularization have demonstrated a protective effect of mild hypothermia in this model of ALF [13], [29], [30] and [31]. Beneficial effects of hypothermia in these animals include delay in the onset of encephalopathy, attenuation of the increase of CSF ammonia concentration, prevention of brain edema and improved brain glucose metabolism [13] and [31]. Mild hypothermia has also proven to be a useful tool in the management of patients with ALF by effectively reducing intracranial pressure and improving cerebral perfusion pressure in patients awaiting orthotopic liver transplantation [32]. In the present study, we demonstrate that mild hypothermia attenuates the increase of both PTBR/IBP mRNA expression and [3H]PK 11195 binding sites caused by hepatic devascularization. In addition, mild hypothermia prevents the increase of pregnenolone levels as well as brain water content observed in the brain of rats with ALF at coma stage of encephalopathy. In view of these findings, it is possible that mild hypothermia exerts some of its protective effects by preventing the synthesis of neurosteroids that contribute to brain edema. It is unclear if the attenuation of PTBR induction is a primary effect of mild hypothermia or is secondary to the decrease brain ammonia concentration previously reported in the same model [13]. Further studies are needed to clarify this issue.

In conclusion, results of the present study demonstrate that mild hypothermia results in attenuation of PTBR induction and prevention of the increase of pregnenolone levels in rats with hepatic devascularization. A better understanding of the various mechanisms by which hypothermia exerts its protective effects may provide a valuable approach to further elucidate the pathogenesis of hepatic encephalopathy and brain edema occurring as a result of acute liver failure.

ACKNOWLEDGEMENTS

Studies were funded by the Canadian Institutes for Health Research (CIHR). Mireille Bélanger is a recipient of the CIHR Doctoral Research Award.

REFERENCES

- [1] M. Bélanger, S. Aboucha, P. Desjardins, R.F. Butterworth Upregulation of peripheral-type (mitochondrial) benzodiazepine receptors in hyperammonemic syndromes: consequences for neuronal excitability L. Hertz (Ed.), *Advances in molecular and cell biology*, Elsevier, Amsterdam (2003)

Bélanger, M. et al., 2005. Mild hypothermia prevents brain edema and attenuates up-regulation of the astrocytic benzodiazepine receptor in experimental acute liver failure. *Journal of Hepatology*, 42(5), p.694–699.

[2] M.J. Woods, D.C. Williams Multiple forms and locations for the peripheral-type benzodiazepine receptor *Biochem Pharmacol*, 52 (1996), pp. 1805–1814

[3] R.R. Anholt, P.L. Pedersen, E.B. De Souza, S.H. Snyder The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane *J Biol Chem*, 261 (1986), pp. 576–583

[4] M.W. McEnery, A.M. Snowman, R.R. Trifiletti, S.H. Snyder Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier *Proc Natl Acad Sci USA*, 89 (1992), pp. 3170–3174

[5] H. Alho, V. Varga, K.E. Krueger Expression of mitochondrial benzodiazepine receptor and its putative endogenous ligand diazepam binding inhibitor in cultured primary astrocytes and C-6 cells: relation to cell growth *Cell Growth Differ*, 5 (1994), pp. 1005–1014

[6] S. Taketani, H. Kohno, T. Furukawa, R. Tokunaga Involvement of peripheral-type benzodiazepine receptors in the intracellular transport of heme and porphyrins *J Biochem (Tokyo)*, 117 (1995), pp. 875–880

[7] M. Castedo, J.L. Perfettini, G. Kroemer Mitochondrial apoptosis and the peripheral benzodiazepine receptor: a novel target for viral and pharmacological manipulation *J Exp Med*, 196 (2002), pp. 1121–1125

[8] P. Casellas, S. Galiegue, A.S. Basile Peripheral benzodiazepine receptors and mitochondrial function *Neurochem Int*, 40 (2002), pp. 475–486

[9] V. Papadopoulos, H. Amri, N. Boujrad, C. Cascio, M. Culty, M. Garnier et al. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis *Steroids*, 62 (1997), pp. 21–28

[10] S.H. Mellon, L.D. Griffin Neurosteroids: biochemistry and clinical significance *Trends Endocrinol Metab*, 13 (2002), pp. 35–43

[11] E.A. Jones Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy *Metab Brain Dis*, 17 (2002), pp. 275–281

[12] A.S. Bender, M.D. Norenberg Effect of benzodiazepines and neurosteroids on ammonia-induced swelling in cultured astrocytes *J Neurosci Res*, 54 (1998), pp. 673–680

[13] C. Rose, A. Michalak, M. Pannunzio, N. Chatauret, A. Rambaldi, R.F. Butterworth Mild hypothermia delays the onset of coma and prevents brain edema and extracellular brain glutamate accumulation in rats with acute liver failure *Hepatology*, 31 (2000), pp. 872–877

[14] S.H. Lee, B. Fisher Portacaval shunt in the rat *Surgery*, 50 (1961), pp. 668–672

[15] A. Marmarou, W. Poll, K. Shulman, H. Bhagavan A simple gravimetric technique for measurement of cerebral edema *J Neurosurg*, 49 (1978), pp. 530–537

[16] U. Nudel, R. Zakut, M. Shani, S. Neuman, Z. Levy, D. Yaffe The nucleotide sequence of the rat cytoplasmic beta-actin gene *Nucl Acids Res*, 11 (1983), pp. 1759–1771

[17] R. Sprengel, P. Werner, P.H. Seeburg, A.G. Mukhin, M.R. Santi, D.R. Grayson et al. Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor *J Biol Chem*, 264 (1989), pp. 20415–20421

[18] D.K. Leong, L. Oliva, R.F. Butterworth Quantitative autoradiography using selective radioligands for central and peripheral-type benzodiazepine receptors in experimental Wernicke's encephalopathy: implications for positron emission tomography imaging *Alcohol Clin Exp Res*, 20 (1996), pp. 601–605

The final publication is available at <http://dx.doi.org/10.1016/j.jhep.2004.12.029>

Bélanger, M. et al., 2005. Mild hypothermia prevents brain edema and attenuates up-regulation of the astrocytic benzodiazepine receptor in experimental acute liver failure. *Journal of Hepatology*, 42(5), p.694–699.

- [19] P. Desjardins, P. Bandeira, V.L. Rao, R.F. Butterworth Portacaval anastomosis causes selective alterations of peripheral-type benzodiazepine receptor expression in rat brain and peripheral tissues *Neurochem Int*, 35 (1999), pp. 293–299
- [20] V.L. Rao, R. Audet, G. Therrien, R.F. Butterworth Tissue-specific alterations of binding sites for peripheral-type benzodiazepine receptor ligand [3H]PK11195 in rats following portacaval anastomosis *Dig Dis Sci*, 39 (1994), pp. 1055–1063
- [21] J. Lavoie, G.P. Layrargues, R.F. Butterworth Increased densities of peripheral-type benzodiazepine receptors in brain autopsy samples from cirrhotic patients with hepatic encephalopathy *Hepatology*, 11 (1990), pp. 874–878
- [22] A. Cagnin, S.D. Taylor-Robinson, D.M. Forton, R.B. Banati In vivo quantification of cerebral ‘peripheral benzodiazepine binding site’ in minimal hepatic encephalopathy *J Hepatol*, 34 (2001), p. 58 [Abstract]
- [23] Y. Itzhak, A. Roig-Cantisano, R.S. Dombro, M.D. Norenberg Acute liver failure and hyperammonemia increase peripheral-type benzodiazepine receptor binding and pregnenolone synthesis in mouse brain *Brain Res*, 705 (1995), pp. 345–348
- [24] Y. Kadota, K. Inoue, R. Tokunaga, S. Taketani Induction of peripheral-type benzodiazepine receptors in mouse brain following thioacetamide-induced acute liver failure *Life Sci*, 58 (1996), pp. 953–959
- [25] V.L. Rao, I.A. Qureshi, R.F. Butterworth Increased densities of binding sites for the peripheral-type benzodiazepine receptor ligand [3H]PK 11195 in congenital ornithine transcarbamylase-deficient sparse fur mouse *Pediatr Res*, 34 (1993), pp. 777–780
- [26] Y. Itzhak, M.D. Norenberg Ammonia-induced upregulation of peripheral-type benzodiazepine receptors in cultured astrocytes labeled with [3H]PK 11195 *Neurosci Lett*, 177 (1994), pp. 35–38
- [27] H. Li, V. Papadopoulos Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern *Endocrinology*, 139 (1998), pp. 4991–4997
- [28] M.D. Norenberg, Y. Itzhak, A.S. Bender The peripheral benzodiazepine receptor and neurosteroids in hepatic encephalopathy *Adv Exp Med Biol*, 420 (1997), pp. 95–111
- [29] P. Traber, M. DalCanto, D. Ganger, A.T. Blei Effect of body temperature on brain edema and encephalopathy in the rat after hepatic devascularization *Gastroenterology*, 96 (1989), pp. 885–891 View Record in Scopus
- [30] N. Chatauret, C. Rose, G. Therrien, R.F. Butterworth Mild hypothermia prevents cerebral edema and CSF lactate accumulation in acute liver failure *Metab Brain Dis*, 16 (2001), pp. 95–102
- [31] N. Chatauret, C. Zwingmann, C. Rose, D. Leibfritz, R.F. Butterworth Effects of hypothermia on brain glucose metabolism in acute liver failure: a H/C-nuclear magnetic resonance study *Gastroenterology*, 125 (2003), pp. 815–824
- [32] R. Jalan, S.W. Damink, N.E. Deutz, A. Lee, P.C. Hayes Moderate hypothermia for uncontrolled intracranial hypertension in acute liver failure *Lancet*, 354 (1999), pp. 1164–1168