Rose, C. et al., 1998. L-ornithine-L-aspartate in experimental portal-systemic encephalopathy: therapeutic efficacy and mechanism of action. *Metabolic Brain Disease*, 13(2), p.147–157.

L-ORNITHINE-L-ASPARTATE IN EXPERIMENTAL PORTAL-SYSTEMIC ENCEPHALOPATHY: THERAPEUTIC EFFICACY AND MECHANISM OF ACTION

C. Rose[1], A. Michalak[1], P. Pannunzio[1], G. Therrien[1], G. Quack[2], G. Kircheis[2] and R.F. Butterworth[1,3]

Neuroscience Research Unit, Centre Hospitaller de 1'Universite de Montreal (Campus Saint-Luc), Montreal, Quebec, Canada Merz + Co.,Frankfurt, Germany To whom correspondence should be addressed at Neuroscience Research Unit, CHUM (Campus Saint-Luc), 1058 St-Denis Street, Montreal, Quebec, Canada, H2X 3J4 Tel.: (514) 281-2444 ext.5759; FAX: (514) 281-2492; Email: butterwr@ere.umontreal.ca

ABSTRACT

Strategies aimed at the lowering of blood ammonia remain the treament of choice in portal-systemic encephalopathy (PSE). L-ornithine-L-aspartate (OA) has recently been shown to be effective in the prevention of ammonia-precipitated coma in humans with PSE. These findings prompted the study of mechanisms of the protective effect of OA in portacaval-shunted rats in which reversible coma was precipitated by ammonium acetate administration (3.85 mmol/kg i.p.). OA infusions (300 mg/kg/h, i.v) offered complete protection in 12/12 animals compared to 0/12 saline-infused controls. This protective effect was accompanied by significant reductions of blood ammonia, concomitant increases of urea production and significant increases in blood and cerebrospinal fluid (CSF) glutamate and glutamine. Increased CSF concentrations of leucine and alanine also accompanied the protective effect of OA. These findings demonstrate the therapeutic efficacy of OA in the prevention of ammonia-precipitated coma in portacaval-shuntedrats and suggest that this protective effect is both peripherally-mediated (increased urea and glutamine synthesis) and centrally-mediated (increased glutamine synthesis).

Keywords: Portal-systemic Encephalopathy; Hepatic Encephalopathy; Ammonia; L-ornithine-L-aspartate; Glutamine synthesis

INTRODUCTION

Of the possible neurotoxins implicated in hepatic encephalopathy (HE), ammonia was the first to be incriminated (Eck, 1877)and finds itself still the leading candidate as a key factor in its pathogenesis (Butterworth et al., 1987, Butterworth, 1994; Lockwood et al., 1991). Recent studies using Positron Emission Tomography (PET) and $^{13}NH_3$ confirm that, in chronic liver failure, brain utilizes ammonia at increased rates and that the blood-brain barrier becomes more permeable to ammonia (Lockwood et al., 1991).

Portal-systemic encephalopathy (PSE) is a common neuropsychiatric disorder resulting from chronic liver disease. Clinical features of PSE include impaired mental function, neurological disturbances such as asterixis or flapping tremor, and altered states of consciousness progressing to stupor and coma. Multiple PSE episodes are common and are associated with precipitating factors such as gastrointestinal bleeding, constipation or sedative use (Sherlock, 1985). Symptoms of PSE are generally reversible suggesting a metabolic etiology.

The final publication is available at http://dx.doi.org/10.1023/A:1020613314572

Ammonia, once taken up by the brain, is transformed into glutamine by glutamine synthetase (GS). However, the ammonia-buffering capacity of GS is limited (Butterworth et al., 1988) in chronic liver failure so that brain ammonia concentrations rise to attain millimolar concentrations which are deleterious to brain function. Neurochemical mechanisms so far proposed to explain the neurotoxic effects of ammonia include direct effects on excitatory and inhibitory neurotransmission (Raabe, 1989) and on cerebral energy metabolism (Hindfelt et al., 1977). Furthermore, astrocytic uptake of glutamate has been found to be inhibited by high levels of ammonia (Bender and Norenberg, 1996) leading to the proposal that PSE may result from glutamatergic synaptic dysregulation (Butterworth, 1996).

Therapeutic strategies aimed at reducing blood and brain ammonia are the mainstay in the prevention and treatment of PSE. However, few treatments of hyperammonemic syndromes have so far been designed to counteract the molecular actions of ammonia. Clinical studies have shown that L-ornithine-L-aspartate (OA) reduces blood ammonia concentrations and improves the clinical symptoms of HE in chronic liver disease (Staedt et al., 1993; Kircheis et al., 1997). However, the mechanism responsible for OA's ammonia-lowering action has not been fully elucidated. The present study was undertaken to assess the efficacy of OA in the prevention of severe encephalopathy precipitated by ammonium acetate treatment of portacaval-shunted rats. The protective effect was studied in relation to changes in plasma and CSF amino acids.

MATERIALS AND METHODS

O-Phthalaldehyde (OPA) reagent solution, 2-mercaptoethanol and amino acid standards were purchased from Sigma Chemical Co., St-Louis, MO; Methanol (HPLC grade), sodium phosphate (monobasic) and tetrahydrofuran (THF, HPLC grade) from Anachemia, Montreal, Quebec, Canada. OA was provided by Merz + Co., Frankfurt, Germany. All reagents and solvents were of reagent-grade purity unless otherwise stated. Double-distilled deionized water was used for preparation of amino acid solutions and buffers. The mobile phase was filtered through 0.45 .|J,m filters (Type GS, Millipore Corporation, Bedford, MA) and degassed under vacuum prior to use.

Portocaval anastomosis (PCA)

Adult male Sprague-Dawley rats weighing 175-200 g were anesthesized with halothane and an end-to-side PCA was performed according to the guidelines of Lee and Fisher (1961). Rats underwent a laparotomy, the inferior vena cava and portal vein were isolated allowing the inferior vena cava to be clamped (anastomosis clamp, Roboz Instruments Inc.), and the removal of an elliptical piece of vein 1.5 times the portal vein diameter. The portal vein was ligated and cut, and an end-to-side anastomosis was performed under a dissecting microscope. Total surgery time was <15 minutes. Following surgery, all animals were housed individually with free access to standard laboratory chow and water under constant conditions of temperature, humidity, and light cycles. Overall perioperative mortality for shunted rats was less than 10%. Four weeks following PCA, rats were pretreated with OA (0.3-1 g/kg body weight/hr, i.v) or isotonic saline 6 hours before ammonium acetate (3.85 mmoles/kg body weight, i.p.) to precipitate severe reversible neurological signs of encephalopathy.

Venous and arterial catheters

Under halothane anesthesia, rats underwent a second laparotomy and two PE-50 catheters were inserted; a venous catheter in the vena cava and an arterial catheter in the aorta. Following recovery from anesthesia, a constant OA infusion was pumped through the venous catheter and blood samples were collected through the arterial catheter.

Cisterna magna catheters

Rats were maintained under isoflurane anesthesia for the duration of the catheter implantation. Cisterna magna catheters were installed as previously described (Swain et al., 1992). The animals head was mounted with the skull in a horizontal position in a stereotaxic apparatus. A 3 cm incision was made in the skin from the back of the head and the overlying connective tissue was removed to expose the skull. A small hole was drilled in the skull using a

The final publication is available at http://dx.doi.org/10.1023/A:1020613314572

dental burr (009) on the midline immediately rostral to the interparietal-occipital bone suture. The hole was drilled in such a way that the occipital bone could be used as a guideline while inserting the cannula (PE-10 tubing, Clay Adams, Parsippany, NJ). The catheter was slowly inserted into the cisterna magna. Correctness of placement of the CSF-inflow window was verified by clearing the CSF outway, at which time a spontaneous flow of CSF occured. When successful implantation had been confirmed, the skull was cleaned and dried and a small amount of dental acrylic cement (Yates and Bird, Chicago, IL) was applied to cover part of the inserted cannula. The whole operation took approximately 20 minutes. Rats were housed individually and were allowed to recover from the operation. Twenty-four hours after this operation, Silastic tubing (Dow Corning, Midland, MI) was connected to the cannula for serial CSF sampling.

Neurological evaluation

Following ammonium acetate administration to rats with PCA, a rapidly progressive deterioration of neurological status was observed which started with lethargy, progressing to loss of righting reflex and ultimately loss of corneal reflex and deep coma, as previously described (Butterworth and Giguere, 1984; Therrien and Butterworth, 1991).

Measurement of plasma amino acids

400 µl samples of blood were withdrawn and collected in heparinized micro-tubes just before the start of infusion of OA, 1 hour after the start of OA infusion and just prior to the administration of ammonium acetate. A last sample was taken from animals from both treatment groups when the saline-treated rats became comatose. Blood samples were centrifuged and the plasma was removed, aliquoted and stored at -80°C.

Amino acids were derivatized with o-phthalaldehyde, separated and analysed using HPLC with fluorescence detection according to the method of Spink et al., 1986. Plasma samples were treated with acidified methanol (1:10), the mixture was left for 20 minutes at 4°C and centrifuged for 10 minutes at 15,400 g at 4°C. Twelve Ml of the supernatant was then withdrawn and mixed with 3 (J.1 of homoserine 0.01 mM as internal standard and reacted at room temperature with 20 (M1OPA reagent for 42 seconds, in a total volume of 35M1. Twenty five Ml of this solution was then injected onto the HPLC column (C18 Pecosphere, Perkin Elmer) with a CMA/200 autosampler. The HPLC system consisted of a solvent delivery system (Perkin Elmer, Series 400) coupled to a fluorometer (Perkin Elmer, LC-10 Fluorescence Detector, excitation 370 nm, emission 418-700 nm). Samples were injected in a 50 M1 loop of a Rheodyne 7125 injector system (Rheodyne, Cotati, CA, U.S.A). Peak area measurements were computed using a programmable recording integrator (Perkin-Elmer LC-100). The chromatograph was run with gradients of increasing organic solvent concentration generated by differential pumping rates of Solvents A and B. Solvent A contained 30 mM disodium phosphate, 30 mM sodium acetate, 2% (v/v) methanol, and 0.05% (w/v) sodium azide adjusted to pH 6.2 with glacial acetic acid. Tetrahydrofuran 1% (v/v) was added just before running the system. Solvent B consisted of a mixture of water: methanol:acetonitrile:tetrahydrofuran in 19:15:15:1 ratio by volume. Each gradient programme ended with a 10 min. hold at 100% solvent B followed by a 5-min reequilibration with 100% solvent A. Amino acid content of plasma was calculated by peak area analysis using standard curves and the internal standard.

Measurement of CSF amino acids

Amino acids were analyzed using a Perkin-Elmer reverse phase HPLC system with fluorescence detection and precolumn o-phthalaldehyde derivatization as previously described (Therrien and Butterworth, 1991). The HPLC system consisted of a solvent delivery system (series 400; Perkin-Elmer; Corwalk, CT) coupled to a filter fluorometer (LC-10 fluorescence detector; Perkin-Elmer; excitation at 370 nm, emission at 418-700 nm). Samples were injected using a 50 M1 loop of a CMA/200 autosampler with derivatization accessories. The column used was a reverse-phase Perkin-Elmer C18.5 m fitted with a Vydec reverse-phase C18 guard column. The chromatogram was run with gradients of increasing organic solvent concentration generated by differential pumping rates of solvents A and B. Solvent A contained 30 mmol/L sodium phosphate, 30 mmol/L sodium acetate, 2% (vol/vol) methanol, and 0.05% (w/vol) sodium azide adjusted to pH 6.2 with glacial acetic acid. Tetrahydrofuran 1% (vol/vol) was added

just before running the system. Solvent B consisted of a mixture of water:methanol:acetonitrile: tetrahydro-furan in 19:15:15:1 ratio by volume. Each gradient program ended with a 10-minute hold at 100% solvent B followed by a 10 minute reequilibration with solvent A. Amino acid content of CSF or brain was calculated by peak area analysis using standard curves and the internal standard.

Measurement of ammonia

Ammonia concentrations were estimated in plasma using a commercial ammonia test kit, which uses an ion-exchange method followed by colorimetric measurement of isolated ammonia nitrogen with the Berthelot phenate-hypochlorate reaction (Dienst, 1961). Ammonia was measured within 24 h of sampling.

STATISTICAL ANALYSIS

Data are expressed as mean ± S.E.M. For comparison of data between OA and saline-treated groups, Student-t-tests with Bonferroni correction were used. P values of <0.05 were considered to be significant.

RESULTS

Protective effect of OA in portacaval-shunted rats administered ammonium acetate

OA (300 mg/kg/h, i.v for 6 hours) afforded complete protection against ammonia acetate-induced encephalopathy in PCS rats. The protective effect observed was highly significant; 12/12 saline-treated PCS controls showed deterioration of neurological status progressing to coma whereas none of 12 OA-treated PCS rats showed deterioration of neurological status. All rats in the saline-treated control group regained consciousness within 3h of ammonium acetateadministration.

Effects of OA on plasma ammonia in ammonium acetate-treated portacaval-shunted rats

Following ammonium acetate administration, the increase of plasma ammonia was significantly reduced in OA-treated PCS rats compared to levels in saline-treated PCS rats at all time points (Figure 1).

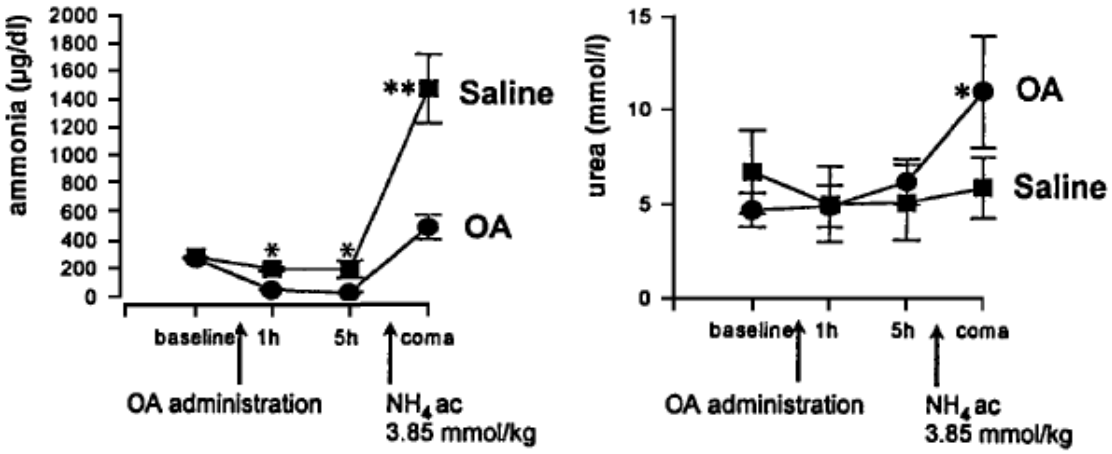

Figure 1. Protective effect of OA (300 mg/kg/h, i.v) in ammonium acetate-induced coma in portacaval-shunted rats in relation to plasma ammonia (left panel) and urea (right panel). Values significantly different from saline-treated controls indicated by *p<0.05, **p<0.01.

Effects of OA on plasma urea in ammonium acetate-treated portacaval-shunted rats

Administration of OA to ammonium acetate-treated PCS rats resulted in a significant increase in plasma urea (11.56±2.1 mM) compared to saline-treated PCS controls at the coma timepoint (5.91±1.6 mM, $p<0.02$) (Figure 1).

Effects of OA on plasma amino acids in ammonium acetate-treated portacaval-shunted rats

The effects of OA treatment (300 mg/Kg/h) on plasma amino acid concentrations measured at times equivalent to those at which saline-treated controls were comatose (ie: had lost their corneal reflexes) are shown in Table 1.

Table 1. Plasma amino acid concentrations in ammonium acetate-treated, portacaval-shunted rats: Effect of OA

	Amino acid concentration (μM)	
Amino acid	Saline-treated	OA-treated
Glutamate	69.6±13.1	2102.1±187.3*
Aspartate	17.1±2.3	5585.5±367.6*
Glutamine	466.5±33.6	1216.8±65.1*
Arginine	150.7±17.6	395.8±51.4*
Tyrosine	111.2±15.3	112.1±11.4
Tryptophan	49.1±3.1	65.3±9.1
Phenylalanine	73.7±7.6	64.1±5.7
Leucine	54.2±5.1	142.8±9.4*
Isoleucine	63.0±10.7	134.1±15.8*
Valine	106.6±17.1	195.0±20.1*
Asparagine	30.4±3.4	74.8±5.7*
GABA	4.9±0.7	15.6±1.7*
Glycine	376.6±22.1	451.6±36.1
Taurine	112.9±12.5	211.2±19.8*
Alanine	188.8±18.3	1087.9±109.8*
Threonine	141.8±27.3	419.9±68.1*
Serine	85.6±17.1	244.5±20.1*

Values represent mean S.E. of duplicate determination from groups of six rats. *$p<0.05$ compared to saline-treated controls by Student t-test

OA treatment prevented rats from becoming comatose and also resulted in 5-fold increases of alanine ($p<0.01$), 3-fold increases in glutamate, glutamine and GABA ($p<0.01$) and 2-fold increases of the branched chain amino acids leucine, isoleucine and valine ($p<0.01$). Two-fold increases of arginine, aspargine, taurine, threonine and serine were also noted after OA treatment.

Effects of OA on CSF amino acids in ammonium acetate-treated portacaval-shunted rats

In contrast to the effects in plasma, OA treatment resulted in less generalized alterations of amino acids in CSF of ammonium acetate-treated portacaval-shunted rats (Table 2).

Table 2. Cerebrospinal Fluid Amino Acid Concentrations in Ammonium Acetate-Treatec Portacaval-Shunted Rats: Effect of OA

	Amino acid concentration (μM)	
Amino acid	Saline-treated	OA-treated
Glutamate	6.2±1.1	17.5±3.1*
Aspartate	12.3±3.9	19.2±2.8
Glutamine	989.1±45.9	1227.3±61.5*
Arginine	34.6±2.7	35.6±5.5
Tyrosine	13.5±2.7	14.9±1.5
Tryptophan	7.6±0.7	7.4±1.3
Phenylalanine	15.4±2.4	15.5±1.7
Leucine	7.3±0.4	10.7±0.9*
Isoleucine	11.9±1.4	12.1±2.5
Valine	11.5±2.4	13.2±1.2
Asparagine	7.9±0.7	9.7±0.7
GABA	0.8±0.1	0.9±0.2
Glycine	20.3±2.2	22.2±3.6
Taurine	17.8±1.9	17.7±1.6
Alanine	44.4±0.7	54.1±3.1*
Threonine	67.1±3.9	63.1±4.5
Serine	72.3±2.9	87.7±6.2*

(Legend as in Table 1)

CSF glutamate was increased 3-fold ($p<0.01$) following OA treatment and significant 30-50% increases of glutamine, leucine, alanine and serine were also noted in the CSF of these animals. OA treatment did not result in significant alterations of other neuroactive amino acids (aspartate, glycine, GABA, taurine) nor of aromatic amino acids (tyrosine, phenylalanine, tryptophan).

DISCUSSION

Results of the present study demonstrate a significant protective effect of OA in ammonia-precipitated coma in rats following PCA. This finding is consistent with clinical studies in which administration of OA results in decreases in plasma ammonia levels and improvement of clinical symptoms in hyperammonemic patients with portal-systemic encephalopathy (Kircheis et al., 1997).

The protective effect of OA in ammonia-induced encephalopathy in shunted rats was accompanied by significant reductions in plasma ammonia and a small but significant increase in plasma urea suggesting that the reduction in plasma ammonia levels by OA is therefore partly the result of increased urea synthesis in liver; both ornithine and aspartate are metabolic substrates for the urea cycle (Figure 2). Increasing the ornithine concentration may stimulate flux via ornithine transcarbamylase (OTC) whereas aspartate affords a second nitrogen donor for the synthesis of urea. Consistent with increased urea cycle flux are the findings of 2-3-fold increases of plasma arginine following OA administration in the present and a previous (Vogels et al., 1995) study .

The final publication is available at http://dx.doi.org/10.1023/A:1020613314572

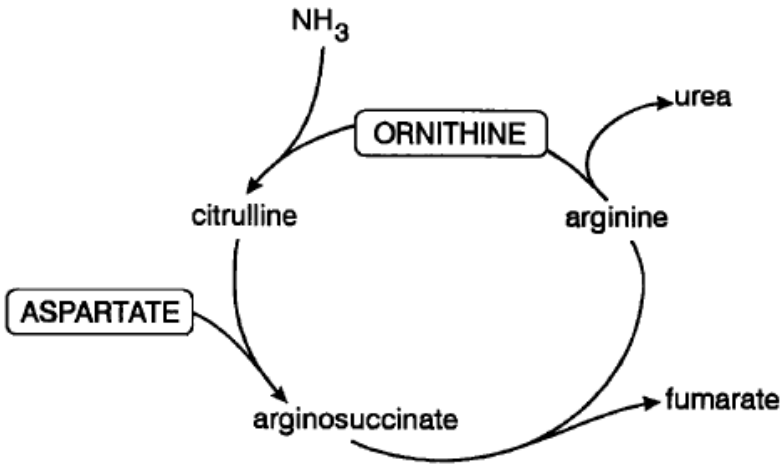

Figure 2. Simplified schematic representation of the urea cycle showing potential points of stimulation of urea cycle flux by L-ornithine and L-aspartate.

Increased urea synthesis by the liver following OA treatment despite the presence of an end-to-side portacaval anastomosis is not surprising. A previous study revealed enhanced urea synthesis and a lowering of blood ammonia following OA administration to cirrhotic rats (Gebhardt et al., 1997).

Other potentially benefical actions of L-ornithine and L-aspartate probably involve their ability to cause increased glutamate concentrations following stimulation of transaminases (Figure 3).

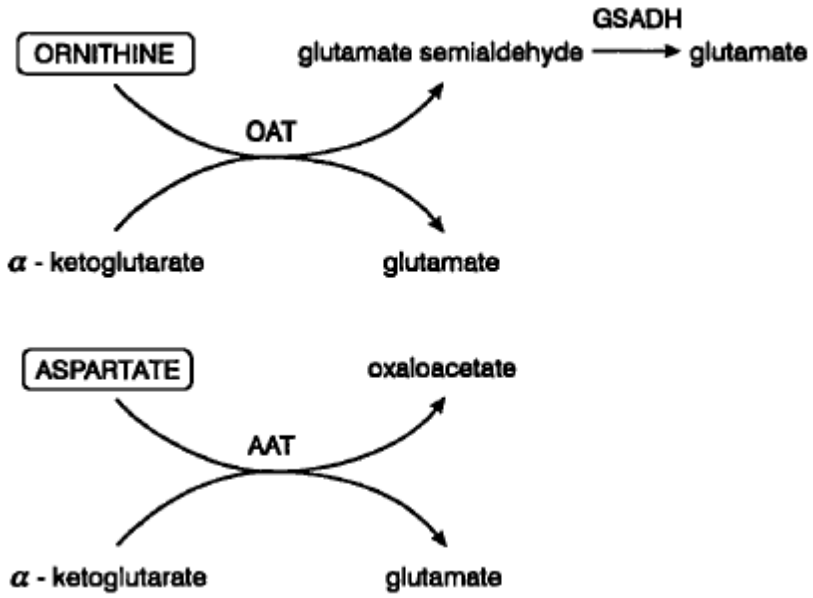

Figure 3. Displacement of transaminase equilibria by OA. OAT: ornithine aminotransferase; AAT: aspartate aminotransferase; GSADH: glutamate semialdehyde dehydrogenase.

Displacement by OA of the ornithine transaminase equilibrium towards glutamate and glutamate semialdehyde (and subsequently glutamate by the action of glutamate semialdehyde dehydrogenase, GSADH) and the aspartate aminotransferase equilibrium towards glutamate formation potentially affords 3 molecules of glutamate. That such mechanisms are operative following OA treatment of PCA shunted rats in the present study is supported by findings

of 3-fold increases of glutamate in body fluids of treated animals (Tables 1,2). The finding of comparable increases of glutamate in both plasma and CSF following OA treatment suggests that the above mechanisms (Figure 3) are operative both in brain and in peripheral tissues. L-ornithine is a substrate for the G^+ transporter at the blood-brain barrier (BBB), which is almost completely impermeable to glutamate or aspartate. Increased BBB uptake of L-ornithine would be expected to result in increased synthesis of glutamate in brain via the transamination reactions (Figure 3).

Increased tissue concentrations of glutamate would then have the potential to stimulate the synthesis of glutamine via glutamine synthesis (GS). GS is present in relatively high specific activities in liver, brain and skeletal muscle. Furthermore, portacaval shunting results in induction of GS in muscle (Girard and Butterworth, 1992). Given the increased availability of enzyme substrate (glutamate) and the possibility of enzyme induction in muscle, it is therefore likely that an important mechanism of ammonia-lowering of OA following portacaval shunting involves increased glutamine synthesis by skeletal muscle. Additional ammonia removal would also be expected via glutamine synthesis in brain. Consistent with these mechanisms are the findings in the present study of significant elevations of glutamine in the blood and CSF of OA-treated, portacaval-shunted rats.

Other potential consequences of increased availability of glutamate would (as expected) include increased GABA synthesis (observed in plasma but not in CSF) resulting from increased flux via glutamic acid decarboxylase as well as increased synthesis of alanine via pyruvate aminotransferase. Consistent with the latter, alanine concentrations were found to be significantly increased both in plasma and CSF of OA-treated animals. In a study of the effects of OA on plasma amino acids in patients with cirrhosis, increases in plasma glutamate and alanine were also reported (Staedt et al., 1993) suggesting that amino acid changes similar to those observed in the present study occur in humans with chronic liver disease following OA treatment. Finally, increased glutamate availability would be expected to stimulate branched-chain amino acid (BCAA) aminotransferases resulting in increased synthesis (or decreased catabolism) of BCAAs. In favour of this mechanism were the findings in the present study of increased plasma concentrations of BCAAs and of leucine in CSF of OA-treated animals. Whether or not increased plasma concentrations of BCAAs contributed to the ammonia-lowering effect of OA is unclear. Data from controlled clinical trials have yielded equivocal results on the ammonia-lowering capacity of BCAAs (Conn and Eriksson, 1989).

In summary, results of the present study clearly demonstrate that OA is effective in the prevention of ammonia-precipitated encephalopathy in portacaval-shunted rats. Findings are consistent with two mechanisms namely: (i) increased urea synthesis (albeit at a reduced rate compared to normal animals) and (ii) displacement of multiple transaminase reactions giving rise to increased glutamate followed by the facilitation of ammonia removal via glutamine synthetase both in peripheral tissues and in brain. Further studies of urea and glutamine synthesis are required in order to confirm these possibilities. OA has the potential to lower blood ammonia in ammoniagenic conditions associated with chronic liver disease and.in this way, is useful in the prevention of PSE. OA prophylaxis could be particularly useful in the prevention of PSE in cirrhotic patients with a history of recurrent gastrointestinal bleeding.

ACKNOWLEDGMENT

These studies were funded by the Medical Research Council of Canada (grant #PG11118) and Merz + Co., Frankfurt, Germany. A. Michalak and G. Therrien were recipients of a Postdoctoral Fellowship and Research Studentship respectively from The Canadian Liver Foundation. The authors thank Dominique D. Roy for her assistance with the preparation of the manuscript.

REFERENCES

Bender A.S., and Norenberg, M.D. (1996). Effects of ammonia on L-glutamate uptake in cultured astrocytes.*Neurochem. Res.* 21:567-573.

Butterworth R.F. and Giguere J.F. (1984). Region-selective glutamine changes in the CNS in relation to function in experimental subacute hepatic encephalopathy. In (Kleinberger P., Ferenci P., Riederer P.and Thaler H., *eds)Advances in Hepatic Encephalopathy and Urea Cycle Disease .* pp 394-401.

Butterworth R.F., Giguere J.F., Michaud J., Lavoie J. and Pomier Layrargues G. (1987). Ammonia: Key factor in the pathogenesis of hepatic encephalopathy. *Neurochem Pathol.* 6:1-12.

Butterworth, R.F., Girard, G. and Giguere J.F. (1988). Regional differences in the capacity for ammonia removal by brain following portacaval anastomosis. *J. Neurochem.* 51:486-490.

Butterworth R.F. (1994). Hepatic encephalopathy. In -(Arias I.M., Boyer J.L., Fausto N., Jakoby W.B., Schachter D.A. and Shafritz D.A., eds) *The liver: Biology and Pathobiology-,* 3rd Ed., Raven Press, Ltd, New York, pp. 1193-1208.

Butterworth R.F. (1996). The neurobiology of Hepatic Encephalopathy. In (A.T. Blei and R.F. Butterworth, eds.) *Seminars in Liver Disease* 16, pp. 281-290.

Conn, H.O. and Eriksson, L.S. (1989). Nutritional therapy of portal-systemic encephalopathy: The branched-chain amino acid story. In (R.F. Butterworth and G. Pomier Layrargues, eds.) *Hepatic Encephalopathy: Pathophysiology and Treatment,* Humana Press, Clifton, N.J., p. 509-528, 1989.

Dienst S.G. (1961). An ion exchange method for plasma ammonia concentration. J *Lab. Clin. Med.* 58 (suppl 71):1-144.

Eck N.V. (1877). Ligature of the portal vein. Voen Med J St-Petersburg (Russo) 130:1-2. Translation by Child C.G.(1953) *Surgery, Gynecology and Obstetrics,* 375-376.

Girard G. and Butterworth R.F. (1992). Effect of portacaval anastomosis on glutamine synthetase activities in liver, brain and skeletal muscle. *Digest. Dis. Sci.* 37:1121-1126

Hindfelt B., Plum, F. and Duffy, T.E (1977). Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. *J. Clin. Invest.* 59:386-396.

Kircheis, G., Nilius, R., Held, C., Berndt, H., Buchner, M, Gortelmeyer, *et al.* (1997). Therapeutic efficacy of L-ornithine-L-aspartate infusions in patients with cirrhosis and hepatic encephalopathy: Results of a placebo-controlled, double-blind study. *Hepatology* 25:1351-1360

Lee S.H. and Fisher B. (1961). Porta-caval shunt in the rat. *Surgery* 50:668-672.

Lockwood A.H., Yap E.W.H. and Wong W.H. (1991). Cerebral ammonia metabolism in patients with severe liver disease and minimal hepatic encephalopathy. *J. Cerebr. Blood Flow and Metab.* 11:337-341.

Gebhardt R., Beckers G., Gauritz F., Haupt W., Jonitza D., Klein S. and Scheja L. (1997). Treatment of cirrhotic rats with L-ornithine-L-aspartate enchance lowers serum ammonia levels. *J. Pharmacol. Exp. Ther.* 283:1-6.

Raabe W. (1989). Neurophysiology of ammonia intoxication. In (R.F. Butterworth and G. Pomier Layrargues, *eds.)Hepatic Encephalopathy: Pathophysiology and Treatment.,* Humana Press, Clifton, New Jersey, 1988, pp.49-77.

Sherlock S. (1985). Diseases of the liver and biliary system, 7th edition, Blackwell Sci., Boston

Spink, D.C., Swann, J.W., Snead, O.C., Waniewski, R.A., Martin, D.L. (1986). Analysis of aspartate and glutamate in human cerebrospinal fluid by high-performance liquid chromatography with automated precolumn derivatization. *Anal. Biochem.* 158:79-86.

The final publication is available at http://dx.doi.org/10.1023/A:1020613314572

Staedt U., Leweling H., Gladisch R., Kortsik C., Hagmuller E. and Holm E. (1993). Effects of ornithine-

aspartate on plasma ammonia and plasma amino acids in patients with cirrhosis. A double-blind, randomized study using a four-fold crossover design. *J. Hepatol.* 19:424-430.

Swain M., Bergeron M., Audet R., Blei A.T. and Butterworth R.F. (1992). Monitoring of neurotransmitter amino acids by means an indwelling cisterna magna catheter: a comparison of two rodent models of fulminant hepatic failure. *Hepatology* 16:1028-1035

Therrien, G., and Butterworth, R.F. (1991). Cerebrospinal fluid amino acids in relation to neurological status in experimental portal-systemic encephalopathy. *Metab. Brain Dis.* 6: 65-74.

Vogels B.A.P.M., Slotbloom J., de Haan J.G., Bovee W.M.M.J., Ouack G. and Chamuleau R.A.F.M. (1995). The effect of L-ornithine-L-aspartate on mild encephalopathy due to portosystemic shunting and hyperammonemia. In (L. Capocaccia et al., eds.) *Advances in Hepatic Encephalopathy and Metabolic Nitrogen Exchange* pp. 423-431.

The final publication is available at http://dx.doi.org/10.1023/A:1020613314572