



UvA-DARE (Digital Academic Repository)

L-ornithine vs. L-ornithine-L-aspartate as a treatment for hyperammonemia-induced encephalopathy in rats

Vogels, B.A.P.M.; Karlsen, O.T.; Maas, M.A.W.; Bovee, W.M.M.J.; Chamuleau, R.A.F.M.

DOI

[10.1016/S0168-8278\(97\)80024-4](https://doi.org/10.1016/S0168-8278(97)80024-4)

Publication date

1997

Published in

Journal of hepatology

[Link to publication](#)

Citation for published version (APA):

Vogels, B. A. P. M., Karlsen, O. T., Maas, M. A. W., Bovee, W. M. M. J., & Chamuleau, R. A. F. M. (1997). L-ornithine vs. L-ornithine-L-aspartate as a treatment for hyperammonemia-induced encephalopathy in rats. *Journal of hepatology*, 26, 174-182.
[https://doi.org/10.1016/S0168-8278\(97\)80024-4](https://doi.org/10.1016/S0168-8278(97)80024-4)

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

L-Ornithine vs. L-ornithine-L-aspartate as a treatment for hyperammonemia-induced encephalopathy in rats

B. A. P. M. Vogels¹, O. T. Karlsen², M. A. W. Maas¹, W. M. M. J. Boveé² and R. A. F. M. Chamuleau¹

¹Academic Medical Center, University of Amsterdam, Department of Experimental Internal Medicine, Amsterdam, and ²Faculty of Applied Physics, Delft University of Technology, Delft, The Netherlands

Background/Aims: The effect of L-ornithine (ORN) and L-ornithine-L-aspartate (OA) therapy on “extracerebral” nitrogen metabolism, brain metabolism and neurotransmission has been investigated in portacaval shunted rats with hyperammonemia-induced encephalopathy.

Methods: One day before ammonium-acetate infusion, a portacaval shunt was performed in three experimental groups: 1 - control rats, 2 - ORN-treated rats and 3 - OA-treated rats. Ammonium-acetate was given as an intravenous bolus injection ($0.4 \text{ mmol}\cdot\text{kg bw}^{-1}$) followed by a constant infusion ($1.9 \text{ mmol}\cdot\text{kg bw}^{-1}\cdot\text{h}^{-1}$) so that steady-state blood ammonia concentrations ($500\text{--}800 \mu\text{M}$) were obtained in the course of 5 h. After 1 h, ammonium-acetate infusion, either L-ornithine or L-ornithine-L-aspartate, was infused for the next 4 h ($3.0 \text{ mmol}\cdot\text{kg bw}^{-1}\cdot\text{h}^{-1}$) in the treated groups. The following parameters were measured: clinical grade of encephalopathy, EEG activity ($n=10\text{--}20/\text{group}$), amino acids in plasma ($n=10\text{--}20/\text{group}$) and brain dialysate ($n=5\text{--}9/\text{group}$), and brain metabolites obtained by *in vivo* cerebral ¹H-MRS ($n=4\text{--}6/\text{group}$).

Results: ORN and OA treatment resulted in significantly lower blood (34% and 39%) and brain

(42% and 22%) ammonia concentrations, significantly higher urea production (39% and 86%) and significantly smaller increases in brain glutamine and lactate concentrations than in controls. These changes were associated with a significantly smaller increase in clinical grade of encephalopathy in ORN- and OA-treated rats, and a significant improvement in EEG activity in ORN-treated rats. OA-treated rats showed a significant increase in aspartate and glutamate concentrations in brain dialysate.

Conclusions: The beneficial effects of both treatments on the manifestations of hyperammonemia-induced encephalopathy can be explained by a reduction in blood and brain ammonia concentrations. It is suggested that when OA is administered, the effect of ornithine is partly counteracted by aspartate, inducing high brain extracellular concentrations of the two excitatory amino acids glutamate and aspartate, and perhaps causing overstimulation of NMDA receptors.

Key words: Hepatic encephalopathy (HE); Hyperammonemia; L-Ornithine; L-Ornithine-L-aspartate; Portacaval shunt; Proton magnetic resonance spectroscopy.

IT IS WIDELY believed that ammonia plays an important role in the multifactorial pathogenesis of hepatic encephalopathy (HE) (1–3). Increased cerebral ammonia concentrations may directly affect inhibitory and excitatory neurotransmission and may

indirectly influence brain energy metabolism (4–6). Furthermore, because glutamine synthase, present in astrocytes, catalyzes glutamine formation from ammonia, hyperammonemia may lead to increased intracellular glutamine concentrations which may promote cell swelling and finally brain edema (7), a common terminal event in fulminant hepatic failure (8,9). In addition, increased intracellular glutamine concentration may stimulate plasma to brain transport of aromatic amino acids (AAA) across the blood–brain barrier (10–12). Since AAA are the di-

Received 4 March; revised 22 April; accepted 2 May 1996

Correspondence: Birgit Vogels, University of Amsterdam, Department of Experimental Internal Medicine, room G2-130, PO Box 22700, 1100 DE Amsterdam, The Netherlands. Tel.: 020–5665910. Fax: 020–6977192.

rect precursors of the monoamines (dopamine, noradrenaline, serotonin, kynurenine and tryptamine), elevated brain concentrations of AAA may affect the synthesis of these amines. Another possible "toxic" effect of raised extracellular ammonia concentrations in the brain is suggested by the observation that in isolated synaptosomes, glutamate re-uptake is inhibited by high ammonia concentrations (13). If this is also the case *in vivo*, this could result in high extracellular glutamate concentrations, possibly leading to overstimulation of N-methyl-D-aspartate (NMDA) receptor activity.

For all these reasons, reducing hyperammonemia in patients with severe liver failure has always been one of the important goals of therapeutic applications. Administration of ornithine and also ornithine compounds has been proved to decrease blood ammonia concentrations (14–16). During the last decades, clinical studies have shown that L-ornithine-L-aspartate reduces blood ammonia concentration, restores amino acid imbalances and may improve the clinical symptoms of HE in patients with mild liver failure (17,18).

In order to study the mechanism of the possible therapeutic effect of L-ornithine alone or in combination with L-aspartate, an experimental study was designed in which the efficacy of a monotherapy of L-ornithine (ORN) was compared with a combined therapy of L-ornithine and L-aspartate (OA) in portacaval shunted rats with hyperammonemia-induced encephalopathy. The question whether the beneficial effects of these agents is solely due to increased "extracerebral" nitrogen metabolism or to alterations in brain metabolism and/or neurotransmission was addressed. The latter was studied by *in vivo* cerebral $^1\text{H-MRS}$ and *in vivo* brain dialysis.

Materials and Methods

Animals

Male Wistar rats (200–300 g, HSD Zeist, The Netherlands; 12 h light cycle: 8 a.m. – 8 p.m.) were used and had free access to standard laboratory chow (RMH 1410, Hope Pharms, The Netherlands) and water ad libitum. Animal welfare was in accordance with institutional guidelines of the University of Amsterdam.

Surgical and experimental procedures

Three experimental groups were studied: 1 – control rats, 2 – ORN-treated rats, and 3 – OA-treated rats. One day before ammonium-acetate was infused, a portacaval shunt operation (PCS) was performed under ether anesthesia in all groups (19). On the day of the infusion, a jugular vein catheter and a carotid

artery catheter were placed in the rats under ether anesthesia in order to infuse the different solutions and to take blood samples, respectively. After recovery of anesthesia, a bolus injection of 0.4 mmol·kg bw⁻¹ ammonium-acetate was given to all groups prior to an ammonium-acetate infusion (1.9 mmol·kg bw⁻¹·h⁻¹) for 5 h (AI-PCS: ammonium-acetate infusion in PCS rats). At t=1 h, a steady-state blood ammonia concentration was obtained and the ORN (L-ornithine-hydrochloride) or OA (L-ornithine-L-aspartate, a generous gift of Dr. G. Quack from Merz & Co. GmbH & Co.) treatment was started in the treated groups: 3.0 mmol·kg bw⁻¹·h⁻¹ intravenously.

In addition to these treated groups, in series 1 and 3 (see below) extra groups were studied: Three AI-PCS rats received a Na-acetate infusion (3.0 mmol·kg bw⁻¹·h⁻¹) with the same osmolarity as the ornithine infusion and another 4 AI-PCS rats received L-aspartate infusion (3.0 mmol·kg bw⁻¹·h⁻¹). The total volume of the infusions was 10 ml per rat in all groups.

Three different series of experiments were performed:

Series 1 – Quantification of severity of encephalopathy (a total of 43 rats used, 10–20 per group). Encephalopathy was graded clinically according to the level of consciousness, using the five grades shown in Table 1. In addition to this, more or less subjective measurement an objective measurement was performed by means of EEG spectral analysis.

Five days before PCS, four golden skull electrodes were implanted (20) in order to measure EEG activity at several time points. EEG spectral analysis was obtained for four EEG frequency band regions within the range of 1–26.5 Hz (21). The EEG left index was calculated as the ratio of the power of the low frequency band (1–7.4 Hz) and high frequency band (13.5–26.5 Hz). Normal values of EEG left index are between 5 and 10. This index increases during the development of encephalopathy to values of 20–30.

Series 2 – Cerebral metabolite concentrations by in vivo $^1\text{H-MRS}$ (a total of 15 rats used, 4–6 per

TABLE 1

Stages in experimental hepatic encephalopathy

Clinical grade 0	normal behavior
Clinical grade 1	mild lethargy
Clinical grade 2	decreased motor activity, poor gesture control, diminished response to pain stimuli
Clinical grade 3	severe ataxia: no spontaneous righting reflex
Clinical grade 4	no righting reflex to pain stimuli
Clinical grade 5	no reaction to pain stimuli

group). To measure the time course of changes in the concentrations of the brain metabolites glutamate, glutamine, lactate, phosphocholine, N-acetyl-aspartate and phosphocreatine, *in vivo* $^1\text{H-MR}$ spectroscopy of rat cerebral cortex was performed, using a SADLOVE (single-shot adiabatic localized volume excitation) sequence with energy reduced, phase compensated 2π pulses for localization. The methodology used is described in Slotboom et al. (22). During the MRS measurements, rats were under inhalation anesthesia: 1% enflurane, 400 ml/min O_2 and 600 ml/min N_2O .

Series 3 – In vivo brain microdialysis (a total of 20 rats used, 5–9 per group). To determine cerebral extracellular amino acid concentrations, *in vivo* brain dialysis was performed as described by Tossman et al. (23) and modified by Bosman et al. (24). Five hours before a PCS operation was performed, a brain dialysis tube (Amicon, Ireland Ltd, Limerick, Ireland) with a 50 kDa molecular mass cutoff was transversally implanted under pentobarbital anesthesia ($45\text{ mg}\cdot\text{kg}\text{ bw}^{-1}$ i.p.) into the cerebral cortex (coordinates: holes were drilled bilaterally 2.0 mm below bregma). A tungsten wire was used for this purpose (Clark electromedical instruments, TW5–3). On the day of the infusion, microdialysis was performed with an iso-osmotic Ringer solution at a flow rate of $5\ \mu\text{l}/\text{min}$. After a stabilization period of 30 min, collection of dialysate samples was started 1 h before the ammonium-acetate infusion and was continued until the end of infusion. Dialysate samples were collected on ice at 30-min intervals in tubes containing $10\ \mu\text{l}$ 0.1 M perchloric acid, which were then directly frozen in liquid nitrogen. These samples were stored at -70°C until required for analysis of their amino acid content (25).

Biochemical parameters

In the first and third series of experiments, blood samples (0.05 ml heparinized blood) were taken every hour from the carotid artery. Blood ammonia concentration was assayed by the Blood Ammonia Checker II (26) and plasma amino acid concentrations measured by means of HPLC (25). The Fischer ratio is defined as the ratio of the plasma concentrations of valine plus isoleucine plus leucine (BCAA) vs. phenylalanine plus tyrosine (AAA). At the end of these experiments, rats were sacrificed under complete ether anesthesia: blood was collected and the brains were quickly removed and frozen in liquid nitrogen. They were stored at -70°C until required for analysis.

Urea was measured in urine (collected during the

5 h of the experiment) and in plasma (at start and end of the experiment) ($n=4/5$ per group). Urea production was defined as:

$$(\text{Urea}_{\text{ur}} \times \text{Vol}_{\text{ur}}) + (\delta \text{Urea}_{\text{pl}} \times \text{Vol}_{\text{H}_2\text{O}})$$

Urea_{ur} : urea concentration in urine,

Vol_{ur} : volume of urine,

$\delta \text{Urea}_{\text{pl}}$: difference in plasma urea concentration between 0 and 5 h

$\text{Vol}_{\text{H}_2\text{O}}$: water compartment (defined as $0.6 \times$ body weight).

Statistical analysis

Results are presented as means \pm SEM. Statistical analysis was performed by the Student's *t*-test for amino acid concentrations in plasma and brain dialysate at the end of the experiment, brain ammonia concentrations and urea production. Other parameters (blood ammonia concentration, clinical grade, EEG left index, brain concentrations of glutamine, glutamate, lactate and P-choline) were analyzed by means of repeated measurement analysis of variance (ANOVA). *p*-Values <0.05 were considered to be significant.

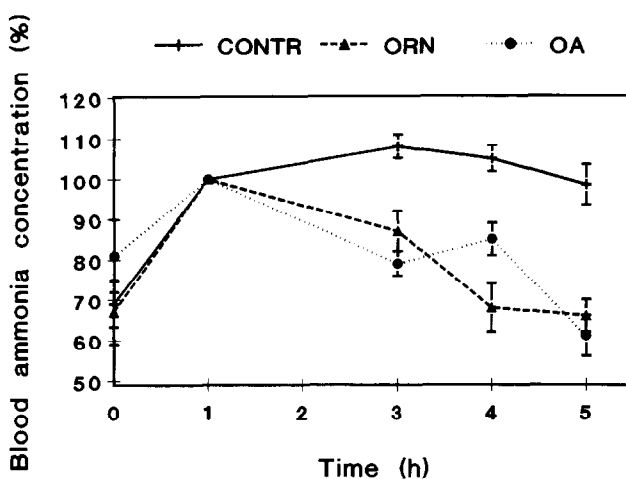


Fig 1. The effect of L-ornithine (ORN) and L-ornithine-L-aspartate (OA) treatment on blood ammonia concentration in AI-PCS rats (PCS rats given an i.v. infusion of ammonium-acetate starting at time 0). L-ornithine ($n=10$) and L-ornithine-L-aspartate ($n=10$) were given as an i.v. infusion, starting 1 h after start of ammonium-acetate infusion. Control rats ($n=20$) were given an ammonium-acetate infusion for 5 h. Ammonia concentration is expressed as percentage of steady-state concentration at $t=1$ h, which amounted to $500\text{--}800\ \mu\text{M}$ and remained constant during the rest of the infusion in the control group. (Values are expressed as mean \pm SEM. Statistical analysis was performed by means of repeated measurement ANOVA. Blood ammonia concentration was significantly higher in controls compared to ORN and OA ($p<0.0001$).

TABLE 2

Amino acid concentration in plasma (μM) at the end of the experiment (mean \pm SEM)

Amino acid	Control <i>n</i> =20	ORN <i>n</i> =15	OA <i>n</i> =10
Aspartate	20.2 \pm 3.7	12.6 \pm 1.2	500 \pm 27.9 [§]
Ornithine	99.8 \pm 13.1	5475 \pm 125 [§]	4255 \pm 124 [§]
Glutamate	48.5 \pm 5.8	64.1 \pm 8.4	554 \pm 32.9 [§]
Glutamine	1065 \pm 64.	1288 \pm 55 [*]	1728 \pm 79 [§]
Citrulline	81.9 \pm 4.3	70.7 \pm 3.6	76.7 \pm 5.5
Fischer ratio	1.89 \pm 0.06	3.06 \pm 0.09 [§]	2.69 \pm 0.09 [§]

Student's *t*-test:

**p*<0.05 treated vs. control rats.

***p*<0.01 treated vs. control rats.

[§]*p*<0.001 treated vs. control rats.

TABLE 3

Amino acid concentration in brain dialysate (μM) at the end of the experiment (mean \pm SEM)

Amino Acid	Control <i>n</i> =9	ORN <i>n</i> =6	OA <i>n</i> =5
Aspartate	2.42 \pm 0.39	1.79 \pm 0.34	30.5 \pm 1.2 [§]
Ornithine	5.48 \pm 1.25	105 \pm 15.4 ^{**}	78.1 \pm 4.8 [§]
Glutamate	5.69 \pm 1.32	4.15 \pm 0.92	16.2 \pm 1.8 [§]
Glutamine	179 \pm 19.0	246 \pm 25.0	235 \pm 18.2
Fischer ratio	1.68 \pm 0.05	2.42 \pm 0.07 [§]	2.36 \pm 0.14 ^{**}

Student's *t*-test:

**p*<0.05 treated vs. control rats.

***p*<0.01 treated vs. control rats.

[§]*p*<0.001 treated vs. control rats.

Results

Series 1 – Quantification of severity of encephalopathy. One hour after the start of the ammonium-acetate infusion a steady-state blood ammonia concentration of 500–800 μM was obtained in all PCS rats. This steady state was sustained for the next 4 h in the control rats (Fig. 1). Blood ammonia concentration in ORN- and OA-treated rats decreased gradually and significantly (*p*<0.0001) with a final decrease of 34% and 39%, respectively. Decreases in blood ammonia were associated with significantly higher urea production rates in both groups of treated rats than in control rats: 3.90 \pm 0.28 mmol/5 h in ORN-treated rats, 5.21 \pm 0.29 mmol/5 h in OA-treated rats vs. 2.80 \pm 0.08 mmol/5 h in controls (*p*<0.05). Ammonia concentration in brain homogenate at the end of the experiment was also significantly decreased after ORN and OA treatment: 1.43 \pm 0.12 $\mu\text{mol/g}$ wet weight in ORN-treated rats, 1.93 \pm 0.18 in OA-treated rats vs. 2.48 \pm 0.17 $\mu\text{mol/g}$ wet weight in control rats (*p*<0.05).

ORN and OA treatment also induced some signifi-

cant changes in several plasma amino acid concentrations (Table 2). As expected, plasma ornithine concentrations became significantly higher in both groups of treated rats than in controls, and aspartate was higher in OA-treated rats than in controls. A significant increase in glutamine and the Fischer ratio, which has a value of about 3.0 in healthy rats, also occurred in treated rats. There was a significant increase in plasma glutamate concentration in OA-treated rats. No significant differences in plasma

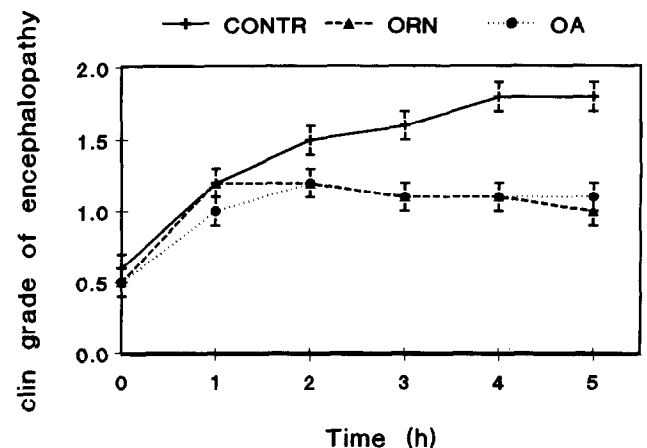


Fig. 2. The effect of L-ornithine (ORN, *n*=10) and L-ornithine-L-aspartate (OA, *n*=10) treatment on clinical grade of encephalopathy in AI-PCS rats. (Values are expressed as mean \pm SEM. Statistical analysis was performed by means of repeated measurement ANOVA. Clinical grade of encephalopathy was significantly higher in controls vs. ORN: *p*<0.0001, and vs. OA: *p*<0.001).

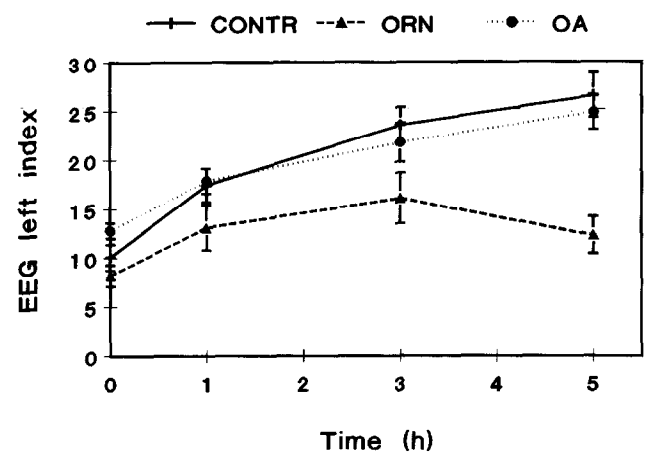


Fig. 3. The effect of L-ornithine (ORN, *n*=10) and L-ornithine-L-aspartate (OA, *n*=10) treatment on EEG left index in AI-PCS rats. (Values are expressed as mean \pm SEM. Statistical analysis was performed by means of repeated measurement ANOVA. EEG left index was significantly higher in controls vs. ORN: *p*<0.001).

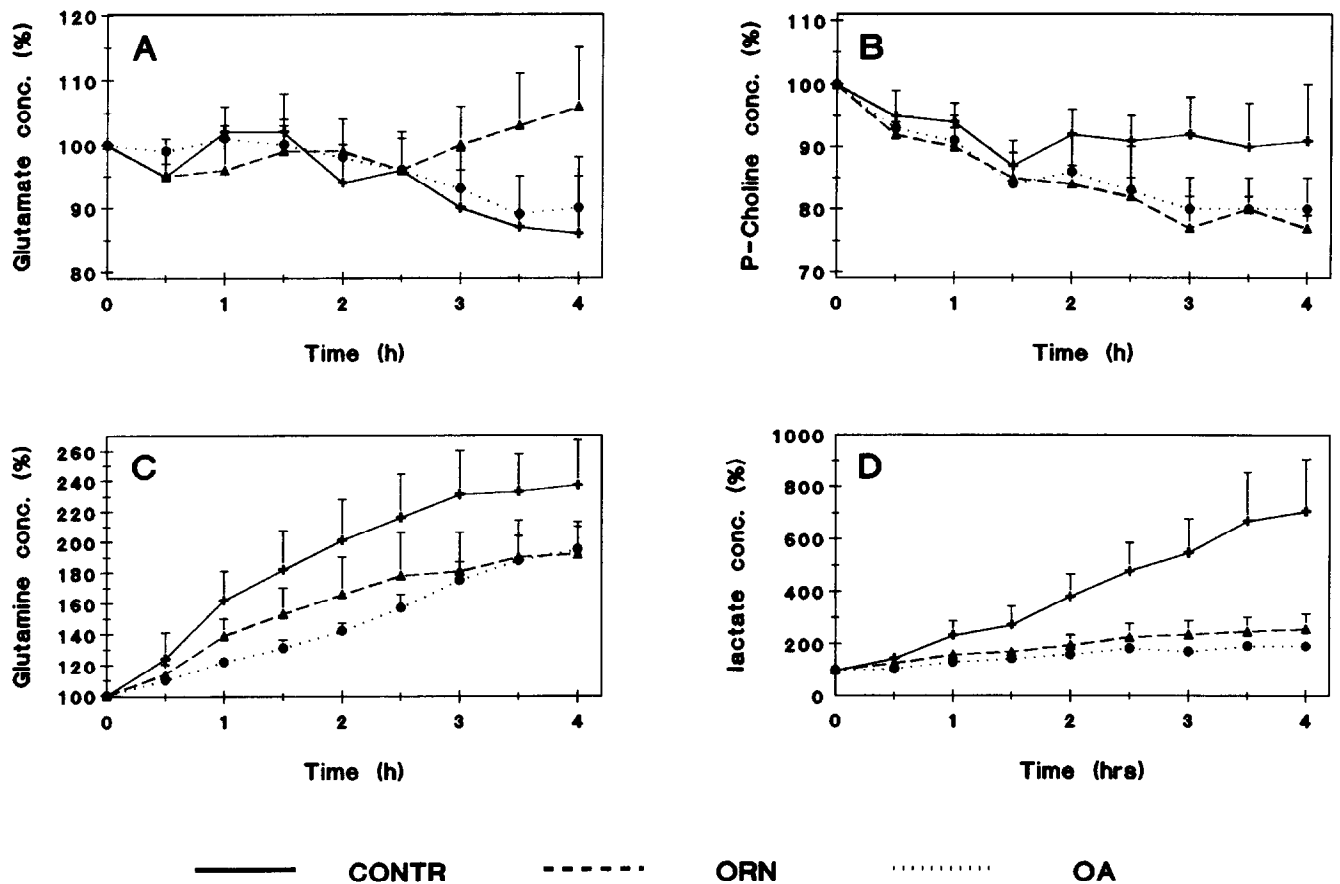


Fig. 4. The effect of L-ornithine (ORN, n=6) and L-ornithine-L-aspartate (OA, n=4) treatment on relative change in brain concentration of (a) glutamate, (b) phosphocholine compounds, (c) glutamine and (d) lactate in AI-PCS rats. The concentrations are measured by means of $^1\text{H-MRS}$ and are expressed as percentage of the concentration at $t=0$ h. (Values are expressed as mean \pm SEM. Statistical analysis was performed by means of repeated measurement ANOVA. Brain concentrations of glutamate and phosphocholine compounds were not significantly different between the groups. Brain glutamine concentrations were significantly higher in controls vs. ORN and vs. OA: $p<0.05$. Brain lactate concentrations were significantly higher in controls vs. ORN and vs. OA: $p<0.001$).

citrulline concentrations were observed between treated and control groups.

Quantification of the severity of encephalopathy showed that ORN treatment significantly reduces clinical grade of encephalopathy (Fig. 2, $p<0.0001$) and EEG left index (Fig. 3, $p<0.001$), indicating improvement of the severity of hyperammonemia-induced encephalopathy. OA treatment was associated with a significant decrease in clinical grade of encephalopathy (Fig. 2, $p<0.001$), but no significant improvement in the EEG left index.

Series 2 – Cerebral metabolite concentrations by in vivo $^1\text{H-MRS}$. In vivo $^1\text{H-MR}$ spectroscopy showed a tendency for brain glutamate concentration to decrease in control and OA-treated rats, whereas glutamate concentration in ORN-treated rats remained constant (Fig. 4a). No significant differences in brain glutamate concentration were observed between the treated and control groups. Cerebral cor-

tex concentration of NAA and phosphocreatine remained constant and no differences between control and treated rats were observed (data not shown). Phosphocholine concentration showed a decrease in all groups, but there was no significant difference between the groups (Fig. 4b). Glutamine and lactate concentrations in cerebral cortex showed a significantly smaller increase in ORN- and OA-treated rats than control rats (Fig. 4c, $p<0.05$ and Fig. 4d, $p<0.001$ respectively).

Series 3 – In vivo brain microdialysis. HPLC analysis of amino acids in brain dialysates showed significantly higher concentrations of ornithine in both groups of treated rats than in controls, whereas significantly increased concentrations of glutamate and aspartate only occurred in OA-treated rats (Table 3). In addition, the ORN-treated rats, just like OA-treated rats, showed a significant increase in the Fischer ratio. No significant differences in cerebral

extracellular glutamine concentration were observed between the groups. Apparently, the difference in increase in brain glutamine concentration between control and treated rats measured by *in vivo* $^1\text{H-MRS}$ (Fig. 4c) is due to an increase in intracellular glutamine concentration.

In the "osmolarity-control" experiments, in which PCS rats were infused with ammonium-acetate infusion plus Na-acetate instead of ornithine, data on blood ammonia concentration and severity of encephalopathy were similar to those in PCS rats infused with ammonium-acetate alone (data not shown). Direct measurement of the plasma osmolarity after infusion showed normal osmolarity values (305–309 mM).

Aspartate infusion in PCS rats with hyperammonemia did not result in a decrease in blood ammonia concentration: steady-state blood ammonia concentration, measured at the end of the experiment (expressed as a percentage of the concentration at $t=1$ h) was $105\pm 13\%$. In addition, severity of encephalopathy was not improved (data not shown), and moreover, *in vivo* brain dialysis showed increased extracellular concentrations of aspartate and glutamate in cerebral cortex (aspartate concentration at the end of the experiment: 22.7 ± 2.0 μM and glutamate concentration: 12.0 ± 1.4 μM).

Discussion

Increased cerebral ammonia concentration is widely considered to be a major factor in the pathogenesis of HE, associated with subacute and chronic liver disease (1–3). Decreasing hyperammonemia may positively affect the manifestations of HE, and many different therapeutic modalities, which may reduce hyperammonemia, have been applied to patients with HE for many decades. Such therapies have included lactulose, benzoic acid, ornithine and arginine (27,28). Their administration has been associated with variable improvement in the clinical manifestations of HE in patients with mild or chronic liver failure. Experimental studies have shown that increased ornithine concentrations in plasma and tissue, obtained by ornithine aminotransferase inhibition by 5-fluoromethylornithine, may be beneficial in (sub)acute liver failure (29).

A combined therapy of L-ornithine and L-aspartate has also been applied as a possible therapy of HE (17,18). The present data show that both ORN as well as OA treatment results in a significant decrease of blood ammonia concentration and a significant increase in urea production. The assumption is that increased plasma concentrations of ornithine will

stimulate urea synthesis in the liver, as has been shown in isolated perfused rat livers (30). Although the increase in urea production might also be explained by the supply of extra nitrogen by means of ORN or OA, we consider this less likely because a stimulation of urea production from externally supplied amino acids would not result in a lowering of plasma ammonia concentrations. In addition, ornithine and ammonia can be transformed in extrahepatic tissue into citrulline via ornithine transcarbamylase. However, we did not observe an increase in plasma citrulline concentrations after ORN or OA treatment, indicating that stimulation of urea synthesis was the main cause of decreasing plasma ammonia levels.

Theoretically, aspartate may also reduce blood ammonia levels, since it is the second nitrogen donor for urea synthesis and it can stimulate glutamine synthesis via increased glutamate synthesis by aspartate-aminotransferase. However, aspartate infusion in our experimental model did not result in a reduction of blood ammonia concentration. This finding is in agreement with a clinical study of Eriksson et al. (31), who showed that aspartate administration to patients with liver failure did not stimulate ammonia elimination. On the contrary, plasma glutamate and glutamine concentrations in the OA-treated group showed a significant increase, which may indicate that aspartate (at least partially) stimulated glutamate synthesis, resulting in an increased glutamine synthesis. Studies have suggested that glutamine-derived ammonia is directly channeled to carbamoylphosphate synthetase (32), a key enzyme of the urea synthesis. This phenomenon affords an explanation for the absence of a decrease in blood ammonia concentration after aspartate treatment and the larger increase in urea synthesis induced by OA compared to ORN treatment. So far, the significant reduction of blood ammonia concentration found in PCS rats with hyperammonemia-induced encephalopathy in this study can mainly be attributed to ornithine.

ORN and OA treatment resulted in an improvement in clinical grade, whereas ORN treatment alone resulted in an improvement in EEG activity. The discrepancy between clinical grade and EEG activity in the OA-treated group can partially be explained by the fact that EEG spectral analysis only reflects electro-activity of the cerebral cortex, whereas clinical evaluation reflects the complete behavior of the rat. Furthermore, clinical evaluation is a less objective method compared to EEG spectral analysis.

Of particular interest was whether the improvement in the severity of hyperammonemia-induced

encephalopathy after ORN and OA treatment was mainly a result of reduction in ammonia concentration in blood and brain. To address this issue, *in vivo* cerebral cortex ^1H -MRS was performed in order to study the total cerebral cortex concentrations of metabolites *in vivo* and in time. Alterations of the total concentration of these metabolites may reflect changes in brain metabolism. In addition, *in vivo* cerebral cortex dialysis was performed to monitor the extracellular cerebral cortex concentrations of amino acids involved in neurotransmission. Alterations in the extracellular concentration of these amino acids may represent changes in neurotransmitter activity.

In vivo ^1H -MRS showed that ORN and OA treatment resulted in a significantly smaller increase in glutamine and lactate concentration in cerebral cortex of the hyperammonemic rats. The increase in brain glutamine concentration in AI-PCS rats, which is generally seen in PCS rats with hyperammonemia (7,10,11), may be either due to an increased glutamine synthesis, inhibition of glutaminase activity or decreased efflux of glutamine from the brain to the blood. Since the glutamine efflux is increased in liver failure (33), glutamine transport is not a very likely explanation. Although it is known that the K_m value of brain glutamine synthase for ammonia is $180\ \mu\text{M}$ (6), the possibility remains that changes in concentrations of other substrates and/or cofactors of the glutamine synthesis are responsible for increased glutamine synthase activity. Inhibition of glutaminase by ammonia is another possible explanation for increased cerebral glutamine and decreased brain glutamate concentrations.

Because glutamine is a well known osmolyte, increased brain glutamine concentrations in hyperammonemia-induced encephalopathy may result in astrocyte swelling. Decreasing intracellular brain glutamine may thus have positive effects on brain edema, a major cause of death in patients with fulminant liver failure (8,9). The smaller increase of brain glutamine concentration in the ORN- and OA-treated rats can be explained by lower brain ammonia concentrations, resulting in less inhibition of glutaminase or a smaller increase in glutamine synthesis.

The increase in brain lactate concentration in PCS rats with hyperammonemia has also been seen in other studies (34,35). Possible explanations of this increase are inhibition of the malate-aspartate shuttle and/or inhibition of the tricarboxylic acid cycle in the brain by high ammonia concentrations. The reduced brain ammonia concentrations detected in ORN- and OA-treated rats may therefore explain the smaller increase in lactate concentration in these rats. Thus,

the changes in brain metabolism can all be explained by lower brain ammonia concentrations.

Both ORN as well as OA treatment resulted in a correction of the Fischer ratio in both brain dialysate and plasma. This observation is consistent with a role for the aromatic amino acids in the pathogenesis of encephalopathy (36). Another observation of interest was the large increase of glutamate and aspartate in brain dialysate, seen only after OA treatment. The presence of high extracellular concentrations of aspartate in the brain may inhibit the re-uptake of glutamate (37), resulting in high extracellular concentrations of glutamate. Increased concentrations of excitatory neurotransmitters may induce NMDA receptor-mediated glutamate neurotoxicity, with resultant cell swelling, neuron degeneration (38,39) and EEG changes. The absence of improvement in EEG activity after OA treatment and the results of aspartate infusion (no improvement in the severity of encephalopathy but an increase in cerebral extracellular glutamate and aspartate concentration) suggest a possible overstimulation of NMDA receptor activity by aspartate.

The increase in brain extracellular concentrations of aspartate and glutamate, amino acids that hardly cross the blood-brain barrier under normal conditions (40), may also be explained by changes in blood-brain barrier transport or integrity due to portacaval shunting and/or hyperammonemia. If this is the case, caution is necessary if aspartate (alone or in combination with ornithine) is used as therapy in patients with porto-systemic shunting.

In summary, this experimental study shows that intravenous administration of ORN and OA to PCS rats with hyperammonemia-induced encephalopathy stimulates urea production significantly, normalizes the Fischer ratio in plasma and brain extracellular fluid, and reduces the increase in brain glutamine and lactate concentration significantly. Since the metabolic changes in the brain induced in this model by ORN and OA can be related to the significant reduction in blood and brain ammonia concentration, the effects of ORN and OA treatment can be explained by the changes they induce in "extracerebral" nitrogen metabolism. In addition, ORN administration might be of therapeutic value in patients with hyperammonemia-induced encephalopathy if their urea synthesis capacity is sufficient.

It is suggested that co-administration of aspartate with ornithine may diminish the beneficial effect of ornithine as a result of high cerebral extracellular glutamate and aspartate concentrations, possibly by causing NMDA-mediated glutamate neurotoxicity.

Acknowledgements

We thank George Jörning kindly for his skillful HPLC analysis of amino acids, and we are very grateful to Dr. Fred Meijer and Dr. Anthony Jones for their critical reading of the manuscript.

References

- Butterworth RF, Giguère JF, Michaud J, Lavoie J, Pomier Layrargues G. Ammonia: Key factor in the pathogenesis of hepatic encephalopathy. *Neurochem Pathol* 1987; 6: 1–12.
- Ferenci P, Puspök A, Steindl P. Current concept in the pathophysiology of hepatic encephalopathy. *Eur J Clin Invest* 1992; 22: 573–81.
- Mousseau DD and Butterworth RF. Minireview. Current theories on the pathogenesis of hepatic encephalopathy. *PSEBM* 1994; 206: 329–44.
- Raabe W, Lin S. Pathophysiology of ammonia intoxication. *Exp Neurol* 1985; 87: 519–32.
- Cooper AJL, Plum F. Biochemistry and physiology of brain ammonia. *Physiol Rev* 1987; 67: 440–519.
- Meijer AJ, Lamers WH, Chamuleau RAFM. Nitrogen metabolism and ornithine cycle function. *Physiol Rev* 1990; 70: 701–48.
- Blei AT, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. *Hepatology* 1994; 19: 1437–44.
- Ware AJ, D'Agostino A, Combes B. Cerebral edema: A major complication of massive hepatic necrosis. *Gastroenterology* 1971; 61: 877–84.
- Ede RJ, Williams R. Hepatic encephalopathy and cerebral edema. *Semin Liver Dis* 1986; 6: 107–18.
- Jeppsson B, James JH, Edwards LL, Fischer JE. Relationship of brain glutamine and brain neutral amino acid concentrations after portacaval anastomosis in rats. *Eur J Clin Invest* 1985; 15: 179–87.
- Hawkins RA, Jessy J, Mans AM, De Joseph MR. Effect of reducing brain glutamine synthesis on metabolic symptoms of hepatic encephalopathy. *J Neurochem* 1993; 60: 1000–6.
- James JH, Ziparo V, Jeppsson B, Fischer JE. Hyperammonemia, plasma amino acid imbalance and blood-brain amino acid transport: A unified theory of portal-systemic encephalopathy. *Lancet* 1979; 2: 772–5.
- Mena EE, Cotman CW. Pathological concentrations of ammonium ions block L-glutamate uptake. *Exp Neurol* 1985; 89: 259–63.
- Greenstein JP, Winitz M, Gullino P, Birnbaum SM, Orey MC. Studies on the metabolism of amino acids and related compounds *in vivo*. III. Prevention of ammonia toxicity by arginine and related compounds. *Arch Biochem Biophys* 1956; 64: 342–54.
- Zieve L, Charboneau D, Lyftogt C. Studies of ammonia loading: Effects of rate of delivery and enhanced removal of NH₄ on blood levels of ammonia and coma induction. *Met Brain Dis* 1989; 4: 113–20.
- Nagao M, Mori T, Tsuchiyama A, Oyanagi K. The role of amino acids and their transport systems in the regulation of ureogenesis in the primary culture of adult rat hepatocytes. *Tohoku J Exp Med* 1989; 158: 309–16.
- Staedt U, Leweling H, Gladisch R, Kortsik C, Hagmuller E, Holm E. 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* 1993; 19: 424–30.
- Kircheis G, Quack G, Erbler H. L-ornithine-L-aspartate in the treatment of hyperammonemia and hepatic encephalopathy. In: Conn HO, Bircher J. *Hepatic Encephalopathy: Syndromes and Therapies*. East Lansing Michigan, 1993: 373–83.
- Lee SH, Fisher B. Portacaval shunt in the rat. *Surgery* 1961; 50: 668–72.
- Popken RJ, Kropveld D, Oosting J, Chamuleau RAFM. Qualitative analysis of EEG power density spectra in experimental hepatic encephalopathy. *Neuropsychobiology* 1983; 9: 235–43.
- Kropveld D, Chamuleau RAFM, Popken RJ, Smit JJH. A computerized EEG spectral analysis system for small laboratory animals. *Neuropsychobiology* 1983; 9: 159–62.
- Slotboom J, Vogels BAPM, de Haan JG, Creyghton JHN, Quack G, Chamuleau RAFM, Bovée WMMJ. Proton resonance spectroscopy study of the effects of L-ornithine-L-aspartate on the development of encephalopathy, using localization pulses with reduced specific absorption rate. *J Magn Resonance* 1994; 105: 147–56.
- Tossman U, Erikson S, Delin A, Hagenfeldt L, Law D, Ungerstedt U. Brain amino acids measured by intracerebral dialysis in portacaval shunted rats. *J Neurochem* 1983; 41: 1046–51.
- Bosman DK, Deutz NEP, Maas MAW, van Eijk HMH, Smit JJH, de Haan JG, Chamuleau RAFM. Amino acid release from cerebral cortex in experimental acute liver failure, studied by *in vivo* cerebral cortex microdialysis. *J Neurochem* 1992; 59: 591–9.
- van Eijk HMH, van der Heyden MAH, van Berlo CLH, Soeters PB. Fully automated liquid chromatographic determination of amino acids. *Clin Chem* 1988; 34: 2510–3.
- Huizenga JR, Gips CH. Determination of blood ammonia using Ammonia Checker. *Ann Clin Biochem* 1983; 20: 187–9.
- Conn HO, Leevy CM, Vlahcevic ZR, Rodgers JB, Maddrey WC, Seeff L, Levy LL. A comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy: a double-blind controlled trial. *Gastroenterology* 1977; 72: 573–83.
- Sushma S, Dasarathy S, Tandon RK, Jain S, Gupta S, Bhist MS. Sodium benzoate in the treatment of acute hepatic encephalopathy: a double blind randomized trial. *Hepatology* 1992; 138–44.
- Seiler N, Sarhan S, Knödgen B. Inhibition of ornithine aminotransferase by 5-fluoromethylornithine. Protection against acute thioacetamide intoxication by elevated tissue ornithine levels. *Pharmacol Toxicol* 1992; 70: 373–80.
- Grunst J, Löffler I, Teilken M, Holl J. Einfluss von Ornithin und Alpha-ketoglutarat auf die Harnstoffbildung aus Ammonia bei normalen und urämischen Rattenlebern. In: Wewlka F, Dragosics B, eds. *Aminosäuren, Ammoniak und Hepatische Enzephalopathie*. Stuttgart: Fischer, 1978; 225–34.
- Eriksson LS. Administration of aspartate to patients with liver cirrhosis. *Clin Nutr Suppl* 1985; 4: 88–96.
- Meijer AJ. Channeling of ammonia from glutaminase to carbamoyl-phosphate synthetase in liver mitochondria. *FEBS Lett* 1985; 191: 249–51.
- de Jong CHC, Deutz NEP, Soeters PB. Cerebral cortex am-

- monia and glutamine metabolism in two rat models of chronic liver insufficiency-induced hyperammonemia: influence of pair-feeding. *J Neurochem* 1993; 60: 1047–57.
34. Hindfeldt B, SiesjöBK. Cerebral effects of acute ammonia intoxication. I. The influence on intracellular and extracellular acid-base parameters. *Scand J Clin Lab Invest* 1971; 28: 353–64.
 35. Therrien G, Giguère JF, Butterworth RF. Increased cerebrospinal fluid lactate reflects deterioration of neurological status in experimental portal-systemic encephalopathy. *Metab Brain Dis* 1991; 6: 225–31.
 36. Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 1976; 80: 77–90.
 37. Balcar VJ, Johnston GAR. The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *J Neurochem* 1972; 19: 2657–66.
 38. Choi DW. Glutamate toxicity in cortical cell culture is calcium-dependent. *Neurosci Lett* 1985; 58: 293–7.
 39. Rothman SM, Olney JW. Excitotoxicity and the NMDA receptor. *Trends Neurosci* 1987; 10: 299–302.
 40. Price MT, Pusateri ME, Crow SE, Buchsbaum S, Olney JW, Lowry OH. Uptake of exogenous aspartate into circumventricular organs but not other regions of adult mouse brain. *J Neurochem* 1984; 42: 740–4.