Hyperammonaemia causes many of the changes found after portacaval shunting

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1. Portacaval shunting in rats results in several metabolic alterations similar to those seen in patients with hepatic encephalopathy. The characteristic changes include: (a) diminution of cerebral function; (b) raised plasma ammonia and brain glutamine levels; (c) increased neutral amino acid transport across the blood–brain barrier; (d) altered brain and plasma amino acid levels; and (e) changes in brain neurotransmitter content. The aetiology of these abnormalities remains unknown. 2. To study the degree to which ammonia could be responsible, rats were made hyperammonaemic by administering 40 units of urease/kg body weight every 12 h and killing the rats 48 h after the first injection. 3. The changes observed in the urease-treated rats were: (a) whole-brain glucose use was significantly depressed, whereas the levels of high-energy phosphates remained unchanged; (b) the permeability of the blood–brain barrier to two large neutral amino acids, tryptophan and leucine, was increased; (c) blood–brain barrier integrity was maintained, as indicated by the unchanged permeability-to-surface-area product for acetate; (d) plasma and brain amino acid concentrations were altered; and (e) dopamine, 5-hydroxytryptamine (serotonin) and noradrenaline levels in brain were unchanged, but 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-hydroxytryptamine, was elevated. 4. The depressed brain glucose use, increased tryptophan permeability-to-surface-area product, elevated brain tryptophan content and rise in the level of cerebral 5-HIAA were closely correlated with the observed rise in brain glutamine content. 5. These results suggest that many of the metabolic alterations seen in rats with portacaval shunts could be due to elevated ammonia levels. Furthermore, the synthesis or accumulation of glutamine may be closely linked to cerebral dysfunction in hyperammonaemia.

INTRODUCTION

Hepatic encephalopathy is a disturbance of brain function that may accompany hepatic insufficiency or portacaval shunting (Hoyumpa *et al.*, 1979; Zieve, 1979; Misra, 1981). Its symptoms are reversible, suggesting that the encephalopathy has a metabolic aetiology. The mechanism of hepatic encephalopathy remains unknown, in spite of much information now being available about associated metabolic abnormalities. Rats with portacaval shunts are a widely used model of latent encephalopathy, with abnormalities similar to those found in human patients (Flynn & Kennan, 1968; Herz *et al.*, 1972a,b; Bircher, 1979). The degree of encephalopathy is reflected by a measurable decrease in the rate of the glucose use (the principal source of energy) throughout the brain (Mans *et al.*, 1983a, 1986; Hawkins & Mans, 1989). Various other characteristic changes are present that may be of aetiological importance. They include: (1) an increase in plasma and brain ammonia (Mans *et al.*, 1984); (2) a pronounced change in the plasma amino acid spectrum, with decreases in levels of the branched-chain amino acids and threonine and increases in the aromatic amino acids (James *et al.*, 1978; Mans *et al.*, 1982); (3) a large rise in the brain content of aromatic amino acids and glutamine (James *et al.*, 1978; Mans *et al.*, 1984); (4) increased permeability of the blood–brain barrier to neutral amino acids and decreased permeability to basic amino acids and monocarboxylic acids (James *et al.*, 1978; Sarna *et al.*, 1979; Hawkins *et al.*, 1981; Mans *et al.*, 1982); and (5) increased brain content of 5-hydroxytryptamine (5-HT; serotonin) [and its metabolite, 5-hydroxyindoleacetic acid [(5-HIAA)] and noradrenaline, but unchanged dopamine levels (Mans & Hawkins, 1986).

The relationship, if any, between these alterations and encephalopathy remains unclear. The onset of hepatic encephalopathy occurs very shortly after portacaval shunting (Mans *et al.*, 1990). Brain energy metabolism begins its downward course within hours and is maximally depressed within 1–2 days (Mans *et al.*, 1990). Most of the other abnormalities described above were also established by 1 day, with the exception of the decrease in plasma levels of branched-chain amino acids and threonine, and the increase in noradrenaline, which took 2 days. The latter changes are, therefore, less likely to be aetiologically important.

One of the primary factors that may be responsible for cerebral dysfunction in liver insufficiency is the increase in circulating ammonia levels (Plum & Hindfelt, 1986). However, definitive studies that examine the degree of encephalopathy, measured quantitatively, as a function of ammonia level, have not been done. The objective of the following experiments was to determine whether, and to what extent, hyperammonaemia alone could be responsible for the decrease in cerebral function as well as the various other abnormalities associated with hepatic encephalopathy. To accomplish this, a rat model was used in which the circulating concentrations of ammonia were raised, and maintained for a period of 48 h by intraperitoneal injections of urease, to values similar to those found after portacaval shunting. Urease injections have been shown by several investigators to induce hyperammonaemia in rats, leading to Alzheimer Type II astrocytosis, similar to that seen in portacaval-shunted animals (Prior & Vissek, 1972; Gibson *et al.*, 1974; Diemer & Laursen, 1977; Bachmann & Colombo, 1983; Batshaw *et al.*, 1986).

Abbreviations used: 5-HT, 5-hydroxytryptamine (serotonin); 5-HIAA, 5-hydroxyindoleacetic acid.

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MATERIALS AND METHODS

Materials

Crystalline jack-bean urease (catalogue no. U-0251) and glutaminase were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. The other enzymes and cofactors were bought from Boehringer Mannheim, G.m.b.H. Biochemica, Mannheim, Germany. L-[5-³H]Tryptophan (28.9 Ci/mmol), D-[6-¹⁴C]glucose (55 mCi/mmol), [1-¹⁴C]acetate (45–60 Ci/mmol) and L-[³H]leucine (135 Ci/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. All other reagents used were of the best available grade.

Rats

Adult male Long-Evans rats were bought from Charles River Laboratories Inc., Wilmington, MA, U.S.A. They were kept in a controlled environment (20 °C, 12 h dark/12 h light) with free access to food and water. The rats weighed 200–300 g at the time of the experiment. The changes in body wt. of the control and experimental rats during the urease treatment were recorded. Immediately after the rats were decapitated the livers were excised and weighed and the liver-to-body wt. ratio was calculated.

Assays and tissue samples

Total (bound plus unbound) tryptophan was estimated separately from the other amino acids by the sensitive fluorimetric method of Eccleston (1973). Other amino acids were measured after h.p.l.c. by the Pico-Tag method. The amino acids were treated with phenylisothiocyanate, as described by the Waters Company (Milford, MA, U.S.A.). Monoamines were determined using h.p.l.c. with electrochemical detection. The brain samples were extracted in HClO₄ containing 2% cysteine and were assayed on the same day. The conditions were as described by Mans *et al.* (1987), except that the pH of the mobile phase was 4.5. Plasma ammonia, brain glutamine and all the other metabolites were assayed enzymically (Bergmeyer, 1974). Plasma was extracted with 0.5 M-HClO₄ and the brain with 1.2 M-HClO₄. The brain and plasma extracts were neutralized with 20% (w/v) KOH in 0.1 M-K₂HPO₄. The brain-blowing technique was used for the determination of intermediary metabolites and high-energy phosphates, because it inactivates brain enzymes within 0.5 s and prevents post-mortem changes in the metabolites estimated (Veech & Hawkins, 1974). The frozen brains were immediately transferred into liquid N₂ and stored at –80 °C until extracted (Veech & Hawkins, 1974).

Experimental design

Four separate experiments were carried out: (1) determination of optimal dose of urease; (2) measurement of glucose use, permeability-to-surface-area product of tryptophan and leucine across the blood–brain barrier and brain monoamine levels; (3) assessment of blood–brain barrier integrity; and (4) determination of various other metabolite concentrations in plasma and brain.

1. Determination of dose of urease and time of experiment

(a) **Determination of optimal dose of urease.** Rats were separated into six groups. One group (control) was given 0.15 M-NaCl solution; the other groups were given 10, 20, 40, 60 or 80 units of urease/kg body wt. intraperitoneally every 12 h and were killed 48 h after the first injection, i.e. each rat received four injections. (One unit of urease produces 1 mg of ammonia nitrogen from urea in 5 min at pH 7 and 30 °C.) There were at least three rats in each group. After decapitation, a blood sample was obtained from the cervical wound. The plasma was separated

by centrifugation. Plasma and brain were extracted and the levels of plasma ammonia and brain glutamine were determined as described above.

(b) **Determination of time course of experiment.** Rats were given the optimum dose of urease (40 units/kg body wt. every 12 h) and decapitated at 0, 12, 24, 36, 48 or 60 h after the first injection of urease. A minimum of 4 rats was used for each time point. Plasma ammonia and brain glutamine were determined as described above.

2. Measurement of glucose use, tryptophan permeability-to-surface-area product and monoamine levels

Experimental rats were given intraperitoneal injections of urease (40 units/kg body wt.) every 12 h, and the controls received equivalent volumes of 0.15 M-NaCl. Catheters were introduced into the femoral artery and vein while the rats were anaesthetized with halothane [1.5–2% halothane in N₂O/O₂ (3:1)], 47 h after the first injection of urease. After a recovery period of 1 h, heparin (200 units) was injected intravenously, an arterial blood sample was withdrawn and the plasma was separated. An injection containing 5 μ Ci of [6-¹⁴C]glucose and 125 μ Ci of [³H]tryptophan/300 g body wt. was given intravenously and washed in with 0.2 ml of 0.15 M-NaCl. (The isotopes were dissolved in fresh plasma obtained from a normal rat, as tryptophan binds to plasma proteins.) Blood samples (100 μ l) were taken at 0.5, 1, 2, 3 and 4.5 min after the isotope injection and kept on ice. An additional blood sample (100 μ l) was taken at each time point and immediately centrifuged to obtain plasma for tryptophan estimation. The rats were killed at 5 min with a lethal dose of pentobarbital. The brains were quickly removed, divided into the two hemispheres and stored at –80 °C until extraction.

Glucose and ¹⁴C were determined in the blood samples and ³H and total tryptophan in the plasma samples obtained at each time point. Ammonia, glucose, tryptophan and amino acids were measured in the plasma sample taken before isotope administration. One brain hemisphere was extracted and ¹⁴C and ³H d.p.m. were determined in a sample of the neutralized supernatant (the net ¹⁴C and ³H d.p.m. in brain were obtained by correcting for 1% blood contamination). Glutamate, glutamine and tryptophan concentrations were measured in the remaining brain extract. The other brain hemisphere was used for the measurement of monoamines. Glucose use was calculated for whole brain as described by Hawkins *et al.* (1985). The brain/plasma glucose ratio, which is necessary for computing the exchange constants (k_2 and k_3) for individual rats, was increased by hyperammonaemia and was correlated with brain glutamine according to the equation: ratio = 0.01 × [glutamine] + 0.17. Therefore this relationship was used to calculate the ratio after urease treatment. For control rats a ratio of 0.22 was used (Hawkins & Mans, 1989). The permeability-to-surface-area product of tryptophan into brain was calculated according to Mans *et al.* (1982).

3. Blood–brain barrier integrity

Hyperammonaemic and control rats were prepared as for experiment 2. A bolus of 25 μ Ci of [1-¹⁴C]acetate and 100 μ Ci of [³H]leucine was injected into the femoral vein and a continuous blood sample was drawn into a pre-cooled syringe, kept on ice. At the end of 2 min the rats were given a cardioplegic dose of pentobarbital and decapitated, and a blood sample was taken from the cervical wound. The brains were removed and separated into the following regions: cortex (frontal and parietal), cerebellum, pons, striatum and thalamus. The radioactivity in the blood and brain samples (dissolved in tissue solubilizer and

subsequently neutralized with acetic acid) was determined. The plasma sample obtained before the bolus injection was used for amino acid and ammonia analysis. Glutamine was measured in the remainder of the brain. The permeability-to-surface-area products for leucine and acetate were calculated as in experiment 2 (Mans *et al.*, 1982).

4. Determination of metabolites in plasma and brain

Urease-treated and control rats were prepared as described for experiment 2. Ketamine (20 mg/kg body wt.) was administered intravenously and the rats were decerebrated by the brain blower within 1 min of the ketamine injection. This dose of ketamine, given for humane reasons, has a minimal affect on brain energy metabolism (Davis *et al.*, 1988). Ammonia, glucose and β -hydroxybutyrate were measured in plasma, and ammonia, glucose, glucose 6-phosphate, pyruvate, lactate, citrate, α -oxoglutarate, malate, ATP, ADP, AMP, phosphocreatine, glutamate and glutamine were estimated in the brain. All assays were carried out on the same day except those for citrate, glutamate, glutamine and β -hydroxybutyrate, which were performed within 1 week.

Statistical analysis

The mean values for the experimental rats were compared with those for the control rats with Student's *t* test. A *P* value of 0.05 or lower was taken to indicate a significant difference.

RESULTS

Establishment of sustained hyperammonaemia

The effect of urease on plasma ammonia and brain glutamine was studied over the range of 10–80 units of urease/kg body wt. injected every 12 h during a 48 h period. As expected, levels of both plasma ammonia and brain glutamine increased in a dose-dependent manner (Table 1) with considerable individual variation. At 40 units/kg, some rats (15%) had seizures and died; higher doses had unacceptably high mortality rates. The brain glutamine values in rats given 40 units/kg were similar to those found in portacaval-shunted rats, although the plasma ammonia concentrations were rather higher. [In portacaval-shunted rats the brain glutamine content and plasma ammonia levels range from 17 to 26 μ mol/g and from 0.24 to 0.46 mM respectively (Mans *et al.*, 1984).] Because the brain glutamine content was considered to be a reliable index of brain ammonia concentration, 40 units of urease/kg body wt. was taken as the optimum dose for further experiments. The levels of plasma ammonia and brain glutamine were examined over 60 h by injecting 40 units of

Table 1. Plasma ammonia and brain glutamine levels after different doses of urease

Values are expressed as means $\pm$ S.E.M., with the numbers of determinations in parentheses. Values from experiments 2 and 4 are included in the 0 and 40 units/kg values. For experimental details, see the Materials and methods section. ***P* < 0.01 compared with 0 units/kg.

Urease (units/kg)	Plasma ammonia (μ mol/ml)	Brain glutamine (μ mol/g)	Survivors (%)
0	0.12 $\pm$ 0.01 (27)	5.80 $\pm$ 0.38 (27)	100
10	0.26 $\pm$ 0.01** (3)	7.00 $\pm$ 0.66 (3)	100
20	0.82 $\pm$ 0.07** (3)	11.00 $\pm$ 4.35** (3)	100
40	1.00 $\pm$ 0.06** (23)	20.00 $\pm$ 1.39** (23)	85
80	1.51 $\pm$ 0.01** (2)	18.00 $\pm$ 1.75** (2)	25

Table 2. Plasma ammonia and brain glutamine levels at different times after urease treatment

Values are expressed as means $\pm$ S.E.M., with the numbers of determinations in parentheses. Plasma ammonia and brain glutamine were estimated at different times after the administration of 40 units of urease/kg body wt. every 12 h. Rats were decapitated 12 h after the last injection. Values from experiments 2 and 4 are included in the 0 and 48 h values. **P* < 0.05, compared with value at 0 h.

Time (h)	Plasma ammonia (μ mol/ml)	Brain glutamine (μ mol/g)
0	0.12 $\pm$ 0.01 (27)	5.8 $\pm$ 0.40 (27)
12	0.54 $\pm$ 0.03* (5)	17.0 $\pm$ 1.9* (5)
24	0.60 $\pm$ 0.07* (5)	10.4 $\pm$ 2.1* (5)
36	1.10 $\pm$ 0.07* (5)	17.6 $\pm$ 1.2* (5)
48	1.00 $\pm$ 0.06* (23)	20.0 $\pm$ 1.4* (23)
60	0.81 $\pm$ 0.07* (4)	23.0 $\pm$ 2.0* (4)

urease/kg intraperitoneally every 12 h (Table 2). Brain glutamine content rose to near-maximal values by 12 h, and plasma ammonia levels reached a plateau after 24 h. There was a correlation between plasma ammonia and brain glutamine levels; however, there was considerable individual variation (Fig. 1). In experiments on portacaval-shunted rats, it was found that most of the characteristic changes were established within the first 2 days (Mans *et al.*, 1990). Therefore this period of hyperammonaemia was chosen for study. Hereinafter, urease treatment refers to hyperammonaemia caused by 40 units of urease/kg body wt. injected intraperitoneally every 12 h with the experiment being conducted at 48 h, i.e. 12 h after the last urease injection.

A decrease in the body wt. of urease-treated rats was observed compared with the controls. The weight change was variable, ranging from +4% to –15%, and was inversely correlated with the brain glutamine content (results not shown). The liver in the experimental rats had a nutmeg appearance that may have been due to glycogen loss. The liver/body wt. ratio was not changed

Fig. 1. Relationship between plasma ammonia and brain glutamine levels in urease-treated rats

The correlation is shown between plasma ammonia and brain glutamine in control rats (○) and rats treated with 40 units of urease/kg body wt. every 12 h and killed 48 h after the first administration (●), i.e. 12 h after the last dose. The two variables are correlated (*P* < 0.01); *r*² = 0.71.

Table 3. Effect of hyperammonaemia on metabolites in plasma and brain

Values are expressed as means $\pm$ S.E.M., with the numbers of determinations in parentheses. %Diff., percentage difference compared with control values. Only statistically significant differences are shown. Brains were removed by pneumatic decerebration and frozen within 0.5 s. For further details see the Materials and methods section. * $P < 0.05$; ** $P < 0.01$ compared with control values.

Metabolite	Control	Urease-treated	% Diff.
Plasma ($\mu\text{mol/ml}$):			
Ammonia	0.12 ± 0.01 (27)	1.00 ± 0.06 (23)	+733**
Glucose	8.73 ± 1.67 (16)	9.47 ± 3.00 (20)	
β -Hydroxybutyrate	0.18 ± 0.01 (7)	0.17 ± 0.01 (7)	
Brain ($\mu\text{mol/g}$):			
Ammonia	0.35 ± 0.03 (7)	0.96 ± 0.06 (7)	+174**
Glutamine	5.78 ± 0.38 (27)	19.98 ± 1.39 (23)	+208**
Glucose	2.19 ± 0.11 (6)	3.33 ± 0.32 (7)	+52**
Glucose 6-phosphate	0.13 ± 0.01 (7)	0.20 ± 0.06 (7)	+54*
Pyruvate	0.08 ± 0.01 (7)	0.15 ± 0.02 (7)	+88 **
Lactate	0.98 ± 0.05 (7)	1.43 ± 0.17 (7)	+46*
Citrate	0.45 ± 0.03 (7)	0.42 ± 0.04 (7)	
α -Oxoglutarate	0.20 ± 0.01 (7)	0.13 ± 0.01 (6)	-35**
Glutamate	8.50 ± 2.80 (7)	10.57 ± 2.37 (7)	
Malate	0.35 ± 0.05 (7)	0.32 ± 0.04 (7)	
ATP	2.77 ± 0.08 (7)	2.93 ± 0.07 (7)	
ADP	0.61 ± 0.02 (7)	0.60 ± 0.03 (7)	
AMP	0.05 ± 0.01 (7)	0.04 ± 0.002 (7)	
Phosphocreatine	4.28 ± 0.12 (7)	4.53 ± 0.27 (7)	

by urease treatment (control 4.0 ± 0.1 % mean $\pm$ S.E.M., $n = 19$; urease-treated 3.8 ± 0.1 %; $n = 20$).

Plasma and brain metabolites

The circulating plasma glucose and β -hydroxybutyrate concentrations were unaltered by urease treatment (Table 3). Thus, although most urease-treated rats lost weight, the plasma glucose and β -hydroxybutyrate concentrations gave no evidence of malnutrition at the time of the experiment. The brain ammonia content of urease-treated rats was very similar to that found in portacaval-shunted rats. Urease treatment raised the brain content of glucose, glucose 6-phosphate, pyruvate and lactate, whereas the α -oxoglutarate concentration was decreased. The increased content of glucose was most probably a consequence of decreased glucose use (see below). The cause and importance, if any, of the other changes was not clear. In any event, there was

Table 4. Hyperammonaemia decreases brain glucose use and increases tryptophan permeability-to-surface-area product

Values are expressed as means $\pm$ S.E.M., with the numbers of determinations in parentheses. Both variables were measured in the same rat. %Diff., percentage difference compared with control values. Tryptophan transport is expressed as the permeability-to-surface area product. For details of experiment, see the Materials and methods section. * $P < 0.05$; ** $P < 0.01$ compared with control values

	Control (10)	Urease-treated (14)	% Diff.
Glucose use ($\mu\text{mol/min per g}$)	0.75 ± 0.04	0.41 ± 0.05	-45*
Tryptophan transport ($\mu\text{l/min per g}$)	11.4 ± 0.8	31.6 ± 2.6	+177**

Fig. 2. Brain glucose use is decreased as cerebral glutamine rises

The correlation is shown between brain glutamine and brain glucose use in control (○) and urease-treated (●) rats. s, stuporous. For further details, see the legend to Fig. 1. A significant inverse correlation ($P < 0.01$) is present between the two variables; $r^2 = 0.73$.

no evidence of an energy lack, since the brain content of high-energy phosphates (ATP, ADP, AMP and phosphocreatine) was unchanged.

Glucose use

On average, whole brain glucose use was depressed by 45 % (Table 4), but there was considerable variability. However, when glucose use was plotted against brain glutamine a significant negative correlation was revealed ($r^2 = 0.73$; Fig. 2). There was also a significant correlation between glucose use and the plasma ammonia concentration, but it was not as close ($r^2 = 0.354$).

Neutral-amino-acid transport

The permeability of the blood-brain barrier to two neutral amino acids, tryptophan and leucine, was measured in separate

Table 5. Effect of hyperammonaemia on leucine and acetate permeability-to-surface-area product

The permeability-to-surface-area product for leucine and acetate was measured using [^3H]leucine and [^{14}C]acetate simultaneously in the same rat. For details, see the Materials and methods section. Values are expressed as means $\pm$ S.E.M. There were six control and nine experimental rats. %Diff., percentage difference compared with control values. Only the statistically significant differences are shown. * $P < 0.05$ compared with control.

Brain region	Permeability-to-surface area product ($\mu\text{l/min per g}$)				
	Leucine			Acetate	
	Control	Treated	% Diff.	Control	Treated
Cortex					
frontal	55 ± 3	108 ± 13	96*	43 ± 2	45 ± 2
parietal	51 ± 2	97 ± 10	90*	41 ± 3	42 ± 2
Thalamus	56 ± 3	95 ± 9	70*	40 ± 4	43 ± 3
Striatum	47 ± 2	97 ± 14	106*	35 ± 2	41 ± 2
Cerebellum	52 ± 3	108 ± 9	108*	40 ± 4	49 ± 2
Pons	48 ± 5	103 ± 11	115*	31 ± 3	43 ± 5

Fig. 3. Tryptophan transport (a), brain tryptophan (b) and 5-HIAA content (c) are correlated with brain glutamine content

○, Control; ●, urease-treated rats. For further details, see Fig. 1. The three variables were significantly correlated with brain glutamine content ($P < 0.01$). (a) $r^2 = 0.64$; (b) $r^2 = 0.64$; (c) $r^2 = 0.57$.

experiments. The permeability-to-surface-area product for tryptophan was increased by 177% (Table 4), while that of leucine was increased by about 100% in each of the six structures studied (Table 5). Transport of neutral amino acids is dependent on the activity of the neutral-amino-acid carrier, as well as on the combined concentrations of the other neutral amino acids that compete for the same carrier. The effect of competition was calculated by taking the sum of the ratios of the concentration of each competing amino acid to its affinity constant for the carrier (see Smith *et al.*, 1987, for details of the calculation). Although some changes in plasma neutral amino acid concentrations were found, the competitor function was nearly identical in both groups. The data showed that competition for tryptophan or leucine transport was not decreased by more than 5% by urease treatment. There was a correlation between tryptophan transport and brain glutamine content (Fig. 3a). A similar correlation

was found for leucine transport (results not shown; $r^2 = 0.36$, $P < 0.01$).

Blood-brain barrier integrity

The increased transport of neutral amino acids into the brain could have been explained if there were a breakdown of the blood-brain barrier. This was examined by measuring the permeability of the blood-brain barrier to acetate, which is transported by the monocarboxylic acid carrier. This carrier has been shown to be unchanged during the first week after portacaval shunting, although after this time its activity may decrease (Sarna *et al.*, 1979; Hawkins *et al.*, 1981). Breakdown of the blood-brain barrier would result in accumulation of label from acetate in brain and an apparent increase in the permeability-to-surface-area product. No indication of an increase was found in the hyperammonaemic rats (Table 5), indicating that blood-brain barrier integrity was maintained.

Plasma and brain amino acids

The plasma amino acid spectrum was altered by urease treatment as follows. The branched-chain amino acids (leucine, isoleucine and valine), threonine, ornithine and proline were depressed, while glutamine was elevated (Table 6). The concentrations of the other amino acids, including the aromatic amino acids, were unchanged. In contrast, the aromatic amino acids were elevated in brain, whereas levels of the other neutral amino acids, with the exception of glutamine, were not different in the treated and control rats. The brain content of tryptophan (Fig. 3), tyrosine ($r^2 = 0.59$), phenylalanine ($r^2 = 0.72$) and histidine ($r^2 = 0.62$) were significantly correlated with brain glutamine levels.

Brain monoamine neurotransmitters

Noradrenaline, dopamine and 5-HT levels were measured and found to be unchanged in urease-treated rats (results not shown). The brain content of 5-HIAA, the metabolite of 5-HT, was elevated by 73% [control 1.50 ± 0.12 nmol/g (8); urease-treated 2.60 ± 0.24 nmol/g (11); means $\pm$ S.E.M. (n); $P < 0.01$], suggesting the possibility of increased 5-HT turnover. The 5-HIAA level was correlated with brain glutamine content (Fig. 3).

DISCUSSION

The purpose of the present study was to evaluate the consequence of sustained hyperammonaemia in an otherwise healthy animal and to compare the observations with those made on portacaval-shunted rats, a widely accepted model of chronic hepatic encephalopathy, during the same period. The time period studied, 48 h, was chosen because most of the changes characteristic of hepatic encephalopathy caused by portacaval shunting are demonstrable within that time (Mans *et al.*, 1990).

In the present study, urease-treated rats had elevated plasma ammonia, with brain ammonia and brain glutamine levels comparable with those seen in portacaval-shunted rats (Mans *et al.*, 1984) and they showed an almost identical pattern of metabolic changes to that observed in portacaval-shunted rats during the first 48 h (Table 7).

Energy consumption and cerebral dysfunction

Humans with hepatic encephalopathy have decreased rates of energy metabolism that are commensurate with diminished cerebral function (Hawkins & Mans, 1989). Rats with chronic encephalopathy due to portacaval shunting show, like humans, a substantial decrease in energy metabolism throughout the brain, as indicated by decreased glucose and oxygen consumption (Eklöf *et al.*, 1974; Nieto *et al.*, 1980; Mans *et al.*, 1983a, 1986).

Table 6. Plasma and brain amino acids in hyperammonaemia

Values are expressed as means $\pm$ S.E.M., with the numbers of determinations in parentheses. For experimental details, see, the Materials and methods section. * $P < 0.05$ compared with control.

	Amino acid (nmol/ml)			
	Plasma		Brain	
	Control	Urease-treated	Control	Urease-treated
Tryptophan	193 $\pm$ 6 (7)	202 $\pm$ 8 (10)	26 $\pm$ 1 (14)	49 $\pm$ 2* (14)
Tyrosine	73 $\pm$ 6 (7)	59 $\pm$ 10 (10)	66 $\pm$ 5 (14)	146 $\pm$ 13* (14)
Phenylalanine	58 $\pm$ 3 (7)	61 $\pm$ 3 (10)	41 $\pm$ 3 (14)	135 $\pm$ 20* (13)
Histidine	50 $\pm$ 2 (7)	48 $\pm$ 1 (10)	40 $\pm$ 2 (10)	125 $\pm$ 19* (13)
Leucine	169 $\pm$ 9 (7)	124 $\pm$ 13* (10)	65 $\pm$ 5 (13)	83 $\pm$ 12 (13)
Isoleucine	99 $\pm$ 5 (7)	77 $\pm$ 8* (10)	36 $\pm$ 5 (13)	51 $\pm$ 7 (13)
Valine	206 $\pm$ 10 (7)	162 $\pm$ 15* (10)	88 $\pm$ 5 (14)	105 $\pm$ 11 (14)
Threonine	282 $\pm$ 3 (7)	214 $\pm$ 21* (10)	904 $\pm$ 152 (14)	965 $\pm$ 155 (14)
Methionine	75 $\pm$ 7 (7)	60 $\pm$ 3 (10)	82 $\pm$ 11 (14)	100 $\pm$ 10 (14)
Ornithine	56 $\pm$ 3 (7)	42 $\pm$ 3* (10)	14 $\pm$ 1 (14)	21 $\pm$ 3* (13)
Glutamine	555 $\pm$ 30 (7)	801 $\pm$ 67* (10)	3820 $\pm$ 216 (14)	13400 $\pm$ 1510* (14)
Proline	206 $\pm$ 9 (7)	132 $\pm$ 16* (10)	59 $\pm$ 5 (14)	94 $\pm$ 16* (14)
Alanine	342 $\pm$ 20 (7)	135 $\pm$ 62* (10)	705 $\pm$ 79 (13)	740 $\pm$ 103 (14)
Glutamate	74 $\pm$ 4 (7)	62 $\pm$ 5 (10)	9720 $\pm$ 517 (10)	9140 $\pm$ 978 (14)
γ -Aminobutyric acid	—	—	3860 $\pm$ 415 (14)	3700 $\pm$ 471 (14)

Table 7. Comparison of the metabolic changes during 48 h of hyperammonaemia and 48 h of portacaval shunting

Observations on the portacaval shunts were taken from Mans *et al.* (1990). $\uparrow$, Elevated; $\downarrow$, decreased; =, normal.

	Portacaval shunt (48 h)	Hyperammonaemia (48 h)
Brain:		
Ammonia	$\uparrow$	$\uparrow$
Glutamine	$\uparrow$	$\uparrow$
Aromatic amino acids	$\uparrow$	$\uparrow$
Branched-chain amino acids	=	=
Energy consumption	$\downarrow$	$\downarrow$
Energy balance	=	=
Neutral amino acid transport across the blood-brain barrier	$\uparrow$	$\uparrow$
Blood-brain barrier integrity	=	=
Noradrenaline	$\uparrow$	=
5-HT	$\uparrow$	=
5-HIAA	$\uparrow$	$\uparrow$
Plasma:		
Ammonia	$\uparrow$	$\uparrow$
Glutamine	$\uparrow$	$\uparrow$
Aromatic amino acids	$\uparrow$	=
Branched-chain amino acids	$\downarrow$	$\downarrow$
Glucose	=	=
Ketone bodies	=	=
Body:		
Liver wt./body wt.	$\downarrow$	=

In the present study, a marked, but variable decrease in cerebral glucose use was caused by hyperammonaemia. There was no detectable alteration in the balance of high-energy intermediates (i.e. ATP, ADP, AMP and phosphocreatine), thereby ruling out direct interference with energy production. Likewise, the decrease in glucose use could not have been a consequence of depressed glucose supply, because the plasma glucose concentrations were normal and the brain glucose content was elevated. Thus the lowered rate of fuel consumption was

most simply explained by a lower demand for energy because of lowered brain activity, similar to the situation that occurs in portacaval-shunted rats and humans (Hawkins & Mans, 1989). The decreased rate of energy consumption caused by ammonia could, as in case of portacaval shunting, be taken as evidence of cerebral dysfunction.

A remarkable finding was that the rate of glucose use was inversely correlated with the brain glutamine content (Fig. 2). Glutamine content seemed to reflect the degree of encephalopathy, supporting similar conclusions relating glutamine concentrations in cerebrospinal fluid to the degree of hepatic encephalopathy in patients (Hourani *et al.*, 1971; Oei *et al.*, 1979). A correlation between plasma ammonia values and cerebral glucose use existed, but there was a much stronger relationship between brain glucose use and cerebral glutamine content. The results suggest that, in the sequence of events occurring between the elevation of plasma ammonia and decreased brain energy consumption, glutamine synthesis (or the increase in brain glutamine content) is more closely related to the actual cause of diminished cerebral activity.

Transport of neutral amino acids across the blood-brain barrier and the increased brain content of aromatic amino acids

Portacaval shunting and hyperammonaemia in the absence of hepatic insufficiency lead to a rise in the brain content of aromatic amino acids (James *et al.*, 1976; Mans *et al.*, 1984; Jonung *et al.*, 1984; Bachmann & Colombo, 1984; Jeppsson *et al.*, 1985; the present study). This seems to be primarily due to an increase in the circulating concentrations of aromatic amino acids (in the case of portacaval shunting) and a much greater activity of the carrier system that transports neutral amino acids across the blood-brain barrier (James *et al.*, 1978; Mans *et al.*, 1979, 1982, 1984; Jeppsson *et al.*, 1985; the present study). The decrease in competing neutral amino acids seems less important (Mans *et al.*, 1982, 1984; Jeppsson *et al.*, 1985).

Hyperammonaemia alone leads to an increased rate of neutral amino acid transport (Mans *et al.*, 1983b; Bachmann & Colombo, 1983; Bachmann *et al.*, 1986). The elevated rate of transport and the increased accumulation of neutral amino acids caused by hyperammonaemia or by portacaval shunting can be reduced by

pretreatment with methionine sulfoximine, an inhibitor of glutamine synthesis (Jonung *et al.*, 1984; Rigotti *et al.*, 1985). It appears, therefore, that the synthesis (or accumulation) of glutamine is responsible for the change in blood-brain barrier kinetics of neutral amino acid transport seen in hyperammonaemia and after portacaval shunting.

Previous observations on portacaval-shunted rats showed that the permeability $\times$ surface area product of tryptophan was increased by about 200%, whereas that for leucine was increased by only 30% (Mans *et al.*, 1982). Similar results were observed in the present study with hyperammonaemia; tryptophan transport was increased by more than was leucine transport, implying a selective increase in the transport mechanism. Furthermore, the increases in both amino acids were correlated with brain glutamine content. These results provide further support for the suggestion that the increased rate of transport seen in shunted rats is a consequence of hyperammonaemia leading to increased rates of glutamine synthesis.

The mechanism by which glutamine is linked with the modulation of neutral amino acid transport is not yet clear (James *et al.*, 1979; Hawkins *et al.*, 1987). Nevertheless, it is established that brain glutamine content is linked to both neutral amino acid transport and the decrease in cerebral energy metabolism. After portacaval shunting the change in neutral amino acid transport occurs very early, beginning within hours, and is correlated with a substantial decrease in cerebral energy consumption (Mans *et al.*, 1990). It seems quite likely, therefore, that the change in neutral amino acid availability is aetiological in the cerebral dysfunction that occurs in response to portacaval shunting or hyperammonaemia.

Monoamine neurotransmitters

Within 48 h of portacaval shunting the brain contents of tryptophan, 5-HT and 5-HIAA were increased, as was the content of noradrenaline (Mans & Hawkins, 1986; Mans *et al.*, 1990). There was no increase in the brain content of the monoamine neurotransmitters in urease-treated rats at 48 h. However, the content of 5-HIAA was significantly elevated, suggesting increased 5-HT metabolism. Similar findings were reported by Bachmann & Colombo (1983). This increase was to be expected, in view of the higher brain content of the precursor tryptophan, which has long been known to influence 5-HT synthesis (Fernstrom & Wurtman, 1971, 1972).

Concluding comment

Many of the metabolic alterations seen in the urease-treated rats are similar to those seen in portacaval-shunted rats, showing that ammonia could be a major factor inducing these changes (Table 7). One of the characteristic abnormalities, elevated neutral amino acid transport, is correlated with a substantial decrease in energy consumption and with an increase in levels of the 5-HT metabolite, 5-HIAA. It seems possible, therefore, that the change in neutral amino acid availability is aetiological in the cerebral dysfunction that occurs in response to portacaval shunting or hyperammonaemia. Since all of these changes are correlated with brain glutamine content, glutamine synthesis may be an important connection in the sequence between hyperammonaemia and brain dysfunction.

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