Effect of ammonia intoxication on cerebral blood flow, its autoregulation and responsiveness to carbon dioxide and papaverine

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SUMMARY Cerebral blood flow (CBF) was measured in anaesthetised cats with ^{133}Xe clearance method under normal conditions and with hyperammonaemia. Elevation of blood ammonia concentration by an intravenous infusion of ammonium acetate caused an increase in CBF and a parallel decrease in cerebrovascular resistance (CVR). These parameters reached, however, plateau at an arterial blood ammonia level exceeding $500\text{ }\mu\text{mol/l}$. Cerebrovascular reactivity to CO_2 diminished following elevation of blood ammonia concentration and at arterial blood ammonia level exceeding $500\text{ }\mu\text{mol/l}$ it was virtually abolished. In contrast, hyperammonaemia influenced neither cerebrovascular responsiveness to papaverine nor autoregulatory properties of the cerebral circulation. It is concluded, therefore, that hyperammonaemia exerts some dilatatory effect on cerebral vessels and severely impairs chemical regulation of CBF but does not elicit cerebral vasomotor paralysis.

A pathogenic role of ammonia in hepatic encephalopathy and coma had already been recognised at the end of the last century.¹ However, the mechanisms by which hyperammonaemia affects the central nervous system are not clear. Especially, the effect of ammonia on regulation of the cerebral blood flow (CBF) still remains to be elucidated.

In 1976 Chandler and Kindt² demonstrated significant elevation of intracranial pressure (ICP) in patients suffering from hepatic encephalopathy of different aetiologies. An increase in ICP by 200–300% of control values has also been found in rhesus monkeys during hyperammonaemia elicited by intravenous infusion of ammonium acetate.³ Altenau and Kindt⁴ using the same experimental model have found that ammonia intoxication causes an increase in CBF and impairs cerebrovascular reactivity to CO_2 and autoregulatory properties of cerebral circulation. Basing on these results, these authors have postulated that ammonia intoxication brings about the cerebral vasomotor paralysis leading to an increase in intra-

cranial blood volume, which in turn would produce an elevation of ICP.

The present study was undertaken to evaluate the effect of elevation of blood ammonia concentration on CBF and its responsiveness to CO_2 . Furthermore, cerebrovascular reactivity to papaverine, the agent known to affect cerebral vessels in a manner different from carbon dioxide, as well as autoregulatory properties of cerebral circulation were examined under normal conditions and with hyperammonaemia in order to elucidate whether an elevation of blood ammonia concentration may result in the vasomotor paralysis of the cerebral vessels.

Materials and methods

The experiments were carried out on 82 cats of either sex weighing between 2.4 and 3.8 kg. Anaesthesia was induced with pentobarbital sodium (Nembutal-Abbott) injected intraperitoneally in a dose of 20 mg/kg and maintained with intravenous alpha-chloralose (60 mg/kg). A tracheostomy was performed and catheters inserted into the left femoral artery and vein for measurement of mean arterial blood pressure (MABP), collection of arterial blood samples and intravenous administration of drugs and solutions. In the experiments, in which autoregulation of CBF was examined the right femoral artery was additionally cannulated for withdrawal of blood to produce controlled hypotension. The

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right cephalic vein was cannulated to induce hyperammonaemia by infusion of ammonium acetate. In order to measure CBF a thin catheter was inserted centripetally through the right lingual artery, so that its tip was located near the external carotid artery. The cat was subsequently mounted in a stereotaxic apparatus. The scalp and temporal muscles were detached to provide an access to frontal, parietal and temporal areas of the calvarium. A 20-gauge needle was introduced into the cisterna magna for a continuous monitoring of ICP. Gallamine triethiodide (Tricuran-Germed) was administered intravenously in a dose of 3–4 mg/kg to cause muscle relaxation and artificial ventilation was maintained throughout the course of the experiment. The respiratory rate and volume were adjusted to achieve arterial carbon dioxide tension (PaCO_2) close to 28 mm Hg. This PaCO_2 level was maintained throughout the experiment except when it was deliberately changed. In the experiments, in which autoregulation of CBF was evaluated arterial oxygen tension (PaO_2) was maintained within the range of 140–160 mm Hg by adjusting the inspiratory oxygen content. Rectal temperature was kept at 37–38°C by external warming with heating pad.

Four series of experiments were performed.

Series I Effect of hyperammonaemia on basal cerebral blood flow and cerebrovascular reactivity to CO_2

This series of experiments was performed on 14 cats. Measurements of CBF under conditions of normal blood ammonia levels were started one hour after completion of the surgical procedure. Subsequently, the infusion of ammonium acetate into the cephalic vein was started. In order to cover a wide range of blood ammonia concentration ammonium acetate was infused at the following rates: 0.39, 0.55, 0.65, 0.86, 1.08, 1.30, 1.74, 1.95, 2.16 and 2.60 mmol/kg/h. In each experiment 2–4 different rates of infusion were used. The blood samples to determine arterial blood ammonia concentration were taken 40 minutes after the onset of each of the infusion rates. This was followed by measurements of CBF.

CBF values to be subjected to further analysis were corrected for the difference between PaCO_2 actually found at the moment of CBF measurement and the level of 28 mm Hg, which according to Fink and Schoolman⁵ corresponds to a normocapnic level in the cat. Corrected values of CBF (cCBF) were obtained using the following equation:

$$\text{cCBF} = \text{CBF} + \text{CI} (28 - \text{PaCO}_2) \text{ ml/min/100g}$$

where CI is an index of the chemical regulation of CBF (see below). Additionally, the cerebrovascular resistance (CVR) was calculated as a ratio of the cerebral perfusion pressure (CPP) to cCBF. CPP was defined as a difference between MABP and mean ICP.

Cerebrovascular reactivity to CO_2 with and without hyperammonaemia was determined on the basis of two successive CBF measurements performed either under normal or hypercapnic conditions. Hypercapnia was achieved by an elevation of CO_2 content in the inspiratory air resulting in PaCO_2 of 43.6 ± 0.8 mm Hg (range 34–55 mm Hg). CBF was measured 5 minutes after induction of hypercapnia. To assess the cerebrovascular reactivity to CO_2 an index of chemical regulation of CBF (CI) was applied.⁶ This index was calculated using the following equation:

$$\text{CI} = \frac{\Delta \text{CBF}}{\Delta \text{PaCO}_2} (\text{ml/min/100g/mm Hg})$$

where Δ CBF is a measure of CBF response to the increment in PaCO_2 (ΔPaCO_2) above the level of normocapnia established in particular experiments.

Series II Examination of relationship between PaCO_2 and cerebral blood flow under conditions of normal blood ammonia and hyperammonaemia

This series of experiments, performed on 20 cats, was designed to determine the relationship between PaCO_2 and CBF under conditions of normal blood ammonia and after elevation of arterial blood ammonia concentration to above 500 $\mu\text{mol/l}$, that is to a level at which severe impairment of the cerebrovascular reactivity to CO_2 was observed (series I). CBF was measured at various levels of PaCO_2 within the ranges of 22.0–58.5 mm Hg and 23.0–53.5 mm Hg under normal conditions and with hyperammonaemia, respectively. Measurements of CBF under conditions of normal blood ammonia levels were started one hour after completion of the surgical procedure. In each experiment 2 or 3 normal blood ammonia CBF measurements at various levels of PaCO_2 were followed by infusion of ammonium acetate into cephalic vein at the rate of 2.16 mmol/kg/h. The blood sample to determine arterial blood ammonia concentration was taken 40 minutes after the onset of ammonium acetate infusion. This was followed by 2 or 3 CBF measurements at various levels of PaCO_2 , similarly as under conditions of normal blood ammonia.

The data obtained in this series of experiments were subjected to the linear regression analysis. Coefficients of the slope of regression lines, corresponding to CI in series I, were used to correct CBF measurements in series III and IV and to present them as normalised values corresponding to PaCO_2 of 28 mm Hg.

Series III Effect of hyperammonaemia on cerebrovascular reactivity to papaverine

This series of experiments was performed on 14 cats. Cerebrovascular reactivity to papaverine under conditions of normal blood ammonia was evaluated one hour after completion of the surgical procedure. Subsequently, an intravenous infusion of ammonium acetate was started at the same rate as in series II. The blood sample to determine arterial blood ammonia concentration was taken 40 minutes after the onset of ammonium acetate infusion. This was followed by determination of the cerebrovascular reactivity to papaverine under conditions of hyperammonaemia. Cerebrovascular responsiveness to papaverine was determined on the basis of CBF measurements performed before and after administration of papaverine and defined as a percentage increase in the base-line cCBF. CBF values were corrected for the difference between PaCO_2 actually found at the moment of CBF measurement and the level of 28 mm Hg, basing on the data obtained in series II. Papaverine hydrochloride (Karlspharma) was infused intravenously at the rate of 1 mg/kg/min and CBF measured 5 minutes after the onset of papaverine infusion.

Series IV Autoregulation of cerebral blood flow under normal conditions and with hyperammonaemia

This series of experiments was performed on two groups of animals. In the first group autoregulation of CBF was examined under conditions of normal blood ammonia. The animals were subjected either to reduction (10 cats) or elevation (15 cats) of MABP. MABP was lowered by means of arterial bleeding reducing the blood volume by 3.0–34.4 ml/kg. The blood was collected into heparinised and warmed syringes and reinfused after measurement of CBF at a desired level of MABP had been completed. Elevation of MABP was achieved by constant intravenous infusion of metaraminol bitartrate (Sharp and Dohme) at the rates varying between 0.31 and 2.08 mg/kg/h. CBF measurements under conditions of normal blood pressure were started one hour after completion of the surgical procedure. In each experiment MABP was either reduced or elevated 2 or 3 times and CBF measured 15 minutes after the new steady level of MABP had been achieved.

In the second group of animals autoregulation of CBF was examined under conditions of hyperammonaemia. The experiments were designed as in the normal blood ammonia group. Infusion of ammonium acetate at the same rate as in series II was started 30 minutes after completion of the surgical procedure. The blood sample to determine arterial blood ammonia concentration was taken 40 minutes after the onset of ammonium acetate infusion. This was followed by CBF measurements under conditions of normotension. Subsequently, the animals were subjected either to gradual reduction (4 cats) or gradual elevation (5 cats) of MABP. In each experiment CBF was measured 2–4 times, 15 minutes after stabilisation of MABP at a new level.

In both groups of animals all CBF values were corrected for the difference between PaCO_2 found during CBF measurement and the level of 28 mm Hg (see series II) and CVR calculated.

Measurements

CBF was measured by the ^{133}Xe clearance technique according to the method described by Bates *et al.*⁷ This technique was recently used by others to measure CBF in cats.^{8,9} A bolus of 0.9% NaCl (80–100 μl) containing 0.2–0.3 mCi of ^{133}Xe was delivered into the lingual artery. The radioactivity was measured by a gamma-scintillation detector placed over the right parieto-occipital region of the skull and directed at a right angle to the bone. To diminish extracranial contamination lead screening was extensively used. CBF was estimated by the initial slope method¹⁰ and expressed in ml/min/100 g. CBF measurements were repeated with intervals of at least 30–40 minutes to exclude inaccuracies which might have been related to residual radioactivity.

MABP and ICP were measured with pressure transducers (Statham P-23Db). Measurements of arterial pH, PCO_2 and PO_2 were performed on blood gas analyser (Radiometer AME-1). Arterial blood ammonia concentration was measured by the colorimetric method.¹¹

Statistical analysis

Means of the data obtained in this study are presented with their standard errors (SE). Differences between mean values were tested by the unpaired *t* test. The data found in series I and IV were analysed using the least squares method to

obtain the trinomial regression equations. Multiple correlation coefficients (*R*) were calculated and their statistical significance evaluated by *F* test. The data obtained in series II were subjected to the linear regression analysis. In this case correlation coefficients (*r*) were calculated and their statistical significance evaluated by *t* test.

Results

Effect of hyperammonaemia on basal cerebral blood flow and cerebrovascular resistance

Control arterial blood ammonia concentration amounted to $130.5 \pm 10.7 \mu\text{mol/l}$ (range 91.5–204.0 $\mu\text{mol/l}$). Intravenous infusion of ammonium acetate produced an elevation of arterial blood ammonia concentration to 257–1085 $\mu\text{mol/l}$ depending on the rate

Fig 1 Corrected cerebral blood flow (cCBF, part a) and cerebrovascular resistance (CVR, part b) as a function of arterial blood ammonia concentration. These relationships are described by the following best fit trinomial equations: a, $y = 20.587 + 4.326 \cdot 10^{-2}x - 5.383 \cdot 10^{-5}x^2 + 2.309 \cdot 10^{-8}x^3$ ($n = 51$, $R = 0.573$, $p < 0.005$); b, $y = 4.448 - 5.457 \cdot 10^{-3}x + 7.364 \cdot 10^{-6}x^2 - 3.453 \cdot 10^{-9}x^3$ ($n = 51$, $R = 0.620$, $p < 0.005$). Open circles: normal ammoniaemia, filled circles: hyperammonaemia.

of infusion. Elevation of blood ammonia concentration caused a gradual increase in CBF and a parallel decrease in CVR (fig 1). However, both parameters reached plateau as blood ammonia level exceeded 500 $\mu\text{mol/l}$. Mean values of CBF, CVR and MABP under conditions of normal blood ammonia and after elevation of blood ammonia concentration to above 500 $\mu\text{mol/l}$ are presented in the table.

Effect of hyperammonaemia on cerebrovascular reactivity to CO_2

In series I increasing blood ammonia concentration caused a gradual decrease in cerebrovascular reactivity to CO_2 (fig 2) and at arterial blood ammonia level exceeding 500 $\mu\text{mol/l}$ responsiveness of CBF to CO_2 was markedly reduced. Under conditions of normal blood ammonia levels mean value of index of chemical CBF regulation was 1.24 ± 0.07 ml/min/100 g/mm Hg, whereas after elevation of blood ammonia concentration to above 500 $\mu\text{mol/l}$ it fell to -0.01 ± 0.06 ml/min/100 g/mm Hg. The change was statistically significant ($p < 0.001$).

Figure 3 shows the relationship between PaCO_2 and CBF obtained in series II under normal conditions and with hyperammonaemia. Within normal blood ammonia levels, at arterial blood ammonia level of 132.9 ± 11.5 $\mu\text{mol/l}$, the slope coefficient of the regression line describing PaCO_2 -CBF relationship was 1.12 ml/min/100 g/mm Hg ($n = 41$, $r = 0.863$, $p < 0.001$). After elevation of arterial blood ammonia concentration to 805.8 ± 37.9 $\mu\text{mol/l}$ the slope coefficient of the regression line decreased about 10 times and amounted to 0.11 ml/min/100 g/mm Hg ($n = 47$, $r = 0.173$, $p > 0.05$). Thus, these results together with those obtained in series I indicate that the cerebrovascular reactivity to CO_2 is virtually abolished at arterial blood ammonia level exceeding 500 $\mu\text{mol/l}$.

Similarly as in series I, hyperammonaemia was not accompanied by any significant changes in MABP, which amounted to 101.2 ± 2.2 mm Hg and $106.0 \pm$

Table Basal cerebral blood flow (cCBF), cerebrovascular resistance (CVR) and mean arterial blood pressure (MABP) found in series I under conditions of normal ammoniaemia and after elevation of arterial blood ammonia level to above 500 $\mu\text{mol/l}$

Parameter	Normal ammoniaemia (n = 12)	Hyperammonaemia (n = 18)
cCBF (ml/min/100g)	25.1 ± 0.9	$32.4 \pm 1.0^*$
CVR (mm Hg/ml/ min/100 g)	3.94 ± 0.09	$3.05 \pm 0.13^*$
MABP (mm Hg)	103.1 ± 3.8	104.4 ± 2.3

* $p < 0.001$.

Fig 2 Cerebrovascular CO_2 reactivity expressed by the index of chemical CBF regulation ($\Delta\text{CBF}/\Delta\text{PaCO}_2$) in relation to arterial blood ammonia concentration. The following best fit trinomial equation describes this relationship:
 $y = 1.923 - 5.873 \cdot 10^{-3}x + 5.001 \cdot 10^{-6}x^2 - 1.018 \cdot 10^{-9}x^3$
($n = 51$, $R = 0.866$, $p < 0.005$). Open circles: normal ammoniaemia, filled circles: hyperammonaemia.

Fig 3 Cerebral blood flow (CBF) as a function of PaCO_2 under conditions of normoammoniaemia (a) and after elevation of arterial blood ammonia level to above 500 $\mu\text{mol/l}$ (b). ns—not significant ($p > 0.05$).

2.5 mm Hg under conditions of normal and raised blood ammonia levels, respectively.

Effect of hyperammonaemia on cerebrovascular reactivity to papaverine

Mean control arterial blood ammonia concentration in this series of experiments was $129.5 \pm 11.6 \mu\text{mol/l}$. Cerebrovascular responsiveness to papaverine was evaluated under conditions of normal ammonia levels and after elevation of arterial blood ammonia concentration to $812.3 \pm 69.0 \mu\text{mol/l}$. Hyperammonaemia did not influence MABP, which amounted to $96.9 \pm 3.3 \text{ mm Hg}$ and $101.2 \pm 3.3 \text{ mm Hg}$ under conditions of normal and raised blood ammonia levels, respectively. Infusion of papaverine caused only a transient drop in MABP by 10–20 mm Hg, lasting 2.5–3.5 minutes.

Hyperammonaemia did not change the cerebrovascular reactivity to papaverine. CBF increased after administration of papaverine by $29.9 \pm 3.9\%$ and $29.2 \pm 3.5\%$ with normal and raised blood ammonia levels, respectively.

Effect of hyperammonaemia on autoregulation of cerebral blood flow

Under conditions with normal ammonia levels autoregulatory properties of CBF were evaluated within the CPP range of 32–183 mm Hg at arterial blood ammonia level of $108.1 \pm 5.8 \mu\text{mol/l}$. The relationship between CPP and CBF demonstrated a typical autoregulatory plateau and CVR was almost linearly related to CPP within the range of CPP between 40 and 120 mm Hg (fig 4).

Under conditions of hyperammonaemia autoregulatory properties of CBF were evaluated within the range of CPP between 43 and 182 mm Hg at arterial blood ammonia level of $802.2 \pm 46.2 \mu\text{mol/l}$. Within the range of CPP between 60 and 135 mm Hg CBF was relatively constant and CVR almost linearly related to CPP (fig 4). Therefore, these results indicate that the autoregulatory properties of cerebral circulation are preserved when blood ammonia concentration is elevated to a level sufficient to abolish the cerebrovascular reactivity to CO_2 .

Comparison of the pressure-flow and pressure-resistance curves under conditions of normal and raised blood ammonia levels revealed that within the range of CPP between 60 and 120 mm Hg, in which autoregulation of CBF could be demonstrated in both experimental situations, hyperammonaemia led to displacement of CPP-CBF curve upwards, whereas CPP-CVR curve was displaced downwards. Within this range of CPP mean values of CBF under normal conditions and with hyperammonaemia amounted to $26.8 \pm 0.9 \text{ ml/min/100 g}$ and $32.1 \pm 1.3 \text{ ml/min/100 g}$, respectively. The corresponding mean values of CVR

were $3.50 \pm 0.15 \text{ mm Hg/ml/min/100 g}$ and $2.92 \pm 0.16 \text{ mm Hg/ml/min/100 g}$. Both changes were statistically significant ($p < 0.01$). Thus, these findings

Fig 4 Relationships between cerebral perfusion pressure (CPP) and corrected cerebral blood flow (cCBF, part a) and cerebrovascular resistance (CVR, part b) under conditions of normal ammoniaemia and hyperammonaemia (arterial blood ammonia concentration exceeding $500 \mu\text{mol/l}$). The following best fit trinomial equations describe above relationships: CPP-cCBF curve in normal ammoniaemia, $y = 6.358 + 0.650x - 7.989 \cdot 10^{-3}x^2 + 3.539 \cdot 10^{-5}x^3$ ($n = 85$, $R = 0.834$, $p < 0.005$) and CPP-cCBF curve in hyperammonaemia, $y = -22.314 + 1.540x - 1.479 \cdot 10^{-2}x^2 + 4.957 \cdot 10^{-5}x^3$ ($n = 47$, $R = 0.886$, $p < 0.005$); CPP-CVR curve in normal ammoniaemia, $y = 0.241 + 3.526 \cdot 10^{-2}x + 1.648 \cdot 10^{-4}x^2 - 1.632 \cdot 10^{-6}x^3$ ($n = 85$, $R = 0.653$, $p < 0.005$) and CPP-CVR curve in hyperammonaemia, $y = 3.178 - 5.307 \cdot 10^{-2}x + 8.173 \cdot 10^{-4}x^2 - 2.972 \cdot 10^{-6}x^3$ ($n = 47$, $R = 0.674$, $p < 0.005$).

confirmed the results obtained in series I that hyperammonaemia causes cerebrovascular dilatation.

Discussion

Basal cerebral blood flow and cerebrovascular resistance

Values of basal cerebral blood flow found in the present study under conditions of normal ammonia levels are lower than those obtained in cats by others⁷⁻⁹ using the same method of CBF measurement. CBF is known to be influenced in different ways by various anaesthetics. In the present study administration of pentobarbital for induction of anaesthesia and its maintenance with chloralose could in part account for low CBF values obtained. The other reason could be a relatively low level of PaCO₂ kept during the experiments. According to the data obtained by Fink and Schoolman⁵ PaCO₂ of 28 mm Hg was considered in the present study to be a normocapnic level in cats, whereas in the other studies it was usually maintained at 40 mm Hg. Using the PaCO₂-CBF relationship with normal ammonia levels, presented in fig 3a, it can be calculated, that in the present study at PaCO₂ of 40 mm Hg basal CBF would be about 41 ml/min/100 g, that is it would not differ from values of CBF found by others⁷⁸ (39–49 ml/min/100 g) in cats anaesthetised with pentobarbital.

Elevation of blood ammonia concentration has been found in this study to cause a progressive increase in the cerebral blood flow accompanied by a progressive decrease in cerebrovascular resistance. Both parameters stabilised, however, at a relatively constant level as arterial blood ammonia concentration exceeded 500 µmol/l. Mean increase in CBF at a plateau level amounted to 29% and was associated with a decrease in CVR amounting to 23% of the mean normal ammonia level value.

These results are in agreement with the data reported by Altenau and Kindt⁴ who found an increase in CBF in ammonia intoxicated rhesus monkeys. Similarly, Gronczewski and Leniger-Follert¹² have found that intracarotid infusion of ammonium acetate in cats produces an increase in local cerebral microflow. On the other hand, James *et al*¹³ have found that hyperammonaemia decreases CBF and cerebral metabolic rate of oxygen (CMRO₂) in dogs. Apart from species differences, the contradictory results obtained in our study and in the study of James *et al* may be possibly explained by differences in the experimental design. In the latter investigation CBF was determined after a short lasting (10 minutes) infusion of ammonium acetate, whereas in our experiments it was measured not earlier than 40 minutes after the onset of ammonium acetate infusion. It is

worthwhile noting that a transient initial decrease in cerebral microflow has been observed in some experiments of Gronczewski and Leniger-Follert¹² during intracarotid infusion of ammonium acetate. Thus, it may be possible that CBF decrease, observed by James *et al*,¹³ is a transient phenomenon followed by its rise during longer lasting hyperammonaemia.

The present results do not explain the mechanisms of the vasodilatory effect of ammonia on the cerebral vascular bed. Gronczewski and Leniger-Follert¹² have suggested that the increase in local microflow, observed by them after intracarotid administration of ammonium acetate, might have been related to the coexisting elevation of extracellular potassium ions activity. These authors have also observed a decrease in extracellular pH following changes in K⁺ concentration, which could augment cerebral vasodilatory response to hyperammonaemia.

Cerebrovascular reactivity to carbon dioxide

The present results demonstrate that elevation of blood ammonia concentration is followed by a progressive decrease in the cerebrovascular reactivity to CO₂ and at arterial blood ammonia level exceeding 500 µmol/l responsiveness of CBF to CO₂ is virtually abolished. These data are in accordance with those obtained by Altenau and Kindt⁴ who found an impairment of the cerebrovascular reactivity to CO₂ in rhesus monkeys at arterial blood ammonia level exceeding 540 µmol/l. Also, the data obtained by Posner and Plum¹⁴ suggest that cerebrovascular reactivity to CO₂ tends to lower in patients with hepatic encephalopathy.

The mechanism whereby hyperammonaemia reduces the cerebrovascular reactivity to CO₂ is not clear. It is known that the responsiveness of CBF to CO₂ is influenced by the cerebral oxygen consumption.^{6,15} A decrease in CMRO₂ during short lasting hyperammonaemia has been reported by James *et al*.¹³

The effect of CO₂ on CBF seems to depend not only on its direct action on brain vessels but also on a central neurogenic mechanism localised in the brainstem.¹⁵⁻¹⁷ With regard to this idea it is worth noting that ammonia intoxication was found to diminish high energy stores in the brainstem of the rat¹⁸ and specifically in the reticular activating system of the mice brain.¹⁹ It is, therefore, possible that changes in metabolism of the brainstem may interfere with the neurogenic mechanism controlling CBF response to CO₂. As is discussed below the loss of the cerebrovascular responsiveness to CO₂ under conditions of hyperammonaemia cannot be explained by the cerebrovascular paralysis, as it was previously suggested by Altenau and Kindt.⁴

Cerebrovascular reactivity to papaverine

Mechanism of the vasodilatory action of papaverine on cerebral vessels is essentially different from that of CO_2 . Papaverine is known as a very potent inhibitor of phosphodiesterase and causes elevation of intracellular level of cyclic AMP, which in turn stimulates an uptake of calcium ions into their storage sites.^{20,21} Takayanagi *et al*²² have found that papaverine not only stimulates Ca^{++} uptake into the microsomal fractions but also inhibits Ca^{++} release from Ca^{++} stores bound to these fractions. Accordingly, papaverine would cause vasodilatation by interference with intracellular concentration of free calcium.

Elevation of arterial blood ammonia concentration to above 500 $\mu\text{mol/l}$, that is to a level at which the cerebrovascular responsiveness to CO_2 is severely impaired, did not cause any significant change in the cerebrovascular reactivity to papaverine. Administration of papaverine elicited almost identical increases in CBF under conditions of normo- and hyperammonaemia. These results indicate, therefore, that abolishment of the responsiveness of CBF to CO_2 at arterial blood ammonia level exceeding 500 $\mu\text{mol/l}$ is not related to the generalised impairment of the cerebral vasomotor activity under conditions of hyperammonaemia.

Autoregulation of cerebral blood flow

The present results demonstrate that elevation of arterial blood ammonia concentration to a level exceeding 500 $\mu\text{mol/l}$ does not impair autoregulatory properties of the cerebral circulation. Under conditions of normal blood ammonia levels ammoniaemia CBF was maintained at a relatively stable level within the CPP range of 40–120 mm Hg. Under conditions of hyperammonaemia the autoregulatory curve was slightly displaced upwards and to the right; however, a relatively stable level of CBF could be demonstrated within the wide range of CPP changes, that is between 60 and 135 mm Hg.

The limits of autoregulation of the cerebral blood flow reported in the literature differ, depending on the species examined and on the experimental conditions.^{23–28} In cat, it has been found that the lower and upper limits of CBF autoregulation (regarding values of MABP) are 60 mm Hg and 160 mm Hg, respectively.^{29,30} In comparison with the above data the autoregulatory curve obtained by us under conditions of normal blood ammonia levels appears to be shifted to the left. However, it should be emphasised that the limits of autoregulation significantly depend on the level of PaCO_2 .^{23,31,32} In our study the pressure-flow relationship with normal blood ammonia levels and hyperammonaemia was examined at a relatively low PaCO_2 level, whereas in the other studies performed on anaesthetised cats it has been

assumed that the normocapnic PaCO_2 level corresponds to 32 mm Hg^{29,30} or even 40 mm Hg.⁷⁸ Recently we have found that autoregulation of CBF in cats subjected to the same anaesthesia as in the present study is maintained within the range of CPP between 70 and 140 mm Hg when PaCO_2 is established at a level of 35 mm Hg (unpublished observations). These limits of autoregulation fit the results obtained in cats by others.^{29,30}

Hyperammonaemia is considered to be one of the factors involved in pathogenesis of hepatic encephalopathy. It has been suggested that the deleterious effect of ammonia may be related to impairment of the control of cerebral blood flow. Altenau and Kindt⁴ have reported that elevation of arterial blood ammonia concentration to a level exceeding 540 $\mu\text{mol/l}$ results in increase in CBF with loss of the cerebrovascular reactivity to CO_2 and impairment of autoregulation of the cerebral circulation. Their conclusion was that ammonia intoxication produces paralysis of the cerebral vessels. Unfortunately, the results supporting this conclusion were reported only in an abstract form without a detailed description of experimental procedure and presentation of numerical data. Therefore, we decided to reinvestigate the effect of ammonia intoxication on cerebral blood flow and its regulation.

Our results confirm the findings of Altenau and Kindt that hyperammonaemia increases CBF and interferes with cerebrovascular reactivity to CO_2 . The present study revealed, however, that CBF responsiveness to directly acting vasoactive agents such as papaverine, is preserved under conditions of hyperammonaemia. Furthermore, in contrast to the latter authors, we were unable to prove that hyperammonaemia impairs autoregulation of CBF. Accordingly, our results do not support the hypothesis that ammonia intoxication brings about cerebral vasomotor paralysis.

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