Intracranial pressure, cerebral blood flow, and cerebrospinal fluid formation during hyperammonemia in cat

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Intracranial pressure (ICP), cerebral blood flow (CBF), and the cerebrospinal fluid (CSF) formation rate were examined in anesthetized cats during ammonia intoxication. Hyperammonemia, evoked by intravenous infusion of ammonium acetate, caused a significant increase in ICP when the arterial blood ammonia level exceeded 400 μmol·liter⁻¹. A progressive elevation of blood ammonia concentration was followed by a gradual rise in CBF, measured by the xenon-133 clearance technique. At an arterial blood ammonia level exceeding 500 μmol·liter⁻¹, the CBF reached a plateau at 30% above the mean control value. Increase in ICP correlated weakly, but significantly, with the increase in CBF (R = 0.489, p < 0.005). Elevation of the arterial blood ammonia level to 780.4 ± 25.5 μmol·liter⁻¹ for 2 hours elicited a significant gradual increase in CSF formation rate, measured by the ventriculocisternal perfusion method with iodine-125-albumin as an indicator substance. A maximum increase in CSF flow of 81% was noted at the end of the ammonium acetate infusion. It is suggested that hyperammonemia increases ICP both by cerebral vasodilatation and by enhancement of the CSF formation rate.

KEY WORDS: ammonia, hyperammonemia, intracranial pressure, cerebral blood flow, cerebrospinal fluid formation, cat

Severe increase in intracranial pressure (ICP) has been observed in the course of encephalopathies associated with liver failure and Reye's syndrome.¹,² It has been proposed that hyperammonemia may account for elevation of ICP in these diseases. Indeed, under experimental conditions, elevation of the blood ammonia concentration by intravenous infusion of ammonium acetate (NH₄Ac) was found to elicit a significant increase in ICP in rhesus monkeys.² Altenau and Kindt⁴ have suggested that ammonia intoxication produces cerebral vasomotor paralysis, resulting primarily in increased intracranial blood volume and secondarily in intracranial hypertension. This conclusion was based on their own observations that hyperammonemia increases cerebral blood flow (CBF) and impairs its autoregulation and responsiveness to CO₂. However, we have recently found that in cats hyperammonemia impairs neither cerebrovascular reactivity to papaverine nor autoregulatory properties of cerebral circulation.⁶ The latter findings negate the hypothesis that intracranial hypertension accompanying hyperammonemia results from the cerebrovascular paralysis.

The present investigation was undertaken to determine whether intracranial hypertension occurring during ammonia intoxication may be related to an increase in CBF or to an elevation of the cerebrospinal fluid (CSF) formation rate.

Materials and Methods

The experiments were performed on 32 cats of either sex, each weighing between 2.5 and 3.8 kg. The animals were initially anesthetized with pentobarbital sodium injected intraperitoneally in a dose of 20 mg·kg⁻¹. Anesthesia was maintained with intravenous alphachloralose in a dose of 60 mg·kg⁻¹.

Experimental Design

Experiment 1. Experiment 1 was developed to study the effect of hyperammonemia on ICP and CBF. In 24 animals, tracheostomy was performed and cath-
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ters were introduced 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. The right cephalic vein was catheterized to induce hyperammonemia by infusion of NH\textsubscript{4}Ac. In order to measure CBF, a thin polyethylene catheter was introduced centripetally through the right lingual artery until its tip was located near the external carotid artery. The cat was subsequently placed in a stereotaxic device. The scalp and temporal muscles were detached to provide access to the frontal, parietal, and temporal areas of the calvaria. A No. 20 needle was introduced into the cisterna magna for continuous monitoring of ICP. Gallamine triethiodide was administered intravenously in a dose of 3 to 4 mg · kg\textsuperscript{-1} to produce muscle relaxation, and artificial ventilation was maintained throughout the course of the experiment. Respiratory parameters were adjusted to achieve a PaCO\textsubscript{2} close to 28 mm Hg. The PaO\textsubscript{2} was maintained within the 100 to 120 mm Hg range by adjusting the inspiratory oxygen content. A rectal thermometer was used to monitor body temperature, which was kept at 37\textdegree to 38\textdegree C using an externally applied heating pad.

Measurements of ICP and CBF under control (normoammonemic) conditions were started 1 hour after completion of the surgical procedure. Subsequently, intravenous infusion of NH\textsubscript{4}Ac was started at a rate varying between 0.39 and 2.60 mmol · kg\textsuperscript{-1} · hr\textsuperscript{-1} to provide a wide range of blood ammonia concentrations. Blood samples were taken 40 minutes after the start of each new infusion rate to determine arterial blood ammonia concentration, followed by measurement of ICP and CBF. In each experiment ICP and CBF were determined at two to four different blood ammonia levels.

Experiment 2. Experiment 2 was designed to test the effect of hyperammonemia on CSF formation rate. The rate of CSF formation was determined in eight cats by a modification of the ventriculocisternal perfusion method described by Pappenheimer, et al.,14 with iodine-125 (\textsuperscript{125}I)-albumin as an indicator substance. The initial surgical procedure was the same as in Experiment 1 except that cannulation of the lingual artery was not performed in this group. The animals were placed in a stereotaxic apparatus in the sphinx position and were immobilized with intravenous gallamine triethiodide (3 to 4 mg · kg\textsuperscript{-1}) and artificially ventilated. Arterial blood gas parameters and rectal temperature were maintained as in Experiment 1. The scalp and temporal muscles were detached, and a stainless steel cannula (outer diameter 0.9 mm) was introduced for infusion into the right lateral ventricle through a burr hole in the skull. The cannula was positioned 3 mm lateral to the sagittal suture and 7 to 9 mm posterior to the coronal suture, with its tip usually located 13 to 14 mm below the bone surface. The outflow cannula (a No. 20 needle) was introduced into the cisterna magna.

The ventriculocisternal system was perfused with artificial CSF at the rate of 80 \( \mu \text{l} · \text{min}^{-1} \). Trace amounts of \textsuperscript{125}I-labeled human serum albumin were added to the perfusion fluid to reach a final specific activity of 40 nCi · ml\textsuperscript{-1}. Intraventricular pressure was continuously monitored and kept at about -2 mm Hg by changing the height of the end of the plastic tubing attached to the outflow cannula.

The rate of CSF formation was calculated by means of the following equation:

\[
V_r = V \left( \frac{C_i}{C_o} - 1 \right),
\]

where C is the specific radioactivity of indicator substance (counts · min\textsuperscript{-1} · ml\textsuperscript{-1}), V is the rate of fluid flow (\( \mu \text{l} · \text{min}^{-1} \)), and subscripts i, o, and f refer to inflow, outflow, and formation, respectively. Radioactivity was measured in aliquots obtained at 15-minute intervals and counted on a well-type scintillation counter. To reduce counting errors each aliquot was counted three times for 1 minute, and the mean was calculated. Volume of aliquots was measured gravimetrically.

The initial ventriculocisternal perfusion was carried out for 2 hours until steady state was reached. During a subsequent 1-hour control (normoammonemic) period the outflow was collected every 15 minutes. This was followed by a 2-hour hyperammonemic period. Ammonium acetate was infused intravenously at a rate of 2.16 mmol · kg\textsuperscript{-1} · hr\textsuperscript{-1} to elevate the blood ammonia concentration to the level that was found in Experiment 1 to cause a definite increase in ICP. During the hyperammonemic period, the outflow was collected at 30-minute intervals.

Measurements

Cerebral blood flow was measured using the xenon-133 (\textsuperscript{133}Xe) clearance technique according to the procedure described by Bates and Sundt.4 In brief, an 80- to 100-\( \mu \text{l} \) bolus of physiological saline containing 0.2 to 0.3 mCi of \textsuperscript{133}Xe was rapidly injected 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. The CBF was estimated by the initial slope method\textsuperscript{13} and expressed in ml · min\textsuperscript{-1} · 100 gm\textsuperscript{-1}.

Intraventricular pressure, MABP, and ICP were measured with pressure transducers.* Measurement of arterial pH, pCO\textsubscript{2}, and pO\textsubscript{2} was performed using a blood gas analyzer.† The arterial blood ammonia concentration was measured by the colorimetric method.11

* Pressure transducer, P-23Db, manufactured by Statham Instruments, Inc., Oxnard, California.
† AME-1 blood gas analyzer manufactured by Radiometer, Copenhagen, Denmark.
TABLE 1

Comparison of physiological variables under control conditions and during hyperammonemia*

<table>
<thead>
<tr>
<th>Physiological Variables</th>
<th>Normoammonemia Levels</th>
<th>Hyperammonemia Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>arterial blood ammonia level (µmol·liter⁻¹)</td>
<td>122.7 ± 7.3 (22)</td>
<td>774.4 ± 31.9† (35)</td>
</tr>
<tr>
<td>ICP (mm Hg)</td>
<td>3.5 ± 0.2 (16)</td>
<td>7.4 ± 0.5‡ (12)</td>
</tr>
<tr>
<td>CBF (ml·min⁻¹·100 gm⁻¹)</td>
<td>25.1 ± 0.5 (22)</td>
<td>32.6 ± 0.5† (35)</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>105.2 ± 3.2 (22)</td>
<td>104.8 ± 1.8 (35)</td>
</tr>
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</table>

* For the hyperammonemia measurements, the arterial blood ammonia concentration exceeded 500 µmol·liter⁻¹. Means ± standard errors are shown. The number of observations is given in parentheses.
† Significantly higher than control value, at p < 0.001.
‡ Measured at the end of the intravenous ammonium acetate infusion.

Statistical Analysis

Results are presented as means with their standard errors. Data found in Experiment 1 were analyzed using the least-squares method to obtain the trinomial regression equations. Multiple correlation coefficients (R) were calculated and their statistical significance was evaluated by F-test. The statistical significance of the differences between the mean values was assessed by unpaired t-test. In Experiment 2 an analysis of variance for the single-factor experiments with repeated measures and Newman-Keuls’ test was applied to evaluate the statistical significance of the CSF formation rate changes.

Experiment 1

Under control conditions, mean ICP in the 24 animals in Experiment 1 was 3.5 ± 0.2 mm Hg. Elevation of blood ammonia concentration elicited a significant increase in ICP; however, in the majority of animals ICP did not change significantly until the arterial blood ammonia level exceeded 400 µmol·liter⁻¹. Mean ICP, measured at the end of the NH₄Ac infusions, was increased 111.4% over the mean normoammonemic value (p < 0.001, Table 1). Control CBF averaged 25.1 ± 0.5 ml·min⁻¹·100 gm⁻¹. Hyperammonemia caused an increase in CBF, which reached a plateau when the arterial blood ammonia concentration exceeded 500 µmol·liter⁻¹ (Fig. 1). Mean increase in CBF at the level of the plateau was 29.9% over the mean normoammonemic value (p < 0.001, Table 1).

Figure 2 illustrates values of ICP plotted against CBF when measured at the same time. A weak but significant correlation (R = 0.489, p < 0.005) was found between these two variables. Thus, the results of Experiment 1 do not give clear evidence for the existence of a causal relationship between the increase in CBF and elevation of ICP.

Experiment 2

Mean CSF formation rate under control conditions was 21.9 ± 1.0 µl·min⁻¹. Intravenous infusion of NH₄Ac increased the arterial blood ammonia concentration from a control value of 135.3 ± 10.2 to 780.4 ± 25.5 µmol·liter⁻¹. Hyperammonemia was associated with an increase in CSF production rate, which was elevated during the entire 2-hour NH₄Ac infusion period (Fig. 3). A maximum increase in CSF flow to 39.7 ± 2.1 µl·min⁻¹ was observed at the end of the NH₄Ac infusion. An analysis of variance applied in five exper-

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![Graph showing relationship between cerebral blood flow (CBF) and intracranial pressure (ICP). The regression equation: Y = 55.582 - 5.567X + 0.199X^2 - 2.236 \times 10^{-3}X^3 (n = 66, R = 0.489, p < 0.005). Filled circles = control conditions; open circles = hyperammonemia.](image)

Fig. 2. Relationship between cerebral blood flow (CBF) and intracranial pressure (ICP). The regression equation: $Y = 55.582 - 5.567X + 0.199X^2 - 2.236 \times 10^{-3}X^3$ ($n = 66, R = 0.489, p < 0.005$). Filled circles = control conditions; open circles = hyperammonemia.

![Graph showing changes in arterial blood ammonia concentration during intravenous infusion of ammonium acetate (NH4Ac) at a rate of 2.16 mmol · kg⁻¹ · hr⁻¹. B: Changes in cerebrospinal fluid (CSF) formation rate during NH4Ac infusion. An analysis of variance applied in five experiments (with an equal number of observations) reveals that the CSF formation rates measured 60, 90, and 120 minutes after the onset of NH4Ac infusion were significantly higher than the mean control value ($p < 0.01$, Newman-Keuls' test) but not significantly different from each other. The MABP did not change significantly during NH4Ac infusion and was 99.4 ± 5.1 and 102.3 ± 2.4 mm Hg under conditions of normo- and hyperammonemia, respectively.](image)

Fig. 3. A: Changes in arterial blood ammonia concentration during intravenous infusion of ammonium acetate (NH4Ac) at a rate of 2.16 mmol · kg⁻¹ · hr⁻¹. B: Changes in cerebrospinal fluid (CSF) formation rate during NH4Ac infusion. An analysis of variance applied in five experiments (with an equal number of observations) reveals that the CSF formation rates measured 60, 90, and 120 minutes after the onset of NH4Ac infusion were significantly higher than the mean control value ($p < 0.01$, Newman-Keuls' test) but are not significantly different from each other. $n =$ number of observations.

Discussion

The results of the present study reveal that elevation of blood ammonia concentration is accompanied by an increase in ICP. These results are in agreement with data obtained by Altenau, et al.,² in rhesus monkeys. However, the ICP increments observed by us were smaller than theirs and we did not note episodes of sudden high ICP waves as described by them. These differences might have been related to the different periods of NH4Ac infusion used and the different blood ammonia levels obtained.

So far, the mechanisms whereby hyperammonemia elicits intracranial hypertension have not been entirely elucidated. It has been found that ammonia intoxication does not affect integrity of the blood-brain barrier nor does it cause cerebral edema.⁹,¹⁰ Altenau and Kindt¹ postulated that hyperammonemia acts by elevating in-
tracranial blood volume as a result of cerebral vasomotor paralysis. This hypothesis was based on their observations that hyperammonemia increases CBF and impairs its autoregulation and responsiveness to CO₂. The hypothesis of ammonia-induced cerebrovascular paralysis was not confirmed by our previous investigation. In the present work, as in our previous study, elevation of blood ammonia concentration was followed by a gradual rise in CBF, which reached a plateau as the arterial blood ammonia level exceeded 500 μmol · liter⁻¹. The mean increase in CBF at the plateau level amounted to approximately 30% of the mean normoammonemic value. However, there was only a weak relationship between the CBF increase and the ICP elevation. It seems unlikely, therefore, that the cerebral vasodilatation could entirely account for the intracranial hypertension observed under conditions of hyperammonemia.

The possibility exists that hyperammonemia increases ICP by enhancing the rate of CSF formation. This assumption was investigated in Experiment 2, in which the blood ammonia concentration was elevated above the level at which a clear-cut increase in ICP was observed in Experiment 1. The mean CSF formation rate under conditions of normoammonemia (21.9 μl · min⁻¹) was similar to those described by other authors who also used the ventriculocisternal perfusion technique to measure CSF production rate in cats. Intravenous infusion of NH₄Ac caused a significant elevation of the rate of CSF production. Enhancement of CSF flow was maintained during the entire 2-hour period of hyperammonemia, reaching a maximum level (81% above the mean normoammonemic value) at the end of NH₄Ac infusion. According to the data of Hochwald and Wallenstein obtained in cats, such an elevation of CSF formation rate may be the cause of a significant increase in ICP.

The mechanism by which hyperammonemia influences the CSF formation rate cannot be explained at present. In this study we found that hyperammonemia increased CBF; however, it is not known whether it also affects choroid plexus blood flow, nor is the relationship between choroid plexus blood flow and the CSF production rate well defined. The data obtained by Ames, et al., suggest that the CSF production rate may depend on the blood supply to the choroid plexus. These authors have observed that changes in the rate of CSF formation on the surface of the exposed plexus tissue are associated with constriction or dilatation of the choroid plexus vessels. On the other hand, Nakamura and Hochwald demonstrated that CSF flow, measured with the ventriculocisternal perfusion method, is not affected by even large decreases in choroid plexus blood flow. Additionally, it should be pointed out that CSF is also elaborated from extrachoroidal sources, although the fraction of CSF derived from those sources and the effect of different experimental conditions have not yet been clearly established.

Among various factors implicated in the control of CSF production is Na⁺-K⁺-activated adenosine triphosphatase (Na⁺-K⁺-ATPase). Inhibition of Na⁺-K⁺-ATPase by cardiac glycosides has been observed to cause a decrease in the CSF formation rate. It is also of interest that ammonia intoxication was found to stimulate Na⁺-K⁺-ATPase in different regions of the mouse brain. Thus, one may speculate that hyperammonemia increases the CSF formation rate by enhancing Na⁺-K⁺-ATPase activity. However, at present there is insufficient evidence to support this assumption, since it is not known whether hyperammonemia influences activity of Na⁺-K⁺-ATPase in the choroid plexus as well.

Our results indicate that hyperammonemia is accompanied by an increase in ICP, CBF, and the CSF formation rate. It appears that an elevation of ICP is caused both by an increased rate of CSF production and by cerebral vasodilatation.

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