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# Elevation of Ammonia Contents in the Cerebral Hemisphere under the Blood-Brain Barrier Opening

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## ABSTRACT

Elevation of ammonia contents was observed 1 hr following infusion of 4% ammonium acetate only in the cerebral hemisphere where reversible opening of the blood-brain barrier was induced by intracarotid injection of 0.1% deoxycholic acid. This finding suggests that ammonia may contribute to hepatic encephalopathy, when the permeability of the blood-brain barrier is altered.

Several factors are known to affect the brain ammonia content, including arterial pH, detoxification of blood-borne ammonia in the brain and the integrity of the blood-brain barrier[1]. In rats with induced fulminant hepatic failure or portacaval shunt, ammonium irons in the brain is much more elevated than in blood[5], because of an increase in ammonia transport across the blood-brain barrier. In our previous study it has been clarified that administration of ammonia during opening of the blood-brain barrier with the osmotic shock produces cytotoxic edema in dogs[4]. These two findings strongly suggest that ammonium ion may participate in the generation of hepatic encephalopathy.

The present study was designed to examine whether or not ammonia contents in the concerned hemisphere of the brain would be increased by the infusion of ammonium acetate, when the blood-brain barrier is opened by an intracarotid injection of bile acid.

Adult Sprague-Dawley rats, weighing 300—400 g, were used after overnight fasting. Animals were anesthetized with an intraabdominal injection of pentobarbital at a dose of 35 mg/kg body weight. Evans blue (1 ml/kg of 2.5%) was administered through the femoral vein cannula to stain the cerebral hemispheres. A four-percent ammonium acetate solution was infused into the femoral vein for 1 hr at a rate of 2 ml/kg body weight/hr (The blood ammonia levels were maintained at about 250 $\mu$g/dl). Thirty min after the start of ammonium acetate infusion, 2 ml/kg body weight of a warmed 0.1% deoxycholic acid solution was injected in less than 1 min into the right internal carotid artery. Saline instead of bile acid was similarly injected as a control. The physiological conditions, including arterial pH, $pCO_2$ and blood sugar levels, did not change during the experiment. Rats were killed by guillotining 30 min after the injection, i.e., 1 hr after the initiation of ammonium acetate infusion. The blood ammonia levels were determined with an enzymatic kit (Kyowa Medix Co., Tokyo, Japan), and brain ammonia contents were similarly measured with the method of Folbergrova et al[3]. The differences were evaluated for statistical significance by the unpaired Student's t-test.

The cerebral hemisphere was stained with Evans blue immediately after but not 2 hr after bile acid infusion, indicating the reversible opening of the blood-brain barrier. The blood ammonia concentrations in bile acid-injected rats

**Table 1.** Ammonia contents in the cerebral hemispheres in rats treated with 4% ammonium acetate during reversible opening of the blood-brain barrier induced by 0.1% deoxycholic acid

| | Blood ammonia levels[a] | Brain ammonia content | |
|---|---|---|---|
| | | Un-injected hemisphere | Injected hemisphere |
| | ($\mu$moles/l) | ($\mu$moles/kg) | |
| Saline (4)[b] | 161 ± 12[c] | 338 ± 83 | 345 ± 61 |
| Deoxycholic acid (4) | 130 ± 20 | 411 ± 119 | 661 ± 79[d] |

a The blood ammonia levels (234—290 $\mu$g/dl)
b ( ) = No. of rats
c Mean ± SD.
d Significantly higher than the un-injected hemisphere in the same rats ($p < 0.02$), and significantly higher than the injected hemisphere in saline-injected rats ($p < 0.01$).

(test) were not significantly different from the levels in saline-injected rats (control) (Table 1). In test rats, the ammonia contents in the right cerebral hemisphere (the bile acid-injected side) (661 ± 79 $\mu$moles/kg brain) were significantly higher than in the opposite hemisphere (411 ± 119) in the same rats and also than in the right hemisphere (the saline-injected side) (345 ± 61) in control rats. The increase in the ammonia contents in the opposite hemisphere in bile acid-injected rats was insignificant as compared with those in saline-injected rats (338 ± 83). This result probably means that a small amount of the injected dexycholic acid flowed into the opposite hemisphere of the brain.

Recent electron microscopic evidence[6] suggests that the tight junction of brain capillary endothelial cells is closed in an animal model of acute hepatic failure. Since transport through the blood-brain barrier does in fact occur, another mode of transport must exist. This mode of transport was shown to be vesicular transport in the same study[6]. In the condition of the increased permeability of the blood-brain barrier frequently found in acute hepatic failure, impermeable ammonium ion ($NH_4^+$) can permeate through the barrier into the brain by virtue of the vesicular transport and the content of ammonia in the brain may reach the concentration high enough to induce encephalopathy. Thus, the concentration of arterial ammonia can not be representative of brain ammonia contents. Therefore, correction is requested on the present belief that ammonia is not an important factor in hepatic encephalopathy accompanying fulminant hepatic failure, based on the finding that ammonia level in blood stays in the normal range or mildly elevates in the rats having neurological symptoms. The present data may also explain the reason why hepatic encephalopathy in some patients with liver cirrhosis occurs without hyperammonemia. The hypothesis that hyperammonemia is a necessary and sufficient etiologic factor for hepatic encephalopathy is supported by phasic relationship between arterial ammonia concentrations and levels of consciousness[2] and also by the present study.

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