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Abstract

The prevention of hepatic encephalopathy by the intravenous infusion of a branched chain amino acid (BCAA)-enriched solution was investigated in methionine and ammonium acetate-treated rats whose liver was already injured with carbon tetrachloride. A BCAA-enriched solution protected the rats from entering a coma. The brain BCAA contents became higher, and the brain methionine and tyrosine levels and the ratio of glutamine to glutamic acid in the brain diminished after administering the BCAA-enriched solution.

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—— BRIEF NOTE ——

PREVENTION OF METHIONINE AND AMMONIA-INDUCED COMA BY INTRAVENOUS INFUSION OF A BRANCHED CHAIN AMINO ACID SOLUTION TO RATS WITH LIVER INJURY

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Abstract. The prevention of hepatic encephalopathy by the intravenous infusion of a branched chain amino acid (BCAA)-enriched solution was investigated in methionine and ammonium acetate-treated rats whose liver was already injured with carbon tetrachloride. A BCAA-enriched solution protected the rats from entering a coma. The brain BCAA contents became higher, and the brain methionine and tyrosine levels and the ratio of glutamine to glutamic acid in the brain diminished after administering the BCAA-enriched solution.

Key words : branched chain amino acid, ammonia, methionine, hepatic encephalopathy.

Various substances such as ammonia, mercaptans and fatty acids seem to be involved in the pathogenesis of hepatic encephalopathy, but an accepted theory accounting for metabolic abnormalities in hepatic encephalopathy has not been established yet (1). A synergistic effect of these substances causing hepatic encephalopathy is more probable than a single effect (2).

Plasma neutral amino acid imbalance is observed in hepatic encephalopathy, and intravenous infusion of branched chain amino acids (BCAA) restores the aminogram to a normal state and improves neuropsychiatric symptoms (3). This clinical observation is noteworthy in consideration of the pathogenesis of hepatic encephalopathy. In the present study, a BCAA-enriched solution was continuously infused intravenously into methionine and ammonium acetate-treated rats with liver injury, and the preventive effect on hepatic encephalopathy was investigated from the aspects of behavior and electroencephalography (EEG). Furthermore, the brain amino acid contents and blood ammonia levels were determined to investigate the mechanism of the BCAA effect.

Male Sprague-Dawley rats weighing 220 to 290 g were used throughout the study. A 20 % carbon tetrachloride (CCl_4) solution in liquid paraffin was given intragastrically at 15 ml per kg body weight to overnight-fasted rats. Six h after the CCl_4 administration, a polyethylene tube was inserted into the cervical vein,

and infusion with a BCAA-enriched solution (leucine, 11.0; isoleucine, 9.0; valine, 8.4 g/l; total amino acid, 83.7 g/l) (3) was continued at a flow rate of 6.6 ml/kg body weight/h until the end of the experiment. During the infusion, rats were unrestrained so that their behavioral changes could be observed easily. Methionine (11.6 mmoles/kg body weight) was administered intragastrically twice, 20 and 22 h following the CCl_4 treatment, and ammonium acetate was injected intraperitoneally 2 h after the 2nd administration of methionine at a dose of 5 mmoles/kg body weight.

The experimental rats were divided into 4 groups as follows : Group I, untreated but saline-infused rats with normal liver; Group II, liver-injured rats infused with saline; Group III, methionine and then ammonia-treated liver-injured rats infused with saline; and Group IV, methionine and ammonia-treated liver-injured rats infused with the BCAA-enriched solution. Some of the rats in Groups III and IV were sacrificed by decapitation 30 min following ammonium acetate injection, and rats in Groups I and II, at the same time. Venous blood ammonia levels were determined by a simple paper test (4). Brain aminogram determinations and EEG recordings were performed according to previous methods (5).

The vital activity of all rats diminished 20 h following CCl_4 administration. They took a sitting position and did not walk; however, they maintained the ability to raise themselves after being laid down. In most saline-infused rats treated with methionine, muscular tension and response to a poking stimulus gradually decreased following the intraperitoneal injection of ammonium acetate. They could not raise their bodies 7 to 13 min following the injection. Thereafter they did not awaken even to a pain stimulus. All rats who fell into a coma recovered 60 to 70 min later. The number of rats suffering from a coma is shown in Table 1. BCAA infusion protected seven out of eight rats from entering a

TABLE 1. PROTECTIVE EFFECT OF A BCAA-ENRICHED SOLUTION ON METHIONINE AND AMMONIUM ACETATE-INDUCED COMA IN CCL_4-ADMINISTERED RATS.

Group	Infusion	No. of rats	Coma*	
			Absent	Present
III	Physiological saline	8	1	7
IV	BCAA-enriched solution	8	7	1

*$p < 0.05$

coma as judged from behavioral changes.

The blood ammonia levels and brain amino acid contents in the four groups are shown in Table 2. When sacrificed, the rats in Group III were all in a coma, but none of the rats in Groups I, II and IV were comatous. Blood ammonia levels in Groups III and IV were significantly higher than those in rats not treated

TABLE 2. BLOOD AMMONIA LEVELS AND BRAIN AMINO ACID CONTENTS IN RATS INFUSED WITH PHYSIOLOGICAL SALINE OR A BCAA-ENRICHED SOLUTION.

Group	I	II	III	IV
Treatment	None	CCl_4	CCl_4+Methionine+ Ammonium Acetate	CCl_4+Methionine+ Ammonium Acetate
Infusion	Saline	Saline	Saline	BCAA-enriched solution
(No. of rats)	(4)	(4)	(5)	(4)
Blood ammonia (μg/dl)	85±17	132±15	334 ± 48** ++	293 ± 32
Brain amino acid (μmoles/kg brain)				
Valine	58±4	83±15	46 ± 4** ++	159 ± 48##
Leucine	63±3	75±7	53 ± 3++	149 ± 37##
Isoleucine	32±4	36±3	25 ± 3++	111 ± 55##
Phenylalanine	41±10	40±2	23 ± 4** ++	18 ± 3
Tyrosine	32±5	39±5	25 ± 2* ++	12 ± 5##
Methionine	24±1	34±5	1684 ± 12** ++	584 ± 103##
Glutamic acid	4873±764	6069±378	4880 ± 543++	5772 ± 540
Glutamine	3240±272	4626±273	6110 ± 868** +	5767 ± 611
Glutamine/Glutamic acid	0.61±0.04	0.77±0.08	1.30 ± 0.24** ++	0.94 ± 0.03#

Mean ± SD.
*: I vs III, +: II vs III, #: III vs IV.
*, + and #: $p<0.05$, **, ++ and ##: $p<0.01$.

with ammonia. A significant difference in blood ammonia levels between Groups III and IV was not observed. Among brain amino acids in rats of Group III, BCAA, aromatic amino acids (AAA) and glutamic acid were lower, and methionine, glutamine and the ratio of glutamine to glutamic acid were higher than those in Group II. By continuous infusion of the BCAA-enriched solution, a 2.8 to 4.4-fold increase in brain BCAA levels and diminished contents of tyrosine and methionine were observed. The ratio of brain glutamine to glutamic acid was lower in BCAA-enriched solution-infused rats (Group IV) than in saline-infused rats (Group III). When saline-infused rats (Group III) became comatous, amplitudes of the background waves of EEGs were depressed, and slow waves of 5-8 Hz with a higher voltage became dominant 60 min after the ammonium acetate injection.

According to Higashi (6), three factors are necessary to yield a reversible coma in rats. In the present study, these factors were liver injury by CCl_4 administration, intragastric feeding of methionine, but not other amino acids, and intraperitoneal injection of ammonium acetate. Coma was not induced by a single treatment of methionine or ammonium acetate to the rats with liver injury. Even if methionine and ammonium acetate were administered serially, a coma was not induced in rats without liver injury. Intragastric feeding and an appro-

priate incubation time were also indispensable for methionine to induce a coma. These results strongly suggest that methionine metabolites from the gastrointestinal tract and ammonia act synergistically in CCl_4-administered rats to induce a coma (6).

Continuous infusion of a BCAA-enriched solution protected the rats from entering a coma and increased the brain BCAA contents while decreasing the brain methionine and tyrosine contents and glutamine to glutamic acid ratio. The results of our previous study showing that hepatic encephalopathy was improved along with an elevation of BCAA levels and decrease of AAA concentrations in cerebrospinal fluid following BCAA drip infusion to cirrhotic patients (7) are consistent with the present results. In comatous rats, blood ammonia levels and brain glutamine contents were mucn higher as a result of ammonia detoxification than in rats of Groups I and II. A BCAA-enriched solution hardly affected blood ammonia levels. Blood ammonia levels, however, may not accurately reflect brain ammonia levels in liver-injured rats (8). As the solution significantly lowered the ratio of glutamine to glutamic acid near to that of the non-comatous rats, it is suggested that ammonia detoxification may be related to BCAA metabolism in the brain. Further investigation of the metabolic fate of BCAA in the brain in relation to ammonia detoxification needs to be carried out in the future.

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