

# *Acta Medica Okayama*

---

*Volume 39, Issue 1*

1985

*Article 1*

FEBRUARY 1985

---

Branched chain amino acid transaminase and  
branched chain alpha-ketoacid dehydrogenase  
activity in the brain, liver and skeletal muscle  
of acute hepatic failure rats

Nobuyuki Takei\*

\*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

# Branched chain amino acid transaminase and branched chain alpha-ketoacid dehydrogenase activity in the brain, liver and skeletal muscle of acute hepatic failure rats\*

Nobuyuki Takei

## Abstract

Branched chain amino acid (BCAA) transaminase activity increased in both the mitochondrial and supernatant fractions of brain from hepatic failure rats, in which a partial hepatectomy was performed 24h following carbon tetrachloride (CCl<sub>4</sub>) administration, although the activity of liver and skeletal muscle was the same as in control rats. The elevation of mitochondrial BCAA transaminase activity in liver-injured rats was partly due to increased activity of brain specific Type III isozyme. Branched chain alpha-ketoacid (BCKA) dehydrogenase in the brain homogenates was not significantly altered in acute hepatic failure rats, while the liver enzyme activity was markedly diminished. BCKA dehydrogenase activity in the brain homogenates was inhibited by adding ATP to the assay system, and was activated in vitro by preincubating the brain homogenate at 37 degrees C for 15 min. These findings suggest that brain BCAA catabolism is accelerated in acute hepatic failure rats.

**KEYWORDS:** branched chain amino acids, branched chain amino acid transaminase, branched chain alpha-ketoacid dehydrogenase, acute hepatic failure, brain

---

\*PMID: 3984777 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 39, (1), 1-10 (1985)

**BRANCHED CHAIN AMINO ACID TRANSAMINASE AND  
BRANCHED CHAIN ALPHA-KETOACID DEHYDROGENASE  
ACTIVITY IN THE BRAIN, LIVER AND  
SKELETAL MUSCLE OF ACUTE  
HEPATIC FAILURE RATS**

Nobuyuki TAKEI

*First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan  
(Director: Prof. H. Nagashima)*

*Received July 23, 1984*

*Abstract.* Branched chain amino acid (BCAA) transaminase activity increased in both the mitochondrial and supernatant fractions of brain from hepatic failure rats, in which a partial hepatectomy was performed 24h following carbon tetrachloride (CCl<sub>4</sub>) administration, although the activity of liver and skeletal muscle was the same as in control rats. The elevation of mitochondrial BCAA transaminase activity in liver-injured rats was partly due to increased activity of brain specific Type III isozyme. Branched chain alpha-ketoacid (BCKA) dehydrogenase in the brain homogenates was not significantly altered in acute hepatic failure rats, while the liver enzyme activity was markedly diminished. BCKA dehydrogenase activity in the brain homogenates was inhibited by adding ATP to the assay system, and was activated *in vitro* by preincubating the brain homogenate at 37 °C for 15 min. These findings suggest that brain BCAA catabolism is accelerated in acute hepatic failure rats.

*Key words :* branched chain amino acids, branched chain amino acid transaminase, branched chain alpha-ketoacid dehydrogenase, acute hepatic failure, brain.

Abnormal metabolism of amino acids and amines in the central nervous system has recently been postulated to be a possible cause of hepatic encephalopathy (1). Elevated methionine and aromatic amino acids (AAA), *e.g.*, tyrosine, phenylalanine and unbound tryptophan, and decreased branched chain amino acids (BCAA), *e.g.*, valine, leucine and isoleucine, are consistently observed in the plasma and cerebrospinal fluid of patients with hepatic failure (1, 2). Fischer and Baldesarini (3) suggested that metabolic abnormalities of AAA, monoamine precursors, in the brain lead to the elevation of pseudoneurotransmitters such as octopamine and phenylethanolamine. Hepatic encephalopathy in severe liver diseases such as liver cirrhosis and fulminant hepatitis was rapidly improved by an intravenous infusion of a BCAA-enriched solution to hepatic coma patients (4, 5). Accelerated transport of BCAA across the blood-brain barrier to the brain has also been reported in an experimental model of encephalopathy (6). The causal relationship between the administration of BCAA and recovery from encephalopathy remains

to be elucidated. In order to investigate the fate of BCAA in the brain during hepatic failure, the activities of BCAA transaminase and branched chain alpha-ketoacid (BCKA) dehydrogenase in the brain, the rate-limiting enzymes of BCAA metabolism, were assayed in acute hepatic failure rats, in which a partial hepatectomy was performed 24 h following  $\text{CCl}_4$  administration.

#### MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 200 to 300 g each, were obtained from Awazu Experimental Animal Co. (Japan). A 20 %  $\text{CCl}_4$  solution in liquid paraffin was administered intragastrically at a dose of 15 ml per kg body weight and D-galactosamine hydrochloride was given intraperitoneally at a dose of 1.5 g per kg body weight to overnight-fasted rats. To create more severe liver damage, a 20 %  $\text{CCl}_4$  solution was administered intragastrically at a dose of 8 ml per kg body weight, followed by a two-thirds partial hepatectomy 24 h after the  $\text{CCl}_4$  administration (7). The rats were starved during the experiment and decapitated 12 h after the hepatectomy. Sham operated rats, which were administered liquid paraffin alone, served as controls.  $\text{CCl}_4$  itself does not produce brain damage, as significant free radical formation from  $\text{CCl}_4$  in the brain is lacking due to low cytochrome P-450 levels in the central nervous system, although rapid entry of  $\text{CCl}_4$  into the brain is observed up to 48 h after intragastric  $\text{CCl}_4$  administration (Shiota, T., unpublished observation). Rats of another group were starved for 2 days and then fed ad libitum on a basal diet (Laboratory Chow MF, Oriental Yeast Co., Japan) or a 5 % BCAA (leucine : valine : isoleucine = 0.8 : 1.1 : 0.6)-supplemented diet for two days.

Brain, liver and skeletal muscle were homogenized with 9 volumes of 0.25 M sucrose solution immediately after the rats were killed by exsanguination. The homogenate, supernatant and mitochondrial fractions were separated according to Hogeboom (8). BCAA transaminase activity was measured at pH 8.6 and 37 °C using 108 mM leucine as the substrate according to Ichihara and Koyama (9). After the 10 min reaction, the amount of ketoacid formed was determined as its hydrazone. The isozyme pattern of the transaminase was investigated by chromatography on a DEAE-cellulose column according to Stephen *et al.* (10).

BCKA dehydrogenase activity was measured at pH 6.8 with 0.75  $\mu$ moles of L-1- $^{14}\text{C}$ -alpha-ketoisocaproate as the substrate in a final volume of 0.33 ml according to Wohlhueter (11). L-1- $^{14}\text{C}$ -alpha-ketoisocaproate was prepared by enzymatic oxidation of L-1- $^{14}\text{C}$ -L-leucine (55 mCi/mmol, Amersham Co., U.K.) according to Wohlhueter's method (11). Incubation tubes were sealed with rubber caps and shaken for 20 min at 37 °C. Blank values were determined by running boiled enzyme through an identical procedure. To trap liberated  $^{14}\text{C}$ - $\text{CO}_2$ , filter paper (10 × 25 mm, No.2, Toyo Roshi Co., Japan), containing 50  $\mu$ l of NCS solubilizer (Amersham Co., U. K.) was suspended from a hook in the rubber cap. The filter papers were transferred into vials containing 6 ml of toluene-PPO-dimethyl POPOP (200 : 10 : 1, v/w/w). Radioactivity was counted in a Beckman scintillation counter (LSC-701, U.S.A.) after standing overnight at room temperature.

Enzyme activity was expressed as nmoles of product formed per min per mg protein. Protein concentrations were determined according to Lowry *et al.* (12). The effects of various compounds including ATP, norepinephrine, dopamine, tyramine, octopamine, and ammonium acetate, on BCKA dehydrogenase activity were examined *in vitro* with the use of brain and liver homogenates at a final concentration of 1 mM. Preincubation of brain and liver homogenates was performed at 37 °C for 15 min before the enzyme assay.

All the results were expressed as the mean  $\pm$  SD. Significant differences between the mean values were determined by Student's t test after analysis of variance.

### RESULTS

The rats, which were partially hepatectomized 24 h after  $\text{CCl}_4$  treatment, had severe hepatic dysfunction, as shown by a marked rise in serum glutamic pyruvic transaminase (GPT) activity and severe prolongation of the hepaplastin test, cerebral edema and electroencephalographic abnormalities (13, 14). Many of the treated rats showed grade I to III of encephalopathy according to Higashi's classification (15). The 48 h survival rate was approximately 50 %.

BCAA transaminase was located in both the supernatant and mitochondrial fractions of the brain, liver and skeletal muscle. BCAA transaminase activity in the brain supernatant fraction was significantly higher than in the liver and skeletal muscle, and the activity in the mitochondrial fraction of the brain was much higher than it was in the liver. In acute hepatic failure rats, the supernatant and mitochondrial BCAA transaminase activity was significantly increased only in the brain (Fig. 1). Similar increases in this enzyme activity were also observed 36 h

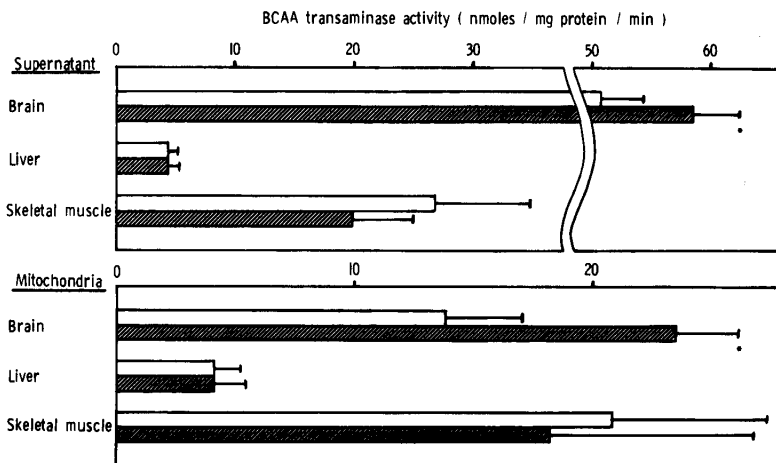


Fig. 1. BCAA transaminase activity in the brain, liver and skeletal muscle in control (□) and acute hepatic failure (▨) rats. Horizontal lines indicate SD of the mean. \* $p < 0.05$ , between control and acute hepatic failure rats. No. of rats = 3.

after an intragastric administration of  $\text{CCl}_4$  and an intraperitoneal injection of D-galactosamine to rats (data not shown). The enzyme in the supernatant of the liver and skeletal muscle was increased by feeding the ordinary diet after 2 days of starvation or the 5 % BCAA-supplemented diet. However, no changes in the brain enzyme were observed after dietary manipulations (Fig. 2). The BCAA

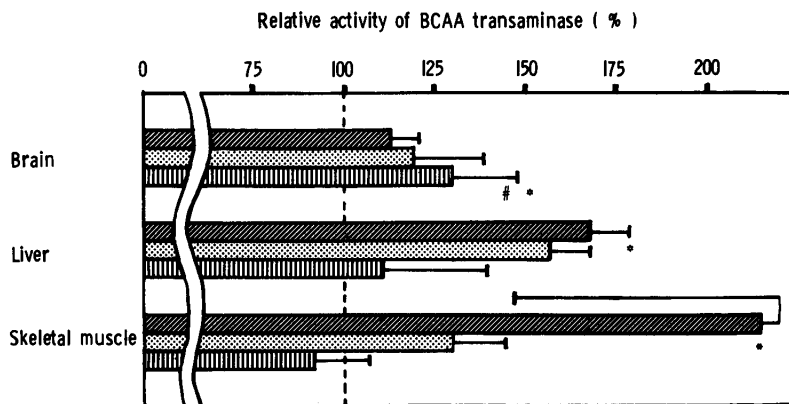


Fig. 2. Supernatant activity of BCAA transaminase in the brain, liver and skeletal muscle under various experimental conditions. The relative enzyme activity (%) was expressed by assuming the activity of 48 h-starved rats in the respective tissues to be 100%. (▨), starved for 48 h and then fed for 24 h, (▩), 5% BCAA-supplemented diet for 48 h and (□), CCl<sub>4</sub> treatment for 36 h. #, No. of rats = 6, No. of rats in the other groups = 3. \*,  $p < 0.05$ , as compared to the fasted rats.

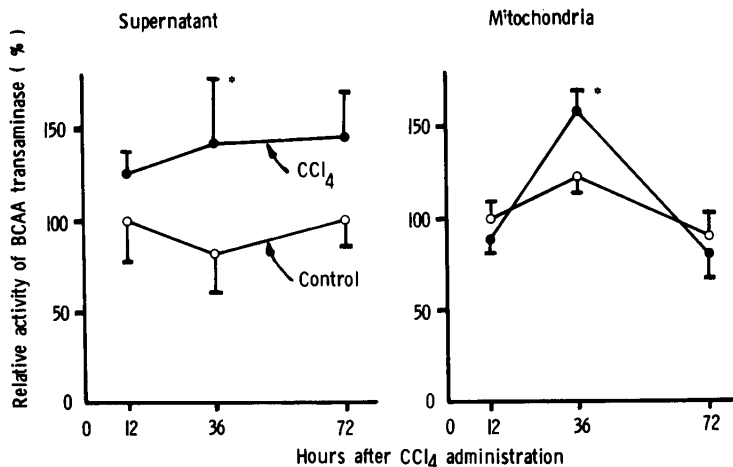


Fig. 3. Time course of brain transaminase activity in the supernatant and mitochondrial fractions following CCl<sub>4</sub> administration. The relative enzyme activity (%) was calculated by assuming the activity 12 h after CCl<sub>4</sub> treatment to be 100%. ○—○, Control and ●—●, CCl<sub>4</sub> treated rats. No. of rats = 3.

transaminase activity in both the supernatant and mitochondrial fractions of the brain increased 36 h following the CCl<sub>4</sub> administration, when liver injury was prominent (Fig. 3).

Isozyme Types I and III were observed in both fractions of the brain, and

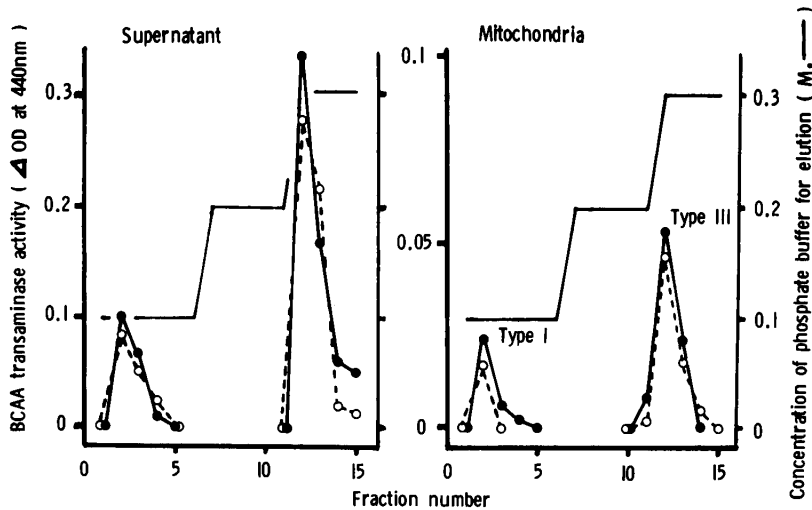


Fig. 4. Isozyme patterns of BCAA transaminase in the brain supernatant and mitochondrial fractions in  $\text{CCl}_4$ -treated rats. Separation of BCAA transaminase isozymes was performed on a DEAE-cellulose column. The isozymes were eluted with 0.1, 0.2 and 0.3 M sodium phosphate buffer. Two-ml fractions were collected.  $\circ$ — $\circ$ , Control and  $\bullet$ — $\bullet$ ,  $\text{CCl}_4$ -treated rats.

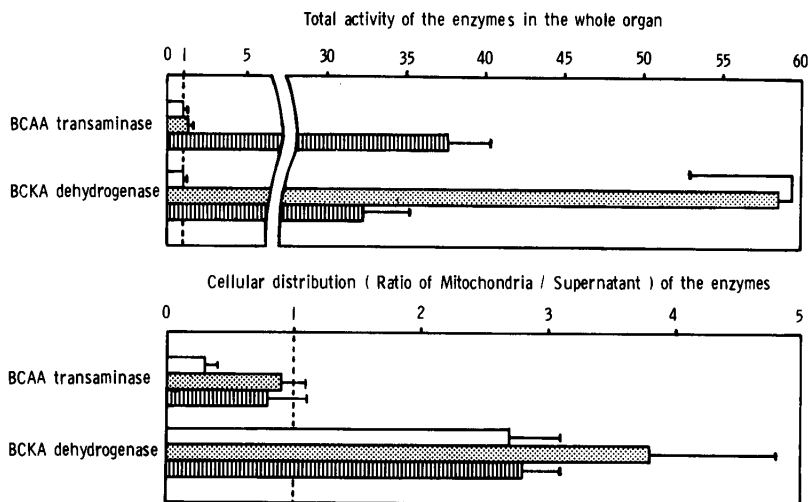


Fig. 5. Total activity of BCAA transaminase and BCKA dehydrogenase in the whole organ, and cellular distribution of the enzymes. Total activity of the enzymes was calculated by weighing the brain and liver and assuming the total weight of skeletal muscle to be 40% of the body weight. Cellular distribution of the enzymes was expressed as the ratio of the mitochondrial fraction to the supernatant fraction. (□), brain, (▨), liver and (▩), skeletal muscle. No. of rats = 3.

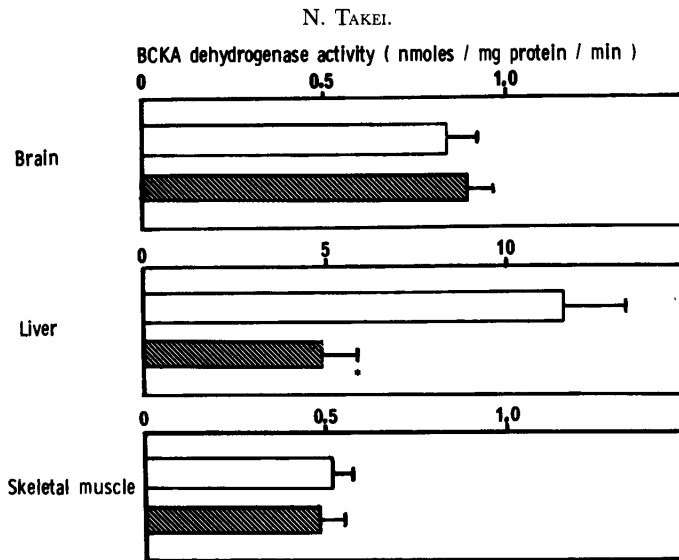


Fig. 6. BCKA dehydrogenase activity in the brain, liver and skeletal muscle homogenates of control (□) and acute hepatic failure rats (▨). The details are as in Fig. 1. No. of rats = 3.

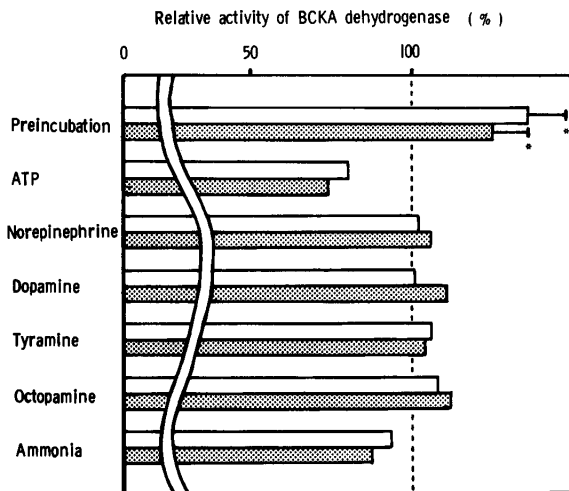


Fig. 7. Effect of various compounds on BCKA dehydrogenase activity. (□), brain and (▨), liver. Preincubation means that the brain and liver homogenates were incubated at 37C for 15min before BCKA dehydrogenase activity was assayed. No. of rats = 3.

their activities were slightly higher in  $\text{CCl}_4$ -treated rats than in control rats. However, no altered isozyme pattern was detected on DEAE-cellulose in  $\text{CCl}_4$ -treated rats (Fig. 4).



Total BCKA dehydrogenase activity was found to be significantly higher in the liver than in the brain and skeletal muscle. The activity was much higher in the mitochondrial fraction of all tissues than in the supernatant fraction (Fig. 5). Total activity of BCAA transaminase of normal rats was highest in the skeletal muscle, and the activity was distributed in both the supernatant and mitochondrial fractions (Fig. 5).

BCKA dehydrogenase activity in the brain and skeletal muscle homogenate was not significantly altered in acute hepatic failure rats, but specific activity of the enzyme was markedly diminished in the liver (Fig. 6).

BCKA dehydrogenase activity in the brain and liver homogenates increased upon preincubation as compared to those without preincubation. The addition of ATP to the brain and liver homogenates resulted in decreased enzyme activity. However, 1 mM of one of the catecholamines, octopamine, tyramine or ammonium acetate, did not alter the enzyme activity (Fig. 7).

#### DISCUSSION

The present study revealed that BCAA catabolism actively takes place in the brain as well as in the skeletal muscle and liver by estimating the activity of BCAA transaminase and BCKA dehydrogenase, the first and second enzymes in BCAA oxidation. Furthermore, increased activity of brain BCAA transaminase and ATP regulation of brain BCKA dehydrogenase were observed in hepatic failure rats, supporting the idea that BCAA catabolism in the brain is accelerated in hepatic failure.

Previous observations (16) showed that tyrosine hydroxylase and aromatic amino acid decarboxylase activity and theoretical dopa contents in the brain of  $\text{CCl}_4$ -injured rats were similar to those in control rats. Similar experimental evidence against the pseudoneurotransmitter hypothesis has accumulated. Zieve and Olsen (17) observed that intraventricular administration of large doses of octopamine failed to produce coma in rats. We have experienced therapeutic efficacy with a special synthetic amino acid solution containing higher amounts of BCAA and lower amounts of AAA and methionine, for hepatic encephalopathy (5). Accelerated transport of BCAA into the brain was demonstrated by determining cerebrospinal fluid (CSF) aminograms of encephalopathic patients with liver cirrhosis who had received an infusion of the BCAA enriched solution (2).

The rate-limiting enzymes for BCAA oxidation, BCAA transaminase and BCKA dehydrogenase, were confirmed to be present in the rat brain (18), and thus active BCAA catabolism might take place in the brain. A previous observation (5), however, suggests that BCAA in the brain is utilized mostly for protein synthesis. There are few studies on BCAA metabolism of the brain, even under normal conditions. Chaplin *et al.* (19) showed that BCAA in the brain is utilized 23 times more for oxidation than for protein synthesis in normal rats. Brain BCAA transaminase activity increased in acute hepatic failure rats, while BCKA dehy-

drogenase activity did not. Accelerated leucine decarboxylation in the brain was observed to be associated with decreased leucine incorporation into brain proteins (20). These changes in leucine metabolism correlated positively with the brain ammonia content. Therefore, BCAA transported into the brain was mostly catabolized, rather than being anabolized to brain proteins in hepatic failure rats. The accelerated catabolism of BCAA in the brain during hepatic failure may reduce the neurotoxicity of ammonia by promoting the synthesis of glutamic acid and glutamine (21). Ammonia transport into the brain may be stimulated in hepatic failure by functional alterations of the blood-brain barrier (22). Ammonia can be incorporated in to the alpha-amido nitrogen of glutamine from glutamic acid, allowing glutamic acid levels to diminish in the brain (13). Low levels of glutamic acid may inhibit the malate-aspartate shuttle and thus lead to the depletion of NADH levels in the astrocyte mitochondria (21). Increased BCAA oxidation due to the enhanced activity of BCAA transaminase may improve ammonia metabolism. BCAA can supply enough glutamic acid for the malate-aspartate shuttle and glutamine synthesis. Mitochondrial supply of NADH by the reaction of BCKA dehydrogenase might also be helpful for ammonia detoxification. The direct relation between accelerated BCAA oxidation and ammonia detoxification in the brain needs to be confirmed by further investigation.

ATP inhibits BCKA dehydrogenase activity in the liver (23). The enzyme activity of the liver supernatant increased after preincubation which caused the ATP concentration to markedly decrease (24). Similar results were confirmed with the brain homogenate. ATP concentrations in the brain from hepatic failure rats are known to be slightly but significantly decreased (25). ATP regulation of BCKA dehydrogenase might contribute to some extent to the accelerated BCAA metabolism in the brain during hepatic failure.

*Acknowledgement.* I wish to thank Prof. Hideo Nagashima and Associate Prof. Akiharu Watanabe for continuously guiding me and giving me many suggestions during this investigation.

#### REFERENCES

1. Fischer, J.E., Yoshimura, N., Aguirre, A., James, J.H. and Cummings, M.G.: Plasma amino acids in patients with hepatic encephalopathy. *Am. J. Surgery* **127**, 40-47, 1974.
2. Watanabe, A., Higashi, T., Shiota, T., Takei, N., Sakata, T., Fujiwara, M., Nakatsukasa, H., Kobayashi, M. and Nagashima, H.: Alterations in neutral amino acid transport across the blood-brain barrier in hepatic failure. *Res. Exp. Med.* **183**, 11-18, 1983.
3. Fischer, J.E. and Baldessarini, R.J.: False neurotransmitters and hepatic failure. *Lancet* **2**, 75-79, 1971.
4. Fischer, J.E., Rosen, H.M., Ebeid, A.M., Abeid, A.M., James, J.H., Keane, J.M. and Soeters, P.B.: The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* **80**, 77-91, 1976.
5. Watanabe, A., Takesue, A., Higashi, T. and Nagashima, H.: Serum amino acids in hepatic encephalopathy -Effects of branched chain amino acid infusion on serum aminogram. *Acta*

- Gastroenterol. Jpn.* **26**, 346-357, 1979.
6. Cardelli-Cangiano, P., Cangiano, C., James, J.E., Jeppsson, B., Brenner, W. and Fischer, J.E.: Uptake of amino acids by brain microvessels isolated from rats after portacaval anastomosis. *J. Neurochem.* **36**, 627-632, 1981.
  7. Higgins, G.M. and Anderson, R.M.: Experimental pathology of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **12**, 186-202, 1931.
  8. Hogeboom, G.H.: Fractionation of cell components of animal tissues. In *Methods in Enzymology*, Vol. I, eds. Colowick, S.P. and Kaplan, N.O. Academic Press, Inc., New York, pp. 16-19, 1981.
  9. Ichihara, A. and Koyama, E.: Transaminase of branched chain amino acids-alpha-ketoglutarate transaminase. *J. Biochem.* **59**, 160-169, 1966.
  10. Stephen, L.R., Robert, D. and Kaji, A.: Changes in leucine aminotransferase isozymes by viral transformation and its correlation with the isozyme changes occurring during differentiation. *Cancer Res.* **37**, 1147-1153, 1977.
  11. Wohlhueter, R.M. and Harper, A.E.: Coinduction of rat liver branched chain alpha-ketoacid dehydrogenase activities. *J. Biol. Chem.* **245**, 2391-2401, 1970.
  12. Lowry, O.H., Rosebrough, N.J. and Farr, A.L.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
  13. Watanabe, A., Shiota, T., Takei, N., Fujiwara, M. and Nagashima, H.: Ammonia detoxification by accelerated oxidation of branched chain amino acids in brains of acute hepatic failure rats. *Hepatology* (in preparation).
  14. Watanabe, A., Higashi, T. and Nagashima, H.: An animal model of fulminant hepatic failure in rat. *Acta Med. Okayama* **33**, 443-450, 1979.
  15. Higashi, T.: Clinical and experimental studies on role of impaired methionine metabolism in pathogenesis of hepatic encephalopathy. *Gastroenterol. Jpn.* **17**, 538-541, 1982.
  16. Takei, N., Watanabe, A., Sakata, T., Hayashi, S., Obata, T., Shiota, T. and Nagashima, H.: Brain tyrosine hydroxylase activity and calculated amount of brain dopa synthesized in carbon tetrachloride-intoxicated rats. *Gastroenterol. Jpn.* **18**, 11-14, 1983.
  17. Zieve, L. and Olsen, R.L.: Can hepatic coma be caused by a reduction of brain noradrenalin or dopamine? *Gut* **18**, 688-691, 1977.
  18. Patel, H.J. and Balazs, R.: Manifestation of metabolic compartmentation during the maturation of the rat brain. *J. Neurochem.* **17**, 955-971, 1970.
  19. Chaplin, E.R., Goldberg, A.L. and Diamond, I.: Leucine oxidation in brain slices and nerve endings. *J. Neurochem.* **26**, 701-707, 1976.
  20. Shiota, T.: Accelerated leucine decarboxylation in the rat brain in relation to increased blood ammonia levels during acute hepatic failure. *Acta Med. Okayama* **38**, 193-205, 1984.
  21. Hindfelt, B., Plum, F. and Duffy, T.E.: Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. *J. Clin. Invest.* **59**, 386-396, 1977.
  22. Ehrlich, M., Plum, F. and Duffy, T.E.: Blood and brain ammonia concentrations after portacaval anastomosis. Effects of acute ammonia loading. *J. Neurochem.* **34**, 1538-1542, 1980.
  23. Odessey, R.: Purification and phosphorylation of branched chain oxoacid dehydrogenase. In *Metabolism and Clinical Implications of Branched Chain amino and Ketoacids*, ed. Walser, M. and Williamson, J.R. Elsevier North Holland, Inc., New York, pp. 23-28, 1981.
  24. Harbhajan, S.P. and Adibi, S.A.: Moderation of mitochondria branched chain alpha Keto acid dehydrogenase activity by ATP. In *Metabolism and Clinical Implications of Branched Chain amino and Ketoacids*, ed. Walser, M. and Williamson, J.R. Elsevier North Holland, Inc., New

York, pp. 91-102, 1981.

25. Hinddfelt, B., Plum, F. and Duffy, T.E.: Effect of acute ammonia intoxication on cerebral metabolism in rats with porta-caval shunts. *J. Clin. Invest.* **59**, 386-396, 1977.