

Acta Medica Okayama

Volume 38, Issue 3

1984

Article 2

JUNE 1984

Accelerated leucine decarboxylation in the rat
brain in relation to increased blood ammonia
levels during acute hepatic failure.

Tetsuya Shiota*

*Okayama University,

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

Accelerated leucine decarboxylation in the rat brain in relation to increased blood ammonia levels during acute hepatic failure.*

Tetsuya Shiota

Abstract

Leucine decarboxylation in rat brain was investigated during acute hepatic failure, induced by partial hepatectomy after carbon tetrachloride (CCl₄) pretreatment of rats. These rats presented metabolic alkalosis, and had significantly higher levels of arterial blood and brain ammonia than control and CCl₄-treated rats. Brain leucine decarboxylation was elevated in rats with hepatic failure. This alteration correlated with arterial blood ammonia concentrations, and probably with elevated brain ammonia levels, as brain ammonia levels were directly related to arterial blood ammonia.

KEYWORDS: leucine, decarboxylation, acute hepatic failure, ammonia

*PMID: 6464803 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 38, (3), 219-225 (1984)

ACCELERATED LEUCINE DECARBOXYLATION IN THE RAT BRAIN IN RELATION TO INCREASED BLOOD AMMONIA LEVELS DURING ACUTE HEPATIC FAILURE

Tetsuya SHIOTA

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

Received December 5, 1983

Abstract. Leucine decarboxylation in rat brain was investigated during acute hepatic failure, induced by partial hepatectomy after carbon tetrachloride (CCl₄) pretreatment of rats. These rats presented metabolic alkalosis, and had significantly higher levels of arterial blood and brain ammonia than control and CCl₄-treated rats. Brain leucine decarboxylation was elevated in rats with hepatic failure. This alteration correlated with arterial blood ammonia concentrations, and probably with elevated brain ammonia levels, as brain ammonia levels were directly related to arterial blood ammonia.

Key words : leucine, decarboxylation, acute hepatic failure, ammonia.

Hepatic encephalopathy is a common, major complication of acute and chronic hepatic failure. This hepato-neurological syndrome is characterized by disturbed consciousness and a high frequency of slow waves in electro-encephalogram (EEG) tracings in addition to severe liver dysfunction (1). Although the pathogenesis of hepatic encephalopathy has not been established firmly, several 'toxic' substances are thought to accumulate in the blood, and their effects on the brain are considered responsible for the neurological abnormalities (2). Ammonia, mercaptans, and fatty acids, among others, have been identified clinically and experimentally as candidates for the toxic substances (3-6).

Besides increased levels of these substances, plasma amino acid imbalances are observed in patients with hepatic failure, and reflect liver damage and a general catabolic state (7). Plasma levels of aromatic amino acids (AAA) (phenylalanine and tyrosine) are elevated in hepatic failure. Increased influx of AAA into the brain may lead to 'false neurotransmitters', an alternate explanation to the 'blood toxin' theory (8). Accelerated neutral amino acid transport into the brain has been observed experimentally and clinically (9, 10), but detailed investigation of brain amino acid metabolism during hepatic insufficiency is lacking. The present study was designed to investigate one of the major metabolic fates of neutral amino acids, leucine decarboxylation, in the rat brain during acute hepatic failure. The effects of impaired ammonia metabolism on these reactions also were examined.

MATERIALS AND METHODS

Chemicals. L-(1- 14 C)-leucine (55 mCi/mmol) were purchased from Amersham Co. (U.K.). Enzymes were purchased from Sigma Chemical Co. (U.S.A.). Other reagents were from Sigma Chemical Co. or Wako Pure Chemical Co. (Japan).

An animal model of acute hepatic failure. Male Sprague-Dawley rats, weighing 200 to 350 g each, were used throughout the study. They were maintained in a constant temperature environment of about 25 °C on Oriental Laboratory Chow MF (Oriental Yeast Co., Japan). To precipitate acute hepatic failure, 20 % carbon tetrachloride (CCl_4) in liquid paraffin (8 ml per kg body weight) was administered intragastrically to overnight-fasted rats. Twenty-four h after the CCl_4 administration, a two-thirds partial hepatectomy was performed by the Higgins and Anderson method (11). The rats were decapitated 12 h after the hepatectomy. Sham operated rats, which were administered liquid paraffin alone, served as controls. Heparinized blood specimens were obtained from the abdominal aorta just before sacrificing. Arterial blood gas was analyzed with a Model 165/2 pH/Blood Gas System (Corning Co., U.S.A.). Plasma was separated for ammonia determination. In the ammonia-loading study, ammonium acetate was injected intraperitoneally at varying doses (1.7 to 5.1 mmol per kg body weight) 12 h following partial hepatectomy. Fifteen min later, the rats were sacrificed by freezing in liquid nitrogen; their brains were fixed *in situ*.

Brain and blood ammonia determinations. Analysis of brain and blood ammonia concentrations was performed according to Folbergrova *et al.* (12). Frozen cerebral tissue was rapidly removed from the skull, and stored at -70 °C until assayed. Frozen specimens, weighing about 50 mg, were transferred to centrifuge tubes and 2 volumes of 0.1 N HCl in 99 % methanol (-20 °C) were added. The samples were crushed with a Teflon pestle, and 5 volumes of ice-cold 0.3 M HClO_4 containing 1 mM EDTA were added. After thorough mixing, this suspension was centrifuged (10,000 rpm in a Hitachi RPR-18 Rotor, 10 min, at 4 °C). The supernatant was neutralized by adding 150 μ l/ml of a solution containing 1.6 N KOH, 0.4 M imidazole, and 0.4 M KCl; and recentrifuged (3,000 rpm in a Hitachi YE Tube-rack, 10 min, 4 °C). Ammonia concentrations of the brain extracts and plasma specimens were determined with an enzymatic kit (Kyowa Medex Co., Japan) (13), in which the reagent mixture consisted of 0.5 ml of sample and 2.6 ml of reactant (100 mM phosphate buffer solution at pH 7.5, 5.4 mM α -ketoglutarate, 0.07 mM $\text{NADPH}_2\text{-Na}_2$, and 12.7 unit/ml glutamate dehydrogenase in 1.2 mM glycerol). Blood ammonia levels were determined within 2 h of sampling.

Leucine decarboxylation. Leucine decarboxylation activity in the rat brain was measured by $^{14}\text{CO}_2$ liberation from L-(1- 14 C)-leucine, with a modification of the method of Paul and Adibi (14). Both cerebral hemispheres, weighing 1.5-1.7 g, were homogenized in a glass tube with a motor-driven glass pestle (10 stroke, 4 °C). The homogenization medium consisted of 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 1 mM ATP, 5 mM MgSO_4 , and 1 mM EDTA. Protein concentrations were determined according to Lowry *et al.* (15). According to preliminary studies, the optimal protein concentration was 4.9 mg/ml (1 : 9 w/v dilution at homogenization). The incubation medium consisted of 137.5 mM NaCl, 1.8 mM MgSO_4 , 0.6 mM KH_2PO_4 , 24.3 mM Na_2HPO_4 , 1.9 mM NaH_2PO_4 (pH 7.4), 2 mM α -ketoglutarate, 4 mM NAD, 0.4 mM L-leucine, 0.5 μ Ci of L-(1- 14 C)-leucine, and 0.25 ml of brain homogenate, in a final volume of 2.5 ml, and contained 1.23 mg of protein. Incubation tubes were sealed with rubber caps and shaken for 60 min (37 °C). To trap liberated $^{14}\text{CO}_2$, 10 $\times$ 25 mm strips of filter paper (No. 2, Toyo Roshi Co., Japan) each containing 50 μ l of NCS Solubilizer were suspended from the rubber caps. The reaction was terminated by adding 0.25 ml of 2.5 N H_2SO_4 . After additional incubation for 1 h, the filter papers were transferred into vials containing 6 ml of

toluene-PPO-POPOP (2000 : 10 : 1, v/w/w). Radio activity was counted in a Beckman scintillation counter (LSC-701, U.S.A.).

RESULTS

Blood and brain ammonia levels. The vital activities of partially hepatectomized rats pretreated with CCl_4 were diminished generally, and responses to pain stimuli were delayed. These rats presented elevated glutamic pyruvic transaminase activities and serum bilirubin concentrations, prolonged prothrombin times, high ampli-

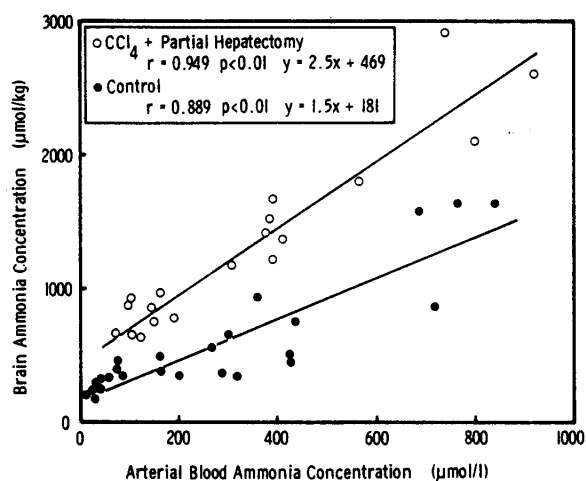


Fig. 1. Correlation between arterial blood and brain ammonia levels in CCl_4 -treated, partially hepatectomized rats and control rats. Doses of ammonium acetate are described in the text.

TABLE 1. ARTERIAL BLOOD AND BRAIN AMMONIA MEASUREMENTS AND ARTERIAL GAS ANALYSES

	Control (4)	CCl_4 (3)	CCl_4 + Partial Hepatectomy (6)
Ammonia			
Blood ($\mu\text{mol/l}$)	40 ± 6	$60 \pm 9^*$	$117 \pm 30^{***} \# \#$
Brain ($\mu\text{mol/kg}$)	263 ± 50	$488 \pm 82^{**}$	$822 \pm 119^{***} \# \#$
Gas Analysis			
pH	7.54 ± 0.05	$7.63 \pm 0.04^*$	$7.73 \pm 0.05^{***} \#$
PO_2 (mmHg)	92.6 ± 17.2	107.0 ± 12.1	$136.1 \pm 6.8^{***} \# \#$
PCO_2 (mmHg)	22.9 ± 2.4	$29.0 \pm 0.8^{**}$	$33.6 \pm 3.5^{***} \#$
HCO_3^- (mEq/l)	19.6 ± 0.8	$33.3 \pm 4.9^{***}$	$46.2 \pm 3.6^{***} \# \#$

Mean $\pm$ SD. () Number of rats.

*, **, *** compared with control ; and #, # # compared with CCl_4 .

* and # $p < 0.05$; ** $p < 0.01$; *** and # # $p < 0.005$.

tude slow waves in EEG, and significant increases in brain water content (data not shown). Marked abnormalities were not detected in control and CCl_4 -treated nonhepatectomized rats. Thus, they were considered to have acute hepatic failure (16).

Arterial blood and brain ammonia levels and arterial blood gas concentrations were measured in these three groups (Table 1). Combined CCl_4 administration and partial hepatectomy induced a three-fold elevation in arterial blood and brain ammonia levels, though CCl_4 administration alone caused only mild elevations.

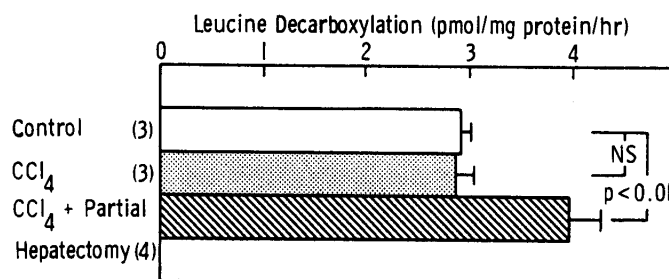


Fig. 2. Leucine decarboxylation in brain homogenates from control, CCl_4 -treated, and acute hepatic failure rats. Horizontal lines at the end of each bar represent SD of the means. The numbers of rats tested are shown in parentheses.

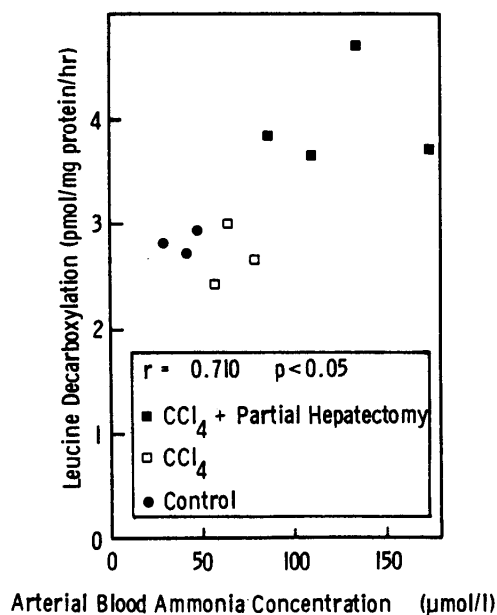


Fig. 3. Correlation between arterial blood ammonia levels and brain leucine decarboxylation.

Blood pH and bicarbonate ion levels increased in the order of control, CCl₄-treated, and hepatic failure rats ; thus, the degree of metabolic alkalosis was in proportion to the severity of hepatic insufficiency.

In order to investigate the correlation between arterial blood and brain ammonia levels, various doses of an aqueous ammonium acetate solution were injected intraperitoneally into the control (1.7 to 5.1 mmol per kg body weight) and hepatic failure (1.7 — 3.4) rats. A strong correlation between blood and brain ammonia levels was observed in both groups (Fig. 1). The regression line of acute hepatic failure rats was steeper. In hepatic failure rats, brain ammonia contents were much higher than in controls even at similar blood concentrations.

Decarboxylation of leucine. Leucine decarboxylation was assayed in the brain homogenates from the three groups (Fig. 2). There was no difference in the rates between control and CCl₄-treated rats. However, CO₂ production in the homogenates from the hepatic failure rats was increased by 36 %. Arterial blood ammonia levels were correlated positively with leucine decarboxylation (Fig. 3).

DISCUSSION

In hepatic failure, severely deteriorated liver function and portal-systemic shunting result in the accumulation of several toxic substances in the circulating blood. These toxic substances, such as mercaptans, fatty acids, and especially ammonia, are thought to be involved in the pathogenesis of hepatic encephalopathy (3-6). However, pharmacological doses of ammonia far in excess of those achieved during liver failure are required to cause experimental encephalopathy (17). Because blood ammonia levels correlate poorly with the severity of hepatic encephalopathy (18), synergistic effects of various toxins with ammonia are postulated to explain the pathogenesis of encephalopathy (19, 20).

Plasma amino acid imbalances are also observed in hepatic failure. Fischer *et al.* (21) suggested that increased entry of plasma AAA into the cerebral environment causes high levels of brain octopamine and tyramine. The explanation of hepatic encephalopathy as due to impaired amino acid metabolism is called the 'false neurotransmitter theory' (8). Intraventricular infusion of octopamine in rats resulted in a 20,000-fold increase in that amine, and a 90 % reduction in brain noradrenaline and dopamine in the absence of coma (22).

Branched chain amino acid (BCAA)-enriched infusions to encephalopathic patients improve their condition (10). The metabolic fates of BCAA in the brain seem to be involved in the pathogenesis or maintenance of hepatic encephalopathy. As to why BCAA infusion improves the neurologic condition, previous authors have reasoned that elevated plasma BCAA levels competitively inhibit the cerebral uptake of AAA, decreasing false neurotransmitters in the brain (8, 21).

In the present study, it was shown that leucine oxidation activities were increased in proportion to blood ammonia levels in rats with acute hepatic failure, and thus the hyperammonemic state probably accelerated leucine oxidation in

the brain homogenates. Leucine is first converted to α -isocaproic acid, which liberates CO_2 in the next step, producing glutamate from α -ketoglutarate in a transamination reaction involving BCAA transaminase (23, 24). Increased leucine oxidation may aid in the detoxification of ammonia, because the glutamate produced by transamination is converted to glutamine by combination with an ammonia molecule. This is the major pathway of ammonia removal in the brain, which lacks the urea cycle. Improvement of hepatic encephalopathy after infusion of a BCAA-enriched solution might be, in part, due to removal of brain ammonia facilitated by BCAA oxidation. The fact that ammonia levels of postmortem brains of cirrhotics, who were treated with high doses of valine, were significantly lower than those in untreated cirrhotics (25) offers clinical support for this hypothesis.

Acknowledgement. I wish to express my sincere thanks to Prof. Hideo Nagashima and Associate Prof. Akiharu Watanabe for continuously guiding me and giving me valuable suggestions during this investigation.

REFERENCES

1. Sherlock, S.: *Diseases of the Liver and Biliary System*. Blackwell, Oxford, pp. 91-106, 1981.
2. Crossley, I.R., Wardle, E.N. and Williams, R.: Biochemical mechanisms of hepatic encephalopathy. *Clin. Sci.* **64**, 247-252, 1983.
3. Walker, C.O. and Schenker, S.: Pathogenesis of hepatic encephalopathy with special reference to the role of ammonia. *Am. J. Clin. Nutr.* **23**, 619-633, 1970.
4. McClain, C.J., Zieve, L., Doizaki, W., Gilbertstadt, S. and Onstad, G.: Mercaptans in portal systemic encephalopathy (PSE) due to alcoholic liver disease. *Gastroenterology* **74**, 1064, 1978.
5. Zieve, L., Nicoloff, D.M. and Mahadevan, V.: Effect of hepatectomy on volatile free fatty acids of the blood. *Gastroenterology* **54**, 1285, 1968.
6. Fagner, P., Delorme, M.L., Dennis, L., Boschat, M. and Oporon, P.: Demonstration of middle molecules in plasma and brain during experimental acute hepatic encephalopathy. *Gastroenterology* **79**, 1014, 1980.
7. Watanabe, A., Takesue, A., Higashi, T. and Nagashima, N.: Serum amino acids in hepatic encephalopathy—Effects of branched chain amino acid infusion on serum aminogram. *Acta Hepato-Gastroenterol.* **26**, 346-357, 1979.
8. Fischer, J.E. and Baldessarini, R.J.: Pathogenesis and therapy of hepatic coma. In *Progress in Liver Diseases*, vol. V, ed. H. Popper and F. Schaffner, Grune and Stratton, New York, pp. 363-397, 1975.
9. Cardelli-Cangiano, P., Cangiano, C., James, J.H., 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.
10. Higashi, T., Watanabe, A., Hayashi, S., Obata, T., Takei, N. and Nagashima, H.: Effect of branched-chain amino acid infusion on alterations in CSF neural amino acids and their transport across the blood-brain barrier in hepatic encephalopathy. In *Metabolism and Clinical Implications of Branched-Chain Amino and Ketoacids*, ed. M. Walser and R. Williamson, Elsevier North Holland, New York, pp. 452-463, 1981.
11. Higgins, G.M. and Anderson, R.M.: Experimental pathology of the liver; I. Restoration

- of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **12**, 186-202, 1931.
12. Folbergrova, J., Passonneau, J.V., Lowry, O.H. and Schulz, D.W.: Glycogen, ammonia and related metabolites in the brain during seizures evoked by methionine sulfoximine. *J. Neurochem.* **16**, 191-203, 1969.
 13. Mondzac, A., Ehlich, G.E. and Seegmiller, J.E.: An enzymatic determination of ammonia in biological fluids. *J. Lab. Clin. Med.* **66**, 526-531, 1965.
 14. Paul, H.S. and Adibi, S.: Assessment of effect of starvation, glucose, fatty acids and hormones on α -decarboxylation of leucine in skeletal muscle of rat. *J. Nutr.* **106**, 1079-1088, 1976.
 15. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
 16. Watanabe, A., Higashi, T. and Nagashima, H.: An animal model of fulminant hepatic failure in the rat. *Acta Med. Okayama* **33**, 443-450, 1979.
 17. Zieve, L. and Doizaki, W.M.: Brain and blood methanethiol and ammonia concentrations in experimental hepatic coma due to injections of various combinations of these substances. *Gastroenterology* **79**, 1070, 1980.
 18. Zieve, L. and Nicoloff, D.M.: Pathogenesis of hepatic coma. *Ann. Rev. Med.* **26**, 143-157, 1975.
 19. Zieve, L., Doizaki, W.M. and Zieve, F.J.: Synergism between mercaptans and ammonia or fatty acids in the production of coma: A possible role for mercaptans in the pathogenesis of hepatic coma. *J. Lab. Clin. Med.* **83**, 16-28, 1974.
 20. Higashi, T.: Impaired metabolism of methionine in severe liver diseases. II. Clinical and experimental studies on role of impaired methionine metabolism in pathogenesis of hepatic encephalopathy. *Gastroenterol. Jpn.* **17**, 84-93, 1982.
 21. Fischer, J.E., Rosen, H.M., Ebeid, A.M., James, J.H. and Keane, J.M.: The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* **80**, 77-91, 1976.
 22. Zieve, L. and Olsen, R.L.: Can hepatic coma be caused by a reduction of brain noradrenaline or dopamine? *Gut* **18**, 688-691, 1977.
 23. Benuck, M., Stern, F. and Lajtha, A.: Regional and subcellular distribution of aminotransferases in rat brain. *J. Neurochem.* **19**, 949-957, 1972.
 24. Meister, A.: *Biochemistry of the Amino Acids*, 2nd Edition, Volume II. Academic Press, New York, pp. 729-757, 1965.
 25. Weiser, M., Riederer, P. and Kleinberger, G.: Human cerebral free amino acids in hepatic coma. *J. Neural Transm. (Suppl.)* **14**, 95-102, 1978.