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Plasma branched chain amino acid abnormalities in sake-treated rats.

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Abstract

Plasma amino acid abnormalities in rats treated with large doses of sake and whisky for 3 days were investigated under adequate nutritional conditions. A significant decrease in plasma branched-chain amino acid (BCAA) levels was observed in sake- but not whisky-treated rats. However, known factors affecting BCAA levels, such as serum insulin and plasma glucagon levels ahd BCAA-metabolizing enzyme (BCAA transaminase and branched chain alpha-ketoacid dehydrogenase) activities in the liver and skeletal muscle, were not significantly altered in the sake group. Furthermore, ethanol-metabolizing enzyme (alcohol and aldehyde dehydrogenases and the microsomal ethanol-oxidizing system) activities in the liver were not altered in the sake group. Other mechanisms need to be considered for explaining the diminished levels of plasma BCAA in sake-treated rats.

KEYWORDS: branched chain amino acid, alcohol, sake, whisky, insulin, glucagon

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—— BRIFE NOTE ——

PLASMA BRANCHED CHAIN AMINO ACID ABNORMALITIES IN SAKE-TREATED RATS

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Abstract. Plasma amino acid abnormalities in rats treated with large doses of sake and whisky for 3 days were investigated under adequate nutritional conditions. A significant decrease in plasma branched-chain amino acid (BCAA) levels was observed in sake- but not whisky-treated rats. However, known factors affecting BCAA levels, such as serum insulin and plasma glucagon levels ahd BCAA-metabolizing enzyme (BCAA transaminase and branched chain alpha-ketoacid dehydrogenase) activities in the liver and skeletal muscle, were not significantly altered in the sake group. Furthermore, ethanol-metabolizing enzyme (alcohol and aldehyde dehydrogenases and the microsomal ethanol-oxidizing system) activities in the liver were not altered in the sake group. Other mechanisms need to be considered for explaining the diminished levels of plasma BCAA in sake-treated rats.

Key words : branched chain amino acid, alcohol, sake, whisky, insulin, glucagon.

A decrease in plasma branched chain amino acid (BCAA) concentrations and an increase in α-amino-n-butyric acid (AANB) levels have been reported in alcoholics with and without liver disease (1-3). Alcohol consumption, nutritional abnormalities and liver injury, which were frequently observed in the chronic alcoholics, might have contributed to the observed abnormalities. We have reported plasma amino acid abnormalities including a decrease in BCAA levels and increase in aromatic amino acid (AAA), methionine and proline concentrations in patients with alcoholic cirrhosis (4). To evaluate the role of alcoholic consumption on plasma amino acid abnormalities in alcoholics, plasma amino acid levels were determined in rats given large doses of sake, a popular Japanese alcoholic beverage, and whisky for 3 consecutive days under adequate dietary conditions. Several factors affecting amino acid metabolism, blood insulin and glucagon concentrations and BCAA-metabolizing enzyme activities in the liver and skeletal muscle were also determined in the alcoholic beverage-treated rats.

Male Sprague-Dawley rats, weighing 200-300 g, were maintained in separate cages on Oriental Laboratory Chow MF and water ad libitum in a room kept at 23 ± 2 ℃ on a 12 h : 12 h light/dark cycle (lights on 07 : 00-19 : 00). Sake

(Gekkeikan, first class, original concentration of 17 % ethanol, Okura Shuzo Co. Ltd., Japan) and whisky (Cutty Sark, special class, 43 % ethanol, Cutty Sark Co., Ltd., Scotland) were administered intragastrically at a dose of 6.7 g ethanol per kg body weight (52.5 ml per kg body weight) in the early morning for 3 consecutive days. An equivalent volume of physiological saline was administered similarly to control rats. Rats got drunk in the daytime but took the diet at night. The dietary intake and body weight were checked every morning, and average daily intake of major nutrients (calories, protein, amino acid and fat) were calculated according to the standard food table. Rats were sacrificed by exsanguination from the abdominal aorta in the morning of the 4 th day of the experiment after 12 h's starvation. Quantitative determinations of plasma amino acid contents were carried out by ion-exchange chromatography with a Hitachi amino acid analyzer Type 034 (Japan) (5). Liver and skeletal muscle tissuse were homogenized in 9 vol of a 0.25 M sucrose solution immediately after sacrifice. BCAA transaminase (EC 2. 6. 1. 6) and branched chain α-ketoacid (BCKA) dehydrogenase (EC 1. 2. 4. 4) activities were determined as previously described (6) and expressed as nmoles of product formed per min per mg protein, and nmoles of product formed per h per g wet tissue, respectively. Serum insulin and plasma glucagon (OAL-123, glucagon antibody) determinations were performed at Otsuka Assay Laboratory by radioimmunoassay. Serum glucose level was determined by an oxidase method (7). Alcohol dehydrogenase (ADH, EC 1. 1. 1. 1) and aldehyde dehydrogenase (ALDH, EC 1. 2. 1. 3) activities in the liver supernatant and mitochondrial fractions were assayed spectrophotometrically at 340 nm (8). The microsomal fraction was used for determination of the activities of the microsomal ethanol-oxidizing system

TABLE 1. PLASMA NEUTRAL AMINO ACID CONCENTRATIONS IN ALCOHOL BEVERAGE-TREATED RATS

	Saline (4)	Sake (4)	Whisky (3)
Nutritional parameter			
Energy (kcal/day)	10 ± 2 (4)	8 ± 1 (4)	8 ± 1 (3)
Body weight change (% of the initial weight)	102 ± 4 (4)	90 ± 7 (4)	91 ± 6 (3)
% Liver weight	4.2 ± 0.8 (9)	3.7 ± 0.5 (8)	4.0 ± 0.3 (6)
Amino acid (μ moles/l)			
Valine	219 ± 24 (4)	131 ± 21** (4)	279 ± 130 (3)
Isoleucine	97 ± 12 (4)	61 ± 9** (4)	127 ± 52 (3)
Leucine	170 ± 14 (4)	112 ± 18** (4)	224 ± 75 (3)
Methionine	65 ± 12 (4)	42 ± 5** (4)	48 ± 15 (3)
Tyrosine	68 ± 12 (4)	52 ± 3* (4)	77 ± 36 (3)
Phenylalanine	62 ± 2 (4)	56 ± 9 (4)	74 ± 9 (3)

Values are given as the mean ± SD obtained with the number of rats given in parentheses. The asterisks denote $p < 0.05$ (*) and $p < 0.01$ (**) over the controls.

(MEOS) and the content of cytochrome P- 450 (8). The activities were expressed as nmoles of product formed per min per mg protein.

The average body weight during the experiment of alcohol rats diminished slightly from the initial body weight by 10 % in sake-treated rats and similarly by 9 % in whisky-treated rats. The average body weight was not diminished in controls (103 %) (Table 1). Daily energy intake was slightly and unsignificantly less in the alcoholic beverage rats than control rats, although 4.7 kcal of energy per 100 g body weight was derived daily from ethanol (1 g ethanol = 7 kcal). Energy intake from sugar and amino acid in the ingested sake (carbohydrate 5 g, protein 0.5 g and amino nitrogen 31 mg per 100 g sake) was less than 1 % of the total daily calory intake. Thus, there was no significant difference in the nutrient intake between sake- and whisky-treated rats (energy 35 kcal, protein 1.9 g and fat 0.3 g/day in both groups). Amino acid intake during the experiment also was not different between alcoholic beverage groups (Val, 775 μ moles/day in the sake group vs 771 in the whisky group; Ile, 631 vs 628, and Leu, 1104 vs 1098).

Plasma neutral amino acid levels were significantly decreased in the sake-treated group, as compared to the control group. However, similar observations were not found in whisky group. Serum proline and lysine concentrations were also slightly decreased in the sake group, but other amino acids such as arginine and histidine were not altered. Thus, the BCAA/AAA ratio was significantly lowered in this group. Plasma AANB levels were not increased in either alcoholic beverage group (date not shown), although the levels of AANB can be used as an empirical biochemical marker for alcoholism (2). BCAA may serve as a significant energy source for the skeletal muscle (9). Dietary protein deficiency results in depressed BCAA and AANB, the magnitude of this depression is related to the duration and severity of the protein deficiency (10). Severe and long-term protein deficiency cannot be considered in the present experiment, and plasma AANB levels were not decreased in either alcoholic beverage group. Therefore, the significant decrease in plasma BCAA levels observed only in the sake group is not due to dietary or protein deficiency. Serum bilirubin concentration, GOT (EC 2. 6. 1. 1) and γ-glutamyl transpeptidase (EC 2. 3. 2. 1) activities were not increased in alcoholic beverage rats. The ratios of liver weight/body weight at the time of sacrifice did not significantly change in the sake and whisky groups. These results suggest that alcohol-induced liver injury did not occur to a great extent under the present experimental conditions. Serum insulin levels in the whisky group were significantly lower than those in the sake and control groups (Table 2). Plasma glucagon levels were higher in the whisky group than those in the control group; the ratio of IRI/IRG was significantly low in the whisky group. Blood glucose levels in all groups were within normal limits. A correlation between plasma BCAA levels and serum insulin levels was not found in either sake ($r = -0.144$) or the whisky ($r = -0.281$) groups. Also, no correlation was observed in the case of glucagon ($r = -0.540$ and -0.766). A close relationship between

TABLE 2. SEVERAL FACTORS INFLUENCING THE PLASMA NEUTRAL AMINO ACID LEVELS IN ALCOHOL BEVERAGE-TREATED RATS

	Saline	Sake	Whisky
Pancreatic hormone			
IRI (nU/ml)	43 ± 28 (4)	46 ± 18 (4)	24 ± 6 (3)
IRG (pg/ml)	127 ± 64 (4)	108 ± 27 (4)	329 ± 160 (3)
Alcohol-metabolizing enzymes (nmoles/mg protein)			
ADH (Cytosole)	286 ± 64 (4)	304 ± 93 (3)	318 ± 81 (3)
ALDH (Mitochondrial)	47 ± 4 (4)	57 ± 14 (3)	51 ± 2 (3)
MEOS	4.6 ± 0.4 (4)	5.1 ± 1.1 (3)	5.0 ± 1.4 (3)
Cytochrome P-450	1.01 ± 0.35 (9)	1.14 ± 0.38 (8)	0.80 ± 0.44 (6)
BCAA-metabolizing enzymes			
BCAA transaminase (nmoles/mg protein)			
Muscle	8.7 ± 1.1 (4)	8.8 ± 1.3 (4)	8.1 ± 6.5 (3)
Liver	6.1 ± 0.6 (4)	0.5 ± 1.0 (4)	6.5 ± 1.8 (3)
BCKA dehydrogenase (nmoles/g tissue/h)			
Muscle	291 ± 44 (4)	287 ± 10 (4)	292 ± 29 (3)
Liver	3847 ± 759 (4)	2460 ± 169 (4)*	2723 ± 296 (3)

Values are given as the mean ± SD obtained with the number of rats given in parentheses. The asterisks denote $p < 0.05$ over controls.

amino acid imbalances and glucagon and insulin concentrations was suggested by Soeters *et al.* (11), but many recent reports (12, 13) are rather contrary to this proposal. BCAA transaminase activities in the liver mitochondria and the skeletal muscle homogenate were not altered in either the sake or the whisky groups. However, BCKA dehydrogenase activities in the liver mitochondria were somewhat diminished in the sake and whisky groups, suggesting some mitochondrial injury. Therefore, the reason why BCAA levels were decreased only in the sake group can not be explained by these known factors which affect BCAA metabolism. The changes might be derived from ethanol metabolism itself in the alcohol beverage groups. However, liver supernatant ADH and mitochondrial ALDH activities were not altered in the sake and whisky groups, and MEOS activity was also not changed. These results suggest that ethanol metabolism following 3 days' treatment with alcohol beverages can not be altered under these conditions. The mechanism of sake-related decreases in plasma BCAA is unkown. β-Phenethyl alcohol, an aromatic alcohol, is abundant in sake at an approximate concentration of 75 mg/l (14), but is scarce in whisky. We have reported that carbon tetrachloride-induced hepatotoxicity was potentiated by pretreatment with β-phenethyl alcohol (15). β-Phenethyl alcohol, n-propanol, and other unanalyzed components (a homolog of ethanol), present in sake may be involved in the decrease in plasma BCAA. The BCAA-decreasing effect might be mediated via some stress mechanism (16). However, one has to consider that a stress reaction is accentuated in some way by sake.

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