Note

Effects of Amino Acids on Alcohol Intake in Stroke-Prone Spontaneously Hypertensive Rats

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Summary Examination was made of the effects of amino acids on alcohol intake in stroke-prone spontaneously hypertensive rats (SHRSP). The animals were divided into two groups according to dietary protein level, normal (15%) and low (5%). The two groups were further divided into two groups according to whether they selected the ethanol solution containing amino acids (non-amino acid group and amino acid group). The 5% ethanol solutions with and without various amino acids were prepared in a water-supplying tube to which the animals had free access for 40 days. The 5% ethanol solution intake in rats fed the normal protein diet was higher than that of the low protein group. Regardless of dietary protein level, 5% ethanol solution intake increased in the amino acid group. Intake of the 5% ethanol solution containing 100mM L-proline, 100mM L-lysine, and 100mM L-threonine was large. For the amino acid group of rats fed normal protein and low protein, plasma glutamate oxalacetate transaminase (GOT) activity was significantly reduced. It is suggested that the alcohol intake may increase by adding amino acid to the alcohol solution.

Key Words amino acids, alcohol intake, stroke-prone spontaneously hypertensive rats (SHRSP), dietary protein level, glutamate oxalacetate transaminase (GOT)

The relationship between taste preference and nutritional state should be investigated. The preference for alcohol was previously found to depend not only on genetic factors, but nutritional status as well. That is, preference for alcohol was higher in spontaneously hypertensive rats (SHR) on a high protein diet than in SHR fed a low protein diet (1). The disappearance of ethanol in blood of rats fed the high protein diet was earlier than that of rats fed the low protein diet following the oral administration of ethanol (1). The efficiency of alcohol metabolism may
thus be reduced owing to protein deficiency. The fed a low protein diet rats were in a condition of essential amino acid insufficiency. The ingestion of essential amino acids may thus be associated with the amount of alcohol intake. Kawada et al. reported that rats ingested both alanine and glutamine due to amelioration and prevention of impairment symptoms induced by the chronic excessive intake of ethanol (2). Also, many investigators (Widmark (1933), Smith and Newman (1959), Kalant (1967), Ward and Jarowski (1971), and Dorato, Lynch, and Ward (1977)) have reported interactions between alcohol and amino acids or protein in biological system (3). These facts suggested that the ingestion of amino acids could have nutritional and physiological functions for rats that consume alcohol.

In the present study, the effects of amino acids on the amount of alcohol intake and hepatic function were investigated for rats on a normal protein or low protein diet with only ethanol solution available for drinking.

Materials and methods. Male stroke-prone spontaneously hypertensive rats (SHRSP, from Simane Medical University (Prof. Yukio Yamori)), 8 weeks old, weighing about 215 g were used. They were divided into two groups \((n = 7)\) according to dietary protein level, 15\% (normal) and 5\% (low) purified whole egg protein (Q. P. Corporation, Tokyo). The two groups were further divided into two groups \((n = 7)\) according to whether they could select 5\% ethanol solution that either contained or did not contain various amino acids (non-amino acid and amino acid groups, respectively). In this present study, the design of behavioral test refers to the experimental method of taste preference which was developed by Torii (Fig. 1) (4). Rats of the non-amino acid group were allowed to ingest 5\% ethanol solution without any l-amino acid. An ethanol solution of 5\% is the most preferable for growing rats; thus, this level was employed in the present study (5). In choice paradigm, the rats had free access to all 5\% ethanol solutions containing different

![Fig. 1. The design of the preference test in SHRSP. Rats \((n = 7)\) were housed together in stainless steel wire-bottom cages throughout the experimental period. One bottle was set up on the cage for the group which was allowed to ingest 5\% ethanol solution (non-amino acid group). Fifteen bottles were set up on the cage for the group which was allowed a free choice among 5\% ethanol solutions containing various amino acids (amino acid group).](image-url)
L-amino acids. The following L-amino acids were added: alanine, glutamine, glutamic acid, proline, and ten essential amino acids for growing rats (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Their concentrations were 100 mM but solutions of amino acids for which 100 mM was not possible, such as glutamic acid (34 mM), arginine (50 mM), histidine (50 mM), isoleucine (50 mM), leucine (75 mM), phenylalanine (45 mM), and valine (35 mM), were prepared at the highest concentration possible (2, 5). Intake of drinking water was assessed using identical drinking tubes of graduated cylinders. The animals were housed in groups of 7 throughout the experiment in stainless steel wire-bottom cages at controlled temperature (25 ± 2°C) and relative humidity (50 ± 5%), with a 12 h light/dark cycle. The consumption of each solution was measured every day and body weight was measured weekly.

Forty days after the onset of behavioral experiment, the animals were fasted overnight and sacrificed by ether anesthesia. Blood was collected from the abdominal aorta in a disposable plastic syringe coated with heparin and transferred to centrifuge tubes and centrifuged at 1,800 × g for measurement of plasma transaminase activity, using a Transaminase CII Test (Wako Pure Chemical Industries, Osaka)(6). Statistical analysis of data was conducted by using the Student's t-test.

Results. Body weight change in rats fed the experimental diets is shown in Fig. 2. Body weight gain in rats fed the normal protein diet was higher than that of rats fed the low protein diet, regardless of amino acid content.

The 5% ethanol solution intake (ml/5 days/cage/100 g BW) in normal protein rats was higher than that of low protein rats (Table 1). In rats in choice paradigm, it was higher than that of the non-amino acid group, regardless of dietary protein level (Table 1).

The intake of 5% ethanol solutions containing various amino acids is shown in Table 2. The intake of 5% ethanol solution containing L-proline was highest in...
Table 1. Effects of dietary protein level and amino acids on 5% ethanol solution intake in SHRSP.

| Experimental group          | Intake (ml/5 days/cage/100 g BW) |
|-----------------------------|----------------------------------|
| Normal protein diet         |                                  |
| Non-amino acid group\(^a\)  | 441.4 ± 30.6                     |
| Amino acid group\(^b\)      | 573.6 ± 22.8*                    |
| Low protein diet            |                                  |
| Non-amino acid group\(^a\)  | 283.1 ± 16.7**                   |
| Amino acid group\(^b\)      | 340.6 ± 15.2***                  |

Values represent mean ± SE (5 days intake of 7 rats/cage). \(^a\) The group was allowed to ingest 5% ethanol solution. \(^b\) The group was allowed a free choice of 5% ethanol solutions containing various amino acids. * \(p<0.01\), compared with non-amino acid group of normal diet protein. ** \(p<0.001\), compared with each group of normal protein diet. *** \(p<0.05\), compared with non-amino acid group of normal protein diet. \(p<0.001\), compared with amino acid group of normal protein diet. \(p<0.05\) compared with non-amino acid group of low protein diet.

Table 2. Intake of each 5% ethanol solutions containing various amino acids in choice paradigm in SHRSP.

| Diet | \(E^b\) (ml/5 days/cage/100 g BW) | \(E/T^c\) (%) |
|------|----------------------------------|---------------|
|      | Normal protein                  | Low protein   | Normal protein | Low protein |
| 1.   | Arg 12.6                         | 1.4           | 2.2           | 0.4         |
| 2.   | His 8.6                          | 1.4           | 1.5           | 0.4         |
| 3.   | Ile 22.4                         | 42.9          | 3.9           | 12.6        |
| 4.   | Leu 10.9                         | 1.7           | 1.9           | 0.5         |
| 5.   | Lys 57.4                         | 74.3          | 10.0          | 21.8        |
| 6.   | Met 14.9                         | 9.5           | 2.6           | 2.8         |
| 7.   | Phe 37.9                         | 1.0           | 6.6           | 0.3         |
| 8.   | Thr 54.5                         | 50.4          | 9.5           | 14.8        |
| 9.   | Trp 8.0                          | 8.2           | 1.4           | 2.4         |
| 10.  | Val 9.8                          | 1.4           | 1.7           | 0.4         |
| 11.  | Ala 37.3                         | 23.5          | 6.5           | 6.9         |
| 12.  | Gln 70.5                         | 6.8           | 12.3          | 2.0         |
| 13.  | Glu 4.6                          | 2.0           | 0.8           | 0.6         |
| 14.  | Pro 211.6                        | 111.7         | 36.9          | 32.8        |
| 15.  | Vehicle\(^a\)                   | 12.6          | 4.4           | 2.2         |

\(^a\) 5% ethanol solution did not contain any amino acid. \(^b\) The intake of each ethanol solution (5 days intake of 7 rats/cage). \(^c\) Each ethanol solution intake is given as a percentage of total consumption.

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Table 3. Effects of dietary protein level and amino acids on plasma transaminase activity in SHRSP.

| Experimental group       | GOT (IU/liter, 25°C) | GPT (IU/liter, 25°C) |
|--------------------------|----------------------|---------------------|
| Normal protein diet      |                      |                     |
| Non-amino acid group     | 105.4±0.4            | 38.5±1.5            |
| Amino acid group         | 98.8±0.2*            | 39.6±0.7            |
| Low protein diet         |                      |                     |
| Non-amino acid group     | 105.5±0.6            | 37.6±0.6            |
| Amino acid group         | 99.7±0.1*            | 38.4±0.9            |

Values represent mean±SE of 7 rats in each group. GOT, glutamate oxalacetate transaminase; GPT, glutamate pyruvate transaminase. *p<0.05, compared with non-amino acid group of each protein level.

both groups of rats fed the normal and low protein diets. With the exception of proline, rats fed the normal protein diet largely ingested ethanol solution containing glutamine, lysine, and threonine. On the other hand, rats fed the low protein diet ingested solutions containing lysine, threonine and isoleucine.

Plasma transaminase activity is shown in Table 3. No change in plasma glutamate oxalacetate transaminase (GOT) activity was observed between the normal protein and low protein groups. In both groups, plasma GOT activity decreased with amino acid ingestion. Plasma glutamate pyruvate transaminase (GPT) activity did not change among the four experimental groups.

Discussion. The results of the present study show that rats ingest more ethanol solution containing amino acids than ethanol solution without amino acids. That is, the alcohol intake may increase by adding amino acids to the alcohol solution (Table 1). A large intake of alcohol is considered to possibly cause malnutrition by displacing other nutrients in a high alcohol content diet, leading possibly to liver disease (i.e. fatty liver, hepatocirrhosis etc.), hyperlipemia, hyperuricemia, and ketoacidosis (7). Torii et al. suggested that amino acids supplementation many possibly prevent malnutrition and metabolic disorder due to chronic alcohol intake (2, 8).

The individual intake of 5% ethanol solution containing each amino acid was found to differ, and both normal and low protein groups showed large intake of ethanol solution with proline, lysine, and threonine. Concerning the relationship between the alcohol metabolism and amino acids, Tieden et al. suggested that alanine and glutamine abundantly supply pyruvic acid that performs the NAD-generating system and thus alcohol metabolism itself can readily progress to the TCA cycle (9, 10). Ethanol oxidation has been shown to be accelerated in the presence of certain L-amino acids, such as proline and lysine (11–14). Proline, lysine and threonine content in ethanol solution may possibly be correlated with

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the capacity for alcohol metabolism in the liver (i.e. elevated alcohol dehydrogenase activity and acetaldehyde dehydrogenase activity). It was also reported that L-lysine lowered blood ethanol levels when ethanol was administered orally, and this decrease in blood ethanol could be the result of poor absorption or increased the elimination (12, 13). To elucidate the role of amino acids (especially proline, lysine, and threonine) in alcohol metabolism in rats on chronic alcohol intake, further study will be required.

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