Comparative Study of Alcohol Metabolism in Stroke-Prone Spontaneously Hypertensive Rats and Wistar-Kyoto Rats Fed Normal or Low Levels of Dietary Protein

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Summary Our previous experiments have shown that the appetite or preference for alcohol is affected by the rat strain and nutritional status, such as dietary protein levels. To determine the affected factors in alcohol preference, the alcohol metabolism in SHRSP (stroke-prone spontaneously hypertensive rats) and WKY (Wistar-Kyoto) rats fed with the standard level (15%) or low level (5%) purified egg protein diet (PEP) was investigated. The animals were kept on the experimental diets for 4 weeks. After 12 h fasting, a 15% ethanol solution was given in a dose of 100 mg ethanol per 100 g body weight with a gastric probe to all animals and the blood ethanol and acetaldehyde levels were determined. Compared with 15% PEP diet-fed SHRSP, WKY showed higher levels of blood ethanol and acetaldehyde. Furthermore, the same results were also observed in SHRSP and WKY fed with 5% PEP diet. On the other hand, regardless of the rat strain, rats fed a low level protein diet showed higher blood ethanol and acetaldehyde levels. We also found that there was no significant change in alcohol dehydrogenase (ADH) activity and acetaldehyde dehydrogenase (ALDH) activity between SHRSP and WKY. However, both SHRSP and WKY fed a 15% PEP diet showed higher ADH and ALDH activity compared with rats fed the 5% PEP diet. These results suggested that the affected factors of preference for alcohol may be correlated with blood ethanol and acetaldehyde levels after alcohol intake.

Key Words appetite or preference for alcohol, stroke-prone spontaneously hypertensive rats (SHRSP), Wistar-Kyoto rats (WKY), dietary protein levels, blood ethanol and acetaldehyde levels, alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH)

There is increasing interest in the genetic factors that determine alcohol preference in animals. The importance of genetic factors has been demonstrated in both mouse and rat strains (1-3). One of the most extensively studied animal
models of human essential hypertension is the spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki, by means of selective inbreeding in a colony of Wistar-Kyoto rats (WKY). Furthermore, rats which show the spontaneously stroke-prone disease were also selectively inbred from SHR, named stroke-prone spontaneously hypertensive rats (SHRSP). Previously, we demonstrated that SHR showed a higher alcohol preference compared with WKY, Sprague-Dawley rats (SD) and Wistar-slc rats (4). On the other hand, we also found that the preference for alcohol seemingly depends on nutritional factors, and the ethanol intake of rats fed a high protein diet was increased, compared with those fed a low protein diet (4). We also observed that alcohol preference in SHRSP was high as well as SHR (5). For clarification of the biochemical basis of the differences due to strain and nutritional status in alcohol preference, one method is that of investigating the ethanol metabolism in animals. In the present study, the effect of different dietary protein levels on ethanol metabolism in SHRSP was compared with those of WKY, the normotensive control of SHRSP.

MATERIALS AND METHODS

Experimental animals. Male SHRSP and WKY received from Shimane Medical University (Prof. Yukio Yamori) and bred by this laboratory were employed as the experimental animals. These rats were habituated by previously feeding a commercially available solid feed (F2, Funabashi Farm Inc., Chiba) to 8 weeks olds and approximately 195g body weight and then subjected to the experiment. The SHRSP and WKY were divided into two groups (n=5), respectively, according to dietary protein level, 15% (normal level) and 5% (low level) purified whole egg protein (Q. P. Corporation, Tokyo). The diet composition is shown in Table 1. They were housed in stainless steel cages at controlled temperature (25±1°C) and relative humidity (50±5%), with a 12 h light/dark cycle. The diets and water were provided ad libitum. The animals were kept on the

Table 1. Composition of experimental diet.

| Ingredients         | Experimental diet |
|---------------------|-------------------|
|                     | 15% protein (%)  | 5% protein (%) |
| PEP<sup>1</sup>     | 15                | 5               |
| Starch              | 58                | 68              |
| Soybean oil         | 6                 | 6               |
| Salt mixture<sup>2</sup> | 6           | 6               |
| Vitamin mixture<sup>2</sup> | 2           | 2               |
| Cellulose powder    | 8                 | 8               |
| Sucrose             | 5                 | 5               |

<sup>1</sup>Purified whole egg protein from Q.P. Corporation, Tokyo Japan.  <sup>2</sup>Oriental Mixture, Oriental Yeast Co., Ltd., Japan.

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Experimental diets for 4 weeks. The body weight of each group was measured once every 3 days and systolic blood pressure in rats were measured weekly by the tail cuff method (Ueda Electronic Works, Ltd., UR-1000).

**Experiment on alcohol metabolism.** In the fifth week, after fasting for 12 h, a 15% ethanol solution was given in a dose of 100 mg ethanol per 100 g body weight with a gastric probe. Blood was collected from the tail vein at constant intervals (30 min, 1, 3, 6 h). The ethanol and acetaldehyde concentrations were determined by the enzymatic method (F kit: ethanol and F kit: acetaldehyde, Boehringer, Mannheim & Yamanouchi, Co., Tokyo) (6).

**Fractionation of rat liver.** In the sixth week, the animals were killed and the livers were removed rapidly. Liver tissue, 0.5 g, was homogenized in 2 ml of 0.25 M sucrose solution containing 10 mM sodium phosphate, pH 7.4, and 2 mM mercaptoethanol by using a Potter homogenizer fitted with a loose Teflon pestle. The homogenate was filtered and centrifuged at 700 \times g for 5 min. The supernatant was centrifuged at 4,500 \times g for 10 min and the pellet was suspended with the sucrose solution again. The suspension was centrifuged for 5 min at 700 \times g and the supernatant was centrifuged at 4,500 \times g for 10 min. The mitochondrial fraction which was contained in the supernatant was suspended a volume of 0.15 M KCl twice the original liver weight and the suspension was treated with 0.3% (w/v) sodium cholate. The suspension was centrifuged at 106,000 \times g for 60 min. The supernatant was used for studies on the mitochondrial alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities (7).

**ADH activity and ALDH activity.** The assay method for ADH activity was essentially the same as described by Zahlten et al. (8), and the ALDH activity was determined by the method of Tottmar et al. (9). Enzyme activity was measured spectrophotometrically by the rate of NADH formation at 340 nm. The standard assay mixture for ADH activity determination contained 0.1 ml of 3M ethanol, 0.5 ml of 0.06 M sodium pyrophosphate, pH 8.5, 0.1 ml of 1.5 mM nicotinamide adenine dinucleotide (NAD\(^+\)), 2.2 ml of distilled water, and 0.1 ml of tissue extract. The assay mixture for ALDH activity contained 0.1 ml of 5 mM (high \(K_m\)) or 0.05 mM (low \(K_m\)) acetaldehyde, 0.5 ml of 0.05 M sodium pyrophosphate, pH 8.8, 0.1 ml of 1 mM NAD\(^+\), 0.1 ml of 0.1 mM pyrazole and 0.1 ml of 2 \(\mu\)M rotenone in methanol, 2 ml of distilled water, and 0.1 ml of tissue extract. The reaction was started by the addition of the substrate and the reaction rate was determined for a 1 min period at 25°C (7–10). Protein content was determined by the method of Lowry et al., with bovine albumin used a standard (8–11). Data were analyzed by Student's t-test (12).

**RESULTS**

**Body weight and systolic blood pressure**

The body weight change in each group is shown in Fig. 1. The body weight gain in rats fed the normal protein diet was higher than that of rats fed the low...
Fig. 1. Effect of dietary protein level on body weight changes in SHRSP and WKY. ●, SHRSP, 5% PEP diet group; ○, SHRSP, 15% PEP diet group; ■, WKY, 5% PEP diet group; □, WKY, 15% PEP diet group.

Fig. 2. Effect of dietary protein level on blood pressure changes in SHRSP and WKY. ●, SHRSP, 5% PEP diet group; ○, SHRSP, 15% PEP diet group; ■, WKY, 5% PEP diet group; □, WKY, 15% PEP diet group.

Changes in blood ethanol and acetaldehyde levels

The changes in blood ethanol concentrations are shown in Fig. 3. Blood ethanol concentration in the 15% PEP diet-fed SHRSP showed the highest level at 30 min (0.68±0.04 g/liter), then decreased rapidly and almost disappeared at 6 h after oral administration of ethanol. When WKY was compared with SHRSP fed with 15% PEP diet, the blood ethanol levels of WKY were higher than those of SHRSP at 1 h (WKY: 1.17±0.24, SHRSP: 0.52±0.04 g/liter, p < 0.05) and at 4 h (WKY: 0.38±0.21, SHRSP: 0.20±0.06, p < 0.05). In the case of the low protein diet, significantly higher ethanol levels were also observed in WKY compared to those of SHRSP. To compare the difference between 15% PEP diet and 5% PEP diet, regardless of SHRSP or WKY. Figure 2 shows the systolic blood pressure in each group. In SHRSP, there was a significant increase in systolic blood pressure compared with WKY. However, there was no significant difference between rats fed the 15% PEP diet and 5% PEP diet.
Fig. 3. Effect of dietary protein level on blood ethanol concentrations following ethanol administration in SHRSP and WKY. SHRSP and WKY were divided into a 5% PEP and a 15% PEP diet group. In the 5th week, all rats fasted for 12 h and then ethanol (100 mg/100 g body weight) was administered with a gastric probe. Blood samples were collected from the tail vein at 30 min, 1, 2, 4, 6 h after oral administration. Each value is the average of four rats. ○, SHRSP, 5% PEP diet group; ●, SHRSP, 15% PEP diet group; ■, WKY, 5% PEP diet group; □, WKY, 15% PEP diet group.

Fig. 4. Effect of dietary protein level on blood acetaldehyde concentrations following ethanol administration in SHRSP and WKY. ○, SHRSP, 5% PEP diet group; ●, SHRSP, 15% PEP diet group; ■, WKY, 5% PEP diet group; □, WKY, 15% PEP diet group.

diet in blood ethanol levels, SHRSP fed with 5% PEP diet were maintained at a higher level and remained significantly higher than those of SHRSP fed the 15% PEP diet at any time after ethanol administration. On the other hand, the same phenomenon was observed in WKY.

Figure 4 shows the changes of blood acetaldehyde concentration in each group. In SHRSP fed a normal protein diet, the blood acetaldehyde reached the maximum level at 30 min after administration, and rapidly decreased. Regardless of the dietary protein level, WKY showed higher blood acetaldehyde levels than those of SHRSP. About the change of blood acetaldehyde concentration owing to the different dietary protein level, the maximum level in SHRSP fed the 5% PEP diet was significantly higher than that of SHRSP fed the 15% PEP diet, and acetaldehyde was detected in the blood, as much as 6 h after being administered.
Table 2. Effect of dietary protein levels on ADH and ALDH activities in SHRSP and WKY.

| Experimental group | Liver weight (g) | Protein content (mg/g liver) | ADH (nmol/min/mg of protein content) | ALDH (nmol/min/mg of protein content) |
|--------------------|----------------|---------------------------|------------------------------------|--------------------------------------|
| SHRSP 5%           | 5.3±0.1**      | 2.0±0.4                   | 21.6±4.1*                          | 11.4±3.4*                            |
| SHRSP 15%          | 8.9±0.3        | 1.9±0.3                   | 33.4±2.7                           | 18.5±2.0                            |
| WKY 5%             | 5.6±0.4**      | 2.1±0.1                   | 22.5±3.2*                          | 10.5±3.2*                            |
| WKY 15%            | 9.0±0.1        | 2.2±0.3                   | 34.2±3.7                           | 16.2±1.7                            |

Values represent mean±SE of 5 rats in each group. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase. *p<0.05, **p<0.01 compared with 15% protein diet group of SHRSP and WKY.

This tendency was observed not only in SHRSP but also in WKY.

The ADH activity and ALDH activity

There was no significant change between SHRSP and WKY fed the 5 or 15% PEP diet (Table 2). However, compared to the 5% PEP diet group, the 15% PEP diet group showed higher ADH and ALDH activity, not only in SHRSP but also in WKY (Table 2).

DISCUSSION

The effects of the animal's strain and dietary protein level on ethanol metabolism were investigated. Regardless of the dietary protein level, SHRSP showed lower blood ethanol levels and acetaldehyde levels after ethanol administration. Ethanol is mainly metabolized by alcohol dehydrogenase (ADH) into acetaldehyde, which is oxidized into acetate by acetaldehyde dehydrogenase (ALDH) (13, 14). Generally, it is considered that the ADH and ALDH activity affected the ethanol and acetaldehyde concentrations in blood. Shiohara et al. reported that the activities of mitochondrial low-$K_m$ and high-$K_m$ ALDH in SHR were higher than those in the WKY (15). However, we could not find any change in ADH or ALDH activity between SHRSP and WKY. Another possible reason for this result is that a decrease in the availability of NAD$,^+$, a coenzyme for ADH and ALDH, may partially contribute to the in high levels of blood ethanol and acetaldehyde in the WKY treated with ethanol compared with those of SHRSP (16). On the other hand, we observed previously that the daily water intake in SHRSP was higher than in that of WKY. Thus, it can not be ignored that there is a relation between lower blood ethanol and acetaldehyde levels and the higher water intake in SHRSP. In addition, SHRSP are genetically predisposed to have kidney trouble. The excretory process of kidneys was possibly related to the low blood ethanol and acetaldehyde concentrations in SHRSP.

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When animals ate the 5% PEP diet, the blood ethanol and acetaldehyde levels increased in comparison with those fed with the 15% PEP diet. It was reported that the intake of low level protein prolonged the elimination of ethanol from the blood (17). We also found that the low-protein diet decreased ADH and ALDH activity (Table 2). From these results, the higher blood ethanol and acetaldehyde levels in the 5% PEP diet group were probably caused by the lower ADH and ALDH activity. On the other hand, we found that the rats fed low-protein diets showed lower levels in their blood amino acids, especially the essential amino acids such as lysine, histidine, phenylalanine, leucine, methionine etc., and higher glutamic acid level (data not shown). It may be that the rats fed a low protein diet were in a condition of essential amino acid deficiency. Therefore, it may be that the deficiency of amino acids, especially essential amino acids, decreased the ability of protein synthesis, thus reducing enzyme activity. Furthermore, it is known that ethanol oxidation accelerates the production of SH-amino acids (i.e. L- and D-cysteine, methionine) which help to generate NAD\(^+\) (18). It was also reported that the ethanol metabolism was accelerated in the presence of certain amino acids such as alanine, lysine, glycine etc. (19–22). We had previously shown that the intake of alcohol can be increased in SHRSP by adding amino acids to ethanol solutions and that the intake of proline, lysine, and threonine was considerably larger (23). We also found that proline and lysine can regulate ethanol metabolism (24). According to these reports, it may be considered that rats fed a low-protein diet had poor ability to metabolize alcohol because of a deficiency of amino acids, and consequently the preference for alcohol decreased.

Acetaldehyde accumulation in the blood leads to several responses such as: flushing of the face, headache, loss of appetite, vomiting, increased heart rate, and changes in blood pressure. It has been reported that a higher acetaldehyde output suggests a greater inhibitory influence upon the metabolism of the brain, which could decisively affect behavior with respect to alcohol preference (25). These results in our study suggest that the blood ethanol and acetaldehyde levels after alcohol administration depended not only on genetic factors (that is, the animal’s strains), but on nutritional status (i.e., dietary protein levels) as well. Thus, we supposed that the different blood ethanol and acetaldehyde concentrations after alcohol intake in rats may be correlated with the preference for alcohol. We also concluded that the blood ethanol and acetaldehyde levels were affected by the nutritional status, i.e., dietary protein levels, the enzyme activity, and liver and kidney function.

REFERENCES

1) Sheppard, J. R., Albersheim, P., and McClearn, G. E. (1968): Enzyme activities and ethanol preference in mice. *Biochem. Genet.*, 2, 205.

2) Eriksson, K. (1968): Genetic selection for voluntary alcohol consumption in the albino rat. *Science*, **158**, 739.

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3) Eriksson, K. (1971): Rat strains specially selected for their voluntary alcohol consumption. *Ann. Med. Exp. Biol. Fenn.*, 49, 67.

4) Kimura, S., Kim, C. H., Ohtomo, I. M., Yokomukai, Y., Komai, M., and Morimatsu, F. (1991): Nutritional studies of the roles of dietary protein levels and umami in the preference response to sodium chloride for experimental animals. *Physiol. Behav.*, 49, 997–1002.

5) Yang, S. C., Ito, M., Morimatsu, F., and Kimura, S. (1992): Nutritional studies in the preference response to alcohol for Stroke-Prone SHR. *Jpn. Heart J.*, 33, 580.

6) Beutler, H. O. (1984): in Methods of Enzymatic Analysis Vol. VI, ed. by Bergmeyer, H. O., Verlag Chemie, Weihem, Deerfield Beach, Florida, pp. 598–606.

7) Nakanisi, S., Shiohara, E., Tsukada, M., and Iijima, Y. (1977): Aldehyde dehydrogenase activities after treatment with phenobarbital in long-Evans rats. *Jpn. J. Stud. Alcohol.*, 12, 5–12.

8) Zahlten, R. N., Michael, E. N., and Jacobson, J. C. (1981): Ethanol metabolism in guinea pig: In vivo ethanol elimination, alcohol dehydrogenase distribution and subcellular localization of acetaldehyde dehydrogenase in liver. *Arch. Biochem. Biophys.*, 207, 371–379.

9) Tottmar, S. O. C., Pettersson, H., and Kissling, K. H. (1973): The subcellular distribution and properties of aldehyde dehydrogenase in rat liver. *Biochem. J.*, 135, 577–586.

10) Morrison, G. R., and Brock, F. E. (1967): Quantitative measurement of alcohol dehydrogenase in the lobule of normal livers. *J. Lab. Clin. Med.*, 70, 116–120.

11) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1953): Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

12) Fisher, R. A. (1970): Statistical Methods for Research Worker, 14th ed., Oliver & Boyd, Edinburgh, Scotland, pp. 140–142.

13) Bosron, W. F., and Li, T. K. (1987): Catalytic properties of human liver alcohol dehydrogenase isoenzymes. *Enzyme*, 37, 19–28.

14) Goedde, H. W., and Agarwal, D. P. (1987): Polymorphism of aldehyde dehydrogenase and alcohol sensitivity. *Enzyme*, 37, 29–44.

15) Shiohara, E., Tsukada, M., Iwatsuki, K., Iijima, F., and Chiba, S. (1984): Activities of NAD+–dependent aldehyde dehydrogenase and alcohol dehydrogenase in the liver of spontaneously hypertensive rats in the process of development. *Clin. Exp. Pharmacol. Physiol.*, 11, 199–207.

16) Cederbaum, A. I., and Rubim, E. (1977): The oxidation of acetaldehyde by isolated mitochondria from various organs of the rat hepatocellular carcinoma. *Arch. Biochem. Biophys.*, 179, 46–66.

17) Kono, H., Bode, J. C., and Maritimi, G. A. (1979): Effect of acute and chronic ethanol administration on the content of coenzymes linked to energy transfer in the liver of rats fed standard or low-protein diet. *Gastroenterol. Jpn.*, 14, 226–232.

18) Tabakoff, B., Eriksson, C. J. P., and Wartburg, J. P. (1989): Methionine lower circulating levels of acetaldehyde after ethanol ingestion. *Alcoholism: Clin. Exp. Res.*, 13, 164–171.

19) Tsukamoto, S., Kanegae, T., Nagoya, T., Shimamura, M., Mieda, T., Nomura, M., Hojo, K., and Okubo, H. (1990): Effects of amino acids on acute alcohol intoxication in mice. *Jpn. J. Alcohol Drug Dependence*, 25, 429–440.
20) Blum, K., Wallace, J. E., and Friedman, R. N. (1974): Reduction of acute alcoholic intoxication by \( \alpha \)-amino acids: Glycine and serine. *Life Sci.*, 14, 557–565.

21) Dorato, M. A., Lynch, V. D., and Ward, C. O. (1977): Effect of lysine and diethanolamine-rutin on blood levels, withdrawal reaction and acute toxicity of ethanol in mice. *J. Pharm. Sci.*, 66, 35–39.

22) Ward, C. O., Lau Cam, C. A., Tang, A. S. M., Breglia, R. J., and Jarowski, C. I. (1972): Effect of lysine on toxicity and depressant effects of ethanol in rats. *Tox. Nad. Appl. Pharmacol.*, 22, 422–426.

23) Yang, S. C., Ito, M., Morimatsu, F., Furukawa, Y., and Kimura, S. (1993): Effects of amino acids on alcohol intake in stroke-prone spontaneously hypertensive rats. *J. Nutr. Sci. Vitaminol.*, 39, 55–61.

24) Yang, S. C., Ito, M., Morimatsu, F., Slamet, B., Furukawa, Y., and Kimura, S. (1994): Stimulation of ethanol metabolism induced by proline and lysine ingestion in prolonged ethanol-administered stroke-prone spontaneously hypertensive rats. *J. Clin. Biochem. Nutr.*, 16, 151–159.

25) Peter Eriksson, C. J., (1973): Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem. Pharmanol.*, 22, 2283–2292.