Pharmacology Biochemistry & Behavior, Vol. 11, pp. 575–579. Printed in the U.S.A.

The Effects of Dietary Niacin and Riboflavin on Voluntary Intake and Metabolism of Ethanol in Rats

LEENA PEKKANEN AND MAIJA RUSI

The Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, Finland

Received 1 August 1979

PEKKANEN, L. AND M. RUSI. *The effects of dietary niacin and riboflavin on voluntary intake and metabolism of ethanol in rats.* PHARMAC. BIOCHEM. BEHAV. 11(5) 575–579, 1979.—The effects of dietary deficiency and excess of niacin and riboflavin on voluntary drinking of 10% (v/v) ethanol were studied in male rats. The effectiveness of dietary deficiency and excess of both niacin and riboflavin on tissue levels of these vitamins was demonstrated by measurements of urinary N¹-methylnicotinamide and blood glutathione reductase (EC 1.6.4.2) activity. A high-niacin diet containing 75 mg niacin/kg food decreased ethanol intake by about 36% compared to the control diet containing 15 mg niacin/kg. Niacin or riboflavin deficiency and a high-riboflavin diet containing 40 mg riboflavin/kg did not affect significantly ethanol drinking. Changes in dietary levels of niacin or riboflavin did not influence on ethanol elimination rate or levels of blood acetaldehyde during ethanol oxidation. Therefore, blood acetaldehyde was not responsible for the decreased ethanol intake of rats fed with a high-niacin diet. It was concluded that the increased ethanol intake caused by dietary deprivation of B-vitamin complex found in earlier studies is not a result of deficiency of niacin or riboflavin but niacin may be involved in the decrease in ethanol drinking, which follows dietary B-vitamin complex supplementation.

Voluntary ethanol intake Niacin Riboflavin Ethanol elimination rate Blood acetaldehyde
Glutathione reductase N¹-methylnicotinamide

VOLUNTARY ethanol consumption has been shown to increase in experimental animals fed diets lacking B-vitamin complex and supplementation of the diets with the same complex appears to reverse this effect [2, 20, 29]. It is not known which members of the B-vitamin complex participate in this effect. Of the single B-vitamins, thiamin, riboflavin, niacin, pyrodoxine and pantothenic acid seem to modify ethanol intake in the same way as B-vitamin complex but the results have not been consistant in all studies [1, 3, 20, 23, 28]. One explanation for the contradictory results could be that in the earlier studies the effects of the experimental diets on the tissue metabolism of the vitamin in question have not been studied. Therefore, in the present experiment the effect of dietary deficiency and excess of niacin and riboflavin on voluntary ethanol intake in rats was studied, and the effectiveness of the experimental diets was estimated by measuring the niacin metabolite, N¹-methylnicotinamide (N-MNA) in urine and the activity of riboflavin-enzyme, glutathione reductase (EC 1.6.4.2), at the end of the study.

Since niacin and riboflavin are precursors for coenzymes essential in ethanol oxidation and ethanol drinking of animals has been assumed to be affected by their capacity to oxidize ethanol [10, 24], the effects of dietary deficiency and excess of niacin and riboflavin on ethanol elimination rates were studied. It has also been hypothetized that ethanol drinking is regulated by blood concentration of the first oxidation product of ethanol, acetaldehyde, which as a toxic substance could cause aversion to ethanol [7, 14, 25, 26]. Therefore, the relationship between vitamin intake and blood acetaldehyde concentrations was also studied during ethanol metabolism.

METHOD

Animals and Apparatus

The animals were 3 month old males of a mixed strain derived from cross-breeding Sprague-Dawley, Wistar and Long-Evans rats in our laboratory [8]. Animals were individually housed in galvanized cages with a mesh bottom. The experiment was carried out in a room under a 12 hr light/12 hr dark cycle with temperatures maintained between 22–24°C. In the free-choice situation 10% (v/v) ethanol, tap water and food were freely available for the animals. Two 100 ml graduated cylinders were used for delivery of fluids, the position of them being interchanged once a week to avoid a possible effect of place affinity on fluid intake.

Diets

The basal diet was made from vitamin-free casein (ICN Pharmaceuticals Inc., Cleveland, Ohio, USA), rice starch and maize oil so that 20% of the total energy was derived from protein, 65% from carbohydrate and 15% from fat. The control diet contained 4 mg of thiamin hydrochloride (Hoffmann and LaRoche, Basle, Switzerland), 8 mg of riboflavin (Merck, Darmstadt, Germany) and 15 mg of

nicotinamide (Hoffman and LaRoche) in each kg of fresh diet, which were the highest levels listed in the nutrient requirement tables for rats [5, 15, 16, 27]. The riboflavin-deficient diet (RD) contained no added riboflavin and the niacin-deficient diet (ND) no added nicotinamide. According to our analyses the basal diet contianed 0.2 mg of riboflavin and 0.4 mg niacin/kg of fresh diet. The high-riboflavin diet (RH) contained 40 mg of riboflavin/kg diet and the high-niacin diet (NH) 75 mg of nicotinamide/kg diet, which were five times the control diet level. The exact content of other vitamins and minerals in the basal diet has been described previously by Pekkanen and coworkers [22].

Procedure

Test groups of 8–9 animals given a free-choice between 10% (v/v) ethanol and water were: ethanol-high-niacin diet (E-NH), ethanol-niacin-deficient diet (E-ND), ethanol-high-riboflavin diet (E-RH), ethanol-riboflavin-deficient diet (E-RD) and ethanol-control diet (E-C). The corresponding water (W) groups were W-RH, W-RD, W-NH, W-ND and W-C. The water-groups were included to dertermine the effects of the test-diets on growth rates, energy and water consumption and ethanol metabolism.

For 3 weeks all animals received the control-diet and tap water and the test diets were given during the next 4 weeks. The first of these test diet weeks was an ethanol habituation period for the ethanol free-choice groups, when the ethanol groups had 10% (v/v) ethanol as their only drinking fluid. For the latter 3 weeks they had a choice between ethanol and water. The fluid intake was recorded daily, food consumption and body weight twice a week.

Analytical Methods

The ethanol elimination rates and blood acetaldehyde concentrations were measured in all animals, first, before the test diets were presented, then after the 1st, 2nd and 4th weeks on the test diets. The rats were injected with 1.5 g/kg ethanol IP as 10% (w/v) solution in saline, and the concentrations of ethanol and acetaldehyde were measured in tail blood haemolyzed with water 30, 100, 140, 180 and 220 min after the injection by head-space gas chromatography [11].

At the end of the study the blood samples for enzyme assay were taken from the hearts of the Nembutal®-anaesthetized animals. Glutathione reductase activity was measured from whole-blood haemolysates according to the method of Glatzle and coworkers [12]. Activities are reported as the change in absorbance at 334 nm per min per g hemoglobin. Activation coefficients to describe the enzyme saturation with FAD were calculated as a quotient of the activity with added FAD/activity without added FAD. Hemoglobin was determined by the cyanmethemoglobin method [13].

Urine was collected from animals kept for 24 hours in metabolic boxes free of food and ethanol. For analysis of N-MNA the urines of all rats in each group were pooled and N-MNA was determined fluorimetrically [4] in a commercial laboratory (Technical Reasearch Centre of Finland, Food Research Laboratory, Espoo, Finland).

RESULTS AND DISCUSSION

Riboflavin Status

In the present study dietary levels of riboflavin had affected the status of the vitamin in the rat tissue. The results in Table 1 show a typical consequence of riboflavin deficiency which is a decreased activity of the FAD-requiring enzyme, glutathione reductase, and high activation coefficient, which indicates the low saturation of the enzyme with FAD *in vivo*. In the E-RD group Student's *t*-test showed a significant decrease in glutathione reductase activity and a significantly higher activation coefficient compared to the E-C group, $t(15) = -3.72$, $p < 0.01$; $t(15) = 5.00$, $p < 0.001$, respectively. In the W-RD group the corresponding differences were not statistically significant, $t(14) = 0.60$, $p < 0.55$; $t(14) = 1.69$, $p < 0.11$, indicating that ingestion of ethanol had potentiated riboflavin deficiency. Correspondingly, there was a trend, even though non-significant, for the high-riboflavin rats to show an increase in glutathione reductase activity both in the E-RH group, $t(14) = 1.80$, $p < 0.09$, and in the W-RH group, $t(14) = 1.87$, $p < 0.08$, compared to the corresponding controls.

TABLE 1

ACTIVITY OF GLUTATHIONE REDUCTASE AND ACTIVATION COEFFICIENT FOR FLAVIN ADENINE DINUCLEOTIDE

Group	Glutathione reductase activity A min/g hemoglobin	Activation coeffieient
E - C* (8)	2.76 ± 0.21	1.12 ± 0.04
E - RH (8)	3.71 ± 0.49	1.20 ± 0.04
E - RD (9)	1.77 ± 0.18†	1.58 ± 0.07†
W - C (8)	2.37 ± 0.32	1.34 ± 0.11
W - RH (8)	3.39 ± 0.45	1.30 ± 0.07
W - RD (8)	2.07 ± 0.36	1.60 ± 0.14

Values are expressed as means ± S.E.M. with the number of animals group in parentheses.

*See text for explanation of abbreviations.

†$p < 0.001$ compared to E - C group.

‡$p < 0.01$ compared to E - C group.

Also the growth rate and food intake of the riboflavin-deficient groups were slightly but not significantly lower than in the other groups. The consumption of water was equal in all groups.

Niacin Status

The urinary output of the niacin metabolite, N-MNA, varies with the degree to which tissue stores are saturated with niacin when urine is collected in fasted animals. Even though it was measured in pooled samples of each group N-MNA tended to be clearly lower in the urine of the E-ND and W-ND groups and higher in the urine of the E-NH group compared to the control groups (Table 2). In the W-NH group the high-niacin diet had not increased niacin stores compared to the W-C group. This could be explained if the level of niacin in the control diet was high enough to saturate tissues maximally, further accumulation of niacin by feeding a high-niacin diet thus being impossible. However, the high-niacin diet had effected tissue stores in the E-NH group: in the control group ethanol ingestion decreased niacin stores (N-MNA excretion in the E-C group was 35% lower than in the W-C group) more than in the high-niacin group (N-MNA excretion in the E-NH group was 13% lower than in the W-NH group) indicating that in the E-NH group a

TABLE 2

EXCRETION OF N'-METHYLNICOTINAMIDE IN URINE PER GROUP OF 8 RATS PER DAY

Group	μg/group/day
E - C*	193
E - NH	255
E - ND	51
W - C	299
W - NH	294
W - ND	83

*See text for explanation of abbreviations.

TABLE 3

EFFECTS OF DIETARY RIBOFLAVIN AND NIACIN ON FREE-CHOICE CONSUMPTION OF ETHANOL DURING 3-WEEK ETHANOL-CHOICE PERIOD

	Ethanol intake/d		
Group	g/kg	Energy from ethanol (% total)	10% (v/v) ethanol intake (% total fluid)
E - C* (8)	2.2 ± 0.6	10 ± 2	31 ± 8
E - RH (8)	1.6 ± 0.4	7 ± 2	26 ± 7
E - RD (8)	1.3 ± 0.2	6 ± 1	22 ± 2
E - NH (9)	0.8 ± 0.2†	4 ± 1	11 ± 3†
E - ND (8)	1.2 ± 0.2	5 ± 1	17 ± 3

Values are expressed as means ± S.E.M. with the number of animals/group in parentheses.

*See text for explanations of abbreviations.

†$p < 0.05$ compared to the E - C group.

high daily intake of niacin compensated for the loss of the vitamin known to be induced by ethanol ingestion [6, 17]. This negative influence of ethanol drinking on vitamin balance can also be seen in the niacin deficient-group (N-MNA excretion in the E-ND group was 38% lower than in the W-ND group).

Voluntary Ethanol Intake

Although dietary levels of niacin and riboflavin had affected the status of these vitamins in this study the changes in voluntary ethanol consumption of the rats were not drastic as shown in Table 3. Of all test-diets, only the high-niacin diet had some effect on ethanol intake: in the E-NH group ethanol intake was significantly lower when expressed as g/kg body wt, $t(14) = 2.19$, $p < 0.05$, and as per cent of total fluid intake, $t(14) = 2.31$, $p < 0.05$, and also slightly lower when expressed as a percentage of the total energy intake, $t(14) = 1.98$, $p < 0.07$, compared to the control group. An increased ethanol consumption as a result of niacin deficiency was not found in this study and the dietary level of riboflavin showed no effect at all on voluntary ethanol consumption.

Blood Acetaldehyde and Ethanol Elimination Rate

Blood acetaldehyde levels after the ethanol test-dose of 1.5 g/kg are presented in Table 4. The matched-pair t-test showed a significant decrease in blood acetaldehyde levels during the study in the E-NH group, $t(7) = 6.10$, $p < 0.001$, and

TABLE 4

THE BLOOD ACETALDEHYDE LEVELS OF RATS 30 min AFTER THE INTRAPERITONEAL INJECTION OF 1.5 g ETHANOL PER kg BODY WEIGHT

	nmol/ml	
Group	Before introducing test-diets	After 4 weeks on test-diets
E - C* (8)	25 ± 4	19 ± 1
E - RH (8)	25 ± 4	18 ± 3
E - RD (9)	21 ± 2	17 ± 2
E - NH (8)	24 ± 3	13 ± 3
E - ND (7)	16 ± 6	13 ± 2
W - C (8)	20 ± 3	15 ± 3
W - RH (8)	24 ± 4	17 ± 3
W - RD (8)	23 ± 4	29 ± 4
W - NH (8)	22 ± 4	7 ± 1
W - ND (8)	26 ± 7	20 ± 3

Values are expressed as means ± S.E.M. with the number of animals/group in parentheses.

*See text for explanation of abbreviations.

in the W-NH group, $t(7)=3.20$, $p<0.05$, and in the E-RH group, $t(7)=2.53$, $p<0.05$, and in the W-RH group, $t(7)=2.86$, $p<0.05$. A non-significant decrease in blood acetaldehyde during the study was also seen in the E-C group, $t(7)=1.62$, $p<0.15$, and in the W-C group, $t(7)=1.05$, $p<0.32$. Therefore, the effects of the test-diet were tested statistically relative to the initial values using Student's t-test for independent variables, the individual changes from the initial values in each group being compared with the changes in the corresponding control groups. This showed no statistically significant differences between the test-groups and the control groups indicating that decreases in blood acetaldehyde levels were not caused by dietary levels of niacin or riboflavin in this study. No changes in ethanol elimination rates during the experiment were found to be caused by dietary deficiency or excess of niacin and riboflavin.

General Comments

The decreased ethanol intake in the group receiving five times more niacin in the diet than the control group cannot be explained by changes in ethanol metabolism. This provides a further evidence that a high level of acetaldehyde in blood is not the only suppressor of voluntary ethanol intake in animals [18, 19, 22].

The B-vitamin complexes, dietary deprivation of which has increased ethanol consumption in the rat and mouse, have contained thiamin, riboflavin, pyridoxine, pantothenic acid, choline and niacin [2], biotin instead of niacin [20], or all the foregoing B-vitamins plus inositol, p-aminobenzoic acid and folic acid [29]. Suppression of ethanol intake to the initial level has been produced by administration of the B-vitamin complex used by Brady and Westerfeld [2] and Williams and coworkers [29]. Of single B-vitamins, a deficiency of thiamin has commonly been regarded as an elevator of voluntary ethanol drinking in experimental animals. Also in our laboratory it has been found that thiamin deficiency increased ethanol intake of rats while dietary excess clearly decreased it even in the rats without previous experience of deficiency [9,21] emphasizing the role of thiamin in the effects on ethanol intake induced by B-vitamin complex.

The results of this report indicate that increased ethanol consumption by deficiency of B-vitamin complex is not caused by the lack of niacin or riboflavin but niacin could be involved in the depression of ethanol intake by supplementation with B-vitamin complex.

REFERENCES

1. Beerstecher, E., J. G. Reed, W. D. Brown and L. J. Berry. The effects of single vitamin deficiencies on the consumption of alcohol by white rats. *Biochemical Institute Studies IV*. Univ. Texas Publ. **5109:** 115–149, 1951.
2. Brady, R. A. and W. W. Westerfeld. The effect of B-complex vitamins on the voluntary consumption of alcohol by rats. *Q. Jl Stud. Alcohol* **7:** 499–505, 1947.
3. Brown, R. V. Vitamin deficiency and voluntary alcohol consumption in mice. *Q. Jl Stud. Alcohol* **30:** 592–597, 1969.
4. Clark, B. R., R. M. Halpern and R. A. Smith. A fluorimetric method quantitation in the picomole range of N'-methylnicotinamide and nicotinamide in serum. *Analyt. Biochem.* **69:** 54–61, 1975.
5. Coates, M. E., P. N. O'Donoghue, P. R. Payne and R. J. Ward. *Laboratory Animal Handbook 2: Dietary Standards for Laboratory Rats and Mice*. London: Laboratory Animals Ltd, 1969.
6. Dastur, D. K., N. Santhadevi, E. V. Quadros and F. R. C. Avari. The B-vitamins in malnutrition with alcoholism. *Br. J. Nutr.* **36:** 143–159, 1976.
7. Eriksson, C. J. P. Ethanol and acetaldehyde metabolism in rat strains gentically selected for their ethanol preference. *Biochem. Pharmac.* **22:** 2283–2292, 1973.
8. Eriksson, K., O. Halkka, J. Lokki and A. Saura. Enzyme polymorphism in feral, outbred and inbred rats (*Rattus norvegicus*). *Heredity* **37:** 341–349, 1976.
9. Eriksson, K., L. Pekkanen and M. Rusi. The effects of dietary thiamin on voluntary ethanol drinking and ethanol metabolism in rat. *Br. J. Nutr.*, in press.
10. Eriksson, K. and P. Pikkarainen. Differences between the sexes in voluntary alcohol consumption and liver ADH-activity in inbred strains of mice. *Metabolism* **17:** 1037–1042, 1968.
11. Eriksson, C. J. P., H. W. Sippel and O. Forsander. The determination of acetaldehyde in biological samples by headspace gaschromatography. *Analyt. Biochem.* **80:** 116–124, 1977.
12. Glatzle, D., H. Weiser, F. Weber and O. Wiss. Correlations between riboflavin supply, glutathione reductase activities and flavin levels in rats. *Int. J. Vit. Res.* **43:** 187–200, 1973.
13. Hainline, A. Hemoglobin. In: *Standard Methods of Clinical Chemistry*, Vol. 5, edited by S. Meites. New York: Academic Press, 1965, pp. 49–60.
14. Horowitz, G. P. and G. Whitney. Alcohol-induced conditioned aversion: genotypic specificity in mice (Mus musculus). *J. comp. physiol. Psychol.* **89:** 340–346, 1975.
15. Jelinek, V. Vitamins in nutrition of laboratory animals. In: *Husbandry of Laboratory Animals*, edited by M. L. Conalty. New York: Academic Press, 1967, pp. 97–114.
16. Joubert, C. J. Total nutritive requirements for small laboratory rodents. In: *Husbandry of Laboratory Animals*, edited by M. L. Conalty. New York: Academic Press, 1967, pp. 133–146.
17. Konttinen, K., E. Oura and H. Suomalainen. Influence of short-term alcohol consumption on the excretion of some components vitamin B complex in the urine of rats. *Annls. Med. exp. Biol. Fenn.* **45:** 63–67, 1967.
18. Li, T. K. and L. Lumeng. Alcohol metabolism in inbred strains of rats with alcohol preference and nonpreference. In: *Alcohol and Aldehyde Metabolizing Systems*, Vol. 3, edited by R. G. Thurman, J. R. Williamson, H. Drott and B. Chance. New York: Academic Press, 1977, pp. 625–634.
19. Lumeng, L., T. D. Hawkins and T. K. Li. New strains of rats with alcohol preference and nonpreference. In: *Alcohol and Aldehyde Metabolizing Systems*, Vol. 3, edited by R. G. Thurman, J. R. Williamson, H. Drott and B. Chance. New York: Academic Press, 1977, pp. 537–544.
20. Mirone, L. Dietary deficiency in mice in relation to voluntary alcohol consumption. *Q. Jl Stud. Alcohol* **18:** 552–560, 1957.
21. Pekkanen, L. The effects of thiamin deprivation and antagonism on voluntary ethanol intake in rats, unpublished.
22. Pekkanen, L., K. Eriksson and M-L. Sihvonen. Dietarily-induced changes in voluntary ethanol consumption and ethanol metabolism in the rat. *Br. J. Nutr.* **40:** 103–113, 1978.
23. Purdy, M. B. and J. G. Lee. The effect of restricted food intake, thiamine deficiency and riboflavin deficiency with voluntary consumption of ethanol by the albino rat. *Q. Jl Stud. Alcohol* **23:** 549–556, 1962.
24. Schlesinger, K. Genetic and biochemical correlates of alcohol preference in mice. *Am. J. Psychiat.* **122:** 767–773, 1966.

25. Schlesinger, K., R. Kakihana and E. L. Bennet. Effects of tetraethylthiuramdisulfate (antabus) on the metabolism and consumption of ethanol in mice. *Psychosom. Med.* **28:** 514–520, 1966.
26. Sheppard, J. R., P. Albersheim and G. McClearn. Aldehyde dehydrogenase and ethanol preference in mice. *J. biol. Chem.* **245:** 2876–2882, 1970.
27. US National Research Council, Food and Nutrition Board. In: *Nutrient Requirement of Domestic Animals no. 10. Nutrient Requirement for Laboratory Animals.* Washington: National Academy of Sciences, 1972.
28. Westerfeld, W. W. and J. Lawroe. The effect of caloric restriction and thiamin deficiency in the voluntary consumption of alcohol by rats. *Q. Jl Stud. Alcohol* **14:** 378–384, 1953.
29. Williams, R. J., L. J. Berry and E. Beerstecher. Biochemical individuality. III. Genototrophic factors in the ethiology of alcoholism. *Archs Biochem.* **23:** 275–290, 1949.