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DIETARY REGULATION OF VOLUNTARY ALCOHOL CONSUMPTION IN RATS

INFLUENCE OF A HIGH PROTEIN DIET AND A METHYLENE BLUE DIET

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Abstract—The importance of glucose homeostatis for high voluntary alcohol consumption was studied in alcohol-preferring (AA) and alcohol-avoiding (ANA) rats fed either a control diet, a protein-rich diet or a control diet supplemented with methylene blue. AA rats on the control diet were found to receive 13.6% of their daily energy intake from alcohol. On the high-protein or methylene blue diet, the alcohol consumption of the AA rats was respectively 40% and 48% higher than on the control diet. The voluntary alcohol consumption of ANA rats corresponded to 0.8–2.3% of their daily energy intake irrespective of diet. The protein diet increased the blood glucose concentration of AA rats by 20% but no increase was observed after the methylene blue diet. The diets had no effect on the blood glucose levels of ANA rats. In AA rats, the protein diet reduced the hepatic concentration of the three major glucogenic amino acids (serine, glycine, alanine) on average by 24%, suggesting an increased utilization for gluconeogenesis. No such reduction was observed in AA rats on the methylene blue diet or in ANA rats on any diet. The utilization of amino acids for maintenance of glucose balance in AA rats is further supported by the observed negative correlation between plasma concentration of urea, the end product of amino acid catabolism, and the sum of the concentrations of the three glucogenic amino acids in the liver, and by the positive correlation between plasma urea and blood glucose concentration. Furthermore, in AA rats, but not in ANA rats, the concentration of alanine, the main amino acid used in gluconeogenesis, correlated negatively with the amount of alcohol consumed. These findings indicate that the maintenance of glucose homeostatis is important for high voluntary alcohol consumption.

Results of several studies support the view that metabolic factors, in addition to the well-documented genetic control [1], are involved in the regulation of voluntary alcohol consumption. Protein-rich diet [2, 3] has been shown to increase and high intake of carbohydrates together with low intake of protein to decrease the amount of alcohol consumed voluntarily by rats [2]. In diabetogenic mice high alcohol intake has been shown to normalize blood glucose level [4] and the results of a recent study suggest that the ability to maintain glucose homeostasis is a prerequisite for high alcohol consumption also in non-diabetic rats [5].

In the presence of ethanol, the blood glucose concentration can be maintained by glycogenolysis and gluconeogenesis. In a recent study [5] it was shown that the effect of ethanol on these two processes differs in AA (Alko Alcohol) and ANA (Alko NonAlcohol) rat strains outbred for their alcohol preference [6]. The AA rats which in a free choice situation prefer alcohol to water were able to maintain their blood glucose concentration by increasing the rate of gluconeogenesis, whereas in the ANA rats which avoid alcohol the contribution of glycogenolysis and gluconeogenesis was equal.

To further study the relationship between glucose homeostasis and voluntary alcohol consumption in the AA and ANA rats, the present study was designed with two specific aims in mind: first we wanted to test whether the availability of glucose precursors alters the voluntary alcohol consumption and second to test the effect of the hepatic redox state on alcohol drinking. The availability of glucose precursors was increased by feeding the rats a high protein diet [7] which is known to increase the concentration of amino acids in the liver [8]. Second the ethanol-induced increase in the hepatic redox state (the $NADH/NAD^+$ ratio) which inhibits gluconeogenesis [9–11] was prevented by an artificial hydrogen acceptor, methylene blue [12].

MATERIALS AND METHODS

Animals and diets. Female AA and ANA rats from the 51st generation [6] were housed individually in a room with a 12-hr light–12-hr dark cycle, constant 55% relative humidity and 22–24° temperature. At the beginning of the experiment the rats weighed 199.0 ± 4.0 g. All the rats had free access to water and a 10% (v/v) ethanol solution throughout the experiment. During the first 3 weeks they were fed normal rat food (R3, Ewos, Södertälje, Sweden). Water, ethanol and food consumption were measured every second day. After 3 weeks both the AA and the ANA rats were divided into three groups

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Table 1. Daily, alcohol, total water, and total energy intake and final body weight of AA and ANA rats on the control diet, high protein diet, and 85 mg/kg methylene blue diet

	Control diet		High protein diet		Methylene blue diet	
	AA (6)	ANA (6)	AA (6)	ANA (6)	AA (7)	ANA (7)
Food (kJ/kg)	1000 ± 20	1210 ± 18	976 ± 43	1093 ± 32	953 ± 27	1095 ± 43
Alcohol (kJ/kg)	159 ± 28	13 ± 5	222 ± 34	9 ± 2	236 ± 19*	26 ± 12
Total energy (kJ/kg)	1168 ± 17	1223 ± 22	1202 ± 18	1102 ± 33	1184 ± 32	1121 ± 48
Water (mL/kg)	45 ± 6	110 ± 7	36 ± 7	78 ± 6†	41 ± 9	75 ± 6†
Total water (mL/kg)	93 ± 9	115 ± 6	111 ± 7	81 ± 3‡	123 ± 9*	84 ± 4‡
Body weight (g)	222 ± 5	208 ± 5	207 ± 6	220 ± 10	199 ± 6	213 ± 7

Total water includes the water and the water in the alcohol solution consumed. The results are means ± SE. Number of animals is given in parentheses.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ when compared to the control of the same strain.

(N = 6–7 in each group) so that the mean ethanol consumption during the preceding 10 days was equal in all three groups. The baseline for the AA rats was between 5.8–6.5 g ethanol/kg × d and between 0.3–0.4 g/kg × d for the ANA rats.

During the test period the two control groups (one AA and one ANA) had access to the Ewos R3 commercial diet, water and ethanol as earlier. The value of metabolizable energy was 13.0 kJ/g for the food and a value of 29.8 kJ/g was used for ethanol in the calculations. Two other groups got a premixed diet of 40% casein, 33% of corn starch, 17% dextrin (all from Yliopiston Apteekki, Helsinki, Finland) and 10% of fat (a commercial mixture of hydrogenated vegetable oils kindly supplied by Paasivaara Ldt, Helsinki, Finland). The calculated energy value of this diet was 15.8 kJ/g. The remaining two groups of AA and ANA rats were fed the Ewos R3 rat food supplemented with 85 mg/kg methylene blue (Merck, Darmstadt, F.R.G.). The rats were fed these diets for 18 days. Body weight, food intake and fluid consumption were measured every second day and the values for the last 10 days were used for the calculations. Statistical comparisons were made by the unpaired *t*-test.

Sampling. Between 9 and 10 in the morning a blood sample for the determination of glucose was taken from the tip of the tail. Thereafter the rats were killed by decapitation, blood was collected into a beaker containing sodium heparin and a piece of the liver was taken and frozen in liquid nitrogen. Plasma and liver were stored at −80° until analysed.

Analytical. Previously published methods were used to analyze concentrations of blood glucose [13], plasma urea [14], blood alcohol [15] and the concentrations of free amino acids in the liver [16].

RESULTS AND DISCUSSION

Ethanol intake reduced the amount of energy obtained from solid food in all three diet groups. The decrease was replaced by energy from ethanol and thus there were no significant differences in the total energy consumption between the two rat strains or the different diet groups (Table 1). The weight gain was also equal in each group. The ANA rats significantly decreased their water consumption when fed either the high protein diet or the methylene blue diet and a similar tendency was seen in the AA rats. However, when the consumption of total water (i.e. water plus water in alcohol solution) was calculated a slight increase was observed in the AA rats.

The voluntary alcohol consumption increased significantly (up 48.4% from 5.34 to 7.92 g/kg × d, $P < 0.05$) in the AA rats fed the methylene blue diet. The AA rats fed the high protein diet also tended to increase their ethanol intake although the effect (39.6%) did not reach the level of statistical significance ($P = 0.194$). The diets had no effect on the voluntary alcohol consumption of the ANA rats. In the control group of the AA rats, 13.6% of the total energy came from ethanol and the respective values for the high protein group and the methylene blue group were 18.4% and 19.9%. For the ANA rats, alcohol contributed only 1–2% to the total energy. The individual variation in the alcohol intake of the AA rats was large (from 3.3 to 10.0 g/kg body weight × d) and according to Eriksson [15] it can be assumed that some of the AA rats in all groups consumed such an amount that was close to their maximum capacity to metabolize ethanol. None of the ANA rats had alcohol in their blood at the time of decapitation, whereas measurable, although low, concentrations were found in the blood of the AA rats (Table 2). The blood ethanol concentration at the time of decapitation was higher in the protein and methylene blue groups than in the controls. These differences in blood ethanol concentration can be due to the amount of alcohol consumed and possible variations in the drinking patterns.

The rate of alcohol oxidation in rats has been found to correlate positively with the amount of protein in the diet [2, 17]. Therefore part of the increase in the voluntary alcohol consumption of the AA rats fed the high protein diet may be due to the enhanced rate of ethanol oxidation. Likewise, administration of methylene blue has been shown to increase the rate of ethanol oxidation in man [18] which may have contributed to the increase in voluntary alcohol consumption in the methylene blue-fed AA rats.

Ethanol has been shown to inhibit gluconeogenesis from many important glucose precursors including lactate and glycerol [9]. Therefore, after ethanol ingestion the role of amino acids in the synthesis of glucose becomes increasingly important. The high

Table 2. Concentration of alcohol, urea and glucose in the blood of AA and ANA rats fed control, high protein, or methylene blue containing diet

	Alcohol (mM)	Urea (mM)	Glucose (mM)
Control diet			
AA (6)	0.03 ± 0.01	5.9 ± 0.1	3.59 ± 0.15
ANA (6)	0	6.9 ± 0.5	3.62 ± 0.11
Protein diet			
AA (6)	0.26 ± 0.09*	8.7 ± 0.4†	4.31 ± 0.27*
ANA (6)	0	10.9 ± 0.5†‡	3.45 ± 0.17
Methylene blue diet			
AA (7)	0.53 ± 0.19*	5.9 ± 0.2	3.73 ± 0.22
ANA (7)	0	7.8 ± 0.2§	3.48 ± 0.19

The blood samples for the analysis were taken in the morning after 18 days period on the diets. Results are the means ± SE. Number of animals in parentheses.

* $P < 0.05$; † $P < 0.001$ when compared to the control of the same strain.

‡ $P < 0.01$; § $P < 0.001$ for the interstrain differences.

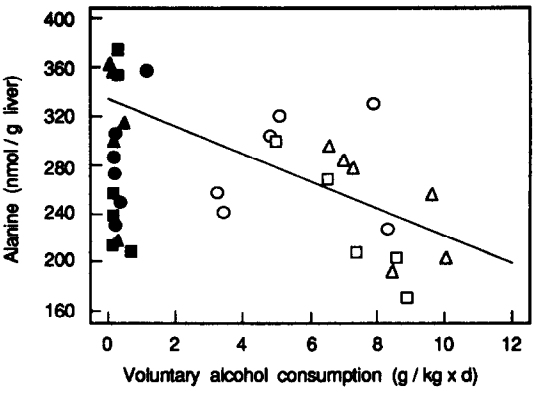

Fig. 1. Correlation of voluntary alcohol consumption with the hepatic alanine concentration. (○) AA rats, control diet; (△) AA rats, high protein diet; (□) AA rats, methylene blue diet; (●) ANA rats, control diet; (▲) ANA rats, high protein diet; (■) ANA rats, methylene blue diet. The regression line for all three groups of the AA rats is shown in the figure.

protein diet has been shown to increase the extraction of glucogenic amino acids by the liver and their utilization in gluconeogenesis [8]. Also the synthesis of urea, the end product of amino acid catabolism, increases in parallel with the synthesis of glucose [19, 20]. When the rats were fed the control diet there was no significant line differences in the concentrations of glucogenic amino acids in the liver (Table 3) or concentrations of glucose and urea in plasma (Table 2), but when the rats were fed the high protein diet or the diet supplemented with methylene blue the utilization of amino acids as indicated by concentration of urea in plasma and amino acids in the liver differed in the AA and the ANA rats. In the AA rats, the plasma concentration of glucose correlated positively with the concentration of urea ($r = 0.473$; $P < 0.05$), whereas no correlation was found in the ANA rats. Furthermore, in the AA rats plasma urea concentration correlated negatively with the sum of the concentrations of alanine, serine, and glycine ($r = -0.523$; $P < 0.05$), which are the three major glucogenic amino acids in the liver. Another index that has been suggested to reflect the use of amino acids for gluconeogenesis [21] is the ratio between the concentrations of branched chain amino acids (valine, leucine and isoleucine) and alanine. The branched chain amino acids are metabolized in the liver only to a minor extent [22], whereas alanine is the major amino acid precursor for gluconeogenesis [9, 23]. This ratio increased in the AA rats fed the high protein diet, but did not change in any other group (Table 3). Together these findings suggest that the use of amino acids for gluconeogenesis increases in AA rats when they are fed the high protein diet. Since the voluntary alcohol

Table 3. Effect of a high protein diet and a methylene blue diet on hepatic concentrations of selected amino acids

	Control diet		Protein diet		Methylene blue diet	
	AA (6) (nmol/g liver)	ANA (6)	AA (6) (% of control)	ANA (6)	AA (7) (% of control)	ANA (7)
Asp	554 ± 49	482 ± 44	62.7	80.1	94.4	124.5
Ser	380 ± 24	336 ± 26	76.6*	82.4	82.6	91.1
Gly	578 ± 38	598 ± 50	71.1†	72.4*	99.7	97.2
Ala	281 ± 18	284 ± 18	83.6	111.6	84.0	94.0
Pro	150 ± 8	157 ± 11	88.7	94.9	79.3*	89.2
Val	331 ± 22	306 ± 27	90.9	101.3	63.1†	90.9
Ile	117 ± 7	109 ± 9	94.9	105.5	82.1*	88.1
Leu	221 ± 13	210 ± 17	96.8	104.8	84.6*	95.2
Glucogenic	1943 ± 131	1856 ± 138	72.3*	84.2	88.4	102.0
$\frac{\text{Val + Leu + Ile}}{\text{Ala}}$	2.38 ± 0.07	2.17 ± 0.35	111.2	94.0	87.7	100.2

Results are means ± SE. The standard errors in the high protein and the methylene blue groups ranged from 6.3 to 9.1%. The number of animals in each group is given in parentheses.

* $P < 0.05$; † $P < 0.01$ from the corresponding control group.

consumption increases simultaneously, it is tempting to speculate that gluconeogenesis is an important regulator of voluntary alcohol consumption. This assumption is further supported by the negative correlation between alanine concentration in the liver and the amount of alcohol consumed during the free choice period that was found in the AA rats ($r = -0.474$; $P < 0.05$), but not in the ANA rats (Fig. 1) and is in keeping with the finding that the AA rats are able to increase their gluconeogenesis in the presence of ethanol [5].

Methylene blue increased significantly the voluntary alcohol consumption of the AA rats. The inhibition of gluconeogenesis by ethanol is believed to be due to the ethanol-induced increase in the redox state [9, 10] which can be prevented by administration of methylene blue [12]. Accordingly, the present finding that methylene blue increases voluntary alcohol consumption can be explained by its ability to maintain the glucose synthesis from amino acids as well as from other precursors like lactate, pyruvate, glycerol.

Taken together, the results of this study suggest that the composition of the diet can increase the voluntary alcohol consumption of the alcohol-preferring AA rats but not that of the alcohol-avoiding ANA rats. The results support the view that both protein and methylene blue normalize the ethanol-disturbed gluconeogenesis thus allowing the rats to consume higher amounts of alcohol. No explanation can be offered for why the ANA rats avoid alcohol. Thus it can be concluded that the regulation of voluntary alcohol consumption is complex and probably involves various mechanisms, one of which is the glucose status of the animal.

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