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HEPATIC AMINO ACID LEVELS IN RATS AFTER LONG-TERM ETHANOL FEEDING

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Abstract—Male Wistar rats were given ethanol to provide 25–33 per cent of the total calories consumed. Control rats were pair-fed either sucrose or lipid. The experiments lasted for 5–9 weeks. In experiment A protein constituted about 15 per cent of the calories and all diets were given until the moment of sacrifice. In experiment B the same diets were fed to similar groups of animals, but a control diet was given to all three groups as the only food the last day before death. Protein provided 10 per cent only of the total calories in this experiment due to a higher intake of ethanol. In experiment C the protein content of the basic diet was enhanced and both ethanol and control rats covered about 25 per cent of their caloric demands by protein. In this experiment, too, a control diet was given to both groups the last day before sacrifice. Long-term ethanol feeding reduced the incorporation of intraperitoneally injected ^{14}C-labelled valine into hepatic protein in all three feeding models. There were, however, no consistent changes in the concentrations of the individual hepatic amino acids when ethanol treated and control rats were compared. It is concluded that reduced hepatic protein synthesis accompanying long-term ethanol consumption is not a direct consequence of lack of a single or several amino acids, and that moderate to severe consumption of ethanol does not disturb the normal pattern of free amino acids in the rat liver.

Long-term feeding of ethanol to rats reduces the rate of incorporation of radioactive amino acids into protein in the livers [1–6]. The mechanism underlying this effect of chronic intake of ethanol is generally unknown. Ethanol may reduce the intestinal absorption of certain amino acids [7,8], and possibly their transport into the liver [9,10]. This suggests that the reduction of protein synthesis mediated by long-term administration of ethanol was a consequence of low intrahepatic levels of one or more amino acids, since it has been shown that the rate of protein synthesis may depend on the hepatic concentration of certain amino acids [11–13]. There are, however, very few data available on the hepatic concentrations of amino acids after chronic consumption of ethanol. In one report [2] the levels of all amino acids determined were actually reduced, supporting the assumption that the reduced hepatic protein synthesis was a consequence of low precursor levels. If so, at least one biochemical effect of ethanol intake might be prevented or treated by nutritional means. The present investigation was undertaken to see if ethanol consumption which caused reduced protein synthesis, was always accompanied by reduced hepatic amino acid levels. This was done by measuring amino acid levels in the livers of various groups of rats pair-fed ethanol in somewhat different ways which all caused reduced rates of incorporation of labelled valine into liver proteins.

MATERIALS AND METHODS

Animals. Male Wistar rats, 210–250 g initial body weight were housed in separate plastic boxes, two animals per box at 22°, 60 per cent humidity on a 12 hr light–12 hr dark cycle from 7 p.m. to 7 a.m.

Ethanol treatment, diets and experimental design. The animals of all groups were offered both a solid synthetic diet *ad libitum* and a liquid diet from small drinking bottles. The solid diet had either a low or a high protein content as detailed previously [6]. The experimental rats (ethanol treated) were fed a liquid diet containing 16 per cent (v/v) ethanol in sucrose, while pair-fed control rats consumed isocaloric amounts of either sucrose or lipid from their drinking fluids. The exact caloric composition of these diets has been published previously [6]. In all experiments the ethanol-consuming group was the leading one with respect to pair feeding, so the amount consumed per cage on one day determined the amount of isocaloric fluid offered to the matched cage in the control groups the next day. Three experiments designed A, B and C were performed, all lasting 5–9 weeks.

In experiment A there was one ethanol group and two control groups, one of these was given sucrose, the other lipid instead of ethanol. The solid diet was of the low protein type. Approximately 15 per cent of all calories consumed were covered by protein in this experiment. The diets were given until the moment of sacrifice.

Experiment B was identical to experiment A with the exception that all liquid diets were replaced by water 24 hr before sacrifice. Due to a higher intake of ethanol in this experiment protein provided only about 10 per cent of the calories.

In experiment C there were only two groups, one experimental and one control in which sucrose replaced ethanol. The solid diet was of the high protein type. Thus these rats obtained approximately 25 per cent of their calories from protein. Twenty-four hours before the end of the experiment the liquid diets were replaced by water and the solid diet by a 20 per cent protein diet.

All rats were weighed twice a week. The final composition of the total diet consumed in the various experi-

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Table 1. Mean daily intake of various diets during long-term feeding*

Expt	Group	Total diet (% of cal)			
		Ethanol	Carbohydrate	Lipid	Protein
A	Ethanol	25	21	40	14
	Sucrose	—	46	40	14
	Lipid	—	20	65	15
B	Ethanol	30	25	35	10
	Sucrose	—	55	35	10
	Lipid	—	23	67	10
C	Ethanol high protein	33	24	19	24
	Sucrose high protein	—	55	20	24

* Rats were given liquid and solid diets, the composition of these has been detailed elsewhere [6]. In experiments B and C the liquid diets were replaced by water the last day and the rats received (% of cal) this day: Carbohydrates 10, Lipid 70, Protein 20.

ments was calculated [6] (Table 1). The daily consumption of ethanol was 8–11 g/kg rat in all experiments and constituted approximately 25–33 per cent of the total calorie intake.

Measurement of hepatic amino acid levels. After exsanguination under light ether anesthesia, the livers were perfused with 10 ml ice-cold 0.9% (w/v) saline through vena porta followed by rapid excision, blotting and weighing of liver samples. The samples were finally frozen in liquid nitrogen. After storage at −80° samples of liver were homogenized in 20 volumes of ice-cold 10% trichloroacetic acid (TCA). After centrifugation of the precipitated proteins the supernatants were extracted with ether to remove TCA and then freeze-dried. The lyophilized amino acids were dissolved in buffer [5] and 1 ml was submitted to analysis in a Jeol JLC-6AH amino acid analyser [14,15] employing a one-column system [16,17] as specified by the manufacturer. Norleucine was added to each sample as internal standard. The transmittance values were transferred by a digital converter to a punched tape and the calculations performed by a Nord-1 computer (Norsk Data-Elektronikk A/S, Oslo Norway) using a program based on that described by Taylor and Davies [18]. Arginine was measured in specimens after ten fold concentration. Tryptophan was determined in separate specimens by the fluorometric method of Denckla and Dewey [19] as modified by Bloxam and Warren [20]. The amounts of the various amino acids were calculated as moles per g fresh liver tissue (wet weight). This was done since none of the feeding schedules caused major changes of liver weight, relative liver weight or hepatic protein concentration (Table 2).

Incorporation of labelled valine. The rats in each

Table 2. Effect of different diets and ethanol feeding on liver composition and on incorporation of labelled valine into liver protein*

Group	Liver		Incorporated into protein (cmp/mg)
	wt (g)	protein (mg/g)	
Exp. A†			
Ethanol	9.01 ± 0.40	250 ± 11	485 ± 38§
Sucrose	10.60 ± 0.66	208 ± 7§	649 ± 60
Lipid	9.47 ± 0.14	233 ± 10	606 ± 43
Exp. B‡			
Ethanol	9.07 ± 0.30	248 ± 8	482 ± 31§
Sucrose	10.12 ± 0.40§	238 ± 6	672 ± 38
Lipid	9.32 ± 0.39	237 ± 8	638 ± 41
Exp. C‡			
Ethanol, high protein	9.92 ± 0.54	246 ± 8	350 ± 27§
Sucrose, high protein	9.81 ± 0.60	235 ± 9	522 ± 30

* Rats were pair-fed for 5–9 weeks as detailed in Table 1, 20 μCi of L-[U-^{14}C]valine per kg rat were injected i.p. 40 min before sacrifice. The results are expressed as mean values ± SEM from six rats.

† The diets were fed until the moment of sacrifice.

‡ The diets were replaced with control diets 24 hr before sacrifice.

§ $\alpha < 0.05$ with respect to the other group(s) in the same experiment.

Table 3. Effect of long-term feeding of ethanol or control diets on the free amino acid concentrations in rat liver *

	Experiment A nmoles/g liver (wet wt)		
	Ethanol	Sucrose	Lipid
Aspartate	1764 ± 289	1140 ± 375	1546 ± 213
Serine	2243 ± 301	2068 ± 271	1714 ± 258
Glutamate	3125 ± 463	2642 ± 510	2804 ± 449
Glycine	1636 ± 24†‡	2260 ± 118	2045 ± 89
Proline	78 ± 10	74 ± 21	100 ± 8
Alanine	1985 ± 108†‡	4166 ± 126	3780 ± 111
Cysteine	76 ± 11†‡	25 ± 8	17 ± 5
Tyrosine	63 ± 5	74 ± 20	76 ± 5
Histidine	893 ± 35†	716 ± 53	770 ± 70
Lysine	1539 ± 52	1323 ± 98	1218 ± 194
Threonine	880 ± 104	944 ± 118	918 ± 86
Valine	204 ± 27	269 ± 38	176 ± 24
Methionine	33 ± 4	34 ± 6	32 ± 3
Isoleucine	107 ± 11	139 ± 22	105 ± 11
Leucine	187 ± 18	197 ± 23	170 ± 19
Phenylalanine	61 ± 9	86 ± 32	65 ± 4
Arginine	10 ± 3	16 ± 4	13 ± 4
Tryptophan	48 ± 5	43 ± 2	40 ± 3

* Control rats were pair-fed isocalorically with leading ethanol consuming rats. All groups were fed until the moment of sacrifice. All values are means ± SEM from 6 rats.

† $\alpha < 0.05$ with respect to corresponding sucrose controls.

‡ $\alpha < 0.05$ with respect to corresponding lipid controls.

group were given intraperitoneal injections of 20 μCi/kg rat L-[U-^{14}C]valine (CFB 75, The Radiochemical Centre, Amersham, U.K.) 40 and occasionally 70 min prior to death to measure the incorporation into protein. Protein was precipitated, washed, dissolved and the radioactivity measured as described earlier [5,6]. The TCA-soluble radioactivity was determined in every sample submitted to incorporation measurement. This radioactivity was shown to represent only labelled valine in experiments where the radioactivity of the valine peak was measured after splitting the eluate from the amino acid analyser column. The specific radioactivity of free valine in liver samples was also determined in such experiments. Since the concentration of free labelled valine was unchanged by any diet or ethanol treatment and since the specific radioactivity of free valine was not influenced either (tested for ethanol and lipid groups only), data on incorporation were likely to represent protein synthesis. Protein concentration was measured according to Lowry *et al.* [21].

Statistics. Wilcoxon's test was used to determine the statistical significance of the results, differences accompanied by α-values above 0.05 were considered insignificant.

RESULTS

Experiment A. In this experiment all diets (ethanol, sucrose control and lipid control) were fed until the moment of sacrifice. The weight gain was about 1.5 g/day in all groups. Samples taken to measure the rate of incorporation of labelled valine into protein revealed 20 to 25 per cent reduction in the ethanol group compared to both control groups (Table 2, Exp. A). This confirmed earlier observations [6]. The amino acid concentrations in the livers are given in Table 3. The concentrations of both glycine and alanine were lower in the ethanol consumption group compared to the control groups. The cysteine level was higher after long-term ethanol intake than after isocaloric consumption of sucrose or lipid control diets. The histidine concentration was also elevated when ethanol treated animals were compared with sucrose consuming controls, but not with respect to lipid fed controls. No significant differences were found with regard to other amino acids measured.

Experiment B. Since control rats consumed their liquid diets faster than the experimental animals, it was obvious that the control rats had consumed a larger percentage of their daily dose of calories just before sacrifice in experiment A. They were thus in a nutritional state different from that of ethanol drinking rats and this might have influenced their hepatic concentration of amino acids. It was therefore of importance to measure amino acids in livers of animals from various groups at more stable nutritional conditions. This was obtained in experiment B by replacing all liquid diets with water 24 hr prior to sacrifice. Incorporation of labelled valine into liver protein was reduced by 25–30 per cent in the ethanol group compared to both control groups, (Table 2, Exp. B) as in previous experiments [6]. In experiment B the weight gain in the ethanol group (1.4 g/day) was somewhat lower than the weight gain in the sucrose group (1.8 g/day) and the lipid group (2.3 g/day). The hepatic amino acid levels are given in Table 4 (three left columns). The glycine

Table 4. Effect of long-term feeding of ethanol or control diets (followed by one day on control diet before sacrifice) on the free amino acid concentrations in rat liver *

	nmoles/g liver (wet wt)				
	Experiment B			Experiment C	
	Ethanol	Sucrose	Lipid	Ethanol high protein	Sucrose high protein
Aspartate	1260 ± 136	1369 ± 79	1318 ± 88	1303 ± 53	1158 ± 88
Serine	1860 ± 228	2263 ± 228	1964 ± 192	1547 ± 88	1612 ± 150
Glutamate	4612 ± 436	4452 ± 128	4392 ± 199	4144 ± 221	4275 ± 296
Glycine	988 ± 128†‡	1627 ± 151	1435 ± 130	901 ± 71	1022 ± 81
Proline	83 ± 12	83 ± 4	96 ± 13	67 ± 6	73 ± 3
Alanine	1864 ± 280†	1254 ± 152	1412 ± 130	1774 ± 115	1538 ± 119
Cysteine	41 ± 9	31 ± 5	30 ± 2	41 ± 5†	31 ± 3
Tyrosine	86 ± 10	88 ± 9	71 ± 4	91 ± 10	86 ± 7
Histidine	837 ± 74	941 ± 44	1023 ± 69	897 ± 50	869 ± 44
Lysine	1152 ± 60†‡	1632 ± 144	1552 ± 92	1356 ± 160	1202 ± 104
Threonine	1064 ± 212	1370 ± 84	1195 ± 82	999 ± 132	965 ± 152
Valine	246 ± 25	234 ± 7	243 ± 13	269 ± 19	310 ± 36
Methionine	36 ± 9	33 ± 3	43 ± 7	26 ± 3	33 ± 5
Isoleucine	145 ± 14	137 ± 6	150 ± 9	152 ± 5	154 ± 12
Leucine	245 ± 18	237 ± 12	254 ± 15	253 ± 13	259 ± 18
Phenylalanine	63 ± 9	55 ± 4	62 ± 5	62 ± 2	64 ± 3

* Control rats were pair-fed isocalorically with leading ethanol consuming rats. All values are means ± SEM from six rats.
† $\alpha < 0.05$ with respect to corresponding sucrose controls.
‡ $\alpha < 0.05$ with respect to corresponding lipid controls.

concentration was again reduced after long-term ethanol intake compared to both control groups, and this time the same was found with respect to the lysine levels. The alanine concentration was not reduced after chronic ethanol consumption in this experiment, this value was in fact increased compared to the sucrose group. No other amino acids measured differed significantly among the various groups.

Experiment C. Since the dietary protein content in experiment B was low in all groups and since long-term intake of ethanol also reduces the incorporation of labelled amino acids into liver protein of animals fed high-protein diets [6], the latter model was also tested with respect to hepatic amino acid levels. The weight increased similarly in the ethanol group and in the control group (sucrose) in this experiment (about 2.3 g/day). The incorporation of labelled valine into liver proteins was reduced by 30–35 per cent in animals drinking ethanol (Table 2, Exp. C). The hepatic amino acid pattern is given in Table 4 (two right columns). The only significant change was an increase in cystine concentration after ethanol intake.

DISCUSSION

Earlier results by Banks *et al.* [2] showed that chronic ethanol consumption was followed by marked reductions in the intrahepatic levels of several amino acids as well as by reduced protein synthesis. These observations could indicate that the former effect had some causal relationship to the latter. However, based on that experiment it was difficult to ascribe these effects to ethanol feeding *per se* since the ethanol drinking animals also exhibited a very low weight gain compared to their controls [2].

This problem was largely circumvented in the present experiments. No reduction of the hepatic concentration of any particular amino acid was present after ethanol intake in all three feeding models used in our experiments. It should be noted that ethanol consumption did not lower the tryptophan concentration in experiment A. A specific regulatory role in hepatic protein synthesis has been ascribed to this amino acid [13, 22]. The sum of the concentrations of the essential as well as the sum of the amino acids shown to be of critical importance for protein synthesis [11,12] were not statistically significantly changed by ethanol consumption in any experiment. Reduced incorporation of amino acids into liver protein in ethanol treated animals was, however, present in all feeding models used. Thus, ethanol consumption caused reduced hepatic protein synthesis without concomitant reduction of any particular amino acid and without any general reduction of amino acid levels. Other causes must therefore be sought to explain why long-term ethanol consumption inhibits protein synthesis in the liver.

If we assume that ethanol feeding really leads to a lowered input of amino acids to the liver from portal blood, several phenomena may have contributed to the observed maintenance of normal amino acid levels in our experiments. Increased proteolysis might have contributed, but lack of elevation of the branched amino acids (leucine, valine, isoleucine) relative to the others, renders this possibility less likely. Reduced intrahepatic amino acid catabolism is another possibility, and the reduction of tryptophan oxygenase and arginase activity after long-term ethanol intake [4, 23–26] supports this possibility. Inhibition of hepatic protein synthesis would also tend to elevate the concentration of amino

acids in the liver. Finally, hormonal effects brought about by the intake of ethanol [4, 27] could have contributed to maintain amino acid homeostasis within the liver.

The present study shows that amino acid measurements following one feeding model only may give the erroneous impression that the concentration of one or more amino acids could be specifically altered by ethanol consumption. Although our results with various models indicate that chronic moderate to severe ethanol intake caused no fundamental changes in the amino acid composition of the rat liver, they do not preclude that such changes may follow for instance more heavy consumption of ethanol, or ethanol given together with other diets, or when given in other ways. In preliminary experiments with an all-liquid diet [28], providing 36 per cent of the calories as ethanol, however, no major changes in hepatic amino acids were found in ethanol drinking rats (J. Mørland and L. Svendsen, unpublished results) thus indicating that long-term ethanol consumption leaves the amino acid composition of the liver mainly unaltered.

Finally, a relationship exists between the free pool of proline and the development of liver cirrhosis [29] and it should be noted that the concentration of liver proline did not increase after ethanol consumption in any of our feeding models. It has been speculated that chronic ethanol consumption elevates hepatic proline levels thereby possibly enhancing collagen biosynthesis [30–32]. Neither our results, nor results on free proline concentrations recently published by other groups [33, 34] support this hypothesis.

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