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Effect of ethanol feeding on fatty acid ethyl ester synthase activity in the liver and pancreas of rats fed a nutritionally adequate diet or a low protein diet

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Alcohol dehydrogenase (ADH) and the microsomal ethanol-oxidizing system (MEOS) are enzymes which catalyse the biological oxidation of ethanol in the liver. Chronic ethanol consumption results in a 2–3-fold enhancement of the MEOS activity [1, 2]. ADH activity has been reported to be increased, decreased and unchanged in the livers of chronic ethanol-fed rats [1–6].

Recently, the presence of non-oxidative ethanol metabolism has been demonstrated in alcohol target organs. In the pancreas, myocardium and brain, where there is little or no ADH activity, the metabolites of this non-oxidative pathway, fatty acid ethyl esters (FAEE), are reported to play an important role in the development of alcoholic organ damage [7–12]. We also reported that ethanol feeding had no effect on pancreatic FAEE synthase activity, but pancreatic FAEE content, increased 5-fold in chronic ethanol-fed rats, correlated inversely with amylase activity [11]. Although FAEE synthase activity is low in the rat liver [11], there is no report about the effect of chronic ethanol feeding on this enzyme activity in liver. In general, alcoholics have low protein intake, so hepatic ADH activity may be decreased [13, 14]. Therefore, the non-oxidative ethanol pathway may work in the liver after ethanol feeding with dietary protein deficiency.

In the present study, we examined the effects of ethanol feeding with a standard or low protein diet on FAEE synthase activity in the liver and pancreas of rats, as well as on hepatic ADH activity.

Materials and Methods

Animals. Twenty-four male Wistar strain rats, weighing about 200 g, were divided into four groups and fed the following diets in individual cages for 7 weeks as described previously [15]. Six rats received a diet in which 18% of the total calories was protein, 35% was fat and 47% was carbohydrate (a standard diet). Six rats received a low protein diet, in which 8% of the calories was protein, 35% was fat and 57% was carbohydrate (a low protein diet). In the ethanol formula, carbohydrate was partially replaced by ethanol. The ethanol amounted to 36% of the total calories in the standard diet (a standard ethanol diet) and also in the low protein diet (a low protein ethanol diet), while the total calories was held constant. All diets contained adequate essential trace elements and vitamins. These experimental diets were purchased from CLEA Japan Inc. (Osaka, Japan). After fasting for 12 hr, the rats were killed by exsanguination from the aorta. The liver and pancreas were removed and subjected to biochemical analysis.

Assay for FAEE synthase activity. FAEE synthase activity was determined by the method of Mogelson and Lange [16] as described previously [11].

Assay for ADH activity. ADH activity was determined by the method of Estival *et al.* [17].

Protein determination. Protein contents were determined by the method of Lowry *et al.* [18].

Statistical analysis. All values were expressed as the mean ± SEM, and the significance of the differences was assessed by one-way analysis of variance.

Results

Body weight, weight gain, organ weights and enzyme activities of the liver and pancreas in the four groups are shown in Table 1. In spite of pair feeding, ethanol feeding resulted in smaller mean weight gain and body weight than the corresponding control groups on both the standard and the low protein diet. However, liver weight per 100 g body weight was greater than the control in the standard ethanol diet group, and did not differ from the control in the low protein ethanol diet group. Rats fed the low protein diet had lower body weight than those fed the standard diet, but liver weight was similar.

The liver ADH activity of rats in the standard ethanol diet did not differ from control. However, these animals did show decreased liver FAEE synthase activity, which was statistically significant when expressed as μmol ethyl oleate/hr/g liver and as μmol ethyl oleate/hr/liver. Rats given the low protein diet had lower liver ADH and FAEE synthase activity than rats given the standard control diet. Liver ADH and FAEE synthase activities in rats given the low protein ethanol diet was very low. It was even lower than the low protein control group.

FAEE synthase activity in the pancreas was remarkably higher than in the liver. There were no significant differences in pancreatic FAEE synthase activity between any of the groups.

Discussion

ADH and MEOS are responsible for the oxidation of ingested ethanol and are abundant in the liver. Ethanol feeding is known to enhance MEOS activity by about 3-fold [19]. After chronic alcohol consumption, liver MEOS activity increases in experimental animals and in humans [6, 20, 21], and the increased rate of ethanol elimination can largely be accounted for by the increase in total liver MEOS activity. However, there is a controversy about the effect of chronic ethanol consumption with nutritionally

Table 1. Effect of ethanol feeding on body weight, weight gain, alcohol dehydrogenase activity and fatty acid ethyl ester synthase activity in liver and pancreas of rats fed a standard or low protein diet

	Standard diet		Low protein diet	
	Control	Ethanol	Control	Ethanol
No. of rats	6	6	6	6
Body weight (g)	413 ± 6	289 ± 7*	338 ± 14*	213 ± 12*‡
Weight gain (g/day)	4.8 ± 0.1	1.8 ± 0.1*	3.5 ± 0.3*	0.8 ± 0.2*‡
Liver				
Weight (g)	13.0 ± 0.7	12.1 ± 0.7	12.2 ± 0.2	7.6 ± 0.4*‡
(g/100 g body wt)	3.1 ± 0.1	4.2 ± 0.2*	3.7 ± 0.1*	3.6 ± 0.1*
Alcohol dehydrogenase				
(μmol NADH/hr/mg protein)	0.45 ± 0.07	0.40 ± 0.10	0.21 ± 0.05†	0.12 ± 0.03*
(μmol NADH/hr/g liver)	20.8 ± 3.3	19.2 ± 5.0	8.0 ± 1.8*	4.4 ± 1.3*
(μmol NADH/hr/liver)	268.5 ± 41.5	226.5 ± 55.3	98.5 ± 22.7*	34.0 ± 11.0*§
(μmol NADH/hr/liver/100 g body wt)	65.1 ± 10.2	79.6 ± 20.1	29.6 ± 6.7†	15.3 ± 4.2*
Fatty acid ethyl ester synthase				
(μmol ethyl oleate/hr/mg protein)	0.09 ± 0.01	0.07 ± 0.01	0.04 ± 0.01*	0.03 ± 0.01*
(μmol ethyl oleate/hr/g liver)	4.4 ± 0.6	2.7 ± 0.4†	2.3 ± 0.3†	1.5 ± 0.3*
(μmol ethyl oleate/hr/liver)	55.7 ± 6.4	32.4 ± 4.2†	27.5 ± 3.9*	11.5 ± 2.1*‡
(μmol ethyl oleate/hr/liver/100 g body wt)	13.5 ± 1.5	11.3 ± 1.5	8.0 ± 1.0†	5.3 ± 0.9*
Pancreas				
Weight (g)	1.4 ± 0.1	1.3 ± 0.0	1.0 ± 0.1†	0.7 ± 0.1*
(g/100 g body wt)	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
Fatty acid ethyl ester synthase				
(μmol ethyl oleate/hr/mg protein)	1.23 ± 0.19	1.03 ± 0.21	1.25 ± 0.18	1.48 ± 0.39
(μmol ethyl oleate/hr/g pancreas)	61.6 ± 10.0	63.8 ± 21.8	70.1 ± 12.0	95.8 ± 24.3
(μmol ethyl oleate/hr/pancreas)	84.1 ± 15.4	81.3 ± 28.3	68.0 ± 11.9	69.1 ± 19.6
(μmol ethyl oleate/hr/pancreas/100 g body wt)	20.4 ± 3.8	27.5 ± 9.2	19.8 ± 3.1	33.2 ± 10.1

Values are means ± SEM.
* $P < 0.01$, † $P < 0.05$ compared with standard control, ‡ $P < 0.01$, § $P < 0.05$ compared with low protein control.
All assays were done in duplicate.

adequate diets on liver ADH activity, although chronic ethanol feeding enhances liver MEOS activity. Increased [2, 4], decreased [1] and unchanged [3, 5, 6] ADH levels have been reported. In the present study, chronic ethanol feeding did not change liver ADH activity in rats fed a standard diet, but a low protein diet significantly decreased liver ADH activity and a low protein ethanol diet led to even more reduction. These reductions of ADH activity may be due to the increased rate of ADH degradation associated with dietary protein deficiency. In fact, it has been reported that food or protein restriction reduces ADH activity by over 50% [13, 14].

Recently, a non-oxidative ethanol metabolizing pathway has been discovered, and metabolites such as FAEE have been reported to play an important role in the development of some forms of organ damage. Since FAEE are neutrally charged and cannot bind to fatty acid binding proteins, they bind to more hydrophobic organelles such as mitochondria. There, the FAEE disturb mitochondrial oxidation, leading to mitochondrial dysfunction [22]. The esterification of free fatty acids with ethanol is catalysed by FAEE synthase, a soluble dimeric enzyme with a molecular weight of 50,000 [16]. This enzyme is distributed in the pancreas, liver, testis and brain in this order in the rat [11], and is distributed in the pancreas, liver, adipose tissue, heart, brain and skeletal muscle in this order in humans [8]. Although the K_m of this enzyme for ethanol is 200 mM, which is higher than that of ADH (2 mM) or MEOS (10 mM) [23], it has been demonstrated that FAEE synthase functions and produces FAEE at physiological concentrations of ethanol from 10 to 200 mM [9].

We previously demonstrated that chronic ethanol feeding had no effect on pancreatic FAEE synthase activity, but pancreatic FAEE content increased and correlated inversely with pancreatic amylase activity. In the present study, pancreatic FAEE synthase activity was unchanged in both the low protein diet group and the low protein ethanol diet group compared with the standard diet group. By contrast, hepatic FAEE synthase activity significantly decreased in the standard ethanol group, the low protein diet group and the low protein ethanol diet group compared with the standard diet group, like ADH activity.

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