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NONOXIDATIVE METABOLISM OF ETHANOL IN THE PANCREAS; IMPLICATION IN ALCOHOLIC PANCREATIC DAMAGE

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Abstract—Alcohol dehydrogenase activity and fatty acid ethyl ester synthase activity were measured in various organs of male Wistar strain rats. The mean (±SE) values of alcohol dehydrogenase activity in liver, testis, pancreas and brain were 223 ± 34, 35 ± 13, 27 ± 17 and 24 ± 15 nmol/hr/mg protein, respectively, but not detectable in heart and skeletal muscle. Fatty acid ethyl ester synthase activity in pancreas, liver, testis and heart were 1348 ± 263, 23 ± 14, 17 ± 3 and 2 ± 1 nmol/hr/mg protein, respectively, but not detectable in brain and skeletal muscle.

Alcohol dehydrogenase activity, fatty acid ethyl ester synthase activity, fatty acid ethyl ester content and amylase activity were measured in pancreas of rat after 7 weeks of ethanol feeding. Compared with control rats, ethanol-fed rats had normal fatty acid ethyl ester synthase activity and alcohol dehydrogenase activity. However, fatty acid ethyl ester content increased five-fold and amylase activity decreased up to 20% of the control group. Fatty acid ethyl ester content was inversely correlated with amylase activity. These results suggest that fatty acid ethyl ester may be responsible for the development of pancreatic damage by ethanol.

It is generally accepted that alcoholic organ damage, for instance liver damage, is induced by the decrease of NAD/NADH ratio and/or the increase of acetaldehyde through the oxidative metabolism of ethanol, other than by ethanol *per se* [1, 2]. We previously showed that prolyl hydroxylase activity and glycylprolyl dipeptidyl-aminopeptidase activity, which were regarded as collagen metabolic enzymes, were significantly increased in pancreas of ethanol-fed rat and these activities were related to amylase depletion, and that these changes were not inhibited by the administration of pyrazole, a specific inhibiter of alcohol dehydrogenase (ADH) [3, 4]. These findings suggest that pancreatic damage by ethanol feeding is not mainly regulated by the oxidative metabolite of ethanol. Chapman and Pattinson reported that ethanol inhibited enzyme synthesis in isolated rat pancreas lobules, but acetaldehyde had no inhibitory effect [5].

Recently, the presence of nonoxidative ethanol metabolism has been demonstrated, the metabolites of which, fatty acid ethyl esters (FAEE), play an important role in the development of some organ damage [6–9]. In the present study, we determined ADH activity, FAEE synthase activity and FAEE content in rat pancreas after 7 weeks of ethanol feeding, and studied the correlation between alcoholic pancreatic damage and nonoxidative metabolism of ethanol.

MATERIALS AND METHODS

Five male Wistar strain rats, weighing about 350 g, which fed on a regular solid diet (CLEA Japan Inc., Tokyo, Japan) *ad libitum*, were used as normal rats. Additionally, 14 male Wistar strain rats, weighing about 200 g, were divided into two groups and received a nutritionally adequate liquid diet (CLEA Japan Inc., Osaka, Japan) for 7 weeks, as described previously [3, 4, 10]. The control diet supplied 18% of the calories as protein, 35% as fat and 47% as carbohydrate (control group). The ethanol diet was similar to the control diet except that ethanol was substituted isocalorically for carbohydrate to provide 36% of the total calories (ethanol group). After fasting for 12 hr, these rats were killed under ether anesthesia by exsanguination from the aorta. The pancreas, liver, testis, brain, heart and skeletal muscle were quickly removed, dissected and freed of fat and connective tissue.

ADH activity was assayed by the method of Estival *et al.* [11]. Briefly, each tissue was homogenized in 10 volumes of 0.3 M sucrose containing 1 mM benzamidine, and centrifuged at 190 *g* for 15 min. The supernatant was taken as the enzyme fraction. The assay mixture contained 2 mM NAD (Oriental East Co. Ltd, Tokyo, Japan), 50 mM ethanol, 75 mM semicarbazide, 75 mM pyrophosphate buffer, pH 9.5 and 6 mM glycine was incubated with enzyme solution at 25°. The production of NADH was measured at 340 nm in the spectrophotometer (U-3200; Hitachi Ltd, Tokyo, Japan). Blank reactions without ethanol were followed simultaneously.

FAEE synthase activity was assayed by the method of Mogelson and Lange [12]. Each tissue was homogenized in 5 volumes of cold 0.01 M Tris–HCl, pH 8.0 containing 1mM 2-mercaptoethanol and centrifuged at 15,000 *g* for 10 min. The supernatant was centrifuged at 48,400 *g* for 1 hr, and the following supernatant was taken as the enzyme fraction. Enzyme fractions were incubated with 0.4 mM [^{14}C]oleate (6600 dpm/nmol; N.E.N., Boston, U.S.A.) and 200 mM ethanol in 60 mM sodium phosphate buffer, pH 7.2 in a total volume of 0.17 ml

at 37° for 1 hr. After the reaction was terminated by the addition of 2 ml cold acetone containing 0.2 μl of carrier ethyl oleate (Sigma Chemical Co., St Louis, MO), volumes were reduced by evaporation under a stream of nitrogen at 37°. Residual lipids were eluted in 50 μl acetone, and whose 2 μl were chromatographed on RP-8F plate (MERCK Co., Darmstadt, F.R.G.), developed with acetonitrile/water/methanol/acetic acid (95/3/2/0.5). After visualization of lipids with iodine vapor, FAEE (ethyl oleate) spots were scraped, and eluted with acetone. After addition of 10 ml ACS-II (Aqueous Counting Scintillant; Amersham Co., Arlington Heights, IL), the radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4640). All assays were done in duplicate. Blank reactions with acetone before incubation were followed simultaneously. Under this condition, FAEE synthase activity increased linearly up to 0.3 mg wet weight of pancreas and up to 3 mg wet weight of liver and testis, but little activity was measured in the heart, brain and skeletal muscle (Fig. 1).

Pancreatic FAEE content was assayed by the method of Kinnunen and Lange [13]. Briefly, removed pancreas was homogenized in 10 volumes of cold acetone and centrifuged at 1,500 *g*. The supernatants were evaporated under a stream of nitrogen at room temperature. After residual lipids were eluted with 50 μl acetone, whose 20 μl were chromatographed on RP-8F plate as described. After visualization of lipids, FAEE spots were scraped, and the lipid was eluted with acetone. After addition of 2 nmol ethyl heptadecanoate (Gaskuro Kogyo Inc., Tokyo, Japan) as the internal standard, lipids in acetone were evaporated under a stream of nitrogen at room temperature. The residual lipids were eluted with 25 μl hexane, and whose 0.5 μl were quantitated by gas chromatograph (GC-14A; Shimadzu Co., Kyoto, Japan) using ULBON HR-SS-10 capillary column (25 mm × 50 m; Shinwakakou Co. Ltd, Kyoto, Japan) (Fig.2). Concentration was calculated using the peak ratio to ethyl heptadecanoate as the internal standard.

Pancreatic amylase activity was measured using the Amylase test (Shionogi Pharm. Co., Osaka, Japan). Protein contents were determined by the method of Lowry *et al.* [14].

All values were expressed as the mean ± SE, and Student's *t*-test was employed in the statistical evaluation of the data. Significance of correlation was assessed using Spearman's test.

RESULTS

Organ distributions of ADH activity and FAEE synthase activity were measured in normal rats. The mean (± SE) of ADH activities in liver, pancreas, testis and brain were 223 ± 34, 27 ± 17, 35 ± 13 and 24 ± 15 nmol/hr/mg protein, respectively, although those in heart and skeletal muscle were not detectable. FAEE synthase activities in liver, pancreas, testis and heart were 23 ± 4, 1348 ± 263, 17 ± 3 and 2 ± 1 nmol/hr/mg protein, respectively, although those in brain and skeletal muscle were not detectable (Fig.3). This data indicates that ethanol was metabolized in the liver by ADH through the oxidative pathway, but it was mainly metabolized in the pancreas by FAEE synthase through the nonoxidative pathway. Accordingly, alcoholic pancreatic damage may be linked to the FAEE which is produced through the nonoxidative pathway.

ADH activity, FAEE synthase activity, FAEE content and amylase activity were measured in rat

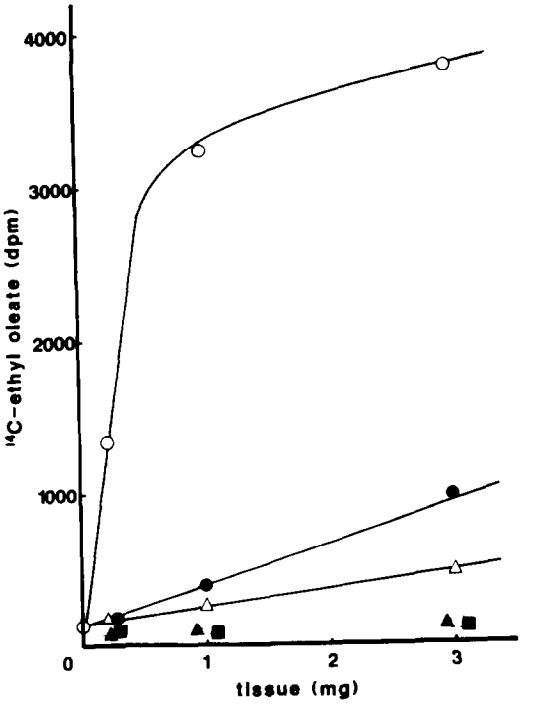

Fig. 1. Relation of fatty acid ethyl ester synthase activity to enzyme solution. ○: pancreas, ●: liver, △: testis, ▲: heart, □: brain, ■: skeletal muscle.

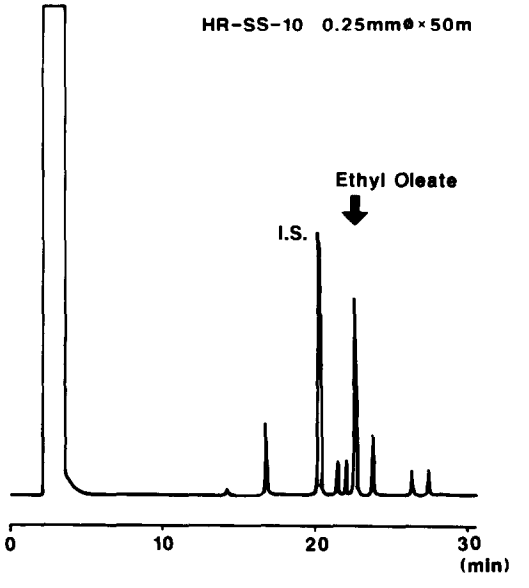

Fig. 2. Fatty acid ethyl ester analysis by gas chromatography following isolation with thin layer chromatography. I.S.: Ethyl heptadecanoate.

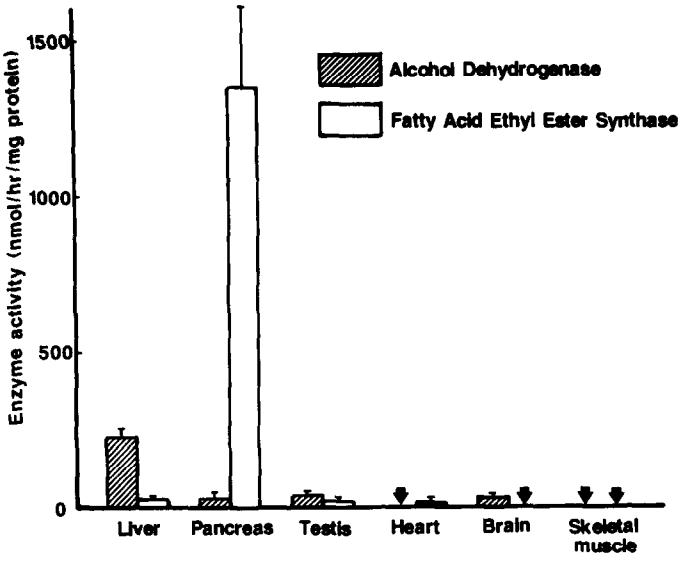

Fig. 3. Distribution of alcohol dehydrogenase and fatty acid ethyl ester synthase in rat organs (mean ± SE).

pancreas after 7 weeks of ethanol feeding (Table 1). Body weight and weight gain were significantly lower in the ethanol group. As we did not use pair feeding, the insufficient intake of the liquid diet in the ethanol group seems to be responsible for the low body weight. In the pancreas, ADH activities were not different between the control group and the ethanol group. The mean of FAEE synthase activities in the control group and the ethanol group were 1287 ± 157 and 987 ± 193 nmol/hr/mg protein, respectively. However, FAEE content in the pancreatic tissue from the control group and from the ethanol group were 1.22 ± 0.25 and 6.83 ± 0.96 nmol/g pancreas, respectively. In the ethanol group FAEE content was increased significantly ($P < 0.01$), and amylase activity was decreased significantly ($P < 0.01$) as compared with the control group. As shown in Fig.4, pancreatic FAEE content was inversely correlated with pancreatic amylase activity ($r = -0.851$, $P < 0.01$).

DISCUSSION

It is proposed that alcoholic organ damage, in which a little ethanol is metabolized through the oxidative pathway, is induced by the nonoxidative ethanol metabolites [7]. Recently, among the nonoxidative ethanol metabolizing enzymes, FAEE synthase has been isolated and purified in rabbit myocardium, and it has a M_r of approximately 50,000 which is a soluble dimer comprised of two nearly identical subunits each with a M_r of 26,000 [12]. Its optimal pH is 6–7, which is nearly the value *in vivo*. K_m for ethanol is 2×10^{-1}M, which is higher than that of ADH (2×10^{-3}M) or microsomal ethanol-oxidizing enzyme (1×10^{-2}M), respectively [15].

Table 1. Effect of ethanol feeding on body weight, alcohol dehydrogenase activity, fatty acid ethyl ester synthase activity, fatty acid ethyl ester content and amylase activity in pancreas of rat*

	Control	Ethanol	P
No. of rats	7	7	
Body weight (g)	408 ± 7	285 ± 7	0.05
Weight gain (g/day)	4.7 ± 0.1	1.7 ± 0.2	0.01
Pancreas			
Weight (g)	1.3 ± 0.1	1.2 ± 0.1	N.S.
Alcohol dehydrogenase (nmol/hr/mg protein)	71 ± 35	100 ± 48	N.S.
Fatty acid ethyl ester synthase (nmol/hr/mg/protein)	1287 ± 157	987 ± 193	N.S.
Fatty acid ethyl ester (nmol/g pancreas)	1.22 ± 0.25	6.83 ± 0.96	0.01
Amylase (IU/mg protein)	93 ± 10	18 ± 3	0.01

* Figures are expressed as mean ± SE.

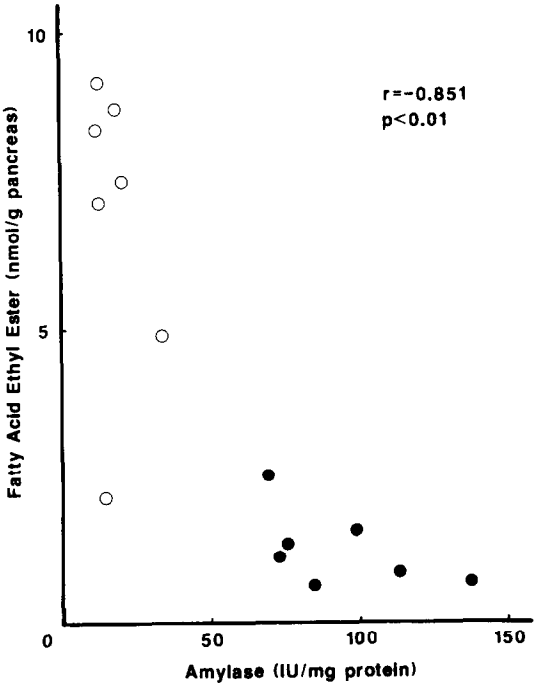

Fig.4. Correlation of pancreatic amylase activity with pancreatic fatty acid ethyl ester content. ●: control diet, ○: ethanol diet.

Accordingly, FAEE synthase seems not to be active in the low ethanol concentrations. However, the partially purified FAEE synthase was active and produced FAEE at ethanol concentrations from 10 to 200 mM [8]. In fact, blood ethanol concentrations from 10 to 40 mM *in vivo* were linearly related to increasing FAEE concentration in tissues [7]. As cholesterol esterase also mediates the esterification of fatty acid with ethanol [16], it is uncertain whether FAEE synthase activity in the present assay system was one enzyme or not.

In the present study, FAEE synthase activity was remarkably high in the pancreas, and a little activity was detected in liver, testis and heart. According to the observation in man, FAEE synthase activity was shown in the pancreas, liver, heart and brain, in this order [7]. Although the distribution of FAEE synthase activity was not identical in rat and man, FAEE synthase activity was remarkably high in the pancreas. As FAEE content in the pancreas was significantly high in the ethanol group, it is conceivable that ethanol is actually metabolized by FAEE synthase in spite of having high K_m for ethanol. In fact, it has been reported that pancreatic FAEE content was high in three-fold in chronic ethanol abusers compared with control subjects [7].

Commonly, nonesterified fatty acids bind to intracellular fatty acid binding proteins. However, since FAEE are neutrally charged, they cannot bind to fatty acid binding proteins and can bind to more hydrophobic organelles like mitochondria [17]. FAEE and/or free fatty acid, thus produced, affect the mitochondrial oxidation leading to mitochondrial dysfunction. It has been reported that ethanol decreases the levels of ATP in rabbit pancreas, and this result is probably linked to mitochondrial dysfunction [18]. In the present study, there is a significant inverse correlation between amylase activity and FAEE content in the pancreas. As pancreatic amylase activity decreased after the administration of ethanol in rats as a biochemical pancreas damage [3, 4, 19–22], the present study confirms that FAEE is one of the factors inducing alcoholic pancreas damage.

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