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DECREASE IN ARACHIDONOYL-CONTAINING PHOSPHATIDYLINOSITOLS IN PANCREAS OF RATS FED AN ETHANOL-CONTAINING DIET

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Abstract—The composition of the glycerophosphatides in pancreas and liver was studied in rats fed an ethanol-containing diet and in pair-fed controls. The fraction of arachidonoyl-containing phosphatidylinositols in pancreas was much lower in the former rats, also when the rats were starved for a final 24 hr period. This fraction was also lower in fed than in starved control rats. The effect was not observed after acute administration of ethanol. It is suggested that the decrease in arachidonoyl-containing phosphatidylinositols was due to chronic pancreatic hyperfunction in the ethanol-fed rats.

Alcoholism is frequently complicated by acute or chronic pancreatitis [1]. The pathogenesis of these diseases is incompletely understood. It has been suggested that the primary event is a change in the composition of the pancreatic juice, resulting from increased parasympathetic tone [1]. An alternative hypothesis is that alcohol is toxic to the pancreas and causes steatosis followed by inflammation and fibrosis [2]. This mechanism would be similar to that suggested for the development of hepatic cirrhosis [3]. This process might be initiated by the metabolism of ethanol in pancreatic cells [4, 5] and a resulting change in the redox state and triglyceride synthesis [4]. A third possibility for the primary change in the pancreatic cell is that the composition of the membrane lipids is changed in the direction of lower fluidity [6, 7]. Therefore the composition of the phospholipids was studied in rats fed an ethanol-containing diet and in pair-fed controls [8].

MATERIALS AND METHODS

Animals. Sprague–Dawley rats were fed the liquid diet described by DeCarli and Lieber [8] for 24 days. The alcohol diet, which provides 36% of the energy from ethanol and the control diet with carbohydrate replacing ethanol, were obtained from Bioserv, Inc. (Frenchtown, NJ, U.S.A.). Alcohol was introduced stepwise, and the controls were pair-fed [8]. The weights before the experiment were 105–115 g (male rats) and 170–185 g (female rats). The controls gained 63 ± 19 g (S.D.) (males, N = 5) and 37 ± 7 g (females, N = 4), whereas the ethanol-treated rats gained 48 ± 11 g (males, N = 5) and 23 ± 5 g (females, N = 4). The rats were killed by cervical dislocation, and the pancreas and liver were taken out, avoiding visible fat. The liver was rinsed in saline and the organs were then homogenized in chloroform–methanol (2 : 1, v/v).

Analytical procedures. Glycerophosphatides and triacylglycerols were quantitatively analyzed as previously described [9], with the exceptions that the 1,2-diacylglycerols obtained by enzymatic hydrolysis of glycerophosphatides were in most cases not subfractionated prior to gas chromatography, and that the ether analogues were not analyzed. The trimethylsilyl ethers of the diacylglycerols obtained from the pancreatic phosphatidylinositols of male rats were analyzed by gas chromatography/mass spectrometry [10] using a 25 m OV-1 fused silica capillary column at 260–300° (4°/min) in a Finnegan 1020 instrument with an electron energy of 40 eV.

Student's *t*-test was used for statistical analysis.

RESULTS

The triacylglycerol content in liver, but not in pancreas, was higher in the ethanol-treated female rats than in the corresponding controls (Table 1). Both the concentration and the relative amounts of phospholipid classes were similar in liver and pancreas, and there were no significant differences between ethanol-treated rats and corresponding controls. The diacylglycerols obtained from the glycerophosphatides were separated according to number of acyl carbons. When this was done with material from livers of female rats, no major differences were observed between ethanol-treated and control rats (Table 2). In contrast, the corresponding analysis of glycerophosphatides from pancreas revealed a pronounced difference (Table 3). Thus, the fraction of phosphatidylinositols with 36 acyl carbons was significantly higher in the ethanol-treated rats than in the control rats. There was a corresponding decrease in the fraction with 38 acyl carbons. Gas chromatography/mass spectrometry of the diacylglycerol trimethylsilyl ether [10, 11] showed that the main component was a stearoyl-eicosatetraenoylglycerol. Thus, ions were seen at *m/z* 433 and 286, indicating an eicosatetraenoyl residue. Ions at *m/z* 267, 341 and 413 indicated a stearoyl residue. Similar,

Table 1. Concentrations (mg/g tissue) of phospholipids and triacylglycerols (including cholesteryl esters) and composition in mol% of phospholipids in liver and pancreas of female rats fed diets with or without ethanol for 24 days

	Liver		Pancreas	
Concentration (mg/g tissue)	Control diet (N=4)	Ethanol diet (N=4)	Control diet (N=3)	Ethanol diet (N=3)
Phospholipids	29.9 ± 2.5	33.3 ± 1.4	27.7 ± 2.6	28.2 ± 1.4
Triacylglycerols	36.8 ± 7.0	81.2 ± 15.8**	68.8 ± 13.9	51.8 ± 16.5
Composition of phospholipids (mol%)				
Sphingomyelins	4.2 ± 0.4	4.0 ± 0.3	4.4 ± 0.4	4.3 ± 1.2
Phosphatidylcholines	55.3 ± 2.9	52.9 ± 1.9	58.6 ± 1.3	58.7 ± 2.5
Phosphatidylethanolamines	23.3 ± 0.7	24.7 ± 2.3	25.0 ± 1.0	23.5 ± 1.5
Phosphatidylinositols	11.5 ± 1.4	11.1 ± 0.7	7.9 ± 1.0	10.3 ± 4.2
Phosphatidylserines	3.4 ± 1.0	3.6 ± 0.4	1.0 ± 0.1	1.1 ± 0.7
Cardiolipins	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
Phosphatidic acids	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
Lysoglycerophosphatides	1.5 ± 0.2	2.9 ± 1.5	2.5 ± 1.0	1.2 ± 0.3
Other phospholipids	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.3

Values are mean ± S.D., ** denotes significant ($P < 0.01$) difference between ethanol and control groups.

Table 2. Percentage composition of molecular species of different glycerophosphatides in liver of female rats fed diets with or without ethanol for 24 days

	Phosphatidylcholines		Phosphatidylethanol amines		Phosphatidylserines		Phosphatidylinositols	
Acyl carbons	Controls	Ethanol diet	Controls	Ethanol diet	Controls	Ethanol diet	Controls	Ethanol diet
32	2.4 ± 0.5	2.0 ± 0.3	< 0.1	< 0.1	2.2 ± 2.4	0.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.1
33	0.3 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	0.3 ± 0.2	< 0.1	< 0.1
34	13.2 ± 2.9	16.1 ± 1.4	3.0 ± 0.2	2.9 ± 0.5	1.7 ± 0.7	2.8 ± 1.8	1.1 ± 0.6	1.4 ± 0.2
35	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	< 0.1	< 0.1
36	29.9 ± 3.2	30.1 ± 1.3	17.9 ± 1.6	16.6 ± 2.0	15.2 ± 4.9	18.6 ± 4.4	8.5 ± 1.8	8.9 ± 1.2
38	52.5 ± 6.0	49.0 ± 2.7	70.7 ± 3.8	71.9 ± 2.8	60.6 ± 5.3	56.9 ± 2.9	88.5 ± 3.4	86.8 ± 2.3
40	1.6 ± 1.2	2.3 ± 2.0	8.2 ± 3.6	8.4 ± 1.3	19.9 ± 5.5	20.9 ± 7.4	1.8 ± 1.0	2.7 ± 1.0

Values are mean ± S.D. (N=4).

Table 3. Percentage composition of molecular species of different glycerophosphatides in pancreas of female rats fed diets with or without ethanol for 24 days

	Phosphatidylcholines		Phosphatidylethanol amines		Phosphatidylserines		Phosphatidylinositols	
Acyl carbons	Controls	Ethanol diet	Controls	Ethanol diet	Controls	Ethanol diet	Controls	Ethanol diet
32	4.6 ± 0.4	3.9 ± 0.3	1.5 ± 0.1	1.8 ± 0.2	1.3 ± 0.1	1.1 ± 0.2	2.3 ± 0.3	1.8 ± 0.1
33	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	< 0.1	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.0
34	40.0 ± 0.8	43.5 ± 2.1	23.1 ± 1.0	22.3 ± 1.3	11.4 ± 2.4	11.1 ± 1.4	17.0 ± 2.4	22.2 ± 1.0
35	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
36	43.4 ± 0.8	41.5 ± 0.8	47.9 ± 1.9	52.2 ± 0.8	58.4 ± 1.0	62.5 ± 1.4	36.2 ± 1.6	53.3 ± 3.0**
38	11.6 ± 0.3	10.5 ± 2.3	26.7 ± 2.2	21.9 ± 1.5	17.2 ± 1.9	15.2 ± 0.4	42.0 ± 6.6	20.4 ± 2.1
40	—	—	0.5 ± 0.8	1.5 ± 1.5	11.1 ± 1.5	9.1 ± 0.6	2.1 ± 2.3	1.8 ± 1.6

Values are mean ± S.D. (N=3), ** denotes significant ($P < 0.01$) difference between ethanol and control groups.

Table 4. Percentage composition of molecular species of phosphatidylinositols in pancreas of male rats

Acyl carbons: double bonds	Major molecular species	Liquid diet 24 days		Control diet 2 days		Liquid diet 23 days starved 1 day		Starved 1 day	
		Pair-fed controls (N=5)	Ethanol diet (N=5)	Starved 1 day (N=4)	Control diet 1 day (N=4)	Pair-fed controls (N=5)	Ethanol diet (N=5)	Water in stomach tube 6 hr (N=4)	Ethanol in stomach tube 6 hr (N=4)
32:1	1-palmitoyl-2-palmitoleoyl	< 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	2.0 ± 0.2	1.2 ± 0.3**	3.1 ± 0.1	3.1 ± 0.4
32:0	1,2-dipalmitoyl	2.2 ± 0.7	1.5 ± 0.2	2.8 ± 0.3	2.0 ± 0.2				
34:2	1-palmitoyl-2-linoleoyl	1.3 ± 0.3	1.4 ± 0.7	3.1 ± 0.7	1.9 ± 0.3				
34:1	1-palmitoyl-2-oleoyl	7.8 ± 1.6	11.0 ± 2.3***	8.0 ± 0.6	10.6 ± 0.8**	13.3 ± 1.3	18.4 ± 1.7**	17.2 ± 1.1	17.6 ± 1.0
34:0	palmitoyl-stearoyl	5.1 ± 1.2	11.8 ± 1.7***	6.4 ± 0.5	9.5 ± 0.7***				
36:4	1-palmitoyl-2-arachidonoyl	6.7 ± 1.7	< 0.1***	3.0 ± 0.6	2.2 ± 0.4				
36:3	oleoyl-linoleoyl	0.9 ± 0.3	3.0 ± 1.1**	1.5 ± 0.4	2.1 ± 0.4				
36:2	1-stearoyl-2-linoleoyl[a]	14.8 ± 2.3	26.3 ± 1.7***	21.4 ± 1.7	23.2 ± 2.1	23.7 ± 2.5	47.8 ± 4.9***	24.5 ± 0.8	24.5 ± 0.4
36:1	1-stearoyl-2-oleoyl	11.7 ± 2.4	26.1 ± 2.0***	12.6 ± 1.8	20.5 ± 2.5**				
38:5	1-oleoyl-2-arachidonoyl	2.3 ± 0.6	<0.1***	4.2 ± 1.3	2.2 ± 0.5				
38:4	1-stearoyl-2-arachidonoyl	45.5 ± 6.6	18.3 ± 4.3***	36.6 ± 2.8	25.1 ± 4.4**	61.0 ± 3.9	31.3 ± 4.6***	55.2 ± 1.9	54.8 ± 1.9
38:3	—	1.6 ± 0.9	0.4 ± 0.5	0.4 ± 0.3	0.6 ± 0.2				

All rats were given pellets *ab lib.* before the experiments. Values are mean ±S.D., ** and *** denote significant ($P < 0.01$ and $P < 0.001$, respectively) differences between corresponding groups.

[a] Contains about 15% 1,2-dioleoyl species.

but much smaller differences were seen in the composition of the phosphatidyletanolamines and the phosphatidylserines.

A similar experiment was performed with male rats. The composition of the molecular species of phosphatidylinositols was analyzed by fractionating the diacylglycerols as trimethylsilyl ethers by reversed-phase chromatography on Lipidex-5000 [11] followed by gas chromatography/mass spectrometry. The diacylglycerols were identified with respect to number of carbons and number of double bonds in each acyl groups as previously described [10, 11]. The results showed that the species containing arachidonic acid constituted a much smaller fraction in the ethanol-treated rats than in the control rats (Table 4). Opposite differences were seen for the major species containing linoleoyl, oleoyl or stearoyl groups at C-2.

Since the ethanol-treated rats could eat continuously whereas the pair-fed controls consumed the food in a few hours after the administration in the morning, the latter rats were actually starved at the time of sacrifice in contrast to the ethanol-treated rats. The effect of starvation was therefore investigated. Male rats (180 g) were given the control diet *ad lib.* for three days or for two days followed by one day of starvation. The composition of the phosphatidylinositols in pancreas of these rats were different (Table 4), indicating that the results from the ethanol-feeding experiments could be at least partly due to the continuous supply of liquid diet. In order to eliminate this, the experiment with ethanol-containing and controls diets was repeated, but the diets were removed 2 hr after the last administration and 24 hr before sacrifice (Table 4). The phosphatidylinositols were analyzed with respect to number of acyl carbons, and the results showed that the difference between ethanol-fed and control rats remained.

The acute effect of ethanol was studied in male rats (180 g) by starvation for 24 hr and feeding 1 ml of water or 1 ml of 20% (w/v) ethanol by stomach tube at 08.00, 10.00 and 12.00 hr, followed by sacrifice at 14.00 hr. The phosphatidylinositols were analyzed with respect to number of acyl carbons, and no differences were seen between ethanol-treated and control rats (Table 4).

DISCUSSION

Feeding animals with ethanol-containing diets has previously been shown to result in an increased concentration of phospholipids in the liver, with decreased arachidonic acid content and increased linoleic acid content [12]. These changes were not observed in the present study, although the hepatic content of triacylglycerols increased as expected [8]. In contrast, the species containing arachidonic acid decreased and those containing linoleic acid increased markedly among the phosphatidylinositols of the pancreas. Possibly there were also small decreases in the arachidonic acid content of the phosphatidylserines and phosphatidylethanolamines. The mechanisms(s) behind the highly specific and pronounced change in the composition of pancreatic phosphatidylinositols is not known. Since the change was not observed in phosphatidylcholines it is unlikely to be compensatory to an increased membrane fluidity caused by the presence of ethanol [6, 7] or that it is due only to decreased formation of arachidonic acid or increased destruction by peroxidation [12]. Stimulation of pancreatic secretion has long been known to be associated with an increased turnover of phosphatidylinositols [13–15], and recent studies have indicated that this is due to preferential formation of prostaglandin E_2 from arachidonic acid released from phosphatidylinositols [16, 17]. This might explain the lower content of arachidonoyl-containing phosphatidylinositols in fed than in starved control rats. Feeding ethanol-containing diet also decreased the content of these species, but this effect remained when the rats were starved. This indicates that food intake and chronic ethanol treatment change the phosphatidylinositol composition by different mechanisms. The chronic hyperfunction of the pancreatic acinar cells that may be caused by ethanol treatment [1, 18] might result in excessive prostaglandin production. If the supply of arachidonic acid is diminished, e.g. by decreased formation or destruction by peroxidation [12], this could explain the decrease in arachidonoyl-containing phosphatidylinositols. A secondary decrease in prostaglandin production might be responsible for the development of pancreatitis, since prostaglandin E_2 has been shown to protect against ethionine-induced pancreatitis [19].

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