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Effect of Ethanol on Rat Liver

III. Lipid Composition of Liver Mitochondria from Rats after Prolonged Alcohol Consumption*

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Long-term feeding of ethanol to rats influences the lipid metabolism of the liver and the structure and function of the liver mitochondria. In order to see if the disturbed lipid metabolism changed the lipid composition of the liver mitochondria, the mitochondrial lipids were quantitatively determined. A slight but non-significant decrease in the content of phospholipids and an increase in that of neutral lipids were observed in mitochondria from alcohol-treated animals, as compared with controls. The composition of the phospholipid fraction was nearly identical in the two groups of animals.

One of the most obvious metabolic effects of ethanol is that on the lipid metabolism. This effect is characterized by changes in the plasma lipids and by the development of fatty liver. The basic effect behind this deranged lipid metabolism has not yet been elucidated. It seems, however, that, in addition to an impaired nutrition, the direct and indirect metabolic actions of alcohol must play some role in the pathogenesis of the fatty liver.[2,3]

Ultrastructural examinations of parenchymal liver cells from human beings and rats after prolonged alcohol consumption have revealed morphological changes in the mitochondria.[4–10] All cellular membrane structures are built up of lipids, chiefly phospholipids, and proteins. It is therefore reasonable to assume that a deranged lipid metabolism may lead to an impairment of the morphology and function of these membranes.

In order to see if the disturbed lipid metabolism in the liver of alcohol-treated rats had an influence on the lipid composition of the liver mitochondria, we quantitatively determined the different mitochondrial lipids.

* A preliminary abstract of this work has been published.[1]

EXPERIMENTAL

Wistar rats from this laboratory's stock were used. The males of each litter were divided into two groups, one being used for ethanol treatment and the other as controls. The controls were drinking water and a sugar solution isocaloric with the ethanol consumed by the ethanol group. Both groups had free access to adequate solid food. The ethanol used was a 15 % (vol./vol.) solution. Each animal was kept in a separate cage and had free access to either water (controls) or ethanol (alcohol-treated group). The animals were kept in this way for 8 months before liver samples were taken.

The liver mitochondria were prepared by the method of Schneider and Hogeboom.[11] The mitochondrial pellet was suspended in 0.25 M sucrose and immediately lyophilized. The mitochondrial suspension was then extracted with a mixture of chloroform and methanol (2:1, vol./vol.) for 30 min by heating to 50°C under reflux. The lipid extract was filtered through a glass filter and the residue washed with the same solvent. The solvent was then evaporated in a vacuum in a stream of nitrogen. The lipids were redissolved in 20 ml of a chloroform-methanol mixture (2:1, vol./vol.). Non-lipid contaminants were removed by phase partition with 5 ml of 0.1 % NaCl solution.

In order to separate the neutral lipids from the phospholipids, the lipid extract was dissolved in a mixture of 8 % diethyl ether in light petroleum (b.p. 60—80°C) and pipetted on a silicic acid column (Bio-Rad silicic acid, minus 325 mesh, freshly activated for 12 h at 110°C). The neutral lipids were eluated with the diethyl ether-light petroleum mixture and the phospholipids with methanol.

The total fat content was determined by evaporating an aliquot of the lipid extracts and weighing the residue. Glyceride-glycerol was determined as formaldehyde by the chromotropic acid color reaction.[12]

The lipid phosphorus was determined by the molybdate reaction after digestion with a mixture of perchloric acid and sulfuric acid.[13] The different phospholipids were separated by thin-layer chromatography on ordinary glass plates. They were coated with silica gel H (E. Merck AG, Darmstadt), by the method of Lees and DeMuria.[14] Before use, the plates were heated at 120°C for 1 h. An aliquot of the phospholipid extract was dissolved in chloroform and applied to the plate with a microsyringe. The chromatography was performed in glass chambers at 4°C in the dark. The solvent used was chloroform-methanol-water (75:25:4, vol./vol.). After separation, the phospholipid spots were made visible by exposing the plates to iodine vapour.[15] The spots with the main phospholipids and the corresponding blank areas were scraped off into test tubes for phosphorus determination.

RESULTS

Table 1 shows the mean values of the amount of total lipids, phospholipids and neutral lipids in liver mitochondria from 13 alcohol-treated rats and 10

Table 1. Lipid composition of rat liver mitochondria. Alcohol = alcohol treated rats; Control = water drinking rats.

	Lipids (mg lipid/100 mg mitochondrial protein)					
	Total lipids		Phospho-lipids		Neutral lipids	
	Alcohol	Control	Alcohol	Control	Alcohol	Control
Mean value	18.1	18.9	15.1	16.2	0.82	0.63
No. of exp.	13	10	13	10	13	10
S.D.	3.8	2.2	3.3	2.6	0.43	0.14
P-value	0.5		0.4		0.2	

control animals. In the alcohol-treated group there are slightly less phospholipids and a little more neutral lipids than in the controls. However, there are no statistically significant differences between the two types of animals. The non-esterified fatty acids, cholesterol, and cholesterol esters were not determined separately. In a few experiments not reported here we found no difference in total cholesterol between the two groups.

The phospholipids in liver mitochondria from alcohol-treated animals and control animals were separated by thin-layer chromatography. The four main phosphorus-containing spots showed the same mobility as sphingomyelin, phosphatidyl ethanolamine, phosphatidyl choline, and cardiolipin. The only ninhydrin-positive spot was that identified as phosphatidyl ethanolamine. The percentual lipid-phosphorus distribution was calculated for these four spots. As can be seen from Table 2, there are no significant differences between the mitochondria from alcohol-treated animals and from controls.

Table 2. Proportions of the main phospholipid fractions of rat liver mitochondria. The lipid phosphorus in each phospholipid fraction is calculated as percentage of lipid phosphorus in the total mitochondrial phospholipids. Alcohol = alcohol treated rats; Control = water drinking rats.

	Phospholipids							
	Sphingomyelin		Phosphatidyl choline		Phosphatidyl ethanolamine		Cardiolipin	
	Alcohol	Control	Alcohol	Control	Alcohol	Control	Alcohol	Control
Mean value	11.4	12.3	45.4	44.2	30.3	29.9	12.9	13.3
No. of exp.	14	13	14	13	14	13	14	13
S.D.	4.1	4.2	3.0	4.5	2.0	3.1	3.5	3.3

DISCUSSION

The lipid composition of rat-liver mitochondria has been thoroughly investigated.[16,17] Nearly all of the mitochondrial lipids are present in the membrane fraction. About 90 % of the membrane lipids are phospholipids; the remainder is largely composed of triglycerides, diglycerides, and cholesterol. Most of the membrane lipids undoubtedly serve a purely structural function in the lipid double-layer core of the membranes. However, the phospholipids in the membrane have also been identified as having quite specific functions in electron transport, translocation mechanisms, and swelling and contraction. Thus, cardiolipin has been shown to be involved in the mechanism of mitochondrial contraction,[18] and lecithin has a specific function in the D-β-hydroxybutyric dehydrogenase.[19] Since the lipids play a vital role in the organization of the complex multi-enzyme system which constitutes the mitochondrion, it seems reasonable to assume that a deranged lipid metabolism may lead to morphological and functional disturbances of the mitochondrion.

The present investigation shows that prolonged alcohol consumption does not change quantitatively the main lipid fractions of liver mitochondria (Tables 1 and 2). We have not investigated the fatty acid composition of the individual mitochondrial lipids. This was, however, recently done by Sheigh *et al.*[20] They found a small and not significant decrease in the amount of the fatty acids 18:2 and 20:4 and a significant rise in 18:1 in rat liver mitochondria after prolonged ingestion of a 15 % ethanol solution.

These changes resemble those observed in liver mitochondria in cases of essential fatty acid deficiency.[21] However, dietary deficiency of essential fatty acids also results in a twofold increase in the neutral lipid content of liver mitochondria.[21] which is not observed to the same extent after prolonged ethanol treatment (Table 1). Moreover the morphological changes of the mitochondria in the livers of animals deficient in essential fatty acids [22] are quite different from those observed after ethanol treatment.[2,7,20]

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