Volume 293, number 1,2, 215–218 FEBS 10426 November 1991

Ethanol and lipid metabolism

Differential effects on liver and brain microsomes

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Received 16 September 1991; revised version received 4 October 1991

We have determined the effect of prolonged ethanol treatment on several enzyme activities related to lipid metabolism in chick-brain and liver microsomes. Ethanol increased microsome cholesterol levels in both organs. The treatment caused a marked increase in the hepatic HMG-CoA reductase and ACAT activities while in the brain a clear decrease was found in these enzyme activities. At the same time the activity of reacylation of phospholipids, was clearly modified in both brain and liver. Thus, while in the liver the turnover of acyl moieties of phosphatidylethanolamine, sphingomyelin and phosphatidylinositol was enhanced by ethanol consumption, in the brain only the reacylation of phosphatidylserine increased to any significant extent. These results indicate that ethanol exerts a differential action in brain and liver, namely cholesterol synthesis and esterification decreased in brain and increased in chick liver. Ethanol also induces faster phospholipid metabolism in both brain and liver microsomes.

ACAT; HMG-CoA reductase; Lipid metabolism; Ethanol treatment; Brain microsome; Liver microsome

1. INTRODUCTION

Prolonged administration of ethanol has a significant effect on lipid metabolism; it increases serum cholesterol levels, especially serum HDL-cholesterol [1], alters hepatic cholesterol metabolism [2,3] and phospholipid metabolism in rat heart, mucose and brain [4–6]. All these change affect, in turn, the lipid levels of various different tissues. There are a large number of reports to the effect that chronic ethanol treatment causes alterations in cholesterol, triglyceride and phospholipid levels in different organs [3,7–10].

The effects of chronic ethanol consumption on several organs may be related in part to its interaction with biological membranes [11]. In spite of such changes being reported by many authors the molecular mechanisms of ethanol–membrane interaction and the consequent biochemical changes have not yet been clearly explained. Ethanol modifies the lipid composition of biological membranes [12–15] and this in turn may well alter the structure and thus the functional capacity of membrane-bound enzymes.

As far as the influence of ethanol on enzymes involved in lipid metabolism is concerned, there are few experimental reports and the results are often contradictory [3,16,17]. The purpose of this study has been to compare the effects of prolonged ethanol ingestion on liver and brain lipid metabolism by analysis of HMG-CoA reductase, ACAT and acyl-CoA transferase activities.

Abbreviations: HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase; ACAT, acyl CoA:cholesterol acyltransferase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin.

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2. MATERIALS AND METHODS

All radiolabelled compounds were supplied by Amersham International (Amersham, Bucks, UK). EDTA, dithiotreitol and silica gel G plates were from Sigma. All the other reagents used were of analytical grade.

Newborn, White Leghorn, male chicks were obtained from a local hatchery and fed ad libitum on a commercial diet (Sanders A-00) in a chamber with a light cycle from 09.00 to 21.00 h and a constant temperature of 31°C. Four-day-old chicks were given a 10% ethanol solution instead of drinking water for a period of 7 days. On day 8 the quantity of ethanol was increased to 15%, and from day 15–35 to 20%. Ethanol consumption averaged 8–10 g/kg of body weight/day. Controls consumed the same diet except for the ethanol, which was replaced isocalorifically by a sucrose solution. Each experimental group contained 20 chicks.

After treatment the chicks were killed by decapitation and their brains and livers were immediately removed, weighed, minced and homogenized in 3 vol. of 50 mM phosphate buffer (ph 7.4) containing EDTA 30 mM, NaCl 250 mM, dithiotreitol 1 mM. Microsomes were obtained as described in [18].

Reductase activity was measured essentially as described by Shapiro et al. [19] with minor modifications [20]. ACAT activity was determined as in [21] using oleoyl CoA as substrate. The reactions were stopped by the addition of 4 ml chloroform/methanol (2/1, v:v) containing cholesteryl oleate as internal standard. Microsomal lipids were extracted according to Folch et al. [22]. Neutral lipids were separated according to Litchestein and Brecher [23]. Radioactivity associated

Table I

Effect of chronic ethanol treatment on total, free and esterified cholesterol levels of microsomes from neonatal chick brain and liver

Cholesterol (μg/mg protein)	Brain		Liver	
	Control	Ethanol	Control	Ethanol
Total	37.40 ± 0.98	51.27 ± 1.00*	33.08 ± 0.28	42.60 ± 0.91**
Free	32.86 ± 0.90	48.30 ± 1.21*	32.76 ± 0.39	39.37 ± 0.25**
Esterified	3.56 ± 0.70	2.42 ± 1.01	0.93 ± 0.01	3.08 ± 0.26**

Results are expressed as μg/mg protein and are means ± SE for 6 determinations. Statistical significances of differences from the controls are indicated by: *$P \leq 0.01$; **$P \leq 0.0001$.

with the cholesterol esters was used to calculate ACAT activity and radioactivity found in free fatty acids was due to oleoyl-CoA hydrolase activity.

The reacylation of phospholipids was measured as follows: a 0.4 mg aliquot of microsomal protein was incubated for 15 min at 37°C with phosphate buffer 0.1 M (pH 7.4), 1.2 mg of fatty-acid-free, bovine-serum albumin and 20 nmol of [^{14}C]oleoyl-CoA (0.15 μCi). The reactions were stopped with 4 ml of chloroform/methanol (2/1, v/v). The phospholipids were separated by TLC according to Higgins' method [24] and the radioactivity found in each phospholipid was used as a measure of the reacylation activity of the phospholipids.

Both total and free cholesterol contents were determined by enzymatic colorimetric methods by using Test Combination Cholesterol and Test Combination Free Cholesterol, respectively (both from Boehringer, Mannheim, Germany). Protein concentration was determined by the method of Lowry et al. [25] using bovine albumin as standard.

3. RESULTS

The effects of chronic ethanol ingestion on the cholesterol levels in brain and liver microsomes are shown in Table I. Ethanol induced a significant increase in the total cholesterol content in both membranes. The increase in total cholesterol in liver microsomes was due to the enhancement of both free and esterified cholesterol, while in brain microsomes this increase was due exclusively to higher free cholesterol levels.

Table II sows the effects of ethanol ingestion on HMG-CoA reductase activity. Compared to the control group there was a clear increase in this enzyme activity in the liver. On the other hand, the mean HMG-CoA reductase activity in brain microsomes from chicks chronically fed on ethanol was significantly lower than in the controls.

The effects of ethanol on ACAT activity are shown in Table II. ACAT activity in the livers of animals fed on ethanol was 40% higher than that of the controls. ACAT activity in the brains, however, was 40% lower. There is a fatty acyl-CoA hydrolase with much higher activity than ACAT associated with the microsomal fraction isolated from chick brain and liver [21,26], and so we tested this hydrolase activity as well to ensure that the differences observed in ACAT activity were not due to any modification in hydrolase activity. As can be seen in Table II, ethanol treatment did not modify oleoyl-CoA hydrolase activity and thus the differences found in cholesterol esterifying activity could not be put down to changes in the availability of oleoyl-CoA used as substrate by ACAT.

In addition we also analyzed the effect of chronic ethanol ingestion on the uptake of oleoyl-CoA by membrane phospholipids and triglycerides. Ethanol significantly increased the level of radiolabelled fatty acid in both lipid components from brain and liver microsomal membranes, although the effect was clearly higher in the triglyceride fraction of hepatic microsomes (Table III). Since the incorporation of radioactivity into each phospholipid is an index of the metabolic turnover of its acyl chains, we also determined the effect of ethanol on the distribution of radioactivity between the different phospholipids (Table IV). As can be seen, the treatment produced different effects in liver and brain. In the liver ethanol enhanced the incorporation of radiolabelled oleoyl-CoA into sphingomyelin, phosphatidylethanolamine and phosphatidylinositol, although only to a significant extent in the two former phospholipids. In

Table II

Alterations induced by ethanol on HMG-CoA reductase, ACAT and oleoyl-CoA hydrolase activities

Enzymatic activity (pmol/ min/mg protein)	Brain		Liver	
	Control	Ethanol	Control	Ethanol
HMG-CoA reductase	1005.4 ± 11.2	774.5 ± 44.5**	1825.0 ± 38.0	2473.0 ± 32.5***
ACAT	22.2 ± 1.1	13.9 ± 0.2***	37.6 ± 1.4	61.6 ± 6.7*
Oleyl-CoA hydrolase	1037.3 ± 26.2	988.7 ± 18.8	917.0 ± 86.6	871.3 ± 9.83

Results are means ± SE for 6 determinations. Statistical significances are indicated by: *$P \leq 0.01$; **$P \leq 0.002$; ***$P \leq 0.0002$.

Table III

Influence of ethanol on the incorporation of oleoyl-CoA into phospholipids and triglycerides from brain and liver microsomes

	pmol/min/mg protein			
	Triglycerides		Phospholipids	
	Control	Ethanol	Control	Ethanol
Liver	36.02 ± 3.72	99.27 ± 7.05**	373.03 ± 12.22	439.52 ± 11.20*
Brain	93.17 ± 4.88	116.12 ± 2.38*	351.80 ± 29.54	428.63 ± 20.06*

Results are expressed as mean ± SE for 6 determinations. Statistical significances of differences from the control are indicated by: *$P \leq 0.01$; **$P \leq 0.0006$.

brain microsomes on the other hand, we found a clear decrease in radioactivity in sphingomyelin while the uptake of acyl chains by phosphatidylserine rose markedly.

4. DISCUSSION

Previous data on the effects of chronic ethanol ingestion on cholesterol synthesis are conflicting. It has been described in rat liver that HMG-CoA reductase activity is increased [3,16] or decreased [17] after chronic ethanol feeding. We have found an increase in chick-liver HMG-CoA reductase activity caused by chronic exposure to ethanol, which may lead to an increase in the rate at which the liver synthesizes cholesterol. To date, no reports have been made concerning the effects of ethanol on HMG-CoA reductase activity of mammalian or avian brain, so the lower activity found in this study provides the first experimental evidence that brain cholesterol metabolism is decreased by the administration of ethanol. We have previously demonstrated that chick brain HMG-CoA reductase is unaltered by dietary treatments which usually modify the activity in the liver [27–29]. Thus our results suggest the high sensitivity of brain HMG-CoA reductase to ethanol.

In the same way as with HMG-CoA reductase activity we found that ethanol induced modifications in the activity responsible for intracellular cholesterol esterification, and that the effects are clearly opposite in brain and liver microsomes. Field et al. [16] have demonstrated in rates an increase in hepatic ACAT activity after ethanol feeding, which they correlated with an increase in the levels of esterified cholesterol. In our study we have observed a clear increase in free cholesterol levels of chick hepatic microsomes after the treatment, therefore the increase in the ACAT activity may reflect both a higher availability of substrate and/or a direct effect of ethanol on the enzyme, leading to an increase in the levels of esterified cholesterol in these membranes.

The effect of ethanol on brain ACAT is clearly different, in that it significantly decreases this activity. Since brain-microsome cholesterol levels increase concomitantly in this situation the decrease in ACAT activity may be attributed either to a specific action of ethanol on brain enzyme or a change in the activity due to an alteration in the lipid microenvironment of the protein and not to changes in the availability of substrate.

We have previously described that chronic ethanol treatment does not produce alterations in the levels of total or individual phospholipids in chick brain and liver microsomes [12], while considerable changes were produced in the fatty acid profiles of different phospholipids [13,30] suggesting changes in the activities involved in the reacylation process of phospholipids. Results obtained in the present study show that ethanol induces a specific increase in the processes of reacylation of phospholipids in both the chick brain and liver. Thus, ethanol alters the uptake of oleoyl-CoA into sphingomyelin, phosphatidylinositol and phosphatidylethanolamine in hepatic microsomes, while in brain membranes only the reacylation of phosphatidylserine, a phospholipid that accounts for no more than 7% of the total phospholipid content, is enhanced. Other authors have also demonstrated an increase in the activ-

Table IV

Influence of ethanol on the incorporation of oleoyl-CoA into different phospholipids of chick brain and liver microsomes

Phospholipid	pmol/min/mg protein			
	Brain		Liver	
	Control	Ethanol	Control	Ethanol
SM	18.35 ± 3.19	7.49 ± 0.15*	3.87 ± 1.17	29.81 ± 2.84***
PC	70.85 ± 8.83	88.25 ± 10.80	103.36 ± 4.66	91.73 ± 9.87
PS	63.70 ± 3.31	116.87 ± 11.20**	89.31 ± 10.39	79.95 ± 9.99
PI	66.48 ± 3.55	76.15 ± 7.22	38.68 ± 6.62	58.24 ± 5.05
PE	58.58 ± 7.03	44.86 ± 7.03	18.72 ± 2.58	44.54 ± 5.83

Results are expressed as mean ± SEM for 6 determinations. Statistical significances of differences from the controls are indicated by: *$P \leq 0.02$; **$P \leq 0.003$; ***$P \leq 0.0002$.

ity of reacylation of rat-brain synaptosomal phospholipids after chronic ethanol administration by the use of [^{3}H]araquidonate [31].

The biochemical mechanism by which ethanol stimulates the uptake of specific fatty acids into the membrane is not clear, although it has been suggested that it may activate phospholipase A_2 activity [32]. The specific effect upon the uptake of fatty acyl-CoA by specific phospholipids in chick brain and liver microsomes found in our study after ethanol administration, suggests that alcohol acts mainly upon the acyltransferase activities involved in the retailoring or remodelling of membrane phospholipids rather than in changes in phospholipase A_2 activity, although further studies are required to clarify the molecular mechanism by which ethanol acts on phospholipid metabolism. Nevertheless, the specific increase in the turnover of acyl moieties induced by ethanol could have an important role in the alterations observed in HMG-CoA reductase and ACAT activities.

Acknowledgements: We thank Dr. John Trout for revising the English text. These studies were supported by Grant 0031.9 from the Junta de Andalucía.

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