Adaptive Changes Induced by Chronic Ethanol Ingestion on Hepatic Mitochondrial and Microsomal Enzyme Activities

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Ethanol exerts its pharmacological effects by altering the physico-chemical properties of biological membranes. Modifications induced by ethanol may result in changes in the activity of membrane-bound enzymes that have been shown to require a specific membrane fluidity and composition for optimal function. In this study we have carried out the analysis of the effects of ethanol on different enzyme activities of hepatic microsomes and mitochondria. Our results show that chronic ethanol treatment causes marked changes to enzyme activity in the mitochondrial and microsomal electron-transport systems in chick liver. The inhibition observed in the mitochondrial enzyme activities studied, indicates that ethanol ingestion depresses the functionality of the respiratory-chain. In microsomes, NADH cytochrome c reductase activity was significantly decreased whilst NADH cytochrome b5 reductase activity was increased after ethanol administration. Hepatic mitochondria and microsomes from control and chronic ethanol-treated chicks were submitted to ethanol in vitro in order to study the possible existence of adaptive changes in the different enzyme systems as consequence of long-term ethanol administration. Incubation of control membranes with different amounts of ethanol induced marked alterations in enzyme activities. In membranes isolated from ethanol-treated chicks, ethanol also produced a similar effect except with cytochrome oxidase and NADH cytochrome b5 reductase, which resisted alteration by ethanol added, suggesting the existence of adaptive changes in these enzyme activities that allow them to remain unaltered after exposure to ethanol in vitro.

Keywords: Ethanol Electron-transport systems Microsomes Mitochondria

INTRODUCTION

Ethanol is an organic solvent which is soluble in water and lipids. This property gives ethanol easy access to all compartments of the body, all tissues being more or less affected by ethanol. The effects of chronic ethanol consumption on several organs may be related in part to its interaction with biological membranes. In fact, many studies have demonstrated that chronic ethanol ingestion causes structural and functional changes in the membrane properties of different tissues. Experimental evidence has shown that ethanol exerts some of its pharmacological effects by modifying the physico-chemical properties of biological membranes by altering their lipid composition and consequently changing their fluidity (Wood and Schroeder, 1988; Beaugé et al., 1988; Sanchez-Amate et al., 1992a). Since many membrane-bound proteins require a specific lipid micro-environment, any changes induced by ethanol in the membrane lipid composition could lead to marked alterations in its functionality. So, in previous reports we have demonstrated that chronic ethanol treatment alters membrane fluidity concomitantly with changes

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in different enzyme activities involved in the lipid metabolism (Sanchez-Amate et al., 1991, 1992b).

It is widely accepted that changes induced after ethanol consumption give rise to membranes that are resistant to the molecular disordering effect of ethanol in vitro (Goldstein, 1987; Hoek and Taraschi, 1988). This behaviour points to an adaptive response of the membrane to the presence of alcohol, a phenomenon known as tolerance. Tolerance has been linked to the lipid components of the bilayer, however, it has been also demonstrated that some membrane proteins exhibit adaptive changes after chronic ethanol administration (Casey et al., 1990; Tuma et al., 1991) although there are few experimental evidences. Therefore, in this study we have examined the effect of chronic ethanol ingestion on several liver membrane-bound enzymes belonging to the enzyme systems involved in the electron-transport processes. We have made a correlated study of these systems in microsomes and mitochondria from chronically ethanol treated-chicks and controls. In addition, we have carried out experiments to investigate the involvement of these membrane enzymes in the adaptive mechanisms to ethanol.

MATERIAL AND METHODS

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 hr and a constant temperature of 31°C. Ethanol treatment was carried out as previously described (Sanchez-Amate et al., 1991). Briefly, 4-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 days 15 to 35 to 20%. Ethanol consumption averaged 8-10 g/kg of body wt/day. Controls consumed the same diet except for the ethanol, which was replaced isocalorically by a sucrose solution. Each experimental group contained 20 chicks.

The isolation of mitochondria and microsomes was performed as previously reported (Sanchez-Amate et al., 1995). After treatment, the chicks were killed by decapitation and the livers were immediately removed, weighed, minced and homogenized in 8 Vol of 0.25 M sucrose. The homogenates were centrifuged at 400 g for 10 min and the supernatants were then recentrifuged at 800 g for 10 min. The supernatants were recentrifuged at 5000 g for 10 min to sediment the mitochondria. Microsomes were sedimented by centrifuging this supernatant at 17,000 g for 10 min and the final supernatant at 105,000 g for 60 min. Protein concentration was determined by the method of Lowry et al. (1951), using bovine-serum albumin as standard.

Membrane incubation with ethanol was carried out as previously described (Sanchez-Amate et al., 1995). Mitochondria and microsomes were incubated with 0, 100 or 400 mM ethanol concentrations for 15 min at 37°C. To remove ethanol, membranes were diluted with 3 Vol of cold 50 mM Tris-HCl buffer, pH 7.4 and were resololated by centrifugation at 20,000 g for 10 min and 150,000 g for 60 min respectively. Mitochondria and microsomes were finally resuspended in the appropriate volume of 50 mM Tris-HCl, pH 7.4, to give a final protein concentration of 0.6 mg/ml, before being used for enzymatic assays.

Cytochrome oxidase activity was determined by the method of Sottocasa et al. (1967). Mitochondrial NADH cytochrome c reductase activity was measured using Hatefi and Rieske's technique (1967). NADH cytochrome c reductase activity of inner membrane was determined by subtracting the activity found in the presence of 0.15 mM rotenone from the total activity. Microsomal NADH cytochrome b\textsubscript{5} reductase activity was analysed by using ferricyanide as electron-acceptor and to determine NADH cytochrome c reductase activity cytochrome c was used instead ferricyanide (Umeki et al., 1984). Microsomal NADPH cytochrome c reductase was analysed using Phillips and Langdon's method (1962).

RESULTS AND DISCUSSION

In the present work, we have examined the effect of chronic ethanol treatment on enzyme activities involved in the microsomal and mitochondrial electron-transport systems. As it can be seen in Table 1, ethanol feeding for 35 days induced marked changes in different enzyme activities, mainly in the mitochondria. So, electron-transport was clearly depressed in the mitochondria of chicks chronically exposed to ethanol. Mitochondria from the alcoholic group exhibited a marked reduction in cytochrome oxidase and rotenone-sensitive NADH cytochrome c reductase activities. There was no difference between control and treated chicks in...
Adaptation of membranes to ethanol

the activity of insensitive-rotenone enzyme, located in the outer mitochondrial membrane. Ethanol-related alterations in several respiratory-chain components of rat liver have often been described. In fact, a decrease in the activity and haem content in cytochrome oxidase ranging from 50 to 70% of control values has been reported (Thayer and Rubin, 1986). Other respiratory components such as the $b$ cytochromes and also the NADH-ubiquinone reductase are dramatically altered as a result of chronic ethanol administration (reviewed by Cunningham et al., 1990). According to the observations above, our results indicate that chick-liver mitochondria are also greatly affected by chronic ethanol consumption. Some of these effects may well be related to a decrease in the synthesis of mitochondrial gene products, as reported by Coleman and Cunningham (1991), but we cannot rule out a relationship between the changes in the physico-chemical state of the membrane induced after chronic ethanol treatment (Sanchez-Amate et al., 1992b) and the decrease in enzyme activity. However, it is interesting to note that the effects of ethanol on mitochondria are different depending on the tissue. So, Cardellach et al. (1991) have demonstrated that chronic ethanol treatment does not produce significant differences in cytochrome content, ATPase activity or the activity of the respiratory chain complexes in skeletal-muscle mitochondria.

In microsomes, the effect of chronic ethanol feeding on the enzyme activities involved in the NADH- and NADPH-dependent electron-transport systems was different depending upon the enzyme analysed (Table 1). Thus, NADPH cytochrome c reductase activity was unaffected, while ethanol consumption alters the two microsomal enzyme activities of the NADH-dependent system, i.e. NADH cytochrome $b$, reductase activity was clearly increased whilst NADH cytochrome $c$ reductase was significantly reduced. As in our assay system cytochrome $c$ is the electron acceptor from cytochrome $b_s$, the inhibition in NADH cytochrome $c$ reductase might be interpreted in terms of a specific decrease in the levels of cytochrome $b_s$, as occurs in rat liver (McCoy et al., 1985). With regard to the activity of NADH cytochrome $b_s$ reductase, it is measured by using ferricyanide as exogenous electron acceptor without the involvement of cytochrome $b_s$, hence, changes in this enzyme activity are not related to the cytochrome $b_s$ levels.

Another important aspect of disturbances induced by ethanol on biological systems is the treatment as an acute or in vitro dose allowing us to ascertain hypothetical adaptive responses (Hoek and Taraschi, 1988; Gonzalez-Calvin et al., 1987). Since in the present study chronic ethanol feeding causes marked alterations in the enzyme activities studied, we could expect that some of the above proteins may be involved in adaptive mechanisms to ethanol and hence exhibit a differential response to ethanol in vitro than that of control membranes.

To test this possibility we have studied the effect of 100 and 400 Mm ethanol concentrations on the different enzyme activities in membranes isolated from both control and ethanol-treated chicks. In a recent report (Sanchez-Amate et al., 1995) we have demonstrated that ethanol in vitro exerts two different effects on the membrane: a fluidizing effect both in the core and at the surface of the membrane which depends on its physical presence, and a rigidization of the surface which occurs after its removal. Directly related to this rigidization we have also detected a persistent alteration in

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<th>Table 1. Ethanol-induced changes in enzyme activities related to the electron-transport systems of chick-liver mitochondria and microsomes</th>
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<td>Mitochondria</td>
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<td>NADH cytochrome $b_s$ reductase</td>
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<td>Each value represents the mean ± SEM of four experiments. Student's $t$-test was used for statistical analyses: *$P \leq 0.02$; **$P \leq 0.003$.</td>
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With regard to microsomes, after ethanol removal, NADH cytochrome c reductase activity was not affected in either control or ethanol-fed animals (results not shown). Similarly, although NADPH cytochrome c reductase activity increased by effect of 100 or 400 mM ethanol amounts, no differences were observed between the membranes isolated from either experimental group (Fig. 2). When ferricyanide replaced cytochrome c as electron-acceptor, ethanol significantly reduced NADH cytochrome b₅ reductase activity in both ethanol-fed and control chicks, the inhibition being significantly more marked in the control membranes.

It is interesting that mitochondrial cytochrome c oxidase and microsomal NADH cytochrome b₅ reductase exhibit a differential behaviour in control and chronic ethanol-treated membranes with respect to ethanol in vitro. In fact, both enzyme activities in membranes isolated from ethanol-treated animals resist the inhibitory effect of ethanol in vitro.
more effectively, indicating the existence of an adaptive change in the membrane induced by chronic ethanol administration. The other microsomal and mitochondrial enzyme activities analysed did not show any differences between control and ethanol-treated membranes, thence, the adaptation of membrane enzyme is not a general phenomenon because it is not exhibited by all membrane proteins but it is restricted to specific proteins. These results can be interpreted as indicating different lipid environment for the different enzymes assayed and thus, they may be differentially sensitive or insensitive to the effects of ethanol added.

In summary, these results taken as a whole, suggest that chronic ethanol administration produces adaptive changes in which membrane-lipid components are probably involved. As a consequence, the lipid-protein interactions are altered and thence specific enzyme activities may be also modified. These adaptive changes render membranes that exhibit enzyme activities with a greater capacity to resist the inhibitory effect of ethanol in vitro.

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REFERENCES


