In Vivo Effect of L-Cysteine on Ethanol-Induced Alterations in Membrane-Bound ATPases of Liver and Kidney of Experimental Rats

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(Received September 21, 1995)

Summary Decreased activities of Na+, K+-ATPase in liver and kidney, Mg2+-ATPase in kidney, and Ca2+-ATPase in liver were observed in alcoholic rats (3.0 g of ethanol/kg body weight, 30% [v/v]) given twice daily for 30 days compared with those of the controls. Increased activities of Ca2+-ATPase in kidney and Mg2+-ATPase in liver were also observed in the alcoholic rats. All the above metabolic alterations induced by alcohol were effectively avoided by L-cysteine (20 mg/100 g body weight) given twice daily for 30 days.

Key Words: ethanol, ATPase, L-cysteine, glutathione

Ethanol toxicity, either acute or chronic, is characterized by a severe derangement of subcellular metabolism and structural alterations of liver cell membranes [1]. The plasma membrane Na+, K+-ATPase is concerned with the maintenance of a low intracellular concentration of Na+ and consequently of cellular water content. Inhibition of this enzymatic activity by ethanol could be responsible for the increased water content that characterizes chronic ethanol toxicity [2]. Decreased activity of Na+, K+-ATPase can lead to a decrease in sodium efflux and thereby alter the membrane permeability [3]. Ca2+-ATPase regulates the calcium pump activity. Intracellular calcium functions as a messenger in the regulation and control of cellular processes that play a central role in mediating muscle contraction, neurosecretion, and other Ca2+-mediated cell functions [4].

Since ATPase are thiol-dependent enzymes, Na+, K+-ATPase, Ca2+-ATPase, and Mg2+-ATPase require SH groups to maintain their structure and function [5, 6]. Therefore L-cysteine, one of the sulphur-containing amino acids, was used to assess its modulatory efficacy on ethanol-induced alterations in membrane-bound ATPases.

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MATERIALS AND METHODS

Wistar strain albino male rats weighing between 160 to 180 g were obtained from the Forensic Science Laboratory, Madras. Twenty-four rats were divided into four groups of six each, defined below, and were maintained in clean cages under controlled illumination (14 h of light/10 h of darkness). Group-I animals were untreated and served as control. The Group-II rats were given ethanol (3.0 g/kg body weight, 30% [v/v]) twice daily by an intragastric tube, while control rats received an equal volume of water [7], for 30 days. Group-III rats were given the same amount of alcohol as in Group II along with an antidote, L-cysteine (20 mg/100 g body weight), twice a day via an intragastric tube for 30 days. Group-IV rats were given L-cysteine alone (same amount as in Group III). Standard rat feed (Hindustan Lever Ltd., Bombay, India) and clean drinking water were given ad libitum to all the experimental rats. After the experimental period of 30 days the rats were killed by cervical decapitation. Liver and kidney were removed from the rats immediately and then washed in ice-cold saline, and a portion of each of the organs were homogenized in 0.1 M Tris-HCl buffer, pH 7.4, and used for the assays.

To examine the extent of the toxic effect of alcohol, we assayed marker enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum, liver, and kidney by the method of King [8]. Na+, K+-ATPase was estimated by the method of Bonting [9]; Mg2+-ATPase, by the method of Ohinishi et al. [10]; and Ca2+-ATPase, by the method of Hjerten and Pan [11]. Glutathione content was determined according to Moron et al. [12]; and protein content, by the method of Lowry et al. [13].

Student's t-test was used for the statistical analysis of each parameter.

RESULTS

The activities of AST and ALT elevated by ethanol treatment of the rats were found to be significantly decreased by L-cysteine administration (Table 1). Decreased activities of Na+, K+-ATPase in liver and kidney, and a decreased activity of Mg2+-ATPase in kidney and of Ca2+-ATPase in the liver were observed in alcoholic rats, whereas increased activities of Ca2+-ATPase in kidney and Mg2+-ATPase in liver were found in these rats. All the above enzymic alterations were avoided by administration of L-cysteine along with the alcohol (Table 2). The level of glutathione was reduced in both liver and kidney of alcohol-treated rats; and, again, co-administration with L-cysteine protected against this loss (Table 3).

DISCUSSION

Increased activities of AST and ALT in serum, liver, and kidney of alcoholic...
ETHANOL-INDUCED ALTERATIONS IN ATPases

Table 1. Levels of aspartate amino transferase (AST) and alanine amino transferase (ALT) in serum, liver, and kidney of control and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Liver</td>
<td>Kidney</td>
<td>Serum</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group I (control)</td>
<td>63.85 ± 3.65</td>
<td>3.44 ± 0.19</td>
<td>2.93 ± 0.14</td>
<td>25.73 ± 3.29</td>
<td>9.30 ± 0.31</td>
<td>7.36 ± 0.36</td>
</tr>
<tr>
<td>Group II (administered alcohol)</td>
<td>83.32 ± 4.87</td>
<td>4.87 ± 0.17***</td>
<td>3.99 ± 0.11***</td>
<td>39.34 ± 4.65***</td>
<td>11.29 ± 0.25***</td>
<td>9.76 ± 0.21***</td>
</tr>
<tr>
<td>Group III (administered alcohol + L-cysteine)</td>
<td>65.26 ± 3.61</td>
<td>3.61 ± 0.18***</td>
<td>3.02 ± 0.20***</td>
<td>27.01 ± 4.30**</td>
<td>9.53 ± 0.24***</td>
<td>7.48 ± 0.31***</td>
</tr>
<tr>
<td>Group IV (administered L-cysteine alone)</td>
<td>62.72 ± 4.04NS</td>
<td>3.34 ± 0.19NS</td>
<td>2.89 ± 0.14NS</td>
<td>25.69 ± 3.00NS</td>
<td>9.29 ± 0.53NS</td>
<td>7.30 ± 0.29NS</td>
</tr>
</tbody>
</table>

Values are expressed as µmol of pyruvate liberated/h/mg protein. Serum values are expressed in units/liter. Values are expressed as mean ± SD for six individual experiments. For statistical evaluation of significant variations, Groups II and IV were compared with Group I, and Group III was compared with Group II. Statistically significant alterations are expressed as **p<0.01; ***p<0.001; NS not significant.

Table 2. Activities of membrane-bound ATPase in liver and kidney of control and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Organs</th>
<th>Na⁺, K⁺-ATPase</th>
<th>Mg²⁺-ATPase</th>
<th>Ca²⁺-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>1.20 ± 0.13</td>
<td>0.096 ± 0.01</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Group I (control)</td>
<td>Kidney</td>
<td>0.69 ± 0.05</td>
<td>0.32 ± 0.03</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Group II (administered alcohol)</td>
<td>Liver</td>
<td>0.26 ± 0.04***</td>
<td>0.14 ± 0.01***</td>
<td>0.11 ± 0.01***</td>
</tr>
<tr>
<td>Group III (administered alcohol + L-cysteine)</td>
<td>Kidney</td>
<td>0.25 ± 0.02***</td>
<td>0.24 ± 0.02***</td>
<td>0.38 ± 0.03***</td>
</tr>
<tr>
<td>Group IV (administered L-cysteine alone)</td>
<td>Liver</td>
<td>0.92 ± 0.12***</td>
<td>0.11 ± 0.02**</td>
<td>0.17 ± 0.02**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.54 ± 0.03***</td>
<td>0.30 ± 0.03**</td>
<td>0.32 ± 0.03**</td>
</tr>
</tbody>
</table>

Values are expressed in units (One unit of enzyme activity is defined as µmol of Pi formed/min/mg protein). Values are expressed as mean ± SD for six individual experiments. For statistical evaluation of significant variations, Groups II and IV were compared with Group I, and Group III was compared with Group II. Statistically significant alterations are expressed as **p<0.01; ***p<0.001; NS not significant.

Rats reflect the severity of the effect of alcohol and were reversed effectively by L-cysteine administration, indicating that L-cysteine acts as a protective agent against hepato and renal toxicity caused by alcohol. ATPase is a lipid-dependent, membrane-bound enzyme involved in active transport processes and has been implicated in the pathogenesis of ethanol-induced liver cell injury [14]. In our study we observed that the activities of Na⁺, K⁺-ATPase in liver and kidney were decreased significantly.

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The observed decrease in Na⁺, K⁺-ATPase activity during chronic ethanol feeding may depend on cell death, intercalation of ethanol into the phospholipid bilayer, reaction of acetaldehyde with membrane phospholipids, and proteins, and the production of free radicals and peroxides [15].

The Mg²⁺-ATPase activity level was elevated in the liver, but suppressed in the kidney, of ethanol-treated rats. Ethanol intoxication was shown earlier to result in increased mitochondrial Mg²⁺-ATPase activity in rat liver [16]. Mg²⁺-ATPase activity is involved in other energy-requiring processes in the cell. Decreased activity of Ca²⁺-ATPase in liver and increased activity in kidney were observed in our alcoholic rats. Decreased Ca²⁺-ATPase activity has been reported during oxidant stress due to hydroperoxides and drugs in hepatocytes [17]. The intracellular concentration of calcium regulates the activity of the Mg²⁺ and Na⁺, K⁺-ATPases. Therefore Ca²⁺ may play a role in the regulation of sodium reabsorption. The steady-state Ca²⁺ concentration is postulated to be regulated only by calcium uptake by this specific Ca²⁺-activated ATPase [18]. Acute and chronic alcohol administration was previously found to induce a marked decrease in hepatic cysteine and glutathione (GSH) levels [19]. Our observations on glutathione agree well with those results. Furthermore, the decreased levels of GSH in liver and kidney were avoided by the co-administration of L-cysteine. Recent studies indicate that some sulphur-containing compounds, such as N-acetyl cysteine, S-adenosyl-L-methionine, and L-methionine, exert various degrees of protection toward ethanol-induced cell injury, which are related to the efficiency of these compounds in maintaining a large pool of reduced GSH [20].

Hence, we conclude that the effect of ethanol intoxication is organ specific and find the differences in the activity of Na⁺, K⁺, Ca²⁺, and Mg²⁺-ATPases in liver and kidney to be really intriguing. Regarding the protective effect of L-cysteine, we suggest that it may be related to the efficiency of L-cysteine in maintaining a large pool of GSH like L-methionine and S-adenosyl-L-methionine.
in the system [20]. Further work however is needed to clarify this point.

Financial assistance given by C.S.I.R., New Delhi, to one of the authors, N.C., in the form of a Senior Research Fellowship is gratefully acknowledged.

REFERENCES

