Alterations in Beta-Adrenergic Receptor Density and Cyclic-AMP Level in the Myocardium of Rats Chronically Treated with Alcohol

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Summary: An impaired function of the myocardial beta-adrenergic receptor system has been reported in patients with end-stage heart failure and this impairment has been postulated to be a factor in further deterioration of cardiac contractile function. As ventricular dysfunction is often associated with prolonged alcohol abuse, we investigated whether or not chronic administration of ethanol could induce alterations in the beta-adrenergic receptor adenylate-cyclase system in rats. Male Wistar rats of 8 weeks of age received 33% ethanol in drinking water for 3 months. As compared with control rats drinking water, the ethanol-treated rats showed weight loss and an increase in the heart/body weight ratio. Chronic ethanol increased myocardial contents of norepinephrine and epinephrine, possibly resulting from sympathoadrenal activation. The beta-adrenergic receptor density (Bmax) of the myocardial membrane was significantly decreased in the ethanol-treated rats (27.7±9.9 vs 39.0±6.0 fmol/mg protein, p<0.01), while the affinity (Kd) did not differ between the two groups. The myocardial content of cyclic-AMP was also reduced in the ethanol rats (865±59 vs 1055±83 pmol/g w.w., p<0.01). These observations indicate that chronic ethanol administration depresses the function of the beta-adrenergic receptor adenylate-cyclase system. The decreased beta-adrenergic receptor density was partly attributed to down-regulation due to increased sympathetic stimulation. This impaired function may contribute to the cardiac contractile dysfunction observed in chronic alcoholics.

Key words: alcoholic myocardial injury — beta-adrenergic receptor — adenylate-cyclase — cyclic-AMP — catecholamines

Introduction

Prolonged and excessive ingestion of alcohol is frequently associated with myocardial damage resulting in alcoholic cardiomyopathy (Rubin, 1979; Katz et al. 1985; Diamond, 1989). Although malnutrition, including protein and thiamine deficiency, in chronic alcoholics could be responsible for the injury to the heart, recent evidence has suggested that alcohol itself could be responsible for most cardiovascular disorders associated with alcohol abuse (Dancy et al. 1985; Diamond, 1989; Urbano-Marquez et al. 1989). The mechanism by which alcohol affects the cardiovascular system is not yet fully understood. Alcohol and its major metabolite, acetaldehyde, are known to perturb the lipid bilayer membrane, with

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resulting changes in ion movement across the membrane (Dancy et al. 1985; Taraschi and Rubin, 1985; Messing et al. 1986; Garrett et al. 1987; Diamond, 1989; Urbano-Marquez et al. 1989; Vasdev et al. 1991) as well as to impair mitochondrial oxidative phosphorylation and mitochondrial respiration (Lange and Sobel, 1983). These molecules can also alter membrane receptors and second messengers (French et al. 1975; Banerjee et al. 1978; Diamond et al. 1987; Saito et al. 1987; Mochly-Rosen et al. 1988; Tabakoff et al. 1988), all of which can disturb cellular and organ function. In a previous study (Sufu, 1991), we observed that chronic ethanol administration in rat impairs fluidity of erythrocyte and myocardial mitochondrial membranes, and depresses mitochondrial respiratory function. Chronic ethanol increased calcium and decreased magnesium content in the myocardium. On the basis of these observations, we proposed that the primary disturbance in the myocardial injury caused by chronic ethanol might be a membrane dysfunction which leads to alterations in ion channels, membrane receptors and membrane-bound enzymes.

The beta-adrenergic receptor adenylate-cyclase system is the major cellular pathway that regulates the myocardial contractile responses to various stimuli. In end-stage heart failure, Bristow et al. (1982) demonstrated decreases in beta-adrenergic receptor density and in adenylate-cyclase activity of the myocardial cells. This could further compromise systolic contractile function of the failing heart. We therefore investigated in the present study whether or not chronic alcohol could induce alteration in the beta-adrenergic receptor adenylate-cyclase system in the rat myocardium.

**Materials and Methods**

**Animals and administration of ethanol**

Male Wistar rats at 8 weeks of age, weighing around 290 g were divided into two groups: a control group and an ethanol-treated group. The control group was given drinking water from the tap, the ethanol group was given 33% vol/vol ethanol in tap water for 3 months. At the end of the experiment, the rats were anesthetized and body weight was recorded. After thoracic cage resection, the hearts were removed, weighed, frozen in liquid nitrogen and stored at −80°C for biochemical analysis.

**Beta-adrenergic receptor assays**

The procedures follow those described by Raum et al. (1983) with some minor modifications. Approximately 1 g of frozen myocardium was thawed, minced and homogenized in 20 ml of homogenation buffer (0.25 M sucrose, 20 mM Tris base, 1 mM MgSO4 and 140 mM NaCl, pH 7.5) for 15 sec using a Polytron PT-10 homogenizer (at setting No.5). The homogenate was filtered through a single layer of cheese cloth, and then centrifuged at 450G for 10 min at 4°C. The pellet was discarded and the supernatant was then centrifuged at 30,000G for 15 min. The supernatant was discarded, and the pellet was washed with incubation buffer (20 mM Tris base, 1 mM MgSO4, 140 mM NaCl, pH 7.5) and then centrifuged at 30,000G for 15 min. After repeating the procedure, the final pellet was resuspended with incubation buffer to give protein concentrations of 0.4–0.8 mg/ml.

For beta-adrenergic receptor assay, aliquots of membrane protein in duplicate were incubated each with one of eight different concentrations (25–500 pM) of 125I-iodohydroxybenzylpindorol (125I-IHYP) (New England Nuclear Corp., Boston) for 60 min at 37°C in the presence or absence of 10−5 M (±) propranolol (Sigma). After incubation, the samples were diluted with 3 ml of washing buffer (10 mM Tris base, 140 mM NaCl, pH 7.5) and filtered.
through Whatman GF/C glass microfiber filters (Whatman International Ltd., England). The filters were washed with an additional 20 ml of washing buffer. The radioactivity retained on the filters was measured in an autowell gamma counter (Aloka JDC-751, Aloka Co., Tokyo). Analyses of the saturation binding assays were performed according to the method of Scatchard (1949). The protein concentrations were determined by the method of Lowry et al. (1951).

Assay of cyclic-AMP
Approximately 1 g of frozen myocardium was thawed, minced and homogenized in 3 ml of 5% trichloroacetic acid using a Polytron PT-10 homogenizer (at setting No.9) for 1 min. After centrifugation of the homogenate at 30,000G for 10 min, the supernatant was washed three times with 10 ml of anhydrous ether. Each residual aqueous layer was placed in a water bath at 50°C for 10 min to remove ether. Cyclic-AMP was determined by using a Yamasa radioimmunoassay kit (Yamasa Shoyu Ltd., Chiba).

Myocardial epinephrine and norepinephrine assay
Approximately 1 g of frozen myocardium was thawed, minced and homogenized in 3 ml of 5% trichloroacetic acid using a Polytron PT-10 homogenizer (at setting No.9) for 1 min. After centrifugation of the homogenate at 3,000G at 4°C for 10 min, the supernatant was used for determination of myocardial epinephrine and norepinephrine contents by high pressure liquid chromatographic method.

Statistical methods
Results were expressed as mean ± S.D. Group means between the ethanol and control groups were compared by Student’s t-test for unpaired data. Statistical significance was determined at a level of p<0.05.

Results
Effect of ethanol on body and heart weights (Table 1)
The initial body weights of control and ethanol groups were not significantly different (294±13 vs 290±14 g). However, the final mean body weight of the rats given ethanol was significantly less than that of the control rats. The mean heart weight in the ethanol rats was significantly lower than that in the control rats. The heart-to-body-weight ratio was significantly increased in the ethanol rats as compared with the control rats.

Effects of ethanol on myocardial catecholamines, beta-adrenergic receptor and cyclic-AMP (Table 2)
The myocardial contents of norepinephrine and epinephrine were significantly increased in the ethanol rats as compared

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ethanol</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>413±41</td>
<td>286±59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.07±0.07</td>
<td>0.85±0.19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart/body weight (10^-3)</td>
<td>2.56±0.14</td>
<td>2.97±0.24</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD.
TABLE 2.

Chronic effects of ethanol on myocardial catecholamines and β-adrenergic receptor system

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>Ethanol</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catecholamines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (ng/g w.w.)</td>
<td>10</td>
<td>544±99</td>
<td>912±211</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E (ng/g w.w.)</td>
<td>10</td>
<td>6.0±2.1</td>
<td>10.5±3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>β-receptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>8</td>
<td>39.0±6.0</td>
<td>27.7±9.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kd (pM)</td>
<td>8</td>
<td>24.6±8.2</td>
<td>27.2±13.8</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>c·AMP (pmol/g w.w.)</strong></td>
<td>6</td>
<td>1055±83</td>
<td>865±59</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SD.
NE: norepinephrine; E: epinephrine; w.w.: wet weight

Discussion

The present study demonstrates that chronic ethanol treatment decreases myocardial beta-adrenergic receptor density (Bmax) in the ethanol rats, while the Kd value did not differ between the two groups. The myocardial level of cyclic-AMP was also decreased in the ethanol rats.

with the control rats. There was a significant reduction in the myocardial beta-adrenergic receptor density (Bmax) in the ethanol rats, while the Kd value did not differ between the two groups. The myocardial level of cyclic-AMP was also decreased in the ethanol rats.

Previous investigators are of equivocal opinions on the effects of alcohol on the beta-adrenergic receptor system in various cell systems (French et al. 1975; Banerjee et al. 1978; Saito et al. 1987; Mochly-Rosen et al. 1988; Tabakoff et al. 1988; Diamond, 1989; Sufu, 1991). In general, short-term treatment with ethanol
increased the production of receptor-dependent cyclic-AMP, but long-term exposure to ethanol led to a decrease in cyclic-AMP. Banerjee et al. (1978) reported a decrease in beta-adrenergic receptor density in brain and heart of rats treated with ethanol for 60 days. A diminished responsiveness of adenylate cyclase activity to stimulation by guanine nucleotides, isoproterenol or norepinephrine was observed in the brain of animals fed ethanol (French et al. 1975; Saito et al. 1987; Mochly-Rosen et al. 1988). In humans with chronic alcoholism, Diamond et al. (1987) observed a reduction of cyclic-AMP level in lymphocytes. Tabakoff et al. (1988) reported reduced cyclic-AMP production by stimulation with cesium fluoride, guanylylimidodiphosphate or prostaglandin E1 in platelet membranes from alcoholics. These investigations are largely consistent with the present results and suggests that ethanol-induced impairment of the beta-adrenergic receptor adenylate cyclase system could be of widespread pathophysiologic importance in chronic alcoholism. In the heart, the decreased beta-adrenergic receptor function could further compromise the contractile function of the myocardium and might contribute to the development of alcoholic cardiomyopathy. Rapid reversal of the cardiac dysfunction of the alcoholic heart disease after discontinuation of ethanol drinking could also be partly explained by up-regulation of the beta-adrenergic receptors which has been reported to occur after alcohol withdrawal (Diamond et al. 1987).

In patients with chronic alcoholism, some degree of undernutrition is present. In order to reproduce the experimental model resembling the clinical condition, we administered 33% ethanol in tap water while the control rats continued to grow. The impaired function of the beta-adrenergic receptor adenylate cyclase system could possibly be the result of the combined effects of alcohol and undernutrition like in clinical patients with alcoholic heart disease. Direct effect of ethanol independent from nutritional conditions remains to be investigated.

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References


