Effect of Ethanol on Mouse Liver Monoamine Oxidase

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Abstract The effects of ethanol and acetaldehyde on monoamine oxidase activity in mouse liver mitochondrial fraction were studied. In vivo, a single dose of ethanol increased the hepatic monoamine oxidase activity compared to control group, and chronic ethanol consumption also increased the enzyme activity using tyramine, benzylamine or serotonin as substrate. Acetaldehyde, the metabolite of ethanol, significantly increased monoamine oxidase activity more than ethanol did. In contrast to the in vivo results, it was found that the monoamine oxidase activity was inhibited in vitro by ethanol or acetaldehyde in the dose-dependent manner.

Keywords Monoamine oxidase, MAO, tyramine, benzylamine, serotonin, ethanol, acetaldehyde.

Monoamine oxidase (MAO, EC 1.4.3.4) is an enzyme located in the mitochondria of various tissues from mammal. The function of the enzyme is the oxidation of endogenous and exogenous monoamines, especially noradrenaline, dopamine, tyramine and serotonin. It is well known that serotonin is a specific substrate for type A MAO and benzylamine is a specific substrate for type B MAO in vitro assay. There have been many reports on the effect of ethanol on MAO activity. However, the effect of ethanol on MAO has not been completely elucidated yet. The present work was undertaken in an attempt to study the effect of ethanol and acetaldehyde on the hepatic mitochondrial MAO activity, using various substrates in vivo and in vitro assay.

MATERIALS AND METHODS

Chemicals
Bovine serum albumin was purchased from Nakarai Chemical, Ltd; ethanol from Fluka Chemical, acetaldehyde from Hayashi Pure Chemical, Ltd., tyramine hydrochloride from Aldrich Chemical Co., serotonin creatinine sulfate from Daichi Pure Chemical, Ltd., benzylamine hydrochloride from Sigma Chemical, Co. All other chemicals used were of reagent grade.

Animals
Male ICR mice weighing 22-27g, were housed in stainless-steel cages in a room maintained 21-23 °C, and acclimatized in an 12hr light-12hr dark cycle animal room for at least 2 weeks prior to use. Animals were allowed free access to food and water but were deprived of food for the 16hr prior to sacrifice.

Preparation of mitochondria
Mice were killed by exsanguination from inferior vena cava. Liver was perfused with cold 0.15M NaCl solution through the portal vein until uniformly pale and quickly excised. After mincing, it was homogenized. The homogenate was centrifuged at 600 × g for 10min and the resulting supernatant was centrifuged at 10,000 × g for 30min. The mitochondrial pellets obtained were suspended in the 0.1M phosphate buffer(pH 7.5) and centrifuged again for 20min at 10,000 × g. The pellets were resuspended in the same buffer and used immediately.
cubation mixtures containing mitochondrial fraction 5 min prior to the addition of substrate. MAO activity was expressed by the amount of ammonia formed, which was measured by the reaction with phenol reagent and hypochlorite solution spectrophotometrically at 625 nm. Protein was determined with bovine serum albumin as standard.15)

Drug administration

In the acute studies, mice were given 25% (v/v) ethanol (1 g/kg) intraperitoneally 90 min before decapitation and 2.5% (v/v) acetaldehyde (100 mg/kg) was given 30 min before decapitation. Control mice received saline. In the chronic studies, mice were fed 5% (v/v) ethanol or water for 2 months and those were maintained during the experimental periods with daily recording body weight.

RESULTS

Effect of ethanol on MAO activity in vitro

Effect of ethanol on the hepatic mitochondrial MAO in vitro was studied using tyramine, benzylamine and serotonin as substrate. As shown in Fig. 1, ethanol had no significant effect on MAO activity to a concentration of 1 mM, whereas above 22 mM it caused significant inhibition in each substrate. For example, MAO activities toward tyramine, benzylamine and serotonin were inhibited by about 50%, 75% and 50% at 0.2 M ethanol, respectively.

![Graph](image1.png)

Fig. 1. Effect of ethanol on MAO activity in vitro.

The assay procedure was described in the experimental methods. Values are mean of 5 experiments. Tyramine, ■—■; benzylamine, ○—○; serotonin, △—△.

![Graph](image2.png)

Fig. 2. Effect of acute ethanol treatment on hepatic MAO activity.

The other conditions are the same as described in Table I. Control, □; ethanol, □. * p < 0.05.

Table I. Change in hepatic mitochondrial MAO activities as a function of time after ethanol treatment

<table>
<thead>
<tr>
<th>Treatment (hour)</th>
<th>Specific activity (NH₃ nmoles / mg protein / min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.64 ± 0.18</td>
</tr>
<tr>
<td>1</td>
<td>2.94 ± 0.21</td>
</tr>
<tr>
<td>1.5</td>
<td>3.43 ± 0.21*</td>
</tr>
<tr>
<td>2</td>
<td>3.41 ± 0.23*</td>
</tr>
<tr>
<td>3</td>
<td>3.37 ± 0.29</td>
</tr>
<tr>
<td>4</td>
<td>3.40 ± 0.35</td>
</tr>
</tbody>
</table>

Mice received ethanol (1 g/kg) intraperitoneally 90 min before sacrifice. The assay procedure was described in the experimental methods. Values are mean ± S.E. for in each group. * p < 0.05.

Effect of acute treatment of ethanol on MAO activity

Table I shows the change in hepatic mitochondrial MAO activities as a function of time after ethanol treatment using tyramine as substrate. MAO activity was increased after ethanol treatment; activity was 115% of the control value 1 hr after treatment, 125% at 1.5 hr, 123% at 2 hr, 119% at 3 hr. Even until 4 hr MAO activity does not returned to control level.

MAO activities toward each substrate 90 min after ethanol treatment are shown in Fig. 2. The enzyme activities were significantly increased toward each substrate as compared to the control group.

Effect of chronic treatment of ethanol on MAO activity

When ethanol was fed chronically, the activities