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Effect of Ethanol on Mouse Liver Monoamine Oxidase

Keun Huh, Sang-Il Lee*, Jong-Min Park and Byung-Su Jang

*Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 713-800 and *Department of Food and Nutrition, Keimyung Junior College, Daegu 705-030, Korea*

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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.[1,2] The function of the enzyme is the oxidation of endogenous and exogenous monoamines, especially noradrenaline, dopamine, tyramine and serotonin.[3-5] It is well known that serotonin is a specific substrate for type A MAO[6,7] and benzylamine is a specific substrate for type B MAO *in vitro* assay.[6-8] There have been many reports on the effect of ethanol on MAO activity.[9-13] 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 Daiichi 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.

Assay of monoamine oxidase activity

MAO activity was assayed by a modification of the method of Nagatsu *et al.*[14] In brief, the reaction mixture contained the same buffer, enzyme solution and each substrate. The reaction was terminated by the addition of 0.767N H_2SO_4 and 2% (w/v) Na_2WO_4. When ethanol or acetaldehyde was studied, ethanol or acetaldehyde was added to in-

cubation mixtures containing mitochondrial fraction 5min 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 625nm. Protein was determined with bovine serum albumin as standard.[15]

Drug administration

In the acute studies, mice were given 25%(v/v) ethanol(1g/kg) intraperitoneally 90min before decapitation and 2.5%(v/v) acetaldehyde(100mg/kg) was given 30min 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 11mM, whereas above 22mM 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 0.2M ethanol, respectively.

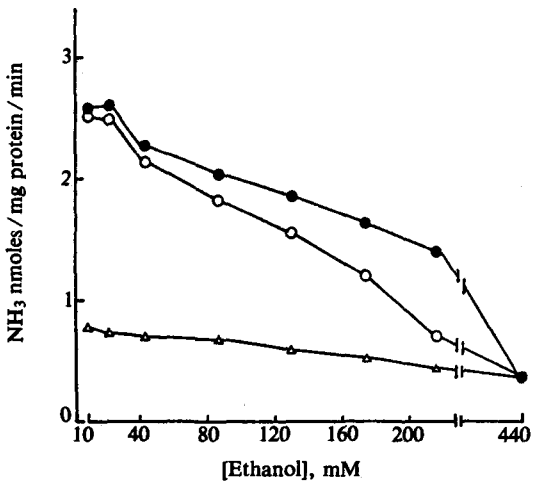

Fig. 1. Effect of ethanol on MAO activity *in vitro*.
The assay procedure was described in the experimental methods. Values are mean of 5 experiments. Tyramine, ●—●; benzylamine, ○—○; serotonin, △—△.

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

Treatment (hour)	Specific activity (NH_3 nmoles/mg protein/min)
0	2.64 ± 0.18
1	2.94 ± 0.21
1.5	3.43 ± 0.21*
2	3.41 ± 0.23*
3	3.37 ± 0.29
4	3.40 ± 0.35

Mice received ethanol(1g/kg) intraperitoneally 90min 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 1hr after treatment, 125% at 1.5hr, 123% at 2hr, 119% at 3hr. Even until 4hr MAO activity does not returned to control level.

MAO activities toward each substrate 90min 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

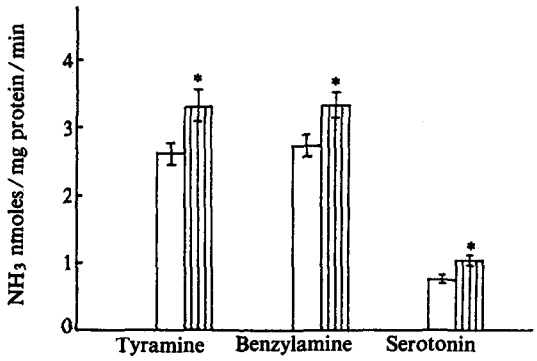

Fig. 2. Effect of acute ethanol treatment on hepatic MAO activity.
The other conditions are the same as described in Table I. Control, □; ethanol, ▥. *, $p < 0.05$.

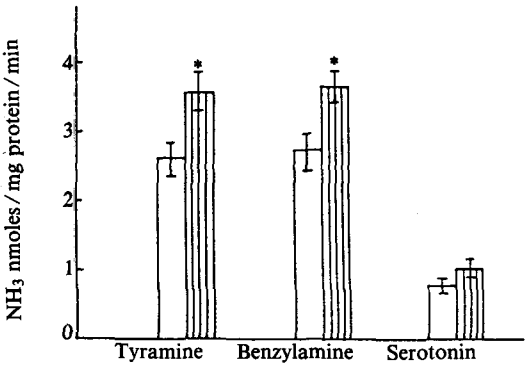

Fig. 3. Effect of chronic ethanol treatment on MAO activity.
The other conditions are the same as described in Fig. 2. Control, □; ethanol, ▥. *, $p < 0.05$.

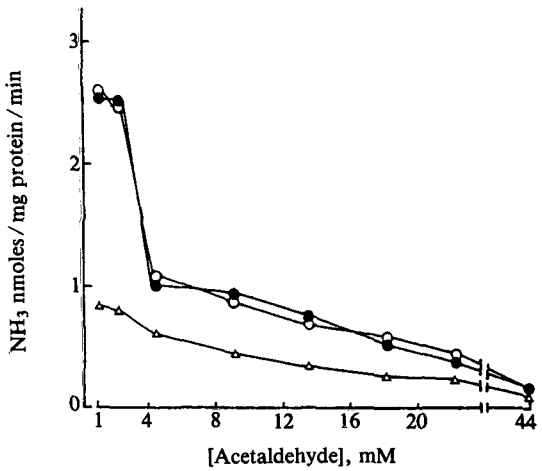

Fig. 4. Effect of acetaldehyde on MAO activity *in vitro*.
The other conditions are the same as described in Fig. 1. Tyramine, ●—●; benzylamine, ○—○; serotonin, △—△.

toward tyramine and benzylamine was significantly increased by about 1.3 fold, whereas the activity toward serotonin was not affected as compared to control group(Fig. 3).

Effect of acetaldehyde on MAO activity in vitro

Fig. 4. shows the effect of acetaldehyde on MAO activity *in vitro*. Addition of acetaldehyde, up to a concentration of 1.1mM, was not influenced the hepatic MAO activity, but the activities toward tyramine, benzylamine and serotonin were significantly inhibited by about 85% at 23mM acetaldehyde.

Table II. Change in hepatic mitochondrial MAO activities as a function of time after acetaldehyde treatment

Treatment (hour)	Specific activity (NH_3 nmoles/mg protein/min)
0	2.64 ± 0.30
1/4	3.37 ± 0.30
1/2	3.82 ± 0.36*
1	3.80 ± 0.33*
2	3.60 ± 0.28*
3	3.50 ± 0.29
4	3.00 ± 0.42

Mice received acetaldehyde(100mg/kg) intraperitoneally 30min before sacrifice. The other conditions are the same as described in Table I. *, $p < 0.05$.

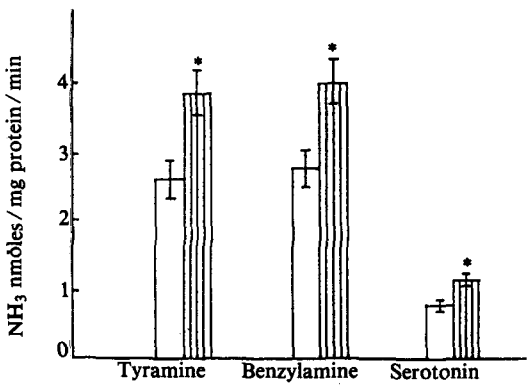

Fig. 5. Effect of acute acetaldehyde treatment on MAO activity.
The other conditions are the same as described in Table II. Control, □; acetaldehyde, ▥. *, $p < 0.05$.

Effect of acute treatment of acetaldehyde on MAO activity

Table II shows the change in hepatic mitochondrial MAO activities as a function of time after acetaldehyde injection. MAO activity was 1.4 fold 30min after acetaldehyde administration as compared to control group, and maintained the steady state level even after 4hr of acetaldehyde treatment. In subsequent experiment, mice were killed at this time.

Effect of acetaldehyde on the MAO activities is shown in Fig. 5. The enzyme activities were significantly increased toward each substrates as compared to the control group.

DISCUSSION

Ingestion of ethanol, either acutely or chronically, causes considerable metabolic derangement as well as changes in the structure and function of hepatocellular organelles.[16-20] Our results indicate that acute and chronic ethanol treatment caused to increase the hepatic mitochondrial MAO activities. Furthermore, these increments were found in all substrates used, tyramine, benzylamine and serotonin. The action of acetaldehyde, metabolite of ethanol, was more powerful than that of ethanol. In the present work, it is evident that the increment of MAO activity can be either due to a change of catalytic activation of existing enzyme or due to an alteration in the quantity of enzyme protein. As we consider that ethanol is one of substrate analogues,[21] a possibility of the substrate induction in ethanol-treated mice may be also considerable. Further experiments to confirm this postulate are under development.

Contrary to these *in vivo* experiments, the enzyme activity was markedly decreased in dose-dependent manner *in vitro*. The data *in vitro*, with ethanol or acetaldehyde, are in good agreement with those of Maynard and Schenker[9] who used liver homogenate as the enzyme source. Whereas, our results are not in agreement with those of Kurosawa,[11] who noted that ethanol increased MAO activity *in vitro* when bovine liver mitochondria is used. McEwen *et al.*[21] suggested that alcohols can act as substrate analogues for MAO, and that the competitive nature of the inhibition of MAO activity by ethanol may be due to this capability.

It was reported that chronic alcoholics appeared to have MAO activity lower than control values.[13] It is therefore possible that the low MAO activity found in alcoholic patients is a secondary effect of the liver damage produced by chronic abuse of alcohol. These different effects of alcohol on MAO activity might be results from the species differences of the experimental animal, the different substrate concentrations and the enzyme preparation.

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