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Immobilization stress increases endogenous monoamine oxidase (MAO) inhibitor in rat liver

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Abstract

Although the physiological role of endogenous monoamine oxidase (MAO) inhibitor still remains unclear, the present study examined whether or not immobilization stress (IMMO) induce MAO inhibitor. An endogenous inhibitor of MAO was separated by gel filtration from 105,000 g supernate in rat liver cytosol following IMMO. The molecular weight of this inhibitor was estimated to be 500–600 by gel filtration. This inhibitor was proved to be heat-stable resistant to protease treatment. IMMO for 2 h significantly decreased MAO. These results suggest that this inhibitor is induced by IMMO. MAO activity in rat liver might be regulated by the level of this inhibitor.

Keywords: Monoamine oxidase (MAO); Immobilization stress (IMMO); Endogenous MAO inhibitor; Benzylamine; Rat liver

1. Introduction

Some evidence indicate that stress can cause a reduction in monoamine oxidase (MAO; EC, 1.4.3.4) activity [1]. Immobilization stress (IMMO) causes an increase in plasma epinephrine and norepinephrine concentration in rat plasma [2]. It is known that endogenous MAO inhibitor in rat brain is significantly increased following stress [3,4]. Our laboratory already reported [5] that endogenous MAO inhibitor have been detected in the cytosol of rat liver. These studies imply that endogenous MAO inhibitor might be important in physiological regulation of MAO activity [6,7]. In the present study, I found an endogenous MAO inhibitor in rat liver cytosol could be induced by subjected to IMMO.

2. Experimental

2.1. Drugs

Benzylamine hydrochloride was purchased from Sigma Chemical Co. (St Louis. MO. USA). The radioactive substrate [7-^{14}C]-benzylamine hydrochloride (1.85–2.29 Gbq/mmol) was obtained from Amersham International (Amersham, England).

2.2. Animals

Male Wistar rats, weighing 200–250 g at the start of the experiments were kept at room temperature (control group). Rats from the IMMO group were immobilized for 1 h or 2 h after being kept under the same conditions as the control group. This study was approved by the Ethical Committee for Animal Experiments, Oita Medical University, Japan.

2.3. Experimental protocol

IMMO was carried out by taping all four limbs of the rat to metal mount attached to a board [8]. The rats were killed by decapitation with a guillotine, and their liver were quickly removed and homogenized in 10 vol. of 10 mM phosphate buffer, pH 7.4 containing 0.25 M sucrose. The homogenate was centrifuged at 105,000 g for 60 min, and the supernate (cytosol fraction) was applied on a Sephadex G-25 column (1.0×60 cm), previously equilibrated with 20 mM phosphate buffer (pH 7.4). The column was eluted with the same buffer at a rate of 10 ml/h and the fractions were collected in 2.5 ml each [5]. An aliquot of each fraction was assayed for MAO inhibition activity, and active

Abbreviations: IMMO; immobilization stress; MAO; monoamine oxidase.
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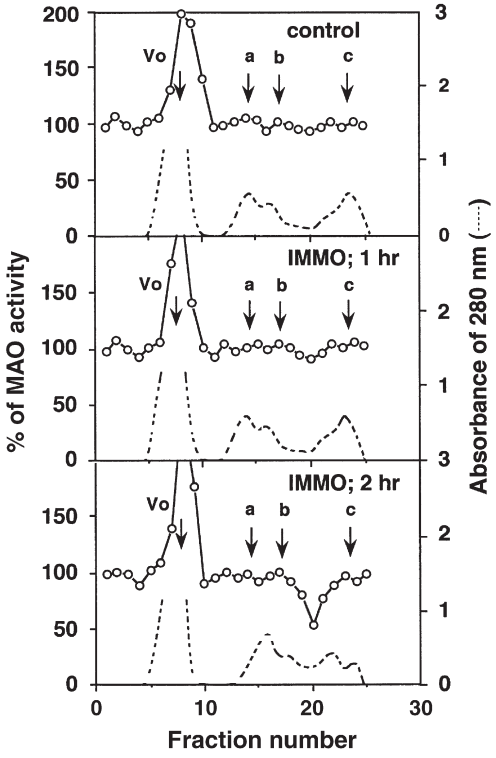

Fig. 1. Gel filtration of rat heart cytosol extract on sephadex G-25 chromatography. Cytosolic fraction (10 mg protein) was applied to a Sephadex G-25 column (1.0×60 cm). The IMMO group (1 h and 2 h) was compared with the control group. The activity of MAO in homogenate was assayed with 100 μM benzylamine as a substrate. Arrows indicate the position of marker compounds. The molecular for markers used were as follows: a, cyanocobalamine; b, FAD; c, DNP-Alanin; Vo; dextran. The broken line shows the absorbance at 280 nm.

fractions were combined and used for further characterization. This fraction is referred to as "endogenous MAO inhibitor (EMI)".

2.4. Assay of MAO activity

The MAO activity was measured using the radioactively labelled [^{14}C]-benzylamine as substrate. Rat liver homogenates were used as source of MAO activity. The 10% (w/v) homogenates of these were prepared in 0.25 M sucrose plus 10 mM phosphate buffer, pH 7.4. MAO activity was assayed radiochemically as reported previously [9,10]. Protein concentrations of the enzyme preparations were measured by the method of [11] with bovine serum albumin as the standard.

2.5. Statistical analysis

All values are presented as means±SE. The significance was determined by using ANOVA with Fisher's post hoc test. A P value of less than 0.05 was regarded being statistically significant.

3. Results and discussion

The gel-filtration of IMMO-treated rat liver cytosol with Sephacryl S-200 column showed that fractions that inhibit activity

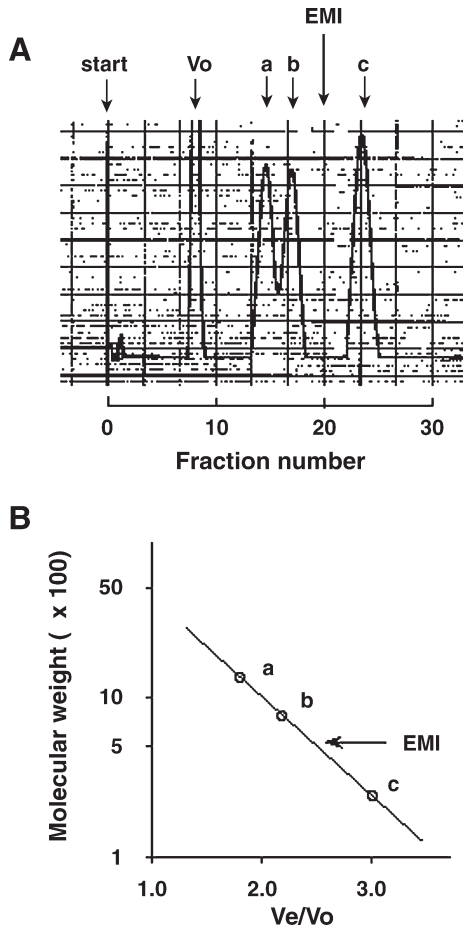

Fig. 2. Sephadex G-25 chromatography of cytosolic fraction from IMMO for 2 h treat rat liver.(A) Separation of a standard mixture. The flow rate was 10 ml/h and effluent fractions were monitored at 280 nm. The arrows shows the mobility of the peak of endogenous MAO inhibitor (EMI). The Ve/Vo for markers used were as follows: a, cyanocobalamine (MW, 1355); b, FAD (MW, 255); c, DNA-Alanin (MW, 255); Vo; dextran (MW, 2,000,000).(B) Estimation of molecular weight of end EMI.

were eluted in a low molecular weight area (<1300) (data not shown). Therefore, I used Sephadex G-25 column to separate the inhibitor and determine the molecular size (Fig. 1). The

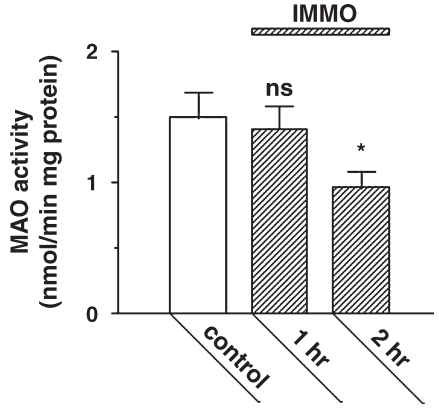

Fig. 3. Effect of immobilization stress (IMMO) on MAO activity in rat liver. MAO activity was assayed radiochemically. Substrate concentration used 100 μM benzylamine, final concentration. MAO activity is expressed as nmol/min/mg protein. Values are mean±S.E.M. for six animals: $^{*}P<0.05$ versus control group. ns, nonsignificant.

molecular weight of the inhibitor was estimated to be 500–600 in IMMO treated rat liver cytosol (Fig. 2). The fractions 19–21 were found to inhibit MAO activity with 100 μM benzylamine as a substrate. Some endogenous MAO activity was observed in fractions 7–10. When similar experiments were repeated, the inhibition activities of IMMO significantly increased from 4.7±2.9% to 48.9±8.8% ($P<0.05$, $n=8$; not illustrated). This activity may be to the contamination of the liver enzyme. When EMI was treated at 100 °C for 10 min, the inhibition activity was not changed. Also, treatment of EMI with subtilism or protease at 37 °C for 12 h did not affect the inhibition activity of EMI. These results suggest that EMI is a non-peptide inhibitor. IMMO for 1 h decreased MAO activity, but this change was not significant. However, IMMO for 2 h significantly decreased MAO activity from 1.54±0.18 to 0.98± 0.13 nmol/min/mg protein ($n=6$, $P<0.05$) (Fig. 3). Therefore, I confirmed the existence of endogenous EMI following IMMO.

I found an endogenous MAO modulator of a low molecular weight (500–600) in liver cytosol of IMMO treated rats. Although the nature of this inhibitor is not clear at present, the present study showed that this compound is heat-stable and resistant to protease treatment. Between stress and MAO inhibitor may account for the function of catecholamine degradation, at least in part. Because MAO has a high affinity for neurotransmitter monoamines such norepinephrine [12], stress might influence the physiological function of the control and peripheral neuron system by catalyzing the deamination of such neurotransmitter monoamines [6,7]. There were no correlations between brain levels and those in the other sources [13]. This inhibitor is involved in MAO inhibiting regulation in the liver [1]. My finding suggested that IMMO-induced modulator may have an important role in MAO activity. I consider that this inhibitor may play some role in regulating the MAO activity in rat liver. While the role of this inhibitor is no doubt important, their mechanism remain to be elucidated. Further in vivo studies using EMI are under way in our laboratory to elucidate the physiological function of MAO in rat liver.

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