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Effects of zinc ion on type A monoamine oxidase in monkey brain mitochondria

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Abstract

The effects of $ZnSO_4$ on types A and B monoamine oxidase (MAO) isozymes in monkey brain mitochondria were investigated, *in vitro*. Type A MAO activity in monkey brain decreased to about 50% with 1 μM $ZnSO_4$ using serotonin as a substrate, and this inhibition was proportional to the concentration of $ZnSO_4$. $ZnSO_4$ had no effect, however, on type B MAO activity in monkey brain using β-phenylethylamine as a substrate. The inhibition by $ZnSO_4$ of type A MAO activity was competitive and reversible. $ZnSO_4$ did not inhibit either type A or type B MAO activity in rat brain mitochondria. Almost similar results were also obtained when $ZnCl_2$ was used, *in vitro*. These results indicate that the inhibiting action of zinc ion differs depending on animal species and organ. Type A MAO in monkey brain mitochondria was highly sensitive to zinc ion, while type B activity was less sensitive.

Keywords: Zinc ion; Type A MAO; Type B MAO; Monkey brain; Type A MAO inhibitor; Rat brain

1. Introduction

Since zinc is required for many enzymes, it is reasonable to speculate that the level of zinc in cells controls the physiological process through the formation and regulation of the activity of zinc-dependent enzymes. Many reports indicate that zinc has antioxidant activity, is a constituent of the cytosolic Cu/Zn-super oxide dismutases [1] and is present in several dehydrogenases, aldolases, peptidases and phosphatases [2], and also has a stabilizing influence on membranes [3]. There has been considerable interest in the roles of zinc in excitatory neuron transmission in the mammalian central nervous system [4,5]. Evidence exists from animal experiments and human studies to support a zinc neurotoxic effect, which is related to metal ions and is mediated by biogenic amines. Since monoamine oxidase (MAO) is a key enzyme that regulates the metabolism of biogenic amines, it has been considered that neurotoxicity caused by metal ions may be due to the inhibition of MAO enzymes, thus inducing an alteration of amine metabolism [6].

There is, however, little information concerning the changes in MAO activities related to biogenic amine metabolism due to the transition metals, zinc. In the present study, we demonstrate that the zinc ion potently inhibits type A MAO activity, but not type B MAO activity in monkey brain mitochondria, *in vitro*.

2. Materials and methods

The chemicals used and their sources were as follows: $ZnSO_4$ and $ZnCl_2$ (purity 99%) from Wako Pure Chemical Industries; all other chemicals from Sigma Chemical Co. The two radiochemical substrates used in this study, 5-hydroxytryptamine binoxalate ([2-^{14}C], 5-HT; 1.48–2.22 GBq/mmol) and β-phenylethylamine hydrochloride, ([ethyl-1-^{14}C], β-PEA; 1.48–2.22 GBq/mmol) were purchased from DuPont NEN (New England Nuclear) Products. Japanese monkeys (*Macaca fuscata*, male, 3–6 years old, N = 3), donated by the Animal Center, Oita Medical University, were euthanized by blood depletion under ketamine (30 mg/kg, i.m.) anesthesia, and their brains were quickly removed. The rats (Wistar, males, 7 weeks old, N = 5) were sacrificed by decapitation and their brains

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were quickly removed. The brains were homogenized in 10 volumes of 0.32 M sucrose solution. Mitochondrial subfractions were obtained by sucrose density gradient centrifugation, as described in a previous report [7]. All procedures were carried out at 4°. This study was performed according to the Oita Medical University guidelines for the care and use of laboratory animals. The MAO activity was measured using the labeled substrates [^{14}C]-5-HT (100 μM) and [^{14}C]-β-PEA (10 μM) as described previously [8]. For investigating the effects of $ZnSO_4$ on MAO activity *in vitro*, the enzyme was preincubated for 20 min at 25° with $ZnSO_4$ at concentrations of 0.01–100 μM before adding the substrates. Reaction was initiated by adding 25 μL of substrate containing labeled 5-HT (substrate for type A MAO) and β-PEA (substrate for type B MAO) and the mixture was incubated for 30 min at 37°. The reaction products were extracted with 2 mL of benzene–ethyl acetate (1:1, v/v). Toluene scintillation liquid (10 mL) containing Triton X-100 was added to 1.0 mL samples of the extract, and the radioactivity was measured by liquid scintillation spectrometry. Enzyme activity was expressed as nmol/min per milligram of protein. Quantitative results are reported as the mean ± SD for triplicate determinations. The effects of $ZnSO_4$ was statistically analyzed by ANOVA techniques with Willliam's method.

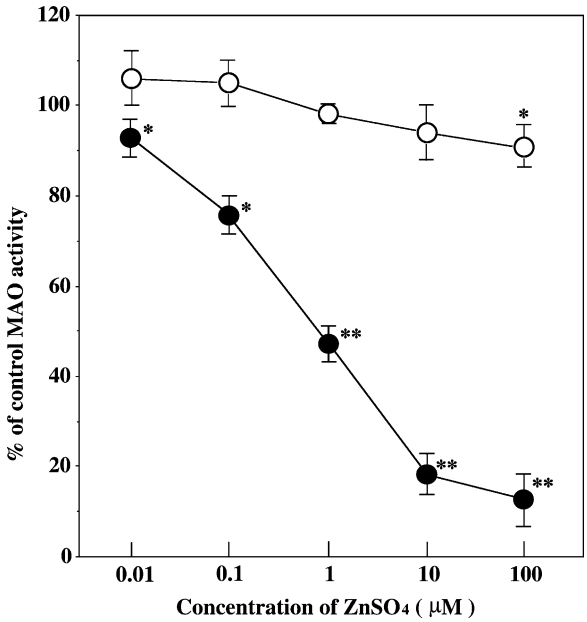

Fig. 1. Effect of $ZnSO_4$ on MAO activities in monkey brain mitochondria. After incubation at 25° for 20 min with 100–0.01 μM $ZnSO_4$, MAO activity was determined with 100 μM 5-HT (●) and 10 μM β-PEA (○) as substrates at 37° for 30 min. The mean ± standard error of the mean (SEM) control values for type A MAO and type B MAO activities were 3.25 ± 0.53 and 4.54 ± 0.98 nmol/min mg protein, respectively. Values are expressed as the percent MAO activity of the control. The results are means of triplicate assays. (*) $P < 0.05$, (**) $P < 0.01$ vs. control (ANOVA with William's test).

3. Results and discussion

As shown in Fig. 1, $ZnSO_4$ at low concentrations (micromolar) potently inhibited type A MAO activity in monkey brain mitochondria. To determine the mechanism of MAO inhibition by $ZnSO_4$, the kinetics of type A MAO inhibition in monkey brain mitochondria upon the addition of the $ZnSO_4$ were investigated. This inhibition was competitive, and reversible. However, $ZnSO_4$ did not inhibit type B MAO activity. The central role of the multiple forms of MAO in the metabolism of biogenic amines and the importance of inhibitors of this enzyme are widely recognized. Previously, inhibitor selectivity has been frequently used as the criterion for the existence of multiple forms of MAO. Two forms of MAO, type A MAO and type B MAO, have been found in the brains of many animals including humans. In monkey brain, type A MAO specifically deaminates 5-HT and is selectively inhibited by clorgyline, while type B MAO acts specifically on β-PEA and is relatively sensitive to deprenyl [7]. In this experiment, MAO activity in monkey brain was highly sensitive to zinc ion with 5-HT and was less so with β-PEA. These results were identical to those of clorgyline as a type A MAO inhibitor and indicate that zinc ion selectively inhibits type A MAO activity, but not type B activity. It is known that the type A and type B MAO have subunit molecular weights of 59,700 and 58,800, respectively, have 70% sequence identity and may be derived from separate genes [9]. In addition, the determination of regions for type A MAO and type B MAO substrate and inhibitor preferences have been examined using chimeric MAO enzymes [10,11]. These results and reports indicate that zinc ions competitively bind to 5-HT binding sites in the substrate binding regions and thus may play a structural rather than a catalytic role.

The effects of $ZnSO_4$ on mitochondrial MAO activity in monkey and rat brain were compared. In monkey brain, $ZnSO_4$ inhibited type A MAO activity, but not type B activity, while in rat brain, neither type A nor type B MAO activity was inhibited by $ZnSO_4$ (Fig. 2). It is not clear how the different MAO-A inhibition by $ZnSO_4$ occurs between monkey and rat. Rat and monkey type A MAOs may be different in their secondary or tertiary structure of 5-HT binding sites in the substrate binding regions in spite of their high sequence identity. In this study, almost similar results were also obtained when $ZnCl_2$ was used as a zinc ion (data not shown).

In this study, we demonstrated that the inhibiting action of zinc ion differs depending on organ and animal species. There is consider able interest in the inhibition of type A MAO activity by zinc ions. It is generally believed that cerebral function in humans is closer to the cerebral function of monkey than of rat, although the molecular cloning of monkey MAO isozymes has not been achieved. It seems most probable that type A MAO activity is inhibited by zinc ions in human brain also. In this study,

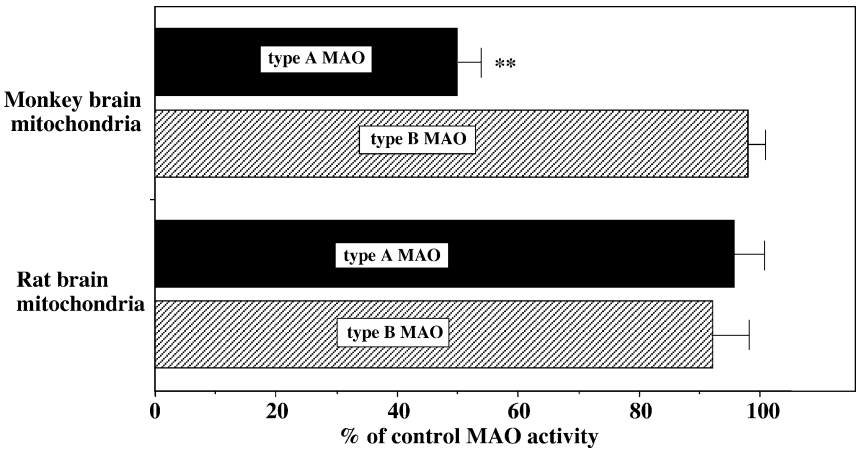

Fig. 2. Effect of $ZnSO_4$ on mitochondrial MAO activities in monkey and rat brain. The effects of $ZnSO_4$ on mitochondrial MAO activity in monkey and rat brain were compared. After incubation at 25° for 20 min with 1 μM $ZnSO_4$, MAO activity was determined with 100 μM 5-HT and 10 μM β-PEA as substrates at 37° for 30 min. The mean ± SEM control values for mitochondrial type A MAO activity in monkey and rat brain were 3.15 ± 0.43 and 1.85 ± 0.77 nmol/min mg protein, respectively, while these of type B MAO were 4.51 ± 0.33 and 2.15 ± 0.12 nmol/min mg protein, respectively. Each value represents the mean percentage (±SEM) of the control MAO activity value in triplicate experiments. (**) $P < 0.01$ vs. control (ANOVA with William's test). Columns: (■) type A MAO; (▨) type B MAO.

since it was demonstrated that type A MAO activity of the brain was inhibited selectively by zinc, this element has the potential to inhibit type A MAO activity when excess zinc is present in the diet. It is considered that this zinc excess may lead to the same results as when a selective type A MAO inhibitor is administered. There are many reports that abnormal behavior including impulsive aggression is associated with a complete and selective deficiency of type A MAO activity in mice models lacking type A MAO as well as in humans [12–14]. From these reports and our data, since zinc is known to be present in brain and in cerebrospinal fluid [15,16], zinc may be an endogenous type A MAO inhibitor in the central nervous system. Further studies are needed to investigate the relationship between the level of zinc ions and the functions of type A MAO in serotonergic neurons in the brain.

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