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CHARACTERIZATION OF EIGHT BIOGENIC INDOLEAMINES AS SUBSTRATES FOR TYPE A AND TYPE B MONOAMINE OXIDASE

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Abstract—Tryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine, 5-hydroxytryptamine (5-HT), 5-hydroxy-*N*-methyltryptamine, bufotenine, 5-methoxytryptamine, and 5-methoxy-*N,N*-dimethyltryptamine were characterized as substrates for type A and type B monoamine oxidase (MAO) in rat liver mitochondria. Experiments on sensitivity to clorgyline and to deprenyl, using two substrate concentrations, showed that tryptamine and its *N*-methylated and *N,N*-dimethylated derivatives were common substrates for both types of MAO at a substrate concentration of 20.0 μM; at 1000 μM, tryptamine and *N*-methyltryptamine were common, but *N,N*-dimethyltryptamine became specific for type B MAO. All the 5-hydroxy- or 5-methoxy-indole derivatives were almost completely specific for type A MAO at a substrate concentration of 20.0 μM; when the concentration was 1000 μM, some of the MAO activity was due to type B MAO for 5-HT, bufotenine and 5-methoxytryptamine. The rat liver mitochondrial enzyme was pretreated with 10^{-7} M clorgyline and 10^{-7} M deprenyl to obtain, respectively, the type B-rich and the type A-rich enzyme. These enzyme preparations were subjected to kinetic analyses for the eight amines. From the kinetic analyses, together with data on inhibitor sensitivity, the following phenomena can be described. *N*-Methylation of tryptamine or of 5-HT did not cause appreciable changes in the specificity of the substrates toward each type of MAO, but it elevated the K_m value of type B MAO when the values for tryptamine and *N*-methyltryptamine were compared. *N,N*-Dimethylation of tryptamine and 5-HT tended to increase the specificity for type B MAO. All the dimethylated compounds had very low activities with either type A or type B MAO. Either the 5-hydroxy- or the 5-methoxy-group contributed to the specificity of the substrates for type A MAO.

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many tissues in two functional forms called type A and type B [1–3]. Various biogenic phenylethylamines have been characterized as substrates for each type of MAO (see a review in Ref. 4). In regard to biogenic indoleamines, 5-hydroxytryptamine (5-HT), tryptamine [5, 6], and 5-methoxytryptamine [5, 7] have been studied *in vitro* as substrates for MAO; the substrate specificity of 5-methoxy-*N,N*-dimethyltryptamine was also studied *in vivo* [8]. To our knowledge, however, other biogenic indoleamines have never been investigated. In the present paper, we have characterized eight biogenic indoleamines as substrates for type A and type B MAO *in vitro*.

MATERIALS AND METHODS

Enzyme. Crude mitochondrial fractions were isolated from the pooled livers of three to ten male Sprague–Dawley rats, weighing 100–200 g, as described previously [9].

Chemicals. Horseradish peroxidase (type II), pargyline–HCl, *N*-methyltryptamine, *N, N*-dimethyltryptamine, 5-HT creatinine sulfate, bufotenine (5-hydroxy-*N,N*-dimethyltryptamine) monooxalate hydrate, 5-methoxytryptamine–HCl and 5-methoxy-*N,N*-dimethyltryptamine were obtained from the Sigma Chemical Co., St. Louis, MO; tryptamine–HCl from Nakarai Chemicals Ltd., Kyoto, Japan; 5-hydroxy-*N*-methyltryptamine oxalate from the Aldrich Chemical Co., Inc., Milwaukee, WI; 2′,7′-dichlorofluorescin diacetate from the Eastman Kodak Co., Rochester, NY; and hydrogen peroxide from Santoku Chemical Industries Co., Ltd., Miyagi, Japan. Clorgyline was supplied by May & Baker Ltd., Dagenham, U.K., and deprenyl was donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

MAO assays. MAO activities were measured photometrically by a slight modification of the method of Köchli and von Wartburg [10]. Details of the procedure are described in our previous paper [11]. For each assay (final volume, 3.0 ml), 0.013 to 1.52 mg of mitochondrial protein was used. The assays were carried out at 37° and pH 7.4 for 10 or 15 min in air. Using these conditions, the assays were linear for at least 20 min of incubation.

Inhibition studies. Clorgyline and deprenyl were dissolved in distilled water, added to the assay mixture without substrate, and preincubated at 37° for 10 min to ensure reproducibility of enzyme inhibi-

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tion; it was confirmed that 10 min of preincubation with 10^{-7} M clorgyline or 10^{-7} M deprenyl resulted in almost the same extent of inhibition as did 30 min of preincubation. Seven different concentrations of each inhibitor were employed over the range of 10^{-10} to 10^{-4} M. It was confirmed that each inhibitor did not interfere with the photometry when hydrogen peroxide was added directly. The inhibition was carried out at substrate concentrations of 20.0 and 1000 μM.

Kinetic studies. The crude mitochondria prepared from the pooled livers of five to eight rats were incubated with 10^{-7} M clorgyline or 10^{-7} M deprenyl at 37° for 30 min and centrifuged at 18,000 *g* for 10 min. The resulting pellet was suspended in 30 ml of 0.25 M sucrose solution and recentrifuged at 18,000 *g* for 10 min. This procedure was repeated once to wash the enzyme completely. The resulting pellet was subjected to kinetic analyses of MAO. Each kinetic constant was determined graphically from Lineweaver–Burk plots using seven or eight substrate concentrations assayed in duplicate.

Protein determinations. Protein was determined by a slight modification [12] of the conventional biuret method, using bovine serum albumin as a standard.

RESULTS

Tryptamine. Figure 1 shows MAO inhibition by clorgyline, a selective inhibitor of type A MAO [1],

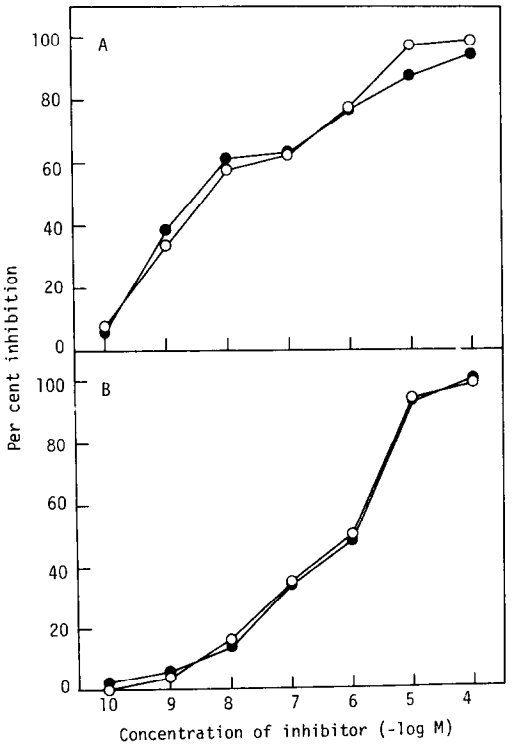

Fig. 1. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of tryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 35.9 and 53.4 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

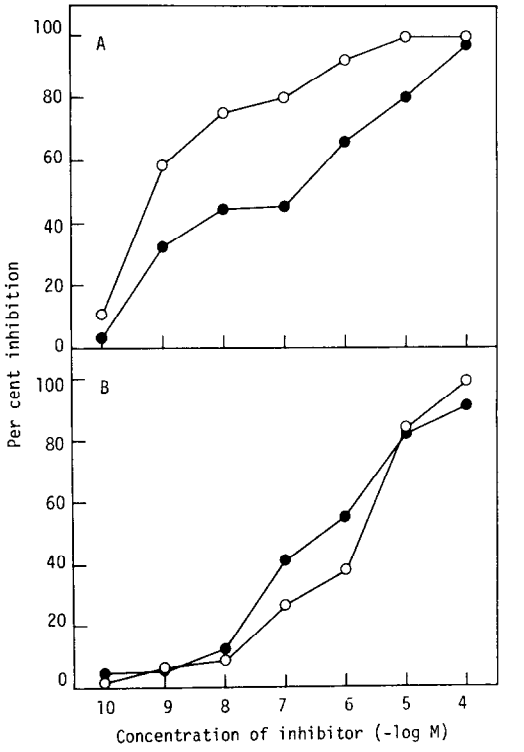

Fig. 2. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of *N*-methyltryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 24.2 and 46.0 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

and by deprenyl, a selective inhibitor of type B MAO [13], using 20.0 and 1000 μM tryptamine as substrate. In the curves with clorgyline (Fig. 1A), there were clear plateaus at 10^{-8} to 10^{-7} M for both concentrations, showing that tryptamine is common for both types. In confirmation of the results with clorgyline, shoulder-like patterns were observed at 10^{-8} to 10^{-6} M deprenyl (Fig. 1B). There were no differences between the inhibition patterns with either inhibitors at the two substrate concentrations.

N-*Methyltryptamine.* Figure 2 illustrates the inhibition by both inhibitors using 20.0 and 1000 μM *N*-methyltryptamine as substrate. The inhibition patterns with clorgyline exhibited clear plateaus at 10^{-8} to 10^{-7} M at both substrate concentrations (Fig. 2A), showing that this substrate is common to both MAO types. The sensitivity to clorgyline with 20.0 μM *N*-methyltryptamine was much higher than with 1000 μM. The susceptibility to deprenyl at 20.0 μM was slightly less than at 1000 μM, confirming the results with clorgyline.

N,N-*Dimethyltryptamine.* Figure 3 shows MAO inhibition by both inhibitors using *N,N*-dimethyltryptamine as substrate. With a 20.0 μM concentration of the substrate, a clear plateau appeared with 10^{-8} to 10^{-7} M clorgyline (Fig. 3A), showing that this substrate was common to both MAO types at this concentration. At 1000 μM, however, the inhibition curve with clorgyline was almost single sigmoidal, showing strong resistance to clorgyline; this result

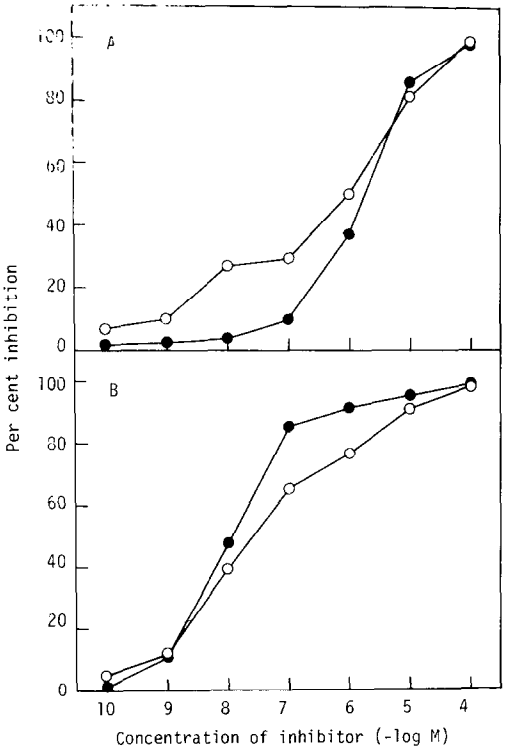

Fig. 3. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of *N,N*-dimethyltryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 2.71 and 9.81 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

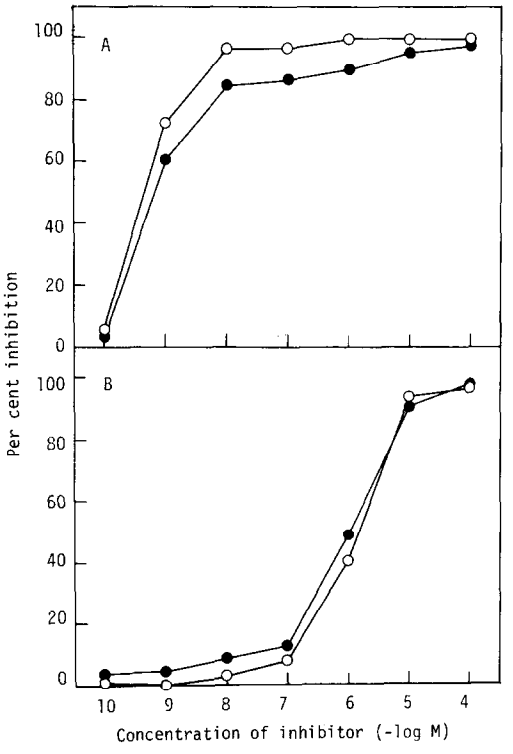

Fig. 4. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of 5-HT as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 24.8 and 51.4 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

indicates that *N,N*-dimethyltryptamine became a specific substrate for type B. The inhibition patterns with deprenyl (Fig. 3B) generally supported the results with clorgyline. The activities measured with this substrate were much lower than those with tryptamine and *N*-methyltryptamine.

5-HT. As shown in Fig. 4, 5-HT was highly susceptible to clorgyline and highly resistant to deprenyl for both substrate concentrations, showing that 5-HT was almost specific for type A MAO. It appeared, however, that there was a slight shift in the inhibition pattern between the two substrate concentrations, suggesting that a minor portion of the MAO activity toward 5-HT was a result of type B MAO when the substrate concentration was 1000 μM.

*5-Hydroxy-*N*-methyltryptamine.* Figure 5 shows the inhibition patterns with 5-hydroxy-*N*-methyltryptamine as substrate. The patterns with clorgyline and deprenyl showed that this substrate was specific for type A MAO at both substrate concentrations. The difference between the patterns at the two substrate concentrations was not clear.

Bufotenine. The inhibition experiments with bufotenine as substrate (Fig. 6) showed that, at 20.0 μM, this substrate almost acted as a specific substrate for type A MAO, but at 1000 μM it became a common substrate. The activities toward bufotenine were very low.

5-Methoxytryptamine. The deamination of 20.0 μM 5-methoxytryptamine by MAO was highly susceptible to clorgyline and highly resistant to deprenyl (Fig. 7), showing that this monoamine was specific for type A MAO at this concentration. At 1000 μM of this substrate, a slight decrease in the sensitivity to clorgyline resulted in a plateau at 10^{-8} to 10^{-7} M clorgyline (Fig. 7A), suggesting that a minor part of the MAO activity toward this substrate was due to type B MAO at a high substrate concentration. The curves with deprenyl (Fig. 7B) were almost single sigmoidal.

*5-Methoxy-*N,N*-dimethyltryptamine.* The inhibition patterns with 5-methoxy-*N,N*-dimethyltryptamine revealed high sensitivity to clorgyline and high resistance to deprenyl (Fig. 8); the patterns with both inhibitors were almost single sigmoidal for both substrate concentrations. These results indicate that this monoamine was specific for type A MAO. The activities toward this substrate were somewhat higher than those toward bufotenine, the *N,N*-dimethylated analog.

Kinetic analyses. Since the uninhibited enzyme is a mixture of type A and type B MAO, rat liver mitochondrial MAO was pretreated with 10^{-7} M clorgyline or 10^{-7} M deprenyl to obtain, respectively, the type B-rich and the type A-rich enzyme. At this concentration, clorgyline inhibited type A MAO, and deprenyl type B MAO, fairly specifically. These

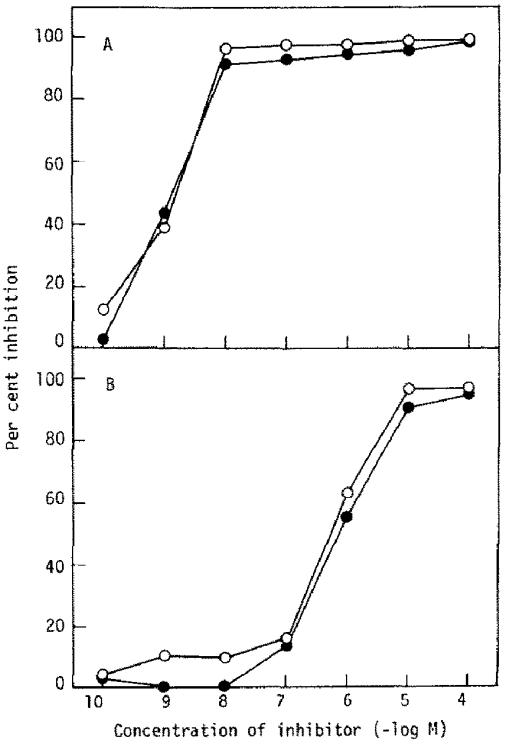

Fig. 5. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of 5-hydroxy-*N*-methyltryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 10.7 and 16.4 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

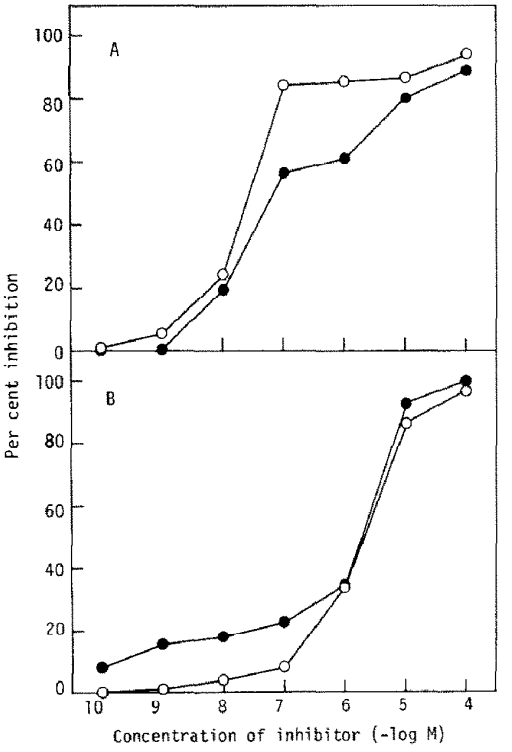

Fig. 6. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of bufotenine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 2.46 and 2.29 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

pretreated enzyme preparations were subjected to kinetic analyses with the eight substrates. The apparent K_m and V_{max} values are summarized in Table 1.

The K_m values of both MAO types for tryptamine were very low. The K_m value of type A MAO for *N*-methyltryptamine was also very low, but the K_m value of type B MAO for it was one order of magnitude higher than that of type A MAO. The K_m value of type B MAO for *N,N*-dimethyltryptamine was even higher than that for *N*-methyltryptamine. The V_{max} value of type B MAO for *N,N*-dimethyltryptamine was very low; the activity of type A MAO toward this substrate was too low to perform kinetic analyses.

The K_m values of both types for 5-HT were much higher than those for tryptamine; the V_{max} value of type A MAO for 5-HT was much higher than that of type B MAO. Both K_m and V_{max} values of type A MAO for 5-hydroxy-*N*-methyltryptamine were lower than those for 5-HT. The V_{max} value of type A MAO for bufotenine, the *N,N*-dimethylated derivative from 5-HT, was extremely low. The activities of type B MAO toward 5-hydroxy-*N*-methyltryptamine and toward bufotenine were too low to perform kinetic analyses.

The K_m value of type A MAO for 5-methoxytryptamine was much lower than that for 5-HT, and thus comparable to that for tryptamine; the K_m value of type B MAO for 5-methoxytryptamine was much higher than that of type A MAO. The V_{max} value of type A MAO for 5-methoxytryptamine was higher than that of type B MAO. The K_m value of type A MAO for 5-methoxy-*N,N*-dimethyltryptamine was slightly higher than that for 5-methoxytryptamine and its V_{max} value was much lower than that for the latter; the activity of type B MAO toward 5-methoxy-*N,N*-dimethyltryptamine was too low to carry out kinetic studies.

DISCUSSION

In the present paper, we have characterized eight biogenic indoleamines as substrates for type A and type B MAO. Tryptamine and its *N*-methyl- and *N,N*-dimethyl-derivatives were common for both MAO types at the substrate concentration of 20.0 μM (Figs. 1–3); at 1000 μM, tryptamine and *N*-methyltryptamine were common, but *N,N*-dimethyltryptamine was specific for type B. All the 5-hydroxy- and 5-methoxy-derivatives were almost specific for type A MAO at 20.0 μM; but, when the substrate concentration was 1000 μM, some portion of the MAO activity was a result of type B MAO, especially that toward 5-HT, bufotenine and 5-methoxytryptamine (Figs. 4–8). 5-HT and tryptamine are known to be specific for type A and common for both types respectively [5, 6]; the data on 5-meth-

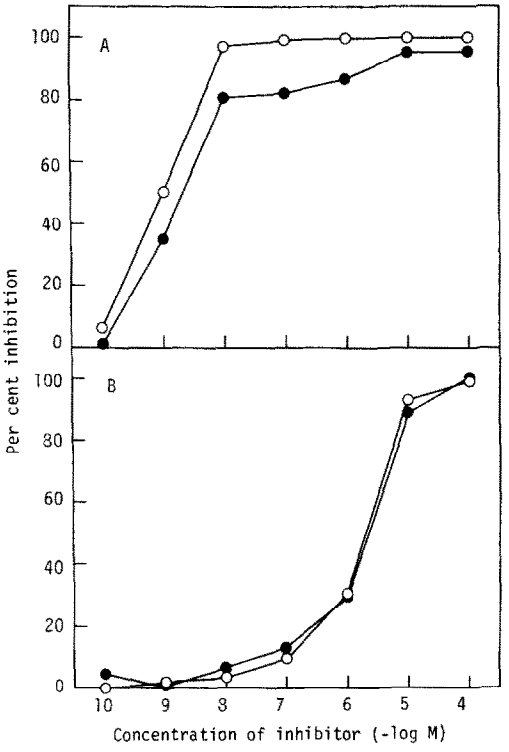

Fig. 7. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of 5-methoxytryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 31.9 and 71.9 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

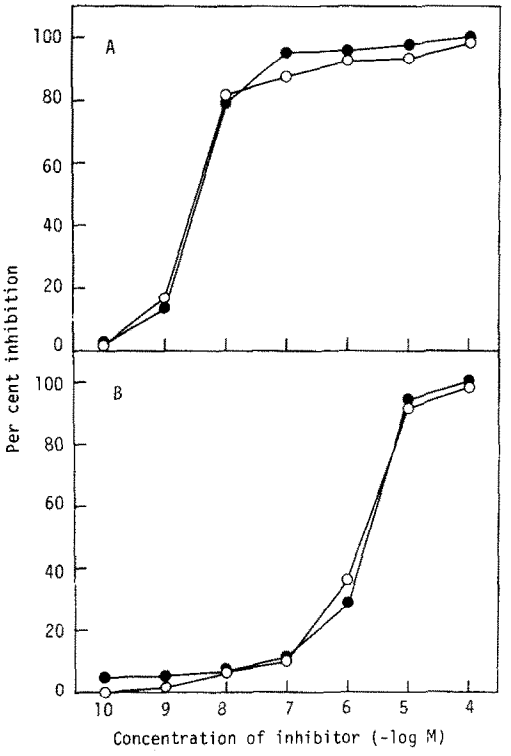

Fig. 8. Inhibition of MAO in rat liver mitochondria by clorgyline (A) and deprenyl (B) using two concentrations of 5-methoxy-*N,N*-dimethyltryptamine as substrate. The concentrations of the substrate were 20.0 (○——○) and 1000 μM (●——●), with specific activities of 4.03 and 10.4 nmoles·(mg protein)$^{-1}$·10 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations.

oxytryptamine were conflicting [5, 7]. 5-Methoxy-*N,N*-dimethyltryptamine was claimed to be specific for type A MAO by *in vivo* studies [8]. The other indoleamines have not been studied yet as substrates for type A and type B MAO prior to the present report.

Since we reported that the inhibition patterns with some substrates, such as β-phenylethylamine [14, 15], phenylethanolamine [9], and *N*-methylphenylethanolamine [16], differed markedly according to the difference in substrate concentration, we have tested, in the present study, two substrate concentrations, viz. 20.0 and 1000 μM. Notable shifts in inhibition pattern with shifts in substrate concentration were observed for *N*-methyltryptamine (Fig. 2), *N,N*-dimethyltryptamine (Fig. 3), and bufotenine (Fig. 6); slight shifts were also noted for 5-HT (Fig. 4) and 5-methoxytryptamine (Fig. 7). These changes in inhibition pattern may be attributable to the different affinities of the substrate for type A and type B MAO as suspected from the K_m values in Table 1.

Since in this study various indoleamine derivatives were characterized as substrates for type A and type B MAO, it is possible to see how a derivatization of an indoleamine affects its specificity. The *N*-methylation of tryptamine resulted in an elevation of the K_m value of type B MAO (Table 1), which probably caused the shift in inhibition pattern (Fig. 2). However, *N*-methyltryptamine, like its precursor tryptamine, was a substrate common to both MAO types (Fig. 2). 5-Hydroxy-*N*-methyltryptamine, the *N*-methylated derivative of 5-HT, showed strong specificity for type A MAO as did 5-HT (Fig. 5). It seems likely, therefore, that *N*-methylation of an indoleamine does not cause remarkable changes in specificity of the monoamine. In this connection, it should be recalled that the *N*-methylation of phenylethylamines did not give notable changes in their substrate specificities [16, 17].

N,N-Dimethyltryptamine (Fig. 3) showed a greater preference for type B MAO than did its precursors, tryptamine (Fig. 1) and *N*-methyltryptamine (Fig. 2); when its concentration was 1000 μM, it was almost specific for type B MAO (Fig. 3). Bufotenine, the *N,N*-dimethylated derivative of 5-HT, became a common substrate, when the substrate concentration was 1000 μM (Fig. 6). These results suggest that *N,N*-dimethylation of an indoleamine tends to increase its specificity for type B MAO.

The 5-hydroxy- and 5-methoxy-groups seem to contribute strongly to the specificity for type A MAO, because all the derivatives having these groups were almost entirely specific for type A MAO, when their substrate concentration was 20.0 μM (Figs. 4–8).

In the present paper, we have studied eight biogenic indoleamines as substrates for type A and

Table 1. Apparent kinetic constants for MAO in rat liver mitochondria pretreated with 10^{-7} M deprenyl or 10^{-7} M clorgyline using various indoleamines as substrates*

Substrate	Deprenyl-treated (type A-rich) MAO†		Clorgyline-treated (type B-rich) MAO†	
	K_m (μM)	V_{max} [nmoles·(mg protein)$^{-1}$·10 min^{-1}]	K_m (μM)	V_{max} [nmoles·(mg protein)$^{-1}$·10 min^{-1}]
Tryptamine	2.48	49.5	6.53	33.1
N-Methyltryptamine	3.14	54.0	24.0	57.8
N,N-Dimethyltryptamine	ND‡	ND	235	10.0
5-HT	117	158	512	13.1
5-Hydroxy-*N*-methyltryptamine	35.7	52.6	ND	ND
Bufotenine	76.9	2.80	ND	ND
5-Methoxytryptamine	3.22	67.1	91.7	39.6
5-Methoxy-*N,N*-dimethyltryptamine	11.1	7.09	ND	ND

*Each kinetic constant was determined graphically from Lineweaver–Burk plots using seven or eight substrate concentrations assayed in duplicate upon a single enzyme source prepared from the pooled livers of five to eight rats. For each assay (final volume, 3.0 ml), 0.024 to 0.237 mg of mitochondrial protein was used. Assays were carried out in air.

† The procedure of the pretreatment is given in the text.

‡ Not determined owing to its low activity.

type B MAO. 5-HT has been widely accepted as a neurotransmitter in the central nervous system. Tryptamine and 5-methoxytryptamine are known to exist normally in various tissues as trace amines (see a review in Ref. 18). *N,N*-Dimethyltryptamine, 5-hydroxy-*N*-methyltryptamine, bufotenine, and 5-methoxy-*N,N*-dimethyltryptamine have been identified in human urine, blood, or cerebrospinal fluid [19–23]. Although the specific MAO types involved in serotonergic or tryptaminergic neurons are not yet known, our present observations may serve to elucidate the *in vivo* metabolism of indoleamines in the future. In addition, our results will provide useful information for studying the topochemistry [24] of the active sites of type A and type B MAO.

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