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CHARACTERISTICS OF MONOAMINE OXIDASE IN MITOCHONDRIA ISOLATED FROM CHICK BRAIN, LIVER, KIDNEY AND HEART

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Abstract—The substrate- and inhibitor-related characteristics of monoamine oxidase (MAO) were studied with mitochondria of chick brain, liver, kidney and heart. The kinetic constants for MAO in these organs were determined, using 5-hydroxytryptamine (5-HT), tyramine and β-phenylethylamine (PEA) as substrates. For all the substrates, the V_{max} values were highest in kidney, followed in decreasing order by brain, liver and heart. For tyramine and PEA, the K_m values were lowest in liver, but for 5-HT it was lowest in heart. Inhibition experiments with clorgyline and deprenyl were carried out on mitochondria of the four organs with the three substrates at their K_m concentrations. From the plateaus observed of inhibition by clorgyline, it was concluded that 5-HT was oxidized by both types of MAO in mitochondria of all the organs; PEA was fairly specific for type B MAO in brain, liver and kidney, but non-specific in heart. In heart mitochondria, appreciable amounts of the activities toward tyramine and PEA were due to an amine oxidase distinct from mitochondrial MAO; 5-HT, however, was oxidized exclusively by mitochondrial MAO in this organ. The above atypical characteristics in substrate specificity found in chick tissues support the idea that the type A and type B concept cannot be applied uncritically to all tissues from all species.

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many animal tissues in two functional forms called type A and type B [1–3], based primarily on its sensitivity to clorgyline [1]. It is clear from recent observations (see reviews in Refs. 4 and 5) that substrate- and inhibitor-related characteristics of type A and type B MAO vary according to tissues and species. Many papers have been published on tissues of various species, such as rats [1–3, 6–10], mice [6, 11, 12], humans [6, 13–17], pigs [6, 18–20], cows [21–23] and rabbits [6, 24]. However, for chick tissues, comparable information is lacking, although a few isolated reports on chick brain [25] and heart [26] have appeared. Therefore, in the present paper, we have studied in much greater detail type A and type B MAO in chick brain, liver, kidney and heart, using 5-hydroxytryptamine (5-HT), tyramine and β-phenylethylamine (PEA) as substrates.

MATERIALS AND METHODS

Enzyme preparations. Young adult chicks of the White Leghorn breed, weighing about 1 kg, were decapitated, and the brains, livers, kidneys and hearts were removed rapidly. They were homogenized with 9 vol. of 0.25 M sucrose, being cooled in an ice bath, and centrifuged at 1500 *g* for 5 min to remove cellular debris. The resulting supernatant fraction was centrifuged at 18,000 *g* for 20 min and the crude mitochondrial pellet was suspended in the sucrose solution. The suspension was recentrifuged at 18,000 *g* for 20 min and the pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.4). These suspensions were used as enzyme sources.

Chemicals. Homovanillic acid and horseradish peroxidase (type II) were obtained from the Sigma Chemical Co., St. Louis, MO; 5-HT creatinine sulfate, tyramine–HCl, PEA–HCl, pargyline–HCl and semicarbazide–HCl from Nakarai Chemicals Ltd., Kyoto, Japan; 2′,7′-dichlorofluorescin diacetate from the Eastman Kodak Co., Rochester, NY; and hydrogen peroxide from Mitsubishi-Gasukagaku Ltd., Tokyo, Japan. Clorgyline, a selective inhibitor of type A MAO [1], was supplied by May & Baker Ltd., Dagenham, England. Deprenyl, a selective inhibitor of type B MAO [27] was donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

MAO assays. MAO activities toward 5-HT were determined by a slight modification of a new photometric assay [28]. In this method, hydrogen peroxide, formed in the MAO reaction, is measured photometrically by converting 2′,7′-dichlorofluorescin to 2′,7′-dichlorofluorescein in the presence of peroxidase. The assay mixture consisted of 0.5 M sodium phosphate buffer (pH 7.4), enzyme solution (0.019–0.237 mg protein), peroxidase solution (0.2 mg), 2′,7′-dichlorofluorescin solution (0.05 mg dissolved in 0.01 N NaOH), 5-HT solution, and water, 0.5 ml of each, to give a final volume of 3.0 ml. After incubation at 37° for 15 or 20 min, the enzyme reaction was terminated by adding 0.1 ml of a solution which contained 0.2 mg pargyline and 0.2 mg semicarbazide. The mixture was subjected to spec-

trophotometric analysis at 502 nm. Blank assays differed from controls only in the respect that the mixture was incubated in the presence of pargyline and semicarbazide. Standards were taken by adding 0.5 ml of hydrogen peroxide solution (4.4 nmoles) to the assay mixture in place of the enzyme solution. Care was taken not to convert more than 10 percent of the substrate to the reaction product. Under these conditions, the assays were linear during incubation at 37° for at least 20 min. The photometric measurements were made in a Shimadzu double beam spectrophotometer UV-200S.

MAO activities toward tyramine and PEA were measured fluorometrically by a slight modification of the method of Guilbault *et al.* [29] and Snyder and Hendley [30]. Details of the procedure were described in a previous paper [31]. For each assay (final volume, 3.0 ml), 0.012–0.268 mg of mitochondrial protein was used. The assays were carried out at 37° and pH 7.4 for 30 or 60 min. Under the conditions used, the assays were linear for at least 60 min of incubation.

Kinetic studies. Five levels of MAO activities with different substrate concentrations were measured in duplicate over the concentration range of 51.5–411 μM for 5-HT, 43.2–288 μM for tyramine and 39.7–317 μM for PEA. The K_m and V_{max} values were determined graphically from Lineweaver–Burk plots.

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 inhibition. Seven different concentrations for each inhibitor were employed over the range of 10^{-10}–10^{-4}M. It was confirmed that each inhibitor did not interfere with the photometry or the fluorometry when hydrogen peroxide was added directly. In most experiments, the inhibition was carried out at the substrate concentrations of their K_m values.

Protein determinations. Protein was determined by a slight modification [32] of the conventional biuret method, using bovine serum albumin as a standard.

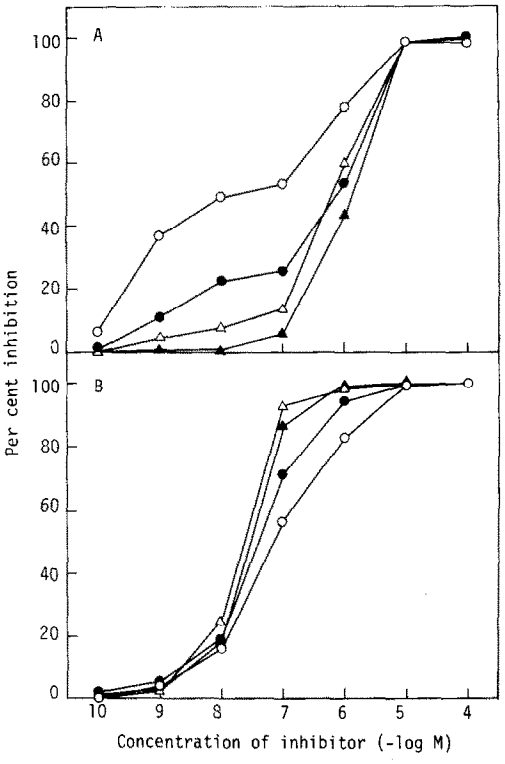

Fig. 1. Inhibition of MAO in chick brain mitochondria by clorgyline (A) and deprenyl (B), using 5-HT (○——○), tyramine (●——●) and PEA (△——△, ▲——▲) as substrates. The concentrations of 5-HT and tyramine were at their K_m values, viz. 345 and 250 μM, respectively. The concentrations of PEA were 64.9 μM (the K_m value, △——△) and 1300 μM (20-fold of the K_m value, ▲——▲). Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled brains of more than ten chicks.

RESULTS

Kinetic constants for MAO. The Michaelis–Menten kinetic constants for mitochondrial MAO in chick brain, liver, kidney and heart were determined from Lineweaver–Burk plots, using 5-HT, tyramine and PEA as substrates. The results are summarized in Table 1. For all the organs, the V_{max} values were highest with 5-HT and lowest with PEA. For all the substrates, the V_{max} values were highest in kidney, followed in decreasing order by brain, liver and heart. The K_m values were highest in brain for all

Table 1. Kinetic constants for MAO in mitochondria isolated from various organs of the chick*

	5-HT		Tyramine		PEA	
Organ	K_m (μM)	V_{max} (nmoles/mg protein/30 min)	K_m (μM)	V_{max} (nmoles/mg protein/30 min)	K_m (μM)	V_{max} (nmoles/mg protein/30 min)
Brain	345	238	250	147	64.9	81.3
Liver	263	182	119	69.9	30.7	29.6
Kidney	333	555	233	400	48.8	210
Heart	161	42.6	196	28.2	56.2	4.76

* Each kinetic constant was determined graphically from Lineweaver–Burk plots using five substrate concentrations assayed in duplicate upon a single enzyme source prepared from the pooled organs of more than ten chicks. For each assay (final volume, 3.0 ml), 0.023–0.237 mg of mitochondrial protein was used. The assay methods are described in the text.

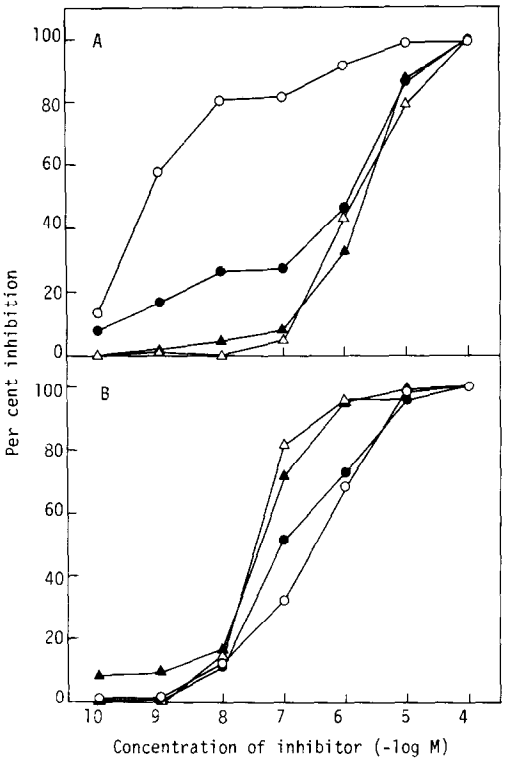

Fig. 2. Inhibition of MAO in chick liver mitochondria by clorgyline (A) and deprenyl (B), using 5-HT (○——○), tyramine (●——●) and PEA (△——△, ▲——▲) as substrates. The concentrations of 5-HT and tyramine were at their K_m values, viz. 263 and 119 μM, respectively. The concentrations of PEA were 30.7 μM (the K_m value, △——△) and 614 μM (20-fold of the K_m value, ▲——▲). Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled livers of more than ten chicks.

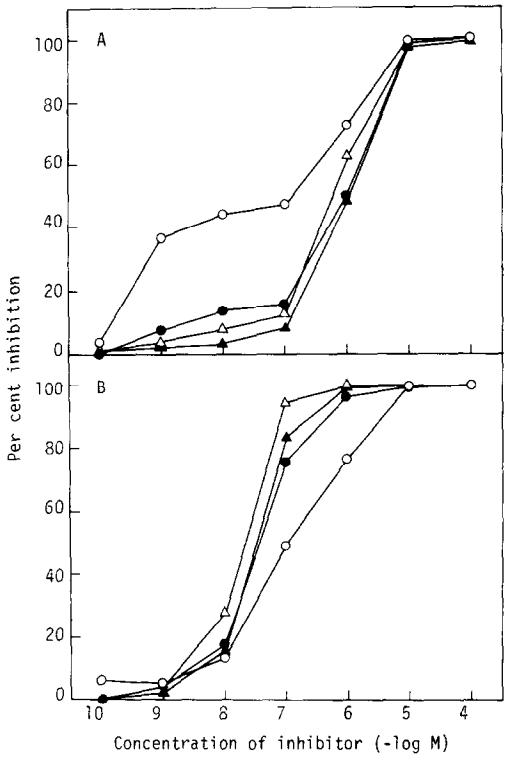

Fig. 3. Inhibition of MAO in chick kidney mitochondria by clorgyline (A) and deprenyl (B), using 5-HT (○——○), tyramine (●——●) and PEA (△——△, ▲——▲) as substrates. The concentrations of 5-HT and tyramine were at their K_m values, viz. 333 and 233 μM, respectively. The concentrations of PEA were 48.8μM (the K_m value, △——△) and 976 μM (20-fold of the K_m value, ▲——▲). Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled kidneys of more than ten chicks.

the substrates. For tyramine and PEA, the K_m values were lowest in liver, but for 5-HT it was lowest in heart.

Inhibition of brain MAO. Figure 1 shows the inhibition of MAO in chick brain mitochondria by clorgyline and deprenyl, using the three substrates. As shown in Fig. 1A, the oxidation of 5-HT was much more susceptible than that of PEA to clorgyline, and the deamination of tyramine was intermediate in sensitivity. There were clear plateaus at 10^{-8}–10^{-7} M of the inhibitor for 5-HT and tyramine. With PEA at its K_m value, a small increase in MAO inhibition was observed at 10^{-9}–10^{-7} M of clorgyline, but this was not true at a PEA concentration 20-fold higher than its K_m value. From the pattern using tyramine as substrate, type A MAO in chick brain is estimated to be 20–30 percent of the total MAO, while type B enzyme is 70–80 percent.

The differentiation observed in the inhibition of chick brain mitochondrial MAO by deprenyl was not as marked as that by clorgyline (Fig. 1B); neither plateaus nor shoulders were observed for all the substrates. However, the substrate susceptibility was in the order consistent with the type A and type B classification.

Inhibition of liver MAO. Figure 2 shows inhibition of MAO in chick liver mitochondria by clorgyline and deprenyl. As shown in Fig. 2A, the susceptibilities of the three substrates were also in the order consistent with the type A and type B concept. Clear plateaus also appeared in the curves with 5-HT and tyramine. For PEA, however, the curves were almost single sigmoidal. From the pattern with tyramine, type A MAO in the liver is estimated to be 20–30 percent and type B MAO 70–80 percent.

The inhibition by deprenyl is illustrated in Fig. 2B. There were no plateaus in these curves.

Inhibition of kidney MAO. Figure 3 shows the inhibition of MAO in kidney mitochondria by clorgyline and deprenyl. All the inhibition patterns were generally similar to those of the brain enzyme; clear plateaus appeared in the inhibition by clorgyline with 5-HT and tyramine, and the curves with PEA were almost single sigmoidal. From the pattern with tyramine, it is estimated that 10–20 percent of the activity was due to type A MAO and 80–90 percent due to type B MAO.

Inhibition of heart MAO. Figure 4 shows the inhibition of MAO in heart mitochondria. The inhibition patterns were quite different from those

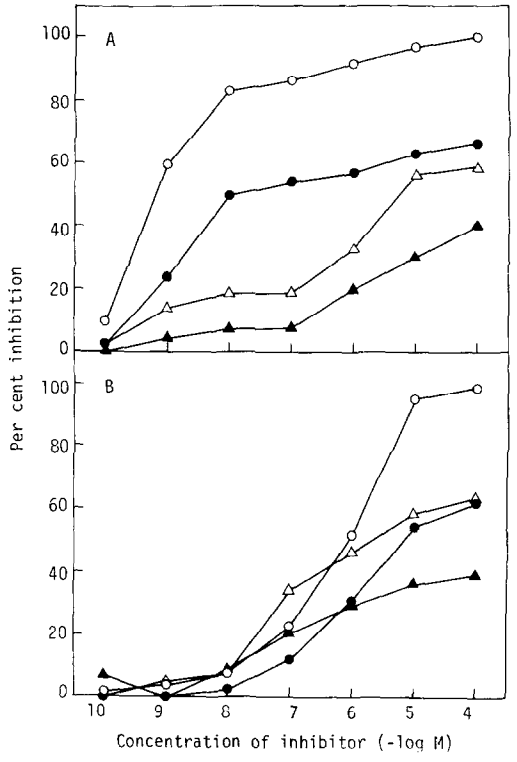

Fig. 4. Inhibition of MAO in chick heart mitochondria by clorgyline (A) and deprenyl (B), using 5-HT (○——○), tyramine (●——●) and PEA (△——△, ▲——▲) as substrates. The concentrations of 5-HT and tyramine were at their K_m values, viz. 161 and 196 μM, respectively. The concentrations of PEA were 56.2 μM (the K_m value, △——△) and 1120 μM (20-fold of the K_m value, ▲——▲). Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled hearts of more than ten chicks.

of the three other enzyme sources. As can be seen in panel A of Fig. 4, the deamination of 5-HT was highly susceptible to clorgyline. The susceptibility of tyramine oxidation to clorgyline was intermediate and that of PEA oxidation was lowest. In the patterns with tyramine and PEA as substrates, the activities, which were not inhibited by 10^{-4}M clorgyline, were detected. Therefore, we tested if semicarbazide (final concentration, 0.60 mM) could inhibit such activities. It was observed that they were completely inhibited by 0.60 mM semicarbazide. In the curves with 5-HT and tyramine, it appeared that there were plateaus at 10^{-8}–10^{-7}M clorgyline. In the curves with PEA, plateaus were much clearer for both concentrations. The deamination of PEA at the K_m concentration was more sensitive to clorgyline than that at the concentration 20-fold higher than its K_m value. From the curve with tyramine, 75–85 percent of the activity sensitive to clorgyline is estimated to be due to type A MAO, while 15–25 percent is due to type B MAO.

The inhibition of heart MAO by deprenyl is also illustrated in Fig. 4B. There were no plateaus for any of the substrates. The 10^{-4} M deprenyl-resistant MAO activities were also observed for tyramine and PEA. The PEA deamination at its K_m value was more sensitive to deprenyl than that at the higher PEA concentration.

DISCUSSION

In this paper, we present the substrate- and inhibitor-related characteristics of MAO in chick brain, liver, kidney and heart.

Since we reported that inhibition patterns varied according to substrate concentration, especially when PEA [33] and phenylethanolamine [31] were used as substrates, the inhibition experiments with all the substrates were carried out at their K_m values. In addition, the concentrations 20-fold higher than the K_m values were also tested for PEA. However, the dramatic changes in inhibition pattern observed in rat mitochondrial MAO could not be demonstrated with any of the chick organs tested.

In the inhibition experiments, some atypical characteristics in substrate specificity were observed. In the inhibition curves by clorgyline with 5-HT as substrate, plateaus were demonstrated with all the tissues, showing that 5-HT is oxidized by both types of MAO in chick tissues. In this connection, it should be recalled that, in beef heart mitochondria [21], in pig brain and liver mitochondria [19] and in chick retina [32], 5-HT is oxidized by either type of MAO.

In regard to PEA, atypical charactistics were also observed: in the inhibition by clorgyline, clear plateaus were observed in the heart mitochondria (Fig. 4A), showing that PEA is oxidized by both types of MAO in this tissue. In other organs, such as the brain (Fig. 1) and kidney (Fig. 3), a small increase in the inhibition could be detected at 10^{-10}–10^{-7} M clorgyline, when a PEA concentration at its K_m value was used. This result agrees well with our previous preliminary report on chick brain [25]. However, in the curves at a PEA concentration 20-fold higher than its K_m value, such a small increase in the inhibition was less marked (Figs. 1A and 3A). Therefore, PEA seems to be fairly specific for type B MAO in chick brain, liver and kidney, while it is non-specific in chick heart. These phenomena may be partly explained by the fact that the ratio of type A to type B in the heart enzyme was much higher than those of other organs, if we take into account that a small portion of MAO activity toward PEA may be catalyzed by type A MAO [8].

Inhibition experiments by deprenyl, a selective inhibitor of type B MAO, were also carried out. The differentiation observed in MAO inhibition by deprenyl was not as marked as that by clorgyline; neither plateaus nor shoulders were observed for all the substrates and tissues. This is also the case for MAO in rabbit tissues [6]. However, the substrate susceptibility was in the order consistent with the type A and type B classification for all the tissues. It seems likely that the percent inhibition of MAO by 10^{-7}M deprenyl reflects the percentage of type B MAO in chick tissues, since an approximate reverse relationship in MAO inhibition between the two inhibitors was obtained at a concentration of 10^{-7}M.

With heart mitochondria, activity, which was

resistant to 10^{-4} M clorgyline or deprenyl and sensitive to 0.60 mM semicarbazide, was demonstrated using tyramine and PEA as substrates, but not using 5-HT (Fig. 4). Fowler and Callingham [26] also reported activity with benzylamine and tyramine in chick heart, which was resistant to 10^{-3} M clorgyline and sensitive to 10^{-3} M semicarbazide, but they did not use PEA as a substrate. Such activity is probably due to an amine oxidase distinct from mitochondrial MAO, which can be classified as EC 1.4.3.6. It should be pointed out that this enzyme is not soluble, since it was prepared after centrifugation twice at 18,000 *g*. It has been reported very recently that benzylamine-oxidizing activity distinct from type B MAO is distributed widely in the organs of humans and rats [34].

In the present paper, we demonstrated some atypical characteristics in substrate specificity of MAO from chick brain, liver, kidney and heart. These results support the idea that the type A and type B classification cannot be applied uncritically to all tissues from all species [4].

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