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CHARACTERIZATION OF *N*-METHYLPHENYLETHYLAMINE AND *N*-METHYLPHENYLETHANOLAMINE AS SUBSTRATES FOR TYPE A AND TYPE B MONOAMINE OXIDASE

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Abstract—*N*-Methylphenylethylamine (MPEA) and *N*-methylphenylethanolamine (MPEOA) were characterized as substrates for type A and type B monoamine oxidase (MAO) in rat brain mitochondria. The inhibition experiments with clorgyline and deprenyl showed that the inhibition patterns with MPEA as substrate were dependent on substrate concentrations but that this amine was a common substrate for both types of MAO at all substrate concentrations tested. When MPEOA was used as substrate, the inhibition patterns differed markedly at different substrate concentrations; at 10.0 μM, MPEOA acted as a specific substrate for type B MAO, but at 100 and 1000 μM it became a common substrate for both types. Kinetic analyses were carried out for MPEA and MPEOA with the uninhibited, the clorgyline-treated (type B MAO), and the deprenyl-treated enzyme (type A MAO). With the uninhibited enzyme, there were downward deflections in the curves of Lineweaver–Burk plots for both MPEA and MPEOA, suggesting the existence of different affinity components derived from type A and type B MAO. By means of the double-reciprocal plots, using the clorgyline- and the deprenyl-treated enzyme, it was confirmed that the high affinity corresponded to that for type B MAO and the low affinity to that for type A MAO for both MPEA and MPEOA. Therefore, the changes in the inhibition pattern at different substrate concentrations may be due to different affinities of the substrate for both types. By comparing the K_m and V_{max} values of both types observed for MPEA and MPEOA, it was pointed out that the β-hydroxylation of MPEA tended to increase the K_m value for type A MAO and to decrease the V_{max} values for both types.

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) can be classified as type A and type B [1–3]. Various biogenic monoamines have been characterized as substrates for both types of MAO (see a review in Ref. 4). In our previous papers [5–7], we reported that the substrate specificity of β-phenylethylamine (PEA) and phenylethanolamine (PEOA) for type A and type B was highly dependent on their substrate concentrations. In the present study, we report that this is also the case for *N*-methylphenylethylamine (MPEA) and *N*-methylphenylethanolamine (MPEOA).

MATERIALS AND METHODS

Enzyme. A crude mitochondrial fraction was isolated from whole brains of male Sprague–Dawley rats, weighing about 200 g, as described previously [6].

Chemicals. MPEA and MPEOA were obtained as gifts from Dr. F. Karoum, Laboratory of Clinical Psychopharmacology, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC. Horseradish peroxidase (type II), homovanillic acid and pargyline–HCl were obtained from the Sigma Chemical Co., St. Louis, MO, 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 determined fluorometrically by the method of Guilbault *et al.* [8] and Snyder and Hendley [9]. For each assay (final volume, 3.0 or 0.6 ml), 0.177–0.352 mg of mitochondrial protein was used. The assays were carried out at 37° and pH 7.4 for 30–90 min. Under the conditions used, the assays were linear during incubation for at least 90 min.

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 the enzyme inhibition. Seven different concentrations of each inhibitor over the range of 10^{-10} to 10^{-4} M were employed. It was confirmed that each inhibitor did not interfere with the fluorometry when hydrogen peroxide was added directly.

Kinetic studies. MAO activities were measured over the range of substrate concentrations of 7.5 to

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1000 μM for MPEA and 10 to 2000 μM for MPEOA. The K_m and V_{max} values were determined graphically from Lineweaver–Burk plots.

For kinetic studies on MAO pretreated with selective inhibitors, the mitochondria 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 MAO assays as described above.

Protein determinations. Protein was determined by a slight modification of the conventional biuret method, using bovine serum albumin as a standard [10].

RESULT

Inhibition of MAO activity toward MPEA. Figure 1 shows MAO inhibition by clorgyline, a selective inhibitor of type A MAO [1], and by deprenyl, a selective inhibitor of type B MAO [11], using various concentrations of MPEA as substrate. In the curves with clorgyline (Fig. 1A), clear plateaus appeared at 10^{-8} to 10^{-7} M for all the substrate concentrations, showing that MPEA is a common substrate for both types over a wide substrate concentration range. The sensitivity to clorgyline with 10.0 μM MPEA was much less than with 1000 μM MPEA; with 100 μM MPEA it was intermediate. The curves with deprenyl (Fig. 1B) did not exhibit plateaus, despite the presence of both types of MAO in rat brain mitochondria. The susceptibility to deprenyl, however, decreased according to the increase in substrate concentration, confirming the results with clorgyline.

Inhibition of MAO activity toward MPEOA. Figure 2 illustrates the inhibition by both inhibitors, using various concentrations of MPEOA as substrate. The inhibition patterns with this substrate differed markedly at different substrate concentrations. At 10.0 μM, the inhibition curves with both inhibitors were almost single sigmoidal; clorgyline, when present in the incubation medium at a concentration of 10^{-7} M, hardly affected the MPEOA deamination, but deprenyl almost completely inhibited its deamination at the same concentration. These data show that MPEOA, at a 10.0 μM concentration, was specific for type B MAO. At 100 μM, however, the pattern was changed dramatically; a clear plateau appeared at 10^{-8}–10^{-7} M clorgyline, showing that at this concentration the amine was a common substrate for both types of MAO. When the concentration was increased up to 1000 μM, the susceptibility to clorgyline was increased even more, showing about 75 per cent inhibition with 10^{-8} to 10^{-7} M clorgyline, indicating that a major part of the activity was due to type A MAO. In the curve with deprenyl, the susceptibility decreased according to the increase in substrate concentration, confirming the results with clorgyline. No plateaus, however, were observed in these curves.

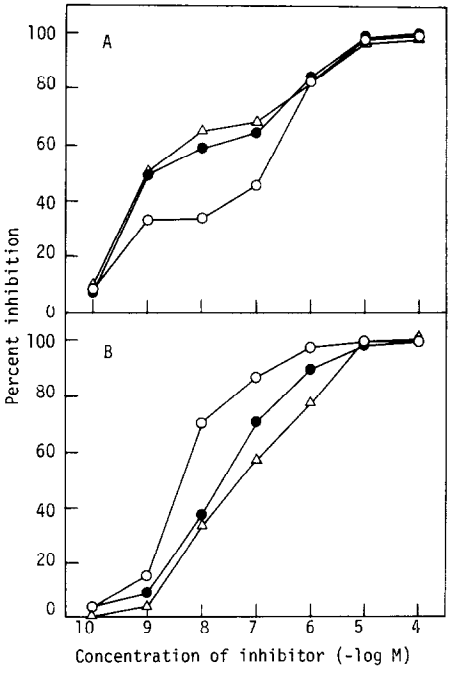

Fig. 1. Inhibition of MAO in rat brain mitochondria by clorgyline (A) and deprenyl (B) using various concentrations of MPEA as substrate. The concentrations of the substrate were 10.0 (○——○), 100 (●——●) and 1000 μM (△——△), with specific activities of 10.5, 26.9 and 32.0 nmoles·(mg protein)$^{-1}$·30 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled brains of six rats.

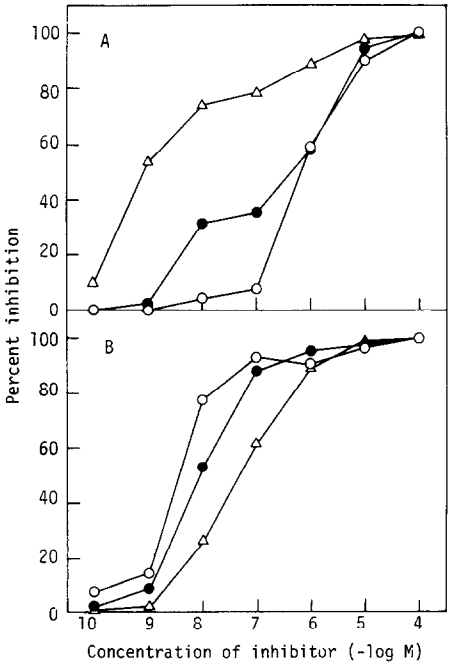

Fig. 2. Inhibition of MAO in rat brain mitochondria by clorgyline (A) and deprenyl (B) using various concentrations of MPEOA as substrate. The concentrations of the substrate were 10.0 (○——○), 100 (●——●) and 1000 μM (△——△) with specific activities of 2.48, 9.60 and 9.88 nmoles·(mg protein)$^{-1}$·30 min^{-1}, respectively, in the absence of inhibitors. Each point is the mean obtained from duplicate determinations upon a single enzyme source prepared from the pooled brains of six rats.

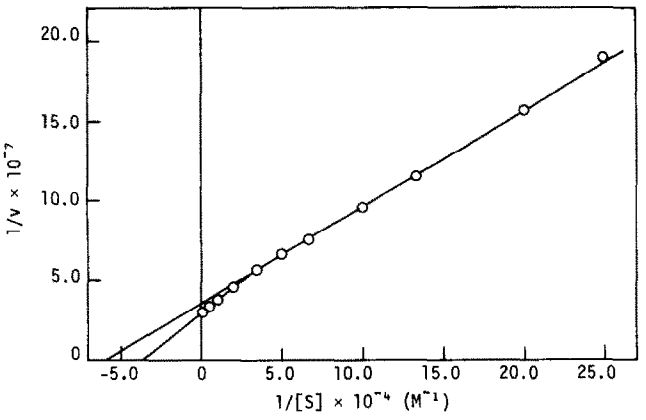

Fig. 3. Lineweaver–Burk plots for uninhibited MAO in rat brain mitochondria with MPEA as substrate. Each point is the mean obtained from duplicate determinations; *v*: moles H_2O_2 formed·(mg protein)$^{-1}$·30 min^{-1}.

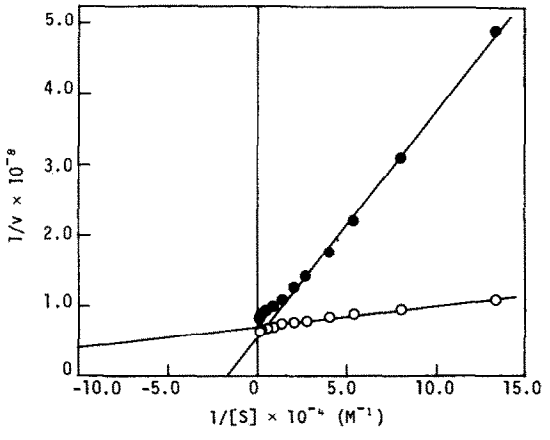

Fig. 4. Lineweaver–Burk plots for MAO in rat brain mitochondria pretreated with 10^{-7} M clorgyline (○——○) or 10^{-7} M deprenyl (●——●) using MPEA as substrate. Each point is the mean obtained from duplicate determinations; *v*: moles H_2O_2 formed·(mg protein)$^{-1}$·30 min^{-1}.

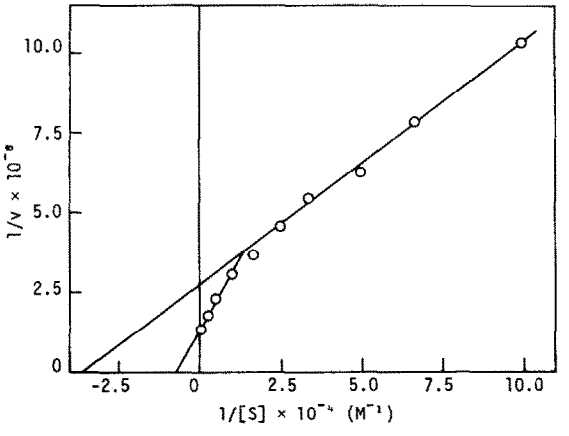

Fig. 5. Lineweaver–Burk plots for uninhibited MAO in rat brain mitochondria with MPEOA as substrate. Each point is the mean obtained from duplicate determinations; *v*: moles H_2O_2 formed·(mg protein)$^{-1}$·30 min^{-1}.

Table 1. Apparent kinetic constants for MAO activities toward MPEA and MPEOA in rat brain mitochondria

Substrate	K_m (μM)	V_{max} [nmoles·(mg protein)$^{-1}$·30 min^{-1}]
MPEA		
High affinity	16.9	27.7
Low affinity	27.7	34.4
Clorgyline-treated*	4.13	14.2
Deprenyl-treated*	58.8	18.5
MPEOA		
High affinity	27.7	3.67
Low affinity	143	7.87
Clorgyline-treated*	13.3	3.24
Deprenyl-treated*	476	4.54

* The procedure of the treatment is given in the text.

Kinetic studies with MPEA. MPEA was further characterized as a substrate for MAO with the uninhibited enzyme by determining the Michaelis–Menten kinetic constants from Lineweaver–Burk plots. As can be seen from Fig. 3, the plots with MPEA revealed a slight downward deflection at the highest substrate concentrations, suggesting another affinity component. The K_m and V_{max} values for the low affinity component calculated from the five plots of the highest concentrations and those for the high affinity component are listed in Table 1.

Since the uninhibited enzyme is a mixture of type A and type B MAO, rat brain mitochondrial MAO was pretreated with 10^{-7} M clorgyline or 10^{-7} M deprenyl to obtain the specific types as described before. At this concentration, clorgyline inhibited type A MAO and deprenyl inhibited type B MAO fairly specifically. As illustrated in Fig. 4, the curve with the deprenyl-treated enzyme (type A MAO) was much steeper than that with the clorgyline-treated enzyme (type B MAO). The respective K_m and V_{max} values are presented in Table 1. The K_m value of the deprenyl-treated enzyme (type A MAO) was higher than that of low affinity component of the uninhibited enzyme, and the value of the clorgyline-treated enzyme (type B MAO) was lower than that of the high affinity component.

Kinetic studies with MPEOA. Figure 5 shows the double-reciprocal plots for MPEOA with the uninhibited MAO. These plots revealed a notable downward deflection at the highest concentrations. The K_m and V_{max} values for the low affinity component calculated from the four plots of the highest concentrations and those for the high affinity component are shown in Table 1.

Figure 6 illustrates the plots for MPEOA with clorgyline-treated (type B MAO) and deprenyl-treated enzyme (type A MAO). As shown in the figure, the downward deflection observed for the uninhibited enzyme completely disappeared after treatment with clorgyline; the high affinity component also disappeared after treatment with deprenyl. The respective K_m and V_{max} values with MPEOA as substrate are presented in Table 1. The K_m value of the deprenyl-treated MAO (type A MAO) was much higher than that of the low affinity component of the uninhibited enzyme; the value of the clorgyline-treated enzyme (type B MAO) was lower than that of the high affinity component.

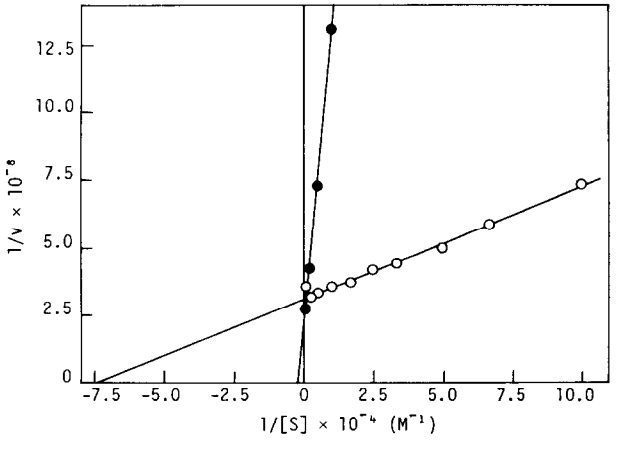

Fig. 6. Lineweaver–Burk plots for MAO in rat brain mitochondria pretreated with 10^{-7} M clorgyline (○——○) or 10^{-7} M deprenyl (●——●) using MPEOA as substrate. Each point is the mean obtained from duplicate determinations; v: moles H_2O_2 formed·(mg protein)$^{-1}$·30 min^{-1}.

DISCUSSION

In the present paper, we have characterized MPEA and MPEOA as substrates for type A and type B MAO. These amines have never been studied as MAO substrates. It was found that MAO inhibition patterns with MPEA and MPEOA differed at different substrate concentrations (Figs. 1 and 2). To clarify the mechanisms of such changes, we performed kinetic analyses using the uninhibited, the clorgyline-treated, and the deprenyl-treated enzyme. Lineweaver–Burk plots with both MPEA and MPEOA, using the uninhibited MAO, revealed downward deflections (Figs. 3 and 5), suggesting two different affinity components of MAO for each substrate. The plots of MAO pretreated with selective inhibitors showed that the low affinity component disappeared after the clorgyline treatment and the high affinity component after the deprenyl treatment (Figs. 4 and 6). These results indicate that the low and the high affinity components correspond to that of type A MAO and type B MAO, respectively. Therefore, it is clear that the changes in the inhibition pattern observed for MPEA and MPEOA are due to different affinities of each substrate for type A and type B MAO.

We reported previously that the inhibition patterns with PEA, the precursor of MPEA, differed markedly according to the difference in substrate concentration [5, 7], and concluded that such changes are predominantly due to a strong substrate inhibition of type B MAO; at low concentrations PEA acts as a specific substrate for type B MAO, but at high concentrations the proportion of type A MAO activity metabolizing PEA increases as a result of the suppression of type B MAO activity by its substrate inhibition. These results on PEA should be compared with those on MPEA in the present study. The changes in the inhibition pattern due to different concentrations of MPEA (Fig. 1), are not as great as those when PEA is used [5, 7]. This can probably be explained by the fact that, with MPEA as substrate, the substrate inhibition of type B MAO was absent (Figs. 3 and 4) and the K_m value of type A MAO for MPEA was only ten times higher than that of type B MAO (Table 1). Another difference to be pointed out in inhibition between MPEA and PEA is that, at 10.0 μM, PEA is a specific substrate for type B MAO [5, 7], whereas MPEA is common for both types at the same concentration (Fig. 1). This difference may be due to the K_m value (58.8 μM) of type A MAO for MPEA (Table 1) being lower than that for PEA (118–143 μM) [7].

The drastic changes in inhibition pattern observed according to the concentrations of MPEOA (Fig. 2) are very similar to those with PEOA [6], the precursor of MPEOA. Such changes can be attributed to a remarkable difference in the K_m value for each substrate between type A and type B MAO; the

values of type A and type B MAO for MPEOA were 476 and 13.3 μM, respectively (Table 1), and those for PEOA were 1490 and 11.4 μM [6].

The relationship betwen monoamine structure and substrate specificity for type A and type B MAO was investigated by comparing the data (Table 1) on MPEA with those on MPEOA. The following effects of the β-hydroxylation of MPEA were noted. First, the β-hydroxylation resulted in the elevation of the K_m value of type A MAO. This is also true when the K_m values for PEA [7] and PEOA [6] are compared with each other. Second, the β-hydroxylation of MPEA caused the decrease in the V_{max} values of both types (Table 1). From our previous data on PEA [7] and PEOA [6], it also can be pointed out that the V_{max} of type B MAO is decreased by the β-hydroxylation of PEA. These effects on both types of MAO make it difficult to conclude whether the β-hydroxylation enhances the preference to one type of MAO.

Since, in our previous papers, we carefully characterized PEA [7] and PEOA [6] as substrates for both types of MAO, using the same method and the same enzyme source, it is also possible to see how the *N*-methylation of a monoamine affects its substrate specificity. The *N*-methylation of both PEA and PEOA tended to decrease the K_m values of type A MAO, and to decrease the V_{max} values of type B MAO (Table 1 and Refs. 6 and 7), suggesting an increase in substrate specificity for type A MAO. These effects of *N*-methylation do not seem remarkable, however, because the inhibition patterns by clorgyline and deprenyl with MPEOA (Fig. 2) were very similar to those with PEOA [6]. In addition, the patterns with synephrine [12], an *N*-methylated compound from octopamine, are very similar to those with octopamine [6].

MPEA was recently identified in human urine [13]. It is well known that MPEOA is also formed from PEOA by phenylethanolamine *N*-methyltransferase [14]. These amines, therefore, may be actively metabolized by MAO *in vivo* in mammalian tissues, and the ratio of deamination of MPEOA by the specific types of MAO may be dependent on its *in situ* concentration in the tissues.

The present study showed that the drastic change in the inhibition pattern observable according to substrate concentration is not limited to PEA [5, 7] and PEOA [6], but also can be found with MPEOA (Fig. 2). Since a single substrate concentration arbitrarily chosen is used in most MAO studies [15–20], it should be stressed again that, in MAO inhibition studies, it is necessary to check the effects of substrate concentrations over a wide range for each substrate and for each enzyme preparation, while suspecting the different affinities of the substrate for type A and type B MAO.

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