STUDIES ON p-PHENYLETHYLAMINE DEAMINATION BY HUMAN PLACENTAL MONOAMINE OXIDASE

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Abstract—Kinetic properties of human placental monoamine oxidase (MAO) were investigated in studies on inhibitors and mixed substrates. MAO activity was determined by a radioisotopic assay. Lineweaver-Burk plots were linear at higher and lower concentrations of PEA, whereas at intermediate substrate concentrations, a downward curving plot was obtained. The Km values of the low- and high-affinity sites for PEA deamination were estimated. Studies with mixed substrates showed that 5-HT was a competitive inhibitor and tyramine a mixed-type inhibitor of deamination at high concentrations of PEA, whereas both were non-competitive inhibitors at lower concentrations of PEA. After pre-incubation of human placental mitochondrial preparations with deprenyl, Lineweaver-Burk plots were completely linear, and the Km value was the same as that obtained at low concentrations of PEA in the absence of deprenyl. Tyramine and 5-HT were competitive inhibitors of PEA deamination by deprenyl-treated MAO. From these results it is concluded that there are two kinds of MAO with high- and low-affinity sites for PEA in mitochondria of human placenta, corresponding to type B and A MAO, and that tyramine, 5-HT and PEA share a substrate-binding site on type A MAO, while tyramine and 5-HT bind to a site on type B MAO that is different from the PEA binding site.

There is much evidence that monoamine oxidase (MAO, monoamine: O2 oxidoreductase [deaminating] EC 1.4.3.4.) exists in more than one form (1–5). Using clorgyline, which is a selective and irreversible inhibitor, Johnston (6) demonstrated the existence of two different types of MAO, type A and B. Type A MAO is sensitive to a low concentration of clorgyline, while type B MAO is sensitive to a low concentration of deprenyl (7). In most tissues serotonin (5-HT) and norepinephrine are metabolized by type A MAO, benzylamine and p-phenylethylamine (PEA) by type B MAO, and tyramine by both types of MAO (6, 8–10). Recent reports (11, 12) have suggested that MAO in human placenta differs from that in other organs in substrate specificity and sensitivity to inhibitors, but that it closely resembles type A MAO.

Our previous studies on MAO of human placenta using mixed substrates (13) indicated that PEA is metabolized by two different catalytic sites. In the present study, using inhibitors and mixed substrates experiments, we examined kinetic properties of PEA deamination by human placental MAO.
MATERIALS AND METHODS

1. Preparations of human placental MAO: Human placenta was washed with saline, separated from coagulated blood and homogenized in a Waring blender with 3 volumes of ice cold 0.25 M sucrose buffered at pH 8.0 with 0.01 M Tris-HCl buffer. The homogenate was centrifuged at 600×g for 10 min at 4°C, and the supernatant was collected. The residue was suspended in sucrose-Tris buffer and recentrifuged as before. The residue was then discarded, and the supernatants were combined and centrifuged at 8,500×g for 20 min at 4°C. The resulting supernatant was discarded, and the precipitate was suspended in sucrose-Tris buffer and recentrifuged. The precipitate (mitochondrial fraction) was suspended in 2 vol. of 0.1 M Tris-HCl buffer (pH 8.0) at a final protein concentration of 6.1 mg/ml, and used as the MAO preparation.

2. Assay of MAO activity: The method for MAO assay was based on that of Wurtman and Axelrod (14). The incubation medium contained radioactive substrates [14C-PEA (1 μCi/ml), 14C-5-HT (2.5 μCi/ml) and 14C-tyramine (1 μCi/ml)] and unlabelled substrates in a total volume of 250 μl of Tris-HCl buffer (0.01 M, pH 8.0). The reaction at 38°C was started by adding 50 μl of enzyme preparation and was stopped after 20 min by adding 0.2 ml of 2N HCl. The medium was then shaken with 6 ml of ether for 15 sec when tyramine and 5-HT were used as substrates, or with 6 ml of toluene for 20 min when PEA was used as a substrate. Four ml of the organic layer was mixed with 6 ml of Aquasol as scintillation liquid and its radioactivity was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer. Activity is expressed in disintegrations per min (DPM).

3. Inhibition studies using deprenyl: For these studies, a mixture of 1 μM deprenyl and enzyme solution was incubated for 30 min at 38°C in a total volume of 5 ml. Then the mixture was rapidly cooled, dialyzed against 1 mM Tris-HCl buffer (pH 8.0) overnight in a cold room and used as deprenyl-treated human placental MAO. A control, not containing deprenyl, was dialyzed in the same way. Remaining enzyme activity, type A MAO activity, was measured as described above.

RESULTS

1. Determination of Km values: The Km values for PEA were calculated from Lineweaver-Burk plots. Figure 1 (left) shows plots for PEA at lower concentrations (5–100 μM), and Fig. 1 (right) those for PEA at higher concentrations (50–400 μM). The plots with PEA as substrate were linear at higher (12.5–400 μM) and lower (5–8 μM) concentrations, with a downward curving region at intermediate substrate concentrations. The Km values of the low- and high-affinity sites for PEA deamination were estimated from the corresponding linear portions of the plots as 13.3 and 44.4 μM respectively. The Km values for tyramine and 5-HT were also calculated in the same way. In contrast to PEA (Fig. 1, left), a single Km value was obtained with tyramine or 5-HT as substrate (Table 1).

2. Mixed substrate experiments: The deaminations of PEA, tyramine and 5-HT by human placental MAO were studied in the presence and absence of different concentrations of other substrates. Initial reaction velocities were measured at various concen-

| Table 1. Km values of mitochondrial MAO from human placenta |
|-----------------|----------------|
| Substrate       | Km (μM)       |
| Tyramine        | 142           |
| 5-HT            | 111           |
| Benzylamine     | 270           |
| PEA             | 44            |
Fig. 1. Reciprocal plots of initial reaction velocities of human placental MAO at different substrate concentrations, using PEA at lower concentrations (5–100 μM, left) and higher concentrations (50–400 μM, right). Abscissa shows 1/substrate concentration in mM and ordinate shows 1/DPM×10⁻⁴. Points are means for duplicate determinations.

Fig. 2. Effects of unlabelled tyramine upon Lineweaver-Burk plots for ¹⁴C-PEA deamination by human placental MAO. Initial reaction velocities were determined using ¹⁴C-PEA at lower concentrations (5–50 μM, left) and higher concentrations (50–400 μM, right) in the presence and absence of different concentrations of tyramine. Tyramine concentrations: O, none; Δ, 100 μM; ▲, 200 μM. Others are as in Fig. 1.

Figure 2 shows the effect of unlabelled tyramine (100 and 200 μM) on PEA deamination. The plots on the left are for lower concentrations of PEA (5–50 μM), and those concentrations of radioactive-substrates, and the effects of the presence of various unlabelled substrates on the deamination were analyzed from double reciprocal plots.
on the right for higher concentrations of PEA (50–400 μM). The data in Fig. 1 suggest that PEA is deaminated by a low- and a high-affinity catalytic site in human placental MAO. At higher concentrations of PEA (66.6–400 μM), tyramine appeared to be a mixed type inhibitor of PEA deamination. However, at lower concentrations of PEA (5–12.5 μM), plots with and without unlabelled tyramine appear to intersect at the same point on the abscissa, indicating that tyramine is a noncompetitive inhibitor of PEA deamination.

Figure 3 shows the effects of unlabelled 5-HT (100 and 200 μM) on PEA deamination. The plots on the left are for lower concentrations of PEA (5–50 μM) and those on the right for higher concentrations of PEA (50–400 μM). As with tyramine, at higher concentrations of PEA (66.6–400 μM), 5-HT appears to be a competitive inhibitor of PEA deamination, whereas at lower concentrations of PEA (5–12.5 μM), the plots with and without unlabelled 5-HT appear to intersect at the same point on the abscissa, indicating that 5-HT is a noncompetitive inhibitor of PEA deamination.

The plots on the left of Fig. 4 show the effects of higher concentrations of unlabelled PEA (500 μM) on tyramine deamination. The plots are linear and those with and without unlabelled PEA intersect at the same point on the ordinate, indicating that tyramine deamination is competitively inhibited by unlabelled PEA. As shown on the right of Fig. 4, the same appears to be true for the effect of PEA on 5-HT deamination.

3. Reciprocal plots in the presence of deprenyl: Human placental mitochondrial MAO samples were preincubated with 1 μM deprenyl for 30 min at 38°C and then dialyzed overnight, as described in the Materials and Methods. A control, not containing deprenyl, was prepared in the same way.

Reaction velocities were measured at various PEA concentrations in the presence and absence of 1 μM deprenyl, and results are shown in Fig. 5. The reciprocal plots using PEA at lower concentrations (5–50 μM) are shown on the left, and those with PEA at

![Fig. 3. Effects of unlabelled 5-HT Lineweaver-Burk plots for 14C-PEA deamination by human placental MAO. Initial reaction velocities were determined using 14C-PEA at lower concentrations (5–50 μM, left) and higher concentrations (50–400 μM, right) in the presence and absence of different concentrations of 5-HT. 5-HT concentrations: ○, none; □, 100 μM; ■, 200 μM. Others are as in Fig. 1.](image-url)
Fig. 4. Left; Effects of unlabelled PEA Lineweaver-Burk plots for ^14^C-tyramine deamination by human placental MAO. Initial reaction velocities were determined using ^14^C-tyramine (33-200 µM) in the presence and absence of PEA. •, absence of PEA; ○, presence of 500 µM PEA. Others are as in Fig. 1. Right; Effects of unlabelled PEA upon Lineweaver-Burk plots for ^14^C-5-HT deamination by human placental MAO. Initial reaction velocities were determined using ^14^C-5-HT (33-200 µM) in the presence and absence of PEA. •, absence of PEA; ○, presence of 1 mM PEA. Others are as in Fig. 1.

Fig. 5. Reciprocal plots of initial reaction velocities at different substrate concentrations for deprenyl-treated and untreated human placental MAO, using PEA at lower concentrations (5-50 µM, left) and higher concentrations (50-400 µM, right). ○, untreated human placental MAO; •, 1 µM deprenyl-treated MAO. Others are as in Fig. 1.

higher concentrations (50-400 µM) are shown on the right. Plots of the control were similar to those in Fig. 1. However, the plots with deprenyl were completely linear,
and they intersected the abscissa at the same point as the plots obtained for the low-affinity component in the absence of deprenyl.

Figure 6 shows the effects of unlabelled tyramine and 5-HT (100 and 200 μM) on PEA deamination by deprenyl-pretreated human placental MAO. Plots for the deprenyl-pretreated enzyme with and without tyramine and 5-HT intersected the ordinate at the same point. These results indicate that tyramine and 5-HT are competitive inhibitors of PEA deamination by deprenyl-pretreated human placental MAO.

**DISCUSSION**

Recent reports have shown that MAO in human placenta is able to deaminate 5-HT, tyramine and PEA and experiments on substrate specificity and inhibitor sensitivity have suggested that human placenta consists of a single form of MAO that closely resembles type A MAO (11, 12). In previous work (13), we obtained non-linear reciprocal plots for PEA deamination in the presence of benzylamine, 5-HT and tyramine, suggesting that PEA is metabolized by two different catalytic sites in human placental MAO.

In the present study, double reciprocal plots for PEA deamination were linear at high and low concentrations of PEA, and the Km values of the high- and low-affinity components of the enzyme were estimated to be 13.3 and 44.4 μM, respectively. Since 1 mM semicarbazide or 1 mM KCN did not affect the deamination at any concentration of PEA used, it is unlikely that the non-linearity was caused by contamination of the MAO preparation with connective tissue or serum. From these results, it is suggested that the MAO which deaminates PEA has high- and low-affinity substrate binding sites for PEA. In recent reports (15, 16), similar curved plots with two linear regions were obtained for MAO of bovine kidney and pig heart, suggesting heterogeneity of the enzyme.
Propargylamine derivatives such as deprenyl, which is a preferential and irreversible inhibitor of type B MAO, and clorgyline, which is a preferential inhibitor of type A MAO, are thought to bind covalently to the flavine adenine dinucleotide cofactor at the active site of the enzyme. It has been proposed that the initial enzyme-inhibitor binding is reversible (17, 18), and competitive, and that it becomes irreversible on prolonged preincubation and on increase in temperature (19). Moreover, irreversible inhibition of MAO by deprenyl was found to be more dependent on the preincubation period and temperature than that by clorgyline. In the present study, when deprenyl and enzyme solution were preincubated for 30 min at 38°C, the inhibitor-bound enzyme became irreversibly inactivated. To investigate the properties of the remaining enzyme that was not inhibited by deprenyl, we dialyzed the reaction mixture overnight to remove the non-covalently bound inhibitor. We found that reciprocal plots for PEA deamination by this pretreated preparation were linear, and that they intersected the abscissa at the same point as the plot of the “low affinity component” of untreated enzyme. Thus the Km value (44.4 μM) for the “low affinity component” of the enzyme for PEA deamination was identical with that for the deprenyl-pretreated enzyme.

To determine whether human placental MAO has different substrate-binding sites for different substrates, we carried out experiments with mixed substrates. When an enzyme shows a double-displacement reaction and one of its substrates is oxygen, as in the case for all MAO preparations so far investigated (20), mixed substrate experiments show apparent noncompetitive inhibition at a low concentration of oxygen, but a more competitive inhibition as the concentration of oxygen is raised (20).

In this investigation, the mixed type kinetics of inhibition by tyramine (Fig. 2, right) may be caused by the presence of type B MAO, since tyramine competitively inhibited PEA deamination by deprenyl-treated enzyme (Fig. 6, right). PEA deamination by “high PEA affinity MAO”, which is sensitive to deprenyl, appeared to be inhibited non-competitively by tyramine and 5-HT, whereas the reaction by deprenyl-resistant MAO was inhibited competitively by 5-HT and tyramine.

These data indicate that PEA, tyramine and 5-HT bind competitively to the same substrate-binding site of type A MAO, “the low PEA affinity site”, whereas tyramine and 5-HT bind to a different site from PEA of type B MAO.

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REFERENCES
1) Neff, N.H. and Yang, H.Y.T.: Another look at the monoamine oxidase and the monoamine oxidase inhibitor drugs. Life Sci. 14, 2061–2074 (1974)
2) Houslay, M.D. and Tipton, K.F.: Multiple forms of monoamine oxidase: Fact and artifact. Life Sci. 19, 467–478 (1976)
3) Jain, M.: Monoamine oxidase: Examination of multiple forms. Life Sci. 20, 1925–1934 (1977)
4) Tipton, K.F. and Mantle, T.J.: Structure and function of monoamine enzyme. Edited by Singer, T.P., Von Korff, R.W. and Murphy D.L., p. 559–585, Macmillan Press (1977)
5) Murphy, D.L.: Substrate-selective monoamine oxidase-inhibitor, tissue, species and functional differences. Biochem. Pharmacol. 27, 1889–1893 (1978)
6) Johnston, M.P.: Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17, 1285–1297 (1968)
7) Knoll, J. and Magyar, K.: Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv. Biochem. Psychopharmacol. 5, 393–408 (1972)
8) Goridis, C. and Neff, N.H.: Monoamine oxidase in sympathetic nerves: A transmitters specific
enzyme type. Brit. J. Pharmacol. 43, 814–818 (1971)

9) Hall, D.W.R., Logan, B.W. and Parsons, G.H.: Further studies on the inhibition of monoamine oxidase by M & B 9302 (clorgyline)-I, substrate specificity in various mammalian species. Biochem. Pharmacol. 18, 1447–1454 (1969)

10) Yang, H.Y.T. and Neff, N.H.: β-Phenylethylamine: A specific substrate for type B monoamine oxidase of brain. J. Pharmacol. exp. Ther. 187, 365–371 (1973)

11) Egashira, T.: Studies on monoamine oxidase XVIII. Enzymic properties of placental monoamine oxidase. Japan. J. Pharmacol. 26, 493–500 (1976)

12) Kikuchi, R. and Kinemuchi, H.: Evidence for existence of type A MAO in mitochondria from human placenta. Folia pharmacol. japon. 74, 763–772 (1978) (Abs. in English)

13) Oguchi, K., Kobayashi, S. and Koide, R.: Studies on human placental monoamine oxidase using mixed substrates. Japan. J. Pharmacol. 30, 157–164 (1980)

14) Wurtman, R. and Axelrod, J.: A sensitive and specific assay for estimation of monoamine oxidase. Biochem. Pharmacol. 12, 1439–1440 (1963)

15) Dugal, B.S.: Localization, purification and substrate specificity of monoamine oxidase. Biochem. Biophys. Acta 480, 56–69 (1977)

16) Lyles, G.A. and Greenawalt, J.W.: Possible heterogeneity of type B monoamine oxidase in pig heart mitochondria. Biochem. Pharmacol. 27, 923–935 (1978)

17) Rando, R.R.: Chemistry and enzymology of K3 inhibitors. Science 185, 320–324 (1974)

18) Olerand, L. and Ekstedt, B.: Soluble and membrane-bound pig liver mitochondrial monoamine oxidase: Thermostability, tryptic digestability and kinetic properties. Biochem. Pharmacol. 21, 2479–2488 (1972)

19) Egashira, T., Ekstedt, B. and Olerand, L.: Inhibition by clorgyline and deprenyl of the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol. 25, 2583–2586 (1976)

20) Fowler, C.J., Ekstedt, B., Egashira, T., Kinemuchi, H. and Oreland, L.: The interaction between human platelet monoamine oxidase, its monoamine substrates and oxygen. Biochem. Pharmacol. 28, 3063–3068 (1979)