Abstract—The activity of mitochondrial monoamine oxidase (MAO) from human placenta was measured with mixtures of labelled and unlabelled tyramine, serotonin (5-HT), benzylamine and β-phenylethylamine (PEA). Tyramine deamination was inhibited by benzylamine and PEA but not by 5-HT, while benzylamine deamination was inhibited by tyramine and PEA, but not by 5-HT. 5-HT deamination was inhibited by tyramine, benzylamine and PEA and PEA deamination was inhibited by tyramine, benzylamine and 5-HT. These results suggest that MAO in human placenta has multiple catalytic sites or consists of different enzymes. Probably, tyramine, benzylamine and PEA are deaminated oxidatively at a common catalytic site while 5-HT is deaminated at another catalytic site. Benzylamine deamination was inhibited in a mixed noncompetitive fashion by tyramine and PEA in air, but benzylamine deamination was competitively inhibited by PEA at higher concentrations of oxygen. The deaminations of other substrates were inhibited competitively by other substrates, in air. Reciprocal plots of PEA deamination with benzylamine, 5-HT and tyramine gave hyperbolic curves.

Monoamine oxidase (MAO) (monoamine: oxidoreductase [deaminating] EC 1.4.3.4.), which catalyzes the oxidation of biogenic amines such as serotonin (5-HT) and catecholamines, has been found to be present in various mammalian tissues. There is much evidence for the existence of multiple types of MAO, which can be distinguished by differences in their sensitivities to inhibitors (1-3), resistances to heat denaturation (4-6), mobilities on polyacrylamide gel electrophoresis (7-9) and antigenic properties (10, 11). Using the selective irreversible inhibitor clorgyline, Johnston (12) 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 less sensitive, but highly sensitive to another specific inhibitor, deprenyl (13). In most tissues, 5-HT and norepinephrine are metabolized solely by type A MAO, benzylamine and β-phenylethylamine (PEA) are metabolized by type B MAO, and tyramine is metabolized by both types of MAO.

Recent reports (13, 14) have suggested that MAO in human placenta differs from MAO in other organs in substrate specificity and sensitivity to inhibitors, and that this MAO activity is due to one single form closely resembling type A MAO. In the present study, we used mixed substrates to examine the properties of MAO in human placenta.

MATERIALS AND METHODS

1. Preparation 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 x g for 10 min at 4.0°C, and the supernatant collected. The residue was suspended in the sucrose-Tris buffer, and recentrifuged as before. The residue was now discarded, and the combined supernatants centrifuged at 8,500 x g for 20 min at 4.0°C. The resulting supernatant was discarded, and the pellet material suspended in the sucrose-Tris buffer and recentrifuged. The pellet (mitochondrial fraction) was suspended in 2 volumes of 0.1 M Tris-HCl buffer (pH 8.0), and used as the MAO preparation.

2. Assay of MAO activity: The method for MAO assay was based on that of Wurtman and Axelrod (15). The incubation medium contained radioactive substrates \([1^4C]\text{-tyramine (1 \(\mu\)Ci/ml), } 1^4C\text{-5-HT (2.5 \(\mu\)Ci/ml), } 1^4C\text{-benzylamine (1 \(\mu\)Ci/ml) or } 1^4C\text{-PEA (1 \(\mu\)Ci/ml)] and unlabelled substrates in a total volume of 250 \(\mu\)l of Tris-HCl buffer (0.01 M, pH 8.0). Incubation was performed at 38°C and was started by adding 50 \(\mu\)l of the enzyme preparation and stopped after 20 min by addition of 0.2 ml of 2 N HCl. The medium was extracted by shaking 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 benzylamine and PEA were used as substrates. Four ml of the organic layer then were mixed with 6 ml of Aquasol as scintillation liquid and the radioactivity measured in a Packard Tri-Carb Liquid Scintillation Spectrometer. Activity is expressed in disintegrations per min (DPM).

The effect of oxygen was examined by the same method, except that the reaction mixtures were flushed with 100% oxygen for 5 min before the incubation.

RESULTS

1. Inhibition of substrate deamination by other substrates: Deamination of labelled substrates was measured in the presence of various concentrations of other monoamines which were not labelled. As shown in Fig. 1, when MAO activity was determined with

![Fig. 1](image-url)
0.5 mM $^{14}$C-tyramine as substrate, addition of 1 mM unlabelled benzylamine or PEA inhibited the activity towards $^{14}$C-tyramine about 50%, whereas the same concentration of unlabelled 5-HT was not inhibitory. Figure 1 also shows that deamination of $^{14}$C-5-HT at a concentration of 0.5 mM was inhibited about 90% by 1 mM unlabelled tyramine or benzylamine, and 55% by 1 mM unlabelled PEA. Figure 2 shows that deamination of $^{14}$C-benzylamine at a concentration of 0.5 mM was inhibited 70% and 40% by unlabelled 1 mM PEA and tyramine, respectively, but only 10% by the same concentration of unlabelled 5-HT. Deamination of $^{14}$C-PEA, at a concentration of 0.05 mM was inhibited 90%, 85% and 60%, respectively, by 1 mM tyramine, benzylamine and 5-HT.

2. Determination of Km values: The Km values for various substrates of human placental MAO were calculated from Lineweaver-Burk plots. The results in Table 1 show that the Km value for benzylamine was highest followed in order by those for tyramine, 5-HT and PEA.

### Table 1. Km values of mitochondrial MAO from human placenta

| Substrate | Km (μM) |
|-----------|---------|
| tyramine  | 142     |
| 5-HT      | 111     |
| benzylamine | 270   |
| PEA       | 44      |

3. Competition experiments: Lineweaver-Burk plots for each substrate in the presence and absence of the other substrates were made to determine whether human placental MAO has different catalytic sites for different substrates or not. Figure 3 (left) shows the effects of unlabelled benzylamine (300 μM) and PEA (300 μM) on $^{14}$C-tyramine deamination. The abscissa shows the reciprocal of the concentration of $^{14}$C-tyramine, and the ordinate...
the reciprocal of activity for tyramine deamination with and without unlabelled benzylamine
and PEA. The three lines intersect at the same point on the ordinate, indicating that the
inhibitions by benzylamine and PEA are competitive.

Figure 3 (right) shows the effects of unlabelled benzylamine (100 $\mu$M), tyramine (100
$\mu$M) and PEA (1 mM) on $^{14}$C-5-HT deamination. The four lines intersect at the same
point on the ordinate, indicating that the inhibitions by benzylamine, tyramine and PEA are
all competitive.

The effects of unlabelled tyramine (1 mM) and PEA (100 $\mu$M) on $^{14}$C-benzylamine
deamination are shown in Fig. 4 (left). Lines with unlabelled tyramine and PEA intersected

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**Fig. 3.** Left: Lineweaver-Burk plot for inhibition of tyramine deamination by PEA
and benzylamine. Various concentrations of $^{14}$C-tyramine were incubated with
or without unlabelled PEA or benzylamine. Unlabelled monoamine: $— △ —$, 0.3 mM PEA; $— △ —$, 0.3 mM benzylamine; $— ● —$, none.
Right: Lineweaver-Burk plot for inhibition of 5-HT deamination by PEA, tyramine
and benzylamine. Various concentrations of $^{14}$C-5-HT were incubated with or
without unlabelled PEA, tyramine or benzylamine. Unlabelled monoamine:
$— △ —$, 1 mM PEA; $— ○ —$, 0.3 mM tyramine; $— △ —$, 0.1 mM benzylamine;
$— ● —$, none.

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**Fig. 4.** Lineweaver-Burk plot for inhibition of benzylamine deamination by PEA and
tyramine at normal (left) and high (right) oxygen concentration. Various concen-
trations of $^{14}$C-benzylamine were incubated with or without unlabelled PEA or
tyramine. Unlabelled monoamine: $— △ —$, 0.1 mM PEA; $— ○ —$, 1 mM tyramine;
$— ● —$, none.
the line without unlabelled substrate in the second quadrant. Thus, benzylamine deamination was inhibited in a mixed-noncompetitive fashion by tyramine and PEA. However, at a high concentration of oxygen, PEA inhibited benzylamine deamination competitively (Fig. 4, right).

Figure 5 (left) shows the effects of unlabelled benzylamine (200 μM), tyramine (500 μM) and serotonin (500 μM) on 14C-PEA deamination. The reciprocal plots for PEA deamination with the three unlabelled substrates gave hyperbolic curves while that without un-

![Graph showing Lineweaver-Burk plots for inhibition of PEA deamination by tyramine, 5-HT and benzylamine at normal (left) and high (right) oxygen concentrations. Various concentrations of 14C-PEA were incubated with or without unlabelled tyramine, 5-HT or benzylamine. Unlabelled monoamine: -○-, 0.5 mM tyramine; -x-, 0.5 mM 5-HT; -△-, 0.2 mM benzylamine; -▲-, 0.5 mM benzylamine; -□-, none.]

**Table 2.** Summary of kinetic data based on Lineweaver-Burk plots on mitochondrial MAO from human placenta

| Substrate | Tyramine | Benzylamine | PEA | 5-HT |
|-----------|----------|-------------|-----|------|
| Tyramine  | inhibition (competitive) | inhibition (competitive) | no inhibition |       |
| Benzylamine | inhibition (noncompetitive) | inhibition (noncompetitive in air, competitive at a high O₂ concentration) | no inhibition |       |
| PEA       | inhibition (curved, but competitive at high concentrations of PEA) | inhibition (curved either in air or at a high O₂ concentration but competitive at high concentrations of PEA) | inhibition (curved, but competitive at high concentrations of PEA) |       |
| 5-HT      | inhibition (competitive) | inhibition (competitive) | inhibition (competitive) |       |

**PEA:** β-phenylethylamine  **5-HT:** serotonin
labelled substrates was linear. Deamination of higher concentrations of $^{14}$C-PEA was inhibited competitively by unlabelled benzylamine, 5-HT and tyramine. On incubations at higher concentrations of oxygen with unlabelled benzylamine (200 and 500 $\mu$M), similar hyperbolic curves were obtained (Fig. 5, right). Results obtained by kinetic study based on Lineweaver-Burk plots on mitochondria MAO from human placenta are summarized in Table 2.

DISCUSSION

In recent reports (13, 14), it was suggested that MAO in human placenta differs in properties from MAO in other organs and that this MAO is a single enzyme closely resembling type A MAO. However, in a previous study (16) on the activity of mitochondrial MAO from human placenta with mixtures of tyramine, 5-HT and benzylamine as substrates, results indicating multiple forms were obtained. The results showed that the deamination of tyramine was competitively inhibited by benzylamine, while the deamination of neither tyramine nor benzylamine was inhibited by 5-HT. The deamination of 5-HT was inhibited both by benzylamine and tyramine. Thus it was suggested that MAO in human placenta may have multiple catalytic sites or consist of two different enzymes, that is, benzylamine and tyramine may be oxidatively deaminated by one catalytic site while 5-HT is deaminated on another.

In the present work, we found that tyramine deamination was inhibited by benzylamine and PEA but not by 5-HT, and that benzylamine deamination was inhibited by tyramine and PEA but not by 5-HT. 5-HT deamination, on the other hand, was inhibited by tyramine, benzylamine and PEA, and PEA deamination was inhibited by tyramine, benzylamine and 5-HT. A similar lack of cross-inhibition was observed with MAO from human brain (17). These results indicate that MAO in human placenta has at least two different catalytic sites. Probably, tyramine, benzylamine and PEA are deaminated oxidatively by one site, while 5-HT is deaminated by another catalytic site, which, however, also has affinity for the other amine substrates. In the present study, tyramine deamination was competitively inhibited by benzylamine and PEA, and 5-HT deamination was competitively inhibited by benzylamine, tyramine and PEA. Furthermore, deamination of PEA at higher concentrations was competitively inhibited by benzylamine, 5-HT and tyramine. These results again indicate that all those substrates were, at least to some extent, bound to a common active site. Benzylamine deamination was inhibited in a mixed non-competitive fashion by PEA and tyramine when incubations were performed under an atmosphere of air, while in cases of higher concentrations of oxygen, the deamination of benzylamine was competitively inhibited by PEA. This finding is also compatible with a common binding site for both these substrates if it is presumed that deamination of monoamines by MAO proceeds in two steps by a ping-pong mechanism or by a double displacement reaction as was shown to be the case for MAO from various other tissues (18–21). Cleland (22) has shown that an inhibitor that binds to both oxidized and reduced forms of an enzyme would cause an apparent non-competitive inhibition but that when the concentration of the second substrate
(in this case oxygen) is raised, the inhibition would become competitive. Thus, also benzylamine and PEA seem to share a common catalytic site on human placental MAO. Roth (23) reported similar results on MAO in human brain, with mutual non-competitive inhibitions between of PEA and benzylamine which became competitive when incubation was carried out under conditions of higher concentrations of oxygen, and concluded that PEA and benzylamine share a common catalytic binding site on human brain MAO. Fowler and Callingham (24) measured the Michaelis constant of rat liver MAO, towards oxygen ($K_O$) with several substrates and found that the $K_O$ value for PEA was about 4 times that for benzylamine. In the present competition experiments, the different inhibitory mechanisms with different pairs of substrates might be due to the different $K_O$ values for these substrates, resulting in different degrees of non-saturation of the second substrate, oxygen, under an atmosphere of air. In summary, these results indicate that all the substrates tested in these competition experiments except for 5-HT, share a common catalytic site on human placental MAO but that also after amines have an affinity for the “5-HT-site”.

Lyles and Greenawalt (25) obtained non-linear reciprocal plots for the metabolism of benzylamine by pig heart MAO and proposed that benzylamine was metabolized by both low-and high-affinity catalytic sites. They suggested that type B MAO in pig heart may be heterogeneous. In our study, the lines obtained in reciprocal plots for PEA deamination with unlabelled 5-HT, tyramine and benzylamine were also non-linear. Deamination of a high concentration of $^{14}$C-PEA appeared to be inhibited competitively by unlabelled substrates. When incubations were performed at higher oxygen concentrations, a similar non-linearity of the reciprocal plots was observed, indicating that the non-linearity of the reciprocal plots for the metabolism of PEA with unlabelled substrates was not due to the reaction of the reduced form of the enzyme under unsaturated conditions. Furthermore, a similar non-linearity of reciprocal plots was obtained when PEA was incubated with 1 mM semicarbazide or 1 mM KCN, indicating that the non-linearity was not caused by contamination with MAO from connective tissue or serum. These results suggest that PEA may be metabolized by two different catalytic sites which have identical $K_m$ value and that 5-HT, tyramine and benzylamine bind competitively to the site which catalyzes PEA at a high but not at low concentrations.

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