STUDIES ON MONOAMINE OXIDASE. XVIII.
ENZYMIC PROPERTIES OF PLACENTAL MONOAMINE OXIDASE

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Abstract—Enzymic properties of partially purified monoamine oxidase (MAO) from human placenta were studied with tyramine, serotonin and benzylamine as substrates. The highest activity was obtained with serotonin and almost no activity was observed with benzylamine. These results are similar to those obtained with rat placental MAO, but different from those with rabbit placental MAO. The Km values for serotonin and tyramine were found to be 0.21 mM and 0.23 mM, respectively and the pH optimum was 8.1 with either substrate. The thermal inactivation curves of this enzyme with the two substrates were identical. The pI curves for inhibition of MAO activity by harmine, pargyline and iproniazid were similar and almost the same pI 50 values for the respective inhibitors were obtained with the two substrates. MAO in human placenta differs from that in other organs, such as liver, brain and plasma from the standpoint of the substrate specificity and the inhibitor sensitivity. The possibility that human placenta contains a single form of MAO is discussed on the basis of the present results.

In 1948 Luschinsky and Tickner (1) reported that human placenta contained a relatively large amount of monoamine oxidase (MAO). The following year Thompson and Tickner (2) confirmed this observation in rat, rabbit and ginea pig placentas. About twenty years later Youdim and Sandler (3) obtained a partially purified preparation of the enzyme from human placenta. Their final product showed a specific activity of 3,500 and 400-fold purification over the original homogenate. There have been many clinical studies (4-8) on human placental MAO obtained at term and postmature deliveries, but characteristics and functional significance of this enzyme are unknown.

In this work the enzymic properties of MAO from mitochondria of human placenta were compared with those of preparations from bovine liver and brain. To determine whether or not MAO in human placenta exists as a single enzyme with a broad substrate specificity or as a multiple form of the enzyme, the substrate specificities, pH optimum, sensitivity to inhibitors and effect of temperature on the enzyme were studied with serotonin and tyramine as substrates.

MATERIALS AND METHODS

Preparation of MAO

Human placental MAO: MAO was partially purified from human placental mitochondria by a slight modification of the method of Youdim and Sourkes (9). Mitochondria were prepared from human placenta by differential centrifugation, suspended in 0.1 M phosphate buffer (pH 7.5) and then were sonicated for 30 min at 20 kHz. Triton X-100 was
added at a final concentration of 1.0% (v/v). The preparation was allowed to stand for 60 min and was then centrifuged at 100,000 × g for 60 min. Saturated ammonium sulfate solution in 0.1 M phosphate buffer (pH 7.5) was added drop-wise to the supernatant, while stirring. The material precipitated at between 25 and 35% saturation was obtained by centrifugation at 10,000 × g for 10 min and dissolved in a minimum amount of 0.1 M phosphate buffer and dialyzed overnight in a cold room against 1.0 mM phosphate buffer (pH 7.5). This preparation was used as the partially purified placental MAO.

**Bovine brain and liver mitochondria:** Bovine brain and liver mitochondria were isolated by a slight modification of the method of Schneider and Hogeboom (10). The materials were homogenized with 0.25 M sucrose containing 10 mM phosphate buffer (pH 7.5) and the mitochondria were collected by centrifugation and suspended in 2 volumes of 0.1 M phosphate buffer (pH 7.5).

**Bovine serum MAO:** Bovine blood, collected at a slaughter house, was centrifuged at 10,000 × g for 30 min and the serum was collected. Saturated ammonium sulfate solution in 0.1 M phosphate buffer was added drop-wise to the supernatant, while stirring, and the material precipitated at between 35 and 45% saturation was collected by centrifugation at 10,000 × g for 10 min, dissolved in a minimum amount of 0.1 M phosphate buffer and dialyzed overnight against 1.0 mM phosphate buffer (pH 7.5). All the procedures described above were performed in a cold room (0-4 °C).

**MAO activity**

MAO activity was determined by measuring oxygen consumption at 38°C, using a Clark oxygen electrode as described previously (11), and expressed as oxygen consumption in μM per min per mg protein of the enzyme preparation. Concentration of 1.0 mM serotonin and tyramine and 3.0 mM benzylamine, β-phenylethylamine, tryptamine and dopamine were used as substrates.

**Protein determination**

Protein concentration was determined spectrophotometrically by measuring the absorbance at 280 nm or by the Biuret method (12), using bovine serum albumin as a standard.

**RESULTS**

**Substrate specificity**

The relative rates of oxidation of various substrates by mitochondrial MAO from human placenta were compared with those by mitochondrial MAO from bovine brain and liver, and partially purified MAO from bovine serum. The results are shown in Table 1. All the substrates tested (i.e. tyramine, serotonin, benzylamine, β-phenylethylamine, tryptamine and dopamine) were oxidized by human placental MAO. Tyramine and serotonin were the best substrates and were oxidized almost equally well. The activity with benzylamine was only 6.2% that seen with tyramine. The activities with dopamine, tryptamine and β-phenylethylamine were 64%, 37.5% and 21.8%, respectively that seen with tyramine. Bovine brain MAO showed a similar substrate specificity, except that the activity was lower
with serotonin and higher with benzylamine. Bovine liver MAO showed a different substrate specificity; its activity was high with benzylamine (105% of that with tyramine) and low with serotonin (14% of that with tyramine). Serum MAO showed a completely different substrate specificity; it did not oxidize serotonin or tryptamine, but had a low activity with tyramine and a high activity with benzylamine.

**MAO activities in the placentas of various animals**

Relative rates of oxidation of different substrates by placental MAO preparation from various animals are shown in Table 2. The results obtained with placental MAO from rats were similar to those obtained with the enzyme from human placenta; both preparations showed high activity with serotonin but little activity with benzylamine. Placental MAO from rabbits oxidized all the substrates rather rapidly and dopamine was oxidized twice as fast as tyramine.

**Effects of inhibitors**

Effects of harmine and pargyline on mitochondrial MAO from human placenta and bovine brain and liver were studied with serotonin as substrate and the results are shown in Figs. 1 and 2. Increasing concentrations of harmine caused progressive inhibition of the

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**TABLE 1. MAO activity in human placenta and bovine brain, liver and serum**

| Substrate       | Human Placenta | Brain | Liver | Serum |
|-----------------|----------------|-------|-------|-------|
| Tyramine        | 100            | 100   | 100   | 8.8   |
| Serotonin       | 103.1          | 60.0  | 14.1  | 0     |
| Benzylamine     | 6.2            | 28.0  | 105.0 | 100   |
| 3-Phenylethylamine | 21.8      | 46.0  | 76.6  | 55.2  |
| Tryptamine      | 37.5           | 50.0  | 56.6  | 0     |
| Dopamine        | 64.0           | 84.0  | 76.6  | 58.0  |

All values are percentages of the MAO activity with tyramine except those for serum MAO which are percentages of MAO activity with benzylamine.

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**TABLE 2. MAO activities in the placenta of various animals**

| Substrate         | Placental MAO activity |
|-------------------|------------------------|
|                   | Human | Rat | Rabbit |
| Tyramine          | 100   | 100 | 100    |
| Serotonin         | 103.1 | 72.6| 86.4   |
| Benzylamine       | 6.2   | 0.6 | 26.9   |
| 3-Phenylethylamine| 21.8  | 20.5| 65.3   |
| Tryptamine        | 37.5  | 37.0| 65.3   |
| Dopamine          | 64.0  | 53.6| 203.8  |

Values are percentages of MAO activity with tyramine. Mitochondria of human placenta and homogenates of rat and rabbit placenta were used as enzyme preparations.
activity of MAO from human placenta, and concentration of $1 \times 10^{-8}$ M and $1 \times 10^{-7}$ M harmine caused about 50% and complete inhibition, respectively. Similar results were obtained with MAO from bovine brain, but a concentration of $1 \times 10^{-8}$ M harmine caused only 30% inhibition of the oxidation of serotonin. On the other hand, harmine in a concentration range of $1 \times 10^{-6}$ M to $1 \times 10^{-10}$ M did not inhibit the activity of MAO from bovine liver. Increasing concentrations of pargyline progressively inhibited the activities of all the MAO preparations. Pargyline completely inhibited the activity of MAO from human placenta with serotonin at a concentration of $1 \times 10^{-4}$ M, and the enzymes from bovine brain and liver at a concentration of $1 \times 10^{-5}$ M. Inhibitions of the preparations by harmine decreased in the order of placenta—brain—liver, and those by pargyline in the order of liver—brain—placenta.

**Purities of the fractions at each step of purification of MAO from human placenta**

Human placental enzyme was partially purified as described in the Materials and Methods. Table 3 shows the MAO activities at each step of purification. The specific activities with serotonin and tyramine at the final step were 31.4-fold and 27.1-fold those of

| TABLE 3. MAO activities at each step of purification |
|---------------------------------------------|
| Specific activity | Purity | Serotonin | Tyramine | Serotonin | Tyramine |
|-------------------|--------|-----------|----------|-----------|----------|
| Homogenate        | 0.05   | 0.04      | 1        | 1         | 1.06     |
| 8,500×g ppt       | 0.56   | 0.47      | 10.9     | 9.8       | 1.19     |
| Sonication & detergent treatment, 100,000×g sup. | 0.60 | 0.49 | 11.7 | 10.2 | 1.23 |
| 25–35% (NH₄)₂SO₄ | 1.60   | 1.30      | 31.4     | 27.1      | 1.23     |

Specific activity is expressed as oxygen consumption (µM) per min per mg protein of the enzymic preparation.
the original homogenate, respectively. The ratios of activities with serotonin and tyramine were similar at each step of purification.

Substrate specificity

Relative rates of oxidation of tyramine, serotonin, benzylamine, β-phenylethylamine, tryptamine and dopamine by purified MAO, obtained at final step of purification, and the original mitochondrial preparation in human placenta are shown in Table 4. The purified preparation oxidized tyramine, serotonin, β-phenylethylamine, tryptamine and dopamine fairly rapidly in comparison with the original mitochondrial MAO but did not oxidize benzylamine.

Table 4. Relative rates of oxidation of various substrates by mitochondrial and partially purified preparation from human placenta

| Substrate         | Mitochondrial preparation | Partially purified preparation |
|-------------------|---------------------------|-------------------------------|
| Tyramine          | 100                       | 100                           |
| Serotonin         | 103.1                     | 122.2                         |
| Benzylamine       | 6.2                       | 0                             |
| β-Phenylethylamine| 21.8                      | 46.6                          |
| Tryptamine        | 37.5                      | 84.4                          |
| Dopamine          | 64.0                      | 95.5                          |

Values are percentages of MAO activity with tyramine.

pH-Activity curve and Michaelis constant

Rates of oxidation of serotonin and tyramine at various pH values were measured. Phosphate buffer was used in the pH range from 5.5 to 7.0, Tris-HCl buffer from pH 7.0 to 8.5 and Clark-Lub's buffer from 8.5 to 9.5. As shown in Fig. 3 (left), the pH optimum was found to be 8.1 with either serotonin or tyramine as substrate.

The Michaelis constants of human placental MAO for serotonin and tyramine deter-

Fig. 3. pH-Activity curves and Km values. Left: pH-activity curves were obtained from the rates of oxidation of serotonin and tyramine at various pH values. Right: Km values were determined from Lineweaver-Burk's double reciprocal plots. Symbols: ○—○ with serotonin as substrate, ●—● with tyramine as substrate.
minded from Lineweaver-Burk's double reciprocal plots were almost identical (Fig. 3, right) being 0.21 mM for serotonin and 0.23 mM for tyramine.

**Effects of inhibitors**

Effects of harmine, pargyline and iproniazid on human placental MAO were studied with serotonin and tyramine as substrates. The results obtained with the partially purified MAO preparation are summarized in Table 5. Complete inhibition of MAO activity was observed with $1 \times 10^{-5}$ M harmine, $1 \times 10^{-4}$ M pargyline and $1 \times 10^{-3}$ M iproniazid, whereas the oxidations of serotonin and tyramine were not affected by $1 \times 10^{-10}$ M harmine, $1 \times 10^{-7}$ M pargyline or $1 \times 10^{-8}$ M iproniazid. The pI 50 values of harmine, pargyline and iproniazid on human placental MAO with tyramine as substrate were $1.8 \times 10^{-7}$ M, $1.0 \times 10^{-5}$ M and $3.8 \times 10^{-5}$ M, respectively and with serotonin were $1.0 \times 10^{-7}$ M, $1.1 \times 10^{-5}$ M and $2.6 \times 10^{-5}$ M, respectively.

**Table 5. Effects of various inhibitors on partially purified MAO activity**

|          | Harmine |           | Pargyline |           | Iproniazid |           |
|----------|---------|-----------|-----------|-----------|------------|-----------|
|          | Serotonin | Tyramine | Serotonin | Tyramine  | Serotonin  | Tyramine  |
| $1 \times 10^{-2}$ M | -        | -        | -        | -        | 0          | 0         |
| $1 \times 10^{-4}$ M | -        | -        | 0        | 0        | 26.7       | 28.3      |
| $1 \times 10^{-5}$ M | 0        | 0        | 69.3     | 69.5     | 64.4       | 66.6      |
| $1 \times 10^{-6}$ M | 3.2      | 10.8     | 93.5     | 95.6     | 79.6       | 73.3      |
| $1 \times 10^{-7}$ M | 48.5     | 56.5     | 100.0    | 100.0    | 98.0       | 97.0      |
| $1 \times 10^{-8}$ M | 71.4     | 76.0     | -        | -        | 100.0      | 100.0     |
| $1 \times 10^{-9}$ M | 93.4     | 95.0     | -        | -        | -          | -         |
| $1 \times 10^{-10}$ M | 100.0    | 100.0    | -        | -        | -          | -         |

Values are percentages of the control activity.

**Thermal inactivation**

Partially purified MAO from human placenta in 0.1 M phosphate buffer (pH 7.5) was heated in test tubes at various temperatures for 5 min. The mixtures were then quickly cooled in ice water and residual activities were determined at 38°C with serotonin and tyramine as substrates. As shown in Fig. 4, MAO activity with serotonin or tyramine as substrate gradually decreased as the temperature was raised, and about 50% of the activity was lost on heating at 45°C. The residual activity was 6.8% with serotonin and 2.0% with tyramine after heating at 50°C and complete inactivation was observed after heating at 55°C for 5 min. The

![Fig. 4. Effect of temperature on human placental MAO activity. Enzyme was incubated at various temperatures for 5 min before measuring MAO activity. Symbols as for Fig. 3.](image-url)
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temperature-inactivation curves for human placental MAO with serotonin and tyramine were similar.

DISCUSSION

Monoamine oxidase (MAO) in mitochondria of different tissues and species is believed to have broad substrate specificity. There is considerable evidence for the existence of several kinds of MAO. In the present study, liver MAO oxidized all the monoamines tested but showed the highest activity with benzylamine as substrate, whereas brain MAO oxidized tyramine most rapidly, and serum MAO oxidized benzylamine, but not serotonin or tyramine. Human placental MAO showed the highest activity with serotonin, but it also oxidized benzylamine slightly. Rat placental MAO showed an almost similar substrate specificity to the enzyme from human placenta. These results implied that the active site of the enzyme in placenta has a different structure from those of other mitochondrial MAO. The substrate specificity of rabbit placental MAO, however, seems to differ somewhat from those of the enzyme in human and rat placenta, and is similar to those of rabbit liver and brain (13). The MAO from bovine thyroid gland (14) is regarded as an enzyme which does not oxidize benzylamine as well as placental MAO.

The MAO activities of human placenta and bovine brain were completely inhibited by addition of $1 \times 10^{-7}$ M harmine, but $1 \times 10^{-6}$ M harmine had no effect on MAO from bovine liver. Moreover, pargyline also had very different effects on the activities of the three enzyme preparations. These results indicate that placental MAO has different properties from mitochondrial MAO from other mammalian organs, such as liver, kidney and brain.

The characters of MAO differ in different species and also in different organs of a single species. Several authors have reported direct or indirect evidence for the existence of multiple forms of MAO, such as marked difference in the heat stabilities of the activities of MAO from rat liver mitochondria for serotonin and tyramine (15), and differences in the affinities for benzylamine and serotonin (16). However, in the present study, the $K_m$ value of placental MAO was found to be 0.21 mM for serotonin and 0.23 mM for tyramine and the optimum pH values with these two substrates were similar. Moreover, there was no difference among the thermal inactivation curves of the enzyme measured with serotonin and tyramine as substrates.

Indirect evidence for multiple forms of mitochondrial MAO has been obtained by selective inhibition with various specific or nonspecific reagents (17-19). However, in the present work, the pH-curves for inhibition of serotonin oxidation by harmine, pargyline and iproniazid were similar to those for inhibition of tyramine oxidation and the respective $pH_{50}$ values for these inhibitors were similar with the two substrates.

These results do not support the existence of two enzymes, such as serotonin oxidase and tyramine oxidase (20). From the results mentioned above, it seems likely that human placental MAO is a single enzyme, like those of pig brain (21) and liver (22). This enzyme seems to be a type A MAO judging from its substrate specificity and inhibitor sensitivity (23). On treatment with Triton X-100, the multiple bands of MAO separated by gel electro-
phoresis disappeared and only one single band was seen (24). The presence of a single enzyme in the preparation of MAO from human placenta may be due to the detergent remaining in the final enzymic preparation. Youdim and Sandler (3) separated at least two bands of enzymic activity from human placental mitochondria by polyacrylamide gel electrophoresis. Further studies are underway in an attempt to purify the enzyme.

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