Studies on Monoamine Oxidase (Report XXIV)

Effect of Harmine on Monoamine Oxidase

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Accepted February 5, 1974

Abstract—Using beef brain and liver mitochondria, the effects of several inhibitors on the oxidation of serotonin and tyramine were studied. Mitochondrial MAO of beef brain showed pH optima of 8.8 with serotonin and 8.0 with tyramine. Pheniprazine had similar effects on the oxidations of serotonin and tyramine by brain MAO at pH 7.4 and pH 8.5. Amphetamine inhibited serotonin oxidation much more than tyramine oxidation by brain MAO at pH 7.4 and pH 8.5. Harmine selectively inhibited serotonin oxidation by brain MAO at pH 7.4. It inhibited serotonin oxidation by brain MAO competitively and tyramine oxidation uncompetitively. However, after heat treatment of mitochondrial MAO of brain at 60°C for 10 min, its effects on the oxidations of serotonin and tyramine were similar. The pI-activity curve of inhibition of oxidation of serotonin coincided with that of tyramine. The effect of harmine on MAO of brain mitochondria differed markedly before and after heat treatment: the pI-activity curve was biphasic before heat treatment but it became sigmoidal after heat treatment. These results suggest that there are at least two types of MAO in beef brain mitochondria: one heat labile, with low affinity for harmine, and the other rather heat stable, with high affinity for harmine. Harmine inhibited liver MAO only very slightly with either serotonin or tyramine as substrate.

Recently, much attention has been focused on the multiplicity of monoamine oxidase (MAO) [EC 1.4.3.4. monoamine: oxygen oxidoreductase (deaminating)], which is involved in metabolism of catecholamine and serotonin (1). Many papers suggest that there are multiple forms of MAO, differing in nature and different enzymes of this group have been found not only in different species but also in different organs of a single species (2).

In 1961, Kamijo (3) reported that the inhibitory effect of p-methylphenyl hydrazine on MAO in guinea pig brain differed from its effect on liver MAO, and so suggested the possible existence of at least two different forms of MAO. Similar results have been reported by others (4,5), and it is now possible to separate several forms of MAO from soluble fractions of mitochondria (6–9).

A previous paper from this laboratory (10), reported that harmine inhibits the oxidation of serotonin much more than that of tyramine by beef brain mitochondria. Thus, it was suggested that harmine affects serotonin metabolism much more than catecholamine metabolism in brain in vivo and, that there are two different forms of MAO in beef brain mitochondria.

This paper describes the effect of the inhibitors, harmine, amphetamine and phenipra-
zine, on the MAO activities of beef brain and liver mitochondria. To obtain more information on multiple forms of MAO in beef brain and liver mitochondria, experiments were performed at both pH 8.5 and pH 7.4, using two substrates, serotonin and tyramine.

MATERIALS AND METHODS

Preparation of beef brain mitochondria

Beef brain tissue was separated from coagulated blood and bone and stored at -20°C. It was homogenized in a Waring blender with 9 volumes of ice cold 0.32 M sucrose containing 0.01 M tris-HCl buffer (pH 8.0). The homogenate was centrifuged at 450 × g for 10 min at 4-6°C, and the supernatant was kept in an ice bath. The precipitate was suspended in the same sucrose-tris buffer, and recentrifuged as before. The precipitate was discarded, and the supernatants were combined, and centrifuged at 8,500 × g for 20 min at 4-6°C. The supernatant was discarded, and the precipitate was suspended in the sucrose-tris buffer and recentrifuged. The precipitate (mitochondrial fraction) was suspended in the same volume of 0.1 M tris-HCl buffer (pH 8.0), and used as the monoamine oxidase preparation.

Preparation of beef liver mitochondria

Beef liver was stored at -20°C. It was homogenized in a Waring blender with 3 volumes of ice cold 0.25 M sucrose containing 0.01 M tris-HCl buffer (pH 8.0). The homogenate was centrifuged at 600 × g for 10 min at 4-6°C in a refrigerated centrifuge, and the supernatant was kept in ice. The precipitate was suspended in the sucrose-tris-HCl buffer and recentrifuged as before. The precipitate was discarded and the supernatants were combined and centrifuged following the same procedure used for beef brain mitochondria. The mitochondrial fraction obtained was suspended in 7 volumes of 0.1 M tris-HCl buffer (pH 8.0), and used as the monoamine oxidase preparation.

Measurement of MAO activity

MAO activity was determined by the standard Warburg manometric method, measuring oxygen consumption at 38°C (11). The reaction mixture containing enzyme solution, 0.05 M tris-HCl buffer (pH 8.5) or 0.05 M phosphate buffer (pH 7.4), substrate at a given concentration and inhibitor solution or distilled water in a total volume of 3.0 ml was equilibrated with 100% oxygen gas for 10 min. The reaction mixture containing the enzyme and inhibitor was pre-incubated for 15 min. Oxygen consumption was followed for 60 min from 10 min after adding the substrate from the side arm. Manometers were shaken 60 times a min in all experiments. MAO activity was expressed as the amount of oxygen consumption (μl) per hour making corrections for oxygen uptake due to non-enzymatic oxidation of substrate and that by enzyme without substrate.

Substrate

Serotonin creatinine sulfate and tyramine hydrochloride were used as substrates at concentrations of 3 × 10^{-4} M, unless otherwise stated.
RESULTS

Effect of pH on MAO activity of brain mitochondria

The effect of pH on MAO activity was examined using serotonin or tyramine as substrate with phosphate buffer from pH 7.0 to 7.5, tris-HCl buffer from pH 7.5 to 9.0, and borate buffer from pH 9.0 to 9.6. As seen in Fig. 1, with serotonin as substrate, activity was maximal at pH 8.8, decreasing gradually at lower pH values and abruptly at higher values. Thus, the pH-activity curve was unsymmetrical. With tyramine as substrate, the pH-activity curve was symmetrical with a maximum at pH 8.0.

Effects of various inhibitors on MAO activity of brain mitochondria

The effects of pheniprazine, amphetamine and harmine on MAO activity were investigated at pH 7.4 (nearly the physiological value) and at pH 8.5 (nearly the optimum for serotonin oxidation).

a) Pheniprazine

The effects of pheniprazine at concentrations of $1 \times 10^{-8}$ M to $1 \times 10^{-3}$ M are shown in Fig. 2. At pH 7.4, the effects

![Fig. 1. pH Optima of MAO in beef brain mitochondria. MAO activities were determined manometrically at 38°C using $3 \times 10^{-3}$ M serotonin and tyramine as substrates. Buffers used were: phosphate buffer (pH 7.0-7.5), tris-HCl buffer (pH 7.5-9.0), and borate buffer (pH 9.0-9.6). 146 mg dry weight of mitochondria were used.]

![Fig. 2. Effects of pheniprazine on the oxidations of serotonin and tyramine by MAO of beef brain mitochondria at pH 7.4 (a) and at pH 8.5 (b). The reaction mixture for (a) contained $3 \times 10^{-3}$ M substrate, 146 mg dry weight of mitochondria and 0.05 M phosphate buffer (pH 7.4) in 3 ml. The reaction mixture for (b) was the same but with 0.05 M tris-HCl buffer (pH 8.5). Incubations were carried out at 38°C. Open circles and solid lines indicate percentages of control MAO activities with $3 \times 10^{-3}$ M serotonin as substrate, and closed circles and broken lines, those with $3 \times 10^{-3}$ M tyramine as substrate.]
on the oxidations of serotonin and tyramine by brain MAO were similar. The pI-activity curves were sigmoidal. Inhibition increased with increase in concentration of the inhibitor above $1 \times 10^{-3}$ M. MAO activity was inhibited completely by $1 \times 10^{-4}$ M pheniprazine, and the value of $I_{50}$ was found to be $7 \times 10^{-6}$ M. Similar results were obtained at pH 8.5 with no difference between the effects of pheniprazine on the oxidations of serotonin and tyramine. The value of $I_{50}$ at pH 8.5 was $3 \times 10^{-6}$ M.

b) Amphetamine

The effects of amphetamine at concentrations of $1 \times 10^{-7}$ M to $1 \times 10^{-2}$ M on brain MAO are shown in Fig. 3. The pI-activity curves for the oxidations of serotonin and tyramine were sigmoidal. With tyramine as substrate, there was no significant inhibition using $1 \times 10^{-5}$ M amphetamine, but 90% inhibition using $1 \times 10^{-2}$ M amphetamine. With serotonin as substrate, there was no inhibition using $1 \times 10^{-6}$ M amphetamine but complete inhibition using $1 \times 10^{-4}$ M amphetamine. The $I_{50}$ values of amphetamine were $1 \times 10^{-3}$ M with tyramine and $1 \times 10^{-4}$ M with serotonin. Thus, at pH 7.4, serotonin oxidation was inhibited approx. 10 times more than tyramine oxidation. At pH 8.5 the difference in the effects of amphetamine on the oxidations of serotonin and tyramine was less. The pI-activity curves with these substrates were sigmoidal and the $I_{50}$ values were $5 \times 10^{-4}$ M with tyramine and $3 \times 10^{-4}$ M with serotonin.

c) Harmane

The effects of harmane at concentrations of $1 \times 10^{-8}$ M to $1 \times 10^{-2}$ M on MAO are shown in Fig. 4. At pH 7.4, its effects on the oxidations of serotonin and tyramine by brain MAO were different. With serotonin as substrate, inhibition increased rapidly with increase in the concentration of harmane from $1 \times 10^{-6}$ M to $1 \times 10^{-4}$ M. Inhibition increased less with increase in concentration from $1 \times 10^{-6}$ M to $1 \times 10^{-4}$ M; that is, the pI-activity curve was rather flat in this concentration range. With tyramine as substrate, there was no significant
inhibition at harmine concentrations of $1 \times 10^{-8}$ M to $1 \times 10^{-2}$ M, and only 55% inhibition with $1 \times 10^{-2}$ M harmine. That is, the p1-activity curve was rather flat. The $I_{50}$ values of harmine were $3 \times 10^{-7}$ M with serotonin and $3 \times 10^{-3}$ M with tyramine. Thus, harmine inhibited serotonin oxidation ten thousand times more than tyramine oxidation.

However, at pH 8.5 there was no marked difference in the $I_{50}$ values for oxidations of these two substrates. Even at a concentration of $1 \times 10^{-2}$ M, harmine caused only 55% and 65% inhibitions of the oxidations of tyramine and serotonin, respectively. The p1-activity curves with serotonin and tyramine were both biphasical and flat in the concentration range of $1 \times 10^{-6}$ M to $1 \times 10^{-3}$ M.

**Effects of various inhibitors on MAO of beef liver mitochondria**

a) Pheniprazine

The effects of pheniprazine at concentrations of $1 \times 10^{-9}$ M to $1 \times 10^{-4}$ M on beef liver
MAO are shown in Fig. 5. At pH 7.4 the pl-activity curves with serotonin and tyramine were both sigmoidal and almost coincided. The oxidations of these substrates were scarcely inhibited by $1 \times 10^{-8}$ M pheniprazine, and completely inhibited by $1 \times 10^{-5}$ M pheniprazine. The $I_{50}$ value for the oxidations of serotonin and tyramine was $1 \times 10^{-6}$ M. At pH 8.5 the pl-activity curves for oxidations of serotonin and tyramine were both sigmoidal, but inhibition of serotonin oxidation was much less than that of tyramine oxidation. The $I_{50}$ values of pheniprazine for the oxidations of serotonin and tyramine at pH 8.5 were $7 \times 10^{-8}$ M and $1 \times 10^{-8}$ M, respectively.

b) Amphetamine

The effects of amphetamine at concentrations of $1 \times 10^{-7}$ M to $1 \times 10^{-2}$ M are shown in Fig. 6. At pH 7.4, amphetamine inhibited the oxidation of serotonin by the liver enzyme somewhat more than that of tyramine, and $1 \times 10^{-2}$ M amphetamine inhibited the oxidations of tyramine and serotonin 90% and 100%, respectively. The $I_{50}$ values of amphetamine for the oxidations of tyramine and serotonin were $7 \times 10^{-4}$ M and $5 \times 10^{-4}$ M, respectively.

At pH 8.5 the inhibitory effects were similar to those at pH 7.4. The pl-activity curves with serotonin and tyramine were sigmoidal, and the $I_{50}$ values of amphetamine for the oxidations of tyramine and serotonin at pH 8.5 were $7 \times 10^{-4}$ M and $3 \times 10^{-4}$ M, respectively.

c) Harmine

Harmine was tested at concentrations of $1 \times 10^{-7}$ M to $1 \times 10^{-2}$ M. As seen in Fig. 7 (left), at pH 7.4, it inhibited oxidation of serotonin much more than that of tyramine at concentrations of $1 \times 10^{-4}$ M to $1 \times 10^{-2}$ M. It had no significant effect at a concentration of $1 \times 10^{-7}$ M, but inhibition gradually increased at higher concentrations. MAO activity with tyramine was inhibited only 35% by $1 \times 10^{-4}$ M harmine. With serotonin as substrate, there was no significant inhibition at harmine concentrations of $1 \times 10^{-7}$ M to $1 \times 10^{-3}$ M, but inhibition gradually increased at higher concentrations of the inhibitor. MAO activity
Fig. 7. Effects of harmine on the oxidations of serotonin and tyramine by MAO of beef liver mitochondria at pH 7.4 (a) and at pH 8.5 (b). Conditions were as for Fig. 5.

was inhibited about 60% by $1 \times 10^{-5}$ M harmine. The pH-activity curves of harmine were much flatter than those of pheniprazine and amphetamine. At pH 8.5 harmine at concentrations of $1 \times 10^{-5}$ M to $1 \times 10^{-3}$ M did not cause significant inhibition of the oxidations of serotonin and tyramine.

Inhibition pattern of harmine

Fig. 8 shows Lineweaver-Burk plots of the inhibition by harmine of the oxidations of serotonin and tyramine by the brain enzyme at pH 7.4, using $7 \times 10^{-4}$ M harmine with serotonin and $7 \times 10^{-4}$ M harmine with tyramine. The $K_m$ values were $1.7 \times 10^{-3}$ M with serotonin and $5.9 \times 10^{-3}$ M with tyramine. As shown in the figure, with serotonin as substrate the $1/v - 1/[s]$ relationships in the presence and absence of harmine were linear and

Fig. 8. Effects of harmine on the oxidations of serotonin and tyramine by MAO in beef brain mitochondria.

(a) Double reciprocal plots of serotonin concentration versus rate of oxidation. Open circles, no harmine (control); closed circles, with $7 \times 10^{-4}$ M harmine.

(b) Double reciprocal plots of tyramine concentration versus rate of oxidation. Open circles, no harmine (control); closed circles, with $7 \times 10^{-4}$ M harmine.

0.05 M phosphate buffer (pH 7.4) was used.

Incubations were carried out at 38°C.
crosed on a point on the ordinate. With tyramine as substrate, however, the lines obtained with and without harmine were parallel. These data show that harmine acts as a competitive inhibitor of serotonin oxidation and as a uncompetitive inhibitor of tyramine oxidation.

**Effect of heat treatment on the activity of the brain enzyme**

The beef brain mitochondria preparation was heated at various temperatures at pH 8.0 for 10 min, after which activity was assayed. Results are summarized in Table 1. After heating at 55°C for 10 min the preparation showed 92.8% of the control activity with serotonin, and 91.6% with tyramine. After heating at 60°C for 10 min it showed 44.6% of the control activity with serotonin and 43.9% with tyramine, while after heating at 65°C for 10 min, the activity with both substrates was lost completely.

|                | Control | 55°C | 60°C | 65°C |
|----------------|---------|------|------|------|
| Serotonin      |         |      |      |      |
| mlO₂           | 93.2    | 86.5 | 41.6 | 1.4  |
| %              | 100     | 92.8 | 44.6 | 1.5  |
| Tyramine       |         |      |      |      |
| mlO₂           | 153.4   | 140.5| 67.3 | 4.4  |
| %              | 100     | 91.6 | 43.9 | 2.9  |

Beef brain mitochondria in 0.05 M phosphate buffer (pH 8.0) were incubated for 10 min at the temp. stated and then cooled in ice. The activities toward serotonin and tyramine were then assayed and expressed as percentages of the activity of the untreated mitochondria. Conditions as in Fig. 2.

**Effect of harmine on heat-treated brain enzyme**

Beef brain enzyme was heated at 60°C and pH 8.0 for 10 min after which the effect of harmine at concentrations of $1 \times 10^{-9}$ M to $1 \times 10^{-5}$ M were investigated at pH 7.4. Re-
sults are shown in Fig. 9. With either serotonin or tyramine heat-treated enzyme showed 100% activity in the presence of $1 \times 10^{-9}$ M harmine, and no activity in the presence of $1 \times 10^{-5}$ M harmine. The effects of harmine on the oxidations of serotonin and tyramine by heat-treated enzyme were similar and the pl-activity curves with serotonin and with tyramine completely coincided. Although the pl-activity curve obtained using native enzyme was biphasical, as described above, the curves with heat-treated enzyme were sigmoidal and the $I_{50}$ values of harmine for oxidations of both substrates were $1 \times 10^{-7}$ M at pH 7.4.

**DISCUSSION**

In 1928, Hare discovered tyramine oxidase, which catalyzes the oxidative deamination of tyramine (12). Later adrenaline oxidase (13) and aliphatic amine oxidase (14) were found by other investigators. Zeller (15) gave the general name, monoamine oxidase, to these enzymes, since they all catalyzed the oxidative deamination of various primary amines by the same stoichiometric reaction as follows: $R\cdot CH_2NH_2 + O_2 + H_2O \rightarrow R\cdot CHO + NH_3 + H_2O_2$. On the other hand, Werle and Roewer (16) suggested that there are different types of amine oxidase with different substrate specificities, one specifically catalyzing the oxidative deamination of aliphatic amines, and another that of aromatic amines. Satake (17) suggested that MAO may be a group of enzymes, each with slightly different substrate specificity. Similar suggestions were made by Hardegg and Heilbronn (18), Gorkin (19), Youdim and Sourkes (20) and Squires (21).

New methods are available for obtaining pure, soluble preparations of MAO, so it is possible to examine whether there are multiple forms of MAO. There are a number of papers on the multiplicity of MAO (8, 9, 22). Finding that various MAO inhibitors specifically changed the concentrations of monoamines in brain (23, 24), Fuller suggested the existence of multiple forms of MAO. Egashira et al. (25) found that sodium nitrite had different effects on the activities of MAO from rat liver and brain mitochondria and concluded that there are multiple forms of MAO in these mitochondria. It was reported in a previous paper from this laboratory (10), that harmine inhibited the oxidation of serotonin by beef brain mitochondria much more than that of tyramine suggesting that there are at least two types of MAO in mitochondria from beef brain. Similar results were observed by Chodera et al. using rat liver mitochondria (26). On the other hand, Gorkin and Tatyanenko reported that harmine caused little inhibition of MAO of beef liver mitochondria and almost none at pH 8.5 (27). Harmine does not appear to inhibit MAO from beef liver mitochondria, even when a highly purified enzyme is utilized. Squires (21) reported that harmine inhibited MAO activity of mouse brain but not that of mouse liver, in vivo. In this study, the inhibitory effects of harmine on the MAO activities of brain and liver mitochondria were compared. Both at pH 8.5 and pH 7.4 harmine inhibited the oxidation of serotonin by the brain mitochondria much more than that by liver mitochondria. This suggests that brain and liver mitochondria contain different types of MAO. There are a few reports on the inhibitions of the oxidations of tyramine and
serotonin by harmine. Zirkle et al. (28) reported that harmine acted as a competitive inhibitor for serotonin oxidation by rat brain MAO. Tipton et al. (29) obtained similar results on its inhibition of tyramine oxidation by pig brain MAO. In this study, harmine was found to inhibit serotonin oxidation by beef brain MAO competitively and tyramine oxidation uncompetitively. Thus mitochondrial MAO of beef brain and pig brain appear to be different.

Oswald et al. (30) found that heat treatment of rat liver mitochondria at 55°C for 10 min decreased MAO activity for tyramine oxidation but not for serotonin oxidation. Tipton et al. (29) reported that the oxidations of both serotonin and tyramine by pig brain mitochondria decreased distinctly after heat treatment of the mitochondria. In this work also, the oxidations of serotonin and tyramine decreased similarly on heat treatment of beef brain mitochondria. As seen in Fig. 9, however, the effect of harmine on MAO of brain mitochondria differed markedly before and after heat treatment: the pI-activity curve was biphasic before heat treatment, and sigmoidal after heat treatment. This suggests that there are at least two types of MAO in beef brain mitochondria: one heat labile, with low affinity for harmine, and the other rather heat stable, with high affinity for harmine. The biphasic pI-activity curve of harmine is explainable if it is assumed that before heat treatment, brain mitochondria contain multiple forms of MAO with different affinities for harmine.

Acknowledgements: Gratitude is extended to Prof. K. Kamijo for guidance and encouragement and to the other members of this laboratory, particularly Prof. Y. Ogura and Dr. S. Sho for their valuable advice and assistance.

REFERENCES

1) Sandler, M. and Youdim, M.B.H.: Pharmacol. Rev. 24, 331 (1972)
2) Youdim, M.B.H.: Advances in Biochemical Psychopharmacology, Edited by Costa, E. and Sandler, M., Vol. 5, p. 67, Raven Press, New York (1972)
3) Kamijo, K.: Folia pharmacol. japon. 57, 192 (1961) (in Japanese)
4) Ragland, J.B.: Biochem. biophys. Res. Commun. 31, 203 (1968)
5) Fujimaki, T.: Folia pharmacol. japon. 64, 393 (1968) (in Japanese)
6) Youdim, M.B.H., Collins, G.G.S. and Sandler, M.: Nature 223, 626 (1969)
7) Collins, G.G.S., Sandler, M., William, E.D. and Youdim, M.B.H.: Nature 225, 817 (1970)
8) Kinemuchi, H.: Japan. J. Pharmacol. 21, 785 (1971)
9) Arai, U., Masamoto, K. and Kamijo, K.: Folia pharmacol. japon. 68, 400 (1972) (in Japanese)
10) Yasuhara, H., Sho, S. and Kamijo, K.: Japan. J. Pharmacol. 22, 439 (1972)
11) Kamijo, K., Koelle, G.B. and Wagner, M.H.: J. Pharmacol. exp. Ther. 117, 213 (1956)
12) Hare, M.L.C.: Biochem. J. 22, 968 (1928)
13) Blaschko, H., Richter, D. and Schlossman, H.: J. Physiol. 90, 1 (1937)
14) Pugh, C.E.M. and Quastel, J.H.: Biochem. J. 31, 286 (1937)
15) Zeller, E.A.: The Enzymes. Edited by Sumner, J.B. and Myrbäck, K., Vol. 2, p. 536, Academic Press, New York (1951)
16) Werle, E. and Rowher, F.: Biochem. J. 50, 320 (1952)
17) Satake, K.: Saktin no Kosokazaku 4, 39 (1955) (in Japanese)
18) Hardeg, W. and Heilbron, E.: Biochim. biophys. Acta 51, 553 (1961)
19) Gorkin, V.Z.: Pharmacol. Rev. 18, 115 (1966)
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20) YOUDIM, M.B.H. AND SOURKES, T.L.: Can. J. Biochem. 43, 1305 (1965)
21) SOURKES, R.F.: Biochem. Pharmacol. 17, 1401 (1968)
22) TIPTON, K.F., YOUDIM, M.B.H. AND SPIRES, I.P.C.: Biochem. Pharmacol. 21, 2197 (1972)
23) FULLER, R.W.: Arch. int. Pharmacodyn. Ther. 174, 32 (1968)
24) FULLER, R.W., WARREN, B.J. AND MOLLOY, B.B.: Biochem. Pharmacol. 19, 2934 (1970)
25) EGASHIRA, T., TAKANO, K., SHIMIZU, K., KUROSAWA, Y. AND KAMIJO, K.: Japan. J. Pharmacol. 21, 274 (1971)
26) CHODRA, A., GORKIN, V.Z. AND GRIDNIEVA, L.I.: Acta biol. med. german. 13, 101 (1964)
27) GORKIN, V.Z. AND TATYANENKO, L.V.: Life Sci. 6, 791 (1967)
28) ZIRKLE, C.L., KAISER, C., TEDESCHI, D.H., TEDESCHI, R.E. AND BURGER, A.: J. Med. Pharm. Chem. 5, 1265 (1962)
29) TIPTON, K.F. AND SPIRES, I.P.C.: Biochem. Pharmacol. 17, 2137 (1968)
30) OSWALD, E.O. AND STRITTMATTER, C.F.: Proc. Soc. exp. Biol. Med. 114, 668 (1963)