*Biochemical Pharmacology*, Vol. 31, No. 8, pp. 1515–1520, 1982.
Printed in Great Britain.

0006-2952/82/081515-06 $03.00/0


# BINDING AND DEAMINATION OF VARIOUS SUBSTRATES BY TYPES A AND B MONOAMINE OXIDASE IN BOVINE BRAIN MITOCHONDRIA

KATSUJI OGUCHI, SHINICHI KOBAYASHI, TADAMASA UESATO and KAZUYA KAMIJO*
Department of Pharmacology, School of Medicine, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142, Japan

(*Received* 15 *May* 1981; *accepted* 17 *September* 1981)

**Abstract**—The binding and deamination of four substrates by type A and type B monoamine oxidase (MAO) in bovine brain mitochondria were investigated in mixed substrate experiments. MAO activity in bovine brain mitochondria, with 5-hydroxytryptamine (5-HT) as substrate, was highly sensitive to clorgyline and less sensitive to deprenyl, while MAO activity with benzylamine or β-phenylethylamine (PEA) as substrate was highly sensitive to deprenyl and less sensitive to clorgyline. On the other hand, when tyramine plus PEA was used as substrate, the inhibition curves of clorgyline and deprenyl were both biphasic. These results indicate that 5-HT and benzylamine were preferentially deaminated by type A MAO and type B MAO, respectively, and that tyramine and PEA were deaminated by both types of MAO. Studies on the inhibition by clorgyline plus deprenyl of tyramine deamination (in the absence and presence of another substrate) showed that the deamination of tyramine by both type A and type B MAO was inhibited by PEA or benzylamine, while only type A MAO was inhibited significantly by 5-HT. The $KA_i$ value, the dissociation constant of the type A MAO and 5-HT complex, and the $KB_i$ values, the dissociation constants of the type B MAO and PEA or benzylamine complex, were almost equal to the $K_m$ values of type A MAO and type B MAO respectively. The $KA_i$ values for PEA and benzylamine were 78 and 58 μM respectively. For the type B MAO–5-HT complex, the dissociation constant $KB_i$ was 1447 μM. These results show that type A MAO deaminates tyramine and 5-HT whereas benzylamine is not deaminated, but only binds to the substrate binding site of type A MAO with almost the same rate as that for deamination by type B MAO; with type B MAO, tyramine, PEA and benzylamine are deaminated, whereas 5-HT is not deaminated and binds to the substrate binding site of type B MAO with low affinity.

There is much evidence that monoamine oxidase [MAO, monoamine: $O_2$ oxidoreductase (deaminating) EC 1.4.3.4], exists as more than one type [1–3]. Using clorgyline, which is a selective and irreversible inhibitor, Johnston [4] demonstrated the existence of two types of MAO, types A and B, in rat brain. Type A MAO is sensitive to a low concentration of clorgyline, while type B MAO is sensitive to a low concentration of deprenyl [5]. 5-Hydroxytryptamine (5-HT) and norepinephrine are deaminated by type A MAO. Benzylamine and β-phenylethylamine (PEA) are deaminated by type B MAO, and tyramine is deaminated by both types of MAO [6–8].

In the present study, using MAO inhibitors and experiments with mixed substrates, the inhibition and deamination of various monoamines, by types A and B MAO in bovine brain mitochondrial preparations, were investigated. The binding and deamination of various substrates by types A and B MAO are discussed.

## MATERIALS AND METHODS

*Preparation of bovine brain MAO.* Cerebral tissue (cortex and medulla) from bovine brain was homogenized in a Waring Blender with 9 vol. of ice-cold 0.32 M sucrose in 0.01 M Tris–HCl buffer (pH 8.0). The homogenate was centrifuged at 600 *g* for 10 min at 4°, and the supernatant fraction was collected. The residue was suspended in the same buffer and recentrifuged. The residue was discarded, and the supernatant fractions were combined and centrifuged at 8500 *g* for 20 min at 4°. The resulting supernatant fraction was discarded, and the pellet material was suspended in the same buffer and recentrifuged. Finally, the pellet (mitochondrial fraction) was suspended in 0.01 M Tris–HCl buffer (pH 8.0), at a protein content of 30 mg/ml, and was used as the MAO preparation.

*Assay of MAO activity.* MAO assay was based on that of Wurtman and Axelrod [9]. The incubation medium contained radioactive susbstrate ([$^{14}$C]5-HT, [$^{14}$C]tyramine, [$^{14}$C]PEA or [$^{14}$C]benzylamine) with unlabeled substrate in a total volume of 275 μl of Tris–HCl buffer (0.01 M, pH 8.0). The final concentrations of substrates were 500 μM for tyramine, 5-HT and benzylamine, and 50 μM for PEA. Incubation was performed at 38°. The reaction was started by adding 25 μl of the enzyme preparation and was stopped after 20 min by adding 0.2 ml of 2 N HCl. The radioactivity was extracted by shaking the medium vigorously 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 and benzylamine were used as substrates. Four ml of the organic layer was mixed with 6 ml of scintillation fluid and

* Author to whom all correspondence should be directed.

the radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. Activity was expressed as disintegration per minute. The enzyme reaction was found to be linear during the incubation time. Protein content was estimated by the modified Biuret method [10] with bovine serum albumin as a standard.

(−)Deprenyl and clorgyline were gifts from Dr. K. Magyar, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary, and from May & Baker Ltd., Dagenham, U.K., respectively. Radiochemical substrates, [2-$^{14}$C]5-hydroxytryptamine binoxalate (44 mCi/mmole), [1-$^{14}$C]tyramine hydrochloride (56 mCi/mmole), and [1-$^{14}$C]$\beta$-phenylethylamine hydrochloride (50.98 mCi/mmole), were purchased from the New England Nuclear Corp., Boston, MA, U.S.A. [7-$^{14}$C]Benzylamine hydrochloride (60.6 mCi/mmole) was purchased from the Amersham Corp., Arlington Heights, IL, U.S.A. All other chemicals used were of the highest commercial grade available.

*Inhibition by clorgyline or deprenyl.* For these studies, enzyme solution was preincubated with clorgyline or deprenyl for 30 min at 38°. After preincubation, remaining enzyme activity was measured as described above. Preparations treated with deprenyl ($3 \times 10^{-8}$ M) and clorgyline ($10^{-7}$ M) were used as preparations of type A and B MAO respectively [11].

*A proposed mechanism of mixed substrate experiments.* When there are two substrates, $S_1$ and $S_2$, in the reaction mixture, the following reaction mechanism seems to account for the data obtained in the present study:

$$E_A + S_1 \rightleftarrows E_AS_1 \rightarrow E_A + P_1 \quad (1)$$
$$E_A + S_2 \rightleftarrows E_AS_2 \rightarrow E_A + P_2 \quad (2)$$
$$E_B + S_1 \rightleftarrows E_BS_1 \rightarrow E_B + P_1 \quad (3)$$
$$E_B + S_2 \rightleftarrows E_BS_2 \rightarrow E_B + P_2 \quad (4)$$

where $E_A$ and $E_B$ are types A and B MAO, $S_1$ and $S_2$ are tyramine and a second substrate, and $P_1$ and $P_2$ are the products respectively. In the deprenyl-inhibited mitochondrial preparation, type A MAO is active and type B MAO is inactive. When substrate for type A MAO is used as the second substrate ($S_2$), only reactions 1 and 2 should occur. The apparent Michaelis constant ($K_{m(app)}$) of oxidative deamination of tyramine at a given concentration of substrate for type A MAO can be expressed as follows:

$$K_{m(app)} = KA_1 (1 + \frac{[S_2]}{KA_2}) \quad (5)$$

where $KA_1$ and $KA_2$ are the true Michaelis constants of tyramine and substrate for type A MAO respectively.

When substrate for type B MAO is used as the second substrate, it is not oxidized by type A MAO but, if it can bind competitively with type A MAO, it can be expressed as follows:

$$E_A + S_2 \overset{KA_i}{\rightleftarrows} E_AS_2 \quad (6)$$

where $KA_i$ is the dissociation constant of type A MAO for type B MAO substrate. The apparent Michaelis constant of oxidative deamination of tyramine ($K_{m(app)}$) at a given concentration of substrate for type B MAO is expressed as follows:

$$K_{m(app)} = KA_1 (1 + \frac{[S_2]}{KA_i}) \quad (7)$$

In the clorgyline-inhibited preparation, only type B MAO is active and type A MAO is inactive. When substrate for type B MAO is used as the second substrate, only reactions 3 and 4 should occur. Then:

$$K_{m(app)} = KB_1 (1 + \frac{[S_2]}{KB_2}) \quad (8)$$

where $KB_1$ and $KB_2$ are the true Michaelis constants for tyramine and for the substrate for type B MAO. If the substrate for type A MAO is not oxidized by type B MAO and it can bind to type B MAO, then:

$$E_B + S_2 \overset{KB_i}{\rightleftarrows} E_BS_2 \quad (9)$$

Where $KB_i$ is the dissociation constant of type B MAO for the substrate for type A MAO, then:

$$K_{m(app)} = KB_1 (1 + \frac{[S_2]}{KB_i}) \quad (10)$$

## RESULTS

*Inhibition of MAO activity by clorgyline and deprenyl.* The effects of various concentrations of clorgyline and deprenyl on MAO activity in the bovine brain mitochondrial preparation are shown

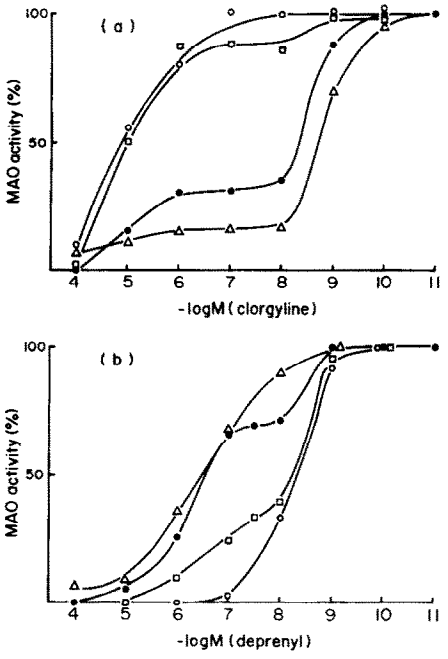


Fig. 1. Inhibition of MAO activity in bovine brain mitochondria by different concentrations of clorgyline (a) and deprenyl (b). The mitochondrial preparation was preincubated for 30 min at 38° with several concentrations of clorgyline and deprenyl. Activity is expressed as percentage of the control activity. Substrate: (△——△) 5-HT (500 $\mu$M); (●——●) tyramine (500 $\mu$M); (□——□) PEA (50 $\mu$M); and (○——○) benzylamine (500 $\mu$M).

Table 1. MAO activity remaining after inhibition of bovine brain mitochondria by deprenyl and clorgyline*

| Substrate | MAO activity (%) Deprenyl-treated mitochondria | Clorgyline-treated mitochondria |
|---|---|---|
| Tyramine (500 μM) | 70 | 32 |
| 5-HT (500 μM) | 88 | 15 |
| PEA (50 μM) | 31 | 91 |
| Benzylamine (500 μM) | 14 | 99 |

* Bovine brain mitochondria were preincubated with deprenyl ($3 \times 10^{-8}$ M) plus clorgyline ($1 \times 10^{-7}$ M) for 30 min at 38°. After preincubation, MAO activity was estimated.

Table 2. $K_m$ values for MAO of untreated, deprenyl-treated and clorgyline-treated bovine brain mitochondrial MAO*

| | $K_m$ (μM) | | |
|---|---|---|---|
| | Untreated mitochondria (types A and B MAO) | Deprenyl-treated mitochondria (type A MAO) | Clorgyline-treated mitochondria (type B MAO) |
| 5-HT | 41 | 38 | |
| Tyramine | 81 | 52 | 104 |
| PEA | 17 | | 9.1 |
| Benzylamine | 83 | | 80 |

* Bovine brain mitochondria were preincubated with either deprenyl ($3 \times 10^{-8}$ M) or clorgyline ($1 \times 10^{-7}$ M) for 30 min at 38° and, then, were used as preparations of types A and B MAO respectively. $K_m$ values were obtained from Lineweaver–Burk plots with various concentrations of labeled substrates.

in Fig. 1. MAO activity, with 5-HT as substrate, was highly sensitive to clorgyline and less sensitive to deprenyl, while MAO activity with benzylamine or PEA as substrate was highly sensitive to deprenyl and less sensitive to clorgyline, although a double-sigmoidal curve was obtained with PEA as substrate. On the other hand, when tyramine was used as substrate, the inhibition curves of clorgyline and deprenyl were both biphasic with a plateau region. These results are in agreement with previous findings [4–6, 8]. The percentages of monoamine oxidase activity remaining after inhibition by $3 \times 10^{-8}$ M deprenyl and $10^{-7}$ M clorgyline are shown in Table 1.

*Determination of* $K_m$ *values.* The $K_m$ values of various substrates for untreated (types A and B MAO), deprenyl-treated (type A MAO) and clorgyline-treated (type B MAO) preparations were calculated from Lineweaver–Burk plots; the results are summarized in Table 2. With the untreated preparation, the $K_m$ values of 5-HT and benzylamine were almost the same as those of type A MAO and type B MAO respectively. The $K_m$ value for PEA was higher than that of type B MAO, and the $K_m$ value for tyramine was intermediate between those of types A and B MAO.

*Inhibition of MAO activity by clorgyline and deprenyl in mixed substrate experiments.* The effects

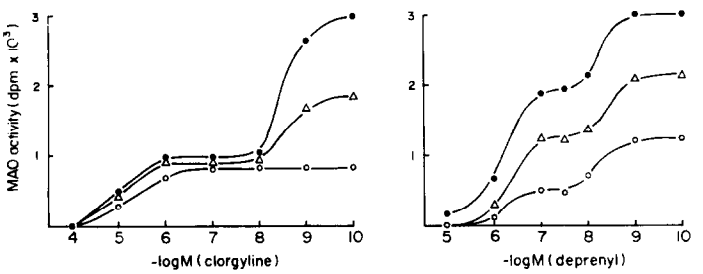


Fig. 2. Inhibition of tyramine (500 μM) deamination in bovine brain mitochondria by different concentrations of clorgyline (left panel) and deprenyl (right panel) with different 5-HT concentrations. Activity is expressed as dpm. 5-HT concentrations: (△——△) 0.3 mM; (○——○) 1 mM; and (●——●) none.

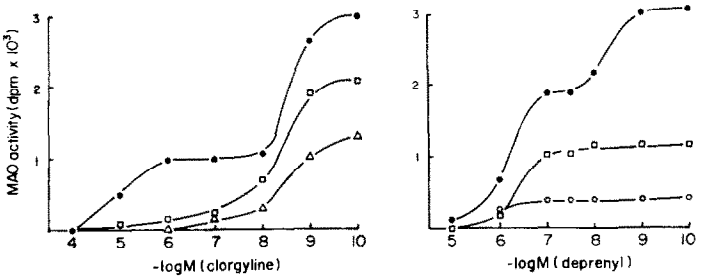


Fig. 3. Inhibition of tyramine (500 $\mu$M) deamination in bovine brain mitochondria by different concentrations of clorgyline (left panel) and deprenyl (right panel) with different PEA concentrations. Activity is expressed as dpm. PEA concentrations: (□——□) 0.1 mM; (△——△) 0.3 mM; (○——○) 3 mM; and (●——●) none.

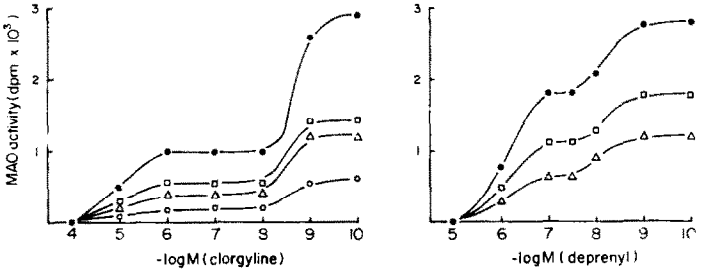


Fig. 4. Inhibition of tyramine (500 $\mu$M) deamination in bovine brain mitochondria by different concentrations of clorgyline (left panel) and deprenyl (right panel) with different benzylamine concentrations. Activity is expressed as dpm. Benzylamine concentrations: (□——□) 0.5 mM; (△——△) 1 mM; (○——○) 3 mM; and (●——●) none.

of various concentrations of clorgyline and deprenyl on [$^{14}$C]tyramine deamination in the absence and presence of different concentrations of unlabeled 5-HT are shown in Fig. 2. With 1 mM 5-HT, no inhibition of type B MAO was observed at concentrations of $10^{-4}$ M to $10^{-8}$ M clorgyline, while type A MAO was almost completely inhibited at concentrations of $10^{-8}$ M to $10^{-10}$ M clorgyline. With deprenyl, tyramine deamination by type A MAO was progressively inhibited with increase in the concentration of added 5-HT, while deamination of tyramine by type B MAO was not affected by concentrations of $10^{-8}$ M to $10^{-10}$ M deprenyl, with or without 5-HT.

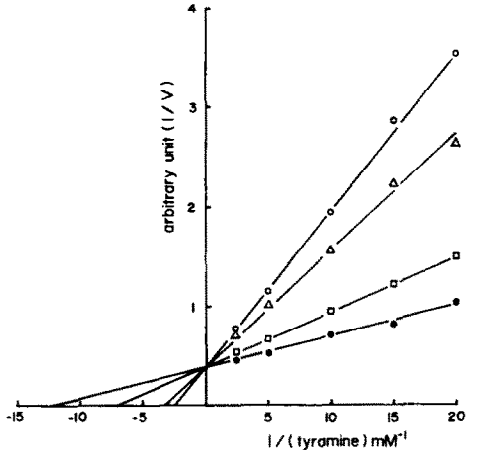


Fig. 5. Inhibition by additional unlabeled substrates of [$^{14}$C]tyramine deamination by bovine brain mitochondrial MAO. Lineweaver–Burk plots of initial velocity of tyramine deamination against tyramine concentration in the presence of unlabeled substrates. Additional unlabeled substrate: (△——△) 5-HT (300 $\mu$M); (□——□) PEA (30 $\mu$M); (○——○) benzylamine (280 $\mu$M); and (●——●) none.

The effects of various concentrations of clorgyline and deprenyl on [$^{14}$C]tyramine deamination in the absence and presence of different concentrations of unlabeled PEA are shown in Fig. 3. With either clorgyline or deprenyl, tyramine deamination of type B MAO was strongly inhibited with increase in the concentration of PEA, while that by type A MAO was also slightly inhibited by PEA.

The effects of several concentrations of clorgyline and deprenyl on [$^{14}$C]tyramine deamination in the absence and presence of different concentrations of unlabeled benzylamine are shown in Fig. 4. With either clorgyline or deprenyl, tyramine deamination by types A and B MAO was also inhibited by increase in the concentration of benzylamine added.

*Competition experiments.* The deamination of [$^{14}$C]tyramine by untreated MAO and types A and B MAO in bovine brain was studied using Lineweaver–Burk plots of activity in the absence and presence of other unlabeled substrates. Figure 5 shows the effects of unlabeled 5-HT, PEA and benzylamine on tyramine deamination by the untreated preparation. Plots of activity, with and without unlabeled substrates, intersect at the same point on the ordinate, indicating that 5-HT, PEA

Table 3. Comparison of $K_m$ values and $KA_i$ or $KB_i$ values for 5-HT, PEA and benzylamine*

| Enzyme preparation | $S_2$ | $Km$ ($\mu$M) | $K_i$ ($\mu$M) | $KA_i$ ($\mu$M) | $KB_i$ ($\mu$M) |
|---|---|---|---|---|---|
| Untreated mitochondria | 5-HT | 41 | 116 | | |
| (types A and B MAO) | PEA | 17 | 39 | | |
| | Benzylamine | 83 | 66 | | |
| Deprenyl-treated mitochondria | 5-HT | 38 | | 52 | |
| (type A MAO) | PEA | | | 78 | |
| | Benzylamine | | | 58 | |
| Clorgyline-treated mitochondria | 5-HT | | | | 1447 |
| (type B MAO) | PEA | 9.1 | | | 5.1 |
| | Benzylamine | 80 | | | 81 |

* Untreated, deprenyl-treated and clorgyline-treated mitochondria were used as enzyme sources, and tyramine was used as the first substrate ($S_1$). $K_m$ values are quoted from Table 1. The dissociation constants, in the presence of the second substrate ($S_2$), were determined from the slopes of double-reciprocal plots, using the data in Figs. 5–7. $KA_i$: the dissociation constant of type A MAO. $KB_i$: the dissociation constant of type B MAO.

and benzylamine are competitive inhibitors of tyramine deamination. The deaminations of [$^{14}$C]5-HT, [$^{14}$C]PEA and [$^{14}$C]benzylamine were also inhibited competitively by tyramine (unpublished observations). Tyramine deamination by type A MAO was competitively inhibited by unlabeled 5-HT, PEA and benzylamine and was strongly inhibited by 5-HT and benzylamine, but weakly inhibited by PEA (Fig. 6). Tyramine deamination by type B MAO was also competitively inhibited by unlabeled 5-HT, PEA and benzylamine. Tyramine deamination was strongly inhibited by PEA and weakly inhibited by 5-HT (Fig. 7).

*Comparisons of* $K_m$ *values and* $KA_i$ *or* $KB_i$ *values for 5-HT, PEA and benzylamine.* The $K_i$, $KA_i$ and $KB_i$ values, the dissociation constants of untreated, deprenyl-treated and clorgyline-treated MAO, for each substrate were determined with [$^{14}$C]tyramine as substrate, as shown in Table 3. The $K_i$, $KA_i$ and $KB_i$ for 5-HT were calculated to be: 116, 52 and 1447 $\mu$M respectively. The $KA_i$ and $K_m$ values of type A MAO for 5-HT were similar. The $K_i$, $KA_i$ and $KB_i$ values for PEA were calculated to be 39, 78 and 5.1 $\mu$M respectively. The $KB_i$ and $K_m$ values of type B MAO for PEA were very similar. The $K_i$, $KA_i$ and $KB_i$ values for benzylamine were calculated to be: 66, 58 and 81 $\mu$M respectively. The $K_m$, $KA_i$ and $KB_i$ values were almost identical.

## DISCUSSION

At least two types of mitochondrial MAO are believed to exist in differing proportions in a variety of animal tissues. In studies on sensitivity to clorgyline, Johnston [4] distinguished two types of MAO (type A MAO and type B MAO). 5-HT is a substrate for type A MAO, PEA and benzylamine are substrates for type B MAO, and tyramine can be deam-

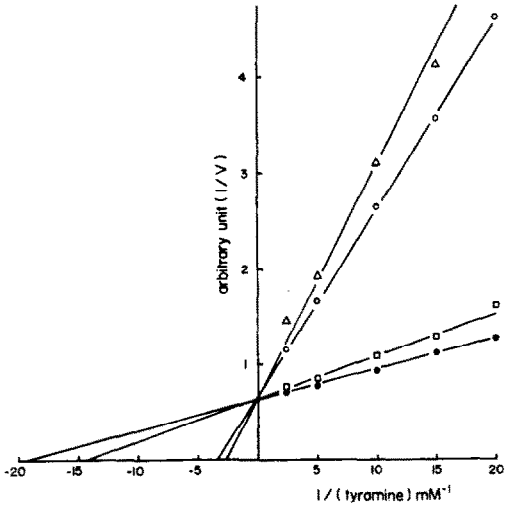


Fig. 6. Inhibition by additional unlabeled substrates of [$^{14}$C]tyramine deamination by deprenyl ($3 \times 10^{-8}$ M)-treated bovine brain mitochondrial MAO. Lineweaver–Burk plots of initial velocity of tyramine deamination against tyramine concentration in the presence of unlabeled substrates. The mitochondrial preparation was preincubated for 30 min at 38° with deprenyl. Symbols are as for those of Fig. 5.

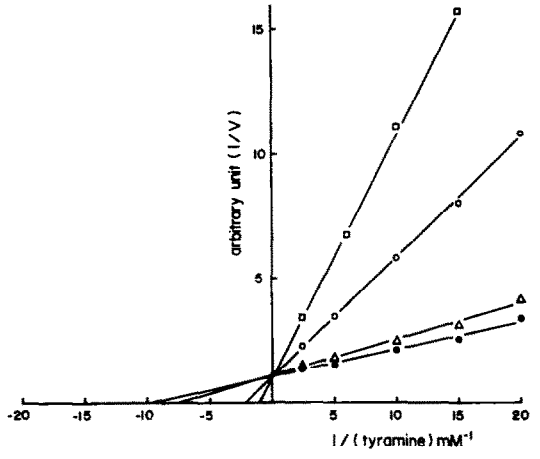


Fig. 7. Inhibition by additional unlabeled substrates of [$^{14}$C]tyramine deamination by clorgyline ($1 \times 10^{-7}$ M)-treated bovine brain mitochondrial MAO. Lineweaver–Burk plots of initial velocity of tyramine deamination against tyramine concentration in the presence of unlabeled substrates. The mitochondrial preparation was preincubated for 30 min at 38° with clorgyline. Symbols are as for those of Fig. 5.

inated by both types of MAO. Types A and B MAO of bovine brain differ in antigenic properties [12].

In the present study, activity with 5-HT was sensitive to clorgyline and fairly resistant to deprenyl, while the activities with PEA and benzylamine were sensitive to deprenyl and fairly resistant to clorgyline. When tyramine was used as substrate, the inhibition curves with clorgyline and deprenyl were both biphasic, with plateau regions at about 70% and 30% inhibition of the enzyme activity, indicating that 70% of the total activity can be attributed to type A MAO and 30% to type B MAO. In addition, when PEA was used as substrate, the inhibition curves of clorgyline and deprenyl were biphasic (Fig. 1). Biphasic curves for inhibition of PEA deamination have been obtained with MAO preparations from bovine brain mitochondria [13] and rat vas deferens [14]. It has been reported that the proportion of the two types of MAO activity varies with the concentration of PEA [15]; with the high PEA concentration, the activity is sensitive to clorgyline, whereas with the low concentration it is highly sensitive to deprenyl. These facts suggest that PEA is deaminated by both types of MAO, mainly by type B MAO, in bovine brain at this concentration of PEA (50 $\mu$M).

The $K_m$ values of various substrates for untreated MAO and types A and B MAO were calculated from Lineweaver–Burk plots (Table 2). With 5-HT, the $K_m$ value of the untreated preparation was the same as that for type A MAO; since the remaining activity of the clorgyline-treated preparation was extremely low, it was not possible to study the deamination of 5-HT by type B MAO (Table 1). With benzylamine, the $K_m$ value of the untreated preparation was similar to those of type B MAO, and the activity of type A MAO for benzylamine was very low (Table 1). With tyramine, the $K_m$ value was intermediate between those of types A and B MAO.

These results indicate that 5-HT is mainly deaminated by type A MAO, that benzylamine is mainly deaminated by type B MAO, and that tyramine and PEA are deaminated by both types of MAO.

In mixed substrate experiments, we previously showed that rat brain mitochondrial MAO has two kinds of binding sites [16]. White and Wu [17] also reported similar results on human brain in mixed substrate experiments with norepinephrine, dopamine, 5-HT, tyramine and tryptamine, although they did not use clorgyline- and deprenyl-treated preparations. In this study, we examined whether tyramine and 5-HT are deaminated at the same active site of type A MAO, and whether tyramine, PEA and benzylamine are deaminated at the same active site of type B MAO, using mixed substrates.

In the case of type A MAO, when 5-HT was used as the second substrate, the $KA_i$ calculated from the inhibition of [$^{14}$C]tyramine deamination with 5-HT (from equation 7), and the $K_m$ were 52 and 38 $\mu$M, respectively, which are very similar. The $KA_i$ for PEA was 78 $\mu$M. This value is the dissociation constant of the $E_AS_2$ complex between PEA and type A MAO, and it was much higher than the $K_m$ in the uninhibited preparation, indicating that PEA binds to type A MAO with lower affinity than to type B MAO. In the case of type B MAO, the $K_m$ values for PEA and benzylamine were 9.1 and 80 $\mu$M respectively. The $KB_i$ values, calculated from the mixed substrate experiment, were 5.1 and 81 $\mu$M respectively (from equation 10). The $K_m$ and $KB_i$ values were almost identical. The $KB_i$ for 5-HT was 1447 $\mu$M, which was much higher than the $K_m$ in the uninhibited preparation. These results indicate that, with type A MAO, tyramine, 5-HT and PEA are deaminated. 5-HT is especially a preferential substrate for type A MAO in bovine brain, whereas benzylamine is not deaminated, but the $K_m$ of type B MAO and the $KA_i$ were identical, indicating that benzylamine binds to the substrate binding site of type A MAO with almost the same rate as that of deamination by type A MAO. With type B MAO, tyramine, PEA and benzylamine are deaminated; benzylamine is especially a preferential substrate for type B MAO and 5-HT binds to the substrate binding site of type B MAO with very low affinity.

*Acknowledgement*—This work was supported in part by the Ministry of Education, Science and Culture of Japan.

## REFERENCES

1. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
2. M. D. Houslay and K. F. Tipton, *Life Sci.* **19**, 467 (1976).
3. M. Jain, *Life Sci.* **20**, 1925 (1977).
4. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
5. J. Knoll and K. Magyar, *Adv. biochem. Psychopharmac.* **5**, 393 (1972).
6. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
7. C. Goridis and N. H. Neff, *Br. J. Pharmac.* **43**, 814 (1971).
8. H-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187**, 365 (1973).
9. R. J. Wurtman and J. Axelrod, *Biochem. Pharmac.* **12**, 1439 (1963).
10. T. Yonetani, *J. biol. Chem.* **236**, 1680 (1961).
11. B. Ekstedt, *Biochem. Pharmac.* **25**, 1133 (1976).
12. R. McCauley and E. Racker, *Molec. cell. Biochem.* **1**, 73 (1973).
13. F. M. Achee and S. Gabay, *Biochem. Pharmac.* **26**, 1637 (1977).
14. E. J. Dial and D. E. Clarke, *Biochem. Pharmac.* **27**, 2374 (1978).
15. H. Kinemuchi, Y. Wakui and K. Kamijo, *J. Neurochem.* **35**, 109 (1980).
16. T. Egashira, Y. Kuroiwa and K. Kamijo, *Archs Biochem. Biophys.* **191**, 714 (1978).
17. H. L. White and J. C. Wu, *J. Neurochem.* **25**, 21 (1975).