Characteristics of Mitochondrial and Synaptosomal Monoamine Oxidase in Monkey Brain

Toru EGASHIRA, Toshinori YAMAMOTO and Yasumitsu YAMANAKA
Department of Pharmacology, Medical College of Oita, 1-1506 Idaigaoka, Hazama-cho, Oita 879-56, Japan
Accepted October 27, 1983

Abstract—Enzymic properties of monoamine oxidase (MAO) from monkey brain were studied. High MAO activity was observed in the mesencephalon and diencephalon of the brain. Highest activity in every region of the brain was found with tyramine as a substrate. Monkey brain mitochondrial MAO showed a different substrate specificity and different \( K_m \) and \( V_{max} \) values than the enzyme from mice, rats, guinea pigs and rabbits. The \( pH \) activity curves were all bell-shaped, but the \( pH \) optima were remarkably different with the various substrates used. The activities of various substrates at \( pH 7.2 \) were compared with those at the \( pH \) optimum. At the \( pH \) optimum, the activity was about 1.2-fold higher with tyramine and dopamine, 2-fold higher with \( \beta \)-phenylethylamine (\( \beta \)-PEA) and 3-fold higher with serotonin (5-HT) and benzylamine. These results were almost similar when synaptosomes from monkey brain were used. MAO activities with 5-HT and \( \beta \)-PEA were strongly inhibited by much lower concentrations of clorgyline and deprenyl, respectively. Plateau-shaped inhibition curves by these inhibitors were obtained with tyramine as the substrate. These results indicate that both the A- and B-form of MAO appear to be uniformly distributed in monkey brain, and the A-form of MAO represents approximately 35% and 50% of the total MAO activity in mitochondria and synaptosomes, respectively.

Most animals possess a high monoamine oxidase (MAO, EC 1.4.3.4) activity in liver, kidney and brain. The important functions carried out by MAO in the animal body probably include the detoxication of poisonous amines produced in the intestine and a major part of the destruction of biogenic amines at nerve endings and elsewhere.

In the relationship of brain function and monoamines, the enzyme MAO is of significant importance. There are many observations on this point, and several investigators have reported brain MAO in rats, mice, dogs, cats, rabbits, guinea pigs and humans (1–3). MAO is closely associated with and tightly bound to the outer membrane of mitochondria. Recently the characteristics and multiplicity of mitochondrial MAO have been extensively studied.

Since Johnston (4) demonstrated the existence of two different functional forms of MAO (A and B forms) in rat brain, many workers have investigated the existence of these two forms of MAO. Studies of this type show that the apparent proportions of the two forms vary widely in different organs (5) and in different animal species (6–8). None of these investigations, however, provides any information about the existence of multiple forms of MAO in monkey brain.

In the present study, the enzymic properties of MAO from the mitochondria of monkey brain were compared with those of MAO from synaptosomes. The determine whether or not MAO in monkey brain exists as the two forms of the enzyme, the substrate specificities, \( pH \) optimum and sensitivity to selective MAO inhibitors were studied.

Materials and Methods

1. Enzyme preparation: Adult monkeys
(Macaca fuscata) were used. The monkeys were anesthetized with ketalar given s.c., and the brains were quickly removed and stored at -60°C until used. Homogenates (10%) were prepared in 0.32 M sucrose, pH 7.2. Mitochondrial fractions were prepared by different centrifugation as described earlier (9). Crude mitochondrial pellets were then resuspended in the homogenization medium, and the mitochondrial subfractions were obtained by sucrose density gradient centrifugation as described by Gray and Whittaker (10) with the following modification (Fig. 1): The suspension of crude mitochondria in 0.32 M sucrose was separated into three fractions by centrifugation at 100,000 x g for 60 min in a discontinuous density gradient consisting of 0.8 M and 1.2 M sucrose. The layer between 0.8 M and 1.2 M sucrose, containing almost all the synaptosomes, was resuspended in 0.32 M sucrose and used as the synaptosomal enzyme preparation. The pellet at the bottom of the density gradient, containing all the mitochondria, was resuspended in 0.32 M sucrose and used as the mitochondrial enzyme preparation. The crude mitochondria, synaptosomes and separated mitochondria were used as the respective enzyme preparations in this experiment.

2. MAO activity: MAO activity was measured with an ammonia selective electrode by a modification (11) of the method of Mayerson et al. (12). The incubation medium consisted of 0.1 M K2PO4 buffer, pH 7.2, 1 mg of protein, various concentrations of substrate and deionized water to a final volume of 2.0 ml. Incubation was carried out for 40 min at 37°C. MAO activity was expressed as nmoles of NH3 formed/min/mg of protein. Substrate concentrations used were 1 mM tyramine and dopamine, 200 μM serotonin (5-HT) and norepinephrine, and 100 μM β-phenylethylamine (β-PEA) and benzylamine, expressed as the final concentrations. When MAO inhibitors (clorgyline, A-form MAO inhibitor and deprenyl, B-form MAO inhibitor) were used (13), the enzymes were preincubated for 60 min at 37°C with 10−12 M to 10−3 M of clorgyline or deprenyl before addition of the substrate.

To determine the MAO activity at various pH values, the following buffers were used: Mcllvain buffer (0.2 M Na2HPO4 and 0.1 M citric acid, pH 5.0–6.0), phosphate buffer (0.1 M KH2PO4 and 0.1 M Na2HPO4, pH 6.0–7.5), Tris-HCl buffer (0.2 M Tris-hydroxy methyl aminomethane and 0.1 M HCl, pH 7.5–9.0), and borate buffer (0.2 M boric acid and 0.1 M NaOH, pH 9.0–10.5).

3. Determination of Michaelis constants: Six concentrations of substrates, each run in duplicate, were utilized for each determination of Km. Other details of the assay conditions were described above. Kinetic constants (Km and Vmax) were determined from Lineweaver-Burk double reciprocal plots of values obtained from graphic representation of kinetic data.

4. Estimation of protein: Protein concentrations of the preparations were measured by the method of Lowry et al. (14) with bovine serum albumin as the standard. The protein concentrations of the enzyme preparations were adjusted to 10 mg/ml.

**Results**

1. MAO activity in various regions of monkey brain using six substrates: MAO activities in various regions of monkey brain were compared using crude mitochondria as the enzyme preparation. The highest activity was observed in the mesencephalon, diencephalon, cerebellum and telencephalon; and the medulla oblongata plus pons exhibited less activity. The substrate tyramine was
found to have the highest activity in all regions of the brain, with less activity observed with dopamine, benzylamine, \( \beta \)-PEA, 5-HT and norepinephrine. However, substrate specificities for each region of the brain were not observed (Table 1).

2. MAO activities in the brain of various animals: The rates of oxidation of various substrates by crude mitochondrial MAO from monkey brain were compared with those by mitochondrial MAO from mouse, rat, guinea pig, rabbit and bovine brains (Table 2). The activities with all substrates tested with monkey brain were lower than those obtained with other brain mitochondria from various animals, except those obtained with bovine brain. The results obtained with bovine brain MAO were similar to those obtained with monkey brain MAO. Mouse, rat, rabbit and guinea pig brain MAO showed a different substrate specificity: these activities were high with tyramine, dopamine and benzylamine.

3. MAO activities of subfractions of crude mitochondria of monkey brain: The relative rates of oxidation of six substrates by mitochondria and synaptosomes obtained by discontinuous density gradient centrifugation are shown in Table 3. The mitochondrial MAO oxidized all substrates fairly rapidly in comparison with the synaptosomal MAO.

   However, significant substrate specificity was not observed when either mitochondria or synaptosomes were used.

4. pH-Activity curve: The rates of oxidation of five substrates at various pH values were investigated using various buffers. When mitochondrial MAO were used as the enzyme preparation, the curves obtained were all bell-shaped, but the pH optima were remarkably different: that is, pH 6.6 with \( \beta \)-PEA, pH 7.8 with tyramine, pH 8.0 with dopamine, pH 8.6 with 5-HT and pH 9.0 with benzylamine (Fig. 2, left). Similar results were obtained when synaptosomes were used (Fig. 2, right).

5. Comparison of MAO activities at optimum pH and pH 7.2: The activities of mitochondrial MAO on various substrates at pH 7.2 were compared with those at the pH of the enzyme preparation.

---

**Table 1.** MAO activity in various regions of monkey brain using six substrates

| Substrate     | Telencephalon | Diencephalon | Mesencephalon | Medulla oblongata | Cerebellum |
|---------------|--------------|-------------|--------------|-------------------|-----------|
| Tyramine      | 1.03±0.03    | 2.02±0.15   | 2.99±0.28    | 0.95±0.05         | 1.79±0.18 |
| Dopamine      | 0.85±0.11    | 0.89±0.10   | 1.62±0.10    | 0.63±0.04         | 1.22±0.06 |
| 5-HT          | 0.21±0.06    | 0.25±0.02   | 0.34±0.06    | 0.07±0.01         | 0.25±0.03 |
| \( \beta \)-PEA | 0.47±0.02    | 0.73±0.04   | 0.62±0.09    | 0.18±0.03         | 0.30±0.07 |
| Norepinephrine| 0.29±0.06    | 0.16±0.01   | 0.22±0.05    | 0.08±0.02         | 0.14±0.08 |
| Benzylamine   | 0.66±0.08    | 0.62±0.08   | 0.97±0.07    | 0.38±0.01         | 0.71±0.07 |

MAO activities in five regions of monkey brain were determined at pH 7.2 using an ammonia electrode at 37°C for 40 min. Substrate concentrations used were 1 mM tyramine and dopamine, 200 \( \mu \)M 5-HT and norepinephrine, and 100 \( \mu \)M \( \beta \)-PEA and benzylamine as a final concentration. Results were expressed as nmole of \( \text{NH}_3 \) formed/min/mg of protein. Values are the mean±S.E. for five separate preparations.

**Table 2.** MAO activity in the brain of various animals

| Substrate     | Monkey | Mouse | Rat | Guinea pig | Rabbit | Bovine |
|---------------|--------|-------|-----|------------|--------|--------|
| Tyramine      | 2.31±0.17 | 2.05±0.12 | 3.33±0.43 | 5.86±1.04 | 3.05±0.07 | 1.86±0.13 |
| Dopamine      | 1.45±0.21 | 1.19±0.15 | 2.88±0.27 | 5.28±0.62 | 2.69±0.08 | 1.34±0.13 |
| 5-HT          | 0.34±0.04 | 1.86±0.08 | 2.47±0.41 | 1.21±0.15 | 1.37±0.04 | 0.38±0.02 |
| \( \beta \)-PEA | 0.53±0.08 | 0.66±0.09 | 0.89±0.14 | 1.50±0.12 | 2.87±0.06 | 0.51±0.05 |
| Norepinephrine| 0.23±0.03 | 0.28±0.03 | 0.54±0.09 | 0.53±0.09 | 0.29±0.08 | 0.26±0.02 |
| Benzylamine   | 0.72±0.11 | 2.48±0.10 | 4.03±0.38 | 1.45±0.35 | 4.00±0.15 | 0.32±0.07 |

Experimental conditions are as for Table 1. Crude brain mitochondria of various animals were used as the enzyme preparations. Values are the mean±S.E. for three separate preparations.
optima: at the pH optima, the activity was about 1.2-fold higher with tyramine and dopamine, 2-fold higher with \( \beta \)-PEA and 3-fold higher with 5-HT and benzylamine than the respective activities at pH 7.2. Similar results were obtained when synaptosomes were used as the enzyme preparation (Table 4).

6. \( K_m \) and \( V_{max} \) values of monkey brain mitochondrial and synaptosomal MAO: The \( K_m \) and \( V_{max} \) values of various substrates for monkey brain MAO were calculated from Lineweaver-Burk double reciprocal plots. The results with tyramine, dopamine 5-HT, \( \beta \)-PEA and benzylamine as substrates are shown in Table 5. Monkey brain mitochondrial MAO showed a higher \( K_m \) value for benzylamine than for the other substrates. The \( V_{max} \) values were high with benzylamine and tyramine and low with 5-HT. Similar results were obtained when synaptosomes were used as the enzyme preparation. However, lower \( K_m \) values for 5-HT, tyramine and dopamine were obtained for synaptosomal MAO in comparison with those for mitochondrial MAO.

7. Inhibition of MAO activity toward \( \beta \)-PEA, tyramine and 5-HT by clorgyline and deprenyl: The effect of increasing concentrations of clorgyline (preferentially A-form inhibitor) on MAO activity in monkey brain mitochondria is shown in Fig. 3. After
incubation at 37°C for 60 min with clorgyline.

MAO activity with 5-HT as substrate (substrate for A-form MAO) was highly sensitive, the activity with 3-PEA (substrate for B-form MAO) was less sensitive and a plateau-shaped curve was obtained with tyramine as substrate (Fig. 3, top). When corresponding experiments were performed with deprenyl (preferentially B-form inhibitor), the opposite results were obtained: 3-PEA oxidation was most sensitive and the 5-HT oxidation was least sensitive to the deprenyl. The activity with tyramine also gave a plateau-shaped curve, indicating that this substrate was oxidized by both the A- and B-forms of MAO (Fig. 3, bottom). Almost similar inhibition curves were obtained when synaptosomes were used as the enzyme preparation (Fig. 4).

The activities of mitochondrial and synaptosomal MAO on various substrates at pH 7.2 were compared with those at the pH optima. The experimental results were expressed as n mole/min/mg of protein. ( ) : Values are percentages of MAO activity with tyramine as the substrate.

### Table 4. Comparison of MAO activities at optimum pH and pH 7.2.

| Substrate | Synaptosomal MAO | Mitochondrial MAO |
|-----------|------------------|------------------|
|           | pH 7.2 | pH 7.2 | Ratio pH/pH 7.2 | pH 7.2 | pH 7.2 | Ratio pH/pH 7.2 |
| Tyramine  | 2.99 (100) | 3.12 (100) | 1.04 | 5.46 (100) | 5.59 (100) | 1.02 |
| Dopamine  | 2.03 (67.8) | 2.61 (83.6) | 1.28 | 3.95 (72.3) | 4.74 (84.7) | 1.20 |
| 5-HT      | 0.46 (15.3) | 1.02 (32.6) | 2.20 | 0.85 (15.5) | 2.65 (47.4) | 3.10 |
| β-PEA     | 0.85 (28.4) | 1.33 (42.6) | 1.56 | 1.51 (27.6) | 2.88 (51.4) | 1.90 |
| Benzylamine | 0.91 (30.4) | 2.38 (76.2) | 2.60 | 1.25 (22.8) | 3.74 (66.9) | 2.90 |

Table 5. K<sub>m</sub> and V<sub>max</sub> values of monkey brain mitochondrial and synaptosomal MAO.

| Substrate | Mitochondrial MAO | Synaptosomal MAO |
|-----------|-------------------|------------------|
|           | K<sub>m</sub> (µM) | V<sub>max</sub> (nmole/min/mg) | K<sub>m</sub> (µM) | V<sub>max</sub> (nmole/min/mg) |
| 5-HT      | 273±9.6 | 2.12±0.02 | 232±4.8 | 0.98±0.05 |
| Tyramine  | 192±9.4 | 16.12±0.30 | 161±6.0 | 18.51±0.7 |
| Dopamine  | 385±3.9 | 8.26±0.43 | 322±7.6 | 3.12±0.08 |
| β-PEA     | 102±9.1 | 6.25±0.16 | 103±1.6 | 3.84±0.11 |
| Benzylamine | 1041±61 | 42.0 ±0.76 | 1176±8.6 | 13.5 ±0.88 |

K<sub>m</sub> and V<sub>max</sub> values are calculated from Lineweaver-Burk plots with six substrate concentrations of each substrate. MAO activity was assayed at pH 7.2 using an ammonia electrode at 37°C for 40 min. Each value is expressed as the mean±S.E.

Discussion

In the present study, we examined the substrate, pH and inhibitor related characteristics of monkey brain MAO. When MAO activities in five selective regions of the brain were determined with various substrates, high activities were observed in the diencephalon and mesencephalon and slight activities were observed in the medulla oblongata and pons. Highest activities were also seen in all regions when tyramine was used as the substrate. However, no significant difference of substrate specificity with the six substrates tested was observed in the different regions of the brain. Moreover, in the present work, the pH-activity curves for each substrate by mitochondrial MAO were similar to those by the synaptosomal
enzyme, and the kinetic constants also were similar with the two enzyme preparations. Recently, Williams et al. (15) demonstrated histochemically the existence of strong MAO activity in rat hypothalamus and hippocampal regions using coronal sections. The investigations by Waldemeier et al. (16) and Demarest et al. (17) also strongly suggested the localization of the type A form of MAO within nigrostriatal dopamine neurons. Others have also reported the distribution of type A and type B MAO in discrete brain regions of the hamster (Edward and Malsburg) (18) in the individual limbic regions and the lower brain stem of the rat (Hirano et al.) (19), and in various areas of the human brain (Collins et al.) (20). From these reports, it should be of interest to investigate the relationship between the localization and function of MAO as related to the absorption and metabolism of amines in these areas.

The rates of oxidation of various substrates by mitochondrial MAO from monkey brain were compared with those by mitochondrial MAO from mouse, rat, guinea pig, rabbit and bovine brain. The activities with all substrates tested with monkey brain were lower than those obtained with the mitochondria from the brains of the other species, except those obtained with bovine brain. Bovine brain MAO and monkey brain MAO exhibited similar substrate specificity; particularly, the activities with 5-HT and β-PEA were low. Mouse, rat, rabbit and guinea pig brain MAO
showed a different substrate specificity; these activities were high with tyramine, dopamine and benzylamine. In the present study, the substrate specificity and specific activity of monkey brain MAO were in broad agreement with earlier reports (21–23) on human brain. However, there have been reports that human brain MAO shows a low activity with dopamine (24) and a high activity with 5-HT (25) or β-PEA (26).

The rates of oxidation of five substrates at various pH values were investigated using several buffers. The curves obtained were all bell-shaped, but the pH optima were remarkably different and also showed that activity was highest with tyramine as the substrate and very low with β-PEA, 5-HT and benzylamine at pH 7.2. However, the activities of various substrates at pH 7.2 were compared with those at the pH optima (see Table 3): at the pH optima, the activity was about 1.2-fold higher with dopamine, 2-fold higher with β-PEA and 3-fold higher with 5-HT and benzylamine than the respective activities at pH 7.2. Similar results were obtained when synaptosomes were used as the enzyme source. From these results, it appears likely that physiological conditions (near pH 7.0) are unsuitable for the oxidation of substrates of the A-form of MAO (5-HT) or the B-form of MAO (β-PEA or benzylamine), but suitable for oxidation of substrates of both forms of MAO (tyramine or dopamine). Previously, MAO activity has usually been determined at approximately pH 7.0, and it has been concluded that tyramine is the best substrate, while 5-HT and β-PEA are poor substrates. However, higher MAO activity could be obtained by assay at the pH optimum even if the MAO preparation had a low content of A-form or B-form MAO. Roth et al. (25) reported that the percentage of inhibition of the A-form of MAO by desmethylchlorpromazine changed only slightly upon increasing the pH from 7.5 to 9.0 with 5-HT as the substrate. This result also indicates that the effects of drugs on MAO activity may be affected by the pH of the incubation medium, in vitro.

The central role of the multiple forms of MAO in the metabolism of biogenic amines and the importance of inhibitors of this enzyme are widely recognized. Recently, inhibitor selectivity has been frequently used as criteria for the existence of multiple forms of MAO. Two forms of MAO, A-form and B-form MAO, have been found in the brains of many species including rats, guinea pigs, rabbits, cats, dogs and cows (6, 7). In rat brain, A-form MAO specifically deaminates 5-HT and is selectively inhibited by clorgyline (4), while B-form MAO acts specifically on benzylamine and β-PEA and is relatively sensitive to deprenyl (27). However, tyramine and dopamine are substrates for both forms of the enzyme and show a plateau-shaped inhibition curve when these two inhibitors are used (28).

In this experiment, MAO activity in monkey brain was highly sensitive to clorgyline with 5-HT and was less sensitive to this inhibitor with β-PEA, while a plateau-shaped inhibition curve was obtained with tyramine as the substrate. When corresponding experiments were performed with deprenyl instead of clorgyline as the inhibitor, β-PEA was highly sensitive and 5-HT was less sensitive to inhibition by deprenyl. Again, the activity with tyramine gave a plateau-shaped curve. These results also indicate the existence of two forms of MAO in monkey brain.

Clorgyline inhibition curves show a distinct difference in percentage inhibition at their plateau with tyramine or kynuramine as the substrate, indicating the relatively different activities of A-form and B-form MAO (7). Using the mitochondrial preparation from monkey brain, a typical plateau-shaped curve was obtained with tyramine (inhibited about 35% of total MAO activity with a low concentration of clorgyline). This indicates that both A- and B-forms of MAO appear to be uniformly distributed, and A-form MAO represents approximately 35% of the total MAO activity in monkey brain mitochondria and approximately 50% of the total MAO activity in monkey brain synaptosomes. However, care should be exercised in the interpretation of studies of this type because the apparent proportions of the two forms vary widely, not only in different organs (8) and in different animal species (6), but also may vary with the conditions of assay of
MAO activity, i.e., the period of preincubation
with MAO inhibitors (13) and the concentra-
tion of substrate used (29, 30).

Acknowledgements: We are grateful to Professor
W.J. Waddell, Department of Pharmacology and
Toxicology, University of Louisville, U.S.A. for
pertinent discussions and advice. We also gratefully
acknowledge the assistance of Miss M. Irie. Deprenyl
was a gift from Professor J. Knoll, Semmelweis
University of Medicine, Budapest, Hungary.
Clorgyline was from May & Barker Ltd., Dagenham,
England.

References
1 Rutledge, C.O., Azzaro, A.J. and Ziance, R.J.:
The role of monoamine oxidase in determining
the amount of monoamine released by drugs in
the central nervous system. Adv. Biochem.
Psychopharmacol. 5, 379–392 (1972)
2 Youdim, M.B.H., Holzbauer, M. and Woods,
H.F.: Physicochemical properties, development
and regulation of central and peripheral mono-
amine oxidase activity. Adv. Biochem. Psycho-
pharmacol. 12, 11–28 (1974)
3 Fuller, R.W. and Hemrick-Luecke, S.K.: An-
tagonism by 1-dopa of the elevation of hypo-
thalamic epinephrine by monoamine oxidase
inhibition in rat. Biochem. Pharmacol. 30, 3023–
3024 (1981)
4 Johnston, J.P.: Some observations upon a new
inhibitor of monoamine oxidase in brain tissue.
Biochem. Pharmacol. 17, 1285–1297 (1968)
5 Egashira, T., Ekstedt, B., Kinemuchi, H., Wiberg,
A. and Oreland, L.: Molecular turnover numbers
of different forms of mitochondrial monoamine
oxidase. Med. Biol. 54, 272–277 (1976)
6 Hall, D.W.R., Logan, B.W. and Parsons, G.H.: Fur-
ther studies on the inhibition of monoamine
oxidase by M & B 9302 (clorgyline). Substrate
specificity in various mammalian species.
Biochem. Pharmacol. 18, 1447–1454 (1969)
7 Squires, R.F.: Multiple forms of monoamine
oxidase in intact mitochondria as characterized
by selective inhibitors and thermal stabi-
lity: A comparison of eight mammalian species.
Adv. Biochem. Psychopharmacol. 5, 355–370 (1972)
8 Houslay, M.D., Tipton, K.F. and Youdim, M.B.H.:
Multiple forms of monoamine oxidase, fact and
artifact. Life Sci. 19, 467–478 (1976)
9 Egashira, T., Kuroiwa, Y. and Kamijo, K.: Multi-
ple catalytic sites of rat brain mitochondrial mono-
amine oxidase. Arch. Biochem. Biophys. 191,
714–718 (1978)
10 Gray, E.G. and Whittaker, V.P.: The isolation of
nerve endings from brain: an electron-micro-
scopic study of cell fragments derived by
homogenization and centrifugation. J. Anat.
96, 79–88 (1962)
11 Egashira, T., Yamamoto, T., Yamakana, Y. and
Kuroiwa, Y.: Preferential inhibition of the B-form
of monoamine oxidase in the liver of rats given
3-methyl-4-dimethylaminoazobenzene in the
diet. Biochem. Pharmacol. 31, 1301–1307 (1982)
12 Mayerson, L.R., McMatry, K.D. and Davis, V.E.:
A rapid and sensitive potentiometric assay for
monoamine oxidase using an ammonia selective
electrode. Anal. Biochem. 86, 287–297 (1978)
13 Egashira, T., Ekstedt, B. and Oreland, L.: In-
hibition by clorgyline and deprenyl of the
different forms of monoamine oxidase in rat
liver mitochondria. Biochem. Pharmacol. 25,
2583–2586 (1976)
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and
Randall, R.J.: Protein measurement with Folin
phenol reagent. J. Biol. Chem. 193, 265–275
(1951)
15 Williams, D., Gascoigne, J.E. and Williams, E.D.:
A tetrazolium technique for the histochemical
demonstration of multiple forms of rat brain
monoamine oxidase. Histochem. J. 7, 585–697
(1975)
16 Waldmeier, P.C., Delini-Stula, A. and Maitre, L.:
Preferential deamination of dopamine by a A
type monoamine oxidase in rat brain. Naunyn
Schmiedebergs Arch. Pharmacol. 292, 9–14
(1976)
17 Demarest, K.T., Smith, D.J. and Azzaro, A.J.: The
presence of the type A form of monoamine
oxidase within nigrostriatal dopamine-containing
neurons. J. Pharmacol. Exp. Ther. 215, 461–468
(1980)
18 Edward, D.J. and Malsburg, C.W.: Distribution of
type A and B monoamine oxidase in discrete
brain regions, pineal and pituitary glands of the
golden hamster. Life Sci. 21, 1009–1014
(1977)
19 Hirano, M., Kim, J.S., Saito, M., Uchimura, H.,
Ito, M. and Nakahara, T.: Monoamine oxidase
activities for serotonin and tyramine in individual
limbic and lower brain stem nuclei of the rat. J.
Neurochem. 30, 263–267 (1978)
20 Collins, G.G.S., Sandler, M., Williams, E.D. and
Youdim, M.B.H.: Multiple forms of human brain
mitochondrial monoamine oxidase. Nature 225,
817–820 (1970)
21 Grofe, S.S., Moses, S.G., Robins, E., Hudgens,
R.W. and Croninger, A.B.: A study of selected
catecholamine metabolizing enzymes; A com-
parison of depressive suicides and alcoholic
suicides with controls. J. Neurochem. 23, 791–
802 (1974)
White, H.L. and Wu, J.C.: Multiple binding sites of human brain monoamine oxidase as indicated by substrate competition. J. Neurochem. 25, 21–26 (1975)

MacKay, A.V.P., Davies, P., Dewar, A.J. and Yates, C.M.: Regional distribution of enzymes associated with neurotransmission by monoamines, acetylcholine and GABA in the human brain. J. Neurochem. 30, 827–839 (1978)

Owen, F., Cross, A.J. and Lofthouse, R.: Distribution and inhibition characteristics of human brain monoamine oxidase. Biochem. Pharmacol. 28, 1077–1080 (1979)

Roth, J.A., Whittemore, R.M., Shakajian, M.P. and Eddy, B.J.: Inhibition of human brain type-A and type-B monoamine oxidase by chlorpromazine and metabolites. Commun. Psychopharmacol. 3, 235–244 (1979)

Russell, S.M., Davey, J. and Mayer, R.J.: The vectorial orientation of human monoamine oxidase in the mitochondrial outer membrane. Biochem. J. 181, 7–14 (1979)

Knoll, J. and Magyar, K.: Some puzzling pharmacological effects of monoamine oxidase inhibitor. Adv. Biochem. Psychopharmacol. 5, 393–408 (1972)

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)

Suzuki, O., Katsumata, Y., Oya, M. and Matsu- moto, T.: Effect of β-phenylethylamine concentra- tion on its substrate specificity for type A and type B monoamine oxidase. Biochem. Phar- macol. 28, 953–956 (1979)

Kinemuchi, H., Wakui, Y. and Kamijo, K.: Substrate selectivity of type A and type B mono- amine oxidase in rat brain. J. Neurochem. 35, 109–115 (1980)