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DEVELOPMENTAL CHANGES IN THE ACTIVITY AND SUBSTRATE SPECIFICITIES OF MOUSE BRAIN MONOAMINE OXIDASE

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Developmental changes in monoamine oxidase (MAO) activity in the mouse brain were investigated with the substrates β-phenylethylamine (PEA), tryptamine, and 5-hydroxytryptamine (5-HT). In the newborn brain, MAO activity towards PEA was found to be much lower than the adult and to be inhibited by clorgyline in a double-sigmoidal fashion. The inhibition curve shifted to a single-sigmoidal pattern with age. MAO activity towards 5-HT as substrate was inhibited by 90% and in a single-sigmoidal manner by clorgyline throughout the postnatal life. Lineweaver-Burk plots with PEA as substrate presented two linear lines (apparent K_m: 28.6 and 4.1 μM) for the newborn and one line (apparent K_m: 11.4 μM) for the adult, respectively. The plot with high K_m value for the newborn brain disappeared in a clorgyline-treated preparation. These findings suggest that age-dependent alterations in the ratio of MAO-A/MAO-B activity affect the substrate specificity of the enzyme.

INTRODUCTION

Mitochondrial monoamine oxidase (MAO) [monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4] exists in two functional forms, MAO-

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A and MAO-B, which differ in substrate specificities and inhibitor sensitivities. The A-form is sensitive and the B-form is resistant to low concentrations of clorgyline (1). It has been generally accepted that 5-HT is a preferential substrate for MAO-A, PEA and benzylamine for MAO-B, and tryptamine and tyramine for both forms of the enzyme. However, the conflicting reports on the differential substrate specificities have recently appeared (2). It has been found, for example, that PEA is deaminated by MAO-A alone in rat heart (3, 4) and 5-HT is metabolized by both forms of MAO in a variety of tissues such as vervet monkey brain (5), beef heart (6), and mouse and rabbit liver (7). However, there is no clear evidence to account for such "unusual" substrate specificities, although some explanations have been forwarded (8, 9).

A number of studies have demonstrated age-dependent changes in MAO activity in rat (10–13), mouse (14–17), and human brain (18, 19). Most of these report an age-dependent relative increase of MAO-B. In this communication, we describe developmental changes in the activity and substrate specificities of MAOs in the mouse brain. The results suggest that the relative proportions of MAO-A and MAO-B may be essential to the substrate selectivity, at least in the mouse brain.

EXPERIMENTAL PROCEDURE

Materials. C3H mice, aged from one day postpartum (newborn) to 6 months, were used. Following decapitation the brain, excluding cerebellum, was dissected and immediately frozen until assayed. The crude mitochondrial pellets (P_2) were prepared by a slight modification of previously described method (11). All steps were carried out on ice or at 4°C. Briefly, the brain tissue was homogenized in 10 volumes of ice cold 0.32 M sucrose with Polytron (Kinematica, Switzerland) and centrifuged at 1,000 *g* for 10 min. The supernatant was recentrifuged at 19,000 *g* for 40 min to obtain the P_2 fraction. Prior to assaying, the samples were resuspended in 0.1 M phosphate buffer (pH 7.5) to adjust to a protein concentration of 1 mg/ml. The enzyme preparation was sonicated for 5 sec with an ultrasonic generator (Kaijo Denki Co., Japan). Protein concentration was determined by the method of Lowry et al. (20).

MAO assay. Radioactive substrates [1-^{14}C] β-phenylethylamine hydrochloride and [2-^{14}C]tryptamine bisuccinate were obtained from New England Nuclear (Boston, U.S.A.); [2-^{14}C]5-hydroxytryptamine creatinine sulphate was from the Radiochemical Center (Amersham, U.K.). Clorgyline hydrochloride was a gift from May & Baker Ltd. (Dagenham, U.K.). All other reagents were standard laboratory reagents of analytical grade wherever possible.

The enzyme activity was determined radiochemically by the methods of Wurtman and Axelrod (21) and McCaman et al. (22) with slight modifications. The methodological aspects have been described in detail elsewhere (23). 5-HT, PEA, and tryptamine were used as substrates for MAO-A, MAO-B, and both, respectively. Unless otherwise stated, the final concentrations of substrates after diluting with non-labeled substrates were 1 mM for 5-HT,

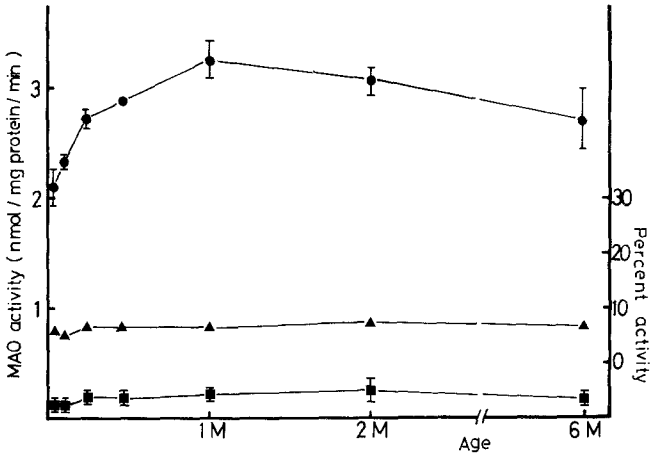

FIG. 1. Developmental changes of mouse brain MAO deaminating 5-HT. Each point is the mean ± SEM of determinations made in 4 mice. Some SEMs were smaller than the point indicators. MAO activity was determined using 1 mM 5-HT in the absence (●) and presence (■) of 10^{-7} M clorgyline. ▲; Percent activity remaining following clorgyline inhibition.

5 μM for PEA and 20 μM for tryptamine. The levels for PEA and tryptamine were near their respective K_m values estimated previously from a Lineweaver-Burk plot.

Aliquots of the enzyme preparation (0.4 ml) were preincubated at 37°C for 10 min before the reaction was initiated by the addition of the substrate solution (0.1 ml). To determine the substrate specificity, the enzyme preparation was preincubated in the presence of different concentrations of clorgyline (37°C for 10 min). After 10 min with substrate, 0.5 ml of 1 N HCl terminated the reaction. Deaminated products were extracted with vigorous shaking into 4.0 ml ethyl acetate when PEA or 5-HT was the substrate, or 4.0 ml toluene when tryptamine was the substrate. If 5-HT was served as substrate, a 3.5 ml aliquot of the organic layer was transferred to a tube containing 1.5 ml of 0.3 N HCl. After centrifugation, a 3.0 ml aliquot of the organic layer was transferred to a scintillation counting vial containing 8 ml of scintillation liquid which contained 5 g of PPO and 0.5 g of POPOP in 1 liter of toluene.

The radioactivity was measured in a liquid scintillation counter. MAO activity was determined in duplicate and expressed as nmol product formed/mg protein/min.

RESULTS

MAO activity with 5-HT as substrate gradually increased during the first month after birth and then slightly decreased (Figure 1). The proportion of MAO activity remaining following inhibition by 10^{-7} M clorgyline was constant throughout postnatal life (less than 10%). Thus the enzyme activity using 5-HT as substrate preferentially indicated MAO-A activity in any ages. On the other hand, MAO activity with PEA as substrate developed rapidly during the first month of postnatal life reach-

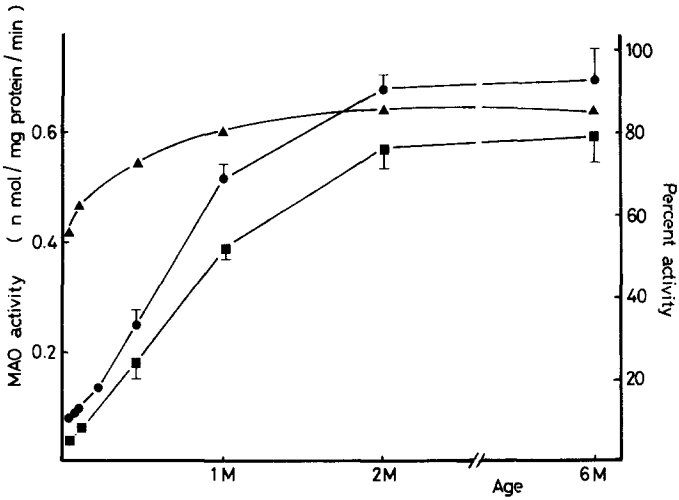

FIG. 2. Developmental changes of mouse brain MAO deaminating PEA. Each point is the mean ± SEM of determinations made in 4 mice. Some SEMs were smaller than the point indicators. MAO activity was determined using 5 μM PEA in the absence (●) and the presence (■) of 10^{-7} M clorgyline. ▲; Percent activity remaining following clorgyline inhibition.

ing a plateau by 2 months (Figure 2). MAO activity as measured by PEA in 2-month-old mice was 9 times higher than that in newborn. The proportion of MAO activity remaining following inhibition by 10^{-7} M clorgyline was 60% in the newborn, and increased with age up to 85% in the 2-month-old mice. Since the activity remaining following clorgyline inhibition is considered to be MAO-B (1), MAO-B activity is 12 times higher in 2-month-old than in newborn mice.

Developmental changes in sensitivity to clorgyline inhibition were further investigated. The inhibition curve of 5-HT deamination represented a single-sigmoidal pattern in all ages (Figure 3). A similar pattern of clorgyline inhibition was obtained using tryptamine as substrate in newborn and 2-week-old mice. This suggested that MAO is predominantly A-form (93.3% when estimated by percent clorgyline inhibition of MAO deaminating tryptamine) in mouse brain soon after birth. However, the inhibition curve of tryptamine deamination changed to a double-sigmoidal pattern in 3-month-old mice, reflecting the existence of both forms of MAO at this stage (MAO-A; 73.2%). On the other hand, MAO activity towards PEA in the newborn mouse was inhibited by clorgyline in a double-sigmoidal fashion, suggesting that PEA was deaminated by both forms

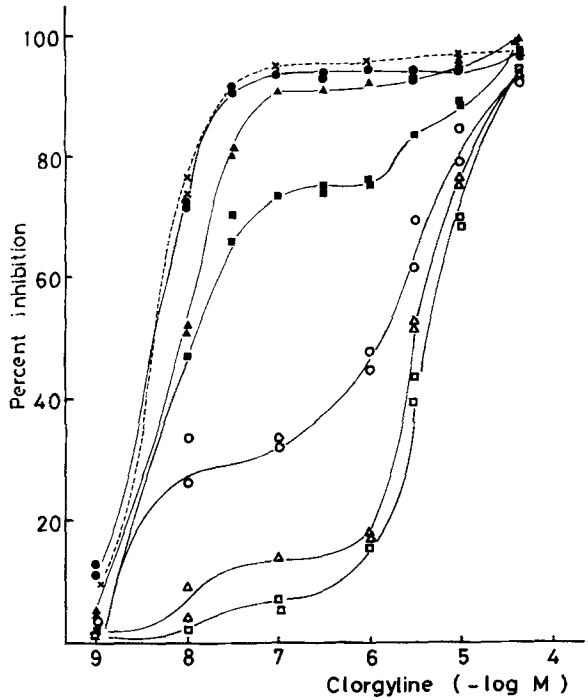

FIG. 3. The effect of three different ages on the inhibition of MAO activity by clorgyline. Samples were preincubated with 10^{-9} to 10^{-4} M clorgyline at 37°C for 10 min before the addition of substrate. 1mM 5-HT (×), 20 μM tryptamine (●; newborn, ▲; 2-week-old, ■; 3-month-old) and 5 μM PEA (○; newborn, △; 2-week-old, □; 3-month-old) were used as substrates. The inhibition curves of 5-HT deamination presented the same pattern in all ages examined.

of MAO at this stage of predominantly MAO-A. However, the inhibition curve became a single-sigmoidal pattern, typical of MAO-B, by 3 months, indicating preferential deamination of PEA by MAO-B as the enzyme activity increased.

The effect of the amount of MAO deaminating PEA on the inhibition curve was investigated. The specific MAO activity towards PEA in the newborn brain was approximately $\frac{1}{9}$ of that in 3-month-old brain under similar protein concentrations. Therefore, the enzyme solution from the 3-month-old mouse was diluted 10-fold, and inhibition curves were derived (Figure 4). While the degree of MAO inhibition by clorgyline was slightly increased in the diluted preparation, the inhibition pattern remained markedly different from that obtained for the newborn. The rel-

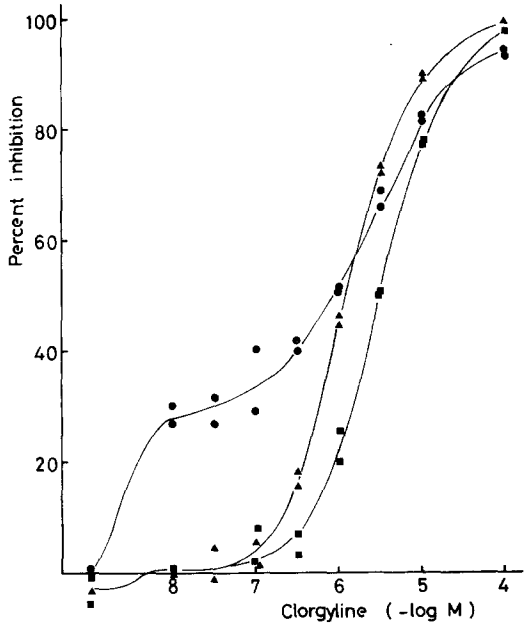

FIG. 4. The effect of the amount of MAO deaminating PEA on the clorgyline inhibition curve. The enzyme solution from the 3-month-old mouse brain (■) was diluted about 10-fold (▲) with 0.1 M phosphate buffer (pH, 7.5) to obtain the similar activity of MAO deaminating PEA as that (●) from the newborn.

age	protein concentration (mg/ml)	MAO activity towards PEA (nmol/mg prot/min)
●; one-day-old	1.25	0.15
■; 3-month-old	1.48	1.29
▲; 3-month-old	0.15	0.18

ative proportion of MAO-B was the same even if absolute activity was diminished.

To determine if kinetic properties of the enzyme change during development, Lineweaver-Burk plots for PEA deamination were compared in newborn and 2-month-old mice (Figure 5). The plots for the adult mouse demonstrated one line, with an apparent K_m of 11.4 μM and substrate inhibition at the concentrations of PEA over 10 μM. On the other hand, Lineweaver-Burk plots for the newborn mouse presented curves composed of two linear portions. The two apparent K_m values were 28.6 μM and 4.1 μM with high range (10 to 100 μM) and low range (1.25 to 10 μM)

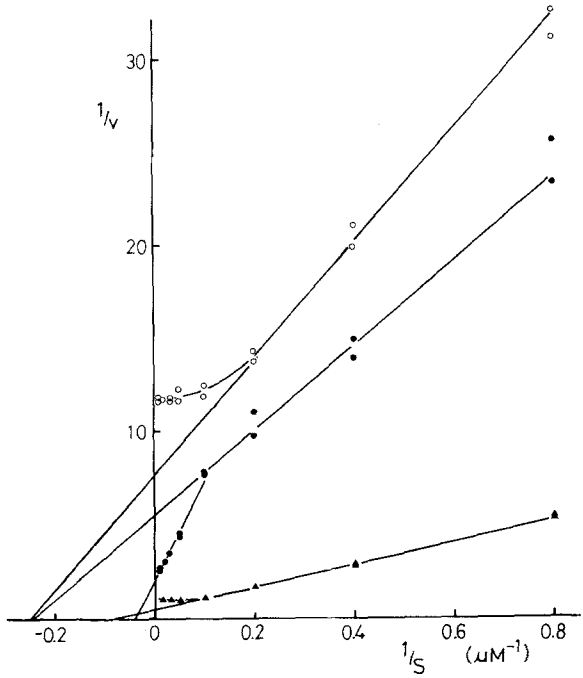

FIG. 5. Lineweaver-Burk plots of MAO deaminating PEA from two-month-old (▲) and newborn mouse brain in the absence (●) and presence (○) of 10^{-7} M clorgyline. The plots from newborn mouse resulted in two linear portions with the apparent K_m values of 28.6 and 4.1 μM and V_{max} values of 0.54 and 0.18 nmol/mg prot/min, respectively. The apparent K_m value for the adult mouse was 11.4 μM (V_{max} 1.90 nmol/mg protein/min).

PEA concentrations, respectively. The linear portion obtained at high PEA concentrations in the absence of inhibitor, disappeared when 10^{-7} M clorgyline was added. Preincubation with clorgyline and 5-HT had little effect on the Lineweaver-Burk plots in the adult mouse brain (Figure 6). This is compatible with the data demonstrating that at this age PEA is deaminated almost exclusively by MAO-B.

Since, in the newborn mouse brain, PEA appeared to be preferentially deaminated by MAO-B at low concentrations (1.25 to 10 μM), and by MAO-A at high concentrations (over 10 μM), the clorgyline inhibition patterns were compared at two different PEA concentrations (Figure 7). Plateaus were reached between 3×10^{-8} M and 10^{-7} M clorgyline in all cases. In the newborn brain 32.5% of PEA deamination was inhibited by 10^{-7} M clorgyline when 5 μM PEA was substrate. This inhibition increased to 50% using 40 μM PEA. Similarly, percent inhibition by 10^{-7}

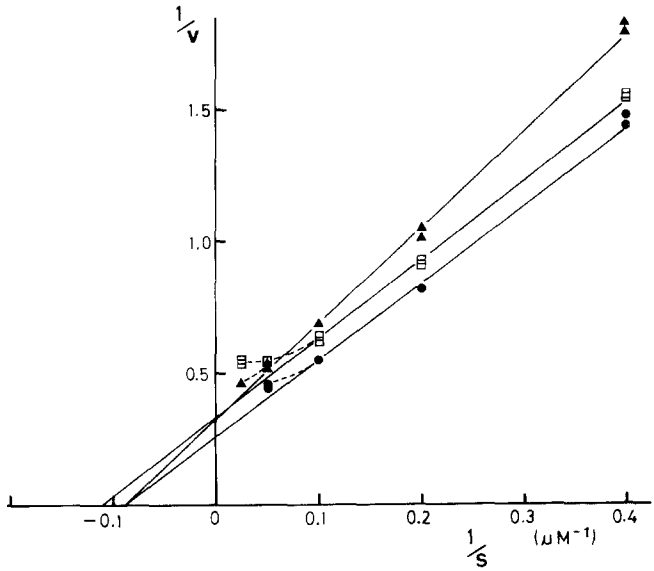

FIG. 6. Lineweaver-Burk plots of MAO towards PEA from two-month-old mouse brain in the absence of inhibitors (●) and in the presence of 10^{-7} M clorgyline (□) or 1 mM 5-HT (▲). Clorgyline was preincubated with the enzyme for 10 min before addition of PEA. 5-HT was added to the reaction mixture at the same time with PEA.

M clorgyline increased from 5% to 13% with increased PEA concentration in the adult brain.

DISCUSSION

In the present study, it was found that the two forms of MAO differ in postnatal development as reported previously (11, 13, 15). Since MAO deaminating 5-HT was exclusively inhibited by 10^{-7} M clorgyline throughout postnatal life, the enzyme activity using this substrate preferentially indicated MAO-A at all ages. Thus, the developmental change in MAO-A activity of the mouse brain followed a gradual increase during the first month after birth and then a slight decrease. A similar change in MAO-A activity with age has been found in the mouse (15, 16) and rat brain (24, 25). On the other hand, the sensitivity of MAO deaminating PEA to clorgyline inhibition changed with postnatal development. The remaining activity following clorgyline inhibition increased with age, in-

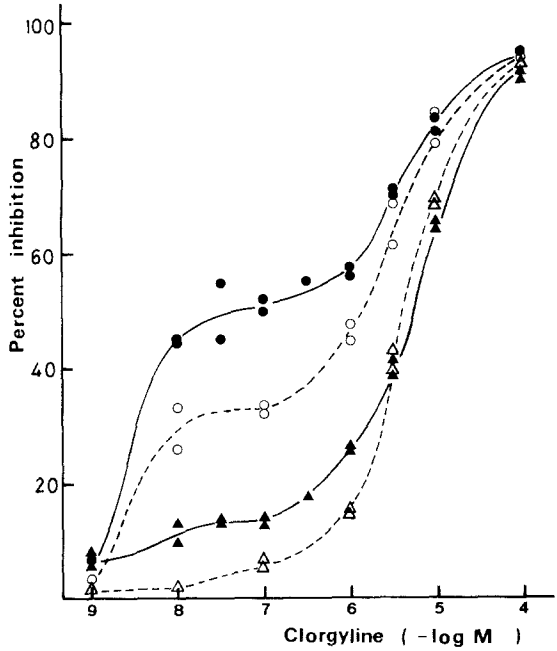

FIG. 7. The inhibition curves of MAO towards PEA at the concentrations of 5 μM and 40 μM by clorgyline. Each plot represents data from the newborn (○; 5 μM PEA, ●; 40 μM PEA) and the adult (△; 5 μM PEA, ▲; 40 μM PEA) mouse brain.

dicating that PEA is deaminated by both forms of MAO in early life and preferentially by MAO-B afterwards. This was confirmed by clorgyline inhibition curve using 5-HT and PEA as substrates (Figure 3). The inhibition curve for tryptamine, known as a common MAO substrate, changed from a single-sigmoidal to a double-sigmoidal pattern with development. Such a shift might be related to an increase in MAO-B after birth. Mantle et al. (10) also observed a rapid increase in the MAO-B/MAO-A ratio with age in the rat brain. They found that the ratio approached the adult value of 32% by the 25th day postnatal.

It has been reported that the inhibition pattern of clorgyline is affected by protein concentration and preincubation time (26), and also by the substrate concentration (27). However, these factors can not be responsible for the differences observed at different stages of development, since the same conditions were used throughout the present study. Other causes of the age-dependent changes should be considered; changes in: 1) the total activity of MAO deaminating PEA, 2) the relative proportion of MAO-B, and 3) the enzyme properties. Since the amount of MAO deam-

inating PEA did not affect the inhibition curve (Figure 4), it is likely that the important parameter for sensitivity to clorgyline is not the absolute amount of MAO-B, but its relative proportion. As to the kinetic properties of the enzyme, it has been reported that the activities of both human brain MAO-B (18) and rat brain MAO-A (25) increased with age. This was due entirely to increased amount of the respective enzyme forms, without changes in either the K_m or molecular turnover numbers. In this study, however, the different K_m values for PEA deamination were obtained from mouse brain in different ages as reported for the rat heart (28). Therefore, we can not rule out that MAO deaminating PEA in the newborn mouse brain has different properties from that in adult mouse brain.

Lineweaver-Burk plots for PEA deamination in the newborn brain presented curves composed of two linear portions with two different apparent K_m values (28.6 μM and 4.1 μM) as shown in Figure 5. The high K_m (low affinity) represented deamination by MAO-A and the low K_m (high affinity) was considered to be related to MAO-B, since the linear portion obtained at higher PEA concentrations (10 to 100 μM) disappeared in the presence of 10^{-7} M clorgyline. Thus the two different lines and K_m values may have indicated involvement of two different enzymes in the newborn brain. MAO-B has approximately 7 times higher affinity for PEA than MAO-A. On the other hand, in the adult mouse the plots using PEA as substrate exhibited one apparent K_m of 11.4 μM. Clorgyline had little effect on this plot. These may explain why PEA was metabolized by both forms of MAO when B-form activity was extremely low and why it became preferentially metabolized by MAO-B as its activity increased. Different affinities to both forms of MAO has been shown in other tissues such as rat heart (4), human brain (29), and rat brain (30, 31). Edwards et al. (28) have obtained similar results of two linear portions of the curve for PEA deamination by heart mitochondria from 4-week-old rat. Their K_m values were 28 and 8 μM, which are similar to the present data. They suggested that PEA was deaminated by both forms of MAO in the young rat heart, being metabolized predominantly by MAO-A with increased MAO-A activity as the rats aged. Thus, the changes observed in rat heart MAO seem to be opposite to those in the mouse brain.

It was found in the present investigation that more PEA is deaminated by MAO-A as the substrate concentration increases. This concurs with previous findings (30, 31) that PEA becomes a common substrate for both forms of MAO at high concentrations (50 to 1000 μM). It seems likely that substrate specificity of MAO towards PEA is determined by substrate concentration under certain conditions.

In conclusion, MAO activity towards 5-HT increased with age, while clorgyline sensitivity and kinetic properties of the enzyme remained con-

stant. And also, MAO deaminating 5-HT was shown to be exclusively MAO-A in all ages examined. This situation suggests sufficient MAO-A throughout the animal's life. On the other hand, MAO activity towards PEA demonstrated marked developmental changes in specific activity, clorgyline sensitivity, and kinetic properties. The finding that PEA was deaminated by both forms of MAO in the newborn mouse was the result of an extremely low MAO-B proportion. An age-related increase in the relative proportion of MAO-B would explain the deserved developmental changes. The effect of the MAO-A/MAO-B ratio and the substrate concentration should be carefully considered when substrate specificity is examined.

REFERENCES

1. JOHNSTON, J. P. 1968. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17:1285–1297.
2. FOWLER, C. J., CALLINGHAM, B. A., MANTLE, T. J., and TIPTON, K. F. 1978. Monoamine oxidase A and B: a useful concept? Biochem. Pharmacol. 27:97–101.
3. LYLES, G. A., and CALLINGHAM, B. A. 1979. Selective influences of age and thyroid hormones on type A monoamine oxidase of the rat heart. J. Pharm. Pharmacol. 31:755–760.
4. LYLES, G. A., and CALLINGHAM, B. A. 1975. Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart. J. Pharm. Pharmacol. 27:682–691.
5. MURPHY, D. L., REDMOND, D. E., GARRICK, N., and BAULU, J. 1979. Brain region differences and some characteristics of monoamine oxidase type A and B activities in the vervet monkey. Neurochem. Res. 4:53–62.
6. MANTLE, T. J., HOUSLAY, M. D., GARRET, N. J., and TIPTON, K. F. 1976. 5-hydroxytryptamine is a substrate for both species of monoamine oxidase in beef heart mitochondria. J. Pharm. Pharmacol. 28:667–671.
7. SUZUKI, O., MIZUTANI, S., KATSUMATA, Y., and OYA, M. 1981. Identification of type A monoamine oxidase in mouse and rabbit liver mitochondria. Experientia 37:18–19.
8. SUZUKI, O., KATSUMATA, Y., and OYA, M. 1982. Substrate specificity of type A and type B monoamine oxidase. Pages 74–86, *in* KAMIJO, K., USDIN, E., and NAGATSU, T. (eds.), Monoamine Oxidase; Basic and Clinical Frontiers, Excerpta Medica, Amsterdam.
9. TIPTON, K. F., FOWLER, C. J., and HOUSLAY, M. D. 1982. Specificities of the two forms of monoamine oxidase. Pages 87–99, *in* KAMIJO, K., USDIN, E., and NAGATSU, T. (eds.), Monoamine Oxidase; Basic and Clinical Frontiers, Excerpta Medica, Amsterdam.
10. MANTLE, T. J., GARRETT, N. J., and TIPTON, K. F. 1976. The development of monoamine oxidase in rat liver and brain. FEBS Lett. 64:227–230.
11. STROLIN BENEDETTI, M., and KEANE, P. E. 1980. Differential changes in monoamine oxidase A and B activity in the aging rat brain. J. Neurochem. 35:1026–1032.
12. LEUNG, T. K. C., LAI, J. C. K., and LIM, L. 1981. The regional distribution of monoamine oxidase activities towards different substrates: effects in rat brain of chronic administration of manganese chloride and of aging. J. Neurochem. 36:2037–2043.
13. BLATCHFORD, D., HOLZBAUER, M., GRAHAME-SMITH, D. G., and YOUDIM, M. B. H. 1976. Ontogenesis of enzyme systems deaminating different monoamines. Br. J. Pharmac. 57:279–293.

14. BAKER, P. C., HOFF, K. M., and SMITH, M. D. 1974. The maturation of monoamine oxidase and 5-hydroxyindole acetic acid in regions of the mouse brain. Brain Res. 65:255–264.
15. JOURDIKIAN, F., TABAKOFF, B., and ALIVISATOS, S. G. A. 1975. Ontogeny of multiple forms of monoamine oxidase in mouse brain. Brain Res. 93:301–308.
16. DIEZ, J. A., and MADERDRUT, J. L. 1977. Development of multiple forms of mouse brain monoamine oxidase in vivo and in vitro. Brain Res. 128:187–197.
17. SAMSA, J. M., BAKER, P. C., and HOFF, K. M. 1979. Monoamine oxidase inhibition and recovery in the mouse brain at various ages after birth. Biol. Neonate 35:249–254.
18. FOWLER, C. J., WIBERG, Å., ORELAND, L., MARCUSSON, J., and WINBLAD, B. 1980. The effect of age on the activity and molecular properties of human brain monoamine oxidase. J. Neural Transm. 49:1–20.
19. ORELAND, L., and FOWLER, C. J. 1980. The activity of human brain and thrombocyte monoamine oxidase (MAO) in relation to various psychiatric disorders. Pages 389–396, *in* SINGER, T. P., VON KORFF, R. W., and MURPHY, D. L. (eds.), Monoamine Oxidase; Structure, Function and Altered Functions, Academic Press, New York.
20. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
21. WURTMAN, R. J., and AXELROD, J. 1963. A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol. 12:1439–1441.
22. MCCAMAN, R. E., MCCAMAN, M. W., HUNT, J. M., and SMITH, M. S. 1965. Microdetermination of monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in nervous tissues. J. Neurochem. 12:15–23.
23. KOIDE, Y., KOIDE, N., ROSS, S., SÄÄF, J., and WETTERBERG, L. 1981. Monoamine oxidase in human platelets—Kinetics and methodological aspects. Biochem. Pharmacol. 30:2893–2900.
24. BOURGOIN, S., ARTAUD, F., ADRIEN, J., HENRY, F., GLOWINSKI, J., and HAMON, M. 1977. 5-hydroxytryptamine catabolism in the rat brain during ontogenesis. J. Neurochem. 28:415–422.
25. NELSON, D. L., HERBET, A., GLOWINSKI, J., and HAMON, M. 1979. [^{3}H]Harmaline as a specific ligand of MAO A-II. Measurement of the turnover rates of MAO A during ontogenesis in the rat brain. J. Neurochem. 32:1829–1836.
26. LYLES, G. A., and GREENAWALT, J. W. 1977. Observations on the inhibition of rat liver monoamine oxidase by clorgyline. Biochem. Pharmacol. 26:2269–2274.
27. FOWLER, C. J., ORELAND, L., and CALLINGHAM, B. A. 1981. The acetylenic monoamine oxidase inhibitors clorgyline, deprenil, pargyline and J-508: their properties and applications. J. Pharm. Pharmacol. 33:341–347.
28. EDWARDS, D. J., PAK, K. Y., and VENETTI, M. C. 1979. Developmental aspects of rat heart monoamine oxidase. Biochem. Pharmacol. 28:2337–2343.
29. SCHURR, A., HO, B. T., and SCHOOLAR, J. C. 1981. Human brain monoamine oxidase: one molecular entity—multiple binding sites? J. Pharm. Pharmacol. 33:165–170.
30. KINEMUCHI, H., WAKUI, Y., and KAMIJO, K. 1980. Substrate selectivity of type A and type B monoamine oxidase in rat brain. J. Neurochem. 35:109–115.
31. SUZUKI, O., HATTORI, H., OYA, M., KATSUMATA, Y., and MATSUMOTO, T. 1979. Oxidation of β-phenylethylamine by both types of monoamine oxidase: effects of substrate concentration and pH. Life Sci. 25:1231–1236.