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SEROTONIN OXIDATION BY TYPE B MAO OF RAT BRAIN

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Abstract—The two MAO types in rat brain can be selectively inhibited by administering intraperitoneal injections of clorgyline or pargyline in suitable doses. Brain mitochondria prepared from such animals exhibit type B or type A MAO activity, respectively. *In vitro* clorgyline and deprenyl dose–response curves confirmed the purity of the enzyme preparations. Specific activities and K_m values of such preparations were determined for tyramine, serotonin and benzylamine. Type B and type A MAO were found to oxidize serotonin and benzylamine, respectively, although they had low affinities. Serotonin oxidation by mitochondria prepared from clorgyline treated animals showed type B characteristics also in its heat inactivation time course.

The two forms of monoamine oxidase (MAO, EC 1.4.3.4), type A and type B, differ in their affinity for various substrates and inhibitors. Serotonin (5-HT) and norepinephrine (NE) are substrates for type A MAO which is sensitive to low doses of the inhibitors clorgyline, Lilly 51641, harmine, harmaline, PCO, etc., while benzylamine and β-phenylethylamine are specific substrates for type B MAO, which is preferentially inhibited by low concentrations of the inhibitors deprenyl, pargyline, etc. Tyramine, tryptamine and dopamine are substrates for both forms of the enzyme [1–9]. Various tissues of rat and other animals were shown to contain varied ratios of A/B [10–13] which, however, do not always fit perfectly into the above scheme of classification. The inhibition with certain selective inhibitors of MAO activity in several rabbit organs [11], rat heart [14] beef brain [15], pig, monkey and human brain, and cat, dog and rabbit liver [16, 17] showed anomalous patterns. The initial outline of substrate specificity and inhibitor sensitivity cannot always be extrapolated to various tissues and species [17] and these selectivities are relative rather than absolute characteristics.

Although 5-HT has been considered a specific substrate for type A MAO [1, 9, 12], there have been reports of 5-HT oxidizing activity resembling type B MAO in various systems such as human platelets [18], beef brain [15], pig brain and liver [19]. With beef heart MAO, Mantle *et al.* [20] observed double-sigmoid inhibition patterns with clorgyline, deprenyl and PCO using 5-HT as substrate and concluded that in the above system the classification of MAO into type A and type B is of limited value. Even in the rat tissues which were originally employed for these studies, Ekstedt [21] observed some slight 5-HT oxidation (2 per cent) by rat liver MAO after it was treated with clorgyline *in vitro*. Green and Youdim [22] proposed from pharmacological studies that although 5-HT is the natural substrate for type A, in rat brain probably some 5-HT oxidation is performed by type B MAO when type A MAO is inhibited. Evidence is presented in the present communication that in rat brain type B MAO can indeed execute 5-HT oxidation, although at a much slower rate than type A MAO.

MATERIALS AND METHODS

Male albino rats (150–175 g) were killed by decapitation and the brains were removed and homogenized in chilled 0.32 M sucrose to give a 10% suspension (w/v). The crude mitochondrial fraction was prepared according to Brody and Bain [23] and the P_2 pellet was freed of the fluffy layer by layering 8 ml of a crude mitochondrial suspension (33% with respect to original tissue weight) in 0.32 M sucrose over 16 ml of 0.8 M sucrose and centrifuging at 50,000 *g* for 30 min in the SW25 rotor of the Spinco ultracentrifuge model L. The fluffy layer at the interphase was discarded and the turbid 0.8 M sucrose layer collected, diluted with sufficient distilled water to make the sucrose concentration isotonic and then centrifuged at 144,000 *g* for 15 min. The pellets obtained are pooled and suspended in 0.32 M sucrose.

In order to prepare rat brain mitochondrial fraction with one type of MAO selectively inhibited, rats were first given i.p. injections of clorgyline (2 mg/kg) for one day or pargyline (2 mg/kg) for 3 days. The animals were killed the next day and mitochondrial fractions prepared as described above. These fractions were satisfactorily pure type B and type A MAO. The purity of the preparations was checked by measuring the inhibition of tyramine oxidation in these enzyme preparations by 10^{-8}M clorgyline *in vitro*. Whereas the enzyme activity in tissue preparations from clorgyline-treated animals was entirely unaffected by the above concentration of clorgyline, enzyme activity from the pargyline-treated animals was completely inhibited. The use of the selective inhibitor *in vivo* as opposed to *in vitro* has the advantage of not retaining much unreacted drug in the tissue preparation. The two enzyme preparations were identical except for their catalytic properties—

the preparations retained both MAO types but only one remained in the active state.

For *in vitro* inhibition studies the enzyme was preincubated with clorgyline or deprenyl for 15 min prior to addition of the substrate. Preliminary experiments indicated that this preincubation period was sufficient for the inhibition by these inhibitors to reach completion and further increase in the preincubation period did not significantly increase the degree of inhibition. MAO activity was assayed by the method of Green and Haughton [24], as described earlier [25]. When benzylamine was used, the enzyme activity was measured spectrophotometrically [26]. Preliminary experiments indicated that under the present experimental conditions, the enzyme activity is linear with respect to time and amount of enzyme employed. Protein was measured according to the method of Lowry *et al.* [27], using serum albumin as standard. The substrate amines were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. and all other chemicals were of analytical grade. Clorgyline, deprenyl and pargyline were kindly supplied by May & Baker Ltd., Dagenham, U.K., Prof. J. Knoll, Budapest and Abbott Laboratories, North Chicago, U.S.A., respectively.

RESULTS AND DISCUSSION

In vitro clorgyline dose–response curves of 5-HT and tyramine oxidation by crude mitochondria of rat brain are shown in Fig. 1. Curves b and c show inhibition patterns of brain mitochondrial MAO of untreated rats by clorgyline, in the presence of 5-HT and tyramine, respectively. 5-HT deamination is not entirely inhibited at 10^{-8}M clorgyline concentration,

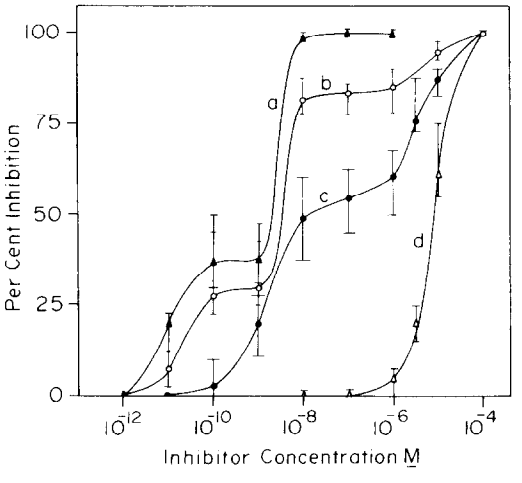

Fig. 1. Inhibition *in vitro* of monoamine oxidase activity in crude mitochondrial fraction of rat brain by clorgyline. Tyramine oxidation in control rats (●——●); 5-HT oxidation in control rats (○——○), in pargyline treated rats (▲——▲), in clorgyline treated rats (△——△). The rats were given i.p. injections of pargyline (2 mg/kg) for 3 days or clorgyline (2 mg/kg) for 1 day while the control rats were given only normal saline. Crude mitochondrial fraction of brain of these animals were prepared as described in the text. The final concentrations of the substrate amines were 0.01 M. Points represent means of 5–6 determinations; bars include all determinations.

which inhibits type A MAO completely in the same system. Some 10–15 per cent of the above enzyme activity remains at the plateau clorgyline doses which is inhibited along with type B MAO. The curve shows another brief break at the region of 10^{-10} to 10^{-9}M inhibitor concentration, which is somewhat in the middle of type A inhibition. It is noteworthy, however, that the type A portion of the tyramine–MAO inhibition curve does not show any corresponding break. Lyles and Greenawalt [28] observed a similar break in the clorgyline inhibition curve with 5-HT as substrate in roughly the same inhibitor concentration region when low enzyme concentrations were employed. This was abolished when enzyme concentrations were raised. In the present studies, however, relatively high enzyme concentrations were employed and a five-fold decrease in enzyme concentration did not alter the pattern. Prolonging the preincubation period from 15 to 30 min did not affect this break either. Whether this brief plateau region indicates the presence of two sub-groups of type A having slightly different inhibitor sensitivities is not clear. The suggestion that type A MAO may not be homogenous has been made also for another system [29]. Thus the clorgyline inhibition curve of crude mitochondrial 5-HT oxidation shows a triple-sigmoid pattern, although the ratio of the three drops in activity is not as inflexible as the A/B ratio of tyramine oxidation in the same preparation. Probably the contributions of the different types towards 5-HT oxidation change slightly from preparation to preparation and under varying enzyme concentrations. It may be recalled that Collins *et al.* [30] reported a triphasic clorgyline inhibition curve with human brain MAO using dopamine as substrate. The clorgyline dose–response curves of 5-HT oxidation by selectively inhibited mitochondrial preparations (Fig. 1, curves a and d) confirm the purity of the preparations. With mitochondrial preparations from pargyline-treated animals, 5-HT oxidation is completely inhibited by 10^{-8}M concentrations of clorgyline, which is specific for complete inhibition of type A MAO of rat brain [25], thus showing that the preparation contains only type A MAO activity. The brief break at 10^{-10} to 10^{-9}M concentrations of clorgyline also remains unaltered in this preparation. The inhibition of 5-HT deamination by the mitochondrial preparation from clorgyline-treated animals occurs at the higher clorgyline concentration region—thus showing type B characteristics. Such characteristics of the preparations are also manifested in Fig. 2, where dose–response curves of deprenyl in presence of 5-HT are illustrated. Curve 2b shows results from brain mitochondria of untreated rats. The latter part of this curve resembles curve 2c, which shows the inhibition pattern of mitochondrial preparation obtained from pargyline-treated rats. With brain mitochondria of untreated rats, low concentrations of deprenyl specific for inhibition of type B MAO cause a slight but distinct inhibition of 5-HT oxidation which accounts for about 15 per cent of the total enzyme activity, corroborating the previous observations with clorgyline. Contrary to the suggestion of Green and Youdim [22] that, in rat brain, type B MAO may oxidize 5-HT when type A MAO is completely

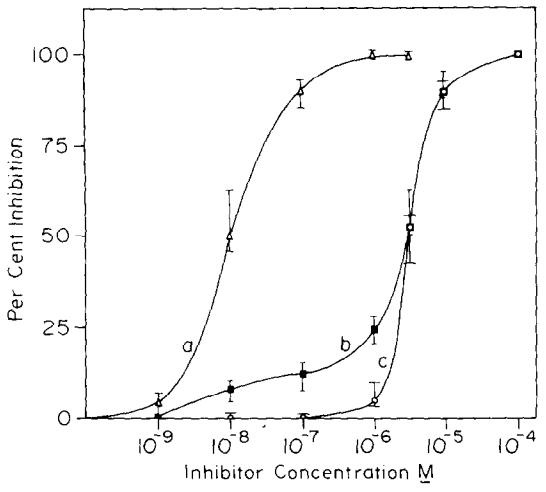

Fig. 2. Inhibiton *in vitro* of 5-HT oxidation by deprenyl in crude mitochondrial fraction of rat brain. Control rats (■——■); clorgyline treated rats (△——△) and pargyline treated rats (○——○). Other details are as in Fig. 1. Points represent means of 5–6 determinations; bars include all determinations.

inhibited, the present results indicate that type B MAO oxidizes 5-HT slightly even when type A MAO is fully active. Curve 2a represents the inhibition pattern of brain mitochondrial MAO prepared from rats given i.p. injection of clorgyline to inhibit selectively type A MAO. This reveals type B characteristics in being completely inhibited by 10^{-6}M deprenyl, which is specific for complete inhibition of type B MAO. The results presented in Table 1 describe the specific activities and K_m values of selectively inhibited rat brain mitochondrial MAO preparations. It is evident from the table that the specific activity of such preparations with type A MAO activity is very low with benzylamine, while the same is true for serotonin when mitochondrial preparations with type B MAO activity are employed.

From the above studies of clorgyline inhibition *in vivo* and *in vitro* it appears that some 10–15 per cent of 5-HT oxidation in crude mitochondrial preparation is carried out by type B MAO. About 45 per cent of total MAO activity in crude mitochondria of rat brain is type B [11], which carried out about 10–15 per cent of the total 5-HT oxidation. It may be mentioned here that with a rat liver preparation Ekstedt [21] observed 2 per cent of total 5-HT deamination after selectively inhibiting the preparation with clorgyline *in vitro* and may possibly have inhibited a little more MAO than just type A. However, this may be a reflection of tissue variation in the same species, which is not uncommon [10, 11]. The observed difference between the present results and those of Ekstedt [21] may also be due to the higher substrate concentration employed in this study. It is known, however, that rat liver contains a somewhat higher proportion of type B MAO than rat brain [10, 11].

Figure 3 depicts the degree of heat inactivation of MAO of brain mitochondrial preparations of untreated rats and rats given clorgyline and pargyline, following preincubation at 55° for different time intervals. The enzyme activity was finally assayed in the presence of tyramine and 5-HT. It has been shown in recent communications [31] that the heat inactivation curves of MAO using different substrates show only a little difference due to thermal treatment at 50°. We feel that rather than using specific substrates to represent the different MAO types, using tyramine (a very good substrate for both forms) with the two selectively inhibited MAO preparations, as used in the present study, will give a clearer picture of heat sensitivity of the two MAO types. The earlier reports of difference in thermal stability between MAO types [2, 11, 13] were followed by other communications in which it was argued that the above difference is largely dependent on purification, dilution factors, etc. [32] and treatment of the purified enzyme preparations with chaotropic agents which abolish the A–B difference in inhibitor sensitivities also abolish completely their difference in thermal stability [33]. The preparations were not subjected to any elaborate fractionation procedure or dilution. Thus those preparative factors were eliminated. As is evident, tyramine oxidation by the two types show a great deal of difference in

Table 1. Specific activities and K_m values of type A and type B MAO of selectively inhibited rat brain mitochondrial preparations*

Substrate	Mitochondria with type A MAO activity		Mitochondria with type B MAO activity	
	Specific activity (nmoles product/hr/mg protein)	K_m (M)	Specific activity (nmoles product/hr/mg protein)	K_m (M)
Tyramine	182.8	4.0×10^{-4}	169.7	2.0×10^{-4}
Serotonin	196.4	3.3×10^{-4}	35.4	2.5×10^{-3}
Benzylamine	34.1	1.0×10^{-3}	172.0	1.4×10^{-4}

* The rats were given i.p. injections of clorgyline or pargyline. Brain mitochondrial fractions were prepared as described in the text and the resulting mitochondrial samples contained either type A or type B MAO activity. For determination of specific activity, 0.01 M of serotonin and tyramine and 0.005 M benzylamine were used. The mitochondrial preparation obtained from brains of untreated rats showed the following specific activities against the substrates given in parentheses: 397.3 (tyramine); 257.1 (serotonin); 185.2 (benzylamine).

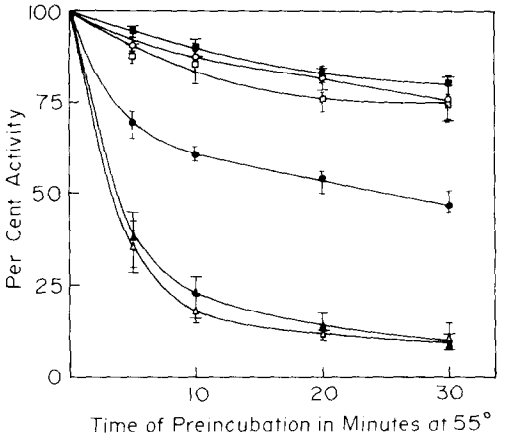

Fig. 3. Heat inactivation time course of MAO types in rat brain mitochondria. Mitochondria from untreated rats: tyramine oxidation (●——●); 5-HT oxidation (○——○). Mitochondria with type A MAO activity (pargyline treated rats): tyramine oxidation (□——□); 5-HT oxidation (■——■). Mitochondria with type B MAO activity (clorgyline treated rats): tyramine oxidation (△——△); 5-HT oxidation (▲——▲). The mitochondrial preparations were preincubated at 55° for different time intervals as specified in the figure before enzyme activity was assayed. Points represent means of 5–6 determinations; bars include all determinations. Other details are as in Fig. 1.

their heat inactivation time course at 55° which is particularly remarkable after 10 min preincubation when 80 per cent inactivation of type B MAO occurs as against 10 per cent of type A. The 5-HT deaminating activity of the clorgyline-treated preparation shows type B characteristics in being more than 75 per cent inactivated within the first 10 min of preincubation at 55°. The fact that the two MAO forms can be selectively inhibited *in vivo* [34, 35] indicates that *in situ* the two forms possibly exist in different lipid microenvironments [33, 36]. The difference in heat sensitivity of the MAO types of selectively inhibited mitochondrial preparations as shown here is possibly a reflection of the difference in the lipid microenvironments of the MAO types.

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