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INHIBITION PATTERNS OF MONOAMINE OXIDASE IN SUB-FRACTIONS OF RAT BRAIN MITOCHONDRIA IN PRESENCE OF SOME SELECTIVE INHIBITORS

CHHANDA MITRA and S. R. GUHA
Indian Institute of Experimental Medicine, Calcutta 32, India

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Abstract—*In vitro* inhibition patterns of MAO activity in different mitochondrial sub-fractions of rat brain in presence of harmine, harmaline and deprenyl with tyramine and serotonin as substrates were investigated. The results indicate that type A and type B MAO are distributed in different ratios among the mitochondrial sub-fractions.

There are at least two forms of the enzyme MAO (monoamine: O_2 oxido-reductase (deaminating), EC 1.4.3.4) designated as type A and type B which differ in their affinity to certain substrates and inhibitors [1–9]. Type A MAO mainly oxidizes serotonin and norepinephrine, and type B MAO oxidizes benzylamine and β-phenyl-ethylamine while tyramine, tryptamine and dopamine are acted upon by both types. Some inhibitors have been called selective inhibitors of MAO since they can inhibit preferentially the activity of one type of MAO thereby blocking the oxidation of particular substrates [4, 6]. On account of biochemical heterogeneity of rat brain mitochondria [10–13] the present work employed sub-fractions of rat brain mitochondria to study the inhibition patterns of MAO activity in presence of three selective inhibitors of MAO e.g. harmine, harmaline and deprenyl of which the first two are known to inhibit type A MAO[4–6] preferentially and the last one is partial towards type B MAO but at higher concentrations deprenyl also inhibits type A MAO [1, 4, 7]. *In vitro* inhibition patterns with the above inhibitors were studied using serotonin and tyramine as substrates.

MATERIALS AND METHODS

Whole brains of adult male albino rats (150–175 g) were used, and mitochondrial sub-fractions were obtained by sucrose density gradient centrifugation according to De Robertis *et al.* [14] with some modifications as described previously [15]. Five fractions to be described as A, B, C, D and E using the terminology of De Robertis *et al.* [14] resulted, of which C, D and E contain more than 90 per cent MAO activity of the original crude mitochondria and these fractions were used for the present study. For the determination of MAO activity tyramine, serotonin or benzylamine was used as substrate and the activity was assayed according to Green and Haughton [16] as described previously [15]. In some experiments the enzyme activity was measured by determining the amount of ammonia formed which was absorbed in Conway microdiffusion cells, according to the method of Conway and Byrne [17], and estimated by nesslerization. The enzyme was allowed to preincubate with the inhibitors for 10 min at 37° prior to addition of the substrate. Any further increase in preincubation period failed to increase the degree of inhibition significantly. All incubations were carried out at 37° for 30 min with air as the gas phase. Preliminary experiments indicated that the reaction proceeds linearly under these conditions with respect to time and enzyme concentration employed. The mitochondrial sub-fractions used in this study had average specific activities expressed as nmoles of ammonia formed/mg protein/min as follows: with tyramine specific activities of fractions C, D and E were 7.4, 12.1 and 13.8 respectively; with serotonin specific activities of fractions C, D and E were 6.3, 7.7 and 4.8 respectively; and with benzylamine the specific activities of fractions C, D and E were 2.5, 7.5 and 11.8 respectively. Protein was determined by the method of Lowry *et al.* [18].

RESULTS AND DISCUSSION

In vitro inhibition patterns of MAO activity in different mitochondrial sub-fractions by harmine are shown in Fig. 1. Harmala alkaloids are known to block the oxidation of serotonin preferentially, so serotonin oxidation is more strongly inhibited than tyramine oxidation with each inhibitor dose employed. In presence of serotonin MAO inhibition curves of all the fractions are very similar. With fraction C, inhibition curve of tyramine oxidation follows that of serotonin oxidation very closely. With fractions D and E the two curves move closely at lower inhibitor concentrations but diverge later at higher doses and the gap between the two inhibition curves widens from fraction C to fraction E. Inhibition curves of tyramine oxidation with fractions C and D show an initial steep drop in activity followed by a sort of plateau which in case of fraction C is only a region of decreased slope leading eventually to almost complete inhibition and with fraction D this region is followed by another drop in activity. In case of fraction E no plateau is discernible; however, there is a steeper drop in activity in the region corresponding to the second phase of inhibition in fraction D.

With harmaline (Fig. 2) similar inhibition patterns were observed. When serotonin is used, MAO activity of fraction C was completely inhibited by a 10^{-5} M

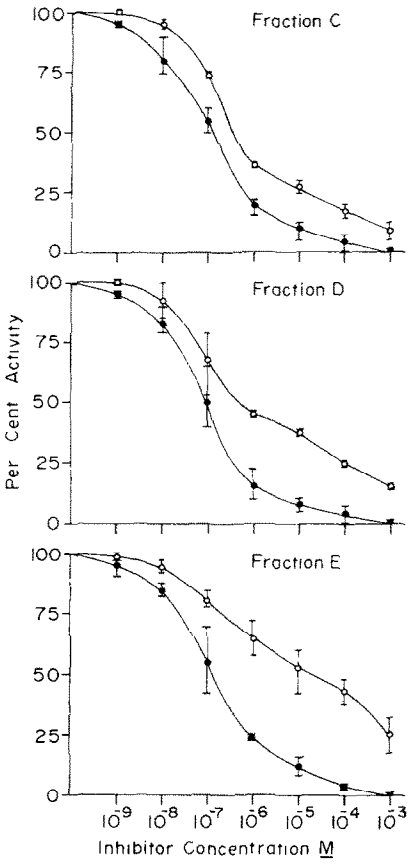

Fig. 1. *In vitro* inhibition of monoamine oxidase activity in sub-fractions of rat brain mitochondria by harmine using serotonin (●) or tyramine (○) as substrate. Each value in the figure is average of 5–6 experiments and the bar encompasses all determinations.

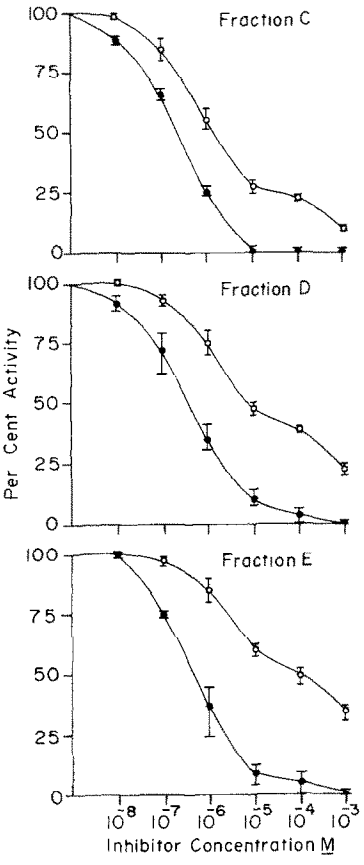

Fig. 2. *In vitro* inhibition of monoamine oxidase activity in sub-fractions of rat brain mitochondria by harmaline using serotonin (●) or tyramine (○) as substrate. Each value in the figure is average of 5–6 experiments and the bar encompasses all determinations.

concentration of the inhibitor and in the case of fractions D and E a slight percentage of activity remained at that inhibitor dose which had to be increased 100 fold to achieve complete inhibition. In presence of tyramine, MAO inhibition curves of all the fractions indicated a plateau at the 10^{-5} M–10^{-4} M region of inhibitor concentration, which is followed by a slight drop in activity in each case. However, there is a considerable difference in the percentage of tyramine deaminating activity remaining uninhibited at the plateau from fraction to fraction.

The inhibition patterns of MAO activity are completely reversed in presence of deprenyl (Fig. 3) and serotonin deaminating activity is very little affected by low doses of deprenyl which however inhibit tyramine oxidation appreciably. Tyramine–MAO inhibition curves show a distinct double sigmoid pattern with a plateau in between, particularly with fractions C and D. The percentage of activity at the plateau varies greatly from fraction to fraction—from 75 per cent with fraction C to about 58 per cent with fraction D and about 35 per cent in the same region with fraction E, which however, does not show a distinct plateau. Thus with this inhibitor also there is a gradual increase in the gap between the two inhibition curves from fractions C to E. With selective MAO inhibitors the position of the plateau is generally taken as an indication of A/B ratio [8]; and from the percentage activity at the plateau region of tyramine–MAO inhibition curves in presence of deprenyl (Fig. 3), A/B ratios of the different mitochondrial sub-fractions are calculated and found to be 75/25, 58/42 and 35/65 for fractions C, D and E respectively. The ratios of specific activities of these fractions with serotonin and benzylamine as substrates give the following comparable values of 71/29, 51/49 and 29/71 for fractions C, D and E respectively. In the present study the results with type A inhibitors and type B inhibitor for each fraction corroborate nicely indicating that type A and type B MAO are distributed in different ratios among the mitochondrial sub-fractions, fraction C being made up mainly of type A MAO and fraction E of mainly type B MAO while fraction D is more or less an even mixture. From the above results it is also apparent that while deprenyl preferentially inhibits type B MAO, it also inhibits type A MAO at a higher concentration. The harmala alkaloids on the other hand inhibit type A MAO at lower doses but type B MAO is quite resistant to them.

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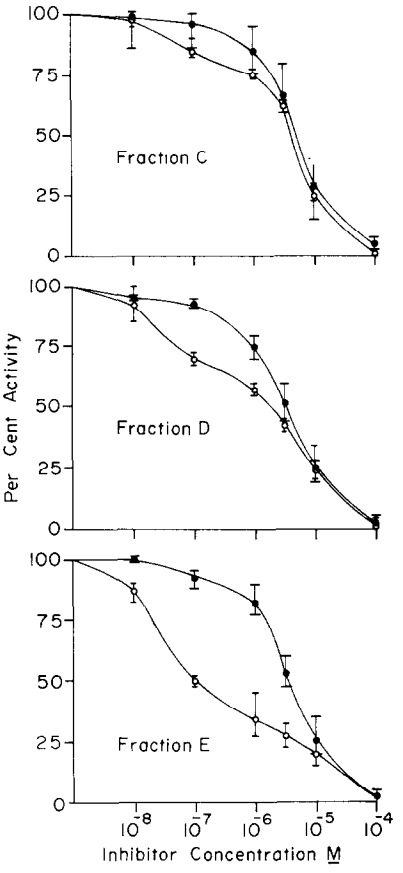

Fig. 3. *In vitro* inhibition of monoamine oxidase activity in sub-fractions of rat brain mitochondria by deprenyl using serotonin (●) or tyramine (○) as substrate. Each value in the figure is average of 5–6 experiments and the bar encompasses all determinations.

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