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INHIBITION OF MONOAMINE OXIDATION IN SUB-FRACTIONS OF CRUDE MITOCHONDRIA OF RAT BRAIN BY CLORGYLINE AND LILLY 51641

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Abstract—There are at least two forms of monoamine oxidase (MAO), designated as type A and type B, which differ in their substrate specificities and sensitivities to certain inhibitors. Inhibition patterns of MAO activity of subfractions of crude mitochondria of rat brain by clorgyline and Lilly 51641 were studied using tyramine and serotonin as substrates. It was observed that type A and type B MAO are distributed in different ratios among the mitochondrial sub-fractions investigated.

Recently there have been reports of biochemical heterogeneity of rat brain mitochondria [1–5]. Various reports indicate that there are at least two forms of monoamine oxidase (MAO) which are designated as type A and type B and differ in their substrate specificity and sensitivity to certain inhibitors [5–14]. Johnston [6] showed that type A MAO is very sensitive to inhibition by low concentrations of clorgyline. The considerably less sensitive form is described as type B MAO. Knoll *et al.* [13] and others [7, 10, 11] showed the presence of these two forms in tissues of various species. It has been shown that type A MAO mainly oxidises serotonin and norepinephrine and that type B MAO oxidises benzylamine and β-phenylethylamine, while tyramine, dopamine and tryptamine are substrates for both forms of the enzyme [8, 9]. The present work was undertaken to characterise MAO activity of the sub-fractions of crude mitochondria of rat brain obtained by the technique of sucrose density gradient centrifugation. Inhibition patterns *in vitro* of MAO activity by clorgyline and Lilly 51641 (chlorophenoxy ethyl cyclopropylamine) were studied using tyramine and serotonin as substrates. The partial separation of MAO types A and B by density gradient centrifugation of crude mitochondria of rat brain, has recently been reported [5, 14].

For each experiment whole brain of four adult male albino rats (150–175 g) were homogenised in 0.32 M sucrose as 10 per cent suspension and centrifuged at 900 *g* for 10 min. The supernatant was then centrifuged at 11,000 *g* for 20 min and the sediment was washed once with 0.32 M sucrose, then recentrifuged at 11,000 *g* for 20 min. The sediment obtained represents the crude mitochondrial fraction which was dispersed in 0.32 M sucrose at 33 per cent of the original tissue weight and subfractionated in a discontinuous sucrose density gradient consisting of 5 ml each of 1.4, 1.2, 1.0 and 0.8 M sucrose. This was described by De Robertis *et al.* [15] with slight variations. The gradient tube was kept at ice-cold condition for 2 hr before use. Five ml of the crude mitochondrial suspension was layered over the gradient and centrifuged for 2 hr at 50,000 *g* in rotor SW 25 of the Spinco Model L ultracentrifuge. The crude mitochondrial layer was fractionated into four layers and a pellet and the terminology used by De Robertis *et al.* [15] is used here. Of these five fractions C, D and E contain more than 90 per cent MAO activity of the original crude mitochondria sub-fractionated and so these three fractions were used for the present studies. Bands C and D were separated, diluted and centrifuged at 100,000 *g* for 30 min. These pellets and the pellet E were resuspended in 0.25 M sucrose. MAO activity was determined according to Green and Haughton [16]. The reaction mixture contained 0.025 M phosphate buffer pH 7.0, 0.01 M tyramine or serotonin, 0.0125 M semicarbazide and 0.5–1.2 mg of mitochondrial protein in a final volume of 2.0 ml. When clorgyline and Lilly 51641 were added, they were incubated with the enzyme for 30 min and 10 min respectively at 37° prior to addition of the substrate. Any further increase in preincubation period failed to produce any significant increase in degree of inhibition. All incubations were done at 37° for 30 min with air as the gas phase. Preliminary experiments with the different fractions indicated that the enzyme activity under these conditions progressed linearly with respect to time and enzyme concentration employed.

Inhibition patterns *in vitro* of MAO activity in different sub-fractions of crude mitochondria of rat brain by Lilly 51641 are shown in Fig. 1. It is evident that serotonin oxidation in all the three fractions is more susceptible to inhibition than tyramine oxidation. This difference in inhibitor sensitivity is most marked in fraction E and tyramine oxidation by fraction E is very little affected by low doses of Lilly 51641. The first phase of inhibition of MAO activity in presence of serotonin and tyramine occurs at inhibitor concentrations of 10^{-8} M and 5×10^{-8} M and further increases of inhibitor doses up to 10^{-6} M produce a gradual increase in inhibition of enzyme activity. These regions of the curves resemble a plateau followed by a sharp drop in enzyme activity when concentration of Lilly 51641 is increased beyond 10^{-6} M. A gradual transition of inhibition patterns from fraction C to fraction E is observed with the gap between the two inhibition curves of serotonin and tyramine oxidation progressively widening.

Tyramine oxidation by fraction E is only slightly

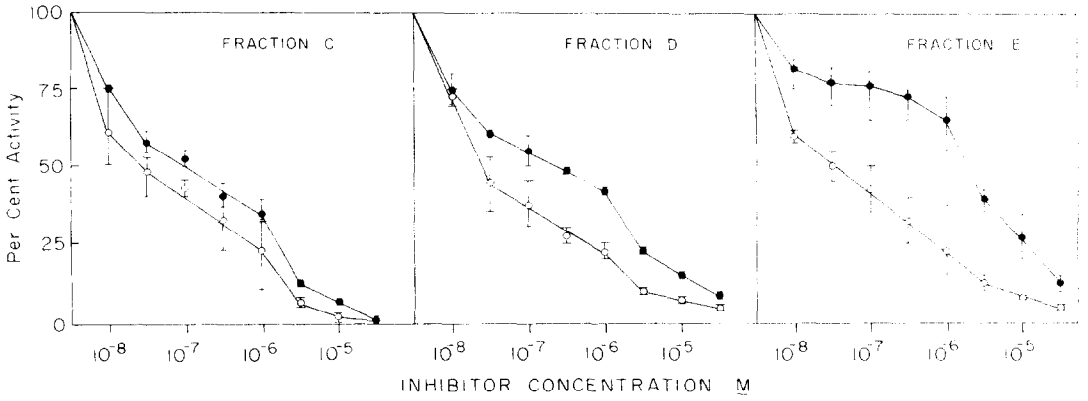

Fig. 1. Inhibition *in vitro* of monoamine oxidase activity in sub-fractions of crude mitochondria of rat brain by Lilly 51641. O——O serotonin, ●——● tyramine. Each value in the figure is average of 5–6 experiments and the bar encompasses all determinations.

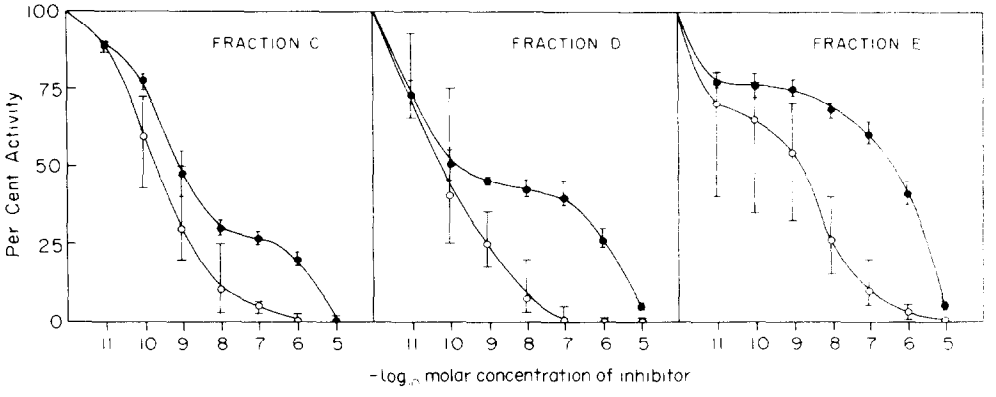

Fig. 2. Inhibition *in vitro* of monoamine oxidase activity in sub-fractions of crude mitochondria of rat brain by clorgyline. O——O serotonin, ●——● tyramine. Each value in the figure is average of 5–6 determinations and the bar encompasses all determinations.

inhibited by low doses of clorgyline (Fig. 2) doses that have been reported to be specific for inhibiting type A MAO [6]. In the region of 10^{-11} to 10^{-9} M concentrations of clorgyline a plateau is observed and the inhibition of MAO activity is only about 25 per cent and further increase of inhibitor concentration is required to produce marked inhibition of enzyme activity. When serotonin is used, MAO activity of fraction E indicated a double sigmoid inhibition curve in presence of clorgyline with a plateau in the region of 10^{-11} to 10^{-9} M inhibitor concentrations. Fraction D in presence of tyramine also shows a typical biphasic inhibition curve with a plateau in between as seen with whole brain MAO [6]. At the plateau a considerable difference in the percentage inhibition of tyramine and serotonin oxidation is observed. Almost 100 per cent inhibition of serotonin is accompanied by about 55 per cent inhibition of tyramine oxidation. The second phase of tyramine–MAO inhibition occurs at clorgyline concentrations of 10^{-7} to 10^{-5} M. Fraction C shows a similar inhibition pattern but the first part of the inhibition curve accounts for about 75 per cent inhibition of tyramine oxidation and it follows the inhibition curve of serotonin oxidation more closely. The remarkable difference among the fractions is that the part of tyramine–MAO sensitive to low concentrations of clorgyline shows a progressive increase in sensitivity from fraction C to fraction E as far as I_{50} value is concerned. It was observed that the first phase of the inhibition is complete and the plateau commences at an inhibitor concentration of 10^{-8} M, in the case of fraction C, which incidentally is similar to that observed with crude mitochondrial fraction (unpublished observation). With fraction D the plateau starts at 10^{-10} M concentration of clorgyline while in the case of fraction E it is 10^{-11} M. Oxidation of serotonin by these fractions is however almost completely inhibited by 10^{-7} M clorgyline. In contrast to serotonin–MAO inhibition curves of fractions C and D which are of sigmoid character, the serotonin–MAO inhibition curve of fraction E showed a double sigmoid appearance which to our knowledge has not been previously reported.

When the different fractions are preincubated with clorgyline for 10 min it was observed although not shown here that the first phase of tyramine–MAO inhibition curve is similar to that obtained with 30 min preincubation period as shown in Fig. 2, indicating that the part of MAO activity sensitive to low clorgyline doses is completely inhibited within 10 min. But the second

phase of the inhibition curve slightly shifts to the left from the region of 10^{-6}–10^{-4} M to 10^{-7}–10^{-5} M on increasing preincubation time from 10 to 30 min, which is understandable considering that this phase representing the more clorgyline-resistant type MAO takes longer preincubation period for the inhibition to be complete.

The above results indicate that type A and type B MAO are distributed in different ratios among the mitochondrial subfractions. Fraction C is predominantly made up of type A MAO and fraction D is more or less an even mixture of both types. Fraction E is predominantly type B MAO with only slight contamination of type A MAO.

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