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Human brain monoamine oxidase: one molecular entity-multiple binding sites?

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Monoamine oxidase (MAO) of human brain cortex was partially characterized by using different substrates and inhibitors. Two K_m values were calculated for each of the three substrates tested, i.e., phenethylamine (PEA), benzylamine (BA) and 5-hydroxtryptamine (5-HT). Clorgyline and 5-HT, both known as MAO-A occupants, were able to abolish the second (high) K_m deamination of PEA. 5-HT, while non-competitively inhibiting the deamination of low BA concentrations, competitively inhibited the deamination of high concentrations of this type B substrate. The kinetics of 5-HT deamination showed positive cooperation which indicates the involvement of subunits in the enzyme structure. The ability of some phospholipids to change the enzyme behaviour was considered as indication that these molecules might play a role in determining the ratio between the so-called A and B types of MAO, and in the regulation of the enzyme's activity.

That the enzyme, monoamine oxidase (EC 1.4.3.4. amine: O_2 oxidoreductase, MAO) exists in multiple forms was first suggested by Johnston (1968), who studied the effects of the irreversible inhibitor clorgyline, on MAO. It has been proposed that MAO can be classified into two types, A and B according to their inhibitor sensitivity and substrate specificity. Type A MAO was found to be solely responsible for the deamination of 5-hydroxtryptamine (5-HT) and shows high sensitivy to clorgyline, while type B metabolizes benzylamine (BA) and β-phenethylamine (PEA) and is less sensitive to clorgyline. Subsequently, it was shown that type B MAO is highly sensitive to the irreversible inhibitor, deprenyl (Knoll & Magyar 1972). Recently, the "multiple forms" concept has been questioned (Jain 1977) mainly because of some exceptions which were contradictory to the early findings (Fowler et al 1978; Lyles & Greenawalt 1978). As an alternative to the "multiple forms" concept, another hypothesis was put forward, insinuating that MAO is an enzyme with multiple binding sites but only one molecular entity (Jain 1977; White & Glassman 1977). Recently, Suzuki et al (1978, 1979) showed that PEA, at relatively high concentrations, is a non-specific substrate for type A and type B MAO of the rat brain. Two K_m values for the deamination of type B substrates by MAO were reported (Dugal 1977; Lyles & Greenawalt 1978). It has been shown (Ekstedt & Oreland 1976) that depletion of the mitochondrial lipids eliminated almost all of the A-activity of pig liver and brain MAO without affecting B-activity.

Speculations about the involvement of lipids in determining the activity and specificity of mitochondrial MAO were made (Houslay & Tipton 1973; Ekstedt & Oreland 1976). A lipid deficient diet in rats has been shown to alter mitochondrial MAO activity quantitatively (decrease in total activity) and qualitatively (changes in A/B ratio) (Kandaswami & D'Iorio 1979). Tong et al (1979) demonstrated higher A/B ratio in obese mice in comparison with lean animals in addition to the total higher MAO activity in the obese mice. In a recent study, Yu & Boulton (1979) were able to isolate a plasma factor (probably phosphatidylethanolamine) which activates platelet MAO. Using A and B substrates, both singly and in combination, and the irreversible inhibitors clorgyline and deprenyl, the present study was designed to characterize the enzyme activity of MAO obtained from human brain cortex.

MATERIALS AND METHODS

Radioactive substrates used were β-phenethylamine hydrochloride-[ethyl-l-^{14}C], 5-hydroxtryptamine creatinine sulphate (methylene-[1,2,^{3}H] (New England Nuclear, Boston, Mass.)) and benzylamine hydrochloride [methylene-^{14}C] (Amersham, Arlington Heights, Ill.). Clorgyline was a gift from May & Baker Ltd. (Essex, England), and deprenyl was synthesized in our laboratories. Bovine brain phosphatidylcholine and egg yolk lysophosphatidyl-

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choline were purchased from Sigma (St Louis, Mo.). All other chemicals were of analytical grade and from commercial sources. Postmortem brain cortices from accidental death victims with no history of mental disorder were used for the preparation of mitochondria as described previously (Schurr et al 1978). Assay of MAO activity was carried out in 3 ml of reaction mixture containing the mitochondrial preparation (0·8–1·25 mg protein), the substrate and 50 μmoles of potassium phosphate buffer, pH 7·4. The mixture was incubated for 30 min at 37 °C, except where otherwise indicated, and the reaction was terminated by adding 0·3 ml of 3 M HCl. When the effect of the phospholipids was studied, liposomes were prepared as described elsewhere (Schurr et al 1978) and were preincubated with the mitochondrial preparation for 20 min before the addition of the substrate. Under these conditions, the assay was linear for at least 40 min. Six ml of toluene or ether, or 5 ml of heptane, were used to extract the deamination products of PEA, 5-HT, and BA, respectively. After centrifugation for 20 min at 1500 g, 4 ml of the organic phase was transferred into a vial followed by the addition of 2 ml methanol and 10 ml of scintillation fluid. (Since ether causes a high degree of quenching, the ether extract was evaporated before the methanol and the scintillation fluid were added). The samples were counted in a Nuclear Chicago scintillation spectrophotometer, Model Mark I, with external standardization. Each experiment was repeated at least three times in duplicates yielding identical patterns. The duplicates agreed with 5% experimental error and representative experiments are shown.

RESULTS

The kinetic behaviour of PEA deamination by human brain cortex MAO is shown in Fig. 1. The two

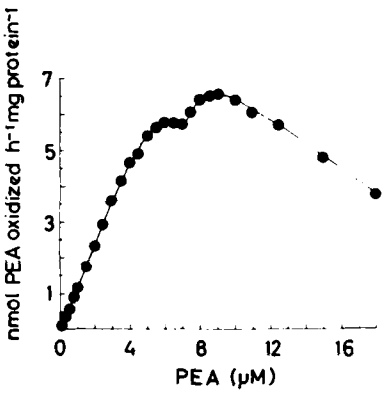

FIG. 1. MAO activity with increasing concentrations of PEA.

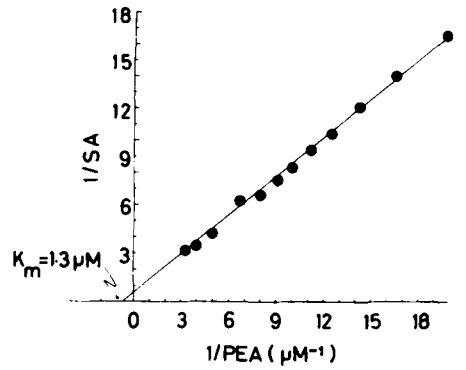

FIG. 2. Lineweaver-Burk plot of MAO activity with low range of PEA concentrations (SA = n moles substrate oxidized h^{-1} mg^{-1} protein).

maxima on the Michaelis-Menten curve represent at least two different K_ms. At the very low range of PEA concentration (0·05–0·3 μM), a K_m value of 1·33 μM was calculated (Fig. 2). As the concentration of PEA increases, the substrate inhibition is apparent. An additional K_m value, which is only roughly calculated because of the limited number of points available, was estimated to be 50 μM (Fig. 3). With BA as a substrate again two maxima on the Michaelis-Menten curve were found (Fig. 4) and hence, two K_m values were calculated (73 and 170 μM) by using the Lineweaver-Burk reciprocal plot (Fig. 6, lower curve). The existence of two different K_ms was reported by Dugal (1977) with bovine kidney MAO and by Lyles & Greenawalt (1978) with pig heart MAO, using *N*-methyl BA and BA as substrates, respectively. These results could be explained by assuming that the type B activity of human brain cortex MAO is heterogeneous (Tipton 1972). An alternative interpretation is that the low K_ms could

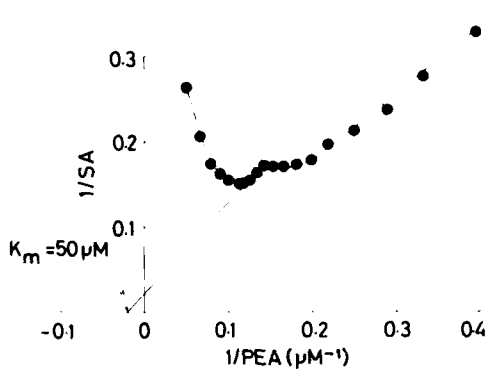

FIG. 3. Lineweaver-Burk plot of MAO activity with 'high' range of PEA concentrations (SA = as in Fig. 2).

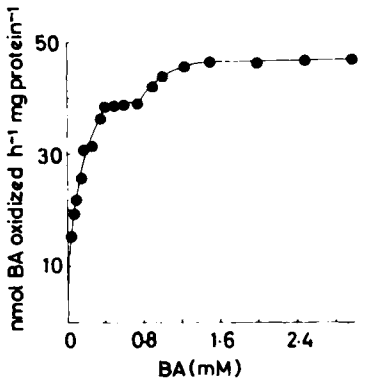

FIG. 4. MAO activity with increasing concentrations of BA.

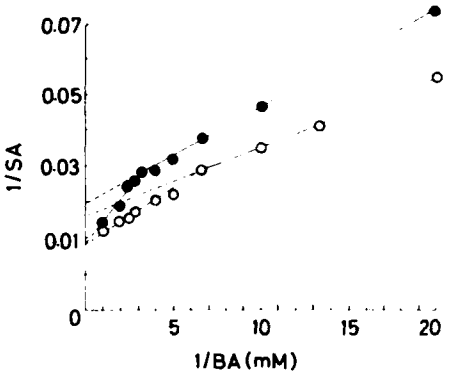

FIG. 6. Lineweaver-Burk plots of MAO activity with BA as a substrate in the absence or presence of 5-HT (SA = as in Fig. 2). ● 5-HT 2×10^{-3}M. ○ Control.

be the result of type B activity, while the high K_m values could be attributed to the type A activity. By using the irreversible inhibitors, clorgyline and deprenyl, or a combination of type A and B substrates, one might be able to differentiate between these two possibilities. PEA deamination in the presence of either deprenyl, clorgyline or 5-HT (as a type A 'occupant') is shown in Fig. 5. After 20 min of preincubation of 10^{-9} M deprenyl with the mitochondrial preparation, the two maxima on the Michaelis-Menten curve are still apparent (Fig. 5A). Such was not the case with clorgyline which at 10^{-9} M caused the disappearance of the second maximum (Fig. 5B). These results indicate that the high K_m value (second maximum on the Michaelis-Menten curve) of PEA deamination is the outcome of type A activity. The ability of 5-HT to mimic this effect of clorgyline (Fig. 5C) supports this idea. The relatively low concentration (10^{-9} M) of deprenyl and clorgyline was chosen mainly because we could not

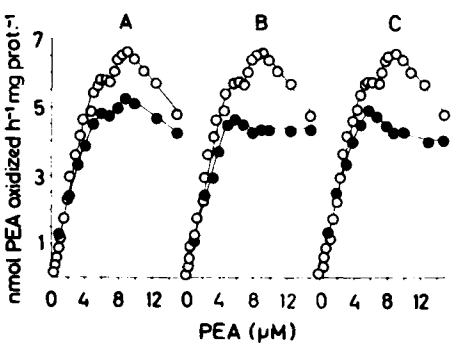

FIG. 5. MAO activity with increasing concentrations of PEA and the effects of (●) A, deprenyl 10^{-9}M; B, clorgyline 10^{-9}M; and C, 5-HT 10^{-4}M. ○ Control. The mitochondrial preparation was preincubated with clorgyline or deprenyl for 20 min before the addition of PEA.

achieve 100% inhibition of one type of MAO activity without substantial inhibition of the other type, using one or another inhibitor. Accordingly, one should consider the results presented in Fig. 5 as qualitative, indicating, but not proving, that the high K_m value of PEA deamination is the outcome of MAO-A activity. Suzuki & Hattori (1980) showed that MAO of chick brain is inhibited almost to the same degree by deprenyl, using either PEA or 5-HT. Because of the substrate inhibition exerted by PEA, we found it somewhat complicated to conduct mixed-substrate experiments with this biogenic amine, and BA was used instead. As can be seen from Fig. 6, BA deamination was inhibited by 5-HT non-competitively at the low K_m range and competitively at the high K_m range of BA concentrations. Such findings further imply that type B substrates can be deaminated also at the A-site of the enzyme, although their affinity to this site is weaker than to the B-site.

Type A activity of human brain cortex MAO was determined by using 5-HT as a substrate. By applying a wide range of concentrations of this amine, a different spectrum from that of the type B activity was found. At very low range of concentrations, the kinetic behaviour of 5-HT oxidation by human brain cortex MAO appeared as a sigmoid curve (Fig. 7, open circles) which can be interpreted as a positive cooperativity (Monod et al 1965; Kirtley & Koshland 1967) between subunits of the enzyme molecule. The Hill coefficient was calculated to be 2·2 (Fig. 9) which indicates the existence of at least three binding sites. As for type B substrates, two K_m values were also found for 5-HT deamination (6·5 and 167 μM). Since lipids have been suggested to be involved in a yet unknown manner with MAO activity (Tipton 1972; Houslay & Tipton 1973; Ekstedt & Oreland 1976; Schurr et al 1978; Yu & Boulton 1979), we

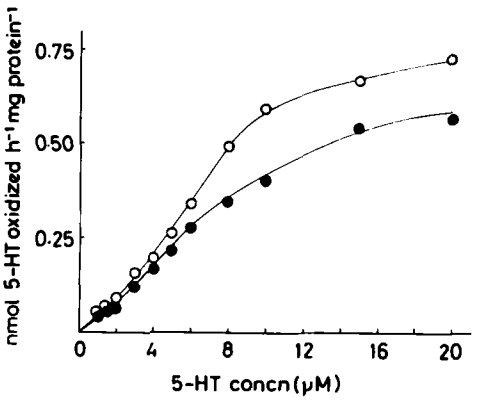

FIG. 7. MAO activity with increasing concentrations of 5-HT at concentration range of 0–20 μM, in the absence (○) and presence (●) of 300 μg PC mg^{-1} protein.

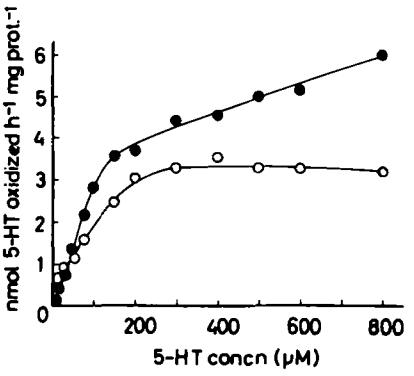

FIG. 8. MAO activity with increasing concentrations of 5-HT at concentration range of 0–800 μM, in the absence (○) and presence (●) of 300 μg PC mg^{-1} protein.

Table 1. Effects of phospholipids on human brain cortex MAO activity.

Addition	MAO activity (% of control) With PEA (10 μM)	With BA (800 μM)	With 5-HT (800 μM)
None	100	100	100
Phosphatidylcholine	51	26	188
Lysophosphatidylcholine	73	23	33

The phospholipids (300 μg mg^{-1} protein) were introduced as liposomes and were preincubated with the mitochondrial preparation for 20 min at 37 °C. Each value is a mean of four determinations.

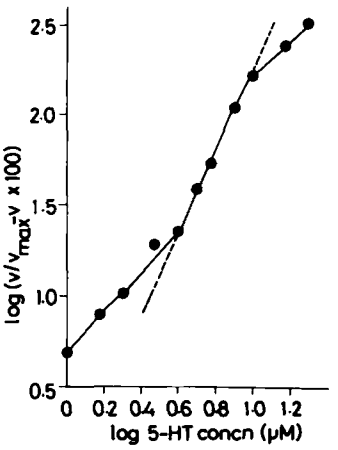

FIG. 9. Hill plot of MAO activity with 5-HT as a substrate. Slope = 2·2.

decided to study the effects of phosphatidylcholine (PC) and Lyso-PC on human brain cortex MAO.

In Table 1, a comparison is made between the effects of PC and Lyso-PC on MAO activity with three different substrates. While Lyso-PC is inhibitory at different degrees in all cases, PC showed stimulation of MAO activity toward 5-HT. Houslay (1978) reported that Lyso-PC is a reversible inhibitor of MAO, while Kandaswami & D'Iorio (1978) showed facilitation of MAO activity with 5-HT and tyramine upon applying diphosphatidylglycerol on purified MAO.

Figure 7 (closed circles) shows that phosphatidylcholine (PC) inhibited the deamination of 5-HT when low concentrations of the substrate were used, while at higher range of 5-HT concentrations, PC caused an increase in the enzyme activity (Fig. 8, closed circles). Moreover, sigmoidity of the Michaelis-Menten curve is apparent in the presence of PC at this range of 5-HT concentration.

DISCUSSION

The existence of at least two functional types, or forms, of mitochondrial MAO was suggested as early as 1968 by Johnston. His interpretation was based on differences in the sensitivity of the enzyme to the irreversible inhibitor clorgyline, while using various substrates. Type A and B MAOs, as they were first designated, were considered to deaminate exclusively 5-HT and BA (and PEA), respectively. Various reports in the literature which deal with human MAO agree with this conception (Tipton 1972; Houslay & Tipton 1973; Suzuki et al 1978, 1979). However, other investigators question the use-

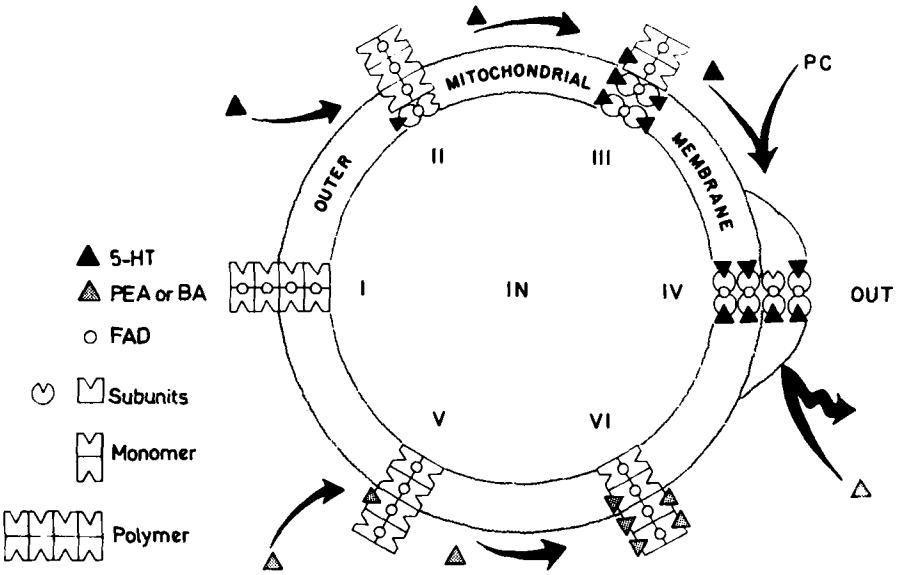

FIG. 10. Schematic model for MAO of human brain cortex (see details in the text).

fulness of this concept and see it as an oversimplification of the real situation (Jain 1977; White & Glassman 1977; Fowler et al 1978; Lyles & Greenawalt 1978). In the present study, we examined the oxidative deamination of type A and B substrates by mitochondrial MAO of human brain cortex in the absence and presence of the irreversible inhibitors clorgyline and deprenyl. At least two K_m values were found for each of the type B substrates, PEA and BA, suggesting the existence of low and high affinity binding sites for these amines. The same was found for 5-HT, long believed to be an ultimate type A substrate of MAO. Recently, Suzuki et al (1978, 1979) showed that PEA, at different concentrations, serves as a substrate for both type B and type A MAO. Clorgyline and deprenyl were then used in order to define these two binding sites, assuming that they are the so-called A and B forms of MAO.

When very low concentrations of the two inhibitors were preincubated with the enzyme it seems, at least qualitatively, that clorgyline (and also 5-HT), but not deprenyl, were able to diminish the high K_m activity of the enzyme with PEA as a substrate. 5-HT, on the other hand, could inhibit competitively the deamination of high concentrations of BA, indicating that the latter is oxidized also by type A MAO. One interpretation of these data could be that the mitochondrial MAO of human brain cortex is a single molecular entity with two of more subunits, each containing active site(s) with high affinity toward either type A or B substrate. Binding of type A amine could also take place at the B-site, although with lower affinity, and vice versa. With 5-HT, the MAO of human brain cortex, at least the low K_m activity, shows some allosteric properties, indicating the existence of co-operative interaction between the enzyme subunits and the substrate, 5-HT. The Hill coefficient of 2·2 may indicate the existence of three or four binding sites. It is assumed that the high K_m activity of 5-HT deamination takes place at the B-site of the enzyme, showing no allosteric properties.

It is tempting to state that the opposite effects of PC on 5-HT deamination are determined by the differences in the environments surrounding the A and B sites of MAO. In the A sites, which are probably embedded in the mitochondrial outer membrane (Russell et al 1979), PC, by changing the protein:phospholipid ratio, decreases the high affinity of the sites to 5-HT. At site B, the less hydrophobic one, PC interacts with MAO to form a 'site A-like' environment which could explain the increase in 5-HT deamination and the lowered affinity toward PEA and BA. This assumption is further supported by the sigmoid shape of the Michaelis-Menten curve at the high range of 5-HT concentrations (Fig. 8, closed circles) in the presence of PC. Yu & Boulton (1979) reported that a factor, probably phosphatidylethanolamine, present in human plasma, can activate human platelet MAO. On the other hand, Lyso-PC probably acts like a detergent, inhibiting all sites of the enzyme, an effect already reported by Houslay (1978).

Recently, Russell et al (1979) proposed a vectorial orientation of human MAO in the outer mito-

chondrial membrane. According to these authors, the 5-HT deamination activity is located on the inside of this membrane while the PEA deaminating activity is on the outer side, suggesting a hydrophobic site for 5-HT and a hydrophilic one for PEA.

The schematic model in Fig. 10 was drawn based on the results reported here and in other recent studies (Ekstedt & Oreland, 1976; Kandaswami & D'Iorio 1978, 1979; Tong et al 1978; Schurr et al 1978; Minamiura & Yasunobu 1978a, b; Russell et al 1979; Suzuki et al 1979). One must keep in mind that such a model is not necessarily the only one possible and is used here only as a scheme in which changes can be made.

In this model, the MAO, composed of four identical monomers (eight subunits), is embedded in the outer mitochondrial membrane leaving two monomers outside (I). There is one FAD moiety for each two subunits (Minamiura & Yasunobu 1978a, b). According to Russell et al (1979), the part of the enzyme that is facing the in-side of the mitochondrial membrane is the 5-HT side, and that which faces the out-side is the PEA side. Binding of 5-HT to one of the subunits facing the in-side of the mitochondrial membrane could cause a conformational change (II) which, in turn, increases the affinity to the substrate at the adjacent subunits (positive cooperativity). When these subunits are fully occupied by the substrate molecules (III), the subunits facing the outer side of the mitochondrial membrane could bind and deaminate excess of this biogenic amine, although at a lower affinity (i.e., a higher K_m value). The 5-HT subunits could also deaminate PEA and BA when these substrates are present in excess (VI). This could explain the second, high K_m value, found for both PEA and BA. The ability of 5-HT and clorgyline to abolish the high K_m of PEA and that of 5-HT to compete with high concentrations of BA support this assumption. Upon preincubation of the MAO preparation with PC liposomes, a patch of phospholipids layer could cover the part of the enzyme facing the outer side of the membrane (PEA subunits), (IV), forming a favourable environment for the binding of 5-HT, while lowering the binding of PEA and BA. Although some of the findings presented here are in contradiction with others (White & Glassman 1977), this could be due, at least in part, to the differences in the origin of the mitochondrial preparations (Lymphoma, Hodgkins disease and alcohol poisoning) used by these authors and our preparation. The results presented here are in agreement with earlier predictions about the involvement of lipids in determining MAO activity toward various substrates. We believe that such an involvement is even more specific and that certain phospholipids might play a role in the enzyme regulation, and in determining the A/B ratio in different tissues and species. Moreover, these data may support the conception that MAO is a single molecular entity with multiple binding sites.

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