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PRELIMINARY COMMUNICATION

EFFECT OF β-PHENYLETHYLAMINE CONCENTRATION ON ITS SUBSTRATE SPECIFICITY FOR TYPE A AND TYPE B MONOAMINE OXIDASE

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The existence of two types of monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO), type A and type B enzyme, has been well documented [1-3]. Although it is not clear if each type of MAO represents a different protein, it is unequivocally accepted that both types of MAO are demonstrable both *in vitro* and *in vivo* [4,5] in their substrate specificity and inhibitor sensitivity. Type A MAO has been shown to be active with 5-hydroxytryptamine (5-HT) and norepinephrine as substrates, and sensitive to inhibition by a low concentration of clorgyline. Type B MAO has been shown to be active with β-phenylethylamine (PEA) and benzylamine (BA), and sensitive to inhibition by a low concentration of deprenyl. Some substrates, such as kynuramine, tyramine, tryptamine and dopamine, are oxidized by either type of MAO. In the present communication, however, we report that PEA loses its substrate specificity for type B MAO at relatively high concentrations.

A crude mitochondrial fraction was isolated from whole brains of male Sprague-Dawley rats as described previously [6], and the suspension was used as an enzyme source. MAO activity toward PEA and BA was determined by the method of Guilbault *et al.* [7] and Snyder and Hendley [8] (Method A). In this method, hydrogen peroxide formed in the MAO reaction is measured fluorometrically by converting homovanillic acid to highly fluorescent compounds in the presence of peroxidase. Another fluorometric method [9] (Method B) was also employed for the confirmation of the results obtained with Method A. In Method B, phenylacetaldehyde, the reaction product from PEA, is measured. PEA-HCl was obtained from Nakarai Chemicals, Ltd., Kyoto, and recrystallized three times from ethanol by adding acetone. MAO activity with 5-HT as substrate was assayed by a radiochemical procedure [10]. Clorgyline, a selective inhibitor of type A MAO [1], was generously supplied by May & Baker Ltd., Dagenham, England. Deprenyl, a selective inhibitor of type B MAO [11], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. It was confirmed that clorgyline and deprenyl neither interfered with the formation of fluorescent compounds nor quenched their fluorescence. For each assay, 0.07 to 1.20 mg of mitochondrial protein was used. The assays were carried out at 37° and pH 7.4 for 30 or 60

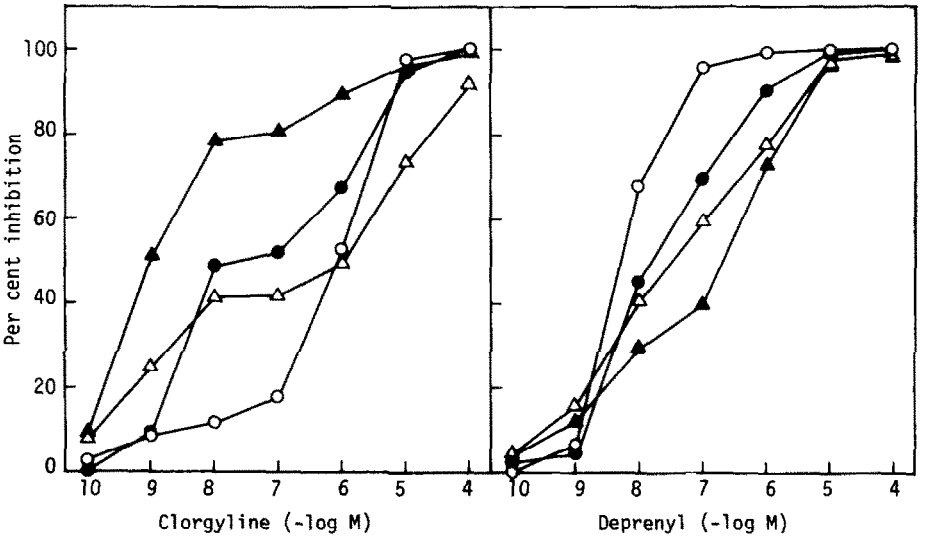

Fig. 1. Inhibition of MAO in rat brain mitochondria by clorgyline and deprenyl using various concentrations of PEA as substrate. The concentrations of the substrate were: 12.5 μM(○—○), 125 μM (●—●, △—△) and 1250 μM (▲—▲). Method A: ○—○, ●—●, ▲—▲; Method B: △—△. The assay mixture was preincubated with the inhibitor at 37° for 10 min. Each point represents the mean obtained from duplicate determinations.

min. The mixture was preincubated at 37° for 10 min to ensure maximal enzyme inhibition. Care was taken not to convert more than 20 per cent of the substrate to reaction product. Protein was measured by the conventional biuret method.

Figure 1 shows MAO inhibition by clorgyline and deprenyl, using various concentrations of PEA as substrate. At 12.5 μM PEA, the inhibition curves obtained with both inhibitors were almost single sigmoidal. The results are compatible with those of previous reports [2,5] and with the widely accepted idea that PEA is a specific substrate for type B MAO. At 125 μM, however, the patterns were changed dramatically: a clear plateau appeared in the curve at 10^{-8}-10^{-7} M clorgyline. The susceptibility of PEA deamination to deprenyl was decreased markedly in parallel with the change in the inhibition pattern with clorgyline, although the plateau was not obvious. These phenomena were confirmed with another MAO assay of a different principle (Method B), as shown in Fig. 1. When the PEA concentration was increased up to 1250 μM, the susceptibility to clorgyline was increased even more, showing about 80 per cent inhibition with 10^{-8}-10^{-7} M clorgyline; the susceptibility to deprenyl was also decreased significantly at this concentration of PEA. These data clearly indicate that PEA is not specific for type B MAO at concentrations of 125-1250 μM.

The inhibition of MAO by both inhibitors, using 12.5 and 1250 μM BA or 5-HT as substrate, is illustrated in Fig. 2. The figure shows almost no difference in the inhibition pattern between the two concentrations for both substrates. Clorgyline, when present in the incubation medium at a concentration of 10^{-8} M, almost completely inhibited the 5-HT deamination. Much higher concentrations were required to inhibit the deamination of BA. In contrast, at the same concentration, deprenyl almost completely blocked the deamination of

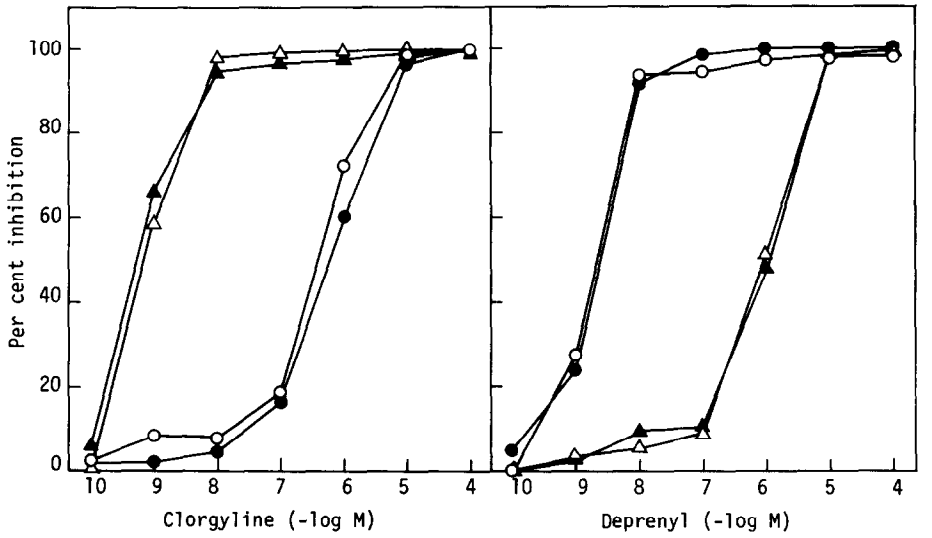

Fig. 2. Inhibition of MAO in rat brain mitochondria by clorgyline and deprenyl using different concentrations of BA and 5-HT as substrates. Key: 12.5 μM BA (○—○); 1250 μM BA (●—●); 12.5 μM 5-HT (△—△); 1250 μM 5-HT (▲—▲). The assay mixture was preincubated with the inhibitor at 37° for 10 min. Each point represents the mean obtained from duplicate determinations.

BA but hardly affected the metabolism of 5-HT unless much higher concentrations were used. These results clearly indicate that BA is highly specific for type B MAO, while 5-HT is specific for type A MAO over a wide concentration range of the substrates.

In the present communication, we demonstrated that in relatively high concentrations (125-1250 μM) PEA became non-specific for type A and type B MAO, but this was not the case for BA and 5-HT. Our results may be related to the K_m values of the substrates, since the value for PEA is much lower than those for BA and 5-HT [12]. However, to explain these phenomena, further investigation is required.

Many workers employ a radiochemical procedure [13] to assay MAO toward PEA. In this method, relatively low concentrations (5-50 μM) of PEA tend to be used to minimize the blank values because some of the labeled PEA is readily extracted into the organic phase in significant quantities despite acidification prior to the extraction [14]. This is probably the reason why they did not discover the phenomena presented in this communication.

Since many reports [15-22] are being published concerning MAO activities using relatively high concentrations (100-1000 μM) of PEA as an indicator of type B MAO, our present observation seems to be of great importance. Further studies on this problem using various substrates and tissues are in progress in our laboratory.

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