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STUDIES ON THE SUBCELLULAR LOCALIZATION OF MONOAMINE OXIDASE TYPES A AND B AND ITS IMPORTANCE FOR THE DEAMINATION OF DOPAMINE IN THE RAT BRAIN

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Abstract—The distribution of the MAO-forms A and B between intra- and extrasynaptosomal rat brain mitochondria was studied with the aid of their known substrate and inhibitor specificities. The activities with the selective substrates serotonin, PEA and benzylamine indicated that intrasynaptosomal mitochondria have about a 3.4-fold higher MAO A:MAO B ratio than extrasynaptosomal mitochondria. However, PEA was found to be a selective substrate for MAO B only at low concentrations (such as 5×10^{-6}M), whereas at higher concentrations (such as 10^{-3}M) it was a substrate for both forms of MAO. The different ratios of the two enzyme forms in the two mitochondrial populations were confirmed when the selective inhibitors clorgyline and deprenyl were used with dopamine or 10^{-3}M PEA. With these two amines, the ratios of MAO A:MAO B activities were 3–4.5 times higher in intrasynaptosomal than in extrasynaptosomal mitochondria. In particular, when the activity with dopamine was measured in intact synaptosomes, deamination being preceded by a specific uptake into these particles, the inhibitor sensitivities clearly showed that MAO activity was almost exclusively attributable to the A-form of the enzyme. Thus, mitochondria in the terminals of dopaminergic neurones have an even more pronounced enrichment in MAO A than the mitochondria obtained by osmotic lysis of a total brain synaptosomal preparation. It was also found that clorgyline and deprenyl have an inhibitory effect on the uptake of dopamine into nerve endings with IC_{50} values in the range of 10^{-5} to 10^{-4}M. These results are discussed in terms of possible physiological significancies of the properties and distribution of the two forms of MAO.

In recent years, the classification of monoamine oxidase (MAO, EC1.4.3.4)* into the two forms A and B on the basis of their inhibitor [1, 2] and substrate [2–5] specificities has been widely investigated and reviewed [6, 7]. The A-form deaminates serotonin and norepinephrine preferentially and is sensitive to low concentrations of the inhibitor clorgyline, whereas the B-form deaminates β-phenylethylamine (PEA) and benzylamine and is inhibited by low concentrations of deprenyl. Dopamine and tyramine are common substrates for both forms of the enzyme. The usefulness of this classification of MAO has been questioned by Fowler *et al.* [8] on the basis of differences in the inhibitor and substrate specificities between species or even between different organs of the same species. However, the expression of the relative activities of the MAO-forms A and B is expected to depend to a large degree on the particular assay conditions which are used. Many of the functional differences between MAO A and B depend upon maintenance of the integrity of the enzyme in the mitochondrial outer membrane, since Houslay and Tipton [9] have pointed out that the two forms of MAO may reflect a different lipid environment of the enzyme in the membrane. Furthermore, the accessibility of the enzyme for a given substrate may be limited *in vivo* by compartmentation, which may or may not be preserved when MAO is measured under *in vitro* conditions. Therefore, a substrate which is, like dopamine, deaminated by both forms of MAO *in vitro* may well be metabolized by only one form *in vivo*. Student and Edwards [10] have reported that synaptosomal rat brain mitochondria have a higher MAO A:B ratio than 'free' extrasynaptosomal mitochondria. The present paper supports this finding with further evidence and investigates its importance for the metabolism of the neurotransmitter dopamine. Furthermore, because inhibition of norepinephrine uptake by deprenyl has been observed earlier [2, 11], effects of clorgyline and deprenyl on the uptake of dopamine in synaptosomes were also studied.

* Abbreviations used: MAO, monoamine oxidase (amine: oxygen oxidoreductase, deaminating. E.C.1.4.3.4.); PEA, β-phenylethylamine.

MATERIALS AND METHODS

Preparation of mitochondria and synaptosomes. Cell fractionation was performed according to Gray and Whittaker [12]. All steps were carried out on ice or at 4°. A 10% homogenate in 0.32M sucrose, obtained from whole brains of male adult Sprague–Dawley rats, was centrifuged at $11{,}000\,g \times$ min, the pellet (P_1) discarded and the supernatant fraction (S_1) recentrifuged at $7.2 \times 10^5\,g \times$ min. The supernatant fraction S_2 was discarded and the crude mitochondrial pellet P_2 was resuspended in 0.32M sucrose to give a final volume of 3 ml suspension per 2g of original brain weight. This material was layered

over a discontinuous density gradient consisting of equal volumes of 1.2 and 0.8M sucrose. The gradient was centrifuged at $6 \times 10^6 g \times$ min in a swingout rotor on a MSE 65 ultracentrifuge. The synaptosomal suspension (at the 0.8M/1.2M interface, fraction B according to the nomenclature of Gray and Whittaker) was collected by aspiration, diluted and either used for uptake and MAO assays or further fractionated in order to obtain synaptosomal mitochondria (see below). The pellet (C, extrasynaptosomal or 'free' mitochondria) was resuspended and dialysed against 5 mM phosphate buffer, pH 7.0, before use for MAO assays.

In order to prepare the mitochondria of intrasynaptosomal origin, fraction B of the density gradient was submitted to osmotic shock with distilled water according to Whittaker *et al.* [13]. The suspension obtained in this way was layered over 1.2M sucrose and centrifuged at $6 \times 10^6 g \times$ min in the swingout rotor of the MSE 65 centrifuge, thus separating synaptosomal mitochondria from membrane fragments, unlysed synaptosomes, vesicles and cytosol [13]. The mitochondrial pellet (I) was treated in the same way as fraction C of the non-synaptosomal mitochondria (see above).

Measurement of accumulation of [^{3}H]*dopamine in synaptosomes.* Incubations of synaptosomes with [^{3}H]dopamine were carried out in a final volume of 5 ml at 37° on a shaking water bath. Each reaction mixture consisted of 0.2 ml of synaptosomal suspension (0.5–1 mg of protein, blank assays contained no synaptosomes) in a Krebs–Ringer phosphate buffer (pH 7.4, 118 mM NaCl, 32 mM Na-phosphate, 4.7 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.3 mM Na-EDTA, 5.6 mM glucose and 1.7 mM ascorbic acid). After a 10-min preincubation (with or without inhibitors present), the reaction was started by addition of small volumes of a stock solution of [^{3}H]dopamine (specific activity 400 Ci/mole), prepared in 0.1 N HCl, to give a final concentration of 10^{-7} M. Equal amounts of 0.1 N HCl did not affect the pH of the assay. The incubation was terminated after 20 min by rapid filtration of the reaction mixture through cellulose nitrate membrane filters (Sartorius SM 11305, ϕ 25 mm) of pore size 0.6 μm under mild vacuum. The filtrate was immediately saturated with NaCl and acidified with 0.1 ml of concentrated HCl, and the synaptosomes remaining on the filters were washed with 5 ml of a cold 0.9 per cent NaCl solution. The filters were then transferred to counting vials for determination of the radioactivity accumulated in the synaptosomes, while the filtrates were used for determination of deaminated metabolites (see below).

Assay of MAO activity. Total deaminated metabolites of [^{3}H]-dopamine were estimated by organic solvent extraction: a 2-ml aliquot of the acidified and salt-saturated filtrate (see above) was extracted with 6 ml of ethyl acetate for 20 min in screw-cap tubes. After centrifugation for separation of the two layers, aliquots of the organic and aqueous phases were removed for liquid scintillation counting. This method was originally described for norepinephrine metabolites [14], but in preliminary experiments we found that dopamine metabolites were also quantitatively extracted into the organic phase, while essentially all of the amine remained in the aqueous phase. The same assay was used for the measurement of MAO activity with dopamine in mitochondria; control experiments showed that the activity was linear with the amount of protein in the assay and with time over the incubation period that was used (20 min).

MAO activities with serotonin, β-phenylethylamine and benzylamine were measured at 25° with the coupled spectrophotometric assay described earlier in detail by Koechli and von Wartburg [15], using the peroxidase-catalysed oxidation of the colourless 2′7′-dichlorofluorescin to the dye 2′7′-dichlorofluorescein (monitored at 502 nm) as an indicator reaction for H_2O_2 formed by monoamine oxidase. The inhibitors clorgyline and deprenyl were preincubated with the mitochondria under assay conditions for 15 min before the reaction was started by addition of the substrate.

Liquid scintillation counting. A 1-ml aliquot of the organic phase from the extraction procedure for determination of dopamine-deaminated metabolites was counted in 15 ml toluene scintillator containing 4 g/l PPO and 0.05 g/l POPOP on a Packard Tri Carb 3320 liquid scintillation counter. In order to measure radioactivity enclosed within synaptosomes (accumulation of dopamine), the membrane filters were dissolved overnight in 1 ml of Protosol. In order to prevent colour quenching, the yellow solutions were bleached by addition of 1 ml of a saturated solution of dibenzoyl peroxide in toluene. As soon as they were colourless, 15 ml of toluene scintillator were added for counting as described above. Liquid scintillation activities were corrected for quenching with the aid of the external standard method, and the dpm values were converted to absolute amounts of dopamine (in nmoles) with the aid of the known specific activity of the radiolabelled substrate.

Protein determinations. Protein concentrations were measured by the method of Lowry *et al.* [16], using crystalline bovine serum albumin as a standard.

Chemicals. All chemicals were of the highest purity commercially available and used without further purification. Dopamine–HCl for isotope dilution was

Table 1. Activities of monoamine oxidase in free and synaptosomal mitochondria*

Substrate	C-Mitochondria	I-Mitochondria	C/I
Serotonin (10^{-4}M)	1.95 ± 0.07 (6)	2.54 ± 0.13 (6)	0.77
PEA (10^{-3} M)	3.37 ± 0.11 (6)	1.80 ± 0.11 (6)	1.87
PEA (5×10^{-6}M)	1.28 ± 0.04 (4)	0.46 ± 0.01 (3)	2.78
Benzylamine (10^{-3}M)	1.40 ± 0.15 (4)	0.54 ± 0.04 (3)	2.59

* Activities of MAO in free (C) and synaptosomal (I) mitochondria with 5-hydroxytryptamine (serotonin), phenylethylamine (PEA) and benzylamine were measured with a spectrophotometric assay as described in Materials and Methods. The results are expressed as nmoles of metabolized amine per minute per mg of mitochondrial protein: means ± S.E. from independent duplicate determinations. Numbers of determinations are given in parentheses.

from Fluka AG, Buchs, Switzerland; [^{3}H]dopamine (labelled in position 2 of the side chain) from New England Nuclear (NEN). Protosol and Liquifluor (concentrate of PPO/POPOP in toluene) were also from NEN. 2′7′-dichlorofluorescin diacetate was purchased from Eastman Kodak, Rochester, NY; horseradish peroxidase grade I from Boehringer Mannheim; serotonin (creatinin sulfate), benzylamine (free base) and β-phenylethylamine (free base) from Fluka AG, Buchs. Clorgyline was obtained from May & Baker, Dagenham, U.K. Deprenyl was a generous gift from Dr. Knoll, Budapest, Hungary.

RESULTS

The specific activities of monoamine oxidase (MAO) were compared in extrasynaptosomal mitochondria (C) and mitochondria obtained from a synaptosomal preparation after osmotic shock (I) (Table 1). Selective substrates for the two MAO forms A (serotonin) and B (phenylethylamine, benzylamine) were used. With 10^{-4}M serotonin, the specific activity was higher in synaptosomal mitochondria, whereas with PEA (5×10^{-6}M) and benzylamine (10^{-3}M) it was higher in extrasynaptosomal mitochondria. Whereas the ratios of specific activities between C- and I- mitochondria with the MAO B substrates 5×10^{-6}M PEA and 10^{-3}M benzylamine were similar, an intermediate ratio between these and the ratio obtained with the MAO A substrate serotonin (10^{-4}M) was found with a higher concentration of PEA (10^{-3}M), indicating that MAO A was also involved in the deamination of 10^{-3}M PEA. The ratio of MAO A : MAO B activities (as measured with serotonin and benzylamine, respectively) was 3.4-fold higher in I-mitochondria (4.70) than in C-mitochondria (1.39). However, these results give only relative information on the distribution of the two enzyme forms, since specific enzyme activities depend on the given substrate and since it cannot be fully excluded that the activities measured with a 'selective' substrate are slightly contaminated by a contribution of the other enzyme form. We therefore extended this study to the use of the selective inhibitors clorgyline (for MAO A) and deprenyl (for MAO B). As is shown in Figs. 1 and 2, the activities with serotonin and benzylamine reported in Table 1 were solely due to MAO A or MAO B, respectively, in both mitochondrial populations. In fact, the inhibition curves with clorgyline and deprenyl reached 100 per cent inhibition without showing the biphasic pattern which has to be expected when both

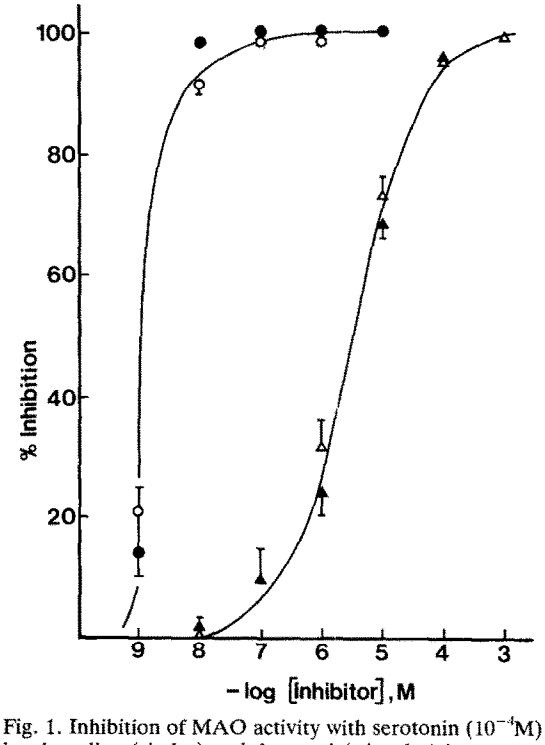

Fig. 1. Inhibition of MAO activity with serotonin (10^{-4}M) by clorgyline (circles) and deprenyl (triangles) in extrasynaptosomal mitochondria (○, △, fraction C according to Materials and Methods) and mitochondria obtained by osmotic lysis of synaptosomes (I, ●, ▲). MAO activity with serotonin was measured with a spectrophotometric assay as described in Materials and Methods. Activities with control samples (containing no drug) are given in Table 1. The data shown are mean values from at least two independent experiments, each run in duplicate, with the S.E.M. when it was not too small to be indicated.

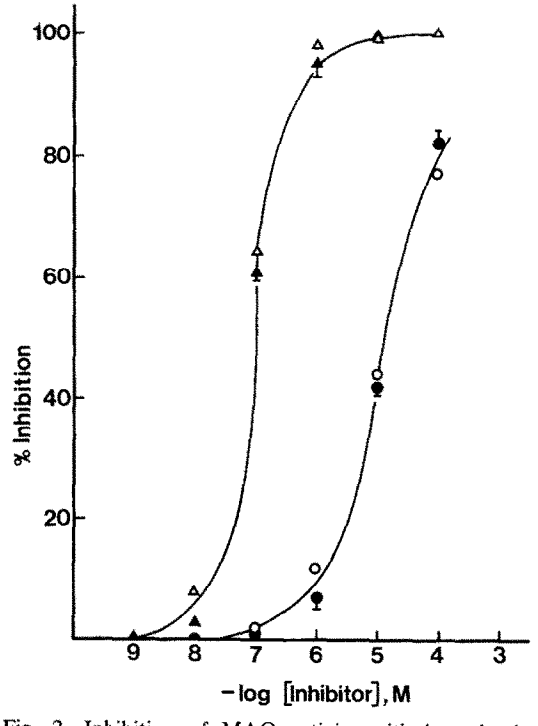

Fig. 2. Inhibition of MAO activity with benzylamine (10^{-3}M) by clorgyline (circles) and deprenyl (triangles) in extrasynaptosomal mitochondria (○, △, fraction C) and mitochondria of synaptosomal origin (●, ▲, I). MAO activity with benzylamine was measured with a spectrophotometric assay as described in Materials and Methods. For reasons of solubility, the highest clorgyline concentration tested was 10^{-4}M. Activities with control samples (containing no drug) are given in Table 1. The data shown are mean values from at least two independent experiments, each run in duplicate, with the S.E.M. when it was not too small to be indicated.

enzyme forms contribute significantly to the deamination of a given substrate. With 5×10^{-6}M PEA, the inhibition curves with the two inhibitors resembled those with benzylamine and showed a monophasic pattern in both mitochondrial preparations (data not shown), indicating that at this low concentration, PEA is a true selective substrate for MAO B. However, at the higher PEA concentration tested (10^{-3}M, Fig. 3), the inhibition curves with clorgyline were clearly double sigmoidal in both intra- and extrasynaptosomal mitochondria. From this finding it can be concluded that MAO A contributes substantially to the deamination of PEA at this concentration, since the type of observed plateaus is known to occur when MAO A is completely inhibited while MAO B is still fully active. The levels of these plateaus indicate that the percentage of the activity due to MAO A was approximately 3-fold higher in I- (49 per cent) than in C-mitochondria (17 per cent), corresponding to a 4.5-fold higher A : B ratio in the former (49:51 vs 17:83). As confirmation, one would expect a corresponding, but reversed pattern (plateau for I—mitochondria at 51 per cent, for C—mitochondria at 83 per cent inhibition) for the MAO B specific inhibitor deprenyl. However, since our measurements with serotonin (Fig. 1) showed an increasing inhibition of MAO A by deprenyl in the critical concentration range, the expected picture appears to be blurred in the curves shown in Fig. 3: they have a vague double sigmoidal shape without exhibiting a clear plateau at the concentrations of 10^{-6} and 10^{-5}M deprenyl. Corresponding inhibition curves were established with dopamine, which is known to be accepted as a substrate by both forms of rat brain MAO (Fig. 4). The plateau levels with clorgyline show that the MAO A : MAO B ratios of activity towards dopamine were about 3:1 (75 per cent maximal inhibition in the low concentration range of clorgyline) in extrasynaptosomal and 9:1 (only 10 per cent of the total activity not inhibited by the low concentrations of clorgyline) in synaptosomal mitochondria. Again, the expected biphasicity in the deprenyl inhibition curves could not be clearly demonstrated because of the above-mentioned effect of deprenyl on MAO A at the critical concentrations.

Since the synaptosomal mitochondria (I) used for these investigations were obtained after osmotic lysis of a total brain synaptosomal preparation, they were

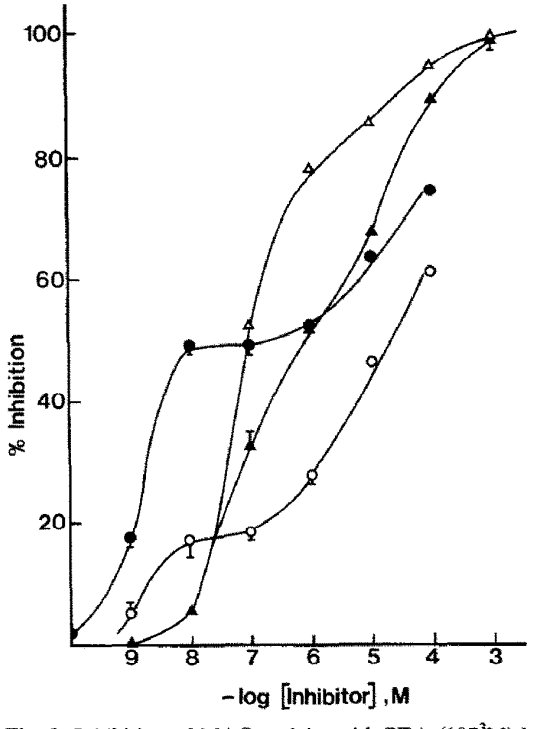

Fig. 3. Inhibition of MAO activity with PEA (10^{-3}M) by clorgyline (circles) and deprenyl (triangles) in extrasynaptosomal mitochondria (○, △, fraction C) and mitochondria of synaptosomal origin (●, ▲, I). MAO activity with PEA was measured with a spectrophotometric assay as described in Materials and Methods. For reasons of solubility, the highest clorgyline concentration tested was 10^{-4}M. Activities with control samples (containing no drug) are given in Table 1. The data shown are mean values from at least two independent experiments, each run in duplicate, with the S.E.M. when it was not too small to be indicated.

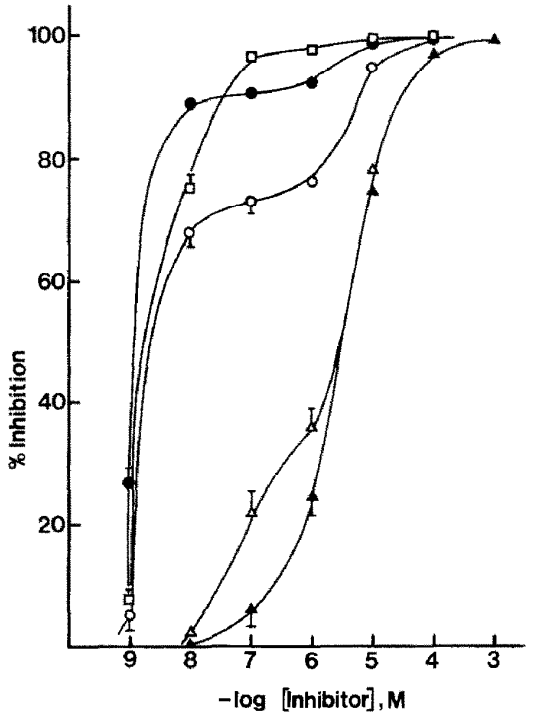

Fig. 4. Inhibition of MAO activity with dopamine by clorgyline (circles) and deprenyl (triangles) in C- (○, △) and I- (●, ▲) mitochondria and intact synaptosomes (fraction B). □: Inhibition with clorgyline in intact synaptosomes. Dopamine concentrations used were 10^{-4}M with mitochondria and 10^{-7}M with synaptosomes. MAO activity was measured with a radioactive assay as described in Materials and Methods. Control activities in the assays containing no drugs were 1.92 ± 0.05 and 1.97 ± 0.07 nmoles per min per mg of mitochondrial protein in fractions C and I, respectively, and $(9.01 \pm 0.7) \times 10^{-2}$ nmoles per min per mg of synaptosomal protein in fraction B. The results shown are mean values from at least two independent duplicate determinations with the S.E.M. when it was not too small to be indicated. The differences between fractions B and I at 10^{-6} and 10^{-7}M clorgyline were highly significant ($P < 0.001$, Student's t-test).

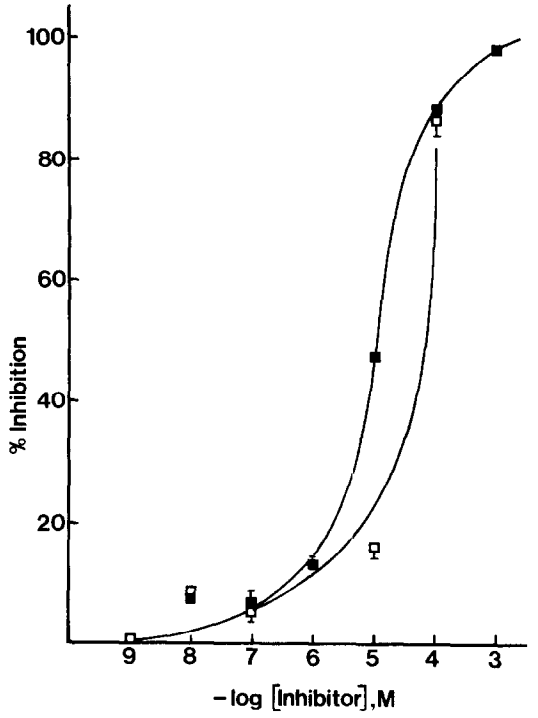

Fig. 5. Inhibition of accumulation of dopamine (10^{-7}M) in synaptosomes by clorgyline (□) and deprenyl (■). The experimental procedure is described in materials and methods. For reasons of solubility, the highest clorgyline concentration used was 10^{-4}M. Accumulation in control samples containing no drug was $(6.62 \pm 0.1) \times 10^{-2}$ nmoles of dopamine per mg of synaptosomal protein. The results shown are the mean values from two independent experiments, each run in duplicate, with the S.E.M. where it was not too small to be indicated.

derived from nerve endings of a variety of different types of neurones. It was of interest to know if the results with dopamine could be confirmed with mitochondria located in the terminals of dopaminergic neurones. We incubated intact rat brain synaptosomes with low external concentrations of dopamine (approximately 1000-fold lower than the K_m of MAO for dopamine); under these conditions the amine can only be metabolized by MAO after it has been taken up into the nerve ending particles by a specific transport mechanism. Deamination of dopamine by free extrasynaptosomal mitochondria, possibly contaminating our synaptosomal preparation to a small extent, should be negligible. The true intrasynaptosomal location of MAO activity was further confirmed in control experiments showing that MAO was indirectly inhibited in the presence of the transport inhibitor cocaine in the synaptosomal fraction (B), whereas it had no direct effect on MAO in the free mitochondria of fraction C (data not shown). The lack of a plateau in the clorgyline inhibition curve with synaptosomes (Fig. 4), and the highly significant ($P < 0.001$) differences between the clorgyline curves with synaptosomes and I-mitochondria indicate that dopamine was metabolized in synaptosomes almost exclusively by the A-form of MAO. The deprenyl inhibition curve with dopamine in synaptosomes was virtually identical with the one shown for I-mitochondria in Fig. 4.

Because under these conditions drugs might exert an indirect inhibitory effect on MAO in synaptosomes by blocking the uptake of its substrate into the nerve endings, we examined the effects of clorgyline and deprenyl on the accumulation of dopamine in synaptosomes. As shown in Fig. 5, both compounds considerably affected the uptake of dopamine in synaptosomes. However, these effects occured in a higher concentration range (IC_{50} values 10^{-5}–10^{-4}M) than the inhibition of MAO and therefore had little if any effect, in the determination of the results with synaptosomes reported in Fig. 4.

DISCUSSION

Several attempts have been made in recent years to obtain information on the physiological significance of the two MAO forms A and B by investigating their subcellular localization. Owen *et al.* [17] reported a heterogeneity of MAO in different populations of rat brain mitochondria. However, these authors used a high PEA concentration which, according to the results reported in this study, does not sufficiently discriminate between the two forms of MAO. Furthermore, some of their reported observations reflect differences in total MAO activity rather than in the A:B ratio. Student and Edwards [10] have found an enrichment in MAO A in synaptosomal compared to extrasynaptosomal mitochondria, but they did not substantiate their results with selective substrates by the use of selective inhibitors with a bifunctional substrate. We therefore further investigated the distribution of MAO A and B between synaptosomal and extrasynaptosomal mitochondria and extended the study to the use of the physiologically active substrate dopamine.

Our results in Table 1 clearly indicate that synaptosomal and extrasynaptosomal mitochondria are different with respect to their relative amounts of MAO A and B, since the specific activities were higher with serotonin (for MAO A solely), but lower with the strict B-substrates PEA (5×10^{-6}M) and benzylamine in I than in C-mitochondria. With 10^{-3}M PEA, an intermediate ratio of activities with I- and C-mitochondria was found, suggesting that 1 mM PEA might be deaminated by both forms of MAO. Inhibition curves with clorgyline and deprenyl (Fig. 3) confirmed that MAO A contributes to the deamination of 1 mM PEA by about 50 per cent in synaptosomal and 17 per cent in non-synaptosomal mitochondria. This indicates about a 4.5-fold higher MAO A:B ratio in I- than in C-mitochondria. This is in agreement with the ratios that can be calculated from the results in Table 1 and with the findings of Student and Edwards [10], who reported a 2.7-fold difference in the relative enzyme amounts in their preparations of synaptic and non-synaptic mitochondria. From our measurements of marker enzymes (data not shown), it seems highly unlikely that cross-contamination of our fractions affected our results to a major extent. In any event, the presence of minor cross-contaminations would imply that the differences in MAO A:MAO B ratios

between C- and I-mitochondria would even be quantitatively more pronounced, not affecting the qualitative statement of a higher enrichment in MAO A in mitochondria of synaptosomal origin.

The finding that PEA is not a selective substrate for MAO B at higher concentrations is in agreement with an earlier report [18] and is probably related to the fact that the K_m of MAO B for PEA is in the micromolar range [19, 20] and thus much lower than the K_m values of MAO for other substrates like serotonin, norepinephrine or benzylamine, which are usually assayed at concentrations near their respective K_m values and might also become non-selective at higher concentrations.

It was of interest to investigate how the ability to deaminate the neurotransmitter dopamine is distributed between the two enzyme forms in our mitochondrial preparations. As the inhibition curves with clorgyline in Fig. 4 show, the higher MAO A : MAO B ratio in intra- (9:1) vs extrasynaptosomal (3:1) mitochondria was again confirmed with the use of dopamine as substrate. However, although dopamine is accepted as a substrate by both forms of MAO *in vitro*, under conditions which allow free access of the substrate to the enzyme, its deamination in the rat brain *in vivo* may well be restricted to only one form of MAO due to compartmentation. We therefore measured MAO activity with dopamine in intact synaptosomes, an approach which is closer to *in vivo* conditions than the use of mitochondrial preparations, as under appropriate conditions it allows a more selective assay of dopamine deaminating activity in terminals of neurones which use this amine as a transmitter. The clorgyline inhibition curve with synaptosomes (Fig. 4) was significantly different from the one with I-mitochondria and showed no signs of a contribution of MAO B to the deamination of dopamine in synaptosomes. It can be seen in Fig. 4 that at low clorgyline concentrations (10^{-8} and 10^{-9}M), the inhibition of dopamine deaminating activity is actually lower in intact synaptosomes than in isolated synaptosomal (I) mitochondria. However, it is very possible that at these low concentrations the repartition of the inhibitor between the synaptosomal compartment and the incubation medium was not complete and that the actual concentrations of inhibitor to which the enzyme was exposed were lower than indicated in the abscissa in Fig. 4. Both clorgyline and deprenyl inhibited the accumulation of dopamine in synaptosomes (Fig. 5). However, this effect occured at much higher concentrations than MAO inhibition (IC_{50} values 10^{-5}–10^{-4}M). Therefore, indirect MAO inhibition in synaptosomes by inhibition of the uptake of dopamine into the nerve endings does not account for the results with synaptosomes shown in Fig. 4.

Thus, the data of the present study suggest that the main sites of dopamine deamination in the rat brain *in vivo*, the terminals of dopaminergic neurones, contain almost only MAO A. With one exception [21], earlier reports attributed dopamine metabolism in the rat brain *in vivo* to MAO A only [11, 22]. While Glover *et al.* [23] found that dopamine is a MAO B substrate in man, Roth and Feor [24] reported that both forms of MAO contribute to the deamination of dopamine in the human brain. However, these studies were performed under *in vitro* conditions which did not consider the possible effects of compartmentation: MAO activities were measured with extrasynaptosomal mitochondria or in synaptosomes using a high substrate concentration which allows passive diffusion and non-specific transport of dopamine and which thus does not distinguish between the nerve endings derived from dopaminergic or non dopaminergic neurones. Furthermore, both groups of investigators [24, 25] have pointed out the difficulties in collecting and storing human post mortem brain samples. In fact, MAO A from human brain was found to be more susceptible to freezing and thawing than MAO B and the diminution of dopamine deaminating activity upon storage of human brain mitochondria was equivalent to the loss of the clorgyline-sensitive MAO activity [24].

Therefore, whereas little doubt seems to exist with regard to the preferential deamination of dopamine by MAO A in the rat brain *in vivo*, the role of the two MAO forms in the metabolism of dopamine in the human brain may require reevaluation. It seems tempting to speculate that the two forms of MAO may represent different physiological functions in the brain. As indicated by its substrate specificities and subcellular distribution, MAO A seems to be especially adapted to the inactivation of neurotransmitter amines in nerve terminals. On the other hand, the substrate specificities of MAO B and the unusually high affinity of this enzyme for PEA indicate that this form of MAO might be directed to the elimination of trace amounts of other aromatic amines such as PEA, which can pass the blood–brain barrier and might act as neuromodulators or 'false' neurotransmitters (for reviews, see Refs. 26 and 27). Since PEA has been associated with various forms of mental illness [27–32], the role of MAO B in the inactivation of this amine may need further investigation.

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