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THE HETEROGENEITY OF MONOAMINE OXIDASE IN DISTINCT POPULATIONS OF RAT BRAIN MITOCHONDRIA

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Abstract—Three populations of rat brain mitochondria were isolated by centrifugation in a Ficoll-sucrose discontinuous gradient. The three fractions showed differential activity towards 5-hydroxytryptamine (a type A MAO substrate) and phenylethylamine (a type B MAO substrate). There were also clear differences in the inhibition characteristics of MAO with the specific inhibitors clorgyline and deprenyl. It is suggested that rat brain mitochondria are not homogeneous with respect to their complements of MAO, and that the differences are not due to artefacts produced by the isolation procedure.

Different preparations of brain mitochondria may be enriched with some enzymes and deficient in others [1, 2, 3, 4, 5]. In addition, it is possible to distinguish between mitochondria derived from neuronal and glial cells [6] and synaptic and non-synaptic mitochondria [1, 4]. The evidence for the heterogeneity of brain mitochondria has been reviewed [7].

Monoamine oxidase (MAO, monoamine-O_2 oxidoreductase, EC 1.4.3.4) is bound to the outer mitochondrial membrane [8]. There is considerable evidence that it exists in more than one form: several bands containing MAO activity have been resolved on polyacrylamide gel electrophoresis of solubilised preparations from rat brain mitochondria [9]. On the basis of *in vitro* studies with the MAO inhibitor clorgyline (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy-propylamine)), Johnstone [10] suggested two forms of MAO, type A and type B, in rat brain. *In vitro* type A MAO is relatively sensitive to inhibition by clorgyline [10] and type B MAO to deprenyl (phenylisopropylmethylpropinylamine) [11]. Noradrenaline [12] and 5-hydroxytryptamine [10] are preferentially deaminated by type A MAO, and benzylamine [13] and β-phenylethylamine [14] by type B MAO. Tyramine [10] is a substrate for both forms of the enzyme.

Tipton and Houslay [15], however, have suggested that the substrate preferences, inhibition characteristics and multiple forms of MAO are attributable to the adherence to the enzyme of differing quantities of lipid or membraneous material.

Lai *et al.* [16] reported a technique for the preparation of three distinct populations of rat brain mitochondria that were relatively free from non-mitochondrial contamination. Two of these populations were derived from synaptosomes, and the remainder were free mitochondria. The mitochondrial populations appear to differ from each other in certain aspects of metabolism of citric acid cycle and related intermediates. In view of this heterogeneity, we investigated the characteristics of monoamine oxidase in the three mitochondrial populations.

MATERIALS AND METHODS

Chemicals. Tyramine, 5-hydroxytryptamine creatinine sulphate, β-phenylethylamine and mannitol were obtained from Sigma (London). [^{14}C]tyramine hydrochloride and [^{14}C]5-hydroxytryptamine creatinine sulphate were obtained from the Radiochemical Centre, Amersham, England, and [^{14}C]β-phenylethylamine hydrochloride from ICN Pharmaceutical Inc., California, U.S.A. Clorgyline was obtained from May and Baker Ltd., Dagenham, Essex and Deprenyl was a kind gift from Dr. A. R. Green, Oxford University. All other chemicals were from British Drug Houses Ltd., Poole, England and of the highest purity available. Ficoll was dialysed against glass-distilled water for at least 5 hr before use.

Preparation of mitochondrial populations. The method employed to prepare the mitochondrial populations was essentially similar to that described by Lai [17] and Lai *et al.* [18], except that the final wash of the three mitochondrial fractions with bovine plasma albumin medium was omitted.

Male Wistar rats (180–200 g) were guillotined, the brains removed and transected at the level of the colliculi. That part of the brain rostral to the transection (ie. the forebrain), with the exception of the olfactory bulbs, was finely minced with scissors. Excess blood was removed by washing the minced brain in isolation medium (0.32 M sucrose, 1 mM potassium–EDTA and 10 mM Tris–HCl pH 7.4) and decanting off the supernatant. A 10% (w/v) homogenate in isolation medium was prepared using a motor-driven MSE homogeniser with a Teflon pestle (all-round clearance of 0.003–0.005 in.) with 3 × 10 passes. The homogenate was then centrifuged at 1300 *g* for 3 min. The supernatant was retained and the pellet was

rehomogenised in 10 ml isolation medium with 16 passes of the pestle, and then centrifuged at 1300 *g* for 3 min.

The pooled supernatants were centrifuged at 17,000 *g* for 10 min to obtain a crude mitochondrial pellet (CM). The remainder of the procedure is presented in Fig. 1. All centrifugation was carried out in an MSE Superspeed 25 centrifuge at 2–4° with a 8 × 50 ml angle rotor except for the two Ficoll-gradient centrifugation steps when a 6 × 16.5 ml swing-out rotor was used. Average *g* values are quoted throughout.

The 7.5% and the 10% Ficoll-sucrose media consisted respectively of 7.5% (w/v) or 10% (w/v) Ficoll, 0.32 M sucrose, 50 μM K-EDTA and 10 mM Tris HCl, pH 7.4. The 4.5% and 6% Ficoll-sucrose media consisted respectively of 4.5% (w/v) or 6% (w/v) Ficoll, 0.24 M mannitol, 0.06 M sucrose, 50 μM K-EDTA and 10 mM Tris-HCl pH 7.4. Ficoll-sucrose medium (3%) was prepared by diluting the 6% Ficoll-sucrose medium 1:1 with distilled water.

Assay of monoamine oxidase activity. Monoamine oxidase activity was assayed by a radiometric technique similar to that developed by Robinson *et al.* [19]. One hundred μl of mitochondrial preparation was incubated at 37° for 30 min at pH 7.2 in the presence of a radioactive substrate (phenylethylamine, 5-hydroxytryptamine or tyramine) in a final volume of 0.5 ml. Substrate concentration was 1 mM and sp. act. of 0.3 μCi/μmole. Reactions were stopped by the addition of 100 μl of 6N HCl to the reaction mixtures and the deaminated products solvent extracted. The deaminated products of phenylethylamine were extracted into heptane (2 ml) and those of 5-hydroxytryptamine and tyramine into ethylacetate:benzene 1:1 (v/v) (2 ml). After centrifugation 1 ml of the

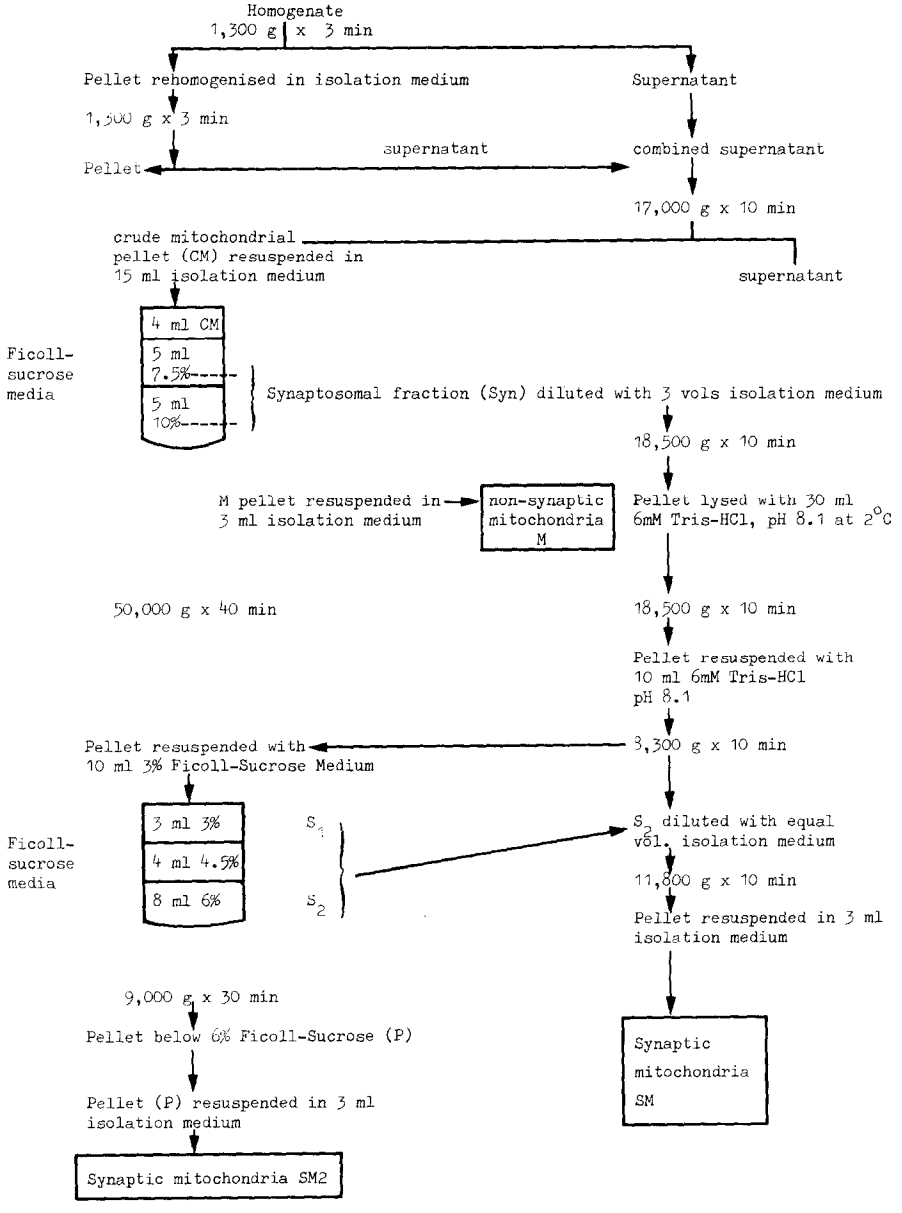

Fig. 1. Preparation of synaptic and non-synaptic rat brain mitochondria.

Table 1. MAO activity of mitochondrial fractions using 5-hydroxytryptamine and phenylethylamine as substrates

	Fraction	5-Hydroxy-tryptamine	Phenylethylamine
8	M	51.6 ± 16.4	15.6 ± 7.5
8	SM	117.5 ± 16.6	31.7 ± 5.8
8	SM2	106.6 ± 3.0	51.8 ± 4.6

(MAO activity, mean ± S.D., expressed as nmoles product/mg protein/30 min).

organic phase was added to 10 ml of Triton X100 based scintillant and the deaminated products quantified by scintillation counting. Blanks consisted of boiled mitochondrial preparations carried through the entire procedure. Mitochondrial protein was measured using a phenol reagent technique [20] and MAO activity was expressed as nmoles of product formed/mg protein/30 min.

Inhibition studies. MAO activity was determined as described with [^{14}C]tyramine as substrate with increasing concentrations of clorgyline (10^{-14}M–10^{-3}M) and deprenyl (10^{-9}M–10^{-3}M). Prior to the MAO assay incubation mixtures were preincubated at 37° for 10 min in the presence of inhibitor but in the absence of substrate.

RESULTS

MAO activity. The specific activities of MAO, with 5-hydroxytryptamine and phenylethylamine as substrates, in the three mitochondrial fractions are presented in Table 1.

From Table 1 it is clear that the three mitochondrial fractions were heterogeneous with respect to MAO activity and fell into three distinct groups when phenylethylamine was used as substrate. Both synaptosomal fractions (SM and SM2) more actively deaminated 5-hydroxytryptamine than did the free mitochondria (M).

Inhibition studies. The effects of increasing concentrations of clorgyline and deprenyl on the MAO activity of the mitochondrial fractions are presented in Figs. 2 and 3 respectively. A double sigmoid curve, indicative of two forms of MAO, resulted in all fractions, with the SM fraction being particularly sensitive to inhibition by clorgyline. The differences in inhibition characteristics between the mitochondrial fractions were not as marked with deprenyl as with clorgyline.

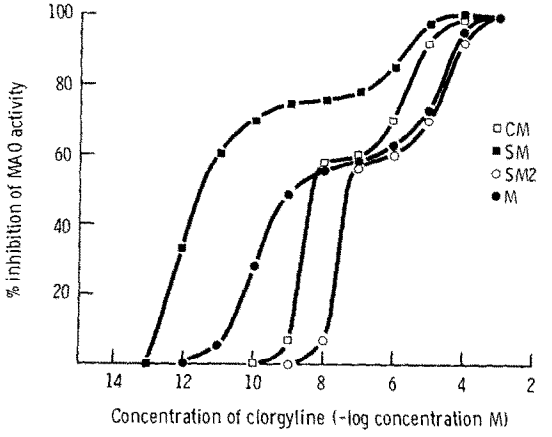

Fig. 2. Effect of clorgyline concentration on MAO activity of rat brain mitochondrial fractions with tyamine as substrate. (□—CM-crude mitochondria, ■—SM-light synaptosomal mitochondria, ○—SM2-heavy synaptosomal mitochondria, ●—M-free mitochondria).

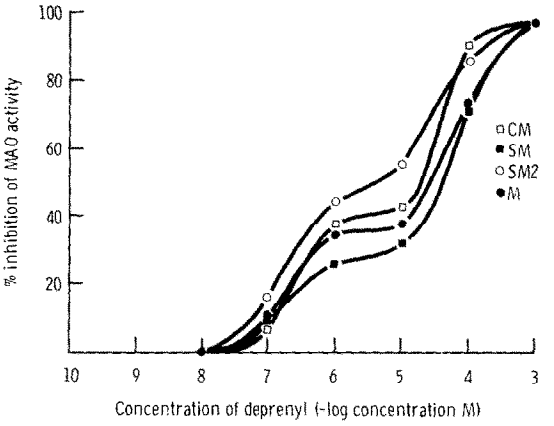

Fig. 3. Effect of deprenyl concentration on MAO activity of rat brain mitochondrial fractions with tyramine as substrate. (□—CM-crude mitochondria, ■—SM-light synaptosomal mitochondria, ○—SM2-heavy synaptosomal mitochondria, ●—M-free mitochondria).

DISCUSSION

The three populations of rat brain mitochondria described by Lai *et al.* [16] differed markedly from each other with respect to monoamine oxidase activity when both the A and B forms of the enzyme were taken into account. It is noteworthy that all the mitochondrial fractions showed considerably more type A activity than type B, particularly the synaptosomal fractions. Serotonin and noradrenaline are preferentially deaminated by type A MAO and recent evidence presented by Braestrup, Anderson and Randrup [21] suggests that this may also be the case for dopamine.

Differential type A and type B activities have been reported in mitochondrial fractions after centrifugation in sucrose gradients [22, 23].

The results of the inhibition studies with clorgyline and deprenyl strongly supported the finding of differential type A and type B MAO activities in the mitochondrial populations. The clorgyline inhibition curves showed distinct differences in percentage inhibition at their plateaus, indicating relatively different activities of type A and type B monoamine oxidase. The deprenyl inhibition curves did not show such striking differences between the three fractions but did support the validity of the clorgyline inhibition curves in as much as that the SM fraction, which was most sensitive to inhibition by clorgyline, was the least sensitive to inhibition by deprenyl and *vice versa* for the SM2 fraction.

It appears, therefore, that not only is monoamine oxidase heterogeneous in preparations of crude mitochondria from rat brain but that the mitochondria themselves are not all similar with respect to their

MAO content. However, as Houslay and Tipton [15] have pointed out these differences need not be the result of different enzyme proteins and may well be due to the presence of different quantities or classes of lipids or type of binding of the enzyme to the mitochondrial membrane. Since intact mitochondria were used in the present study, it seems unlikely that the results obtained were due to artefacts produced by preparation of the fractions, and differential MAO activity towards a variety of substrates is likely to be an *in vivo* reality. The differential *in vivo* metabolism of brain amines after administration of specific MAO inhibitors has been demonstrated by Neff *et al.* [22].

Whether or not the two synaptosomal mitochondrial populations are representative of mitochondria from different anatomical regions of the rat brain is at present unknown, although the technique used in the present investigation could be scaled down and made suitable for investigating this possibility.

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