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SUBCELLULAR LOCALIZATION OF TYPES A AND B MONOAMINE OXIDASE IN RAT BRAIN*

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Abstract—The subcellular localization of the A and B forms of MAO in rat brain was examined. Although both enzymatic forms were found to be present in synaptosomal fractions, the B:A ratio in the synaptosomal fraction was one-half that in the extrasynaptosomal mitochondrial fraction obtained from whole brain. The synaptic mitochondria, isolated following lysis of the synaptosomes, had the same B:A ratio as the nonlysed synaptosomes, suggesting that (1) MAO-B activity is, indeed, associated with synaptosomes and (2) that this activity is located in the mitochondria. The relative activities of the A and B enzymes in synaptosomal fractions were found to be greatly different among discrete brain regions, even though only small variations are evident in the whole homogenates. We conclude from these data that the activity of MAO-B may be nonuniformly located among different populations of synaptic endings.

Monoamine oxidase (MAO: E.C. 1.4.3.4.) is a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines [1]. Recent evidence points to the existence of multiple forms of MAO having different substrate specificities. Studies originally reported by Johnston [2] demonstrated that two forms of MAO could be differentiated on the basis of their sensitivity to inhibition by clorgyline. The form which was inhibited by low concentrations of clorgyline was designated as type A MAO, and the form which was relatively insensitive to inhibition by low concentrations of clorgyline was designated as type B MAO. It was subsequently found that type B MAO was selectively inhibited by low concentrations of deprenyl [3]. The putative neurotransmitters, serotonin (5HT) [2] and norepinephrine [4], are preferred substrates for MAO-A. On the other hand, benzylamine (Bz) [5] and phenylethylamine (PE) [6] are preferred substrates for MAO-B, whereas dopamine, tryptamine (Trypt) and tyramine serve as substrates for both forms of MAO [7]. The two forms of MAO could also be demonstrated *in vivo* following intravenous injection of low doses of clorgyline or deprenyl [8].

There is evidence to suggest that types A and B MAO are associated with specific cell types. Goridis and Neff [4] reported that denervation of the rat pineal gland by superior cervical ganglionectomy resulted in a marked decrease in type A but not type B MAO activity. The relative content of types A and B MAO activity in the same cell type may also vary among species. Human blood platelets contain only type B MAO activity, whereas rabbit platelets contain both types A and B MAO [9]. These data suggest that the presence or absence of a particular type of MAO in a certain type of cells may be importantly related to the physiological function of that cell and its disposition of particular biogenic amines.

Little is known, however, concerning the cellular localization of multiple forms of MAO in brain. In both rat and human brain homogenates, very little variation in the relative activities of types A and B MAO have been detected [10, 11]. Nevertheless, the ratio of MAO-B:MAO-A was slightly, but significantly, higher in the cerebellum and olfactory bulb than in other regions of the rat brain [12]. Hirano *et al.* [13] have reported that the ratio of MAO activities measured with the substrates serotonin and tyramine varied 4.4-fold among several hypothalamic nuclei, but, unfortunately, no B-specific substrate was used. Yang and Neff [6] found that brain mitochondria sedimenting at different buoyant densities on a continuous sucrose gradient displayed different MAO-B:MAO-A enzymatic activities, but, since whole brain homogenates had been used, it was not possible to determine the cellular origin of either the MAO-A or MAO-B-rich mitochondria. Bourne *et al.* [14] have reported that two populations of mitochondria derived from rat brain synaptosomes are distinguished from free mitochondria on the basis of their MAO-A:MAO-B ratio. In the present studies, we have found that the ratio of MAO-B:MAO-A in the synaptosomal fraction is one-half that in the free mitochondria isolated from whole rat brain, and that this ratio varies among discrete brain regions. From these studies, it is apparent that the relative activities of MAO-A and MAO-B in brain homogenates do not necessarily reflect these activities at specific subcellular locations.

MATERIALS AND METHODS

Subcellular fractionation. Male Sprague–Dawley rats (Zivic-Miller Labs, Pittsburgh, PA), 39–45 days

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old, weighing 150–200 g were used. From six to twelve animals were decapitated and the brains were removed and placed on ice within 30 sec. The brains were homogenized in 9 vol cold 0.32 M sucrose, using 10 vertical strokes in a teflon-glass tissue grinder (0.025 cm clearance) at a speed of 600 rpm.

When experiments were performed on the brain regions, cerebellum, striatum and cerebral cortex were removed according to the method of Glowinski and Iversen [15].

The methodology used for subcellular fractionation and subsynaptosomal fractionation was adapted from Autilio *et al.* [16], Morgan *et al* [17], and Cotman and Matthews [18]. A crude nuclear fraction (P_1) was obtained by centrifugation at 1000 *g* for 10 min. The resulting supernatant (S_1) was centrifuged at 14,000 *g* for 15 min to yield the crude mitochondrial pellet (P_2) and the supernatant, S_2. Centrifugation of S_2 at 70,000 *g* for 1 hr gave a microsomal fraction (P_3) and a cell soluble fraction (S_3).

The crude mitochondrial pellet (P_2) was washed 3 times (14,000 *g* 15 min) by resuspending in 0.32 M sucrose, and, after centrifugation, the final pellet (P_2') was resuspended in isoosmotic sucrose, 3 ml/g of original tissue. Volumes of 2–4 ml were then layered on Ficoll-sucrose discontinuous gradients. (These were previously prepared by layering 7 ml each of solutions containing 5, 7.5, 10 and 13% Ficoll in 0.32 M sucrose and then allowing them to equilibrate 1–2 hr at room temperature and finally at 4° for another 1–2 hr.) The gradients were centrifuged in a Spinco SW 25.1 rotor at 13,000 rev/min (for $R = R_{av}$: 19,000 *g*) for 75 min. The two layers containing myelin (My) (on top of the 5% layer and at the 5–7.5% interface) were collected and pooled, by pipetting off all material from the top of the gradient through half of the 7.5% Ficoll-sucrose layer. The synaptosomal (S) fractions were collected and pooled by pipetting off all material from the middle of the 7.5% layer to the middle of the 13% layer. The mitochondrial pellet (M) was suspended in the remaining 13% Ficoll-sucrose solution. Since Ficoll was found to interfere with the Lowry protein determinations, the myelin, synaptosomal, and mitochondrial fractions were diluted with 0.32 M sucrose and centrifuged at 29,000 rpm for 45 min. The resulting pellets were then suspended in 0.32 M sucrose.

Subsynaptosomal fractionation. The synaptosomal fraction which was obtained as described above was suspended in 20–25 ml of 0.32 M sucrose and pelleted in a Spinco No. 30 rotor at 25,000 rpm (for $R = R_{av}$: 54,450 *g*) for 30 min. The washed synaptosomes (S') were subjected to osmotic shock in 6 mM Tris–HCl, pH 8.4, for 60–90 min at 4°, according to the procedure of Cotman and Matthews [18].

The lysed synaptosomes were then centrifuged at 14,000 *g* for 15 min. Only material not sedimenting under these conditions was considered synaptic plasma membranes (PM), in order to avoid contamination by subcellular particles whose sedimentation properties were not changed by the shocking procedure [17]. The pellet was resuspended in 0.32 M sucrose to a final volume of 2 ml, of which 1.8 ml was layered on a discontinuous sucrose gradient, which was composed of 14 ml each of 35% and 38% sucrose and which had been equilibrated as described above. The gradient was centrifuged 90 min 22,500 rpm (for $R = R_{av}$: 44,100 *g*).

The pellet obtained from this centrifugation contained synaptic mitochondria (SM) which were separated from synaptic plasma membranes and from unbroken synaptosomes. After pipetting off the sucrose solutions, the synaptic mitochondrial fraction was resuspended in 20–25 ml of 0.32 M sucrose.

Finally, the synaptic mitochondrial suspension, and the suspension of the synaptic plasma membrane fraction (PM) were centrifuged 12 hr at 25,000 *g* and the resulting pellets were resuspended in 0.32 M sucrose.

All samples were stored at −20° and assays were carried out within 4 days.

Assays. MAO was assayed by a modification of the radiochemical procedure of Wurtman and Axelrod [19], according to Edwards and Burns [20], using the substrates ^{14}C tryptamine (6.17×10^{-5} M), ^{14}C PE (4.88×10^{-6} M), and ^{14}C benzylamine (2.19×10^{-4} M). For the substrate ^{14}C 5HT (10^{-4} M), MAO was assayed by a modification of the procedure of McCaman *et al.* [21]. All reactions were carried out in a volume of 2 ml for 1 hr at 37°, during which time the assay was linear as long as not more than 10% of the substrate was converted to product. After stopping the reactions by the addition of 0.2 ml of 2 N HCl, the deaminated products were separated from the remaining substrate by solvent extraction and determined by liquid-scintillation counting.

Rotenone-insensitive NADH: cytochrome *c* reductase was assayed spectrophotometrically at 30° in a Gilford model No. 240 spectrophotometer, by following the reduction of added cytochrome *c* at 550 nm [22].

Protein was estimated by the method of Lowry *et al.* [23], which was automated using a Technicon Auto Analyzer.

Drug inhibition studies. In order to characterize further the MAO activity in the subcellular fractions, studies were carried out with clorgyline and deprenyl. For *in vitro* studies, clorgyline was added to the assay mixture to a final concentration of 10^{-10}–10^{-5} M. The assay was carried out with either PE or 5HT as substrate and was initiated by the addition of enzyme. For *in vivo* studies, animals were given an i.v. injection of either clorgyline (1 mg/kg) or deprenyl (1 mg/kg) 2 hr before they were killed. Subcellular fractions of the brains were then obtained as described above and assayed for MAO activity.

Electron microscopy. Pellets were fixed in 1% osmium tetroxide in phosphate buffer, pH 7.2. The fractions were dehydrated in ethanol and embedded in Epon and Araldite. Sections from various locations in the pellet were cut using a Porter–Blum Microtome MT-1 and stained with uranyl acetate and post-stained with lead citrate-Reynolds. Several representative sections of each pellet were examined with a Phillips 300 electron microscope.

Materials. Density gradient grade sucrose (Schwarz–Mann) was used throughout. Ficoll (Pharmacia) was dialyzed for 12 hr against two changes of glass-distilled water and stored 12 hr at 4° until use. NADH, NADPH, cytochrome *c* and serotonin creatinine sulfate were obtained from Sigma Chemical Co. Phenylethylamine-HCl and benzylamine-HCl were purchased from K and K Laboratories. Tryptamine-

HCl was obtained from Aldrich Chemical Co. The substrates [side chain-2-^{14}C]tryptamine, [ethyl-1-^{14}C]phenylethylamine and [side chain-2-^{14}C]serotonin were purchased from New England Nuclear. [7-^{14}C]Benzylamine was obtained from Mallinckrodt.

Clorgyline (*N*-methyl-*N*-propargyl-3(2,4-dichlorophenoxy)propylamine hydrochloride) was a gift from May and Baker, Ltd. England. Deprenyl was kindly provided by Dr. Knoll, Budapest.

RESULTS

Electron microscopy. Electron micrographs of the synaptosomal and mitochondrial fractions were derived from a gradient layered with an unwashed crude mitochondrial fraction. In both cases, there was a large number of the intact representative particles. The electron micrographs of the synaptic mitochondria show a dense population of particles which look very different from the extrasynaptosomal mitochondria (i.e., they are smaller and have a larger number of cristae-like structures). This difference may be due to alterations occurring after exposure to 6 mM Tris–HCl at pH 8.4.

Subcellular fractionation of whole rat brain. Table 1 includes data combined from two experiments. The large amount of protein (69 per cent) and MAO activity (55–57 per cent) that sedimented in the first centrifugation step (P_1) was a result of the mild homogenizing conditions used in order to isolate intact synaptosomes. The pellet P_1 presumably contains many unbroken cells, reducing the apparent recovery of enzymatic activity in other subcellular fractions.

The crude mitochondrial fraction (P_2) contained 16.5 and 14 per cent of the total activities of MAO-A and MAO-B, respectively, in the whole homogenate (Table 1). Although 14 per cent of the total brain protein appeared in the P_2 fraction, it should be noted that the amount of MAO activity which could be accounted for through this step of the procedure (i.e., $\%P_1 + \%P_2 + \%S_2$) was 74.5 and 71.1 per cent for the A and B forms, respectively, whereas 99.4 per cent of the total protein could be accounted for. The lower recovery of MAO activity than of total protein may be due to a partial denaturation of active enzyme or to a selective loss of enzymic protein. However, the activities of the A and B enzymes show similar losses of activity. The specific enzymatic activities for MAO in the crude mitochondrial fraction were only slightly or not at all higher than that for the whole homogenate. This is probably due to the combination of a loss of recovered activity during the preparation of P_2 in addition to a large degree of contamination of this fraction by other cellular components.

When the crude mitochondrial pellet (P_2) was fractionated on a Ficoll-sucrose gradient, 21–26 per cent of the total MAO activity, determined with either A- or B-specific substrates, was recovered in the myelin fraction of the gradient. This would appear to be due to a contaminant rather than to any intrinsic MAO activity associated with myelin. First, when the crude mitochondrial pellet was washed three times (to obtain P_2') before being fractionated on a Ficoll-sucrose gradient, only 5–9 per cent of the MAO activity was recovered in the myelin fraction (Table 1). Secondly, based on the activity of rotenone-insensitive NADH:cytochrome *c* reductase, an enzymatic marker for outer mitochondrial membranes, we estimate that about 7 per cent of the protein in the myelin fraction is contained in outer mitochondrial membranes, assuming that the outer mitochondrial membrane contains 10 per cent of the total mitochondrial protein [17]. These results suggest, therefore, that the MAO activity in the myelin fraction could be accounted for by contamination by sheared off outer mitochondrial membranes [17].

The distributions of the A and B forms of MAO in the synaptosomal and mitochondrial fractions were found to be significantly different (Table 1). For MAO-A, 37 per cent of the total activity recovered from the gradient was in the synaptosomal fraction

Table 1. Subcellular fractionation of whole rat brain*

Fraction	*n*	Per cent recovered			Specific enzymatic activity (nmoles/hr/mg protein)		
		Protein	MAO-A	MAO-B	MAO-A	MAO-B	MAO-B/MAO-A
Homogenate	3	100	100	100	136 ± 4	32.2 ± 1.2	0.238 ± 0.010
P_1	6	69.1	55.0	56.6	108 ± 13	24.8 ± 0.4	0.230 ± 0.031
S_1	6	38.3	25.1	22.5	90.2 ± 5.4	19.2 ± 1.1	0.214 ± 0.004
S_2	6	16.3	3.0	3.5	30.1 ± 6.2	8.4 ± 1.8	0.278 ± 0.012
P_3	6	3.7	2.3	2.5	96.4 ± 17.6	25.4 ± 4.4	0.266 ± 0.008
S_3	6	9.6	0.20	0.35	4.0 ± 1.0	1.9 ± 0.6	0.413 ± 0.046
P_2	6	14.0	16.5	14.0	159 ± 6	32.2 ± 1.9	0.204 ± 0.013
P_2'	3	11.7	15.5	12.2	180 ± 5	33.9 ± 5.5	0.193 ± 0.041
Myelin	6	0.932	0.598	0.294	95.1 ± 14.3	11.0 ± 1.5	0.120 ± 0.006
Synaptosomes (S)	6	1.85	2.58	1.50	187 ± 17	27.7 ± 4.0	0.148 ± 0.021
Mitochondria (M)	6	2.20	4.82	4.34	234 ± 21	63.1 ± 4.9	0.274 ± 0.029
Washed synaptosomes (S')	3	1.59	2.56	1.17	218 ± 17	23.3 ± 4.1	0.105 ± 0.010
Synaptic mitochondria (SM)	2	0.7	1.5	0.6	309	31.2	0.102
Synaptic plasma membranes (PM)	2	0.5	0.17	0.16	81.9	11.0	0.238

* Data are combined from two experiments. In each experiment, 6 animals were used and two whole brains were pooled for each tissue sample carried separately through the fractionation procedure. Values are the means ± S.E.M. for the total number of tissue samples indicated. Recovery of total protein and enzymatic activity is given relative to the whole homogenate. The specific enzymatic activities are expressed as nmoles product formed/hr/mg protein. MAO-A and MAO-B were assayed with the substrates 5HT and phenylethylamine, respectively. Each SM and PM was obtained by pooling 3 S' fractions.

Table 2. Ratios of the specific activities of monoamine oxidase with four substrates in subcellular fractions of rat brain regions*

Brain region	n	MAO-PE / MAO-5HT	MAO-PE / MAO-Bz	MAO-Trypt / MAO-5HT
			Homogenate	
Cerebellum	5	0.323 ± 0.030	0.687 ± 0.082	0.716 ± 0.067
Striatum	5	0.205 ± 0.031	0.640 ± 0.050	0.625 ± 0.054
Cortex	5	0.243 ± 0.037	0.767 ± 0.077	0.793 ± 0.099
			Synaptosomes	
Cerebellum	4	0.271 ± 0.070	0.533 ± 0.114	0.785 ± 0.127
Striatum	5	0.119 ± 0.020	0.587 ± 0.084	0.657 ± 0.074
Cortex	3	0.0678 ± 0.0070	0.410 ± 0.041	0.614 ± 0.093
			Mitochondria	
Cerebellum	5	0.350 ± 0.068	0.565 ± 0.078	0.796 ± 0.106
Striatum	5	0.182 ± 0.033	0.556 ± 0.058	0.674 ± 0.079
Cortex	3	0.144 ± 0.014	0.474 ± 0.054	0.712 ± 0.153

* Mean ± S.E. of data pooled from five experiments.
Substrates used were PE, 5HT, benzylamine (Bz) and tryptamine (Trypt).

and 54 per cent was in the mitochondrial fraction. On the other hand, only 25 per cent of the MAO-B activity was found in the synaptosomal fraction and 71 per cent in the mitochondrial fraction. The ratio of MAO-B activity to the MAO-A activity was 1.9-fold higher in the mitochondrial (0.274) than in synaptosomal (0.148) fractions ($P < 0.005$).

As can be seen from Table 1, the relative specific enzymatic activities compared to the homogenate for MAO-A and MAO-B were 1.72 and 1.96, respectively, in the mitochondrial fraction and 1.38 and 0.86, respectively, in the synaptosomal fraction. These results are comparable to results observed by other investigators using similar techniques for subcellular fractionation of brain tissue. For example, Jones *et al.* [24] found that the specific activities of MAO (substrate not specified) were 2.8- and 1.4-fold higher in mitochondrial and synaptosomal fractions, respectively, than in the whole homogenate.

After the synaptosomal fractions were lysed by osmotic shock, the isolated synaptic mitochondria contained 58 and 55 per cent of the activities of MAO-A and MAO-B, respectively, and 44 per cent of the protein (as compared with the washed synaptosomal fraction, S'). The ratio of MAO-B:MAO-A activity of this fraction (0.102) was similar to that observed for the intact synaptosomes (S') (0.105). The synaptic plasma membrane fractions, on the other hand, contained 6 per cent and 14 per cent of the MAO-A and MAO-B activities, respectively, and 29 per cent of the protein. Thus, there appears to be no selective localization of either type of MAO in synaptic plasma membranes to account for the relatively low MAO-B:MAO-A ratio in synaptosomal fractions.

The specific activity of rotenone-insensitive NADH: cytochrome *c* reductase was actually higher in the synaptic plasma membrane fraction (34.7 ± 7.8 μmoles/hr/g protein) than in either the nonsynaptosomal mitochondrial (M) (17.6 ± 2.3 μmoles/hr/g protein) or the synaptic mitochondrial fractions (SM (23.0 ± 11.0 μmoles/hr/g protein)). This suggests that the small amount of MAO activity present in the synaptic plasma membrane fraction may be due to contamination by outer mitochondrial membrane fragments.

Subcellular distribution of MAO activity in three brain regions. Table 2 demonstrates that the ratio MAO-B:MAO-A varies as much as 4-fold in synaptosomal fractions obtained from different brain regions. Of the three brain regions examined, the cerebellar synaptosome fraction was found to have the highest MAO-B:MAO-A (0.271), whereas the synaptosomal fraction of the cerebral cortex was found to have the lowest ratio (0.068). In striking contrast, relatively small variations in this ratio were observed for the whole homogenates of these brain regions. For comparison, Table 2 also shows that the ratios, MAO-PE:MAO-Bz and MAO-Trypt:MAO-5HT, i.e. the ratio of MAO activities determined with the substrates PE and benzylamine (both specific substrates

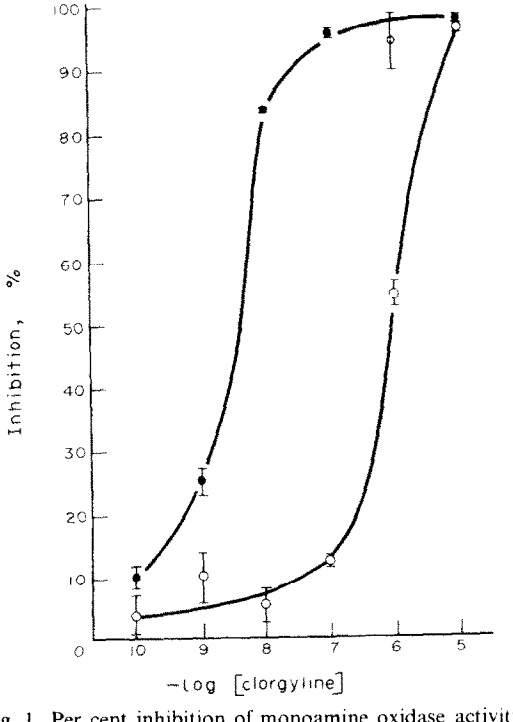

Fig. 1. Per cent inhibition of monoamine oxidase activity in the synaptosomal fraction of rat brain vs the clorgyline concentration with 5HT, ●, or phenylethylamine, ○, as substrate. Data presented are the means of duplicate determinations on the same synaptosomal fraction.

for MAO-B) or with tryptamine (which is primarily deaminated by MAO-A) and 5HT (a specific substrate for MAO-A) have little variation among different brain regions, for either whole homogenates or for subcellular fractions.

Sensitivity to MAO inhibitors. Synaptosomal MAO-A activity was inhibited *in vitro* 84 per cent by 10^{-8} M clorgyline, while the MAO-B activity was inhibited less than 8 per cent (Fig. 1).

When clorgyline was administered *in vivo* (1 mg/kg), only 2 per cent of the MAO-A activity was retained in the isolated synaptosomal fraction, while 81 per cent of the MAO-B activity remained (Table 3). On the other hand, deprenyl injected *in vivo* (1 mg/kg) resulted in retention of 75 per cent of the MAO-A activity and only 7 per cent of the MAO-B activity in the synaptosomal fraction.

Table 3. *In vivo* effects of clorgyline and deprenyl (1 mg/kg, i.v.) on MAO activity in subcellular fractions of rat brain

	MAO activity Per cent of control			
	+Clorgyline		+Deprenyl	
	MAO-A	MAO-B	MAO-A	MAO-B
Myelin	2.8	96.7	80.6	8.1
Synaptosomes	1.8	81.3	75.7	7.6
Mitochondria	3.6	99.6	73.1	6.7

MAO-A and MAO-B were assayed with the substrates 5HT and PE, respectively. All activities are expressed as per cent of activity in the corresponding subcellular fraction obtained from the control animals.

DISCUSSION

Our results indicate that MAO activities which have the substrate preferences characteristic of the A and B forms may be distinguished by their subcellular distribution in rat brain. We have found approximately 2-fold differences between synaptosomal and mitochondrial fractions in the ratio of MAO activity determined with the A- and B-specific substrates, 5HT and PE, respectively (Table 1). This difference is consistent with previous reports that MAO in distinct populations of rat brain mitochondria differ in their relative activity towards multiple substrates. For example, the pattern of MAO activity of rat brain mitochondria centrifuged on a continuous sucrose density gradient varied depending on the substrate used [25]. Yang and Neff [6] in using the preferred substrates for MAO-A and B, reported that the enzyme in mitochondria having a higher buoyant density had a greater ratio of PE to 5HT deamination. However, in most previous studies, the conditions used for homogenization of the tissue did not permit the isolation of intact synaptosomes, so that it was not possible to directly relate differences in substrate preferences of the enzyme to a particular anatomical site (e.g. synaptosomal vs. extrasynaptosomal mitochondria) and hence to a possible functional significance. Although one recent report did indicate that, in cat brain, MAO in synaptosomes vs free mitochondria had different substrate preferences, no specific substrate for MAO-B was used [26].

In addition, although synaptosomes have a lower ratio of MAO-B:MAO-A than do free mitochondria, they, nevertheless, have a significant amount of the B enzyme. However, since the synaptosomal MAO activity measured with the substrate PE was only 1.5 per cent of the total activity in the original homogenate (Table 1), it was necessary to confirm that this activity was representative of MAO-B in the whole homogenate. Indeed, this enzymatic activity showed the expected insensitivity to inhibition by clorgyline *in vitro* (Fig. 1) and *in vivo* (Table 3).

Although the procedure for subcellular fractionation was designed to obtain synaptosomes free of contamination by other cellular particles, it is difficult to quantitate the purity of a synaptosomal preparation. Marker enzymes are of little value for estimating contamination of the synaptosomal preparation by extrasynaptosomal mitochondria as there are no known enzymes unique to extrasynaptosomal mitochondria. In spite of the difficulties involved in isolating synaptosomes free from contamination by other cellular componants, such contamination would not appear to account for the MAO-B activity in our synaptosomal fraction. The specific activity of MAO-B in the washed synaptosomal fraction (S') was 23.3 nmoles/hr/mg, while the specific activity in the extrasynaptosomal mitochondria (M) was 63.1 nmoles/hr/mg. If the MAO-B activity in the synaptosomal fraction is due to contamination by extrasynaptosomal mitochondria, then 37 per cent of the synaptosomal fraction would be extrasynaptosomal mitochondria. This number of free mitochondria are not evident in electron micrographs of the synaptosomal fraction. Moreover, some of the free mitochondria in the synaptosomal fraction are likely a result of synaptosomal lysis and are, therefore, synaptosomal in origin.

The data also suggest that the MAO-B activity in the synaptosomal fraction is not due to contaminating outer mitochondrial membranes derived from extrasynaptosomal mitochondria. Contaminating outer mitochondrial membranes in the synaptosomal fraction would not be expected to pellet through the 35 and 38 per cent sucrose gradient designed to separate synaptic plasma membranes from synaptic mitochondria. One would expect a disappearance or a large decrease in specific activity of MAO-B in the isolated synaptosomal mitochondria as compared with the intact synaptosomes, if this activity were due entirely to contaminating outer mitochondrial membranes. Instead, the values in Table 1 for the specific activity of MAO-B increases from washed synaptosomes (23.3 nmoles/hr/mg) to synaptic mitochondria (31.2 nmoles/hr/mg).

Thus, while one cannot eliminate entirely the possibility that the MAO-B activity in the synaptosomal fraction is due to contamination by other cellular fractions, the data suggest that at least a large proportion of the MAO-B activity is, indeed, contained by synaptosomes.

Our results would appear to rule out any selective extramitochondrial localization of either MAO-A or MAO-B. Although we observed the microsomal fraction (P_3) to contain a small amount of MAO activity, about 2 per cent of the total homogenate activity, the ratio of MAO-B to MAO-A activity was similar to that found for the free mitochondrial fraction (Table 1). We believe that based on the marker

enzymes that this activity is due to contamination by shearing of outer mitochondrial membranes, as suggested by Jarrott and Iversen [27]. Lowe *et al.* [28] reached a similar conclusion that, based on histochemical localization, the apparent activity of MAO in rat heart microsomes [29] may also be due to a shearing of outer mitochondrial membranes during homogenization.

The small amount of MAO activity in the synaptic plasma membrane fraction also appeared to be due to contamination by outer mitochondrial membrane fragments, based on the specific activity of the outer mitochondrial membrane marker, rotenone-insensitive NADH: cytochrome *c* reductase. Although Morgan *et al.* [17] and Tabakoff *et al.* [30] previously observed MAO activity in their synaptic plasma membrane fractions, Tabakoff *et al.* [30] could also account for this activity by contaminating outer mitochondrial membranes.

The more than 2-fold lower ratio of MAO-B to MAO-A in synaptosomes (0.105) and synaptic mitochondria (0.102) as compared with free mitochondria (0.274) could be accounted for by assuming that all synaptic mitochondria have this low B/A ratio and thus are enzymatically distinct from other brain cell mitochondria. Alternatively, this low ratio of MAO-B/MAO-A could also be due to synaptic mitochondria in certain populations of nerve endings having either a lack or relatively low amounts of MAO-B, such that the resulting overall MAO-B to MAO-A ratio in the synaptic mitochondrial fraction is relatively low. Since it has been reported that there is a complete absence of MAO in rat brain cholinergic nerve endings [31], it is conceivable that synaptosomes from other nerve ending populations may lack only the type B enzyme.

The latter notion that the ratio of MAO-B/MAO-A varies among different synaptic endings is supported by our results obtained for discrete brain regions. Although the ratio of MAO activity determined with the substrates PE and 5HT is slightly but significantly higher in the cerebellum than in either the striatum or cortex, in confirmation of our previous findings [12], the differences in this ratio among different brain regions become even more dramatic when subcellular fractions are examined. For example, the B/A ratio was only 1.3-fold higher in the whole homogenates of cerebellum as compared to cerebral cortex, but this ratio was 4.0-fold higher in the cerebellum than in the cerebral cortex when the synaptosomal fractions were compared (Table 2). Intermediate differences which were found in the B:A ratios in these brain regions for the free mitochondrial fractions (e.g., 2.4-fold for cerebellum vs cerebral cortex) may be a result of some of the free mitochondria in the fractions originating from broken synaptosomes.

Clearly, these results demonstrate that the activity of MAO in homogenates of brain tissue may give a poor reflection of the enzymatic activity in synaptic endings or in particular types of synaptic endings. Thus, a substantial reduction of activity at one particular cellular location (e.g. in the synaptic endings) or in specific cell types may be obscured by measurement of the activity in tissue homogenates.

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REFERENCES

1. M. B. H. Youdim, in *M.T.P. International Review of Science* (Ed. H. Blaschko) p. 169. Butterworths Ltd., London (1975).
2. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
3. J. Knoll and K. Magyar, in *Monoamine Oxidase: New Vistas* (Eds E. Costa and M. Sandler) Vol. 5, p. 383. Raven Press, New York (1972).
4. C. Goridis and N. H. Neff, *Neuropharmac.* **10**, 557 (1971).
5. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
6. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187**, 365 (1973).
7. N. H. Neff and H.-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
8. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **189**, 733 (1974).
9. D. J. Edwards and S.-S. Chang, *Life Sci.* **17**, 1127 (1975).
10. M. A. Schwartz, R. J. Wyatt, H.-Y. T. Yang and N. H. Neff, *Archs gen. Psychiat.* **31**, 557 (1974).
11. O. Suzuki and K. Yagi, *Experientia* **32**, 13 (1976).
12. D. J. Edwards, in *Trace Amines and the Brain* (Eds E. Usdin and M. Sandler) p. 59. Marcel Dekker, Inc., New York (1976).
13. M. Hirano, H. Uchimura and M. Saito, *Brain Res.* **93**, 558 (1975).
14. R. Bourne, J. C. K. Lai and F. Owen, *Br. J. Pharmac.* **55**, 298p (1975).
15. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
16. L. A. Autilio, S. H. Appel, P. Pettis and P.-L. Gambetti, *Biochemistry* **7**, 2615 (1968).
17. I. G. Morgan, L. S. Wolfe, P. Mandel and G. Gombos, *Biochim. biophys. Acta* **241**, 737 (1971).
18. C. W. Cotman and D. A. Matthews, *Biochim. biophys. Acta* **249**, 380 (1971).
19. R. J. Wurtman and J. Axelrod, *Biochem. Pharmac.* **12**, 1439 (1963).
20. D. J. Edwards and M. O. Burns, *Life Sci.* **15**, 2045 (1974).
21. R. E. McCaman, M. W. McCaman, J. M. Hunt and M. S. Smith, *J. Neurochem.* **12**, 15 (1965).
22. G. L. Sottocasa, B. Kuylenstierna, L. Ernster and A. Bergstrand, *J. Cell Biol.* **32**, 415 (1967).
23. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
24. L. R. Jones, H. R. Mahler and W. J. Moore, *J. biol. Chem.* **250**, 973 (1975).
25. M. C. Kroon and H. Veldstra, *FEBS Lett.* **24**, 173 (1972).
26. M. B. H. Youdim and B. Holman, *J. Neural Transm.* **37**, 11 (1975).
27. B. Jarrott and L. L. Iversen, *Biochem. Pharmac.* **17**, 1619 (1968).
28. M. C. Lowe, D. D. Reichenbach and A. Horita, *J. Pharmac. exp. Ther.* **194**, 522 (1975).
29. J. de Champlain, R. A. Mueller and J. Axelrod, *J. Pharmac. exp. Ther.* **166**, 339 (1969).
30. B. Tabakoff, L. Meyerson and S. G. A. Alivisatos, *Brain Res.* **66**, 491 (1974).
31. G. R. Arnaiz and E. D. P. De Robertis, *J. Neurochem.* **9**, 503 (1962).