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Monoamine oxidase in rat arteries: evidence for different forms and selective localization

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Summary

1. Two forms of monoamine oxidase activity were differentiated in rat mesenteric and femoral artery by means of substrate and inhibitor specificities: one form deaminated tyramine, 5-hydroxytryptamine and noradrenaline and was highly sensitive to pargyline and clorgyline but resistant towards carbonyl reagents. This form resembled type A monoamine oxidase previously described. The other deaminated tyramine but not 5-hydroxytryptamine or noradrenaline and was inhibited by carbonyl reagents but not by clorgyline or pargyline.

2. About one third of the total monoamine oxidase in homogenates of rat mesenteric artery was recovered in a 10^5 *g* supernatant. Both forms were partially soluble, but relatively less of the type A activity was recovered in the soluble fraction.

3. Chemical sympathectomy with 6-hydroxydopamine resulted in a loss of 59% of monoamine oxidase activity in the mesenteric artery. There was a selective loss of type A activity, as revealed by the 70% decrease in 5-hydroxytryptamine deaminating ability and by the marked decrease in clorgyline sensitivity. The second monoamine oxidase species was resistant to 6-hydroxydopamine. The soluble activity was not affected by chemical sympathectomy. Most of the transmitter-specific monoamine oxidase of the arterial wall was localized within the adrenergic nerve endings. Our observations are consistent with the hypothesis that extraneuronal monoamine oxidase plays only a minor role in metabolizing noradrenaline in sympathetically innervated tissues.

4. Plasma amine oxidase might originate from the arterial wall since it has similar characteristics to that found in the mesenteric artery.

Introduction

In many organs there seem to be several forms of amine oxidase activity, which differ in substrate specificity (Johnston, 1968; Youdim, Collins & Sandler, 1969), inhibitor sensitivity (Squires, 1968) and electrophoretic mobility (Collins, Youdim & Sandler, 1968; Sierens & D'Iorio, 1970). Since the relation between the various amine oxidases occurring in different tissues and species are not understood, and since no consistent nomenclature exists, we will call 'monoamine oxidase' (MAO) all those amine oxidases which deaminate tyramine and we will refer to different forms or types of this activity.

There is now compelling evidence that different forms of MAO are associated with specific cell types (Goridis & Neff, 1971a ; Jarrott, 1971). The MAO associated with the sympathetic nerves (type A MAO) of the pineal gland differs from the extraneuronal form in that it is completely inhibited by 0·1 μM clorgyline, is relatively heat-stable and deaminates tyramine, 5-hydroxytryptamine (5-HT) and noradrenaline. By contrast, 0·1 mM clorgyline is required to inhibit the extraneuronal MAO which is heat labile and does not appreciably deaminate 5-HT or noradrenaline (Goridis & Neff, 1971b ; Yang, Goridis & Neff, 1972).

In the rabbit ear artery, the only MAO which can be demonstrated histochemically appears to be located extraneuronally and is distributed throughout the media (De La Lande, Hill, Jellett & McNeil, 1970). On the other hand, conflicting results concerning the relative importance of the intra- and extraneuronal MAO in transmitter catabolism have been obtained in physiological experiments on aortic strips (Kalsner & Nickerson, 1969) or the perfused ear artery (De La Lande & Jellet, 1972).

The present study was undertaken to determine whether selectively-distributed multiple forms of MAO, as found in rat pineal gland, could also be demonstrated in the smaller arteries, and to assess the distribution of these forms between intra- and extraneuronal tissue.

Methods

Male Wistar rats (120–180 g) were used. The superior mesenteric artery and the femoral arteries were dissected out, freed from adhering connective or fat tissue and rinsed free of blood. Arteries (4–10) were pooled and homogenized (2 ml glass homogenizer with Teflon pestle) in 50 mM sodium potassium phosphate buffer, pH 7·2 (150 μl/artery). When heart, brain or salivary gland MAO was to be assayed, a 1:20 (w/v) homogenate was prepared. The crude homogenates were centrifuged for 10 min at 750 *g* and the supernatant used as enzyme source. For subcellular fractionation studies, the arteries were homogenized in 0·25 M sucrose. The 750 *g* supernatant (10 min) was centrifuged for 30 min at 12×10^3 *g* to provide the 'mitochondrial' fraction which was washed once with 0·25 M sucrose. The post-mitochondrial supernatant was centrifuged for 1 h at 10^5 *g* to give the 'microsomal' and the 'soluble' fractions. Brain and salivary gland homogenates were fractionated in the same way.

6-Hydroxydopamine hydrobromide (6-OHDA) was injected intravenously into rats twice on day 1 (50 mg/kg) and twice on day 7 (100 mg/kg). The animals were killed on day 9 (Thoenen & Tranzer, 1968). Difficulties in producing chemical sympathectomy of vascular tissues have been reported (Goldman & Jacobowitz, 1971 ; Berkowitz, Spector & Tarver, 1972). The latter authors determined MAO activity in mesenteric artery on day 15 of the injection schedule (Thoenen & Tranzer, 1968) and did not find a significant reduction following 6-OHDA. We assayed MAO activity at different times and found a gradual recovery of MAO between day 10 and day 14 which was virtually complete on day 15 (Coquil & Goridis, unpublished results). We might conclude that the adrenergic nerve terminals of the arterial wall regenerate rapidly after 6-OHDA. This view is supported by the results of Finch, Haeusler, Kuhn & Thoenen (1972), who studied the recovery of adrenergic nerve function in the vascular system after 6-OHDA and observed a gradual recovery, beginning on day ten.

MAO activity was measured with tyramine, 5-HT or noradrenaline as substrate as described previously (Goridis & Neff, 1971a, 1971b). When the effect of different inhibitors was studied, the enzyme source was incubated with the inhibitor at room temperature for the times indicated in the legends to figures and tables before adding substrate.

Drugs

6-Hydroxydopamine hydrobromide (6-OHDA) was obtained from AB Biotec, Stockholm (Sweden) and pargyline hydrochloride from Abbott Labs North Chicago (Ill.). Semicarbazide hydrochloride and hydroxylamine hydrochloride (both analytical grade) were purchased from E. Merck, Darmstadt (Germany). Clorgyline (M&B 9302), *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propylamine hydrochloride) was generously supplied by Dr. D. R. Maxwell of May & Baker Ltd.

Results

Selective inhibition of arterial monoamine oxidase by various inhibitors

When the inhibition of brain MAO by pargyline with either tyramine or 5-HT as substrate, was plotted semi-logarithmically as a function of time (Fig. 1), a straight line was obtained. In contrast, a biphasic curve was obtained when a homogenate of rat mesenteric artery was used as enzyme source with tyramine as substrate. With 5-HT as substrate, the inactivation of arterial MAO could be fitted to a curve virtually identical to that obtained for brain MAO. For subsequent investigations we assumed that the biphasic curve was due to the presence of two enzymes acting on tyramine; one rapidly inactivated, and a second either not inhibited by pargyline or with a very low affinity for the inhibitor. By extrapolating the biphasic curve to zero time, 35–40% of the tyramine deaminating activity could be attributed to the pargyline-insensitive MAO. Since a linear relationship was obtained with 5-HT as substrate, apparently only the rapidly inactivated form was able to metabolize 5-HT.

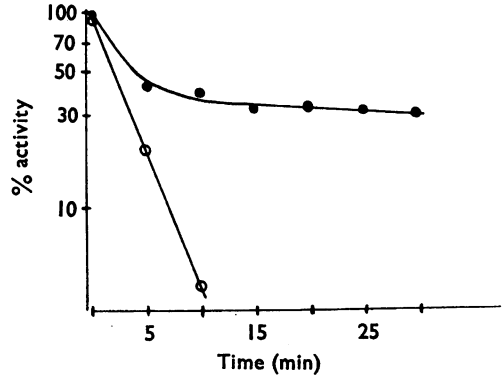

FIG. 1. Inhibition of brain and mesenteric artery monoamine oxidase (MAO) by pargyline. Mesenteric arteries were pooled and assayed for MAO activity with tyramine as substrate as described in **Methods**. A piece of cerebral hemisphere was processed in parallel. The rates of inhibition were determined by incubation of the reaction mixtures at room temperature for various times before adding substrate. Each point represents the mean of two samples from the same homogenate. The differences between duplicate determinations were $<\pm 6\%$. The control values did not change with different times of preincubation. ●, Mesenteric artery MAO; ○, brain MAO.

When the MAO activity of rat mesenteric artery was determined in the presence of clorgyline with tyramine as substrate, the inhibition of the enzyme could be represented by a sigmoid-shaped curve which levelled off at a maximum of about 60% inhibition. With 5-HT or noradrenaline as substrate, complete inhibition could be attained with clorgyline (0·1 mM) or with pargyline (0·05 mM). This enzyme has the same characteristics as the type A MAO found in sympathetic nerves (Goridis & Neff, 1971a, b).

The inhibition of the enzyme affecting tyramine and 5-HT by various concentrations of clorgyline is shown in Figure 2. About 40% of the activity of the enzyme metabolizing tyramine could not be inhibited by clorgyline or pargyline and it was evidently not able to deaminate 5-HT. The tyramine metabolizing enzyme could be inhibited 33% with the carbonyl reagent semicarbazide (1·0 mM) which, at this concentration, was almost without effect when 5-HT or noradrenaline was used as the substrate. Complete inhibition of enzyme activity for all the substrates could be obtained by combining semicarbazide (1·0 mM) and pargyline (6·05 mM). The figures for the various combinations of inhibitors and substrates are given in Table 1.

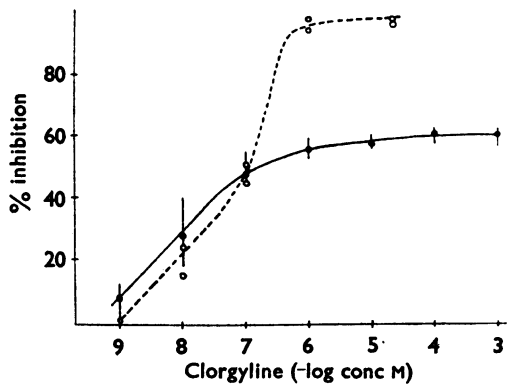

FIG. 2. Inhibition of mesenteric artery monoamine oxidase (MAO) by clorgyline. Homogenates of mesenteric arteries (750 *g* supernatant) were incubated in the presence of various concentrations of clorgyline for 15 min at room temperature and the remaining activity assayed with either tyramine (—●—) or 5-HT (- -○- -) as substrates. In the case of tyramine, the mean and the range of determinations on 3–5 homogenates are shown; with 5-HT as substrate only two homogenates were assayed.

TABLE 1. *Percentage inhibition of monoamine oxidase activity in rat mesenteric artery measured with different substrates*

	Substrate		
	Tyramine	5-Hydroxytryptamine	Noradrenaline
Control value (nmol/mg protein)/h ±S.E.M. (*n*)	64·5±5·1 (10)	25·5±1·4 (6)	0·67±0·09 (4)
Clorgyline 10^{-4} M*	61 (58–64)	98 (96–100)	100 (100)
Pargyline 5×10^{-5} M*	64 (61–66)	95 (94–97)	100 (100)
Semicarbazide 10^{-3} M*	33 (29–35)	9 (3–12)	0 (0)
Semicarbazide 10^{-3} M*+pargyline 5×10^{-5} M	100 (100)	98 (97–100)	100 (100)

* Percentage inhibition. Mesenteric arteries were pooled and homogenized, and aliquots of the 750*g* supernatants assayed for MAO activity as described in **Methods**. The rates of inhibition of MAO were determined by incubation of the reaction mixtures at room temperature for 15 min (clorgyline) or 30 min (pargyline and semicarbazide) before addition of substrate. For the rates of inhibition the mean and the range of duplicate determinations on three homogenates is given.

Subcellular distribution of monoamine oxidase activity in mesenteric and femoral arteries

The results of the subcellular fractionation of a homogenate from mesenteric or femoral artery are presented in Tables 2 and 3. A considerable part of the MAO activity with tyramine or 5-HT as substrate was recovered in the 10^5 *g* supernatant (' soluble ' fraction). Brain or salivary gland tissue were fractionated identically, no ' soluble ' activity could be detected (Table 3). About 40% of the MAO activity recovered in the different fractions of the mesenteric arteries was found in a 10^5 *g*

TABLE 2. *Subcellular distribution of arterial monoamine oxidase*

Exp. no.	% activity in 'Mitochondria'	'Microsomes'	'Soluble fraction'
1	28	40	32
2	26	41	33

Homogenates from mesenteric arteries were fractionated and assayed with tyramine as substrate as described in **Methods**. The values are given as the percentage activity recovered in the fraction, taking the sum recovered in the three fractions as 100%.

TABLE 3. *Distribution of monoamine oxidase activity between a crude mitochondrial and a soluble fraction*

Tissue (substrate)	% activity in 'Mitochondrial' fraction	'Soluble' fraction
Mesenteric artery (tyramine)	42·7±0·98 (8)*	57·1±1·6 (8)*
Mesenteric artery (5-hydroxytryptamine)	56·1±1·5 (10)	43·9±1·5 (10)
Femoral artery (tyramine)	30	70
Brain (tyramine)	100	0
Salivary gland (tyramine)	100	0

The values are presented as the percentage activity recovered in the fraction, taking the sum recovered in the two fractions as 100%. The values for the mesenteric arteries are given as the mean±S.E.M. (*n*). * $P<0{\cdot}01$ for the differences between the activity measured with tyramine and the one measured with 5-HT as substrate. In the other cases only a duplicate determination on one preparation was made.

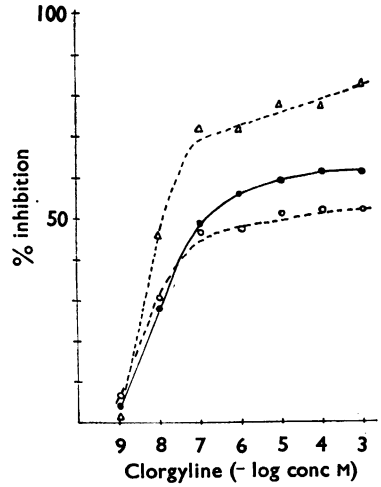

FIG. 3. Effect of clorgyline on mesenteric artery monoamine oxidase activity in a 750 *g* supernatant (—●—), a mitochondrial preparation (- -△- -) or a soluble fraction (- -○- -) with tyramine as substrate. The inhibition rates for the 750 *g* supernatant are taken from Figure 2.

pellet (Table 2). At present we do not know whether this indicates a microsomal MAO, or whether the microsomal fraction contains mitochondrial fragments. When MAO was measured with two substrates, a different distribution of the two activities was seen. The activity towards 5-HT was more concentrated in the mitochondrial ' fraction than was the activity towards tyramine. In addition, the ' mitochondrial ' activity was more sensitive to clorgyline than the ' soluble ' MAO supporting our conclusion, that the ' mitochondrial ' fraction was relatively rich in type A MAO (Figure 3).

Effect of various inhibitors on arterial monoamine oxidase

The effects of different inhibitors on the tyramine deaminating ability of various preparations are summarized in Table 4. Whereas brain MAO is almost completely inhibited by 0·1 mM clorgyline and 0·05 mM pargyline, MAO in mesenteric or femoral artery is only 61 to 70% inhibited. In contrast, the carbonyl reagents, which were devoid of any activity towards brain MAO, partially inhibited the enzyme of the arterial wall. Cyanide, an inhibitor of soluble aorta amine oxidase from beef and rabbit (Rucker & O'Dell, 1971 ; Rucker & Goettlich-Reimann, 1972) did not inactivate arterial MAO. The ' mitochondrial ' activity was more sensitive to clorgyline or pargyline than the ' soluble ' MAO, whereas the ' soluble ' activity was inhibited more by semicarbazide. These results confirm that relatively more of the 5-HT deaminating activity (sensitive to clorgyline and pargyline and resistant to semicarbazide) was recovered in the ' mitochondrial ' fraction.

TABLE 4. *Effect of various inhibitors on monoamine oxidase activity in different preparations*

Preparation	Clorgyline 10^{-4} M	Pargyline 5×10^{-5} M	Semicarbazide 10^{-3} M	Hydroxylamine 10^{-3} M	NaCN 10^{-3} M
			% inhibition (range)		
Mesenteric artery					
Crude homogenate	61 (60–62)	70 (64–74)	31 (30–33)	32 (29–33)	0
'Mitochondria'	74 (70–77)	86 (80–91)	21 (14–28)	n.d.	0
'Soluble'	44 (36–52)	54 (52–58)	42 (40–44)	n.d.	0
Femoral artery					
Crude homogenate	62 (59–64)	64 (61–67)	32 (31–33)	n.d.	n.d.
'Mitochondria'	71 (68–73)	n.d.	n.d.	n.d.	n.d.
'Soluble'	50 (44–57)	n.d.	n.d.	n.d.	n.d.
Brain					
Crude homogenate	96 (90–100)	100 (99–100)	0 (0)	0 (0)	0

Mesenteric and femoral arteries were pooled, fractionated and assayed with tyramine as substrate as described in **Methods**. A piece of cerebral hemisphere was processed in an identical manner. The rates of inhibition were determined by incubation of the reaction mixtures at room temperature for 15 min (clorgyline) or 30 min (all other inhibitors) before adding substrate. The values are given as the mean and the range for determinations on 3–5 preparations. n.d.=not determined.

Effect of chemical sympathectomy on monoamine oxidase of rat mesenteric artery

The 5-HT deaminating ability of the mesenteric artery was reduced by 70% in 6-OHDA treated rats (Table 5). With tyramine as substrate, the MAO activity declined 59% (Table 5 and Figure 4). However, the ' soluble ' activity did not change significantly after 6-OHDA. There was also no significant change in the

MAO activity of the heart (Table 5) confirming earlier studies in which either chemical (Jarrott, 1971) or immunosympathectomy (Klingman, 1966) was used.

TABLE 5. *The effect of 6-hydrodopamine (6-OHDA) on the metabolism of 5-hydroxytryptamine and tyramine in the mesenteric artery and in the heart*

	Mesenteric artery 750*g* supernatant		%	Mesenteric artery 105*g* supernatant		Heart 750*g* supernatant	
	Control	6-OHDA	Fall	Control	6-OHDA	Control	6-OHDA
5-HT (nmol/h)/artery or mg heart	12±0·4	3·6±0·7*	70			2·2±0·2	2·4± 0·3
5-HT (nmol/h)/mg protein	26±0·8	9·0±2·0*	65	2·2±0·2	2·7±0·7	19·0±2·2	23·0± 2·9
Tyramine (nmol/h)/artery or mg heart	27±3·3	11·0±2·5*	59			6·8±0·8	5·9± 0·9
Tyramine (nmol/h)/mg protein	60±7·0	36·0±4·0*	40	11·0±3·3	16·0±3·2	45·0±4·0	54·0±10·0

Four mesenteric arteries were pooled for each determination, and the result was divided by four to provide a value for one artery. From each group of rats one heart was taken. The values shown are the mean ± S.E.M. for three determinations. 6-hydroxydopamine (6-OHDA) was administered to rats twice on day 1 (50 mg/kg i.v.) and twice on day 7 (100 mg/kg i.v.) and the animals killed on day 9. Controls were injected with carrier solution (ascorbate 2 mg/ml) only. * Statistically significant as compared to control animals ($P<0·01$).

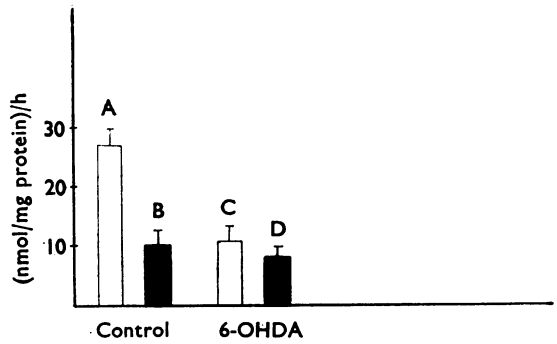

FIG. 4. Effect of 6-hydroxydopamine (6-OHDA) on mesenteric artery monoamine oxidase. 6-OHDA was administered to rats as described in **Methods** and Table 5. Four arteries were pooled for each determination, the results of three determinations ±S.E.M. are given. Aliquots of arterial preparations (750 *g* supernatants) were incubated with (solid bars) or without (open bars) 10^{-3}M clorgyline for 15 min at room temperature and the remaining activity assayed with tyramine as substrate. A *vs* C, $P<0·01$; B *vs* D, $P>0·05$.

When the inhibition of arterial MAO by increasing concentrations of clorgyline was followed, a marked drop in the sensitivity towards clorgyline was seen in animals treated with 6-OHDA (Figure 5). As shown in Fig. 4, where the absolute activities rather than per cent inhibition are compared, this change in inhibitor sensitivity was due to a selective loss of the clorgyline sensitive MAO, i.e. type A. The clorgyline-resistant activity was not reduced to a significant extent after chemical sympathectomy (Fig. 4, closed bars) despite the marked drop of total MAO (open bars).

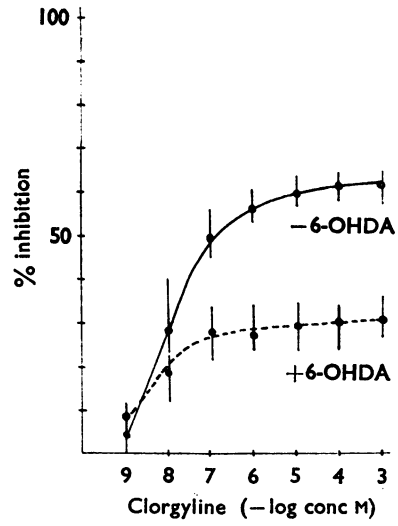

FIG. 5. Inhibition of mesenteric artery monoamine oxidase by clorgyline in control (—●—) and 6-hydroxydopamine (6-OHDA)-treated rats (- -●- -). Details of the procedure as in Fig. 4 except that different inhibitor concentrations were used. The values are shown as the mean and the range for 3 determinations.

Discussion

Evidence has accumulated that sympathetic neurones contain a species of MAO, arbitrarily called type A, which is very sensitive to clorgyline and is capable of deaminating noradrenaline and 5-HT *in vitro* (Jarrott, 1971; Goridis & Neff, 1971a, b; Goridis & Neff, 1972). But this MAO type is not confined to sympathetic nerves, since it can be found in rat brain and liver (Hall, Logan & Parsons, 1969). As shown in the present study, 60% of the MAO activity in the mesenteric or femoral arteries can be attributed to this or a similar form (Fig. 2, Table 1). In contrast, the second MAO type found in the arterial wall does not occur in rat brain, salivary gland (Fig. 1, Table), pineal gland (Goridis & Neff, 1971a), vas deferens (Jarrott, 1971) or liver (Hall, Logan & Parsons, 1969). This MAO form oxidizes tyramine but neither 5-HT nor noradrenaline and is inhibited by semicarbazide but resistant to pargyline and clorgyline. A tyramine-oxidizing activity sensitive to carbonyl reagents, has been found in rabbit aorta (Rucker & Goettlich-Reimann, 1972) and in the plasma of man (McEwen, 1965) and pig (Buffoni, 1966). It appears from our results that a similar enzyme also occurs in the wall of smaller rat arteries. However, cyanide, an inhibitor of the rabbit aorta and pig plasma enzyme, does not affect the MAO from rat mesenteric artery.

Chemical sympathectomy by 6-OHDA results in the loss of 70% of the 5-HT oxidizing MAO from the arterial wall (Table 5). With tyramine as substrate, the change is less pronounced, but a marked drop of the sensitivity to clorgyline is observed (Figure 5). These results are consistent with the view that the overwhelming majority of the 5-HT deaminating, clorgyline-sensitive activity (type A MAO) is found within sympathetic nerve endings or in structures dependent upon the nerves. However, we cannot conclude that type A activity of the arterial wall is found exclusively in the adrenergic nerve endings. Since the 'soluble' MAO, which acts on 5-HT, was not affected by 6-OHDA treatment, at least this

part of the type A activity does not depend on intact adrenergic innervation. On the other hand, the clorgyline-resistant activity, which did not change significantly after 6-OHDA (Fig. 4) appears to be localized entirely outside the adrenergic nerves.

We have shown in the present study (Table 1) as well as for other tissues (Goridis & Neff, 1971b) that type A MAO is responsible for the deamination of noradrenaline. The selective loss of type A activity after chemical sympathectomy implies that in the arterial wall the extraneuronal activity is of little importance in noradrenaline catabolism. De La Lande & Jellett (1972) arrived at essentially the same conclusion based on physiological experiments on the rabbit ear artery. Our results would suggest that the MAO activity observed histochemically throughout the media corresponds to the plasma-like amine oxidase activity.

Our previous work with MAO from normal and denervated pineal gland has shown that almost all of the type A activity, the transmitter specific enzyme, is associated with the sympathetic nerve endings within the gland (Goridis & Neff, 1971a, b) and similar findings have been reported by Jarrott (1971) working on MAO from rat vas deferens. Apparently, the differential distribution of the transmitter specific MAO between the two sites of the neuroeffector junction that we postulated as a result of our findings on the pineal gland, is a more general feature of sympathetically innervated tissues.

Further studies of MAO activity in normal and chemically sympathectomized arteries were carried out to determine the subcellular distribution. In contrast to salivary gland and brain MAO, about one third of the activity with tyramine as substrate was recovered in a 'soluble' fraction (Tables 2 and 3). Surprisingly, not only the activity sensitive to carbonyl reagents and resistant to clorgyline and pargyline, i.e. the plasma amine oxidase-like enzyme, but also type A MAO was found to be partially soluble. Type A MAO however was somewhat more concentrated in the 'mitochondrial' fraction (Tables 3 and 4). The results after chemical sympathectomy indicate that the soluble activity is entirely extraneuronal. Further studies are needed to elucidate the relation between the soluble and the particulate activities of the arterial wall and between the soluble enzyme and the plasma amine oxidase. At this stage, we can only postulate that the plasma amine oxidase might originate from the walls of the vasculature rather than from the connective tissue as has been previously suggested (Ruckler & O'Dell, 1971).

Please send reprint requests to Dr. C. Goridis.

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