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Monoamine oxidase in sympathetic nerves: a transmitter specific enzyme type

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Summary

1. When rat brain or superior cervical ganglion monoamine oxidase was incubated with increasing concentrations of clorgyline, using tyramine as substrate, the inhibition of the enzyme could be represented by a pair of sigmoidal curves joined by a horizontal region where inhibition was constant. Tyramine appeared to be metabolized by two enzymes, one of which was highly sensitive to clorgyline, designated A, whereas the other enzyme, designated B, was less sensitive to clorgyline.

2. The ratio of A/B activity for brain was 6/4 while in the ganglion it was 9/1.

3. When the experiments were repeated using noradrenaline as the substrate, the inhibition of the enzyme followed a simple sigmoidal curve where deamination was inhibited by low concentrations of clorgyline as observed with enzyme A.

4. We conclude that tyramine is deaminated by both A and B enzymes whereas noradrenaline is deaminated only by enzyme A, the enzyme which is most active in the ganglion. Our observations are consistent with the hypothesis that a specific intraneuronal monoamine oxidase plays an important role in the catabolism of noradrenaline in sympathetic nerves.

Introduction

Rat brain and liver mitochondrial monoamine oxidase (MAO) appears to occur in several forms which differ in their electrophoretic mobility (Collins, Youdim & Sandler, 1968; Youdim, Collins & Sandler, 1969; Sierens & D'Iorio, 1970) and inhibitor sensitivity (Johnston, 1968). For example, Johnston (1968) reported that a plot of percentage inhibition of tyramine deamination by rat liver or brain MAO versus the log of clorgyline (M+B 9302, N-methyl-N-propargyl-3-(2,4-dichlorophenoxy) propylamine hydrochloride) concentrations results in a pair of sigmoid curves, joined by a horizontal region where inhibition remains essentially constant. With 5-hydroxytryptamine (5-HT) as the substrate a single sigmoid curve is obtained. He interpreted these curves as indicating that there are two species of MAO present in rat liver and brain. One enzyme species, which he designated as enzyme A, was very sensitive to clorgyline and catalysed the oxidation of tyramine and 5-HT; and another species, enzyme B, which was less sensitive to clorgyline and oxidized tyramine, but not 5-HT. In contrast to rat liver and brain, where both enzyme activities are present in equal proportions, rat superior cervical ganglion

contains about 90% of type A enzyme, the enzyme which deaminates 5-HT (Goridis & Neff, 1971). We present evidence that type A MAO in sympathetic nerves is also the form which metabolizes noradrenaline.

Methods

Male Sprague-Dawley rats (160–180 g) were used in all experiments. Cervical ganglia were pooled and homogenized in 0·25 M sucrose (75 μl/cervical ganglion) in a 1 ml homogenizer fitted with a Teflon pestle. The homogenate was centrifuged for 10 min at 700 *g*. We discarded the sediment and centrifuged the supernatant at 12,000 *g* for 20 min to prepare a mitochondrial fraction. The mitochondria were washed once with 0·25 M sucrose. The pellet was suspended in 0·25 M sucrose (50 μl/cervical ganglion). Mitochondria were prepared from cerebral hemispheres in a similar manner. The mitochondrial fractions were assayed for MAO activity with tyramine or 5-HT as described previously (Goridis & Neff, 1971). When noradrenaline was used as substrate, the incubation mixture consisted of 30 μl of the mitochondrial preparation (0·03–0·04 mg protein), 0·2 μmol ascorbic acid and 0·5 nmol 1-noradrenaline-(methylene-^{14}C) (Amersham/Searle, 190 mCi/mmol) with about $1{\cdot}5 \times 10^5$ d.p.m., in a total volume of 170 μl. All reagents were made in sodium-potassium phosphate buffer 0·067 M, pH 7·2. The mixture was incubated at 37° C for 30 minutes. The reaction was stopped by adding 20 μl each of 0·25 M $ZnSO_4$ and 0·20 M $Ba(OH)_2$. Noradrenaline was separated from its deaminated metabolites by cation exchange chromatography as described for tyramine (Goridis & Neff, 1971). The accumulation of radioactive products, as the measurement of enzyme activity, was proportional to the amount of protein and linear with time of

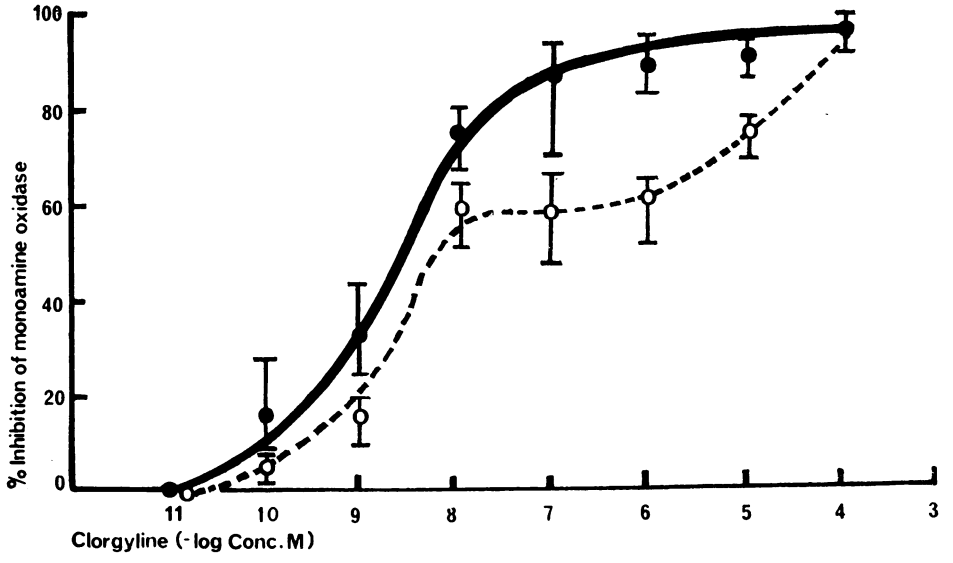

FIG. 1. Inhibition of brain mitochondrial MAO by increasing concentrations of clorgyline with tyramine and noradrenaline as substrates. The values are presented as mean and range for determinations from four or more homogenates. ●, Noradrenaline ; ○, tyramine.

incubation up to 45 minutes. Neither the addition of aldehyde dehydrogenase and NAD^+ nor the presence of a detergent had any effect on the oxidation of noradrenaline in the test system used. When the inhibition of MAO by clorgyline was investigated, the mitochondrial preparation was preincubated with clorgyline at 22° C for 15 minutes. The assay conditions using normetanephrine (DL-normetanephrine-7-^3H, New England Nuclear Co., 0·25 mCi/μmol) as substrate were essentially the same as described above for noradrenaline ; about 5×10^5 d.p.m. were added to each sample.

Results

When the MAO activity of rat brain mitochondria was determined in the presence of clorgyline with tyramine as substrate, the inhibition of the enzyme could be represented by a double sigmoidal-shaped curve with a plateau at about 55% inhibition of enzyme activity (Fig. 1). With noradrenaline as substrate a single sigmoidal-shaped curve was obtained (Fig. 1). Using the terminology of Johnston (1968) we can conclude that there are two types of MAO, enzyme A and enzyme B,

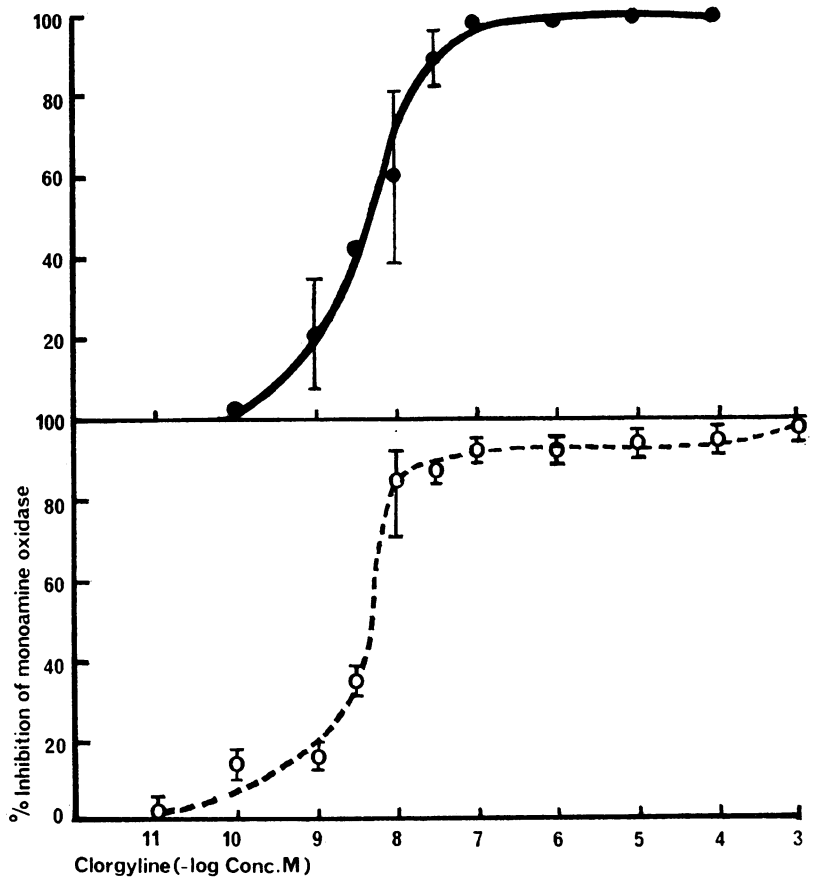

FIG. 2. Inhibition of superior cervical ganglion mitochondrial MAO by increasing concentrations of clorgyline with tyramine and noradrenaline as substrates. The values are presented as mean and range for determinations from four or more homogenates. ●, Noradrenaline ; ○, tyramine.

both of which oxidize tyramine. In contrast, it appears that the activity towards noradrenaline is primarily due to enzyme A. The slope of the curve at high concentrations of inhibitor using noradrenaline as substrate may indicate that enzyme B has some affinity for noradrenaline in our test system. When mitochondria from rat superior cervical ganglion were incubated together with clorgyline and tyramine, the curve representing the enzyme inhibition showed a plateau at about 90% inhibition of the total MAO activity indicating that 90% of the activity can be attributed to enzyme A (Fig. 2). As in brain, noradrenaline was deaminated preferentially by enzyme A, the predominant enzyme in the ganglion (Fig. 2). The MAO activity of mitochondria from the cervical ganglion and the cerebral hemispheres with three of the substrates studied and the ratios of the enzyme activities in the tissues are shown in Table 1. When normetanephrine was used as substrate in the presence of increasing concentrations of clorgyline the inhibition followed a curve which could be superimposed on the curve obtained with noradrenaline as substrate, whether mitochondria from cerebral hemispheres or from cervical ganglia were used as the enzyme source.

Discussion

Intraneuronal MAO plays an important role in the catabolism of noradrenaline in sympathetic nerves (Kopin & Axelrod, 1963). Evidence has accumulated that sympathetic neurones contain a species of MAO called type A which is very sensitive to clorgyline and is capable of oxidizing 5-HT *in vitro* (Jarrott, 1971 ; Goridis & Neff, 1971). Apparently type A MAO also metabolizes noradrenaline in our test system. Since 5-HT is not detectable in sympathetic ganglia (Snyder, Axelrod & Zweig, 1965) type A MAO in sympathetic nerves appears to be primarily responsible for the degradation of noradrenaline. Two lines of evidence are presented to support this hypothesis. (1) The inhibition of MAO by clorgyline with tyramine as substrate, shows that 90% of the total activity in superior cervical ganglion can be attributed to the type A enzyme (Fig. 2). Noradrenaline appeared to be metabolized in our test system nearly exclusively by type A MAO (Figs. 1 & 2) ; similar curves were obtained for both brain and ganglia with noradrenaline as substrate. (2) The MAO activity per mg tissue with noradrenaline and 5-HT as substrates are greater in the ganglion than in the cerebral hemispheres, whereas the opposite is true for the tyramine metabolizing activity. The ratio of the activity in the ganglion to that in the brain has almost the same value whether noradrenaline or 5-HT are used as substrates (Table 1), again indicating that both substrates can be deaminated by the same species of MAO. Our previous work with MAO from normal and

TABLE 1. *Comparison of mitochondrial monoamine oxidase activity from superior cervical ganglion or cerebral hemispheres using different substrates*

Tissue	Substrate (nmol/mg tissue)/h ± S.E.M. Tyramine	Noradrenaline	5-Hydroxytryptamine
A. Cerebral hemispheres	11·0±0·9 (4)	0·027±0·004 (8)	6·7±1 (6)
B. Superior cervical ganglion	8·5±0·5 (4)	0·048±0·006 (6)	13·0±3 (4)
$\frac{B}{A}$	0·7	1·8	1·9

Mitochondria were isolated and assayed for MAO activity as described in **Methods.** Number of animals in parentheses.

denervated pineal glands has shown that almost all of the type A MAO activity in the pineal gland is associated with the sympathetic nerve endings while type B MAO is associated with the pineal cells (Goridis & Neff, 1971). Apparently, the transmitter noradrenaline is preferentially deaminated within the adrenergic nerve endings, at least in the pineal gland. Since catechol-*O*-methyl transferase appears to be mainly located extraneuronally (Carlsson & Hillarp, 1962; Iversen, Glowinski & Axelrod, 1966) we determined which type of MAO oxidized the product of the methylation reaction, normetanephrine. The catecholamine and its methylated derivative were deaminated by the same species of MAO, type A enzyme, which appears to be located intraneuronally.

Further studies are needed to elucidate whether the type of MAO found in sympathetic nerves in the periphery is also found in central monoaminergic neurones and whether the distribution of the transmitter specific enzyme between the two sides of the synaptic junction, as shown for the pineal gland, can be demonstrated for other tissues.

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