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INHIBITION BY PARGYLINE OF CARDIOVASCULAR AMINE OXIDASE ACTIVITY*

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Abstract—Pargyline, a monoamine oxidase inhibitor, is an antihypertensive agent. We report on the ability of pargyline to inhibit the oxidative deamination of norepinephrine (NE) and 2-phenylethylamine (PEA) by rat heart and mesenteric artery. The cardiovascular tissues appear to contain amine oxidases that are quite different from those of brain. For example, pargyline, when tested *in vitro* or administered to rats, did not differentially block the deamination of NE and PEA as it does in brain. Moreover, there is a PEA oxidase activity that is blocked by pargyline and an activity that is resistant to pargyline but blocked by semicarbazide or cuprizone. PEA oxidase activity can be differentiated from NE oxidase activity by thermal stability. The pargyline-resistant PEA oxidase activity may be related to a connective tissue amine oxidase.

Multiple forms of monoamine oxidase (monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4.; MAO) exist in mammalian liver and brain which have been characterized by their sensitivity to inhibitor drugs and their specificity for substrates [1]. The MAO inhibitor drugs are indicated for the treatment of some forms of mental depression as well as for some forms of hypertension [2]. The properties of the MAO's of the cardiovascular system are not as well documented as those of brain and in this report we present some of the properties of the enzymes of rat heart and mesenteric artery. Pargyline, an inhibitor of MAO which is indicated for the treatment of moderate hypertension, was evaluated for its ability to block the deamination of 2-phenylethylamine (PEA), a substrate for type B MAO [3], and norepinephrine (NE), a substrate for type A MAO [4]. Pargyline is a preferential inhibitor of type B MAO of brain [5, 6]. We found that the enzymes of the cardiovascular system have different characteristics from the enzymes of brain. Moreover there is a PEA oxidase present in the cardiovascular system that cannot be inhibited by pargyline. Some of the properties of the pargyline-resistant enzyme activity were studied.

MATERIALS AND METHODS

Preparation of samples and enzyme assay. Male Sprague–Dawley rats, 200–250 g, obtained from Zivic-Miller Laboratories (Allison Park, PA) were decapitated and their hearts and anterior mesenteric arteries removed. Samples were cleared of adhering tissue and rinsed free of blood with saline. Arteries were homogenized with 0.25 ml and hearts with 20 vol. 67 mM phosphate buffer, pH 7.2. The supernatant fraction from a 750 *g*, 10 min, centrifugation was used as the enzyme source.

When NE was the substrate, three mesenteric arteries were pooled, homogenized with buffer and centrifuged. Supernate solutions, 0.1 ml, and radioactive NE (*l*-noradrenaline-methylene-^{14}C, Amersham/Searle, Arlington, Heights, IL), 12 μM, were incubated at 37° for 90 min in a total volume of 0.25 ml buffer. Ascorbic acid (2.3 mM) was added to the buffer when NE was the substrate.

When PEA was the substrate, one mesenteric artery was processed as described and incubated for 60 min with radioactive PEA (2-phenylethylamine-1-^{14}C, New England Nuclear, Boston, MA), 0.2 mM. When the enzyme activity of heart was assayed 0.1 ml of homogenate was incubated with substrate as described for mesenteric artery. In one series of experiments radioactive tyramine (tyramine-1-^{14}C, New England Nuclear), 2.1 mM was used as substrate. A detailed description of the assay is described elsewhere [4]. In brief, the homogenates were incubated using the conditions already described and the reactions stopped by adding 25 μl each of Zn So_4 (0.25 M) and $Ba(OH)_2$ (0.2 M) when NE was the substrate or 50 μl of 60 per cent w/v perchloric acid when PEA or tyramine was the substrate. The radioactive deaminated reaction products were separated from the substrates by cation exchange chromatography on Rexyn 101 (Fisher Scientific Co., Fair Lawn, NJ). Radioactivity was counted in a Beckman LS-250 Liquid Scintillation System with automatic quench correction. The enzyme assay was linear with protein and time of incubation. Protein was determined by the method of Lowry *et al.* [7]. The specific radioactivity of the substrates were adjusted so that 1–4 × 10^3 cpm of product was found during the incubation of untreated samples which represented less than 10 per cent conversion of the substrates. When studying the deamination of NE it was necessary to

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use the radiolabelled NE as supplied by the manufacturer (57 mCi/mmol) without dilution with stable NE because of low enzyme activity. The final concentrations of tyramine and PEA used gave optimal enzyme activity with our reaction conditions.

When the inhibitory properties of pargyline (generously supplied by Abbott Labs., North Chicago, IL), clorgyline (generously supplied by May and Baker, Ltd., Dagenham, England), semicarbazide (Aldrich, Milwaukee, WI) or cuprizone (Aldrich) were studied *in vitro*, they were preincubated with an enzyme preparation at 22° for 15 min before adding substrate.

In one series of studies, pargyline was injected i.v. and the rats were killed 2 hr later. Samples were processed as already described.

Enzyme thermostability. A large volume of a 750 *g* supernatant fraction from mesenteric artery or heart was placed in an incubator maintained at 50° and samples were withdrawn at various time intervals and cooled in an ice bath. The samples were then assayed at 37° for enzyme activity using NE or PEA as substrate.

Identification of phenylacetic acid. To confirm that PEA was indeed deaminated in the presence of pargyline (1 mM) by homogenates of mesenteric artery, aldehyde dehydrogenase [8] was added to convert the radiolabelled phenylacetaldehyde reaction product to phenylacetic acid which was then extracted and identified by thin layer chromatography. It was necessary to convert the aldehyde to the acid because phenylacetaldehyde as well as phenylethanol are volatile and are lost when the homogenate extract is dried in preparation for thin layer chromatography. Four mesenteric arteries were homogenized in 67 mM phosphate buffer, pH 7.2. The initial incubation mixture consisted of homogenate, 40 μl; aldehyde dehydrogenase, 5–8 units; nicotinamide, 5 μmoles; NAD^+, 0.12 μmoles; and pargyline, 1 mM, in a total volume of 70 μl. After 15 min of preincubation at 22°, radiolabelled PEA, 0.1 ml, was added resulting in a final concentration of 0.2 mM. This mixture was incubated for 2 hr at 37°. The reaction was stopped by adding 1 ml of 1 N hydrochloric acid containing 0.1 mg phenylacetic acid as a carrier. The product of the reaction was extracted into 1 ml of diethyl ether. The organic phase, 0.5 ml, was evaporated under nitrogen and the residue was dissolved in 0.2 ml of ethanol which was then applied to a silica gel H thin layer chromatogram plate (Analtech, Inc., Newark, DE). Standards of phenylacetic acid were applied to the plate and the plate was developed with 1-butanol–acetic acid–water (60:15:25). Phenylacetic acid was located by exposing the plate to iodine vapor. Areas corresponding to phenylacetic acid were scraped from the plate into Aquasol (New England Nuclear) and radioactivity was determined. The recovery of radiolabelled phenylacetic acid (Amersham/Searle, Arlington Heights, IL) carried through the incubation, extraction and chromatography procedures was 80 per cent with a range between 76–87 per cent for 5 samples.

RESULTS

Inhibition of NE and PEA deamination by pargyline or clorgyline. Preparations from rat heart and mesenteric artery deaminated NE and PEA (Table 1). The activity towards NE appeared similar in both tissues, however, the PEA oxidase activity was about 10-fold greater in mesenteric artery than in heart. The ratio of PEA to NE activity is a measure of the relative abundance of the types of enzymes associated with the tissues. The control enzyme specific activities for Figs 1–5 were similar to the values shown in Table 1.

In vitro, the deamination of NE could be almost completely blocked by 10 μM pargyline when studied in either mesenteric artery (Fig. 1) or heart (Fig. 2). Moreover, a dose of 10 mg/kg, i.v. pargyline almost completely blocked NE deamination in both tissues when evaluated 2 hr after treatment. In contrast to NE deamination, PEA deamination could not be completely blocked in either tissue by the presence of 1 mM pargyline (Figs 1 and 2). Furthermore, similar results were found when pargyline was administered intravenously. Approximately 80 and 20 per cent of the deaminating activity in mesenteric artery and heart, respectively, were resistant to inhibition by pargyline. The intravenous dose of pargyline could not be increased beyond 20 mg/kg because of its toxicity.

Johnston [9] and other investigators [5, 10] have described a biphasic or double S-shaped curve for the inhibition of tyramine deamination by brain and liver using the drug clorgyline. The curve takes this form because of the presence of multiple forms of MAO in the tissues that have different sensitivities to inhibition by clorgyline. In heart, we could not demonstrate the presence of multiple forms of enzyme activity using tyramine together with clorgyline (Fig. 3). Moreover, clorgyline, like pargyline (Figs 1 and 2) was not capable of completely blocking the deamination of PEA when present at high concentrations. About 30 per cent of the activity towards PEA was evident even in the presence of 1 mM clorgyline.

Formation of a deaminated metabolite of PEA by mesenteric artery in the presence of pargyline. To verify the formation of a deaminated product of PEA in the presence of 1 mM pargyline, homogenate of mesenteric artery was incubated together with alde-

Table 1. Amine oxidase activity of rat heart and mesenteric artery

Tissue	nmol product/mg prot/hr mean ± S.E.M. [6]		Ratio
	NE	PEA	PEA/NE
Heart	0.30 ± 0.01	3.5 ± 0.5	12
Mesenteric artery	0.30 ± 0.01	34 ± 8	113

Tissues were incubated with substrate as described in Methods. The substrate concentrations were NE, 0.012 mM and PEA, 0.2 mM.

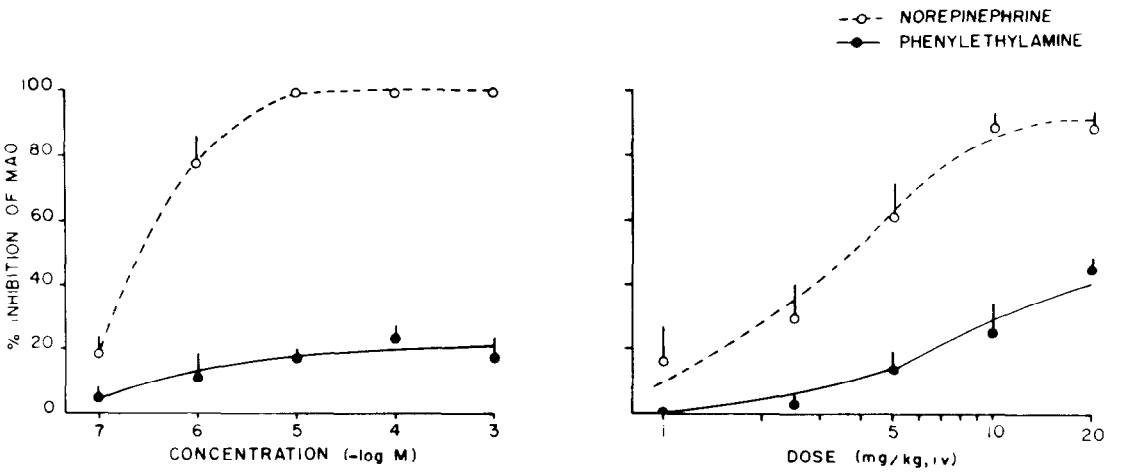

Fig. 1. Inactivation of the amine oxidase activity of mesenteric artery by pargyline tested *in vitro* or following its administration to rats. For *in vitro* studies pargyline was preincubated for 15 min at 22° with homogenate before adding phenylethylamine or norepinephrine and the incubation was continued at 37° as described in Methods. Rats were killed 2 hr after pargyline was injected i.v. and a homogenate was prepared and assayed as described in Methods. Results are expressed as mean ± S.E.M. for 3 or 4 samples.

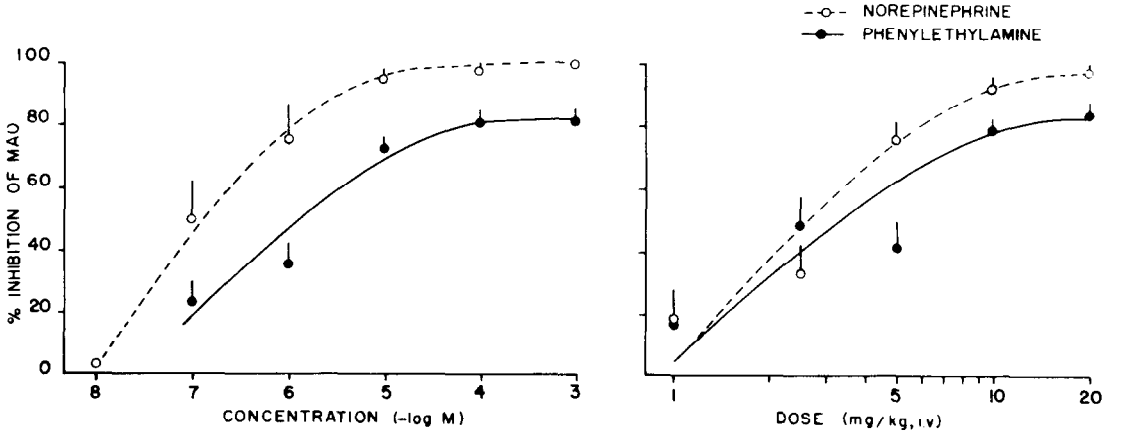

Fig. 2. Inhibition of the amine oxidase activity of heart by pargyline tested *in vitro* or following its administration to rats. Experimental details are as for Fig. 1. Results are expressed as mean ± S.E.M. for 3 or 4 samples.

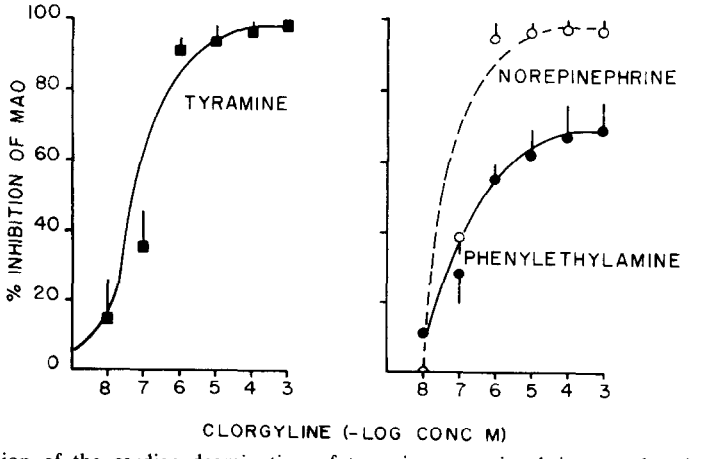

Fig. 3. Inhibition of the cardiac deamination of tyramine, norepinephrine or phenylethylamine by clorgyline. Clorgyline was preincubated with homogenate of rat heart for 15 min at 22° before adding substrates and continuing the assay as described in Methods. Results are expressed as the mean ± S.E.M. for 3 or 4 samples.

Table 2. Failure of pargyline to prevent the oxidative deamination of 2-phenylethylamine by the mesenteric artery

Treatment	Phenylacetic acid formed cpm/sample
Boiled homogenate	825
Homogenate	4091
Homogenate + 1 mM pargyline	3729

Homogenate of rat mesenteric artery was incubated for 2 hr at 37° with radioactive PEA, 0.2 mM, in the presence of aldehyde dehydrogenase. Pargyline was preincubated with the enzyme source for 15 min at 20° before adding the substrate. The acid formed was extracted and isolated by thin-layer chromatography as described in Methods.

hyde dehydrogenase to convert the phenylacetaldehyde formed to phenylacetic acid. As shown in Table 2, phenylacetic acid was apparently a product of the reaction. Similar results were obtained in another study when the developing solution was 1-butanol–pyridine–water (1:1:1).

Inhibition of the pargyline-resistant amine oxidase by semicarbazide or cuprizone. With the substrates NE and PEA and the inhibitor drugs pargyline, semicarbazide and cuprizone at least three types of amine oxidase activity can be postulated to exist in the cardiovascular tissues (Table 3). Apparently there is an enzyme(s) present that deaminates NE which is inhibited by pargyline (0.1 mM) and only minimally inhibited by semicarbazide or cuprizone. PEA is apparently deaminated by at least two enzymes, an enzyme(s) that is blocked by pargyline (0.1 mM) and an enzyme(s) that is blocked by semicarbazide (1.0 mM) or cuprizone (0.1 mM). By combining pargyline and semicarbazide almost all enzyme activity is inhibited. These enzyme activities are apparently present in both heart and mesenteric artery. The mesenteric artery contains about 90% of the pargyline-resistant PEA oxidase, the heart only 30%.

Thermostability of the amine oxidase of heart and mesenteric artery. The thermostability of the amine oxidase activity towards NE and PEA apparently differed in heart and mesenteric artery (Figs 4 and 5). In heart and mesenteric artery the activity towards NE declined exponentially with time at 50°, although the decline appeared somewhat faster in mesenteric artery. The decline of PEA oxidase activity appeared to be biphasic in heart and linear in mesenteric artery.

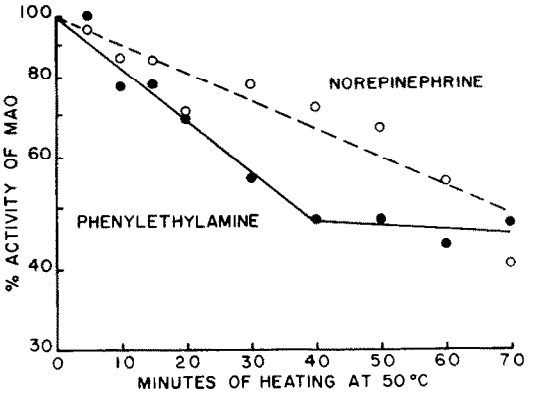

Fig. 4. Thermal inactivation of heart amine oxidase activity. Homogenate of heart was heated and portions removed, cooled and assayed for enzyme activity using norepinephrine and phenylethylamine as substrates as described in Methods. Results are expressed as the mean for duplicate determinations.

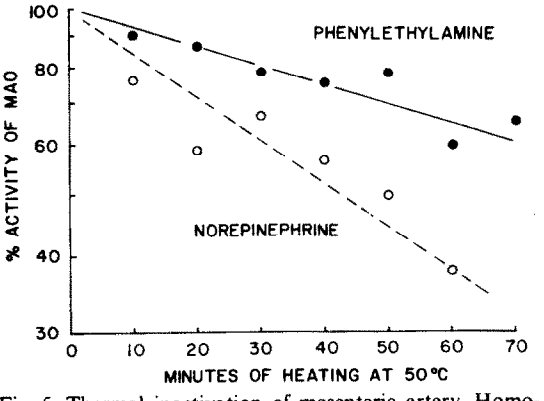

Fig. 5. Thermal inactivation of mesenteric artery. Homogenate of mesenteric artery was heated and portions removed, cooled and assayed for enzyme activity using norepinephrine and phenylethylamine as substrates as described in Methods. Results are expressed as the mean for duplicate determinations.

DISCUSSION

The MAO inhibitor pargyline is indicated for the treatment of some forms of hypertension, however,

Table 3. Inhibition of the oxidative deamination of norepinephrine (NE) and 2-phenylethylamine (PEA)

Inhibitor	Concentration mM	% Inhibition ± S.E.M. [4] Heart NE	Heart PEA	Mesenteric artery NE	Mesenteric artery PEA
Semicarbazide	1.0	0 ± 0	30 ± 12	11 ± 2	87 ± 5
Pargyline	0.1	98 ± 1	66 ± 5	97 ± 3	17 ± 10
Semicarbazide Plus Pargyline	1.0 + 0.1	98 ± 1	96 ± 4	96 ± 2	100 ± 0
Cuprizone	0.1	0 ± 0	36 ± 6	10 ± 7	70 ± 5

Homogenates from heart or mesenteric artery were preincubated at 20° for 15 min with the inhibitor. After adding the substrate, incubation was continued at 37° for 90 min (NE) or 60 min (PEA).

its mechanism of action is unknown. Several hypotheses for its antihypertensive activity have been suggested all of which imply that its acts peripherally rather than centrally to lower blood pressure [11–14]. Our objective was to provide information about the types of enzymes associated with the cardiovascular system and the enzymes inhibited by pargyline.

There is now evidence that the properties of the amine oxidases of the cardiovascular system are different from those of the brain [15, 16]. The enzymes of brain, which are the classical mitochondrial MAO's can be differentiated into two types of activity by using inhibitor drugs and specific endogenous substrates. Clorgyline is a preferential inhibitor of type A MAO [9] while pargyline is a preferential inhibitor of type B MAO [5, 6], NE is a specific substrate for type A MAO [4] while PEA is a specific substrate for type B MAO [3]. For example, pargyline when tested *in vitro* or administered to rats selectively blocks type B enzyme activity of brain at concentrations that have minimal effects on type A enzyme activity [6]. The response to pargyline is not the same in cardiovascular tissues. Pargyline is more active toward inhibiting the deamination of NE than in inhibiting the deamination of PEA. Moreover, PEA deamination is not completely inhibited by high concentrations of pargyline as is the deamination of PEA by brain [6]. Thus the PEA oxidases of the cardiovascular systems are quite different in their response to pargyline when compared to the oxidases of brain. The pargyline-resistant enzyme is blocked by the carbonyl reagent semicarbazide and the copper chelator cuprizone, properties which are similar to connective tissue amine oxidase [17, 18]. Furthermore, the pargyline-resistant enzyme activity of mesenteric artery is not associated with mitochondria [15]. This amine oxidase may play a role in the cross-linking of collagen and elastin in blood vessels [17, 18].

In support of the view that different amine oxidases exist in the cardiovascular tissue, we observed a difference in the thermostability of the enzyme activity toward PEA and NE. With a preparation from heart we found that PEA deamination was lost rapidly at first and then more slowly by heating at 50° which suggests that several enzymes are responsible for the deamination of PEA by heart. With a preparation from mesenteric artery we found that the loss of PEA deaminating activity was monophasic and slower than the loss of NE deaminating activity. The inactivation of PEA oxidase activity of mesenteric artery may appear to be monophasic because it consists primarily of pargyline-resistant PEA oxidase activity. The relatively slow inactivation phase of PEA oxidase activity of heart by heating at 50° and the relatively slow loss of PEA oxidase activity in mesenteric artery at 50° may be the consequence of destroying a similar enzyme in both tissues.

Clorgyline could not be used to demonstrate the presence of multiple forms of MAO in heart as it has been used with brain [9]. In contrast to brain where a double sigmoidal curve is observed with clorgyline and the substrate tyramine, only a simple inhibition curve was evident in our study with heart. Moreover, clorgyline inhibited the deamination of NE and PEA equally well except that PEA deamination could not be blocked by more than 70 per cent in the presence of 1 mM clorgyline, a finding that is consistent with our studies with pargyline.

Our results and reports by others [16] suggest that at least three types of amine oxidase activity are associated with cardiovascular tissue. There is a mitochondrial enzyme activity that deaminates NE and is blocked by the MAO inhibitors clorgyline and pargyline [15]. In the mesenteric artery the enzyme that deaminates NE is associated primarily with sympathetic nerves as treatment with 6-hydroxydopamine almost abolishes this activity [15]. There are at least two enzyme activities that deaminate PEA. One enzyme activity is inhibited by pargyline or clorgyline, but the drugs cannot be used to readily differentiate this activity from the activity that deaminates NE. The enzyme activities, however, can be differentiated by their thermostability. Finally, there is a PEA oxidase that is resistant to pargyline but can be inhibited by semicarbazide and cuprizone, a finding suggesting that this activity may be related to a connective tissue amine oxidase or to a plasma amine oxidase [19]. The mesenteric artery has a greater proportion of the pargyline-resistant PEA oxidase than the heart. The relationship between the pargyline-resistant enzyme activity and the hypertensive state of an animal, as well as correlating the inhibition of the various amine oxidases with reduction of blood pressure following treatment with pargyline may give insight into the etiology of hypertension.

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