Metabolism of amines in the isolated perfused mesenteric arterial bed of the rat

Jonathan Elliott, Brian A. Callingham & Dennis F. Sharman

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ

1 Semicarbazide-sensitive amine oxidase (SSAO) activity has been demonstrated in the isolated mesenteric arterial bed of the rat *in vitro* by studying the metabolism of benzylamine (Bz) and tyramine (Tyr) added to the perfusing fluid.

2 Pretreatment of rats with (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL72145), a potent inhibitor of SSAO in rat mesenteric blood vessels, reduced the amount of metabolites, following the addition of Bz (25 μ M) or Tyr (100 μ M) to the perfusing fluid, by 83% and 52% respectively. Inactivation of monoamine oxidase type A (MAO-A) by the addition of clorgyline (10 μ M) to the perfusing fluid, had little effect on the appearance of metabolites from Tyr.

3 The presence of 3 μ M cocaine in the perfusing fluid increased the amount of metabolites produced from Tyr.

4 The metabolites of Tyr appearing in the perfusion fluid from control preparations were 85% *p*-hydroxyphenylacetic acid and the remainder consisted of a mixture of *p*-hydroxyphenylacetaldehyde and, possibly, *p*-hydroxyphenylethanol.

5 The metabolism of Tyr by homogenates of the rat mesenteric vascular bed was carried out by SSAO (60%) and MAO-A (40%) with very little contribution from MAO-B. Homogenates from rats pretreated with MDL 72145 showed metabolism of Tyr by MAO-A only.

6 These data indicate that SSAO is capable of metabolizing amines present in the fluid perfusing blood vessels to metabolites that are readily released. Histochemical evidence has shown that whereas MAO-A is present in the mitochondria of smooth muscle cells and nerve endings, SSAO is located in the plasma membrane of the smooth muscle cells. This subcellular distribution may explain the differences found between metabolites released from intact vessels and the metabolism seen in homogenates. The identity of the Tyr metabolizing activity in intact vessels that is resistant to both MDL 72145 and clorgyline remains to be determined.

Introduction

Rat blood vessels possess an amine oxidase activity capable of metabolising tyramine (Tyr), which has different properties from monoamine oxidase (MAO; EC 1.4.3.4) enzymes (Coquil *et al.*, 1973). This enzyme activity is insensitive to inhibition by clorgyline but is inactivated by carbonyl reagents such as semicarbazide and, as a result, is called semicarbazide-sensitive amine oxidase (SSAO). It is considered to be a member of the group of enzymes classified as EC 1.4.3.6 (see Callingham & Barrand 1987, for review). SSAO has been shown to reside in the plasma membrane of vascular smooth muscle cells (Wibo *et al.*, 1980; Lyles & Singh, 1985).

Homogenates of blood vessels have been widely used to examine the biochemical properties of this enzyme activity (Lewinsohn *et al.*, 1978; Clarke *et*

al., 1982; Precious *et al.*, 1988; Elliott *et al.*, 1989b). The preferred substrate appears to be benzylamine (Bz) but a number of aromatic and aliphatic amines also have been shown to be substrates. In spite of all the work done with homogenates, this enzyme has not been studied in intact blood vessels of the rat and its physiological significance is poorly understood.

Studies with intact rat caudal artery confirm the long held view that neuronal uptake mechanisms are important for the inactivation of noradrenaline. Catechol-*O*-methyltransferase and extraneuronal uptake make a more significant contribution when the amine enters from the luminal side of the blood vessel rather than the extraluminal side (Venning & de la Lande, 1984; 1985). Amines, that are substrates

for SSAO, and which enter the blood vessel from the luminal side may be inactivated in a different manner.

The presence of SSAO in the plasma membrane of vascular smooth muscle cells might suggest that this enzyme has ready access to amines present in the circulation without the need for specialised uptake systems (Callingham *et al.*, 1983). There is however, no direct evidence to support this. In order to examine such a possibility, the isolated perfused mesenteric arterial bed of the rat was chosen to determine whether, or not, SSAO is capable of metabolizing substrates present in the fluid perfusing these vessels. Tyr was chosen as a suitable amine for study because it is metabolized by both SSAO and MAO-A in homogenates of these vessels (Coquil *et al.*, 1973). The relative contribution of these two enzymes to the production of metabolites appearing in the perfusate could then be assessed. Preliminary results from these studies have been given elsewhere (Elliott & Callingham, 1988).

Methods

Male Wistar rats (200–350 g), supplied by A. J. Tuck & Son, Rayleigh, Essex were used throughout these studies. Heparin (500 i.u.) was administered by i.p. injection 10 min before the rats were anaesthetized with urethane (2 g kg⁻¹; i.p.). The mesenteric vascular bed was isolated and perfused as described by McGregor (1965). Briefly, the cranial mesenteric artery was cannulated with a polyethylene catheter (1.02 mm o.d., Portex Ltd., Hythe, Kent) and the ileocolonic arterial branches were ligated. Perfusion with modified Krebs-Henseleit solution, maintained at 37°C and gassed with 95% O₂ and 5% CO₂, was started as soon as possible after the blood flow had ceased. A constant flow rate of 2 ml min⁻¹ was delivered by a Harvard 1203A peristaltic pump. The preparation, once isolated from the rat, was placed in a methacrylate dish resting on a block warmed by circulating water and covered with paraffin film for protection against evaporation. The effluent from the preparation was collected. Perfusion pressure was recorded by a Washington PT400 pressure transducer connected via an amplifier to a Kipp & Zonen BD41 two channel X/T pen recorder. Two preparations were tested at the same time. The resting perfusion pressure was consistent between the various preparations. For example, with 36 preparations, in which Tyr was used, it was 8.6 ± 0.28 mmHg, (mean $\pm$ s.e.mean). Each preparation was allowed a 45 min equilibration period before the metabolism studies were begun.

Some rats were injected (i.p.) with either 1 or 2 mg kg⁻¹ (E)-2-(3',4'-dimethoxyphenyl)-3-fluoro-

allylamine (MDL 72145) 1 h before the dissection. This drug is a potent irreversible inhibitor of SSAO (Lyles & Fitzpatrick, 1985). When used in this way, it has been shown to inhibit SSAO selectively without affecting MAO-A in rat blood vessels (Elliott *et al.*, 1989a). Although this inhibitor also inhibits MAO-B (Zreika *et al.*, 1984), evidence from homogenates of rat mesenteric blood vessels suggests there is little or no MAO-B present in this tissue (Coquil *et al.*, 1973; Fuentes & Neff, 1977; see results below). In those preparations where the inhibition of MAO-A was to be studied, clorgyline (10 μ M) was present in the perfusing Krebs solution for 30 min during the equilibration period, after which time, perfusion with drug-free solution was resumed. Clorgyline was used in this way instead of by *in vivo* administration to the rats as we have noted some inhibition of SSAO following clorgyline given *in vivo* (unpublished observations) similar to that reported with pargyline (Elliott *et al.*, 1989a). In those preparations where the effect of cocaine on amine metabolism was examined, cocaine (3 μ M) was added to the perfusing fluid after the equilibration period and remained present throughout the experiment. The effects of these drugs on the amine oxidase activities in the mesenteric blood vessels were assessed by assay of MAO-A and SSAO activities in homogenates of the preparations following the perfusion experiments as described below.

Metabolism studies

At the start of each experiment the preparations were stimulated with 10 nmol of noradrenaline administered in Krebs solution (200 μ l) through an injection port into the perfusing fluid. This was repeated 5 times at 5 min intervals and served to ensure that the preparations were responding consistently. Subsequently, 10 ml of 25 μ M [¹⁴C]-Bz (5 μ Ci μ mol⁻¹) or 20 ml of 100 μ M [³H]-Tyr (6.6 μ Ci μ mol⁻¹) in Krebs solution were perfused. Totals of 30 and 100 ml of perfusate were collected in 0.5 and 1 ml fractions in the Bz and Tyr experiments, respectively. Each fraction was sampled and its total radioactivity measured in a Packard A2020 liquid scintillation spectrometer. Corrections for the efficiency of counting were made by the use of automatic external standardization allowing the amounts of metabolites to be expressed in absolute terms. Deaminated products were extracted into 5 volumes of a water-saturated mixture of ethyl acetate and toluene (1:1) following acidification with 3 M HCl (25 μ l or 50 μ l). The amount of unchanged Tyr extracted by this method was estimated and found to be less than 0.05% of the total Tyr present. Each fraction was corrected for this when calculating the amount of metabolites extracted. The Tyr concentra-

tion used in these studies ($100\text{ }\mu\text{M}$) was chosen as it is in the same range as the K_m value for the deamination of this amine ($86\text{ }\mu\text{M}$, unpublished observations) in crude homogenates of mesenteric blood vessels.

Chromatographic studies

In some experiments with Tyr, fractions 12 to 36 (6 to 18 min; the period when maximum output of metabolites occurred) were pooled before extraction. The organic solvent was evaporated down to dryness with nitrogen. The residue was dissolved in 0.5 ml ethyl acetate and the metabolites were chromatographed and compared with reference compounds on Whatman no. 1 paper. The phenolic compounds were detected by spraying the paper with a solution of Fast Scarlet GG salt (1 mg ml^{-1} in 0.1 M HCl), followed by 1 M sodium carbonate solution. Radioactivity was located by cutting the chromatogram into 1 cm bands, then eluting with ethoxyethanol and counting. The solvent systems and R_F values of the reference compounds are shown in Table 2.

Amine oxidase activities in homogenates

Following each perfusion experiment, the mesenteric blood vessels were cleaned of adhering fat and frozen until needed. Each mesentery was homogenized in 20 w/v in 1 mM potassium phosphate buffer ($\text{pH } 7.8$) with a mechanical homogenizer (Polytron mark 5, Kinematica) on the day of the assay. The resulting homogenate was centrifuged at $600g$ for 10 min to separate unbroken cells and nuclei. A modification of the radiochemical method of Callingham & Laverty (1973) was used to measure the MAO-A and SSAO activity in the supernatant as described elsewhere (Elliott *et al.*, 1989b). The enzyme activity of each sample is the mean of triplicate estimations. The substrates used were 5-hydroxytryptamine (5-HT, $500\text{ }\mu\text{M}$), to measure MAO-A activity, and Bz ($10\text{ }\mu\text{M}$) to measure SSAO activity. The protein content of each homogenate was estimated by the method of Lowry *et al.* (1951). The results are expressed in nmol of product formed $\text{h}^{-1}\text{mg}^{-1}$ protein. Some homogenates were pre-incubated at 37°C for 30 min with various concentrations of clorgyline (0.1 nM to 0.5 mM) and the metabolism of Tyr ($100\text{ }\mu\text{M}$) assayed in the resulting homogenates. In these experiments, the metabolism was expressed as a percentage of a control, which had been preincubated in the absence of clorgyline.

Drugs and solutions

The perfusion solution (modified Krebs-Henseleit solution) had the following composition (mM): NaCl

118, KCl 4.57, CaCl_2 1.27, KH_2PO_4 1.19, MgSO_4 1.19, NaHCO_3 25 and glucose 5.55. Heparin injection BP was obtained from Paines & Byrne Ltd., Greenford, Middlesex, and urethane from BDH, Ltd. The radiochemicals used were $[7\text{-}^{14}\text{C}]$ -benzylamine hydrochloride and $[G\text{-}^3\text{H}]$ -tyramine hydrochloride from Dupont U.K. Ltd., Stevenage, Herts., and 5-hydroxy- $[G\text{-}^3\text{H}]$ -tryptamine creatinine sulphate from Amersham International plc., Amersham. Unlabelled amines, *p*-tyramine hydrochloride, 5-hydroxytryptamine creatinine sulphate and (–)-nor-adrenaline bitartrate and *p*-hydroxyphenylacetic acid were obtained from Sigma Chemical Company Ltd.,

Figure 1 Shows the profile of metabolites extracted from the perfusate of the isolated perfused mesenteric arterial bed of the rat following the addition of radiolabelled benzylamine (Bz) or tyramine (Tyr) to the Krebs solution perfusing the vessels. The deaminated metabolites were extracted following acidification of each fraction of perfusate. Each point represents the mean amount (nmol) of metabolite extracted (s.e. mean shown by vertical bar when larger than the symbol) and has been plotted against the fraction number. Metabolites from control preparations (●) and from preparations from rats treated with MDL 72145 (▽). In (a) $25\text{ }\mu\text{M}$ $[^{14}\text{C}]$ -Bz was infused into the preparations for 5 min and fractions of 0.5 ml of perfusate collected for 15 min (30 ml); in (b) $100\text{ }\mu\text{M}$ $[^3\text{H}]$ -Tyr was perfused through each preparation for 10 min and 1 ml fractions of perfusate collected for 50 min (100 ml).

Poole, Dorset. Benzylamine was purchased from Sigma as the free base and the hydrochloride prepared and crystallised. The alcohol metabolite of tyramine, *p*-hydroxyphenylethanol was obtained from Aldrich Chemical Company, Gillingham, Dorset. The aldehyde, *p*-hydroxyphenylacetaldehyde was prepared, as the Wanzlick derivative, by the method described by Sharman (1987) (m.p. 194° to 195°C, uncorrected, open capillary). This was dissolved in 1 M HCl, the free aldehyde was then extracted into ethyl acetate and the organic solution was used for the chromatography experiments. Clorgyline hydrochloride was a gift from May & Baker Ltd., (now Rhône-Poulenc) Eccles, Manchester, and MDL 72145 was a gift from Merrell Dow Research Institute, Strasbourg, France. All other reagents were of analytical grade where possible.

Statistical analysis of results

Statistical significance was evaluated by Student's *t* test, in which each group was compared with control.

Results

When Bz was added to the fluid perfusing rat mesenteric vascular beds, metabolites appeared in the perfusate within 2 min. The time course for the appearance of the metabolites of Bz in the perfusate is shown in Figure 1a and the quantities of radioactivity recovered and metabolites extracted are given in Table 1. Of the administered radioactivity, 70%

was recovered in the perfusate and this did not differ significantly between control preparations and preparations from rats treated with MDL 72145. There was, however, an 83% reduction in the amount of metabolites extracted following MDL 72145 treatment. The profiles of tyramine metabolites appearing from control preparations and preparations from rats treated with MDL 72145 are shown in Figure 1b. The amount of radioactivity recovered and metabolites extracted, from all 5 groups of preparations where the metabolism of Tyr was examined in this way, are given in Table 1. The recovery of radioactivity from these preparations was 80% and again did not differ significantly between the groups. Treatment of the preparations with clorgyline (10 µM) did not reduce the amount of metabolites extracted, whereas treatment of the rats with MDL 72145 caused a reduction of more than 50%. A combination of these two inhibitors gave a small further reduction in the amount of metabolites down to 42% of control. In the presence of cocaine (3 µM), a significant increase in the amount of metabolites was seen.

Table 2 gives the chromatographic data for the metabolites of tyramine appearing in fractions 12 to 36 from control preparations. The recoveries of the radioactivity chromatographed were 78% for solvent system 1 and 68% for solvent system 2. All the radioactivity recovered from chromatograms made with solvent system 1 was found in one peak with an *R_F* value of 0.91. When solvent system 2 was used, two peaks of radioactivity were recovered, with 83.3% of the recovered activity having the same *R_F* value as the acid metabolite.

Table 1 Amine metabolism in the isolated perfused mesenteric arterial bed of the rat

Amine	Group	(n)	Total radioactivity recovered (nmol)	Amount of metabolites extracted (nmol)
Benzylamine (25 µM)	Control	(4)	174.7 ± 3.8	30.8 ± 1.0
	MDL	(4)	176.4 ± 6.5	5.4 ± 0.4**
Tyramine (100 µM)	Control	(8)	1562 ± 53	69.2 ± 3.5
	Clorgyline	(6)	1616 ± 55	70.6 ± 4.1
	MDL	(6)	1574 ± 40	33.8 ± 2.4**
	MDL + Clorg	(4)	1549 ± 21	29.2 ± 2.9**
	Cocaine	(6)	1675 ± 40	92.6 ± 9.3*

The isolated perfused mesenteric vascular bed of the rat was perfused with radioactive amines, benzylamine (Bz) and tyramine (Tyr) in modified Krebs-Henseleit solution. Fractions of perfusate collected (0.5 ml or 1 ml) were assessed for their total radioactive content and, following acidification, the amount of radioactivity extracted into an organic solvent. Totals of 30 and 100 ml of perfusate were collected following Bz and Tyr infusion respectively. The values given in the table are the sums of the amounts found in each fraction. The preparations studied were from control rats (control), rats treated with 1 or 2 mg kg⁻¹ MDL 72145 1 h before use (MDL), control rats where clorgyline (10 µM) had been added to the perfusing fluid for 30 min during the equilibration period (Clorg), rats treated with MDL 72145 where the preparation was also perfused with clorgyline (MDL + Clorg) and from control rats where cocaine (3 µM) was present in the perfusing fluid throughout the experiment.

(**P < 0.001; *P < 0.05 vs control by Student's *t* test).

Table 2 Chromatography of tyramine and its metabolites

Compound	R_F value		Colour reaction
	Solvent 1	Solvent 2	
<i>p</i> -Hydroxyphenylacetic acid	0.91	0.54	pink
<i>p</i> -Hydroxyphenylethanol	0.92	0.91	orange
<i>p</i> -Hydroxyphenylacetaldehyde	0.94	streaked	red
<i>p</i> -Tyramine	0.58	0.85	yellow
Radioactive metabolites	0.91	(a) 0.54 (83.3%) (b) 0.94 (16.7%)	

Tyramine and its metabolites were chromatographed on Whatman no. 1 paper by ascending chromatography. The solvent systems used were: (1) *n*-butanol-acetic acid-water (25: 4: 10) and (2) propan-2-ol-ammonia-water (28: 1: 6). The standard compounds were dissolved in ethylacetate (except tyramine which was dissolved in water). The aldehyde was prepared from its Wanzlich derivative on the day of the experiment. The metabolites collected from control mesenteric preparations perfused with radiolabelled tyramine were pooled and extracted as described in the methods section. Each chromatogram was allowed to run to at least 25 cm and the standard compounds detected by spraying the paper with a solution of Fast GG scarlet salt (1 mg ml^{-1} in 0.1 M HCl) followed by $1 \text{ M Na}_2\text{CO}_3$. The radioactivity was located by cutting the chromatogram into 1 cm bands and counting by liquid scintillation. The recovery of the radioactivity applied to the chromatogram was 78% from solvent 1 and 68% from solvent 2. The values given in the table are the mean for the metabolites extracted from four preparations and the percentage found at both sites on the chromatogram using solvent system 2 are shown.

The amine oxidase activities detected in homogenates of mesenteric blood vessels from the tyramine experiments are shown in Figure 2 and demonstrate the selectivity of the inhibitors used. Figure 3 shows the effects of clorgyline on the metabolism of Tyr by

homogenates of mesenteric blood vessels. Tyr metabolism was inhibited by 40% with $0.1 \mu\text{M}$ clorgyline, with very little further inhibition occurring at higher clorgyline concentrations. The metabolism of Tyr by preparations from MDL 72145-treated rats was completely inhibited by $0.1 \mu\text{M}$ clorgyline.

Figure 2 The specific activities of amine oxidase enzymes have been measured in homogenates of the mesenteric vascular beds following the perfusion experiments. Monoamine oxidase-A activity (open columns) was measured by incubating the homogenates with $500 \mu\text{M}$ [^3H]-5-hydroxytryptamine. Semicarbazide-sensitive amine oxidase activity (solid columns) was measured by the ability of the homogenates to deaminate [^{14}C]-benzylamine. The groups of mesenteric arterial beds examined in this way are the same as those given in Table 1 and the same abbreviations have been used here.

Discussion

These studies have shown that a significant amount of Bz is metabolized as it passes through the mesenteric arterial bed of the rat. The enzyme primarily responsible for this metabolism is SSAO demonstrating that this amine oxidase can function in intact blood vessels as well as in tissue homogenates. These findings complement those made with homogenates of this tissue, where almost all the metabolism of Bz could be attributed to SSAO. The lack of hydroxyl substituents on the Bz molecule make this amine extremely lipophilic (Mack & Bönisch, 1979) and able to enter and leave cells of the blood vessel wall with ease. Bz is the amine for which vascular SSAO has the highest affinity (K_m $2.06 \pm 0.26 \mu\text{M}$, $n = 4$ in mesenteric homogenates) and the one which is turned over at the fastest rate by this enzyme activity (Elliott *et al.*, 1989b).

Addition of an hydroxyl group in the *para* position of phenylethylamine results in a marked decrease in lipophilicity at physiological pH (Mack & Bönisch, 1979) and therefore in the ability to cross cell membranes. Tyr has been shown to be a substrate for both MAO-A and SSAO in tissue homogenates in rat blood vessels with little or no contribution from MAO-B (Coquil *et al.*, 1973;

Figure 3 Shows the effect of clorgyline on the deamination of tyramine by homogenates of the mesenteric vascular bed of the rat to reveal the relative proportions of monoamine oxidase-A and semicarbazide-sensitive amine oxidase. Homogenates of this tissue were made following perfusion experiments and they were preincubated with various concentrations of clorgyline for 30 min at 37°C. The resulting metabolism of tyramine (100 μ M) was measured radiochemically (see Methods). The metabolism has been measured in homogenates from control rats (■) and from rats treated with MDL 72145 (Δ). The metabolism found, following preincubation with clorgyline in control and MDL 72145-treated rats, has been expressed as percentages of the deamination in the absence of clorgyline and plotted against negative log clorgyline concentration. Each point is the mean value from 4 experiments; vertical bars show s.e.mean.

Clarke *et al.*, 1982). At the concentration used here (100 μ M), 60% of the metabolism could be attributed to SSAO and 40% to MAO-A when tissue homogenates were examined. The metabolites of Tyr were readily released from the intact mesenteric arteries following infusion of the amine. Inhibition of SSAO reduced the metabolites appearing to 48% of control. MAO-A seemed to contribute very little to the formation of these metabolites even when SSAO had been inhibited. It has been assumed, that since MAO-B contributes very little to the metabolism of Tyr in these tissue homogenates, it is unlikely to contribute to the production of metabolites in the intact mesenteric arterial bed.

SSAO has been shown to be located in the medial part of the blood vessel wall (Lyles & Singh, 1985) and its activity is not affected by sympathectomy (Coquil *et al.*, 1973). MAO-A is an intracellular enzyme situated on the outer membrane of mitochondria (Cotzias & Dole, 1951; Hawkins, 1952; Greenawalt & Schnaitman, 1970). In tissues that have a sympathetic nerve supply, MAO has been shown to be present both intraneuronally and extraneuronally (Jarrott & Iversen, 1971) while the nerve endings are located at the adventitial-medial junction (Hodge & Robinson, 1972). Chemical sympathectomy caused a 70% decrease in MAO-A activity in rat femoral and mesenteric arteries (Coquil *et al.*, 1973). Blockade of neuronal uptake with cocaine increased the amount of metabolites formed during Tyr infusion. At the concentration used here, cocaine has been shown to potentiate the effects of directly acting sympathomimetic amines and to inhibit the responses to indirectly acting agents, such as Tyr, in this preparation (George & Leach, 1973; Elliott *et al.*, 1989c) indicating that it effectively blocked neuronal uptake. The fact that this resulted in an increase in metabolites leaving the tissue could indicate that the nerve endings in this tissue represented a significant site of accumulation of Tyr from which deaminated metabolites were not readily released. Blocking this site increased the availability of Tyr for the tyramine-metabolizing sites in the rest of the tissue. These experiments provide further evidence that neuronal MAO-A does not seem to contribute to the production of the metabolites collected from this preparation. However, the pressor response to Tyr was significantly reduced in the presence of cocaine resulting in a smaller rise in intraluminal hydrostatic pressure. It is possible that changes of this nature could have an influence on the efflux of metabolites. A possible way to exclude any such influence would be the use of an agent such as prazosin (Venning & de la Lande, 1985).

Following deamination of the amines, the aldehyde products can be metabolized to the corresponding acid or alcohol by aldehyde dehydrogenase (ALDH; EC 1.2.1.3.) or aldehyde reductase (EC 1.1.1.2) (Weissbach *et al.*, 1957; Leeper *et al.*, 1958; Goldstein *et al.*, 1961). The major metabolite extracted from the perfusate in these studies was the acidic metabolite of Tyr *p*-hydroxyphenylacetic acid (*p*-HPAA). Tacker *et al.* (1970) showed that when [^{14} C]-Tyr was given to rats by i.p. injection, 70% was excreted in the urine as *p*-HPAA. This is in contrast to the situation for noradrenaline, the metabolism of which in the whole rat and in isolated blood vessels produced alcohols as the principal metabolites (Kopin & Gordon, 1962; Venning & de la Lande, 1985). It seems that β -hydroxylated phenylethylamines are preferentially metabolized to the

corresponding alcohol by the rat, whereas phenylethylamines which lack the β -hydroxyl group are metabolized to the corresponding acid (Breese *et al.*, 1969). Mack & Bönisch (1979) demonstrated that the alcohol metabolites of hydroxylated amines were relatively lipophilic whereas the acid metabolites, at physiological pH, were present mainly in the dissociated form and were much less able to cross biological membranes. When the metabolism of noradrenaline was studied in isolated rat blood vessels, only small amounts of the acid metabolite were released from the tissue (Levin, 1974). By contrast, in these studies the acid metabolite of Tyr appeared very rapidly in the perfusing fluid. The presence of SSAO in the plasma membrane of smooth muscle cells may explain this anomaly. The subcellular location of aldehyde dehydrogenase in this tissue is not known. In brain and liver ALDH has been found in the mitochondrial and soluble fractions (Deitrich, 1966). Within the mitochondria, ALDH is to be found on the inner membrane (Pettersson & Totmar, 1982), which is conveniently positioned to act on aldehydes formed by MAO. Microsomal ALDH has been demonstrated in the liver and the presence of this enzyme in the plasma membrane of vascular smooth muscle cells close to SSAO might be postulated from these results. Preliminary studies in which dianilino-ethane was used to precipitate the aldehyde from the metabolites collected (Wanzlick & Löchel, 1953) have confirmed that only a small proportion (less than 10%) of the metabolites collected, remain as the aldehyde.

The identity of the metabolites of Tyr and the activity responsible for their formation following inactivation of both SSAO and MAO-A (42% of the metabolism in the absence of inhibitors), remains to

be determined. Some activity might be attributed to incomplete inhibition of SSAO in the intact tissue. In tissue homogenates from rats treated with MDL 72145, SSAO activity was reduced to 5% of the control value and no Tyr metabolism could be detected when MAO-A was inhibited as well. It would seem therefore, that the homogenization procedure has led to inactivation of the residual activity. It is possible that homogenization releases MDL 72145 from sites where it has been trapped in the intact preparation. It is unlikely that MAO-B is responsible for the residual metabolism of Tyr in the intact preparations from the rats treated with MDL 72145 since no Tyr metabolism due to this form of MAO could be detected in homogenates. In addition, MDL 72145 is a potent inhibitor of MAO-B (Zreika *et al.*, 1984) and the dose administered in our studies gave rise to almost complete inhibition of MAO-B in liver homogenates (data not shown).

The fact that radioactivity was extracted into the organic solvent from the acidified perfusate indicates that the amine group had been altered. An alternative way that this could be achieved is by acetylation of the amine moiety. Following inhibition of MAO and SSAO with iproniazid, the metabolism of dopamine and noradrenaline in the rat *in vivo* involved the formation of N-acetylated metabolites (Goldstein & Musacchio, 1962; Smith & Wortis, 1962). At present, this possibility remains to be explored.

In summary, SSAO can act in intact blood vessels on amines present in the perfusing fluid. The rapid release of acidic metabolites may indicate that these are formed at the cell surface and entry into the cells is not a prerequisite for metabolism.

J.E. is a Wellcome Veterinary Scholar.

References

- BREESE, G.R., CHASE, T.N. & KOPIN, I.J. (1969). Metabolism of some phenylethylamines and their β -hydroxylated analogs in brain. *J. Pharmacol. Exp. Ther.*, **165**, 9–13.
- CALLINGHAM, B.A. & BARRAND, M.A. (1987). Some properties of semicarbazide-sensitive amine oxidases. *J. Neural. Trans.* [Suppl.], **23**, 37–54.
- CALLINGHAM, B.A. & LAVERTY, R. (1973). Studies on the nature of the increased monoamine oxidase activity in the heart following adrenalectomy. *J. Pharm. Pharmacol.*, **37**, 329–335.
- CALLINGHAM, B.A., OGUCHI, E. & OGUCHI, K. (1983). Monoamine oxidase activities in rat mesenteric arteries and veins. *Br. J. Pharmacol.*, **79**, 302P.
- CLARKE, D.E., LYLES, G.A. & CALLINGHAM, B.A. (1982). A comparison of cardiac and vascular clorgyline resistant amine oxidase and monoamine oxidase. *Biochem. Pharmacol.*, **31**, 27–35.
- COQUIL, J.F., GORIDIS, C., MACK, G. & NEFF, N.H. (1973). Monoamine oxidase in rat arteries: evidence for different forms and selective localisation. *Br. J. Pharmacol.*, **48**, 590–599.
- COTZIAS, G.C. & DOLE, V.P. (1951). Metabolism of amines II. Mitochondrial localisation of monoamine oxidase. *Proc. Soc. Exp. Biol.*, **78**, 157–160.
- DEITRICH, R.A. (1966). Tissue and subcellular distribution of mammalian aldehyde-oxidising capacity. *Biochem. Pharmacol.*, **15**, 1911–1922.
- ELLIOTT, J. & CALLINGHAM, B.A. (1988). Amine metabolism in the isolated perfused mesenteric arterial bed of the rat. *Br. J. Pharmacol.*, **95**, 576P.
- ELLIOTT, J., CALLINGHAM, B.A. & BARRAND, M.A. (1989a). *In vivo* effects of (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine on amine oxidase activities in the rat. Selective inhibition of semicarbazide-sensitive amine oxidase in vascular and brown adipose tissue. *J. Pharm. Pharmacol.*, **41**, 37–41.
- ELLIOTT, J., CALLINGHAM, B.A. & SHARMAN, D.F. (1989b). Semicarbazide-sensitive amine oxidase of the rat aorta:

- interactions with naturally occurring amines and their structural analogues. *Biochem. Pharmacol.*, **38**, 1507–1515.
- ELLIOTT, J., CALLINGHAM, B.A. & SHARMAN, D.F. (1989c). The influence of amine metabolising enzymes on the pharmacology of tyramine in the isolated perfused mesenteric arterial bed of the rat. *Br. J. Pharmacol.*, **98**, 515–522.
- FUENTES, J.A. & NEFF, N.H. (1977). Inhibition by pargyline of cardiovascular amine oxidase activity. *Biochem. Pharmacol.*, **26**, 2107–2112.
- GEORGE, A.J. & LEACH, G.D.H. (1973). The effects of changes in ionic environment and modification of adrenergic function on the vascular responses to sympathomimetic amines. *J. Pharm. Pharmacol.*, **25**, 521–529.
- GOLDSTEIN, M.A., FRIEDHOFF, A.J., POMERANTZ, S. & CONTRERA, J.F. (1961). The formation of 3,4-dihydroxyphenylethanol and 3-methoxy-4-hydroxyphenylethanol from 3,4-dihydroxyphenylethylamine in the rat. *J. Biol. Chem.*, **236**, 1816–1821.
- GOLDSTEIN, M. & MUSACCHIO, J.M. (1962). The formation *in vivo* of N-acetyldopamine and N-acetyl-3-methoxydopamine. *Biochim. Biophys. Acta*, **58**, 607–608.
- GREENAWALT, J.W. & SCHNAITMAN, C. (1970). An appraisal of the use of monoamine oxidase as an enzyme marker for the outer membrane of the rat liver mitochondria. *J. Cell. Biol.*, **46**, 173–179.
- HAWKINS, J. (1952). The localisation of amine oxidase in the liver cell. *Biochem. J.*, **50**, 577–581.
- HODGE, R.L. & ROBINSON, S.M. (1972). The action of clonidine on isolated arterial preparations. *Aust. J. Exp. Biol. Med. Sci.*, **50**, 517–526.
- JARROTT, B. & IVERSEN, L.L. (1971). Noradrenaline metabolising enzymes in normal and sympathetically denervated vas deferens. *J. Neurochem.*, **18**, 1–6.
- KOPIN, I.J. & GORDON, E.K. (1962). Metabolism of norepinephrine- H^3 released by tyramine and reserpine. *J. Pharmacol. Exp. Ther.*, **138**, 351–359.
- LEEPER, L.C., WEISSBACH, H. & UDENFRIEND, S. (1958). Studies on the metabolism of norepinephrine, epinephrine and their o-methyl analogs by partially purified enzyme preparations. *Arch. Biochem. Biophys.*, **77**, 417–427.
- LEVIN, J.A. (1974). The uptake and metabolism of ^3H -L- and ^3H -DL-norepinephrine by intact rabbit aorta and by isolated adventitia and media. *J. Pharmacol. Exp. Ther.*, **190**, 210–226.
- LEWINSON, R., BÖHM, K.-H., GLOVER, V. & SANDLER, M. (1978). A benzylamine oxidase distinct from monoamine oxidase B – widespread distribution in man and rat. *Biochem. Pharmacol.*, **27**, 1857–1863.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- LYLES, G.A. & FITZPATRICK, M.S. (1985). An allylamine derivative (MDL 72145) with potent irreversible inhibitory actions on rat aorta semicarbazide-sensitive amine oxidase. *J. Pharm. Pharmacol.*, **37**, 329–335.
- LYLES, G.A. & SINGH, I. (1985). Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta. *J. Pharm. Pharmacol.*, **37**, 637–643.
- MCGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177**, 21–30.
- MACK, F. & BÖNISCH, H. (1979). Dissociation constants and lipophilicity of catecholamines and related compounds. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **310**, 1–9.
- PETERSSON, H. & TOTTMAR, O. (1982). Aldehyde dehydrogenases in rat brain. Subcellular distribution and properties. *J. Neurochem.*, **38**, 477–487.
- PRECIOUS, E., GUNN, C.E. & LYLES, G.A. (1988). Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. *Biochem. Pharmacol.*, **27**, 1857–1863.
- SHARMAN, D.F. (1987). The metabolism of dopamine in the blood of ruminant animals: Formation of 3,4-dihydroxyphenyl acetaldehyde. *Comp. Biochem. Physiol.*, **86C**, 151–155.
- SMITH, A.A. & WORTIS, S.B. (1962). Formation and metabolism of N-acetylnormetanephrine in the rat. *Biochim. Biophys. Acta*, **60**, 420–422.
- TACKER, M., McISAAC, W.M. & CREAVER, P.J. (1970). Metabolism of tyramine- $1-^{14}C$ by the rat. *Biochem. Pharmacol.*, **19**, 2763–2773.
- VENNING, M.G. & DE LA LANDE, I.S. (1984). Effects of uptake and surface of entry on the responses of the rat caudal artery to noradrenaline, adrenaline and methoxamine. *Blood Vessels*, **21**, 149–155.
- VENNING, M.G. & DE LA LANDE, I.S. (1985). Effect of inhibition of neuronal uptake on the efflux of noradrenaline into the lumen of the rat tail artery. *Blood Vessels*, **22**, 244–246.
- WANZLICK, H.-W. & LÖCHEL, W. (1953). 1,2-Dianilino-äthan als Aldehydreaktions. *Chem. Ber.*, **86**, 1463–1466.
- WEISSBACH, H., REDFIELD, B.G. & UDENFRIEND, S. (1957). Soluble monoamine oxidase; its properties and actions on serotonin. *J. Biol. Chem.*, **229**, 953–963.
- WIBO, M., DUONG, A.T. & GODFRAIND, T. (1980). Subcellular location of semicarbazide-sensitive amine oxidase in rat aorta. *Eur. J. Biochem.*, **112**, 87–94.
- ZREIKA, M., McDONALD, I.A., BEY, P. & PALFREYMAN, M.G. (1984). MDL 72145, an enzyme-activated irreversible inhibitor with selectivity for monoamine oxidase type B. *J. Neurochem.*, **43**, 448–454.

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