J. Pharm. Pharmacol. 1985, 37: 637–643
Received March 11, 1985

Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta

GEOFFREY A. LYLES* AND IQBAL SINGH

Department of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Hospital, Dundee DD1 9SY, UK

Several methods have been used to study the distribution of the semicarbazide-sensitive amine oxidase (SSAO) within the wall of the rat aorta. After separation of the smooth muscle-containing layers of the tunica media from the connective tissue of the tunica adventitia, much higher specific enzyme activity (measured with 1 μM benzylamine) was found in homogenates of the media than of adventitia. Similar results were obtained for MAO-A (with 1 mM 5-HT as substrate). SSAO activity was also considerably higher in homogenates of cells (predominantly smooth muscle) isolated from medial tissue by enzymatic dissociation with collagenase and elastase compared with homogenates of cells (mostly of connective tissue origin) from the adventitia. Histochemical staining resulting from SSAO activity (with benzylamine as substrate) occurred predominantly and intensely over the tunica media in rat aortic sections, although some occasional staining of adventitial sites was also observed. Staining was prevented by the SSAO inhibitors hydroxylamine (1 μM) and semicarbazide (1 mM), but not by the MAO inhibitor, clorgyline (1 mM). These results indicate that SSAO is associated predominantly, although not exclusively, with the smooth muscle cells in the rat aorta. Our findings that β-aminopropionitrile (BAPN) is a reversible, competitive inhibitor (K_i around 2×10^{-4} M) of SSAO, in contrast to the irreversible inhibition of the connective tissue lysyl oxidase by BAPN reported by others, provides further evidence that these enzymes are not identical.

In aorta homogenates of a variety of animal species including rat (Clarke et al 1982), rabbit (Rucker & Goettlich-Riemann 1972), pig (Buffoni et al 1976), ox (Rucker & O'Dell 1971) and man (Lewinsohn et al 1978), evidence has been found for the metabolism of monoamines, particularly benzylamine, by an amine oxidase activity which is sensitive to inhibition by semicarbazide and other carbonyl reagents. Consequently, this enzyme may belong to the group of amine oxidases believed to contain pyridoxal phosphate as cofactor (see Blaschko 1974).

Inhibition by carbonyl reagents also helps to distinguish the semicarbazide-sensitive amine oxidase (SSAO) from the mitochondrial flavin-containing enzyme, monoamine oxidase (MAO) also found in many tissues. The latter enzyme can in fact be subdivided into two distinct forms, called MAO-A and MAO-B, based on differences in the relative sensitivities of these forms to inhibition by the acetylenic drugs clorgyline (MAO-A selective) and selegiline (deprenyl) (MAO-B selective) (see Fowler & Ross 1984 for review). In contrast, concentrations of these acetylenic agents which are sufficient to inhibit both MAO activities completely, have little or no effect on SSAO, thus providing a further method for distinguishing between these different enzymes. Other names which have been used for SSAO include benzylamine oxidase and clorgyline–or pargyline-resistant amine oxidase (e.g. Lewinsohn et al 1978; Barrand & Callingham 1982; Urdin & Fuentes 1983).

Although SSAO activity has also been studied in other rat tissues including heart (Lyles & Callingham 1975), mesenteric and femoral arteries (Coquil et al 1973; Callingham et al 1983), lung and skull (Andree & Clarke 1982), brown adipose tissue (Barrand & Callingham 1982), anococcygeus muscle (Callingham 1982), duodenum, kidney and adrenals (Guffroy & Strolin Benedetti 1984), its physiological importance is far from clear. From a survey of its specific activity in many rat and human tissues, Lewinsohn and co-workers concluded that the enzyme may be localized predominantly within vascular and non-vascular smooth muscle (Lewinsohn et al 1978; Lewinsohn 1981). In addition, histochemical studies showed strong staining for SSAO activity in the walls of blood vessels of human placenta (Ryder et al 1979). In this respect, our

* Correspondence.

recent studies involving separation of different cell types from rat heart and skeletal muscle after tissue dispersal with collagenase, also have suggested that SSAO may be associated with vascular elements in these particular tissues (Lyles et al 1984; Archer & Lyles 1984). To investigate further the cellular localization of SSAO in vascular tissue, we have used both biochemical and histochemical techniques to study the distribution of the enzyme within different layers and cellular constituents of the rat aortic wall. Furthermore, the possibility that SSAO may be related to the amine oxidase (lysyl oxidase) involved in cross-linking of collagen and elastin in the formation of connective tissue, has been considered by examining the effects of the potent irreversible lysyl oxidase inhibitor, β-aminopropionitrile (Tang et al 1983) on SSAO in homogenates of rat aorta.

Preliminary details of this work have been communicated previously (Lyles & Singh 1984: Lyles 1984a).

MATERIALS AND METHODS

Materials

[Methylene-^{14}C]benzylamine hydrochloride and [G-^{3}H]5-hydroxytryptamine (5-HT) creatinine sulphate were obtained from Amersham International PLC (Amersham, UK).

The following reagents were purchased from Sigma London (Poole, UK): collagenase (type 1A), elastase (type 1), horseradish peroxidase (type II), bovine serum albumin, semicarbazide hydrochloride, hydroxylamine hydrochloride, β-aminopropionitrile fumarate, benzylamine hydrochloride and 3-amino-9-ethylcarbazole. Clorgyline hydrochloride (M & B 9302) was a generous gift from May & Baker Ltd (Dagenham, UK).

Male or female rats, 250–400 g, were obtained from our Departmental breeding colony, Animal Services Unit, University of Dundee.

Methods

Preparation of homogenates from adventitial, medial and whole tissue from rat aorta

Rats were killed by stunning followed by cervical dislocation. For preparation of separated adventitial and medial layers of the rat aorta, the thoracic segment of the vessel was quickly removed, immersed in isolation buffer (g litre^{-1}: NaCl 6·8, KCl 0·4, glucose 0·9, Na_2HPO_4 0·21, $Na_2HPO_4.H_2O$ 0·06, pH 7·4) and trimmed of larger pieces of adherent connective or fat tissue, and also visible residual side branches of blood vessels joining the aorta. Each aorta was then rolled gently onto a short polythene cannula support which fitted the vessel lumen and extended just beyond either end of the vessel. The tissue was then transferred to a small glass vial and immersed in isolation buffer containing 1 mg ml^{-1} collagenase. After incubation at 37 °C in a shaking water bath for about 1 h, the outer adventitial layer of the aorta was loosened sufficiently by this treatment to enable it to be stripped by 'teasing' it gently with fine-pointed forceps away from the underlying intact media. The separated adventitia and media were then washed and stored at −20 °C in a small volume of 1 mM potassium phosphate, pH 7·8.

Homogenates of these tissues were prepared in 1 ml of 1 mM potassium phosphate buffer pH 7·8, using a hand-held ground glass homogenizer. After centrifugation at 60*g* for 10 min, supernatant fractions were used for assays.

Homogenates of intact rat aortae were used for the inhibition studies involving β-aminoproprionitrile (BAPN). Here, tissues were homogenized as above at a tissue (mg): buffer (μl) ratio of 1:40. The supernatant fractions were diluted six-fold for assay of SSAO, or used undiluted for subsequent assay of MAO-A to ensure linear metabolite production with time in the assays described below.

Preparation of isolated cell fractions

In some experiments, the adventitial and medial tissue obtained was further incubated with shaking at 37 °C for 3–4 h in isolation buffer containing 1 mg ml^{-1} collagenase and 0·5 mg ml^{-1} elastase. This procedure resulted in almost complete dispersal of the tissue samples. It was necessary in these experiments to pool adventitial or medial tissue, respectively, from 2 rats at a time to provide adequate material in the eventual cell fractions for enzyme assays. At the end of the incubation period, samples were centrifuged at 6*g* for 10 min, resulting in almost complete removal of any residual undispersed tissue. The supernatants were then centrifuged at 550*g* for 10 min to sediment the isolated cells released from the tissues. The resulting adventitial pellet was found (by light microscopy) to consist largely of connective tissue cells such as fibroblasts, with a few residual strands of collagen within very small segments of undigested tissue. In contrast, the medial pellets consisted predominantly of smooth muscle cells. There was little visible evidence for a significant endothelial cell component in this fraction. It is likely that insertion of the cannula support through the blood vessel lumen would, in any case, produce significant damage to the endothelial lining of the tunica intima.

All cell pellets were resuspended and centrifuged immediately at 875*g* for 5 min in 1 mM potassium phosphate buffer, pH 7·8. Resulting pellets were then resuspended in 1 ml of this buffer for storage (generally overnight) at −20 °C. Samples were then thawed, homogenized in this storage buffer and centrifuged as above to provide supernatant fractions for enzyme assays.

Enzyme assays

Amine oxidase activities were assayed by the method of Callingham & Laverty (1973) as described fully by Lyles & Callingham (1982), using 1 μM [^{14}C]benzylamine (sp. act. 10 μCi μmol^{-1}) as substrate for SSAO, and 1 mM [^{3}H]5-HT (sp. act. 2 μCi μmol^{-1}) for MAO-A. Preliminary experiments with homogenates prepared either from tissue samples or isolated cell fractions showed that preincubation for 20 min at 37 °C with 1 mM clorgyline failed to inhibit benzylamine metabolism, whereas 5-HT metabolism was completely prevented by 10^{-6} M clorgyline. The results confirmed that the assay conditions with these substrates were valid for assessment of SSAO and MAO-A activity in the different homogenates.

Protein concentrations of homogenates were estimated by the method of Lowry et al (1951) using bovine serum albumin as standard.

Differences between mean specific activities of experimental groups were tested for statistical significance by the Wilcoxon Rank Sum method (2-tailed).

Histochemical studies

Cryostat-cut transverse sections (20 μm) of rat aorta were stained for SSAO activity by use of the coupled peroxidatic oxidation method described by Ryder et al (1979). Slight modifications involved the use of benzylamine as substrate in the incubation medium at a final concentration of 50 μM rather than 8 mM to reflect the much lower K_m for benzylamine of the rat enzyme (around 5 μM; Clarke et al 1982) than the human enzyme (around 150 μM; Hayes et al 1983), and to avoid the inhibition of rat SSAO by high substrate concentrations (Lyles & Callingham 1975). Also a longer incubation time (2–3 h) was used here. For inhibitor studies, tissue sections were preincubated (15 min at 37 °C) with the inhibitor diluted from an aqueous stock solution (2×10^{-2} M) to an appropriate concentration in 0·05 M potassium phosphate buffer, pH 7·6, followed by subsequent incubation in the histochemical reaction medium, in which the same inhibitor concentration was also incorporated. Preparation of 'no substrate' and 'cold-acetone treated' control sections were as previously described, as also were the washing, post-fixation and final mounting of all sections in glycerol jelly (Ryder et al 1979). Sections were observed and photographed in a Leitz Ortholux II/Orthomat photomicroscope using Ilford FP4 35 mm black and white film.

RESULTS

Amine oxidase activities of tissue and cell fraction homogenates

The SSAO and MAO-A activities of homogenates from the adventitial and medial layers of the rat aorta are shown in Table 1. These results, indicated that the specific activity of SSAO was approximately ten-fold higher in the medial than in the adventitial homogenates. In contrast, MAO-A activity was found in the medial homogenates but could not be detected under these conditions in the adventitial layers.

Table 1. Amine oxidase activities of homogenates and dissociated cells from separated adventitia and media of rat aorta. SSAO was measured with 1 μM benzylamine and MAO-A with 1 mM 5-HT. Values for homogenates show means ± s.e.m. from triplicate determinations on individual aortae (number in parentheses), whereas similar determinations were made on dissociated cell preparations, each derived from the appropriate pooled tissue of two rats.

Source	Activity (n mole substrate h^{-1} (mg prot.)$^{-1}$) SSAO	MAO-A
Adventitial homogenates	6·3 ± 1·4 (6)	ND
Medial homogenates	65·3 ± 11·3* (6)	71·8 ± 5·0 (5)
Adventitial cells	2·8 ± 1·1 (7)	ND
Medial cells	42·9 ± 7·7** (7)	ND

*$P < 0·01$; **$P < 0·001$ comparing corresponding medial with adventitial samples. ND = not detectable (see text).

Corresponding activities of SSAO in homogenates derived from isolated cell fractions are also shown in Table 1. For these experiments, it was necessary to pool appropriate cell fractions from two rats at a time to provide sufficient material for assay. Again, the specific activity of SSAO in cells derived from the blood vessel media was considerably higher than in those from the adventitia. The assay of MAO-A activity in these cell fractions was more difficult and enzyme activity was barely above blank values in either medial or adventitial cell fractions pooled from only two rats. In contrast, in a single experiment in which medial cell fractions from six rats were pooled to provide more material for homogenization, MAO-A activity of 167 nmol h^{-1} mg^{-1} protein

was measured in the resulting homogenate. However, since our primary purpose throughout the current work was to examine the distribution and activity of SSAO in the aorta, no further studies on MAO-A activity in cell fractions were carried out at this stage in view of the likely necessity of requiring large numbers of animals to obtain reliable data.

Histochemical studies

No histochemical staining of rat aorta was found in tissue sections incubated in the reaction medium lacking the substrate, benzylamine. In contrast, those sections incubated in the presence of 50 μM benzylamine showed marked enzymatic staining over the whole of the tunica media of the vessel (Fig. 1). This stain comprised a strong diffuse reddish-brown background over the smooth muscle cells lying between the elastic laminae of the media. In addition, a more intense dotted appearance to the stain was evident in places against the more general background staining. In contrast to the reaction product formation occurring over the media, the adventitia was mostly devoid of any reaction. However, very occasionally small patches of staining were apparent over parts of the adventitia, an example of this being shown in Fig. 1. In some other sections (not shown), staining could also be found associated with small amounts of residual adipose tissue remaining attached to the outer adventitial surface. As reported by Ryder et al (1979), treatment of tissue sections with cold acetone before incubation had no noticeable effect on the development of the reaction product associated with these structures, thus indicating that lipid solubility of the reaction product was unlikely to have significantly affected the staining patterns observed.

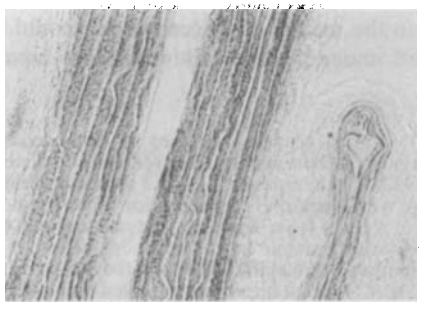

FIG. 1. Incubation in medium containing benzylamine (50 μM). Cutting of section has resulted in diametrically opposite walls of the vessel being in close apposition, with intimal surfaces almost in contact (centre). Also, at bottom right, is a transverse section through another smaller vessel, probably representing a residual portion of a side-branch from the aorta, remaining after dissection. Staining found predominantly over tunica media of vessel walls. Note, however, the small patch of adventitial staining on outer surface of left-hand wall (Mag: 560×).

Preincubation of sections with semicarbazide (10^{-3} M) or hydroxylamine (10^{-6} M), followed by incubation in reaction medium containing these SSAO inhibitors completely prevented the development of tissue staining (see Fig. 2, for hydroxylamine). In contrast, the MAO inhibitor clorgyline (10^{-5} M) had little if any inhibitory effect upon reaction product formation (Fig. 3).

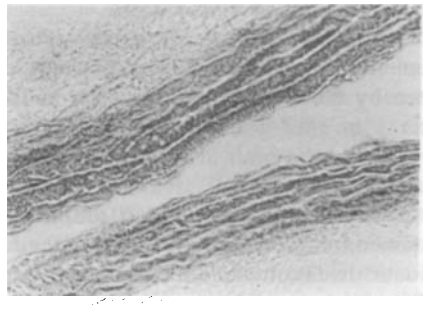

FIG. 2. Pretreatment with hydroxylamine (1 μM), incubation in medium containing benzylamine and hydroxylamine. Intimal surfaces of walls again almost in contact (as in Fig. 1). No staining observed (Mag: 560×).

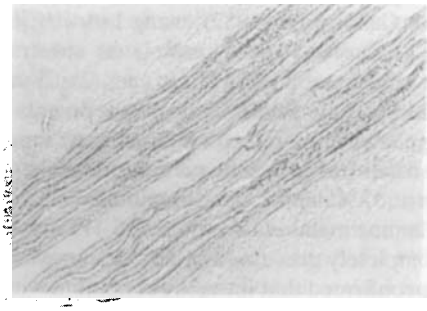

FIG. 3. Similar to Fig. 2 except clorgyline (1 μM) used as inhibitor. Staining present over tunica media of vessel walls (Mag: 560×).

Inhibition of rat aorta SSAO by β-aminopropionitrile (BAPN)

Other studies were carried out to investigate possible inhibitory effects of BAPN upon SSAO. Homogenates from intact rat aorta were used for these experiments.

In preliminary studies, BAPN (10^{-4} to 10^{-2} M) was preincubated with homogenate samples for 20 min at 37 °C, followed by assay of remaining SSAO activity with 1 μM benzylamine. Enzyme activity was inhibited in a dose-dependent manner with an estimated IC50 of approximately 5×10^{-4} M

(Fig. 4). Subsequent studies revealed however that inhibition by 10^{-3} M BAPN was not dependent upon time of preincubation (up to 1 h) and was fully reversible by dialysis (not shown).

The nature of this reversible inhibition was studied by measuring the effects of BAPN (0·1, 0·5 and 1·0 mM), without preincubation, upon initial reaction velocities for SSAO obtained at varying benzylamine concentrations (0·5 to 4 μM). The Lineweaver Burk plot in Fig. 5 shows that BAPN was a competitive inhibitor of SSAO in this representative experiment with a K_i value (estimated from the linear slope replot) of $2·5 \times 10^{-4}$ M. Identical conclusions were also obtained in replicate experiments performed on two other aorta homogenates, yielding a mean K_i of $2·2 \pm 0·4 \times 10^{-4}$ M (n = 3) for the inhibition. From the control data in these plots for benzylamine oxidation in the absence of BAPN, the K_m for benzylamine as substrate for rat aorta SSAO was found to be $1·7 \pm 0·3$ μM (n = 3).

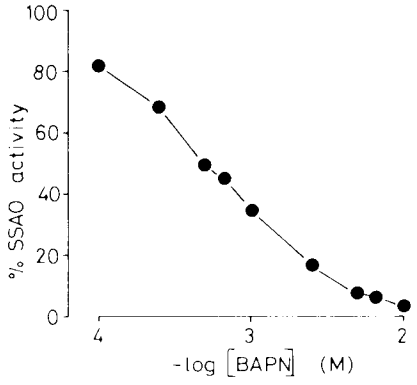

FIG. 4. Inhibition of rat aorta SSAO by BAPN. Homogenate samples were preincubated (20 min, 37 °C) with various concentrations of BAPN, and remaining SSAO activity was assayed with 1 μM benzylamine. Activities are expressed as percentages of control samples preincubated without BAPN. Each point is the mean of triplicate determinations on two different aorta homogenates (control specific activities of 42·5 and 45·8 nmol h^{-1} $mg\ prot.^{-1}$).

DISCUSSION

Our results support previous suggestions that SSAO is associated with vascular smooth muscle, thus providing an explanation for the relatively high specific enzyme activity found in blood vessels compared with other organs of rat and man (Lewinsohn et al 1978; Lewinsohn 1981). For instance, after separation of tunica media from adventitia, we found considerably higher specific activity of SSAO in homogenates of the medial vascular layers, in support of similar findings reported by Lewinsohn (1981) on a single human aorta in which these layers were separated. The only cell type found in the tunica media of the aorta of many mammals, including the rat, is the smooth muscle cell (Cliff 1976), which would thus appear to be a major source of the enzyme found in aorta homogenates. This conclusion was further supported by the presence of SSAO activity in the isolated smooth muscle cell

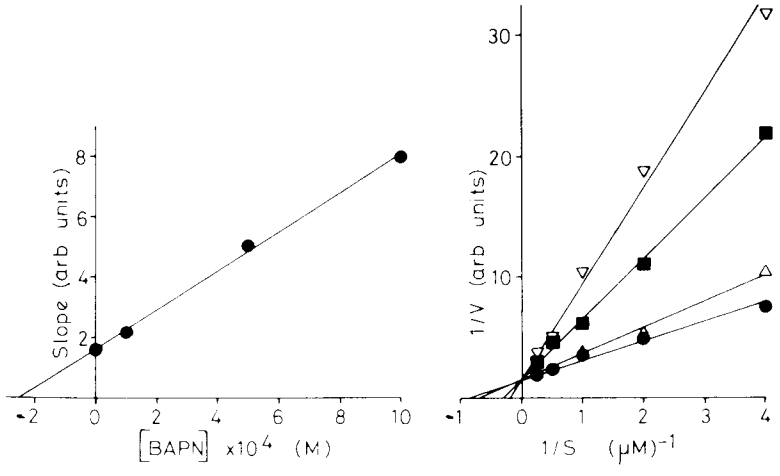

FIG. 5. Lineweaver-Burk plot (at right) for inhibition of rat aorta SSAO by BAPN. Initial reaction velocities (V) in arbitrary units were measured at different concentrations of benzylamine as substrate (S), and in the presence of BAPN at the following concentrations: zero (●), 0·1 (△), 0·5 (■) and 1·0 mM (▽). Each point is the mean of triplicate determinations. Slope replot of data shown at left.

fraction obtained from medial tissue, whereas cell fractions from the adventitia, containing predominantly connective tissue cells showed much lower activity. Furthermore, our histochemical results which showed strong staining over the tunica media provided direct visual evidence for the association of SSAO with the vascular smooth muscle layers, in agreement with similar studies on human placental blood vessels (Ryder et al 1979). In our experiments, this staining was dependent upon the presence of the substrate benzylamine, could be prevented by SSAO inhibitors (semicarbazide and hydroxylamine), but was insensitive to high concentrations of the MAO inhibitor clorgyline. Although benzylamine is a substrate for MAO-B (and sometimes MAO-A) in many animal tissues (Parkinson et al 1980), the inhibitor specificity of our histochemical results is consistent with biochemical evidence that no significant benzylamine metabolism is brought about by MAO activities in rat aorta (Clarke et al 1982). We have also recently used this histochemical method successfully to show the presence of SSAO on larger blood vessels within rat skeletal muscle (Archer & Lyles 1984).

Although SSAO is clearly associated with vascular smooth muscle, the significance of this finding and the potential importance of the inhibition of SSAO by various therapeutic agents (see Lyles 1984b) remains unclear. The difference in K_m values for benzylamine deamination by the rat (Clarke et al 1982) and human enzyme (Hayes et al 1983) suggests that species-related variations in enzymic properties may also be a relevant factor. In addition to the synthetic amine benzylamine, SSAO (in the rat) can metabolize some endogenous biogenic amines such as β-phenylethylamine, tyramine, tryptamine and dopamine which could thus serve as physiological substrates for the enzyme. SSAO appears to be associated with cell plasma membrane fractions from rat aorta (Wibo et al 1980) indicating that in contrast to the intracellular mitochondrial enzyme MAO, SSAO may conceivably deaminate extracellular amines.

Lewinsohn (1981) has provided evidence that SSAO may also be found on non-vascular smooth muscle in some human tissues. However, its localization does not appear to be restricted to smooth muscle alone. For instance, SSAO has been found in isolated fat cells from brown adipose tissue (Barrand et al 1984) and indeed, some evidence for histochemical staining of adipose tissue was found in the current work. We also found very sparse patches of staining over the adventitia of the rat aorta, and in view of our ability to find SSAO activity (albeit low) after assay of homogenates of adventitial tissue or isolated cells from this source, these findings suggest that some connective tissue component or cell(s) may also contain the enzyme. However, we are unable to identify this source definitively from the present results. The presence of SSAO in human lymphocytes and granulocytes isolated from blood has also recently been described (Banchelli et al 1983). Hence, the unknown functional importance of this enzyme may encompass a role shared by divergent types of cell.

The maturation of connective tissue within blood vessels requires the catalytic activity of lysyl oxidase to promote cross-link formation within collagen and elastin molecules. Cultured smooth muscle cells from rabbit aorta have been shown to produce and secrete lysyl oxidase (Gonnerman et al 1981). However, previous evidence has indicated that SSAO is not identical to lysyl oxidase, since for example benzylamine is not considered to be a substrate for the latter enzyme (Shieh et al 1975). Also, intratracheal administration of bleomycin to rats to produce lung fibrosis, results in an increase in lysyl oxidase but not SSAO activity in the lung (Hayes et al 1982). In the current experiments, these previous conclusions were supported by our findings that BAPN is a reversible competitive inhibitor of SSAO, in contrast to its well-documented potent (at μM concentrations) irreversible inhibition of lysyl oxidase (Tang et al 1983).

Some limited results on the distribution of MAO-A activity in the rat aorta are also presented here. MAO-A was measurable in homogenates from medial but not adventitial tissue, indicating that smooth muscle is also a likely source of this enzyme. The rat aorta is virtually devoid of sympathetic innervation (Patil et al 1972) and thus adrenergic nerve endings are unlikely to provide a significant source for MAO-A activity in this tissue. Trevithick et al (1981) also concluded that MAO-A was the predominant activity associated with cultured porcine smooth muscle cells. Although we obtained an indication that MAO-A activity could be recovered in the rat smooth muscle cells isolated in the present experiments, the scarcity of material available prevented detailed investigation at this stage.

In conclusion, we have demonstrated that in the rat aorta, SSAO is associated predominantly with the smooth muscle cells of the tunica media. These findings may provide further clues to the unknown physiological function of this enzyme in vascular tissue.

Acknowledgements

The support of I. S. by a Medical Student Vacation Research Grant from the Scottish Home and Health Department during part of this work is gratefully acknowledged. We also thank Mr G. Coghill (Department of Pathology) for help with the histochemical studies, and both Susan Fitzpatrick and Marion Hamilton of our Department for technical and secretarial assistance, respectively.

REFERENCES

Andree, T. H., Clarke, D. E. (1982) Biochem. Pharmacol. 31: 825–830

Archer, D. R., Lyles, G. A. (1984) Br. J. Pharmacol. 83: 421P

Banchelli, G., Fantozzi, R., Ignesti, G., Pirisino, R., Raimondi, L., Buffoni, F. (1983) Biochem. Int. 7: 89–94

Barrand, M. A., Callingham, B. A. (1982) Biochem. Pharmacol. 31: 2177–2184

Barrand, M. A., Callingham, B. A., Fox, S. A. (1984) J. Pharm. Pharmacol. 36: 652–658

Blaschko, H. (1974) Rev. Physiol. Biochem. Pharmacol. 70: 83–148

Buffoni, F., Marino, P., Pirisino, R. (1976) Ital. J. Biochem. 25: 191–203

Callingham, B. A. (1982) in: Kamijo, K., Usdin, E., Nagatsu, T. (eds) Monoamine oxidase. Basic and clinical frontiers. International Congress Series 564, Excerpta Medica, Amsterdam, pp 100–111

Callingham, B. A., Laverty, R. (1973) J. Pharm. Pharmacol. 25: 940–947

Callingham, B. A., Oguchi, E., Oguchi, K. (1983) Br. J. Pharmacol. 79: 302P

Clarke, D. E., Lyles, G. A., Callingham, B. A. (1982) Biochem. Pharmacol. 31: 27–35

Cliff, W. J. (1976) Blood Vessels. Cambridge Univ. Press, Cambridge, UK

Coquil, J. F., Goridis, C., Mack, G., Neff, N. H. (1973) Br. J. Pharmacol. 48: 590–599

Fowler, C. J., Ross, S. B. (1984) Medicinal Res. Rev. 4: 323–358

Gonnerman, W. A., Ferrera, R., Franzblau, C. (1981) Biochemistry 20: 3864–3867

Guffroy, C., Strolin Benedetti, M. (1984) Life Sci. 34: 535–545

Hayes, B. E., Counts, D. F., Kelley, J., Clarke, D. E. (1982) Biochem. Pharmacol. 32: 2347–2350

Hayes, B. E., Ostrow, P. T., Clarke, D. E. (1983) Exp. Mol. Pathol. 38: 243–254

Lewinsohn, R. (1981) J. Pharm. Pharmacol. 33: 569–575

Lewinsohn, R., Bohm, K.-H., Glover, V., Sandler, M. (1978) Biochem. Pharmacol. 27: 1857–1863

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Lyles, G. A. (1984a) J. Pharm. Pharmacol. 36 (workshop supplement): 22W

Lyles, G. A. (1984b) in: Tipton, K. F., Dostert, P., Strolin Benedetti, M. (eds) Monoamine Oxidase and Disease. Academic Press, London, pp 547–556

Lyles, G. A., Callingham, B. A. (1975) J. Pharm. Pharmacol. 27: 682–691

Lyles, G. A., Callingham, B. A. (1982) Biochem. Pharmacol. 31: 1417–1424

Lyles, G. A., Singh, I. (1984) 9th International Congress of Pharmacology, London, abstract 396P

Lyles, G. A., McAuslane, J. A. N., Fitzpatrick, C. M. S. (1984) Biochem. Pharmacol. 33: 2569–2574

Parkinson, D., Lyles, G. A., Browne, B. J., Callingham, B. A. (1980) J. Pharm. Pharmacol. 32: 844–850

Patil, P. N., Fudge, K., Jacobowitz, D. (1972) Eur. J. Pharmacol. 19: 79–87

Rucker, R. B., Goettlich-Riemann, W. (1972) Proc. Soc. Exp. Biol. Med. 139: 286–289

Rucker, R. B., O'Dell, B. L. (1971) Biochim. Biophys. Acta 235: 32–43

Ryder, T. A., Mackenzie, M. L., Pryse-Davies, J., Glover, V., Lewinsohn, R., Sandler, M. (1979) Histochemistry 62: 93–100

Shieh, J. J., Tamaye, T., Yasunobu, K. T. (1975) Biochim. Biophys. Acta 377: 229–238

Tang, S.-S., Trackman, P. C., Kagan, H. M. (1983) J. Biol. Chem. 258: 4331–4338

Trevithick, M. A., Olverman, H. J., Pearson, J. D., Gordon, J. L., Lyles, G. A., Callingham, B. A. (1981) Biochem. Pharmacol. 30: 2209–2216

Urdin, M. D. C., Fuentes, J. A. (1983) Ibid. 32: 2345–2347

Wibo, M., Duong, A. T., Godfraind, T. (1980) Eur. J. Biochem. 112: 87–94