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Properties of a Semicarbazide-Sensitive Amine Oxidase in Human Umbilical Artery

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Abstract—The metabolism of some aromatic amines by amine oxidase activities in human umbilical artery homogenates has been studied. The inhibitory effects of clorgyline showed that 5-hydroxytryptamine (5-HT) and tryptamine, 1 mM, were predominantly substrates for monoamine oxidase (MAO) type A, whereas MAO-A and B were both involved in the metabolism of β-phenylethylamine (PEA), 100 μM, and tyramine, 1 mM. About 20–30% of tyramine and PEA metabolism was resistant to 1 mM clorgyline, but sensitive to inhibition by semicarbazide, 1 mM, indicating the presence of a semicarbazide-sensitive amine oxidase (SSAO). Benzylamine, 1 mM, appeared to be metabolized exclusively by SSAO with a K_m (161 μM) at pH 7·8 similar to that found for SSAO in other human tissues. Tyramine and PEA were relatively poor substrates for SSAO, with very high apparent K_m values of 17·6 and 13·3 mM, respectively, when determined in the presence of clorgyline, 10^{-3} M, added to inhibit any metabolism of those amines by MAO activities. However, kinetic studies with benzylamine indicated that clorgyline, 10^{-3} M, also appears to inhibit SSAO competitively such that the true K_m values for tyramine and PEA may be about 60% of those apparent values given above. No evidence for the metabolism of 5-HT or tryptamine by SSAO was obtained. The aliphatic amine methylamine was recently shown to be a specific substrate for SSAO in umbilical artery homogenates. We have used benzylamine and methylamine as SSAO substrates in histochemical studies to localize SSAO in tissue sections. Both amines promoted tissue staining which occurred predominantly over the medial layers of the vessel wall, and this staining was prevented by the presence of semicarbazide, 1 mM, but not by pargyline, 1 mM, in the reaction medium. The results support the notion that smooth muscle cells are an important site of SSAO activity in human blood vessels, and reinforce the possibility that methylamine, an endogenously-occurring amine, may be a better candidate as a physiological substrate for SSAO in man, than several aromatic biogenic amines so far examined.

It is now known that more than one type of amine oxidase activity can deaminate various physiologically-active aromatic monoamines in animal tissues. In this respect, much attention has been given to the properties of monoamine oxidase (MAO), a flavoprotein enzyme found on the outer mitochondrial membrane in two distinct molecular forms called MAO-A and B with different substrate and inhibitor specificities, and the potential therapeutic uses of MAO-A or B selective inhibitors in the treatment of depression and Parkinson's Disease, for example, have been reviewed by Fowler & Ross (1984).

Less clear is the physiological importance of another amine oxidase found in addition to MAO in many tissues, and distinguished from the latter not only by a resistance to inhibition by relatively high concentrations (0·1–1 mM) of the MAO-inhibiting acetylenic compounds clorgyline, selegiline and pargyline, but also by its sensitivity to inhibition by semicarbazide, 1 mM. The inhibition of this semicarbazide-sensitive amine oxidase (SSAO) by various carbonyl reagents has led to suggestions that its cofactor could be pyridoxal phosphate, or possibly pyrroloquinoline quinone. Although the enzyme is present in various cell types including adipocytes (Barrand et al 1984), chondrocytes (Lyles & Bertie 1987), granulocytes and lymphocytes (Banchelli et al 1983), particularly high activity is associated with smooth muscle cells of vascular (Ryder et al 1979; Lyles & Singh 1985) and non-vascular origin (Lewinsohn 1981), where the enzyme appears to be a constituent of plasmalemmal and/or microsomal membranes (Wibo et al 1980; Barrand & Callingham 1982). However, the discovery of a functional role for SSAO in these tissues has so far proved elusive. Consequently, although SSAO is known to be sensitive in-vitro to inhibition by a number of drugs in current clinical use, including the hydrazine derivatives phenelzine, hydralazine and benserazide (reviewed by Lyles 1984), it is not yet clear if this property has any beneficial or adverse implications for the treatment of patients with such compounds.

Although possible clues to the physiological importance of SSAO may be obtained from a clearer perspective of its substrate specificity, and especially of its ability to metabolize endogenous amines, this approach has been complicated to some extent by the discovery first, that a non-physiological substrate, benzylamine, appears to have a lower K_m than those naturally-occurring amines so far examined, and second, that the properties of SSAO differ considerably between species. For example, in rat tissues such as the aorta, the K_m for benzylamine metabolism (around 5 μM) (Clarke et al 1982) is lower than those values (10–70 μM) reported for the dietary or trace endogenous amines β-phenylethylamine, tyramine and tryptamine (Guffroy et al 1985; Lyles & Taneja 1987). Under comparable assay conditions, the K_m for benzylamine in human aorta is much higher than in the rat (e.g. 143 μM, Hayes et al 1983; 295 μM, Suzuki & Matsumoto 1984), and furthermore, many endogenous aromatic monoamines, including those trace amines above, are deaminated at much lower rates than benzylamine. The neurotransmitter amines noradrenaline and 5-hydroxytryptamine, and the

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hormone adrenaline do not appear to be likely candidates as physiological substrates for SSAO since current evidence suggests that they are metabolized slightly, if at all, by the enzyme in either man or rat.

To extend our knowledge of the properties of SSAO in vasculature of human origin, we have studied the deamination (and its sensitivity to inhibition) of several aromatic monoamines in homogenates of human umbilical artery, with a view to determining the kinetic constants for metabolism by SSAO of those amines found to be substrates for the enzyme. In addition, we present histochemical evidence that, as in the rat aorta (Lyles & Singh 1985) and in other human blood vessels (Ryder et al 1979), the SSAO activity in the human umbilical artery is associated in major part with the smooth muscle layers of the tunica media. Some of these results have been communicated earlier (Lyles & Wollage 1987).

Materials and Methods

Materials

Umbilical cords were provided by the Maternity Unit, Ninewells Hospital, usually from deliveries involving Caesarean sections.

Radiochemical amines purchased from Amersham International plc, UK were: 5-hydroxy [G-^{3}H]tryptamine creatinine sulphate, [7-^{14}C]benzylamine hydrochloride, [G-^{3}H]tryptamine hydrochloride and 2-phenyl [1-^{14}C]ethylamine hydrochloride. [Ring-^{3}H]tyramine hydrochloride was obtained from NEN Research Products, Du Pont (UK) Ltd, Stevenage, UK.

The following reagents were purchased from Sigma (Poole, UK): horseradish peroxidase (type II), 3-amino-9-ethylcarbazole, bovine serum albumin and the hydrochlorides of benzylamine, methylamine, semicarbazide and pargyline. Clorgyline hydrochloride was a generous gift from May and Baker Ltd (Dagenham, UK).

Methods

Tissue homogenates. Arteries were dissected from fresh umbilical cords, washed extensively with saline (0·9% NaCl, w/v) to remove contaminating blood as a potential source of plasma amine oxidase, and then stored at −20 °C for use within a few days. Thawed tissues were homogenized in 1 mM potassium phosphate buffer (pH 7·8) at various tissue (g): buffer (mL) ratios (see below) depending upon the substrate to be used in subsequent assays. These ratios were chosen on the basis of preliminary experiments to determine assay conditions under which metabolite formation was linear during appropriate assay incubation times. Homogenates were centrifuged at 600 *g* for 10 min before the resulting supernatants were used in the assays below.

Radiochemical assays. The radiochemical assays, which usually involved 25 μL homogenate, 25 μL water, or aqueous inhibitor solution, and 50 μL radioactive substrate in 0·2 M potassium phosphate buffer pH 7·8, were based on the method of Callingham & Laverty (1973) as described by Lyles & Callingham (1982) and Precious et al (1988). Specifically, the current studies with umbilical artery involved the use of 1:40 tissue homogenates and assay incubation times of 15 or 30 min, respectively when [^{14}C]benzylamine (sp. act. 0·5 μCi μmol^{-1}) or [^{3}H]5-hydroxytryptamine (2 μCi μmol^{-1}) were used as substrates. 1:10 homogenates were used with [^{3}H]tryptamine (2μCi μmol^{-1}), [^{14}C] β-phenylethylamine (2 μCi μmol^{-1}) and [^{3}H]tyramine (1 μCi μmol^{-1}) at respective incubation times of 30, 45 and 60 min.

The inhibitory effects of semicarbazide or clorgyline were studied by preincubating homogenates for 20 min at 37 °C with aqueous solutions of clorgyline or solutions of semicarbazide prepared in 0·2 M potassium phosphate buffer, pH 7·8, followed by the addition of the appropriate radioactive amine to assay for remaining enzyme activity. Comparisons were made with control samples preincubated with inhibitor vehicle alone.

The possibility of semicarbazide affecting the organic solvent extractability of metabolites in the assay was tested as described by Lyles & Shaffer (1979) by comparing activities in assays where semicarbazide was present throughout, with those in which semicarbazide was absent during the assay incubation but was instead added at the end of the incubation period, and immediately before the solvent extraction of accumulated metabolites. These assays were carried out with the appropriate constituents in the normal 100 μL assay volume described above, but involved the addition of either 25 μL semicarbazide solution or water at the end. Control samples in these experiments were subjected to the same protocol, except that semicarbazide was omitted from all solutions.

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

Histochemistry. Cryostat-cut transverse sections (20 μm) of umbilical artery were stained for amine oxidase activity by a coupled peroxidatic oxidation technique (Ryder et al 1979; Lyles & Singh 1985) in which either benzylamine or methylamine were used as substrates at final concentrations of 2 or 5 mM, respectively, in the reaction medium, which was incubated with sections for 2–3 h at 37 °C. Effects of inhibitors were assessed by preincubating sections for 15 min at 37 °C with inhibitor solutions prepared in 0·05 M potassium phosphate buffer pH 7·6, followed by subsequent incubation in reaction medium in which the corresponding inhibitor had also been included.

Results

Inhibitor studies

The effects of increasing clorgyline concentrations on the metabolism of 5-hydroxytryptamine (5-HT) and tryptamine, 1 mM, in umbilical artery homogenates is shown in Fig. 1. The complete inhibition of 5-HT metabolism by relatively low clorgyline concentrations (e.g. 10^{-8} M) indicates that 5-HT is a substrate for MAO-A alone in the tissue. Similarly, tryptamine deamination was largely attributable to MAO-A, although the additional inhibitory effects with clorgyline, 10^{-7}–10^{-4} M, indicate the possibility of a small contribution by MAO-B also. These inhibitor curves gave no suggestion of any metabolism of these amines by an enzyme activity (e.g. SSAO) resistant to clorgyline, 10^{-3} M.

Fig. 1 also illustrates the effects of clorgyline and semicar-

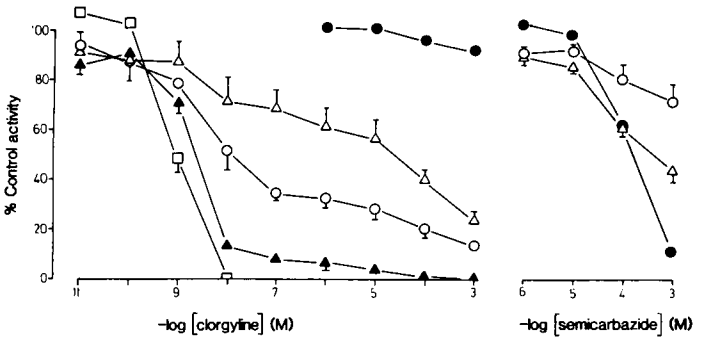

FIG. 1. Inhibition of the metabolism of 1 mM 5-hydroxytryptamine (5-HT, □), 1 mM tryptamine (TRYPT, ▲), 100 μM β-phenylethylamine (PEA, △), 1 mM tyramine (TYR, ○) and 1 mM benzylamine (BZ, ●) by clorgyline or semicarbazide. Activities were expressed as percentages of corresponding control samples without inhibitor. Each point is the mean (±s.e. of the ratio where exceeding symbol size) of 4–11 homogenates, each assayed in triplicate. Mean specific activities of controls were 40·6 (5-HT), 34·9 (TRYPT), 4·7 (PEA), 25·4 (TYR) and 329·9 (BZ) nmol (mg prot.)$^{-1}$h^{-1}.

bazide as inhibitors of β-phenylethylamine (PEA) 100 μM, tyramine 1 mM, and benzylamine, 1 mM, metabolism. Use of clorgyline resulted in biphasic inhibition curves with PEA and tyramine as substrates, indicating the involvement of both MAO-A and B in the deamination of these amines at the substrate concentrations tested. In addition, clorgyline, 10^{-3} M, failed to inhibit completely the deamination of these amines, with approximately 14% of tyramine and 24% of PEA metabolism remaining at this inhibitor concentration, therefore suggesting some possible contribution of SSAO to their metabolism. Further evidence for this was provided by the ability of semicarbazide (especially at 10^{-3} M) to inhibit a significant proportion of the total tyramine and PEA degradation. Clorgyline had only a small inhibitory effect upon benzylamine metabolism (3 and 7% inhibition, respectively with 10^{-4} and 10^{-3} M clorgyline) whereas a considerable proportion (89%) of total benzylamine deamination was inhibited by semicarbazide, 10^{-3} M. These results suggest therefore that benzylamine metabolism in umbilical artery is brought about almost completely if not exclusively by SSAO, with possibly a very small contribution by MAO-B.

Several experiments were carried out subsequently to investigate the possibility that the apparent inhibition of amine metabolism by semicarbazide could conceivably result from the carbonyl reagent interfering with the organic solvent extraction of amine metabolites in the assay, perhaps by forming Schiff bases with the aldehyde metabolite. To test this, normal assay incubations which were completed without semicarbazide being present, had the drug added to the reaction mixtures subsequently and immediately before the solvent extraction step. Table 1 shows that under those circumstances, little if any inhibition of amine metabolism was apparent when compared with appropriate control samples to which inhibitor vehicle was added at the end. On the other hand, in assays run concurrently in which semicarbazide was present during the normal incubation period, similar inhibitory effects of semicarbazide to those seen in Fig. 1 were again obtained. These data are therefore consistent with semicarbazide producing direct inhibition of an enzyme activity which can metabolize tyramine, PEA and benzylamine.

Kinetic constants

Kinetic constants for benzylamine metabolism by umbilical artery homogenates were estimated by linear regression from double reciprocal plots obtained from the use of 50 μM to 1 mM benzylamine. Data in Fig. 1 illustrate that an increasing proportion of the metabolism of benzylamine (and also of tyramine and PEA) is inhibited by increasing clorgyline concentrations up to 10^{-3} M. In many earlier studies, that clorgyline concentration has been useful for completely inactivating MAO activities in animal tissue homogenates, while having little if any apparent inhibitory effect upon SSAO (e.g. Clarke et al 1982; Lyles 1984). Consequently, in an attempt to exclude any possible influence of MAO activities upon the determination of kinetic constants for benzylamine metabolism by SSAO, some umbilical artery homogenates were pretreated with 10^{-3} M clorgyline (for 20 min at 37 °C) before addition of substrate and comparisons were made with the corresponding homogenate samples preincubated with inhibitor vehicle (distilled water) instead.

Fig. 2 shows kinetic data from one such homogenate, representative of two others studied identically. In the example shown, the K_m for benzylamine (205 μM) obtained in the presence of clorgyline was greater (by 47%) than the value (141 μM) obtained without clorgyline. A similar result

Table 1. Effects of semicarbazide on assays of amine metabolism in human umbilical artery.

Substrate (concentration)	Semicarbazide concentration (M)	Activity (% of control) (+)	(−)
5-HT (1 mM)	10^{-4}	100±6	92±7
	10^{-3}	94±1	93±6
TYR (1 mM)	10^{-4}	95±1	102±3
	10^{-3}	84±4	98±3
PEA (100 μM)	10^{-4}	82±2	103±1
	10^{-3}	68±4	94±4
BZ (1 mM)	10^{-4}	27±1	96±2
	10^{-3}	5±2	100±2

The column marked (+) denotes activities determined in homogenates which were preincubated *with* semicarbazide before the substrate addition to the assay, whereas activities denoted (−) were obtained from homogenates assayed *without* semicarbazide present in the assay incubation but instead the drug was added just prior to solvent extraction of amine metabolites. Activities are expressed as percentages of appropriate controls subjected to identical experimental procedures, but using solutions lacking semicarbazide throughout. Each value represents the mean±s.e. of 3 different homogenates, each assayed in triplicate.

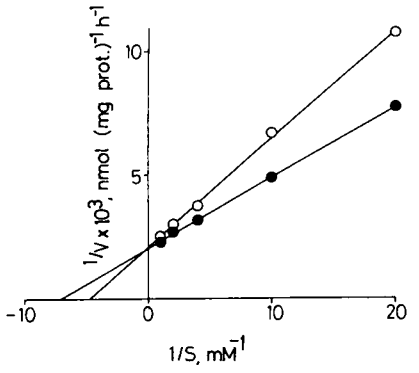

FIG. 2. Lineweaver-Burk plots for metabolism of benzylamine determined in the absence (●) and presence (○) of clorgyline, 10^{-3} M. Data are from a single experiment representative of 2 others with different homogenates. Each point is the mean of triplicate determinations. Kinetic constants, estimated by linear regression are given in the Text.

was observed with two other homogenates in which K_m values without clorgyline (171 and 180 μM) were increased respectively, by 81% and 75% (to 309 and 315 μM) in clorgyline-treated samples. In contrast, V_{max} values were relatively unchanged. For example, in Fig. 2, clorgyline treatment resulted in a small (3%) decrease in V_{max} (from 491 to 475 nmol (mg prot.)$^{-1}$h^{-1}), whereas with two other homogenates the corresponding V_{max} values without clorgyline (370 and 813) were increased slightly by 10 and 9%, respectively (to 408 and 884 nmol (mg prot.)$^{-1}$h^{-1}). These results showing increases in apparent K_m after clorgyline with little, if any, change in V_{max}, are indicative of an apparent competitive action of clorgyline upon the total benzylamine metabolizing activity. This does not support the notion that clorgyline is selectively inactivating a small MAO-B component contributing to the total activity, but rather suggests that a competitive action of clorgyline upon SSAO is responsible predominantly, if not exclusively, for the results obtained.

Therefore, in the absence of any firm evidence for a significant MAO-B component here, additional determinations of kinetic constants were made without clorgyline in several other tissues. Mean values (±s.e., n=9) for the complete series of experiments, were $K_m = 161 \pm 21$ μM, $V_{max} = 365 \pm 67$ nmol (mg prot.)$^{-1}$h^{-1}.

In attempts to determine kinetic constants for the metabolism of tyramine and PEA by SSAO, there are clearly very large contributions by MAO activities to the total metabolism of these amines (Fig. 1). Consequently, although for the reasons above clorgyline, 10^{-3} M, may not be totally selective at inhibiting MAO, nevertheless we felt that an approach to estimating these constants required at least the inactivation of MAO-A and B by preincubating homogenates with that concentration of clorgyline. In preliminary studies, we also found it necessary to reduce the specific radioactivities of the substrates to 0·1 μCi μmol^{-1} (PEA) or 0·2 μCi μmol^{-1} (tyramine) to use these amines at the relatively high concentrations (2–40 mM) required for determination of their apparent K_m values. Fig. 3 shows representative individual kinetic plots for each substrate. Mean values (±s.e. for 4 arteries) were K_m (mM): 17·6±1·9 (tyramine) and 13·3±1·7 (PEA); V_{max} (nmol (mg prot.)$^{-1}$h^{-1}): 90±19 (tyramine) and 303±51 (PEA).

In Fig. 1, 5-HT is a substrate for MAO-A alone. Kinetic studies (not shown) with 5-HT, 50 μM to 1 mM, yielded mean (±s.e. for 6 arteries) values of $K_m = 151 \pm 23$ μM and $V_{max} = 36 \pm 10$ nmol (mg prot.)$^{-1}$h^{-1}, for that MAO-A activity.

Histochemistry

The histochemical method used relies upon hydrogen peroxide, a product of amine oxidase reactions, oxidizing 3-amino-9-ethylcarbazole in the presence of exogenous horseradish peroxidase to an insoluble red-brown deposit which stains the tissue sections. Results presented earlier in this paper with benzylamine, and elsewhere with methylamine (Precious et al 1988), have indicated that these amines are relatively specific substrates for SSAO in human umbilical artery and hence we have used them in the histochemical

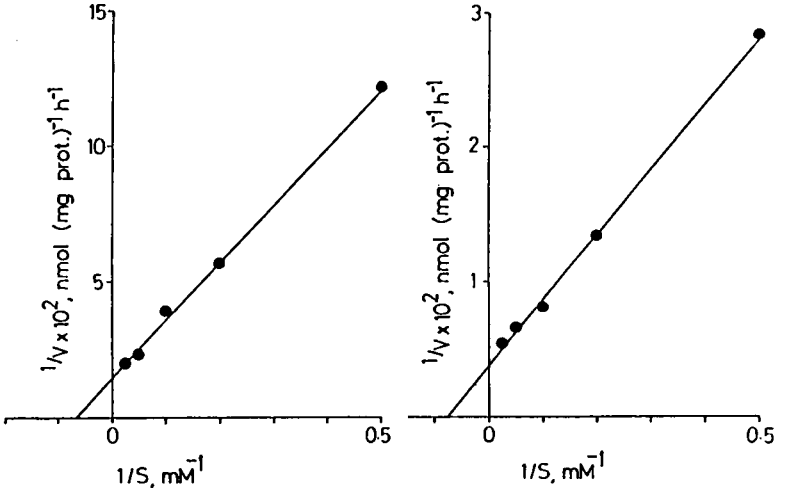

FIG. 3. Lineweaver-Burk plots for metabolism of tyramine (TYR, at left) and β-phenylethylamine (PEA, at right) by SSAO, after pre-incubation of homogenates with clorgyline, 10^{-3} M, to inhibit MAO activities. Data are from single experiments representative of 3 others. Each point is the mean of triplicate determinations on individual homogenates. Kinetic constants estimated by linear regression from the data shown were K_m (mM): 14·5 (TYR), 13·0 (PEA); V_{max} (nmol (mg prot.)$^{-1}$h^{-1}): 67·1 (TYR), 264·2 (PEA).

reaction medium at final concentrations approximately ten-times higher than their estimated K_m values.

The use of either amine was found to result in staining, predominantly over the medial layers of the blood vessel. This staining was usually stronger over the outer medial layers and consisted of a diffuse reddish-brown background within the tissue section, but containing a more intense patchy dotted appearance over the background stain. The presence of pargyline (10^{-3} M) in the histochemical reaction medium to inhibit MAO activities produced no discernible reduction of staining intensity with either amine as substrate. Examples of these results are shown in the photomicrographs Figs 4 and 5.

No staining occurred when amines were omitted from the reaction medium. In addition, staining was prevented by including semicarbazide, 10^{-3} M, in the amine-containing medium. In fact, unstained tissue sections were highly transparent and many structural features of the whole vessel wall were difficult to observe clearly through the microscope. Fig. 6 shows an example of a semicarbazide-treated section, where methylamine was the substrate. Although the internal elastic membrane shows up as a dark feature, neither this nor any other parts of the vessel wall were stained the characteristic red-brown colour found in corresponding control sections lacking semicarbazide.

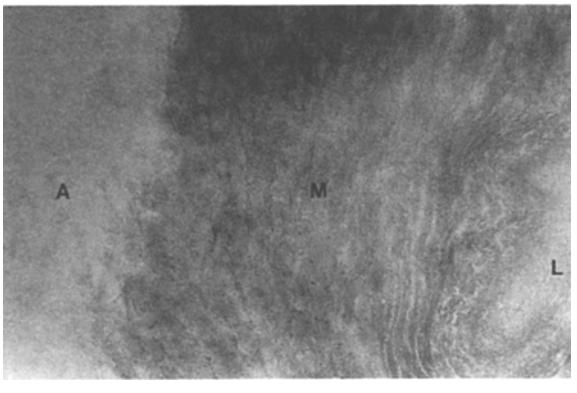

FIG. 4. Human umbilical artery (transverse section). Benzylamine (2 mM) as amine oxidase substrate. L, lumen of vessel; M, media; A, adventitia. Note strong staining throughout smooth muscle containing layers of media (Mag: 70 ×).

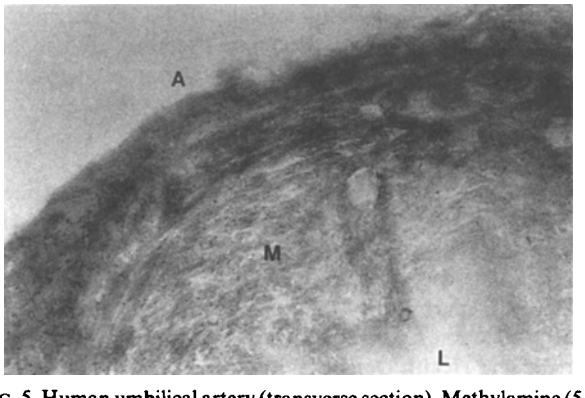

FIG. 5. Human umbilical artery (transverse section). Methylamine (5 mM) as amine oxidase substrate in presence of pargyline, 10^{-3} M. L, vessel lumen; M, media; A, adventitial tissue (almost transparent in this section). Note again, strong staining of media, especially in outer layers. (Mag: 70 ×).

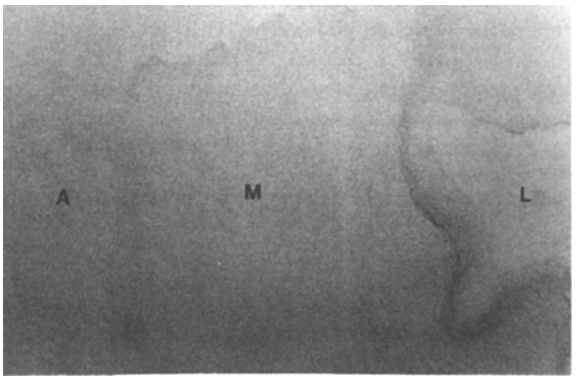

FIG. 6. Human umbilical artery (transverse section). Methylamine (5 mM) as amine oxidase substrate in presence of semicarbazide, 10^{-3} M. L, lumen; M, media; A, adventitial tissue. (Mag. 70 ×).

Discussion

Our results have provided evidence for the existence of MAO-A, MAO-B and SSAO in the human umbilical artery. These conclusions are consistent with the work of Lewinsohn & Sandler (1982) on umbilical vessel homogenates in which 5-HT metabolism was assumed to be due to MAO-A activity, a result we confirmed by its high sensitivity to inhibition by clorgyline. Those authors also found that selegiline (deprenyl) 4×10^{-7} M, a concentration selective for inhibition of MAO-B, produced little if any inhibition of benzylamine metabolism, and they concluded that virtually all this selegiline-resistant enzyme activity was attributable to SSAO (called benzylamine oxidase in that report). This is again supported by our detailed inhibitor data with clorgyline and semicarbazide. The latter in turn indicated that MAO-A, MAO-B and SSAO each contribute to the total metabolism of tyramine, 1 mM, and PEA, 100 μM, whereas tryptamine, 1 mM, was predominantly a substrate for MAO-A.

Our interpretation that MAO-B is present in umbilical artery is based upon the data in Fig. 1 showing that tyramine and PEA metabolism are increasingly inhibited by clorgyline concentrations from 10^{-6} to 10^{-3} M. On the other hand it was surprising that no clear evidence for any significant contribution of MAO-B towards benzylamine metabolism was obtained, especially since it is normally an excellent substrate for MAO-B in animal tissues. In fact, we found that the small inhibitory effect of clorgyline, 10^{-3} M, on the oxidation of benzylamine, 1 mM, was more readily explained by a competitive inhibition of SSAO by the drug (see Fig. 2) rather than by the selective inactivation of a MAO-B component. One possible explanation for these findings could be the following. If SSAO were present in much greater amounts than the MAO activities in umbilical artery, it might be extremely difficult in these inhibitor plots to detect clearly a small contribution of MAO-B towards total benzylamine metabolism, when compared with the very large activity provided by SSAO, an enzyme which on current evidence deaminates benzylamine readily. In contrast, tyra-

mine and PEA are good MAO substrates (with PEA deaminated preferentially by MAO-B) in animal tissues (Strolin Benedetti & Dostert 1985) and this may favour a much greater proportional contribution of MAO activities to their metabolism at the substrate concentrations used in Fig. 1, especially since our kinetic data (see below) indicate that tyramine and PEA are, relatively speaking, very poor (i.e. high K_m) substrates for SSAO in umbilical artery under these conditions.

Our overall intention in this study was to provide further information about the biochemical properties of vascular SSAO in man, and in particular to determine its activity, if any, against certain aromatic amines which might possibly be endogenous physiological substrates. Earlier reports have tended to assess the substrate specificity of SSAO in man by comparing the deamination of various biogenic amines with that of the non-physiological amine benzylamine at single substrate concentrations (Hayes et al 1983; Suzuki & Matsumoto 1984, 1987), whereas we hoped to determine kinetic constants for appropriate substrates.

The K_m (161 μM) found here for benzylamine (determined in the absence of clorgyline in the radiochemical assay) is similar to other reported values in human tissues, studied under comparable pH conditions (Lewinsohn et al 1978; Kinemuchi et al 1982; Hayes et al 1983; Lewinsohn 1984; Suzuki & Matsumoto 1984, 1987) including our earlier studies using a spectrophotometric assay with benzylamine in umbilical artery (Precious et al 1988). We also found, in agreement with some of these earlier reports, that tyramine and PEA can also be deaminated by SSAO, but in contrast, we were unable to demonstrate tryptamine metabolism by this enzyme as some of these workers have done. In this respect, however, Sullivan et al (1986) also failed to detect tryptamine oxidation by SSAO in several human tissues. Despite some of these discrepancies, our data (especially with benzylamine) suggests that SSAO in umbilical artery, a readily available source of fresh human vascular tissue, is at least broadly similar in its substrate and inhibitor characteristics to the enzyme studied in various post mortem human sources.

Estimations of apparent kinetic constants for SSAO metabolism of tyramine and PEA, carried out in the presence of clorgyline, 10^{-3} M, to remove any influence of MAO activities upon these determinations, yielded relatively high K_m values of 17·6 and 13·3 mM, respectively. However, as noted above, this concentration of clorgyline may inhibit SSAO competitively such that these apparent K_m values may overestimate the true values to some extent. If the apparent K_i for competitive inhibition of SSAO by clorgyline were to be the same whichever substrate is used to assay the enzyme, then the ratio of K_m values obtained in the presence and absence of clorgyline would be constant. The mean ratio for benzylamine (from 3 experiments) was 1·68 (see Results). Adjustments of the apparent constants for tyramine and PEA by this ratio would imply true K_m values of around 10·5 and 7·9 mM, respectively. Ideally, the most precise kinetic constants for these amines would be obtained in the presence of a clorgyline concentration with *absolute* selectivity to inhibit MAO activities completely, with no action upon SSAO, whereas such a concentration has proved difficult to identify in the current study. Nevertheless, our data point to the qualitative conclusion that the metabolism of tyramine and PEA by SSAO is carried out with K_m values considerably higher than those usually found for MAO activities in animal tissues (Strolin Benedetti & Dostert 1985) and this casts doubt upon the likelihood of SSAO playing any major role in metabolizing these particular amines at physiological concentrations in-vivo in man. On the other hand, the corresponding K_m values for metabolism of tyramine, PEA and tryptamine by SSAO in rat tissues are much lower than those in man, and are in the same range as those of MAO activities against these amines (see Introduction). Thus, species differences in the properties of SSAO may be important when the physiological relevance of SSAO is being investigated.

One consistent finding from the current histochemical work, which agrees with similar previous studies with human (Ryder et al 1979) and rat (Lyles & Singh 1985; Lyles & Archer 1986) vasculature, is the predominant association of SSAO with the smooth muscle layers of blood vessels. Histochemical staining occurred after including either benzylamine or methylamine in the reaction medium, the latter being an endogenously-occurring aliphatic amine in man and other species, having properties as a highly specific and active substrate for SSAO in human umbilical artery and rat aorta (Precious et al 1988). The prevention of staining here with semicarbazide, 10^{-3} M, and the lack of effect of pargyline, 10^{-3} M, are indeed consistent with this staining arising from benzylamine or methylamine deamination by SSAO in the tissue.

We recently reported a K_m of 780 μM for metabolism of methylamine by SSAO in umbilical artery (Precious et al 1988), well below the apparent K_m values found in the present work for the biogenic aromatic amines examined. In addition, the V_{max} for methylamine in the earlier study was approximately 70% greater than that of benzylamine, indicating a more rapid turnover of methylamine by human SSAO. It was therefore of interest that, albeit from a purely qualitative inspection, the intensity of histochemical staining produced here by methylamine 5 mM, appeared to be as great, if not greater than that produced by benzylamine, 2 mM, which is consistent with previous biochemical evidence for a relatively strong deaminating activity of SSAO towards methylamine.

As a whole, these results strengthen the possibility that methylamine may be a more plausible candidate as an endogenous physiological substrate for SSAO (in man, at least) than various aromatic amines examined to date, and this suggestion is under further investigation in our laboratory.

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References

Banchelli, G., Fantozzi, R., Ignesti, G., Pirisino, R., Raimondi, L.,

Buffoni, F. (1983) The amine oxidase of human blood lymphocytes and granulocytes. Biochem. Int. 7: 89–94

Barrand, M. A., Callingham, B. A. (1982) Monoamine oxidase activities in brown adipose tissue of the rat: some properties and subcellular distribution. Biochem. Pharmacol. 31: 2177–2184

Barrand, M. A., Callingham, B. A., Fox, S. A. (1984) Amine oxidase activities in brown adipose tissue of the rat: identification of semicarbazide-sensitive (clorgyline-resistant) activity at the fat cell membrane. J. Pharm. Pharmacol. 36: 652–658

Callingham, B. A., Laverty, R. (1973) Studies on the nature of the increased monoamine oxidase activity in the rat heart after adrenalectomy. Ibid. 25: 940–947

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

Fowler, C. J., Ross, S. B. (1984) Selective inhibitors of monoamine oxidase A and B: biochemical, pharmacological, and clinical properties. Medicinal Res. Rev. 4: 323–358

Guffroy, C., Boucher, T., Strolin Benedetti, M. (1985) Further investigation of the metabolism of two trace amines, β-phenylethylamine and p-tyramine by rat aorta semicarbazide-sensitive amine oxidase. In: Boulton, A. A., Maitre, L., Bieck, P. R., Riederer, P. (eds). Neuropsychopharmacology of the Trace Amines. Humana Press, Clifton, N.J. pp 39–50

Hayes, B. E., Ostrow, P. T., Clarke, D. E. (1983) Benzylamine oxidase in normal and atherosclerotic human aortae. Exp. Mol. Pathol. 38: 243–254

Kinemuchi, H., Morikawa, F., Tajima, H., Kamijo, K. (1982) Enzymic characteristics of a clorgyline- and deprenyl-resistant amine oxidase in human placenta. In: Kamijo, K., Usdin, E., Nagatsu, T. (eds) Monoamine Oxidase. Basic and Clinical Frontiers. International Congress Series 564, Excerpta Medica, Amsterdam, pp 112–124

Lewinsohn, R. (1981) Amine oxidase in human blood vessels and non-vascular smooth muscle. J. Pharm. Pharmacol. 33: 569–575

Lewinsohn, R. (1984) Mammalian monoamine-oxidizing enzymes, with special reference to benzylamine oxidase in human tissues. Brazilian J. Med. Biol. Res. 17: 223–256

Lewinsohn, R., Bohm, 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

Lewinsohn, R., Sandler, M. (1982) Monoamine-oxidizing enzymes in human pregnancy. Clin. Chim. Acta 120: 301–312

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. (1984) The interaction of semicarbazide-sensitive amine oxidase with MAO inhibitors. In: Tipton, K.F., Dostert, P., Strolin Benedetti, M. (eds) Monoamine Oxidase and Disease. Academic Press, London, pp 547–556

Lyles, G. A., Archer, D. R. (1986) Monoamine oxidase activities in dissociated cell fractions from rat skeletal muscle. J. Pharm. Pharmacol. 38: 288–293

Lyles, G. A., Bertie, K. H. (1987) Properties of a semicarbazide-sensitive amine oxidase in rat articular cartilage. Pharmacol. Toxicol. 60 (Suppl. 1): 33

Lyles, G. A., Callingham, B. A. (1982) In vitro and in vivo inhibition by benserazide of clorgyline-resistant amine oxidases in rat cardiovascular tissues. Biochem. Pharmacol. 31: 1417–1424

Lyles, G. A., Shaffer, C. J. (1979) Substrate specificity and inhibitor sensitivity of monoamine oxidase in rat kidney mitochondria. Ibid. 28: 1099–1106

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

Lyles, G. A., Taneja, D. T. (1987) Effects of amine oxidase inhibitors upon tryptamine metabolism and tryptamine-induced contractions of rat aorta. Br. J. Pharmacol. 90: 16P

Lyles, G. A., Wollage, E. (1987) Some properties of amine oxidases in human umbilical artery. Ibid. 90: 175P

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. 37: 707–713

Ryder, T. A., MacKenzie, M. L., Pryse-Davies, J., Glover, V., Lewinsohn, R., Sandler, M. (1979) A coupled peroxidatic oxidation technique for the histochemical localization of monoamine oxidase A and B and benzylamine oxidase. Histochemistry 62: 93–100

Strolin Benedetti, M., Dostert, P. (1985) Stereochemical aspects of MAO interactions: reversible and selective inhibitors of monoamine oxidase. TIPS 6: 246–251

Sullivan, J. P., McDonnell, L., Hardiman, O. M., Farrell, M. A., Phillips, J P., Tipton, K. F. (1986) The oxidation of tryptamine by the two forms of monoamine oxidase in human tissues. Biochem. Pharmacol. 35: 3255–3260

Suzuki, O., Matsumoto, T. (1984) Some properties of benzylamine oxidase in human aorta. Biogenic Amines 1: 249–257

Suzuki, O., Matsumoto, T. (1987) Properties of benzylamine oxidase in human small intestine. Ibid. 4: 45–53

Wibo, M., Duong, A. T., Godfraind, T. (1980) Subcellular location of semicarbazide-sensitive amine oxidase in rat aorta. Eur. J. Biochem. 112: 87–94