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DEAMINATION OF METHYLAMINE BY SEMICARBAZIDE-SENSITIVE AMINE OXIDASE IN HUMAN UMBILICAL ARTERY AND RAT AORTA

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Abstract—The deamination of methylamine (MA) by amine oxidase enzymes has been studied and compared with that of benzylamine (BZ) in homogenates of rat aorta and human umbilical artery by means of a radiochemical assay to estimate the radiolabelled deaminated metabolites produced, and also a spectrophotometric assay to measure H_2O_2 formation during the metabolism of these substrates. The effects of various inhibitors used in these assays suggest that a semicarbazide-sensitive amine oxidase (SSAO) is predominantly if not wholly responsible for the deamination of both MA and BZ in these tissues. MA was found to have a relatively higher apparent K_m (102 μM in aorta; 779 μM in umbilical artery) than BZ (6.8 μM in aorta; 207 μM in umbilical artery) for metabolism by SSAO in these tissues. However, these large differences between species in the apparent K_m values for each amine indicate that the biochemical properties of SSAO in human and rat vasculature are not identical. SSAO in human umbilical artery was particularly active towards MA, with a V_{max} which was approximately 70% greater than that for BZ as substrate, whereas in rat aorta the V_{max} for MA was around 60% of that for BZ. MA is known to occur endogenously in man and other species, and the possibility that it may be a physiological substrate *in vivo* for SSAO is discussed.

Blood vessels of various species contain a semicarbazide-sensitive amine oxidase (SSAO) whose physiological importance remains to be determined. In the vascular wall, smooth muscle cells are a particularly rich source of this enzyme [1, 2], which may be found on the plasma membrane [3]. SSAO is inhibited by semicarbazide and other reagents which react with carbonyl groups, suggesting that pyridoxal phosphate or pyrroloquinoline quinone could be the enzyme cofactor. This inhibitor specificity also helps to distinguish SSAO from the mitochondrial flavoprotein enzyme, monoamine oxidase (MAO), which can exist in two forms called MAO-A and B in many animal tissues. MAO activities are insensitive to inhibition by semicarbazide at concentrations (around 1 mM) which are generally sufficient to inhibit SSAO completely. Conversely, SSAO is largely resistant to inhibition by acetylenic inhibitors of MAO such as clorgyline, deprenyl and pargyline, when used at concentrations (also around 1 mM) which totally abolish MAO activities (reviewed in Ref. 4).

In rat and human tissues, SSAO has a characteristically high activity and low K_m for metabolism of the synthetic amine benzylamine as substrate compared with various physiological amines, and consequently the name "benzylamine oxidase" has also been attributed to this enzyme by some investigators (e.g. [5]). In this respect, SSAO is similar to the soluble plasma amine oxidases found in a variety of animal species [6]. Major species differences in the properties of SSAO have been demonstrated, indicating that different forms of the enzyme may exist. For example, the K_m for benzylamine metabolism by SSAO in the rat is around 5 μM (see Ref. 4), whereas much higher K_m values (between 100 and 300 μM) have been found in human blood vessels [7–9]. In addition, although several biogenic amines (β-phenylethylamine, tyramine and tryptamine) are metabolized by SSAO in rat aorta, with K_m values between 10 and 70 μM [10, 11], these amines appear to be exceedingly poor substrates for the human aortic enzyme [7, 8]. For example, K_m values of approximately 3 and 15 mM for tyramine and β-phenylethylamine, respectively were obtained recently in human umbilical artery [9].

The possibility that SSAO may metabolize aliphatic amines, such as methylamine (MA), has received little previous attention. The latter amine can be produced and absorbed as a result of gut bacterial degradation of dietary creatinine, lecithin and choline, and indeed significant increases in the normal urinary excretion of MA have been measured after feeding these precursors to man and rats [12, 13]. MA may also arise from endogenous metabolic degradation of sarcosine and creatinine [14], and it is known to be a product of the intracellular deamination by MAO of the secondary amine adrenaline [15].

It is generally accepted that MA is not a substrate for MAO [16, 17]. However, urinary excretion of endogenous MA in rats was reported to increase substantially after the administration of phenipazine, a hydrazine-based MAO inhibitor [14]. In addition, Dar *et al.* [18] found that the related MAO inhibitor iproniazid markedly enhanced the urinary

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recovery of (^{14}C)-MA administered to rats. These findings may be reconciled on the basis that many hydrazine-based MAO inhibitors are also effective inhibitors of SSAO [4], suggesting that the latter could in fact be the enzyme responsible for MA metabolism in mammalian tissues.

In the current study, we have used both radiochemical and spectrophotometric amine oxidase assays to provide evidence for the deamination of MA in homogenates of rat aorta and human umbilical artery. By employing selective inhibitors, our results indicate that MA is metabolized predominantly, if not exclusively, by SSAO in these blood vessels. However, the properties of the rat and human enzymes are not identical in this respect, providing further indications of species differences in SSAO.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (285–570 g) were obtained from our Departmental breeding colony, Animal Services Unit, University of Dundee. Umbilical cords were provided by the Maternity Unit, Ninewells Hospital, usually from deliveries carried out by Caesarean section.

The radiochemicals (^{14}C)-methylamine hydrochloride (sp. act. 59 mCi/mmol), (7-^{14}C)-benzylamine hydrochloride (51 mCi/mmol) and 5-hydroxy-(G-^{3}H) tryptamine creatinine sulphate (14 Ci/mmol) were purchased from Amersham International plc, Little Chalfont, U.K.

Various amine oxidase inhibitors and their commercial sources were: Propargylamine hydrochloride (Aldrich Chemical Co., Gillingham, U.K.); pargyline hydrochloride, isoniazid, iproniazid phosphate, hydralazine hydrochloride and semicarbazide hydrochloride (Sigma, Poole, U.K.). Clorgyline hydrochloride was generously donated by May & Baker Ltd. (Dagenham, U.K.).

The following reagents required for the spectrophotometric assays were obtained from Sigma: 2,2′-azino-bis(3-ethylbenzthiazoline sulphonic acid) (ABTS, diammonium salt), horseradish peroxidase (type II), sodium azide, and the hydrochlorides of methylamine (MA) and benzylamine (BZ).

Methods

Preparation of tissue homogenates. Rats were killed by cervical dislocation and aortae (abdominal and thoracic portions) and livers (in some experiments) were removed for immediate use in assays. Human umbilical arteries were dissected from umbilical cords as they were made available, and stored at −20° for use within a few days.

Tissues were always washed extensively with saline (0.9% NaCl, w/v) to eliminate blood as a potential source of contaminating plasma amine oxidase activity, before homogenization of tissues in 1 mM potassium phosphate buffer (pH 7.8) in a hand-held ground glass homogenizer. In order to ensure the linear production with time of metabolite formation from MA and BZ as substrates in the different types of assay used below, it was necessary to use several different tissue (g):buffer (ml) homogenization ratios. These were chosen on the basis of preliminary studies and are given in the following sections. All homogenates were centrifuged at 600 *g* for 10 min and the resulting supernates used as the enzyme source in the assays below.

Radiochemical assays. These involved the method of Callingham and Laverty [19] as described fully in Ref. 20.

Assays contained 25 μl homogenate, 25 μl water (or aqueous solution of inhibitor, when appropriate) and 50 μl radioactive substrate (in 0.2 M potassium phosphate buffer, pH 7.8). During inhibitor studies, homogenate and inhibitor aliquots were preincubated for 20 min at 37° prior to substrate addition. Mixtures containing substrate were then incubated at 37° for predetermined times which ensured linear metabolite formation. Assays were stopped with 10 μl 3 N HCl, and radiolabelled deaminated metabolites were extracted into 0.6 ml ethyl acetate/toluene (1:1, v/v) before liquid scintillation counting of 0.4 ml of this extract (see Ref. 20).

In some experiments, 1:240 (g:ml) homogenates of rat aorta were prepared, for subsequent incubation (for 5 min) with 1 μM (^{14}C)-BZ (sp. act. 10 μCi/μmole) as a specific substrate for SSAO in this tissue.

Deamination of various concentrations of (^{14}C)-MA (sp. act. 1 μCi/μmole) was determined with 1:15 aorta homogenates and 1:40 umbilical artery homogenates with incubation times of 1 hr and 15 min respectively. On the basis of our previous work with the latter tissue [9], 1 mM (^{14}C)-BZ (sp. act. 1 μCi/μmole) was used in 15 min assays with 1:40 homogenates to determine SSAO activity in this human blood vessel.

MAO-A and B activities were determined by 5 min incubation of rat liver homogenates (1:10) with 100 μM (^{3}H)-5-hydroxytryptamine (2 μCi/μmole) and 100 μM (^{14}C)-Bz (1 μCi/μmole) respectively, as specific substrates for these enzymes.

Spectrophotometric assays. We recently modified the MAO assay described by Szutowicz *et al.* [21] to study the metabolism of unlabelled amines by SSAO in rat aorta, and full details are given elsewhere.* This assay follows the peroxidase-catalyzed oxidation of ABTS by the H_2O_2 formed in the amine oxidase reaction, and it contains sodium azide (3.1 mM final concentration) to inhibit the breakdown of this H_2O_2 by any catalase present in the tissue homogenates. We previously established that this concentration of sodium azide has no inhibitory effect on rat aorta SSAO, since the metabolism of 1 μM (^{14}C)-BZ was unchanged by the presence of this concentration of azide in radiochemical assays. Of relevance for the current study, we have also found no effect of 3.1 mM sodium azide on the metabolism of 1 mM (^{14}C)-BZ by SSAO in human umbilical artery homogenates. Consequently this azide concentration is also suitable for inclusion in the spectrophotometric assay of SSAO in human blood vessels. This result is in contrast to a previous report, involving a fluorometric assay, that SSAO in human aorta was inhibited almost completely by

* G. A. Lyles, C. M. S. Marshall, I. A. McDonald, P. Bey and M. G. Palfreyman, *Biochem. Pharmac.* in press.

10^{-3} M sodium azide [8]. We have no obvious explanation for this discrepancy at present.

Preliminary studies were carried out to establish incubation times and homogenization conditions which allowed linear formation of H_2O_2 with time upon incubation of tissue homogenates with MA or BZ. As a result, 1:30 homogenates of aorta were assayed for 15 min (BZ) or 60 min (MA), and 1:10 homogenates of umbilical artery were incubated for 30 min with both amines.

We previously reported that the SSAO inhibitors semicarbazide and hydroxylamine cannot be used in this assay, since they interfere with the peroxidase-catalyzed oxidation of ABTS, by causing rapid bleaching of the coloured oxidized form of ABTS as soon as it is formed. We have now found that a number of other drugs with inhibitory activity towards amine oxidases also have this effect. These include the hydrazine derivatives phenelzine, iproniazid, isoniazid and hydralazine when used in the assay at concentrations of 10^{-4}–10^{-3} M. The latter effect was demonstrated by the ability of these drugs to interfere with the colour produced as a result of adding standard solutions of H_2O_2 to assays containing the inhibitors, in the absence of tissue homogenates and amine substrates. In contrast, the acetylenic amine oxidase inhibitors were without this effect. Thus, in addition to clorgyline (10^{-4} M) and pargyline (10^{-3} M) which were found in earlier studies to be appropriate for use, we have now found that propargylamine (10^{-3} M) can also be used in this assay. We were unable to increase clorgyline concentrations above 10^{-4} M since an increasing insolubility of clorgyline in the assay buffer was found to cause a slight cloudiness of the assay solution, leading to a background absorbance which became superimposed on that due to the colour reaction of the enzyme assay, and which thus led to inaccurate estimates of the latter.

In summary, therefore, the inhibitor studies described below involved the preincubation of homogenates with acetylenic compounds, before initiation of the assay reaction by the addition of amine substrate.

Protein assays. Protein concentrations of homogenates were determined by the method of Lowry *et al.* [22], using bovine serum albumin as standard.

RESULTS

Inhibition of (^{14}C)-MA *metabolism by clorgyline and semicarbazide*

The deamination of 1 mM (^{14}C)-MA was determined in homogenates of rat aorta and human umbilical artery which had been preincubated with clorgyline or semicarbazide. In concentrations below and including 10^{-4} M, clorgyline had little if any inhibitory action on metabolism of MA (Fig. 1). With 10^{-3} M clorgyline, around 20–30% inhibition of control activities was found. Semicarbazide caused a marked concentration-dependent inhibition of MA metabolism, resulting in around 90% inhibition being produced over the range 10^{-6}–10^{-3} M of the drug. This inhibitor sensitivity therefore suggests strongly that SSAO is the predominant enzyme responsible for metabolism of MA in these tissue homogenates.

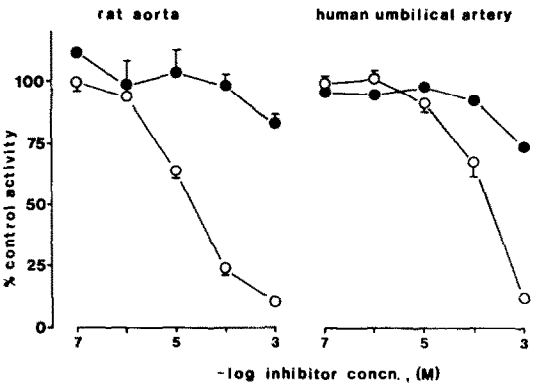

Fig. 1. Inhibition of 1 mM methylamine (MA) metabolism in homogenates of rat aorta and human umbilical artery. Homogenates were preincubated with clorgyline (●) or semicarbazide (○) at indicated concentrations for 20 min at 37° before addition of ^{14}C-MA to assay remaining deaminating activity. Activities are expressed as percentages of corresponding control samples preincubated without inhibitor. Each point is the mean (±SE of the ratio where exceeding symbol size) of 4 homogenates assayed in triplicate.

Kinetic studies on (^{14}C)-MA *metabolism*

MA metabolism was determined with various concentrations of MA (50 μM–1 mM in aorta, 100 μM–2 mM in umbilical artery) and results were analyzed by the Lineweaver–Burk method. Figure 2 shows

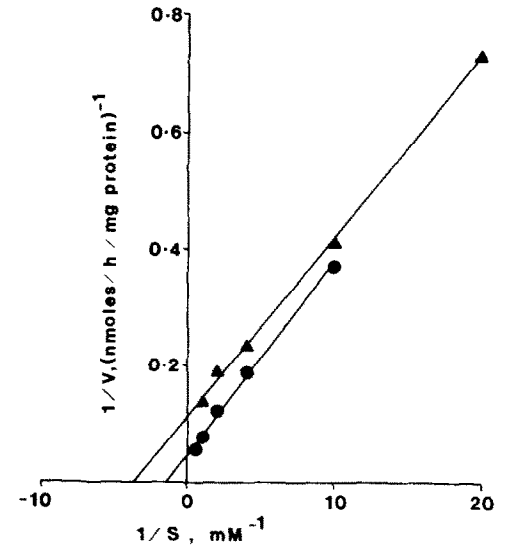

Fig. 2. Lineweaver–Burk plot for metabolism of ^{14}C-methylamine by rat aorta (▲) and human umbilical artery (●) homogenate. Data are from single experiments representative of 2 or 4 others respectively. Each point is the mean of triplicate determinations. Apparent kinetic constants, determined by linear regression, in this experiment were K_m (μM): 274 (aorta), 698 (umbilical artery); V_{max} (nmoles/hr/mg protein): 8.9 (aorta), 21.2 (umbilical artery).

representative plots obtained with each tissue in individual experiments. Mean values for apparent kinetic constants of MA metabolism from this whole series of experiments with different tissue samples were as follows: aorta (N = 3), $K_m = 302 \pm 14\ \mu M$, $V_{max} = 14.5 \pm 5.1$ nmoles MA metabolized/hr/mg protein; umbilical artery (N = 5), $K_m = 999 \pm 213\ \mu M$, $V_{max} = 35.9 \pm 6.8$ nmoles MA/hr/mg protein.

Effects of propargylamine on SSAO and MAO activities

Previous reports have indicated that propargylamine can inhibit the flavoprotein MAO [23] as well as non-flavin linked amine oxidases such as plasma amine oxidase [24]. These findings, in addition to our demonstration of the suitability of acetylenic compounds for use as potential inhibitors in the spectrophotometric assay, suggested to us that propargylamine could possibly be a useful inhibitor of these enzymes for our studies. Preliminary studies were therefore carried out to assess the inhibitory activity of this compound against SSAO in rat aorta and human umbilical artery, and also against MAO-A and B in rat liver. Appropriate selective concentrations of radiolabelled substrates specific for these enzymes were added to radiochemical assays in which tissue homogenates were first preincubated with various concentrations of propargylamine. Figure 3 shows the results obtained.

The drug was highly active as an inhibitor of 1 μM BZ metabolism by SSAO in rat aorta, with an estimated IC_{50} from the inhibition curve of 1.8×10^{-8} M. It was a much weaker inhibitor of MAO activities, with an apparent selectivity for MAO-B compared with MAO-A. IC_{50} concentrations for metabolism of 100 μM BZ (by MAO-B) and 100 μM 5-HT (by MAO-A) were 2.5×10^{-5} M and 1.25×10^{-4} M, respectively. SSAO in human umbilical artery, assessed by its metabolism of 1 mM BZ, was less sensitive than the enzyme in rat aorta to the inhibitory effects of propargylamine (IC_{50} of 6.3×10^{-7} M), and required concentrations of around 10^{-5}–10^{-4} M to achieve complete inhibition of SSAO in the human blood vessel.

Inhibition of MA and BZ metabolism by acetylenic compounds in the spectrophotometric assay

Homogenates of rat aorta and human umbilical artery were preincubated with clorgyline (10^{-4} M), pargyline (10^{-3} M) and propargylamine (10^{-6} M with aorta and 10^{-4} M with umbilical artery). Concentrations of propargylamine used here were chosen as being likely to inhibit SSAO completely on the basis of the data in Fig. 3. The effects of these inhibitors on MA and BZ metabolism are shown in Table 1. H_2O_2 production resulting from the deamination of these amines was inhibited little, if at all, by clorgyline and pargyline. In fact, clorgyline appeared to increase slightly the metabolism of BZ in rat aorta, although we have no obvious explanation for this result at present. On the other hand, the use of propargylamine resulted in virtually complete inhibition of MA and BZ deamination in both tissues.

Kinetic studies on MA and BZ metabolism in the spectrophotometric assay

Deamination of various concentrations of MA and BZ in homogenates of rat aorta and human umbilical artery was determined and apparent kinetic con-

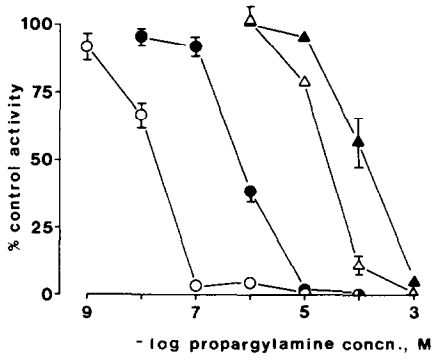

Fig. 3. Inhibitory effects of propargylamine upon SSAO and MAO activities. Key to enzymes and tissues (substrates used in parentheses): (○) rat aorta SSAO (1 μM benzylamine), (●) human umbilical artery SSAO (1 mM benzylamine), (▲) rat liver MAO-A (100 μM 5-HT), (△) rat liver MAO-B (100 μM benzylamine). Tissue homogenates were preincubated with propargylamine at the indicated concentrations for 20 min at 37° before addition of radiolabelled substrate to assess remaining enzyme activity. Activities are expressed as percentages of corresponding control samples preincubated without inhibitor. Each point is the mean (±SE of the ratio where exceeding symbol size) of 3 homogenates each assayed in triplicate.

Table 1. Inhibition of methylamine (MA) and benzylamine (BZ) metabolism by acetylenic compounds in homogenates of rat aorta and human umbilical artery

		Deaminating activity (% of controls)	
Aorta: Inhibitor		MA (1 mM)	BZ (50 μM)
10^{-4} M clorgyline	(4)	94 ± 6	132 ± 13
10^{-3} M pargyline	(4)	91 ± 5	108 ± 11
10^{-6} M propargylamine	(4)	1.7 ± 0.9	0.4 ± 0.4
Umbilical artery: Inhibitor		MA (1 mM)	BZ (1 mM)
10^{-4} M clorgyline	(7)	93 ± 2	92 ± 5
10^{-3} M pargyline	(7)	94 ± 2	91 ± 3
10^{-4} M propargylamine	(4)	0.8 ± 0.4	0.5 ± 0.3

Tissue homogenates were preincubated for 20 min at 37° with aqueous inhibitor solutions before addition of amine substrate at final concentrations indicated. Resulting H_2O_2 formation was determined by spectrophotometric assay. Deaminating activities are expressed as percentages of corresponding control samples preincubated with distilled water alone. Each value is the mean (±SE) of determinations on a number of different samples (in parentheses after inhibitors), each assayed in triplicate.

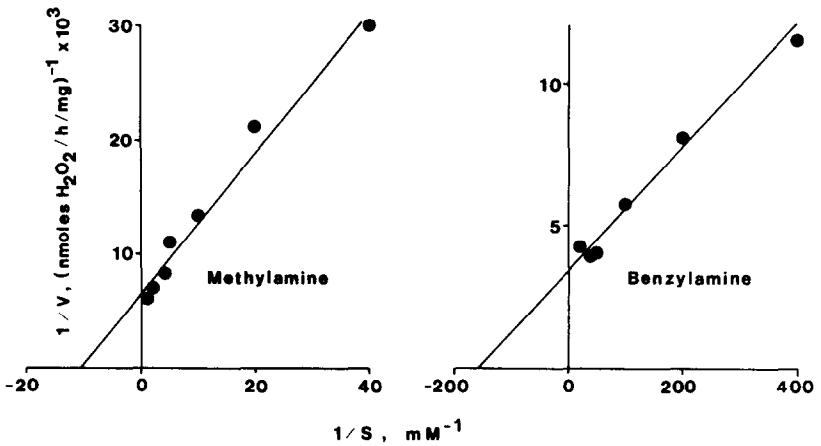

Fig. 4. Lineweaver–Burk plots for H_2O_2 formation from metabolism of methylamine (MA) and benzylamine (BZ) in the same homogenate of rat aorta. Data are from a single experiment representative of 5 or 3 others, respectively. Each point is the mean of triplicate determinations. Kinetic constants, determined by linear regression, in this experiment were K_m (μM): 94 (MA), 6 (BZ); V_{max} (nmoles H_2O_2/hr/mg protein): 152 (MA), 287 (BA).

stants for amine metabolism were estimated from the data by the method of Lineweaver–Burk. Representative plots from single experiments with the tissues are shown in Figs 4 and 5.

Mean values for kinetic constants derived from this complete series of experiments are shown in Table 2.

DISCUSSION

The metabolism of MA by amine oxidase activities in rat aorta and human umbilical artery has been studied by radiochemical assay using (^{14}C)-MA, or by a spectrophotometric method determining H_2O_2 formation. As a whole, the deamination of MA was insensitive to the MAO-selective inhibitors clorgyline and pargyline but was inhibited completely by the well-established SSAO-selective inhibitor semicarbazide, and also by propargylamine, a compound demonstrated here to be selective for SSAO by its inhibitory activity against (^{14}C)-BZ metabolism in these blood vessels. Consequently, MA appears to be metabolized predominantly if not exclusively by SSAO in these blood vessels. A similar spectrum of inhibitor selectivity against BZ metabolism in the spectrophotometric assay is also consistent with previous radiochemical studies concluding that BZ is a substrate almost entirely for SSAO in these tissues [9, 25].

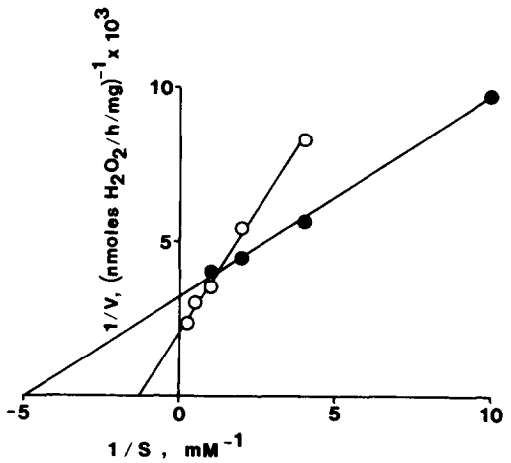

Fig. 5. Lineweaver–Burk plot for H_2O_2 formation from metabolism of methylamine (MA, ○) and benzylamine (BZ, ●) in the same homogenate of human umbilical artery. Data are from a single experiment representative of 3 others. Each point is the mean of triplicate determinations. Apparent kinetic constants, determined by linear regression, in this experiment were K_m (μM): 788 (MA), 201 (BZ); V_{max} (nmoles H_2O_2/hr/mg protein): 495 (MA), 311 (BZ).

In the radiochemical assays carried out here, 1 mM (^{14}C)-MA metabolism was inhibited partially (around 20%) by 10^{-3} M clorgyline. Although this could represent a small contribution of MAO-B to MA oxidation, this seems unlikely since little, if any, inhibitory effect was seen with 10^{-4} M clorgyline, which should produce substantial inhibition of MAO-B. Alternatively, it may be the case that some inhibition of SSAO occurs with clorgyline at 10^{-3} M and higher concentrations, an effect described by others [26] but not previously a consistent finding in our laboratory.

The kinetic studies of MA and BZ metabolism carried out here with the spectrophotometric assay reinforce existing evidence that the properties of SSAO in rat and human tissues are not identical [5]. The apparent K_m for BZ in rat aorta (6.8 μM) was considerably lower than that in human umbilical artery (207 μM). These results agree very well with K_m values for BZ determined by radiochemical methods in these tissues [9, 25]. Similarly, MA was also metabolized with a lower apparent K_m in aorta (102 μM) than in umbilical artery (779 μM). Leaving aside the species differences, it is also apparent that the K_m of MA for metabolism by SSAO is higher than that of BZ in each tissue.

Some interesting findings also emerged when com-

Table 2. Kinetic constants determined by spectrophotometric assay for metabolism of methylamine (MA) and benzylamine (BZ) in homogenates of rat aorta and human umbilical artery

Tissue	Substrate		K_m (μM)	V_{max} (nmoles H_2O_2/hr/mg protein)
Aorta	MA	(6)	102 ± 14	146 ± 20
	BZ	(4)	6.8 ± 2.6	241 ± 40
Umbilical artery	MA	(4)	779 ± 76	505 ± 40
	BZ	(4)	207 ± 27	295 ± 16

Assays involving incubation of various concentrations of amines with tissue homogenates were carried out in triplicate, and the resulting data from each homogenate were plotted as individual Lineweaver–Burk plots. Apparent kinetic constants were then estimated by linear regression from each plot. Each value above is the mean (±SE) of values from several different samples (number in parentheses) of each tissue. With umbilical artery, the same 4 samples were studied concurrently with both MA and BZ, to provide direct comparative data. In aorta, kinetic constants for MA in those 4 samples which produced the BZ data above, gave a corresponding mean K_m of 101 ± 19 μM and V_{max} of 119 ± 17 nmoles/hr/mg for MA.

paring V_{max} values for these amines, with the V_{max} for MA being approximately 60% in aorta, and 170% in umbilical artery, of the corresponding values for BZ. Therefore it would seem that in the human blood vessel, turnover of MA by SSAO is faster than that of BZ, despite the lower K_m of BZ for the enzyme. These results with MA in human umbilical artery are of considerable interest since a number of biogenic *aromatic* amines have been reported to be extremely poor substrates for SSAO in human vasculature [7–9]. Consequently MA may be a better candidate as a potential physiological amine substrate for the enzyme in man. In relation to this McEwen [27] reported some time ago that among a series of short-chain aliphatic amines, MA was metabolized with particularly high activity, comparable with that towards BZ, by the soluble amine oxidase of human plasma, an enzyme similar to SSAO with respect to its substrate and inhibitor sensitivity [5], although no kinetic constants for MA were determined in that study.

The radiochemical assay used here provided estimates of K_m values for MA metabolism by SSAO in rat aorta and human umbilical artery which were qualitatively in agreement with those determined colorimetrically. However, a major discrepancy between the radiochemical and spectrophotometric methods arose with determinations of V_{max} values, which were around tenfold lower in the former assay. While it is true that these comparisons are being made with different samples of each tissue in the two methods, nevertheless this extremely large apparent difference in V_{max} values warrants some possible explanation. We have not yet attempted to identify those deaminated metabolites of MA which are produced, although formaldehyde is the likely immediate metabolic product of the deamination reaction. Since this is a relatively polar molecule, its partition into the organic solvent used for metabolite extraction in the radiochemical method may correspondingly be fairly low, such that the present assay may be considerably underestimating the amount of metabolite actually formed. Further work is in progress to examine this possibility. Despite this, the radiochemical assay used so far does possess the advantage of allowing the inhibitory effects of hydrazides such as semicarbazide upon the metabolism of MA to be examined, whereas the spectrophotometric assay, measuring H_2O_2 production, is likely to provide a more accurate estimation of the total deaminating activity towards MA.

In conclusion, therefore, our results suggest that a possible physiological function of SSAO may be the metabolism of MA in man and other species, a role which on current evidence may not be shared by MAO. Although MA is an endogenous amine (see Introduction), its physiological significance is not clear. It has been found in high concentrations in uraemic plasma, and since it is capable of crossing the blood–brain barrier, it has been proposed that MA may act as a contributory toxin towards the neurological disorders often seen in uraemic patients [28, 29]. If so, SSAO in brain microvasculature, for example, could be important in controlling access of this amine to the central nervous system. Effects of MA which have so far been demonstrated *in vitro* include its cytotoxicity towards cultured neurones and fibroblasts [30, 31], as well as an ability (at low mM concentrations) to inhibit the involvement of endocytotic and lysosomal activity in the regulation of intracellular processing and recycling of certain plasma membrane receptors [32, 33]. Inhibition of insulin release from pancreatic cells by MA has also been demonstrated [34]. Whether such effects occur *in vivo* with normal or pathological concentrations of MA is unclear. Baba *et al.* [28] reported plasma levels (in μg/100 ml) which correspond to normal and uraemic concentrations of around 0.7 and 8 μM respectively. Of course, if vascular SSAO (and also plasma amine oxidase?) were to represent an efficient mechanism for MA degradation *in vivo*, normal circulating concentrations of MA may correspondingly be maintained at relatively low levels.

Although it may be premature to describe SSAO, especially in man, as a "methylamine oxidase", the possibility that this enzyme may play a protective role in preventing exposure to excessive plasma and tissue concentrations of this amine would appear to be worthy of further consideration, and studies to examine this are in progress in our laboratory.

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