J. Pharm. Pharmacol. 1990, 42: 322-338
Received December 22, 1989

© 1990 J. Pharm. Pharmacol.

Further Studies on the Metabolism of Methylamine by Semicarbazide-sensitive Amine Oxidase Activities in Human Plasma, Umbilical Artery and Rat Aorta

GEOFFREY A. LYLES, ANDREW HOLT AND C. M. SUSAN MARSHALL

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

Abstract—An ion exchange radiochemical assay has been developed to study the deamination of [^{14}C]methylamine (MA) in homogenates of rat aorta and human umbilical artery, as well as in samples of human plasma. MA metabolism was found to be inhibited almost completely by 1 mM semicarbazide, but virtually unaffected by 0·1 mM clorgyline, suggesting that MA is a substrate for the semicarbazide-sensitive amine oxidase (SSAO) activities which also metabolize benzylamine (BZ) in these sources. Mean K_m values for MA metabolism by aorta, umbilical artery and plasma were 182, 832 and 516 μM, respectively, with corresponding V_{max} values in aorta and umbilical artery of 100 and 590 nmol (mg prot.)$^{-1}$ h^{-1}, and in plasma of 48 nmol (mL serum)$^{-1}$ h^{-1}. Kinetic constants determined for [^{14}C]BZ metabolism in plasma (by an organic solvent extraction assay) and in umbilical artery (by the ion exchange assay) yielded mean K_m values of 225 μM (plasma), 222 μM (umbilical artery), and V_{max} values of 28 nmol (mL serum)$^{-1}$ h^{-1} (plasma) and 377 nmol (mg prot.)$^{-1}$ h^{-1} (umbilical artery). The deamination of [^{14}C]MA was inhibited competitively by unlabelled BZ, with K_i values in umbilical artery and plasma of 220 and 172 μM, respectively. Also, metabolite formation from mixtures of [^{14}C]BZ (200 μM) and [^{14}C]MA (800 μM) was extremely close to that predicted for a single enzyme capable of metabolizing two alternative substrates in a competitive fashion. β-Aminopropionitrile was found to be a reversible, competitive inhibitor (K_i of 165 μM) of [^{14}C]MA metabolism in umbilical artery, inhibitory properties characteristic of those found previously for the effects of β-aminopropionitrile upon BZ-metabolizing SSAO activities in other tissues. The possibility that vascular and plasma SSAO activities may be involved in the endogenous turnover of the biogenic amine MA is discussed.

Recent studies have shown that the aliphatic amine methylamine (MA) is a substrate in-vitro for the membrane-bound semicarbazide-sensitive amine oxidase (SSAO) found in human and rat blood vessels (Precious et al 1988; Precious & Lyles 1988). This enzyme, which may contain pyridoxal phosphate or pyrroloquinoline quinone as cofactor, is believed to exist on the plasma membrane of smooth muscle cells in the vasculature, and while exhibiting a relatively high activity and affinity towards the synthetic aromatic amine substrate benzylamine (BZ), and a characteristic sensitivity to inhibition by semicarbazide and other carbonyl reagents, the physiological significance of SSAO and its role in metabolizing possible endogenous substrates remains to be established (reviewed by Lewinsohn 1984; Lyles 1984; Callingham & Barrand 1987).

MA is a biogenic amine which is absorbed from the diet or after gut bacterial degradation of dietary precursors, as well as originating as a product of certain mammalian metabolic pathways (refs in Precious et al 1988). This amine is not a substrate for either the A or B forms of the flavin-dependent mitochondrial monoamine oxidase (Precious et al 1988; Yu 1989), and hence we have suggested that SSAO may be involved in the endogenous turnover of MA. To support this, we found that the daily urinary excretion of MA in rats was enhanced by treatment of the animals with the SSAO inhibitors semicarbazide and hydralazine, but was not influenced by the MAO-selective inhibitor pargyline (Lyles & McDougall 1989).

Correspondence to: G. A. Lyles, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, UK.

In our earlier studies on MA deamination in vascular homogenates, two different assays were used, the first being a colorimetric method to detect hydrogen peroxide formation during the deaminating reaction, while in the second, a radiochemical technique, organic solvents were employed in an attempt to extract deaminated metabolites of [^{14}C]MA from assay mixtures incubated with tissue homogenates (Precious et al 1988). Although both assays provided valuable data, the study of certain amine oxidase inhibitors in the colorimetric assay was prevented by their bleaching action on the oxidized assay chromogen, whereas absolute specific enzyme activities for MA metabolism were considerably lower when estimated by the radiochemical than by the colorimetric method, suggesting that the solvent extraction was providing a relatively poor recovery of deaminated metabolites. In an attempt to overcome these deficiencies, we have developed a more sensitive and quantitative radiochemical assay in which ion exchange chromatography is used instead to separate [^{14}C]metabolites from [^{14}C]MA. This assay has been used to investigate further the properties of MA metabolism by vascular homogenates, as well as to establish if MA is a good substrate for human plasma amine oxidase, a possibility suggested by brief observations of McEwen (1965), but to our knowledge not studied in any detail.

Materials and Methods

Materials

Umbilical cords were supplied by the Maternity Unit, Ninewells Hospital, usually from deliveries involving Caesarean sections. Male Wistar rats (300–500 g) were obtained

from the Departmental breeding colony, Animal Services Unit, University of Dundee.

Radiochemicals purchased from Amersham International plc, UK were [^{14}C]methylamine hydrochloride (55 μCi μmol^{-1}) and [7-^{14}C]benzylamine hydrochloride (51 μCi μmol^{-1}). Unlabelled amine was added to give 100 mM stock solutions at final specific activities of 1 (MA) and 0·5 μCi μmol^{-1} (BZ) which were stored frozen between experiments.

BDH Ltd (Poole, UK) provided Amberlite resin CG-50 (Type III, 400 mesh) in addition to the following reagents for preparing Bray's scintillant: naphthalene, 2,5-diphenyloxazole (PPO), 1,4-di-2-(5-phenyloxazolyl)benzene (POPOP), *p*-dioxan, methanol.

Reagents for the spectrophotometric assay (horseradish peroxidase, sodium azide, methylamine hydrochloride and 2,2′-azinobis(3-ethyl benzthiazoline-6-sulphonic acid) were purchased from Sigma (Poole, UK), as were also semicarbazide hydrochloride, β-aminopropionitrile fumarate, benzylamine hydrochloride and bovine serum albumin. Clorgyline hydrochloride was obtained from May and Baker Ltd (Dagenham, UK).

Methods

Enzyme Sources. Umbilical arteries dissected from umbilical cords and aortae removed from rats killed by cervical dislocation were washed with saline (0·9% NaCl, w/v) to remove blood, and then stored at −20°C for use within a few days. After thawing, blood vessels were homogenized in 1 mM potassium phosphate buffer, pH 7·8, at a tissue (g): buffer (mL) ratio of 1:40, centrifuged at 600 *g* for 10 min and the resulting supernatants were used as tissue homogenates in the assays below.

For studies on human plasma, blood (approx. 20 mL) was collected by antecubital venepuncture from four healthy male volunteers (age range 22–38 years) into glass tubes without anticoagulant, allowed to clot and then centrifuged (1700 *g* for 10 min). The serum was removed, divided into several smaller portions and stored as above for subsequent use in experiments.

Colorimetric amine oxidase assay. General details of this spectrophotometric method, modified from that of Szutowicz et al (1984) are given fully elsewhere (Lyles et al 1987) and its specific application to studying MA metabolism is described in Precious et al (1988). In the current studies, assay incubations of 30 min were used with the homogenates prepared above.

Radiochemical amine oxidase assay. The assay developed for studying [^{14}C]MA metabolism was adapted from that described by Tipton & Youdim (1976) for aromatic amine metabolism by MAO activities. Assays, carried out in triplicate, were set up in ice-cooled glass tubes containing 25 μL enzyme source, 25 μL distilled water (or aqueous inhibitor solution, in inhibitor studies) and 50 μL appropriate [^{14}C]MA concentration (prepared in 0·2 M potassium phosphate buffer pH 7·8). In some inhibitor studies, samples were preincubated with inhibitors for 20 min at 37°C before further ice-cooling and addition of substrate. Samples were then incubated at 37°C for either 30 min (umbilical artery), 60 min (aorta) or 120 min (plasma), these times being chosen from preliminary experiments establishing linear rates of metabolite production in the presence or absence of inhibitors over these periods. Following this, tubes were again ice-cooled rapidly, 1 mL distilled water added to each, before transfer of contents of each tube onto individual freshly-packed columns (3 cm length in Pasteur pipettes) of Amberlite CG-50 ion exchange resin. Each column was washed with a further 1 mL water, and total eluates were collected into scintillation vials, mixed with 10 mL Bray's solution (prepared according to Tipton & Youdim (1976)), and counted for radioactivity with quench correction by external standardization. Blank assays were carried out by passing samples through columns immediately after substrate addition and without incubation. These blanks were generally around 100–150 d min^{-1} at the lowest MA concentration (50 μM) and 4500–5000 d min^{-1}, at the highest concentration (2mM) used in these experiments. An assay producing d min^{-1} double values those of the blank at 50μM MA would correspond to approximately 50 pmol product formation. In practice, radioactivity recovered from assays with tissue homogenates or plasma in the absence of inhibitor drugs varied from 3 to 9 times the corresponding blank values at 50 or 100 μM MA, and remained well above twice the blank at the highest MA concentration used.

Samples (50 μL) of [^{14}C]MA solutions used were also counted separately in 10 mL Bray's in each experiment. Comparison of the radioactivity associated with these standards, with that of the eluate from blank assays in which equivalent amounts of MA were passed through the column, showed that 98·5–99% of MA was consistently retained on these columns.

The study of [^{14}C]BZ metabolism by samples of human plasma was carried out by the use of a solvent extraction assay described in detail before (e.g. Precious et al 1988). However, we also set up the ion exchange assay method for determining [^{14}C]BZ metabolism in umbilical artery homogenates, with the ultimate objective of being able to use this method for studying metabolism of [^{14}C]BZ alone, and also in mixtures with [^{14}C]MA (see Results). It was found in preliminary experiments that the assay described above for [^{14}C]MA could also be used without modification with [^{14}C]BZ as substrate, product formation from the latter remaining linear for 20 min assay incubations. This time was used in all experiments involving [^{14}C]BZ in the ion exchange assay.

Dialysis studies. Samples (0·5 mL) in triplicate of umbilical artery homogenate were incubated for 60 min at 37°C with either 0·5 mL 2 mM β-aminopropionitrile (BAPN) prepared in water, or with 0·5 mL water alone (controls). At the end of this period, some mixtures were dialysed for 24 h at 4°C against 1L 0·5 mM potassium phosphate buffer with a fresh buffer change after 3 h. An equivalent set of samples (also in triplicate) was kept at 4°C without dialysis during this period. Aliquots (50 μL) of each dialysed and undialysed sample were then assayed in triplicate with 50 μL 2 mM [^{14}C]MA by the ion exchange method above.

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

Results

Comparison of MA metabolism determined by colorimetric and radiochemical assay

Initial studies were carried out to determine if the ion exchange radiochemical assay developed here gave estimates of MA deamination similar to those of the colorimetric assay. Three homogenates from different umbilical arteries were assayed concurrently by the two methods at a final MA concentration of 1 mM. Mean (±s.e.) specific enzyme activities for the group were 281 ± 32 nmol H_2O_2 (mg prot.)$^{-1}$ h^{-1} for the colorimetric assay and 366 ± 31 nmol MA metabolized (mg prot.)$^{-1}$ h^{-1} for the radiochemical assay.

Effects of clorgyline and semicarbazide upon MA metabolism in rat aorta, human umbilical artery and plasma

Tissue homogenates or plasma samples were preincubated with inhibitor concentrations from 0.1 μM to 1 mM, and remaining enzyme activity was determined with 1 mM [^{14}C]MA as substrate in the ion exchange assay, and compared with activity in control samples preincubated without inhibitor. We have previously published similar earlier inhibitor studies carried out with rat aorta and human umbilical artery and using a solvent extraction-based radiochemical assay, in which little or no inhibition of MA deamination was produced by clorgyline concentrations up to 0·1 mM, whereas semicarbazide produced an increasing degree of inhibition, resulting in virtually complete abolition of MA deamination at 1 mM semicarbazide (Precious et al 1988). Essentially identical results were obtained in the current study with the ion exchange method, and for this reason are not reproduced here.

On the other hand, inhibitory effects of these drugs upon MA metabolism in human plasma have not been studied before, and these results are shown in Fig. 1. Also shown are the results of identical studies carried out with 1 mM [^{14}C]BZ, an established substrate for human plasma amine oxidase. It can be seen that the deamination of both MA and BZ was largely resistant to inhibition by clorgyline concentrations up to 1 mM, whereas similar concentrations of semicarbazide produced a progressive degree of inhibition which resulted in virtually complete inhibition of MA metabolism, and around 80% inhibition of BZ metabolism at 1 mM semicarbazide. These results suggest that MA is predominantly, if not exclusively, a substrate for the soluble amine oxidase in human plasma.

Kinetic constants for MA metabolism in rat aorta, human umbilical artery and plasma

The metabolism of [^{14}C]MA was measured at final assay concentrations of 0·05–1 mM in rat aorta, and 0·1–2 mM in human umbilical artery homogenates. Kinetic constants for MA metabolism were determined by linear regression of data plotted by the Lineweaver-Burk method. Mean (±s.e.) values from experiments with different samples of each tissue type were for K_m (μM): 182 ± 23 (rat aorta, n = 7), 832 ± 80 (umbilical artery, n = 11); corresponding values for V_{max} (nmol (mg prot.)$^{-1}$ h^{-1}) were: 100 ± 20 (aorta) and 590 ± 76 (umbilical artery). Examples of typical data for the metabolism of MA by umbilical artery at the concentrations above are shown in the control plots of the inhibition studies depicted in Figs 3 and 4. Although not shown, plots for MA metabolism in rat aortic homogenates,from which the kinetic constants above were determined also showed a similarly high degree of linearity.

Kinetic constants for MA (0·1–1 mM) and BZ (0·05–1 mM) metabolism were also determined in human plasma samples. A representative experiment is shown in Fig. 2. Mean values (±s.e.) from four different samples were: K_m (μM) of 516 ± 74 (MA) and 225 ± 36 (BZ): V_{max} (nmol (mL serum)$^{-1}$ h^{-1}) of 48 ± 5 (MA) and 28 ± 3 (BZ).

Inhibition of [^{14}C]MA metabolism in human umbilical artery and plasma by unlabelled BZ

Assays of [^{14}C]MA metabolism (0·2–2 mM in umbilical artery, 0·1–1 mM in plasma) were carried out in the presence of unlabelled BZ at final assay concentrations of 100, 200 or

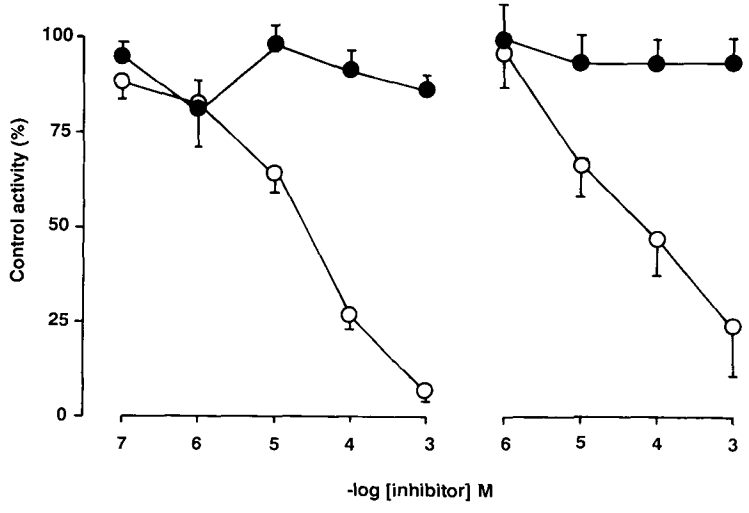

FIG. 1. Effects of clorgyline (●) and semicarbazide (○) upon the metabolism of 1 mM [^{14}C]MA (left panel) and [^{14}C]BZ (right panel) in human plasma. Plasma samples were preincubated with inhibitor drugs for 20 min at 37°C before addition of radiolabelled substrate. Deaminating activities are expressed as a percentage of those in control samples preincubated without inhibitor. Each point is the mean (±s.e.) of four different plasma samples, each assayed in triplicate.

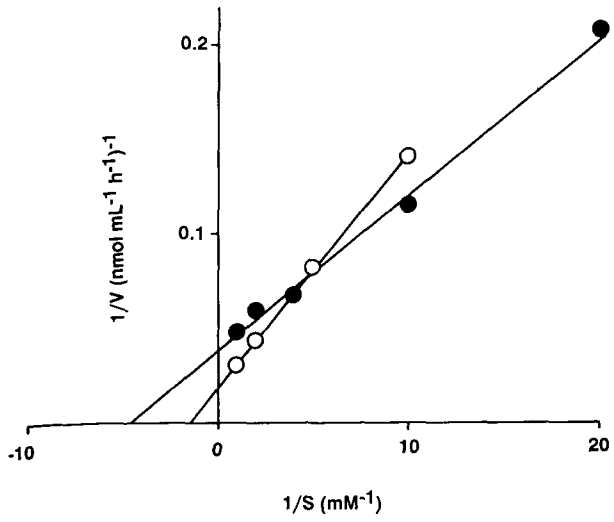

FIG. 2. Lineweaver-Burk plot for metabolism of [^{14}C]MA (○) and [^{14}C]BZ (●) in human plasma. Each point is the mean of triplicate determinations. Kinetic constants determined by linear regression in this representative experiment were K_m (μM): 672 (MA), 224 (BZ); V_{max} (nmol mL^{-1} h^{-1}): 55 (MA), 27 (BZ).

400 μM. A representative plot for data obtained with umbilical artery is shown in Fig. 3 although similar results were obtained with plasma also. In each case, BZ was found to be a competitive inhibitor of MA metabolism. K_i values were estimated by linear regression of slope replots (see Fig. 3) of data originally plotted by the method of Lineweaver-Burk. Mean values (±s.e.) for K_i (μM) from experiments with different samples of each enzyme source were 220±10 (umbilical artery, n=3) and 172±27 (plasma, n=4).

Kinetic constants for [^{14}C]BZ metabolism in human umbilical artery and product formation from mixtures of [^{14}C]BZ and [^{14}C]MA

The deamination of [^{14}C]BZ at 0·1, 0·2, 0·5 and 1 mM was determined in umbilical artery homogenates by the ion exchange method, and kinetic constants for BZ were determined by linear regression from Lineweaver-Burk plots (not shown) of the data. Mean values (±s.e.) from experiments on four arteries were $K_m = 222 \pm 22$ μM; $V_{max} = 377 \pm 23$ nmol (mg prot.)$^{-1}$ h^{-1}.

In subsequent experiments, we determined the [^{14}C] metabolite formation from 200 μM [^{14}C]BZ and 800 μM [^{14}C]MA, these substrates being used both individually and also as a mixture with each homogenate tested. The substrate concentrations were chosen to approximate closely to their K_m values found in the earlier work reported here. If a single enzyme metabolizes two different substrates at the same catalytic site, it can be shown that (see Segel 1975):

$$v_T = \frac{v_A\left(1 + \frac{[A]}{K_A}\right) + v_B\left(1 + \frac{[B]}{K_B}\right)}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} \quad (1)$$

where v_T = product formation from a mixture of the two substrates (A and B); v_A, v_B = product formation from A and B tested individually; [A], [B] = assay concentrations of A and B; K_A; K_B = Michaelis constants (K_m) for A and B.

If the substrates are used at exactly their K_m concentrations, then $v_T/(v_A + v_B) = 0{\cdot}67$. In four experiments carried out here with BZ and MA, the mean ratio (±s.e.) obtained was 0·71±0·02. However, Table 1 provides a more rigorous analysis based upon equation (1) above, for comparing experimentally determined values of v_T with those values predicted from inserting K_m values of 222 and 832 μM, respectively, for BZ and MA into the equation, these being the actual mean K_m values found earlier for the substrates and reported above in this paper. Here, it can be seen that there was a very good correspondence between the predicted and experimental values for v_T in these experiments.

Effects of β-aminopropionitrile (BAPN) upon MA metabolism in human umbilical artery

In preliminary experiments, preincubation of umbilical artery homogenates with 1 mM BAPN for periods between 5

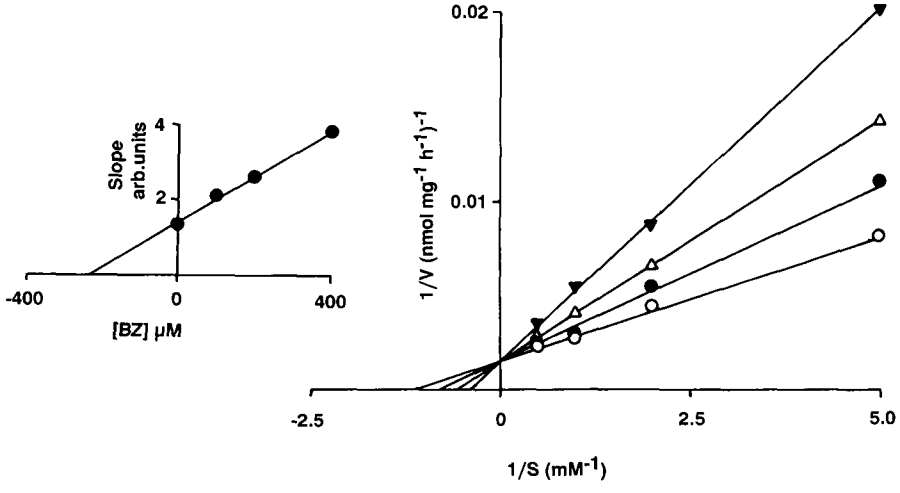

FIG. 3. Lineweaver-Burk plot (at right) showing inhibition of [^{14}C]MA metabolism by unlabelled BZ in human umbilical artery homogenate. Final BZ concentrations (μM) were 0 (○), 100 (●), 200 (△), 400 (▼). Each point is the mean of triplicate determinations. The Ki for BZ, determined by linear regression from the slope replot (at left) in this representative experiment was 235 μM.

Table 1. Comparison of predicted and actual metabolite formation in mixtures of [^{14}C]BZ and [^{14}C]MA.

Expt. no.	v_A d min^{-1}	v_B d min^{-1}	Predicted v_T d min^{-1}	Actual v_T d min^{-1}	Ratio $\left(\frac{\text{Actual } v_T}{\text{Predicted } v_T}\right)$
1	623	3526	2832	2743	0·97
2	577	3460	2757	2822	1·02
3	858	5401	4274	4759	1·11
4	721	4481	3552	3681	1·04
				Mean ratio = 1·04 ± 0·03	

v_A and v_B represent [^{14}C]metabolite formation (in d min^{-1}) from 200 μM BZ and 800 μM MA, respectively, in assays containing the appropriate amine individually. The predicted metabolite formation (v_T) from a mixture of the two amines if metabolized by the same enzyme was determined as described in the text, and compared with actual experimentally determined values. Experiments were carried out on 4 different arteries, assays on each artery involving concurrent determinations (in triplicate) of BZ and MA metabolism alone, and in a mixture.

and 60 min, produced an approximately constant inhibition (of around 65–70%) of 1 mM MA deamination. (Note that subsequent addition of substrate reduces the final BAPN concentration by half in the assay.) Dialysis of homogenates preincubated for 60 min with 1 mM BAPN revealed that this inhibition was almost completely, if not totally, reversible. Thus, in experiments with triplicate samples of two different homogenates, it was found that BAPN produced a mean percentage inhibition before dialysis of 63·5 and 62·1% in the two samples studied, whereas corresponding percentage inhibitions after dialysis were 7·0 and 5·0%, respectively, when compared with activities in samples treated identically but lacking BAPN.

Final studies examined the effects of 100, 200 and 500 μM BAPN upon the deamination of 0·2–2 mM MA. No preincubation step was used in these experiments. Fig. 4. shows a representative Lineweaver-Burk plot obtained from one such experiment. BAPN was found to be a competitive inhibitor of MA metabolism, and by linear regression of slope replots of the data (see Fig. 4) from three separate experiments with different arteries,the mean (±s.e.) K_i for BAPN was 165 ± 15 μM.

Discussion

In the present study we have introduced the use of an ion exchange radiochemical assay for studying the metabolism of [^{14}C]MA by amine oxidase activity in tissue homogenates or plasma samples. Whereas a previously used organic solvent extraction-based radiochemical method indicated rates of MA deamination (in vascular homogenates) approximately ten times lower than those determined by the colorimetric method (Precious et al 1988), the ion exchange method used here indicated activities similar to, if not a little higher than those determined colorimetrically in the same samples. This quantitative improvement in the radiochemi-

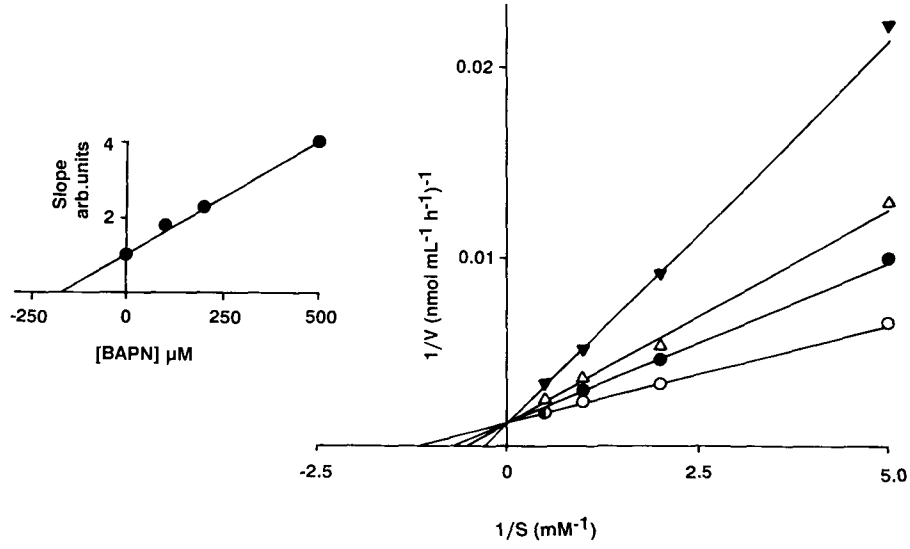

FIG. 4. Lineweaver-Burk plot (at right) showing inhibition of [^{14}C]MA metabolism by β-aminopropionitrile (BAPN) in human umbilical artery homogenate. Final BAPN concentrations (μM) were 0 (○), 100 (●), 200 (△), 500 (▼). Each point is the mean of triplicate determinations. The K_i for BAPN, determined by linear regression from the slope replot (at left) in this representative experiment was 176 μM.

cal method probably reflects a much better recovery of deaminated metabolites of [^{14}C]MA after chromatography than after solvent extraction, since the initial product of the deamination reaction, formaldehyde, is a relatively polar molecule. The recovered metabolites may also include [^{14}C]formate, since preliminary spectrophotometric studies in our laboratory have shown that umbilical artery homogenates contain a glutathione-dependent aldehyde dehydrogenase activity which metabolizes low micromolar concentrations of added formaldehyde, with the concurrent reduction of the coenzyme NAD. Furthermore, we have found that incubation of umbilical artery homogenates with MA also produces a glutathione-dependent reduction of NAD, presumably resulting from the initial amine oxidase-induced conversion of MA to formaldehyde, and the subsequent oxidation of formaldehyde by the dehydrogenase in the homogenates (unpublished results).

Studies were carried out with clorgyline and semicarbazide to assess the inhibitor sensitivity of MA metabolism in rat aorta, human umbilical artery and plasma. In all cases, 1 mM MA metabolism was resistant to inhibition by the acetylenic drug clorgyline, when used at concentrations up to 0·1 mM which are sufficient to inhibit MAO activities selectively. On the other hand, MA metabolism was inhibited almost completely by 1 mM semicarbazide, and these results are consistent with the conclusion that MA is a substrate predominantly, if not exclusively, for SSAO activity in these sources. The effects of these inhibitors upon MA metabolism in plasma were very similar to their effects upon metabolism of BZ, a recognized substrate for plasma amine oxidase (Lewinsohn 1984), and consequently, this confirms McEwen's (1965) original findings that MA is a substrate for the plasma enzyme.

Estimates of K_m values for MA metabolism by SSAO in vascular homogenates, determined here with the ion exchange assay (182 μM, rat aorta; 832 μM, human umbilical artery) are in fairly close agreement with those determined earlier with other assay methods, and confirm our findings of species-related differences in K_m values for MA metabolism by human compared with rat blood vessels (Precious et al 1988). In plasma, MA was found to have a higher K_m (516 μM) than BZ (225 μM), a situation similar to that seen in earlier comparisons with these amines as substrates for umbilical artery SSAO (Precious et al 1988). This plasma K_m for BZ, in turn, is similar to values reported by other workers under comparable assay conditions (Murphy et al 1976; Lewinsohn 1984). Interestingly, we previously showed that the maximum turnover rate for MA metabolism by umbilical artery SSAO was approximately 70% greater than that of BZ (Precious et al 1988). When comparing V_{max} values for metabolism of these amines by plasma SSAO, a virtually identical result was obtained here, illustrating further the similarity with respect to a number of biochemical properties between the soluble plasma SSAO and the membrane-bound vascular enzyme in man (Lewinsohn 1984). Indeed, it has been suggested that the vasculature could be the source of the plasma enzyme, and some support for this has come from findings that porcine aortic smooth muscle cells in culture appear to secrete a soluble benzylamine oxidase activity into the medium (Hysmith & Boor 1987).

Further support for the conclusion that the oxidative deamination of MA studied here was carried out by the BZ-metabolizing SSAO activities was provided by experiments showing that BZ was a competitive inhibitor of [^{14}C]MA metabolism in umbilical artery and plasma, with estimated K_i values of 220 and 172 μM, respectively. These values were very similar to those K_m values for BZ metabolism by SSAO determined here using the ion exchange assay (with umbilical artery) and the solvent extraction assay (with plasma). Additional evidence that MA and BZ are probably metabolized by the same catalytic moiety (in umbilical artery, at least) was obtained in the studies quantitating metabolite formation from mixtures of [^{14}C]BZ and [^{14}C]MA, where the total amine metabolism found was extremely close to that predicted for a situation where BZ and MA would be competing for deamination at a single enzyme active site. However, an alternative but less likely possibility cannot be ruled out in which the two amines are metabolized exclusively by separate enzymes, but each amine can inhibit competitively the other's metabolizing enzyme, without itself being metabolized by the latter. If the K_i for the competitive inhibition shown by each amine coincidentally happened to be identical to the K_m for its metabolism by its own degrading enzyme, then the kinetic results obtained would be indistinguishable from those for one enzyme metabolizing two substrates competitively (Segel 1975). Purification of SSAO to homogeneity, and the demonstration of BZ and MA deaminating activity on the same purified enzyme protein would ultimately help to resolve this issue.

The rationale for studying possible effects of BAPN upon MA metabolism was based upon evidence that BAPN (at low micromolar concentrations) is an irreversible inhibitor of the connective tissue crosslinking enzyme lysyl oxidase (Tang et al 1983), but a reversible competitive inhibitor of the BZ-metabolizing SSAO activities of pig plasma (Page & Benditt 1967), chick aorta and bone (Bird et al 1966; Rucker et al 1969), and rat aorta (Lyles & Singh 1985), the latter properties due at least in part to BAPN being a substrate for some or all of these benzylamine oxidases (Page & Benditt 1967; Raimondi et al 1985). In view of a report by Trackman et al (1981) that calf aorta lysyl oxidase could metabolize MA at high (10 mM) in-vitro concentrations, it was envisaged that an investigation of BAPN would indicate whether or not lysyl oxidase in the vasculature might conceivably be responsible for the MA metabolism measured in the current study. In fact, BAPN was a reversible, competitive inhibitor (K_i of 165 μM) in umbilical artery, kinetic results which were not consistent with lysyl oxidase being involved in MA metabolism here. The K_i for BAPN in human umbilical artery was similar to that (220 μM) previously determined for inhibition by BAPN of rat aorta SSAO activity towards BZ as substrate (Lyles & Singh 1985).

In conclusion, the vasculature and plasma contain SSAO activities which can metabolize MA in-vitro. These enzymes are probably the same as those previously called benzylamine oxidases and, in fact, MA would appear to be a more selective substrate for assaying these activities than BZ, since the latter is potentially also a substrate for MAO activities in tissue homogenates (Lyles 1984), whereas MA does not seem to be degraded significantly by MAO. Whether or not SSAO activities are involved in the *endogenous* metabolism of MA in various tissues (e.g. vasculature) and blood remains to be

determined. With respect to this, investigations of possible physiological or toxicological influences of MA (see Precious et al 1988 for discussion) and its deaminated metabolite formaldehyde (Boeniger 1987; Heck & Keller 1988) upon cellular function could be of interest. Studies on the characteristics of MA metabolism in various animal tissues, by assay methods such as those described here, should allow the role of amine oxidases in MA breakdown to be explored further.

Acknowledgements

We gratefully acknowledge the help of the staff of the Maternity Unit, Ninewells Hospital in making available umbilical tissue for this study.

References

Bird, D. W., Savage, J. E., O'Dell, B. L. (1966) Effect of copper deficiency and inhibitors on the amine oxidase activity of chick tissues. Proc. Soc. Exp. Biol. Med. 123: 250–254

Boeniger, M.F. (1987) Formate in urine as a biological indicator of formaldehyde exposure: a review. Am. Ind. Hyg. Assoc. J. 48: 900–908

Callingham, B. A., Barrand, M. A. (1987) Some properties of semicarbazide-sensitive amine oxidases. J. Neural Transm. 23 (Suppl.): 37–54

Heck, H.d' A., Keller, D. A. (1988) Toxicology of formaldehyde. ISI Atlas of Science (Pharmacology) 2: 5–9

Hysmith, R. M., Boor, P. J. (1987) In vitro expression of benzylamine oxidase activity in cultured porcine smooth muscle cells. J. Cardiovasc. Pharmacol. 9: 668–674

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

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., 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., McDougall, S. A. (1989) The enhanced daily excretion of urinary methylamine in rats treated with semicarbazide or hydralazine may be related to the inhibition of semicarbazide-sensitive amine oxidase activities. Ibid. 41: 97–100

Lyles, G. A., Marshall, C. M. S., McDonald, I. A., Bey, P., Palfreyman, M. G. (1987) Inhibition of rat aorta semicarbazide-sensitive amine oxidase by 2-phenyl-3-haloallylamines and related compounds. Biochem. Pharmacol. 36: 2847–2853

McEwen, C. M. (1965) Human plasma monoamine oxidase. I. Purification and identification. J. Biol. Chem. 240: 2003–2010

Murphy, D. L., Wright, C., Buchsbaum, M., Nichols, A., Costa, J. L., Wyatt, R. J. (1976) Platelet and plasma amine oxidase activity in 680 normals: sex and age differences and stability over time. Biochem. Med. 16: 254–265

Page, R. C., Benditt, E. P. (1967) Molecular diseases of connective and vascular tissues. II. Amine oxidase inhibition by the lathyrogen, β-amino-propionitrile. Biochemistry 6: 1142–1148

Precious, E., Lyles, G. A. (1988) Properties of a semicarbazide-sensitive amine oxidase in human umbilical artery. J. Pharm. Pharmacol. 40: 627–633

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

Raimondi, L., Banchelli, G., Bertocci, B., Lodovici, M., Ignesti, G., Pirisino, R., Buffoni, F., Bertini, V., De Munno, A. (1985) Reaction of pig plasma benzylamine oxidase with beta-aminopropionitrile. Agents Actions 16: 95–98

Rucker, R. B., Rogler, J. C., Parker, H. E. (1969) The partial characterization of an amine oxidase in bone tissue. Proc. Soc. Exp. Biol. Med. 130: 1150–1155

Segel, I. H. (1975) Enzyme Kinetics. John Wiley & Sons, New York, p 113

Szutowicz, A., Kobes, R. D., Orsulak, P. J. (1984) Colorimetric assay for monoamine oxidase in tissues using peroxidase and 2,2′-azinodi(3-ethylbenzthiazoline-6-sulfonic acid) as chromogen. Analyt. Biochem. 138: 86–94

Tang, S.-S., Trackman, P. C., Kagan, H. M. (1983) Reaction of aortic lysyl oxidase with β-aminopropionitrile. J. Biol. Chem. 258: 4331–4338

Tipton, K. F., Youdim, M. B. H. (1976) Assay of monoamine oxidase. In: Monoamine oxidase and its inhibition. Ciba Fdn. Symp. 39 (New Series), Elsevier, Amsterdam, pp 393–403

Trackman, P. C., Zoski, C. G., Kagan, H. M. (1981) Development of a peroxidase-coupled fluorometric assay for lysyl oxidase. Analyt. Biochem. 113: 336–342

Yu, P. H. (1989) Deamination of aliphatic amines of different chain lengths by rat liver monoamine oxidase A and B. J. Pharm. Pharmacol. 41: 205–208