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# THE METABOLISM OF AMINOACETONE TO METHYLGLYOXAL BY SEMICARBAZIDE-SENSITIVE AMINE OXIDASE IN HUMAN UMBILICAL ARTERY

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**Abstract**—The aliphatic amine aminoacetone has been described previously as a product of mitochondrial metabolism of threonine and glycine. Here, aminoacetone is shown to be deaminated to methylglyoxal by supernatants obtained by low speed centrifugation (600 *g*/10 min) of human umbilical artery homogenates, and also by membrane fractions isolated by high speed centrifugation (105,000 *g*/60 min) of these supernatants. Metabolism of 100 $\mu$M aminoacetone was completely inhibited by 1 mM propargylamine and MDL 72145, drugs which are capable of inhibiting the membrane-bound semicarbazide-sensitive amine oxidase (SSAO) activity found in vascular smooth muscle cells, whereas 1 mM pargyline and deprenyl which are inhibitors of monoamine oxidase, were without inhibitory effect. Estimated kinetic constants (at pH 7.8) for aminoacetone metabolism were $K_m$ = 92 $\mu$M; $V_{max}$ = 270 nmol/hr/mg protein. In addition, aminoacetone was a competitive inhibitor ($K_i$ = 83 $\mu$M and 128 $\mu$M in low speed supernatants and high speed membrane fractions, respectively) of [$^{14}$C]benzylamine metabolism by SSAO in this tissue. Aminoacetone would appear to be an endogenously occurring amine with a $K_m$ for metabolism by SSAO far lower than other aliphatic and aromatic biogenic amines examined previously as potential physiological substrates for the human vascular enzyme and possible implications of this are discussed.

Mammalian vascular smooth muscle cells contain a monoamine-metabolizing amine oxidase which is thought to exist in the plasma membrane and/or microsomes. The physiological importance of this enzyme is unknown. It is usually distinguished from both the A and B subtypes of the outer mitochondrial membrane flavoprotein monoamine oxidase (MAO†) by being resistant to inhibition by the acetylenic MAO inhibitor drugs clorgyline, pargyline and selegiline (deprenyl), but conversely by being inhibited by semicarbazide and some related compounds which are ineffective, or less potent as MAO inhibitors. The organic cofactor present in this semicarbazide-sensitive amine oxidase (SSAO) remains to be identified, although possible candidates are pyridoxal phosphate or pyrroloquinoline quinone [1–4].

SSAO is also characterized and frequently assayed on the basis of its relatively high deaminating activity towards the aromatic amine benzylamine, and this has led to the enzyme being called benzylamine oxidase by some investigators (e.g. Ref. 3). However, benzylamine is a synthetic amine which is not believed to occur naturally *in vivo*, and consequently several attempts, to ascribe a possible physiological role for SSAO in amine metabolism have involved studying the *in vitro* deaminating activity of the enzyme towards a number of different amines of endogenous or environmental origin. In the latter respect, it has been noted that the substrate specificity can vary markedly between different species. For example, in vascular homogenates from the rat, it has been observed that $\beta$-phenylethylamine, tryptamine and tyramine, which are aromatic amines produced by endogenous metabolic pathways and which are also present in certain foodstuffs, are deaminated readily by SSAO with estimated $K_m$ values between 10 and 70 $\mu$M [5, 6], whereas these amines are exceedingly poor substrates ($K_m$ > 1 mM) for SSAO in human blood vessels [7].

We became interested in the metabolism of *aliphatic* amines of endogenous and/or dietary origin when we demonstrated that SSAO in rat and human vascular homogenates can readily deaminate methylamine to formaldehyde [7–9], a catalytic property which does not appear to be shown by MAO activities [8, 10]. These findings prompted the current study of aminoacetone. This aliphatic amine has been identified in rat and human urine [11] and is a product of mitochondrial metabolism of glycine and threonine [12–14]. Earlier studies have indicated that aminoacetone can be deaminated to methylglyoxal by amine oxidase activities present in homogenates of various guinea pig tissues [12], and in goat and ox plasma [15, 16]. Furthermore, Ray and Ray [17] purified an "aminoacetone oxidase" from goat liver which had high activity also towards the polyamines spermine and spermidine, but with no detectable activity towards benzylamine. In order to obtain further information about the nature of those amine oxidases capable of metabolizing aminoacetone we have examined it as a potential substrate for SSAO in human umbilical artery, and have found that it is deaminated with a $K_m$ value

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† Abbreviations: MAO, monoamine oxidase; SSAO, semicarbazide-sensitive amine oxidase; DNP, 2,4-dinitrophenylhydrazine.

much lower than found for other physiologically occurring monoamines studied previously as potential substrates for the human vascular enzyme.

## MATERIALS AND METHODS

*Materials.* Aminoacetone hydrochloride was synthesized and crystallized by the method of Hepworth [18]. 2,4-Dinitrophenylhydrazine, methylglyoxal (aqueous solution) and pargyline hydrochloride were purchased from the Sigma Chemical Co. (Poole, U.K.). Propargylamine hydrochloride was obtained from the Aldrich Chemical Co. (Gillingham, U.K.). The hydrochlorides of L-deprenyl (selegiline) and MDL 72145 ((*E*) - 2 - (3,4 - dimethoxyphenyl) - 3 - fluoroallylamine ) were gifts from Professor J. Knoll, Semmelweis University (Budapest, Hungary) and Dr M. G. Palfreyman, Merrell Dow Research Institute (Cincinnati, OH, U.S.A.), respectively. [7-$^{14}$C]Benzylamine hydrochloride (51 mCi/mmol) was obtained from Amersham International (Amersham, U.K.).

Umbilical cords were supplied by the Maternity Unit, Ninewells Hospital. Arteries were dissected and stored frozen before use in subsequent experiments within 1–2 weeks. They were then homogenized in 1 mM potassium phosphate buffer pH 7.8 (1 *g* tissue/30 mL), centrifuged at 600 *g* for 10 min, and the supernatants from this low-speed centrifugation were used as enzyme source for some experiments. In other studies, these supernatants were further centrifuged (105,000 *g* for 60 min) to prepare membrane fractions which were then suspended in the same buffer in a volume of 1 mL/40 mg original tissue wet weight. These membrane fractions generally had a protein content of 0.8–1.0 mg/mL, determined by the method of Lowry *et al* [19].

*Estimation of methylglyoxal formation from aminoacetone metabolism.* Since hydrogen peroxide formation is a product arising from the metabolism of amines by amine oxidase enzymes, we hoped initially to follow aminoacetone oxidation by assay methods which detect hydrogen peroxide. The spectrophotometric methods tried depended upon coupling hydrogen peroxide formation to the oxidation of either 2,2′-azinobis (3-ethylbenzthiazoline sulphonic acid) [20] or *o*-dianisidine [17], in the presence of exogenously added horseradish peroxidase as the catalytic agent. However, in preliminary studies it was found that in the *absence* of tissue fractions in the assays, the mixing of 100 μM aminoacetone with these other reagents produced visible colour reactions with 2,2′-azinobis (3-ethylbenzthiazoline sulphonic acid) and with *o*-dianisidine, and these occurred at the appropriate wavelengths for their oxidized forms, suggesting that oxidation of these indicators can be induced by aminoacetone itself. Although we do not know the chemical basis of these effects, they indicated that such actions would preclude using these and possibly similar peroxidase-based assays from accurately detecting hydrogen peroxide formation. These results are at variance with those of Ray and Ray [17] who used an *o*-dianisidine method to determine aminoacetone metabolism by a purified amine oxidase from goat liver. We are unaware at present of the reason for this discrepancy.

Consequently, we adopted a different approach designed to determine methylglyoxal formation from aminoacetone, by modifying an analytical method described previously by Cooper [21]. Assay tubes contained 100 μL tissue fraction, 300 μL 1 mM potassium phosphate buffer pH 7.8, and 100 μL aminoacetone prepared at required concentrations in 200 mM potassium phosphate buffer pH 7.8. Blank assays omitted aminoacetone from the latter buffer. Tubes were incubated for 30 min at 37°, this time being chosen from preliminary experiments with a range of aminoacetone concentrations (25–125 μM) to determine linearity of rate of product formation without utilizing greater than 20% of available substrate. Preliminary experiments also determined that metabolite formation over this time was also proportional to varying amounts (25–100 μL) of tissue fraction used in the assay. Then, 165 μL 2,4-dinitrophenylhydrazine (DNP, 0.1% w/v in 2 M HCl) was added to each tube, and the formation of the methylglyoxal–DNP adduct was promoted by incubation for 15 min at 37°. The violet-blue coloured derivative of this adduct in alkali was then produced by addition of 835 μL 10% (w/v) NaOH. Tubes were centrifuged for 10 min at 600 *g* before measurement of absorbance at 550 nm in supernatants. The latter centrifugation step was adopted to precipitate some of the membrane protein and thus reduce the turbidity of samples before spectrophotometry. Under the assay conditions used, the estimated extinction coefficient for the adduct was $4.4 \times 10^4\ cm^{-1}\ M^{-1}$, determined from standard curves obtained by derivatizing standard concentrations of methylglyoxal in the presence of equivalent tissue samples to those used above. Since aminoacetone itself produces a small background absorbance at this wavelength, this was corrected for by including in each experiment an identical set of enzyme assays to those described above which, however, did not receive the initial 30-min incubation, in order to prevent aminoacetone metabolism, and this background was subtracted as appropriate.

In studies with amine oxidase inhibitors, solutions of these were prepared at required concentrations in 1 mM potassium phosphate buffer and were included in assays containing 100 μL membrane fraction, 100 μL inhibitor solution, 200 μL 1 mM potassium phosphate buffer. Control assays contained, all constituents except inhibitor. Assays were preincubated for 20 min at 37° before addition of 100 μL aminoacetone as described earlier.

*Effects of aminoacetone upon* [$^{14}$C]*benzylamine metabolism.* The kinetics of the interaction between aminoacetone and [$^{14}$C]benzylamine metabolism by SSAO was studied by the radiochemical method described more fully in Precious *et al.* [8]. Briefly, these assays containing 25 μL tissue fraction, 25 μL aminoacetone (at desired concentrations in 200 mM potassium phosphate buffer pH 7.8) and 50 μL [$^{14}$C]-benzylamine (prepared in the same buffer) were incubated for 30 min at 37°, before organic solvent extraction of radiochemical metabolites, which were estimated by liquid scintillation spectrometry.

Table 1. Inhibitor effects upon aminoacetone metabolism in human umbilical artery membrane fractions

| Inhibitor | Deaminating activity (% of control) |
|---|---|
| Propargylamine (1 mM) | 0.7 ± 0.7 |
| MDL 72145 (1 mM) | 0.9 ± 0.9 |
| Pargyline (1 mM) | 98.4 ± 2.4 |
| L-Deprenyl (0.1 mM) | 115.0 ± 8.4 |
| L-Deprenyl (1 mM) | 114.7 ± 3.7 |

Membrane fractions were preincubated with inhibitors for 20 min before assay of aminoacetone (100 $\mu$M) metabolism.

Values are means ± SE of 3 or 4 tissues studied in triplicate.

Mean ± SE activity of all control tissues used ($N = 7$) was 138 ± 8 nmol methylglyoxal produced per hour per milligram protein.

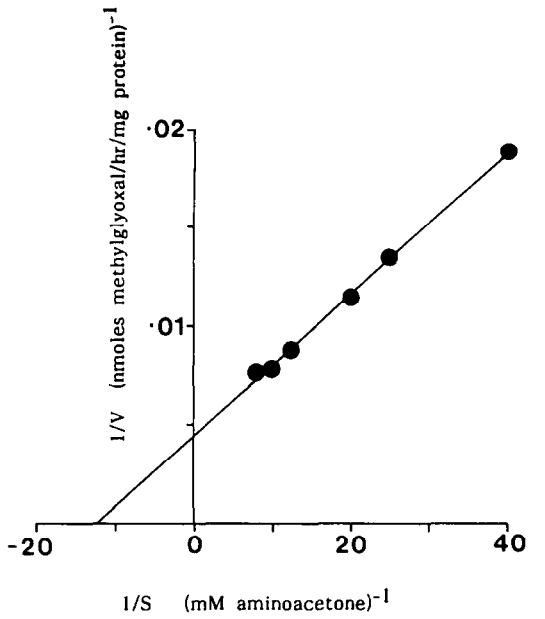


Fig. 1. Representative Lineweaver–Burk plot for aminoacetone (25–125 $\mu$M) metabolism by a high-speed membrane fraction from one human umbilical artery. Linear regression was used to fit the line to the data points which are each the mean of triplicate determinations.

## RESULTS

In preliminary experiments, it was established that low-speed supernatants from human umbilical artery homogenates were capable of converting aminoacetone to methylglyoxal which could be detected by the DNP method. However, in separate studies, it was also noted that incubation of these supernatants with exogenously added methylglyoxal (a total of 5 $\mu$mol in the assay) in place of aminoacetone, resulted in a gradual time-dependent loss of detectable methylglyoxal (by approximately one half) at the end of a 30-min incubation. These results are probably explained by the presence of a significant activity of the methylglyoxal-degrading "glyoxalase" enzyme system which is found predominantly in the cytosol of cells [22]. This factor could lead to a marked underestimation of methylglyoxal formation from aminoacetone in our assays. However, no such loss of exogenous methylglyoxal was detected when incubated as above with high-speed membrane fractions from the tissue, so the latter were used in all subsequent studies which measured aminoacetone deamination directly.

### *Effects of inhibitors upon aminoacetone metabolism*

Table 1 shows the effects of various amine oxidase inhibitors upon the metabolism of 100 $\mu$M aminoacetone in membrane fractions. Pargyline and L-deprenyl which are selective inhibitors at 0.1–1 mM of both MAO-A and -B activities without affecting SSAO, did not influence aminoacetone deamination. However, propargylamine and MDL 72145, which although capable of inhibiting MAO are also inhibitors of SSAO [8, 20], were found to block metabolism completely. We were unable to use semicarbazide itself as a selective inhibitor of SSAO because in preliminary studies it was found to interfere with the quantitative determination of standard concentrations of methylglyoxal by the DNP method. This presumably is because semicarbazide is itself a hydrazine derivative which may interact with the carbonyl groups of methylglyoxal hindering the formation of the DNP adduct. We also attempted to use 1 mM clorgyline as a potential MAO inhibitor in these studies but this drug was found to produce an unacceptably high background turbidity in the samples, which was traced to the slight insolubility of clorgyline in the assay buffer used. These problems were not encountered with any of the other amine oxidase inhibitors employed.

### *Kinetic constants for aminoacetone metabolism*

Figure 1 shows a representative Lineweaver–Burk plot for aminoacetone metabolism by membrane fractions. These studies were routinely carried out with aminoacetone concentrations between 25 and 125 $\mu$M. In preliminary studies (not shown) with concentrations above 125 $\mu$M up to 1 mM, it was found that the rate of deamination began to decrease markedly in a concentration-dependent manner, presumably due to "high-substrate" inhibition. Mean apparent kinetic constants for aminoacetone metabolism from 25–125 $\mu$M, derived from separate experiments on seven different tissues, were 92 ± 21 $\mu$M ($K_m$) and 270 ± 49 nmol methylglyoxal produced per hour per milligram protein ($V_{max}$).

### *Effects of aminoacetone upon [$^{14}$C]benzylamine metabolism by SSAO*

It has been shown previously that benzylamine is a substrate for SSAO alone in human umbilical artery [7]. The metabolism of benzylamine (0.1–1 mM) was determined in the absence and presence of aminoacetone (50,100 and 200 $\mu$M). Results were depicted as shown in the representative Lineweaver–Burk plot in Fig 2. Aminoacetone produced a competitive pattern of inhibition against benzylamine

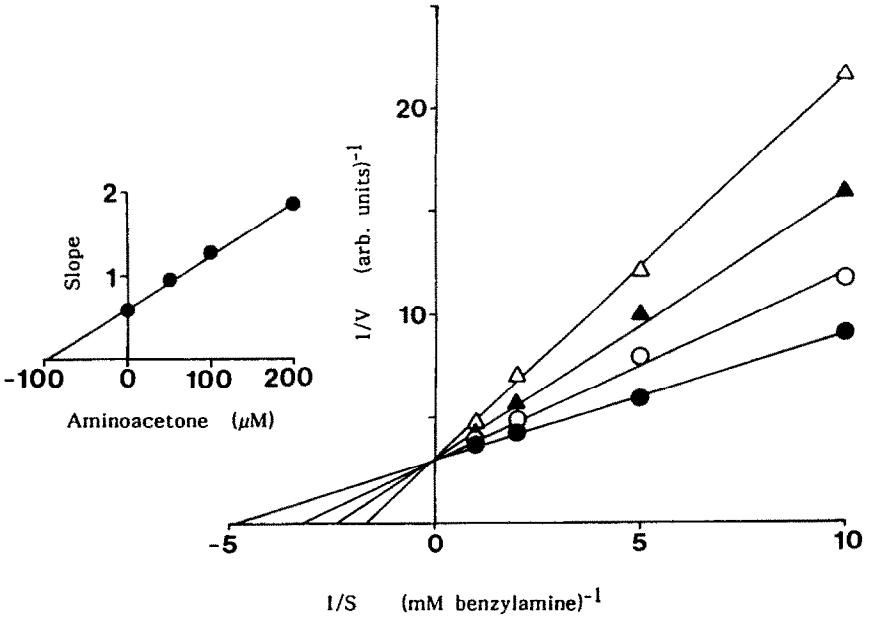


Fig. 2. Representative Lineweaver–Burk plot (at right) showing inhibition of [$^{14}$C]benzylamine metabolism by aminoacetone in human umbilical artery homogenate. Final aminoacetone (μM) concentrations were 0 (●), 50 (○), 100 (▲) and 200 (△). Each point is the mean of triplicate determinations. The $K_i$ for aminoacetone was determined by linear regression from the slope replot (at left).

Table 2. Kinetics of benzylamine metabolism and its inhibition by aminoacetone

| | Low-speed supernatants | Membrane fractions |
|---|---|---|
| Benzylamine $K_m$ (μM) | 263 ± 19 | 275 ± 73 |
| Benzylamine $V_{max}$ (nmol/hr/mg) | 425 ± 30 | 806 ± 198 |
| Aminoacetone $K_i$ (μM) | 83 ± 9 | 128 ± 31 |
| Ratio of $K_i/K_m$ | 0.32 ± 0.04 | 0.48 ± 0.04 |

Kinetic constants were estimated as shown in Fig. 2 using low-speed supernatants (N = 5) and membrane fractions (N = 7) from different umbilical arteries.

Values shown are means ± SE.

metabolism, and its $K_i$ value was determined by the slope-replot method indicated.

A series of these kinetic experiments was carried out with both low-speed supernatants and subsequently with high-speed membrane fractions. A summary of the mean kinetic constants ($K_m$, $V_{max}$) for benzylamine metabolism and the $K_i$ for aminoacetone inhibition is shown in Table 2. It can be seen that the $K_m$ found for benzylamine (around 250 μM) is higher than the $K_m$ for aminoacetone (around 100 μM) reported earlier in this paper. The $K_i$ values for aminoacetone determined here in low-speed supernatants and also in membrane fractions were quite close to this earlier $K_m$ value for aminoacetone. In each individual experiment carried out, the aminoacetone $K_i$ was found to be considerably lower than the corresponding $K_m$ for benzylamine, and this is reflected in the mean values reported in Table 2 for this ratio between each corresponding aminoacetone $K_i$ and benzylamine $K_m$.

## DISCUSSION

The present study has demonstrated that aminoacetone is deaminated to methylglyoxal in homogenates and high-speed membrane fractions from human umbilical artery. This study has not specifically investigated the subcellular localization of this deaminating activity, although the high-speed membrane fractions used will contain mitochondrial, microsomal and plasmalemmal components.

However, the enzyme involved is clearly membrane-bound. Two main findings suggested that SSAO activity is involved in this metabolism. First, aminoacetone degradation was inhibited by drugs which block SSAO activity (propargylamine, MDL 72145), but was unaffected by the MAO inhibitors pargyline and deprenyl. Second, aminoacetone inhibited competitively the metabolism of [$^{14}$C]-benzylamine, a substrate only for SSAO in human umbilical artery [7], and the $K_i$ value (around 100 $\mu$M) for this competitive inhibition was very similar to our separate estimates of the $K_m$ for aminoacetone conversion to methylglyoxal. This $K_m$ for aminoacetone is considerably lower than values found previously under comparable assay pH conditions for other aromatic and aliphatic amines studied as possible endogenous substrates for SSAO in man and, in addition, is lower than that of benzylamine,the synthetic substrate frequently used for studying the human enzyme [7–9].

Although this competitive interaction between aminoacetone and benzylamine is consistent with a single enzyme being capable of metabolizing both amines, it does not rule out an alternative possibility that each amine has a specific metabolizing enzyme which, however, binds the other amine competitively but without metabolism. One method which can be used to help distinguish between these possibilities compares total product formation, obtained with mixtures of two substrates, with product formation when the substrates are studied individually (e.g. Ref. 9). We therefore investigated the feasibility of determining methylglyoxal (as its DNP-derivative) in the presence of similar derivatives of either benzaldehyde or formaldehyde, which are respective products of benzylamine and methylamine metabolism by SSAO. In preliminary studies (not shown), standard samples of benzaldehyde or formaldehyde (at 100 $\mu$M final concentration) were converted to DNP-adducts by the method used for methylglyoxal, and were then scanned by difference spectroscopy to determine their absorption spectra. These derivatives showed broad absorption peaks at around 470 nm (benzaldehyde) and 430 nm (formaldehyde), but there remained some residual absorbance above background at 550 nm, the wavelength used for methylglyoxal determination. This overlap between the spectral peaks for the different aldehydes indicates that it would be difficult to assess accurately the relative contributions of two different aldehydes in a mixture to the total absorbance measured at a given wavelength, particularly if their DNP-derivatives have different extinction coefficients. Consequently, this potential approach for carrying out "mixed substrate" assays with SSAO was not pursued further. In addition, as indicated earlier (in Materials and Methods) it was not possible to use hydrogen peroxide formation as an assay for aminoacetone metabolism thus preventing the use of peroxidase-based assays as another approach for carrying out "mixed substrate" experiments. Perhaps a more definitive indication of whether or not a single SSAO-type enzyme metabolizes aminoacetone, methylamine and benzylamine would result from the eventual isolation and purification to homogeneity of SSAO enzyme protein from umbilical artery membrane fractions.

The possibility that methylglyoxal formation in animal tissues may have effects upon cellular function has been of interest for many years, since this aldehyde is known to have cytotoxic actions at high concentrations [22]. Methylglyoxal can be further metabolized in animal cells by combination with reduced glutathione, and enzymatic conversion (by glyoxalase I) of the glutathione adduct to *S*-D-lactoylglutathione, a compound implicated recently as a possible physiological regulator of cytoskeletal function in various cell types [23]. Whether or not metabolism of aminoacetone by SSAO in blood vessels could represent a significant pathway for methylglyoxal (and lactoylglutathione) formation *in vivo* remains to be investigated. However, aminoacetone has been described as a constituent of human urine [11], and we were able to confirm this finding in experiments (not shown) with urine from an adult male and female by use of the colorimetric analytical procedure of Urata and Granick [12]. Most investigations of possible sources of aminoacetone formation have focussed upon *in vitro* mitochondrial metabolism of glycine and threonine, especially in the liver [12–14], although some other tissue homogenates (including kidney, adrenals, brain) also formed detectable amounts of aminoacetone [12]. However, there appears to be no information as to whether vascular cells can produce aminoacetone by these pathways and, in addition, we are not aware of any reports detecting (and quantifying) aminoacetone in blood, so the origins of urinary aminoacetone are unclear.

Other workers have demonstrated previously that aminoacetone is a substrate for the plasma amine oxidase activity found in goats and oxen [15, 16] but not for the enzyme in pig plasma [24], although all of these enzymes are capable of metabolizing benzylamine. Goat liver appears to possess an aminoacetone-oxidizing enzyme with high activity towards polyamines, but with no detectable activity towards benzylamine [17]. Urata and Granick [12] proposed that MAO may be involved in aminoacetone degradation since the disappearance of aminoacetone upon incubation with guinea pig liver mitochondrial membrane fractions was inhibited by various substrates (benzylamine, $\beta$-phenylethylamine) and inhibitor drugs (amphetamine, aminoguanidine, iproniazid, isoniazid) of amine oxidase activity. However, these compounds are all capable of interacting with SSAO activity [25] and their overall specificity suggests that mitochondrial MAO may not necessarily have been responsible for some or any of the aminoacetone metabolism in that study. In our work, here, with umbilical artery, we have found no indication that MAO contributes to aminoacetone deamination, even though both A and B forms of MAO can be demonstrated with other substrates in this tissue [7].

In conclusion, the present study with aminoacetone has identified an endogenously occurring amine which is deaminated readily by an enzyme which appears to be SSAO in human umbilical artery, and has a much lower $K_m$ than monoamines examined previously as potential physiological substrates.

Taken in conjunction with our previous studies with methylamine [7–9], it would appear that human vascular SSAO may preferentially metabolize endogenous aliphatic rather than aromatic amines producing aldehydes with potentially cytotoxic properties. Whether or not these findings may provide additional clues about possible physiological or toxicological consequences arising from the location of SSAO in blood vessels remains to be seen.

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