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Oxidative deamination of aliphatic amines by rat aorta semicarbazide-sensitive amine oxidase

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Abstract—Rat aorta semicarbazide-sensitive amine oxidase (SSAO) exhibits very high affinity in the deamination of an homologous series of aliphatic amines of 1 to 18 straight chain carbon atoms. The K_m value decreases substantially as the chain length of these amines increases. The V_{max} values are higher for the short chain amines. Diamines are poor substrates for SSAO or are not acted upon by the enzyme. The substrate preference for SSAO differs from that for monoamine oxidase.

Blood vessels of various species contain an amine oxidase, namely semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6), which is resistant to inhibition by clorgyline, a type A monoamine oxidase (MAO, EC 1.4.3.4) inhibitor, but quite sensitive to semicarbazide (Clarke et al 1982; Callingham & Barrand 1987) and (E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) (Lyles & Fitzpatrick 1985; Elliott et al 1989a). This enzyme can oxidize different endogenous as well as various exogenous amine substrates. It is known that the enzyme is particularly rich in the vascular wall and smooth muscle cells. While MAO is located subcellularly on the outer mitochondrial membrane (Yu 1986), SSAO is found to be heterogenously distributed, i.e. it is present in either the cytoplasm or plasma membrane (Wibo et al 1980). The function of SSAO is not yet well understood (Fuentes & Neff 1977; Lewinsohn et al 1980). Recently is has been found that methylamine (CH_3NH_2) and allylamine ($CH_2=CHCH_2NH_2$) can be deaminated by SSAO, leading to the formation of the toxic compounds formaldehyde (HCHO) and acrolein ($CH_2=CHCHO$), respectively (Boor & Nelson 1982; Nelson & Boor 1982; Precious et al 1988). It has been suggested that SSAO might play a role in certain cardiovascular or smooth muscle disorders. n-Pentylamine can also be deaminated by vascular SSAO (Guffroy et al 1983). Although SSAO is distinctly different from MAO, the substrate spectra of both enzymes appear to be largely overlapping. Recently it has been reported that straight chain saturated aliphatic amines can be oxidized with quite high affinity by MAO-B (Yu 1989). It would be interesting to examine whether vascular SSAO would oxidize other aliphatic amines and to compare its kinetic parameters regarding these substrates with that of MAO.

Materials and methods

SSAO was prepared from the rat aorta as previously described (Clarke et al 1982). Rats (male Wistar, 180–200 g) were killed by cervical dislocation and the aorta dissected out. The aorta was then rinsed in physiological saline to clean away any adhering blood, and freed of surrounding fat. The tissues were then frozen (−20°C) until assayed. Tissues were homogenized by means of a

Polytron homogenizer (PT-10-35, setting 5 for four periods of 5 s on ice) in 1 mM potassium phosphate buffer (pH 7·5). The crude homogenates were centrifuged at 600 *g* for 10 min and supernatant fractions of 1 or 2 mL were frozen in glass vials until use. Before assay the aorta SSAO preparations were thawed, homogenized with a Brown-sonic ultrasonicator at 75 W for several 5 s intervals. In the kinetic analysis of SSAO, the enzyme preparations were further incubated with 0·1 mM clorgyline at room temperature (20°C) for 30 min to inactivate MAO activities.

A sensitive fluorometric method based on the formation of an intense fluorophore formed from homovanillic acid and the hydrogen peroxide released during the oxidation of the amines was adopted for the measurement of SSAO activity (Snyder & Hendley 1968; Yu & Boulton 1980). The SSAO preparations (30–40 μg protein) were incubated at 37°C for 10 min in the presence of amine substrate in a total volume of 200 μL of 0·05 M phosphate buffer (pH 7·5) containing 50 μg homovanillic acid and 0·82 units of horseradish peroxidase. The fluorescence intensity was measured in a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 315 nm and emission wavelength 425 nm. The enzyme reactions were linear both with respect to time (for at least 15 min) and the amount of the enzyme used.

Protein concentrations were determined by the Bradford method (Bradford 1976) with bovine serum albumin as standared. The kinetic parameters were analysed according to Wilkinson (1961).

Results and discussion

The effects of clorgyline, an MAO-A inhibitor, and semicarbazide, an SSAO inhibitor, on the deamination of several different aliphatic amines and *p*-tyramine are compared in Fig. 1. Clorgyline did not inhibit the rat aorta enzyme in the deamination of methylamine and it only slightly inhibited the oxidation of the longer chain aliphatic amines, such as hexylamine and nonylamine. The oxidation of *p*-tyramine is also only partially blocked by clorgyline. Semicarbazide, however, inhibits completely the deamination of methylamine, but only partially inhibits the deamination of *p*-tyramine and the longer chain aliphatic amines. These results indicate that methylamine is deaminated only by SSAO, while the longer chain aliphatic amines are deaminated by both MAO and SSAO. This is consistent with the finding that rat liver mitochondrial MAO is able to catalyse the deamination of aliphatic amines with chain lengths extending from 3 up to 18 carbons (Yu 1989).

In order to examine the kinetic properties of rat aorta SSAO, therefore, it is necessary to inhibit MAO activity. Thus the enzyme was incubated with clorgyline (1×10^{-4} M) to ensure that neither MAO-A nor MAO-B activities remained present in the SSAO preparations. It is well known that clorgyline at such high concentration blocks both MAO-A and MAO-B activities (Yu 1986). In Table 1, a systematic comparison of the kinetic parameters of SSAO with respect to unbranched aliphatic amine substrates containing from one to 18 carbons as well as several other amines are listed. Unlike MAO, aorta SSAO can readily oxidize methylamine and ethylamine. The K_m values obtained with methylamine agree quite well with the previous values (Precious et al 1988). Interestingly, as the carbon chain length increased, so did the affinity of the substrates for the enzyme. Nonylamine, decylamine and octylamine clearly exhibited the highest affinity for SSAO. A 90-fold difference in K_m values between methylamine ($K_m = 2{\cdot}5 \times 10^{-4}$ M) and nonylamine ($K_m = 2{\cdot}8 \times 10^{-6}$ M) was observed. The V_{max} values, however, appeared to be only slightly different, with lower V_{max} values being observed for the longer chain aliphatic amines. Thus although dodecylamine and octadecylamine can be oxidized by SSAO, they cannot be considered good substrates; part of the reason for this, however, must be the limited solubility of such long chain aliphatic amines in aqueous solution. MAO exhibits a relatively high affinity for aliphatic amines with chain lengths of 5 to 10 carbons; the K_m values for these substrates ranged from 1·3 to $1{\cdot}8 \times 10^{-5}$ M (Yu 1989). SSAO also possesses very high

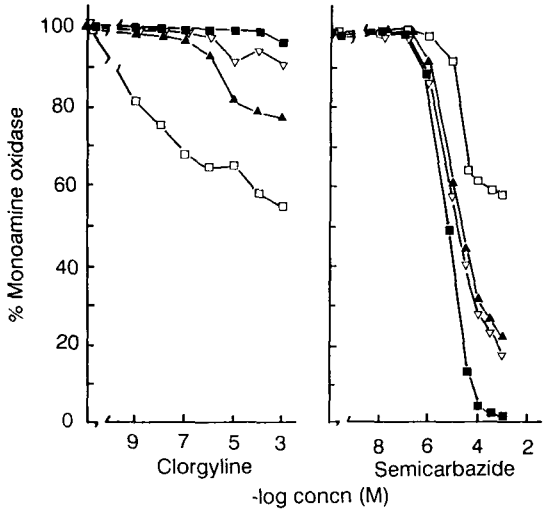

FIG. 1. Inhibition by clorgyline and semicarbazide of the deamination of amines catalysed by rat aorta homogenates. Substrates used were methylamine (■), n-hexylamine (▽), n-nonylamine (▲) and *p*-tyramine (□). The rat aorta enzymes were preincubated with the inhibitors for 10 min at room temperature, and the enzyme reactions were initiated by the addition of the substrates (1 mM), followed by further incubation at 37°C for 10 min. Activities were expressed as percentages of corresponding experiments conducted in the absence of inhibitor. Each point is the mean of two experiments using different enzyme preparations.

Table 1. Kinetic parameters of rat aorta semicarbazide-sensitive amine oxidase with respect to aliphatic amines of different chain length, diamines and arylalkylamines.

	V_{max} (nmol min^{-1} mg^{-1})	K_m (μM)	V_{max}/K_m × 100
Methylamine	1·53 ± 0·05	246·7 ± 21·4	0·6
Ethylamine	0·86 ± 0·03	393·4 ± 39·5	0·3
n-Propylamine	1·07 ± 0·07	234·1 ± 31·8	0·5
n-Butylamine	1·44 ± 0·12	143·9 ± 28·9	1·0
n-Pentylamine	0·83 ± 0·04	40·5 ± 8·2	2·1
n-Hexylamine	0·98 ± 0·06	27·8 ± 5·7	3·5
n-Heptylamine	0·78 ± 0·02	13·9 ± 0·9	5·6
n-Octylamine	0·89 ± 0·07	5·5 ± 1·0	16·2
n-Nonylamine	0·75 ± 0·07	2·8 ± 0·8	26·8
n-Decylamine	0·79 ± 0·09	4·2 ± 0·9	18·8
n-Dodecylamine[b]	0·43 ± 0·02	11·0 ± 0·3	3·9
n-Octadecylamine[b]	0·43 ± 0·06	793·5 ± 161·3	0·1
Benzylamine	1·66 ± 0·09	6·2 ± 0·7	26·8
--Phenylethylamine	0·62 ± 0·03	16·2 ± 1·6	3·8
Histamine	1·21 ± 0·08	582·9 ± 88·4	0·2
Spermidine	1·81 ± 0·08	1914·5 ± 145·7	0·1
Spermine	0·45 ± 0·04	2881·0 ± 329·1	<0·1
Cadaverine	—[a]	—	
Putrescine	—[a]	—	
Allylamine	1·44 ± 0·05	145·2 ± 17·5	1·0

[a] Enzyme activity was undetected. [b] The substrates were not totally soluble in the incubation mixture. A homogenous suspension of the substrate was obtained by ultrasonication with a Brown-sonic 1510 for 1 min at 75 W using a needle probe tip.

affinity for aliphatic amines, yet it appears to have a much narrower optimal range of carbon chain lengths for optimal affinity. Nonylamine, for example, exhibits the highest affinity. Its K_m value is even significantly lower than that of benzylamine, which is known to be the best substrate for SSAO.

Other amines of interest, such as allylamine (Table 1), an unsaturated amine, are known to be converted to toxic products by SSAO, such as acrolein in the case of allylamine (Boor & Nelson 1982). Kinetic data regarding this amine have not been reported. Its K_m value was found to be somewhat higher than those of longer chain aliphatic amines, but comparable to that of propylamine. The V_{max} value for allylylamine, however, is quite high. It is interesting that rat liver MAO is unable to oxidize allylamine (result not shown), although propylamine, a saturated amine with the same number of carbon atoms, can serve as an MAO substrate (Yu 1989).

Diamines, such as putrescine $[NH_2(CH_2)_4NH_2]$ and cadaverine $[NH_2(CH_2)_5NH_2]$ are not oxidized by SSAO. Spermine $[NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_2NH_2]$ and spermidine $[NH_2(CH_2)_4NH(CH_2)_2NH_2]$, however, are oxidized, but rather poorly (Table 1). These findings agree with an earlier observation that rat aorta SSAO possesses a low affinity towards diamines (Elliott et al 1989b). However the data in this latter study was obtained in a rather indirect manner, i.e. the competitive effect of various amines on the deamination of benzylamine by SSAO was analysed kinetically (Elliott et al 1989b). Whether rat aorta SSAO should be placed in the diamine oxidase (EC 1.4.3.6) family remains an interesting question (Callingham & Barrand 1987).

Aliphatic amines such as methylamine, dimethylamine, ethylamine, n-propylamine, n-butylamine, and ethanolamine, etc. have been reported to occur normally in urine or brain tissues from human and other species (Blau 1961; Perry et al 1965; Ishitoya et al 1973). The longer chain aliphatic amines are possibly not biological substrates for SSAO. Nevertheless, the high affinity of SSAO towards these amines indicates that SSAO can deaminate these amines from either endogenous or exogenous sources in-vivo. Whether the aldehyde products formed as a result of the deamination of the aliphatic amines are as potentially hazardous as that of acrolein from allylamine, particularly in the vascular system, where SSAO is distributed, would seem to be a worthwhile problem to investigate.

A pentylamine derivative, namely, 2-n-pentylamino-acetamide (Milacemide), has been shown to possess anticonvulsant activity (van Dorsser et al 1983). MAO-B is involved in the oxidation of this drug which releases glycine in the central nervous system, thus, contributing to its pharmacological activity (de Varebeke et al 1988). It is quite possible that SSAO could also be involved in the metabolism of Milacemide, especially since the affinities, i.e. the K_m values, regarding pentylamine are similar for both MAO-B and SSAO (Gufrroy et al 1983). However, SSAO-catalysed deamination probably occurs only in the vascular system, where the enzyme is located (Wibo et al 1980; Yu 1986). Semicarbazide, an SSAO inhibitor, however, did not seem to effect the level of milacemide in the rat urinary excretion (Strolin Benedetti et al 1988). The total SSAO activity should be much lower than the total MAO activity. The contribution of SSAO in the overall in-vivo deamination of amines, i.e. by examining the amines and their metabolites in the urine, would be insignificant. SSAO can be very important in certain tissues, such as in the vascular smooth muscles.

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