A Role for a New Vascular Enzyme in the Metabolism of Xenobiotic Amines

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Although it has long been thought that environmental toxins may play an underlying role in vascular diseases such as atherosclerosis, this concept is not supported by any clear-cut experimental evidence of toxic metabolism by cardiovascular enzymes. In this study, we demonstrate that allylamine, a selective cardiovascular toxin in vivo, is actively metabolized in vitro by a purified vascular enzyme (semicarbazide-sensitive amine oxidase), which has been localized recently to vascular smooth muscle cells. Oxidative deamination of allylamine to a highly toxic aldehyde, acrolein, was blocked through enzyme inhibition by semicarbazide-sensitive amine oxidase inhibitors, which obviate allylamine’s in vivo toxic manifestations. This unique demonstration of metabolism of a known in vivo cardiovascular toxin by semicarbazide-sensitive amine oxidase suggests that this vascular enzyme’s physiological role may include metabolism of exogenous amines. (Circulation Research 1990;66:249–252)

The highly selective cardiovascular toxin allylamine is a highly reactive unsaturated allylamine that has been used industrially in the organic synthesis of commercial products including rubber, pharmaceuticals, and plastics. The extent of industrial exposure to this chemical is difficult to ascertain, although several reports suggest that occasional occupational exposure and environmental contamination occur in this and other countries (see Reference 1 for review). Since the 1930s, there has been sporadic experimental interest in allylamine due to this compound’s extraordinary and selective ability to induce progressive acute myocardial necrosis and chronic vascular lesions.2,3

The first suggestion that vascular smooth muscle was involved in the toxicity of allylamine came in 1974 when Lalich and Paik4 showed that allylamine-induced lesions were markedly diminished in severity by the concomitant feeding of hydralazine, an antihypertensive agent with direct smooth muscle effects. We later showed a similar striking protective effect of other compounds5 and subsequently correlated this protective in vivo effect with the ability of these compounds to inhibit the metabolism of allylamine to a highly toxic aldehyde.6

A common feature of the compounds that protect against allylamine cardiovascular toxic damage is the inhibition by these compounds of the vascular enzyme semicarbazide-sensitive amine oxidase (SSAO), also known as “benzylamine oxidase” or “connective tissue amine oxidase.” We hypothesized that this little-studied vascular enzyme, which is produced by vascular smooth muscle cells, is responsible for the toxic metabolism of allylamine by vascular tissue.

In this study, using several sources of vascular SSAO (including purified enzyme), we demonstrate the oxidative deamination of allylamine to the aldehyde acrolein as a clear example of the capacity of vascular tissue to metabolize exogenous compounds. We further demonstrate that SSAO inhibitors that are capable of obviating in vivo and in vitro lesions act through enzyme inhibition and not through trapping the distal aldehyde metabolite. One underlying function of the vascular enzyme SSAO may be deamination of environmental amines.

Materials and Methods

Allylamine was incubated with three sources of SSAO: 1) homogenates of cultured porcine aortic smooth muscle cells procured and maintained by standard methods as previously described,7 2) media from cultured smooth muscle cells, which contain high SSAO activity,7 or 3) SSAO purified8 from smooth muscle cell culture media by established chromatographic methods. Controls consisted of blanks and homogenates of porcine aortic endothelial cells that contain no SSAO activity. Incubations were similar to the standard assay of SSAO activity9 except for the substitution of allylamine for benzylamine as substrate; enzyme source was preincubated in 0.1 M phosphate buffer in the presence of 0.22 mM
deprenyl 20 minutes before addition of allylamine (1.4 mM final concentration, 21°C, 240 μl total volume). Reactions were stopped at 5, 10, and 30 minutes by the addition of an equal volume of saturated solution of 2,4-dinitrophenylhydrazine (2,4-DNP) in 2 M sulfuric acid. The product of allylamine oxidative deamination, acrolein, was measured as the trapped 2,4-DNP adduct by previously described high-performance liquid chromatography (HPLC).10 HPLC was performed on an isocratic liquid chromatograph (model 330, Beckman Instruments, Fullerton, California) with an ultraspHERE ODS column (5-μm particle size, 25 cm×4.6 mm i.d.). Acetonitrile-water (1:1 vol/vol) was used as solvent at a flow rate of 1 ml/min. The column eluent was monitored at 356 nm. A volume of 40 μl was injected in each case. Comparisons of recovered product were made between groups by analysis of variance.

Experiments were also performed with semicarbazide, which has been shown to protect against allylamine-induced myocardial lesions in vivo,5 and phenelzine, which is a known inhibitor of SSAO and affords in vitro protection against allylamine-induced injury to vascular smooth muscle cells.11,12 The complete set of experiments described above were repeated except that the 20-minute preincubation (before addition of allylamine substrate) contained either semicarbazide or phenelzine (2.2 μM).

Results

Acrolein was actively produced by all samples tested, with brisk, time-dependent production by smooth muscle cell media and purified SSAO (Table 1). Indeed, during incubation of samples with purified SSAO, the odor of acrolein was evident, suggesting that a significant portion of the highly volatile aldehyde product (acrolein) was lost. Nevertheless, on a molar basis, the conversion of allylamine to acrolein by purified SSAO, based on recovered aldehyde, was high (24% at 5 minutes; 33% at 10 minutes; 50% at 30 minutes). Also, recovery between the three groups by use of different enzyme sources was significantly different at 10 and 30 minutes (p<0.01). Control homogenates of cultured porcine endothelial cells (which contain no SSAO activity)7 or blanks gave no detectable acrolein. After preincubation with the SSAO inhibitors semicarbazide or phenelzine (2.2 μM), no acrolein was detected in

incubations of allylamine with the three enzyme sources (lower limit of acrolein detection, 1 ng).

The possibility exists, however, that these inhibitors could actually be trapping aldehyde product rather than inhibiting the SSAO-mediated conversion of allylamine to acrolein. Such a mechanism could also attenuate the in vivo toxicity of allylamine4,5 by binding the distal toxic metabolite. Both semicarbazide and phenelzine readily form adducts with aldehydes, as does the other chemical with a previously demonstrated protective effect against allylamine-induced cardiovascular toxicity, hydralazine.5

To test this possibility, authentic standards of semicarbazide- and phenylethylamine-acrolein adducts (the latter as a potential product of phenelzine) were synthesized, and their chemical structures were confirmed by mass spectroscopy and nuclear magnetic resonance. The proton nuclear magnetic resonance spectra (Figure 1) of acrolein semicarbazone (I in Figure 1) gave the following (250 MHz, δ, D2O): 8.14 ppm (1H, d, J=9.16 Hz, C2-H), 7.14 ppm (1H, m, C3-H), and 6.16 ppm (2H, dd, J=10.3, 5.2 Hz, C4-H) (NH3 was exchanged with D2O). The proton nuclear magnetic resonance spectra of the acrolein phenethylamine adduct gave a mixture of two isomers Ia (10%) and IIb (90%) (250 MHz, δ, CDCl3): 9.98 ppm (1H, bs, CHO), 7.54 ppm (5-aromatic protons), 5.4–6.1 ppm (4H, m), 3.6 ppm (2H, dd, J=10.5 and 3.54 Hz), and 3.31 ppm (2H, dd, J=10.55 and 3.5 Hz).

HPLC methodology for detection of both adducts was established with a lower detection limit of 16 ng. No adduct was detected in any of the samples preincubated with semicarbazide or phenelzine. Hence, we conclude that the action of these compounds in obviating the biotransformation of allylamine to acrolein is due to direct inhibition of the enzyme SSAO and not secondary to trapping of the aldehyde.

Discussion

The concept that environmental chemicals, or xenobiotics, may play an underlying toxic role in causing human atherosclerotic disease is an older idea that, despite the numerous toxic substances in our environment, has had very little experimental confirmation.13 Although cardiovascular tissues such as myocardium, vascular smooth muscle, and endothelium are metabolically active insofar as they possess a variety of enzymes involved in the metabolism of
endogenous substances, no clear example of the metabolism of a xenobiotic vascular toxin by a cardiovascular enzyme exists. The mode of enzymic “detoxification” and “toxification” of xenobiotics has been studied and debated extensively in recent years, primarily with regard to a variety of hepatic enzyme preparations. A few such enzyme systems have been identified with relatively low activity in cardiovascular tissues, but studies of their potential significance have been rare.

SSAO is an enzyme that is distinct from other forms of monoamine oxidase (such as MAO A or MAO B) and has highest activity in blood vessels. MAO A and MAO B, which are present in liver, brain, and heart, have entirely different inhibitor characteristics from SSAO and apparently are not capable of metabolizing allylamine in vitro.

Although the metabolic function of SSAO is unknown, it has widespread distribution in mammalian species, and recently the enzyme has been localized to the smooth muscle cells of vessels. We have shown that when grown in vitro, porcine vascular smooth muscle cells express SSAO activity and release enzyme into their culture media; cultured endothelial cells or adventitia-derived fibroblasts do not have SSAO activity; thus, SSAO is further identified as a vascular smooth muscle enzyme.

In this study, we have definitively shown that the highly selective in vivo cardiovascular toxin allylamine is metabolized by SSAO. Enzymes localized in vascular tissue, such as SSAO, have been singularly neglected and are worthy of further study. Very recently, vascular SSAO has been shown to metabolize methylamine, an endogenous amine that may result as a breakdown product of creatinine, sarcosine, and catecholamines. In that study, the investigators propose that one physiological role of SSAO may be cellular protection from such endogenous amines. Our unique demonstration of enzymic biotransformation of a known in vivo cardiovascular

**FIGURE 1.** Chemical structures and 1H-NMR spectra of adducts expected from reaction of acrolein with semicarbazide-sensitive amine oxidase inhibitor semicarbazide (I) and phenylethylamine amine (II). Substances were synthesized and structures were confirmed by mass spectroscopy and proton nuclear magnetic resonance (see text for details); proton nuclear magnetic resonance spectra of the phenylethylamine-acrolein adduct gave a mixture of two isomers IIA (10%) and IIB (90%). High-performance liquid chromatography of incubated samples in repeated experiments showed that neither adduct was present (lower detection limit, 16 ng) after incubation; this absence confirms that enzyme inhibition is the mechanism through which semicarbazide-sensitive amine oxidase inhibitors obviate the metabolism of allylamine to acrolein, rather than “trapping” of the aldehyde. PPM, parts per million.
toxin suggests an additional role for this vascular enzyme, that is, the metabolism of exogenous amines in our environment with potentially deleterious cardiovascular effects.

References
11. Hysmith RM, Boor PJ: Role of benzylamine oxidase in the cytotoxicity of allylamine toward aortic smooth muscle cells. Toxicology 1988;51:133–145

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