

Semicarbazide-Sensitive Amine Oxidase Substrates Potentiate Hydralazine Hypotension: Possible Role of Hydrogen Peroxide

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ABSTRACT

The relation between inhibition of semicarbazide-sensitive amine oxidase (SSAO) and vasodilation by hydralazine (HYD) was evaluated in chloralose/urethane-anesthetized rats pretreated with various substrates of the enzyme and subsequently administered a threshold hypotensive dose of the vasodilator. The SSAO substrates benzylamine, phenethylamine, and methylamine potentiate the hypotensive response to HYD. Methylamine, which was studied in greater detail because of its status as a possible endogenous SSAO substrate, does not influence the response to the reference vasodilator pinacidil; it does enhance HYD relaxation in aortic rings obtained from pretreated rats. Experiments designed to identify the product of SSAO activity responsible for potentiation by methylamine suggest involvement of hydrogen peroxide (H_2O_2), as evidenced by

the findings that such potentiation is abolished by additional pretreatment with the H_2O_2 -metabolizing enzyme catalase, and that the plasma concentration of H_2O_2 is increased by methylamine and decreased by HYD. These results are interpreted as a substantiation of the relation between the known SSAO inhibitory effect of HYD and its vasodilator activity. Pretreatment with the SSAO substrates would increase production of H_2O_2 in vascular smooth muscle and thus magnify the influence of this vasoconstrictor agent on vascular tone. In these conditions, the decrease in H_2O_2 production and hence in vascular tone caused by SSAO inhibition by HYD would also be magnified. It is speculated that inhibition of vascular SSAO could represent a novel mechanism of vasodilation.

The mechanism of the vasodilator action of hydralazine (HYD) has been under continuous scrutiny since its introduction as an antihypertensive agent in the early 1950s, but to this date, no incontrovertible hypothesis to that effect has been forthcoming. Currently, the drug is thought to relax vascular smooth muscle by diminishing intracellular Ca^{2+} concentration through a mechanism different from that of traditional blockers of L-type Ca^{2+} channels (Orallo et al., 1991), either by opening $K_{Ca-1.1} K^+$ channels (Bang et al., 1998) or by inhibiting Ca^{2+} release from sarcoplasmic reticulum (Ellershaw and Gurney, 2001). The main limitation of this hypothesis is that it is based on *in vitro* experiments in which, at best, high micromolar concentrations of HYD are used, whereas hypotension can be detected *in vivo* with doses yielding much lower concentrations of the drug. For example, Ogiso et al. (1985) state that a plasma concentration of 0.02 $\mu\text{g/ml}$ (100 nM) lowers blood pressure by around 20 mm Hg in the awake rat. It is argued that such *in vitro/in vivo* differences can be accounted for, considering that after systemic administration, HYD is concentrated in the vicinity of vas-

cular smooth muscle (Baker et al., 1992), although the extent of this process in relation to plasma levels is unknown.

Based on observations of potentiation of HYD hypotension by isoniazid and other hydrazine derivatives (Vidrio, 1994), we have recently postulated inhibition of semicarbazide-sensitive amine oxidase (SSAO) as a possible mechanism of HYD vasodilation (Vidrio et al., 2002). Although the physiological or pathological role of SSAO is not completely known (Callingham et al., 1995), some of the characteristics of the enzyme make this supposition tenable. HYD, like other hydrazine derivatives, is a potent irreversible inhibitor of SSAO, with an IC_{50} in the nanomolar range when tested in rat aorta (Lyles and Callingham, 1982) and rat heart (Lyles et al., 1983). The enzyme is particularly abundant in vascular smooth muscle (Lyles and Singh, 1985), where it is located in the plasmalemma (Wibo et al., 1980). Such distribution would favor interaction with HYD, which is bound to extracellular protein in cultured vascular smooth muscle cells (Baker et al., 1992). Despite its vascular localization, involvement of SSAO in vascular function is not clear. One of the products of enzymatic activity is hydrogen peroxide (H_2O_2) (Callingham et al., 1995), and we have postulated that it is through this agent that SSAO could influence vascular tone

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(Vidrio et al., 2002). The ability of H_2O_2 to contract vascular smooth muscle has been documented in isolated preparations such as rat pulmonary artery (Jin and Rhoades, 1997), rat aorta (Rodríguez-Martínez et al., 1998), and human umbilical artery (Okatani et al., 1997). Multiple mechanisms appear to be involved in this effect (Yang et al., 1998; Shen et al., 2000). It is therefore conceivable that H_2O_2 formed in blood vessels by SSAO could contribute to vascular tone in these structures and that a decrease in product generation by in situ inhibition of the enzyme by HYD could reduce this tone, thereby producing hypotension.

The viability of this hypothesis was assessed in the present work by exploring the influence of pretreatment with various SSAO substrates, particularly methylamine (Lyles, 1995), on HYD hypotension in anesthetized rats. It was reasoned that increased availability of substrate would enhance production of H_2O_2 by SSAO, thereby increasing the proportion of vascular tone due to this agent. In these circumstances, a decrease in H_2O_2 production by HYD through SSAO inhibition would lead to a greater fall in pressure. Involvement of H_2O_2 in this potentiation was assessed by the use of the H_2O_2 -metabolizing enzyme catalase as well as by determining the influence of SSAO substrate and HYD on plasma concentration of this analyte. Enhancement of HYD action by pretreatment with an SSAO substrate was also explored in vitro in rat aortic rings.

Of the three SSAO substrates tested, benzylamine, phenethylamine, and methylamine, emphasis was placed on the last agent. Although methylamine shows the lowest affinity for SSAO (Lyles, 1995), it is not a substrate for monoamine oxidase (MAO), as benzylamine and specially phenethylamine are (Blaschko, 1952), thus making interpretation of results more straightforward. In addition, methylamine is a naturally occurring compound and is thought to be the endogenous substrate for SSAO (Lyles, 1995).

Materials and Methods

Animals. Studies were carried out in male Wistar rats weighing between 200 and 300 g, bred in the animal facilities of the Faculty of Medicine, Universidad Nacional Autónoma de México. They were subjected to a 12-h light/dark cycle and were maintained at 21–23°C; tap water and food pellets (5001 Rodent Laboratory Chow; Agribrands Purina Canada, Woodstock, ON, Canada) were available freely. Animals were brought daily to the laboratory for the experiments, which were conducted according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication 85–23, revised 1996).

Experiments in Anesthetized Rats. Animals were anesthetized with a mixture of chloralose, 50 mg/kg, and urethane, 800 mg/kg, administered i.p. Cannulas were placed in the trachea to facilitate spontaneous breathing, in a femoral artery for blood pressure recording, and in a femoral vein for drug administration. The arterial cannula was filled with heparin, 50 U/ml, to prevent clotting; no anticoagulant was administered systemically. Blood pressure was recorded on a Grass model 79 polygraph with a Gould-Statham P23ID transducer (Grass Instrument Division, Astro-Med, West Warwick, RI). High frequencies of the transducer signal were electronically filtered to obtain a smooth tracing approximately equivalent to mean blood pressure. Heart rate was simultaneously recorded on another channel of the polygraph with a Grass 7P4 cardiograph triggered by the pulse waves from the unfiltered transducer signal. Rectal temperature of the rats was maintained between 36.8° and 37°C with a Barnant temperature controller (Barnant Co., Bar-

rington, IL). After a stabilization period of at least 20 min, the test agents HYD or pinacidil were injected i.v. as a bolus, and recordings were continued for 60 min thereafter; pressure and heart rate values were tabulated at 10-min intervals. All experimental groups consisted of six rats.

Responses to HYD, 0.1 mg/kg, were obtained in control untreated groups and in groups pretreated with benzylamine, 400 mg/kg, phenethylamine, 100 mg/kg, or methylamine, 200 mg/kg, all administered i.p., 2 h before HYD. Metabolism of phenethylamine by MAO was prevented by additional pretreatment with pargyline, 30 mg/kg i.p., administered 30 min before phenethylamine. A control HYD group was run in parallel with each of these pretreatment groups; initial blood pressure values of each control group were matched as close as possible with its corresponding pretreatment group to make meaningful comparisons possible. Additional controls included a group pretreated with pargyline alone and two others, one untreated and one pretreated with methylamine as above, in which pinacidil, 0.2 mg/kg, was used as the test vasodilator instead of HYD. Three other sets of experiments involving methylamine pretreatment and HYD as the test vasodilator were conducted. In one experiment, metabolism of the amine was prevented with the SSAO inhibitor isoniazid, 10 mg/kg i.p., administered 30 min before methylamine. In the other two, accumulation of the methylamine metabolite H_2O_2 was blocked with the H_2O_2 scavenger catalase, 200,000 U/kg i.v., 15 or 60 min before HYD. Finally, the influence of the pretreatment agents methylamine and catalase by themselves on blood pressure and heart rate was determined. Recordings after methylamine were continued for 2 h, which was the pretreatment interval used for this agent.

Experiments in Rat Aortic Rings. Rats were anesthetized with sodium pentobarbital, 20 mg i.p. total dose, and subsequently sacrificed by cervical dislocation. The thoracic aorta was removed, and segments 0.5 cm long were obtained and suspended in a jacketed 20-ml organ chamber between two nickel/chromium wire hooks. One of the hooks was fastened to the bottom of the chamber and the other was attached to a Grass FT03 force transducer, which was connected in turn to a Grass model 79 polygraph (Grass Instrument Division). The baths contained Krebs-Henseleit solution of the following composition: 119 mM NaCl; 4.7 mM KCl; 1.1 mM $MgSO_4$; 1.2 mM KH_2PO_4 ; 2.5 mM $CaCl_2$; 25 mM $NaHCO_3$; 11 mM glucose; 0.02 mM EDTA; 0.6 mM ascorbic acid. The solution was kept at 37°C and was bubbled with a mixture of 95% O_2 and 5% CO_2 . The preparations were subjected to a resting tension of 2g, which was maintained constant throughout the experiments. After a stabilization period of at least 90 min during which the preparations were stimulated several times with 30 nM norepinephrine, integrity of the vascular endothelium was assessed by verifying that the contracted rings relaxed by at least 50% when challenged with 1 μM acetylcholine. Endothelium was removed in some rings by rubbing intraluminally with a 20-gauge hypodermic needle; in these preparations, absence of the endothelium was confirmed by a less than 10% relaxation upon acetylcholine challenge.

All rings were contracted with 30 nM norepinephrine and when responses induced by this agonist reached a plateau, cumulative concentration-response curves to HYD (3 μM to 1 mM) or pinacidil (300 nM to 100 μM) were constructed in groups of eight or nine rings with or without endothelium. Some preparations were obtained from control untreated rats and others from animals pretreated 15 h before sacrifice with methylamine, 400 mg/kg i.p. For comparison, blood pressure and heart rate responses to HYD, 0.1 mg/kg, were determined in anesthetized rats subjected to this pretreatment schedule.

Determination of H_2O_2 in Plasma. Rats were anesthetized with chloralose/urethane as described above, and blood was obtained by cardiac puncture. Groups of 12 animals received either no pretreatment, methylamine, 200 mg/kg i.p. 2 h before anesthesia, HYD, 0.1 mg/kg, or catalase, 200,000 U/kg, the latter two agents injected in the penile vein while under anesthesia, 15 min before cardiac punc-

ture. A combination of methylamine and HYD, at the doses and intervals noted, was also tested. Of the blood obtained from each rat, two 1-ml aliquots were deposited in conical polypropylene centrifuge tubes containing 100 μ l of heparin, 100 U/ml; the tubes were maintained over ice. To one of the tubes of each pair, 1.5 ml of acetone was added to precipitate proteins; these tubes were used for direct determination of H_2O_2 (see below). All tubes were centrifuged at 6000 rpm for 30 min. A 500- μ l aliquot of plasma or plasma/acetone mixture was deposited in an electrolysis cell containing 2 ml of 0.1 Tris buffer, pH 7.8, from which dissolved O_2 had been previously removed by bubbling with N_2 for 10 min. Determinations were carried out by differential pulse polarography as described previously (Díaz-Arista et al., 2002) using a Metrohm 622 polarograph with an integrated recorder (Metrohm Ltd, Herisau, Switzerland). The analyte was determined directly in the plasma/acetone samples and indirectly by treating the plasma samples without acetone with 50 μ l of catalase, 20,000 U/ml, and measuring the O_2 thus released from the H_2O_2 contained in the sample. For each analytical run, calibration curves were constructed with dilutions of a standard solution of H_2O_2 , in which the actual concentration of the analyte was determined by normalization with $KMnO_4$ in H_2SO_4 and oxalic acid. In the case of curves for indirect determinations, O_2 was released by mixing for 10 min with appropriate amounts of catalase.

Drugs and Chemicals. The hydrochlorides of benzylamine, methylamine, β -phenylethylamine, pargyline, (\pm)-norepinephrine, and HYD, as well as heparin sodium, acetylcholine chloride, isoniazid, chloralose, and urethane were obtained from Sigma-Aldrich (St. Louis, MO). Pinacidil was purchased from Sigma/RBI (Natick, MA). Doses refer to the salts where applicable. All other chemicals were reagent grade.

For in vivo experiments, drugs were dissolved in 0.9% NaCl solution, aided in the case of pinacidil by a drop of 0.1 N HCl; the volume of injection was 1 ml/kg. For in vitro work, stock solutions of HYD (100 mM), acetylcholine (1 mM), and pinacidil (10 mM) were prepared daily in Krebs-Henseleit solution, in the case of pinacidil with the addition of HCl. Norepinephrine (1 mM) was dissolved in 0.1% ascorbic acid. In all cases, appropriate dilutions from these stock solutions were made with Krebs.

Data Presentation and Statistical Analysis. Results are presented as means \pm S.E.M. Mean blood pressure and heart rate are depicted in mm Hg and beats/min, respectively; aortic ring relaxation is reported as percentage of inhibition of norepinephrine contraction, and H_2O_2 plasma content as micromolar concentration. Pressure and heart rate values at each 10-min tabulation period of pretreated rats were compared with the corresponding controls by unpaired *t* tests or one-way analysis of variance followed by Dunnett's test for single or multiple comparisons, respectively. In cases of nonhomogeneous variances among groups, the nonparametric Mann-Whitney or Kruskal-Wallis procedures were used. Relaxation of aortic rings at each drug concentration as well as plasma H_2O_2 in the pretreated and control groups were also compared by these tests. In the experiments with methylamine and catalase alone, all post-drug values were compared with time 0 figures by repeated-measures analysis of variance and Dunnett's test. In all cases, a probability level of less than 0.05 was accepted as significant. The aortic ring results were additionally evaluated by subjecting individual concentration-response curves to nonlinear regression analysis to calculate the negative logarithm of the EC_{50} (pEC_{50}). Statistical procedures and regression analyses were carried out with a GraphPad Prism 3.0 package (GraphPad Software Inc., San Diego, CA).

Results

Experiments in Anesthetized Rats. In control unpretreated animals, the test dose of HYD decreased mean blood pressure by not more than 15 mm Hg (Figs. 1, 2, 3, and 6). Maximum effects tended to occur 10 min after administration, with a slow subsequent recovery evident in some groups. The accompanying changes in heart rate were minimal. This pattern of response was unchanged after pretreatment with pargyline alone (Fig. 1B). Pressure responses to this same dose of HYD were considerably enhanced at practically all observation times, after pretreatment with benzylamine (Fig. 1A), phenethylamine (Fig. 1B), or methylamine (Fig. 2A); in contrast, the effect of the reference

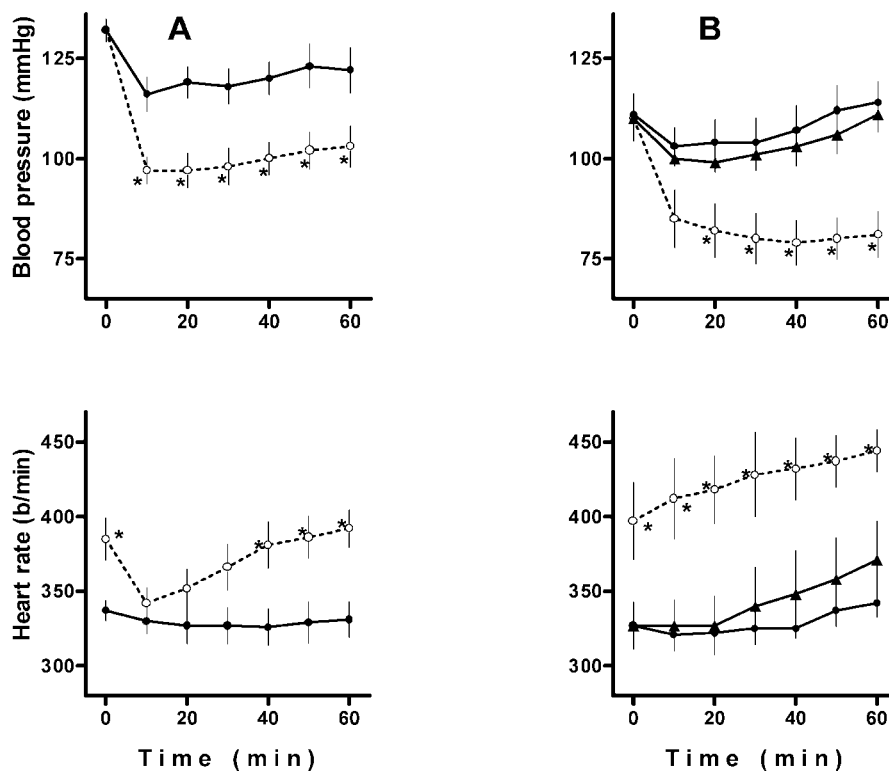


Fig. 1. Mean blood pressure and heart rate after administration of HYD, 0.1 mg/kg i.v. to control rats (solid circles) or to rats pretreated 2 h previously (open circles) with benzylamine, 400 mg/kg i.p. (A) or phenethylamine, 100 mg/kg i.p. (B). Animals in panel B also received pargyline, 30 mg/kg i.p., 30 min before phenethylamine; an additional group received pargyline alone (solid triangles). Symbols correspond to means of six animals; vertical lines are S.E.M. Asterisks indicate significant differences from control. Abscissae denote time in minutes after HYD injection; ordinates, blood pressure and heart rate in mm Hg and beats/min, respectively.

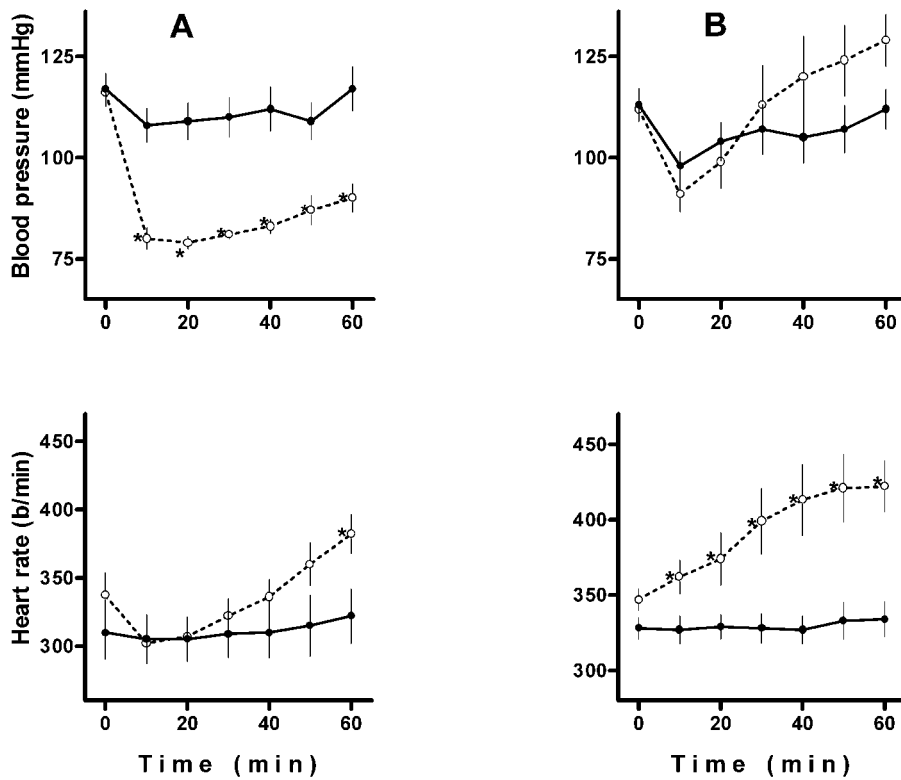


Fig. 2. Mean blood pressure and heart rate after administration of HYD, 0.1 mg/kg i.v. (A) or pinacidil, 0.2 mg/kg i.v. (B) to control rats (solid circles) or to rats pretreated 2 h previously with methylamine, 200 mg/kg i.p. (open circles). Symbols correspond to means of six animals; vertical lines are S.E.M. Asterisks indicate significant differences from control. Abscissae denote time in minutes after vasodilator injection; ordinates, blood pressure and heart rate in mm Hg and beats/min, respectively.

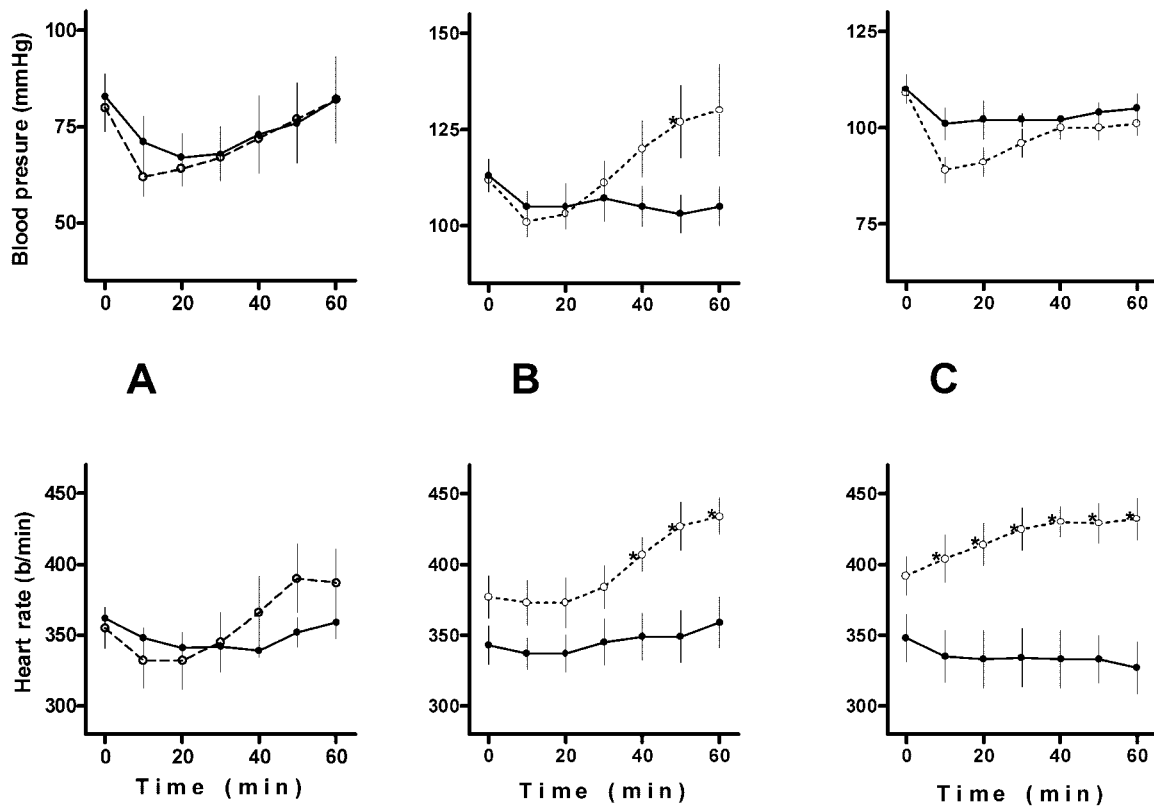


Fig. 3. Mean blood pressure and heart rate after administration of HYD, 0.1 mg/kg i.v. to control rats (solid circles) or to rats pretreated with methylamine, 200 mg/kg i.p. 2 h previously (open circles). In addition, animals received isoniazid, 10 mg/kg i.p. 30 min before methylamine (A) or catalase, 200,000 U/kg i.v., either 15 min (B) or 60 min (C) before HYD. Symbols correspond to means of six animals; vertical lines are S.E.M. Asterisks indicate significant differences from control. Abscissae denote time in minutes after HYD injection; ordinates, blood pressure and heart rate in mm Hg and beats/min, respectively.

vasodilator pinacidil was unchanged after methylamine (Fig. 2B). Heart rates, which were increased at time 0 in the benzylamine and phenethylamine groups, tended to rise after all pretreatments upon injection of HYD or pinacidil. The potentiation of HYD hypotension by methylamine was prevented by previous administration of isoniazid (Fig. 3A), as well as by pretreatment with catalase, administered either 15 or 60 min before the vasodilator (Fig. 3, B and C). Isoniazid but not catalase also diminished the slowly developing tachycardia after HYD. Methylamine, administered alone at the HYD potentiating dose of 200 mg/kg, lowered blood pressure within 10 min of injection, an effect which tended to subside over the next 2 h. Heart rate also decreased initially, but later increased persistently (Fig. 4A). Catalase alone produced a late fall in blood pressure, accompanied by a progressive increase in heart rate (Fig. 4B).

Experiments in Rat Aortic Rings. The pretreatment schedule used for methylamine in the above experiments, i.e., 200 mg/kg 2 h previously, did not influence the relaxant effects of HYD in rings obtained from animals thus pretreated (results not shown). Based on exploratory tests, the dose used in the definitive experiments was increased to 400 mg/kg and the pretreatment interval to 15 h. Such pretreatment did not influence contractile responses to norepinephrine, either in rings with endothelium (control, 1.84 ± 0.08 g; pretreated, 1.92 ± 0.06 g), or without endothelium (control, 1.92 ± 0.06 g; pretreated, 1.90 ± 0.08 g). Intact or rubbed preparations from rats receiving methylamine showed enhanced relaxant responses to HYD, but not to pinacidil (Fig. 5). Table 1 shows the pEC₅₀ values derived from these concentration-response curves. Values for HYD were increased by approximately 0.4 log, whereas those for pinacidil showed no significant change. As shown in Fig. 6, methylamine at this pretreatment schedule also potentiated HYD hypotension.

Determination of H₂O₂ in Plasma. Concentrations of H₂O₂ in the different experimental groups are shown in Table 2. Direct measurements of the analyte agreed closely with indirect determinations based on O₂ released by catalase. Methylamine increased and HYD decreased H₂O₂ levels; the combination of both agents yielded values similar to control. As expected, catalase lowered concentrations of H₂O₂.

Discussion

The present study was carried out in anesthetized rats using the hypotensive response to HYD as an index of the vasodilator activity of the drug. It is well known that responses to cardiovascular agents in general are modified by anesthesia, making results difficult to interpret. In the case of HYD, blood pressure responses in conscious animals are accompanied by marked reflex cardiac stimulant reactions that greatly modify the pressure effects. In anesthetized subjects, this factor is eliminated, as shown in the present experiments by the unchanged heart rate after HYD in the control groups, so that changes in blood pressure more accurately reflect changes in vascular dynamics.

The metabolism of benzylamine, phenethylamine, and methylamine by SSAO has been well studied in various *in vitro* conditions (Precious and Lyles, 1988; Elliott et al., 1989; Lyles et al., 1990). *In vivo* information is available only for methylamine and is restricted to documentation of increases in the urinary excretion of the substrate after inhibition of SSAO (Lyles and McDougall, 1989) or of formaldehyde, one of the products of enzyme activity after administration of exogenous amine (Deng et al., 1998). In the absence of data on the time course and extent of *in vivo* metabolism of these amines by SSAO, the pretreatment intervals as well as the doses used in the present study were chosen on the basis of results

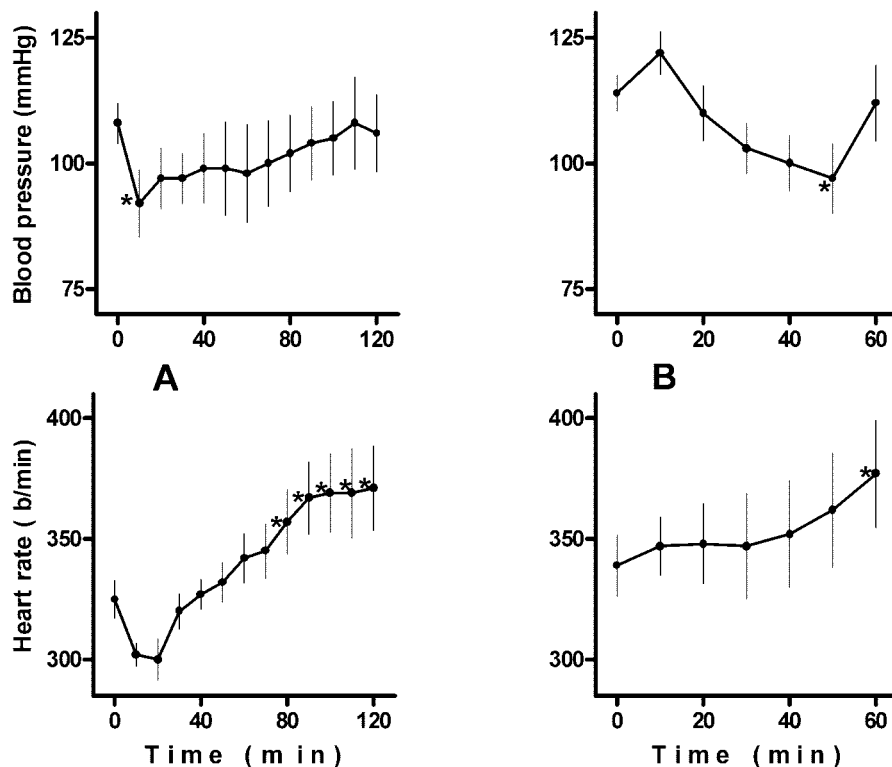


Fig. 4. Mean blood pressure and heart rate after administration of methylamine, 200 mg/kg *i.v.* (A) and catalase, 200,000 U/kg *i.v.* (B) to control unpretreated rats. Symbols correspond to means of six animals; vertical lines are S.E.M. Asterisks indicate significant differences from zero time values. Abscissae denote time in minutes after injection; ordinates, blood pressure or heart rate in mm Hg and beats/min, respectively.

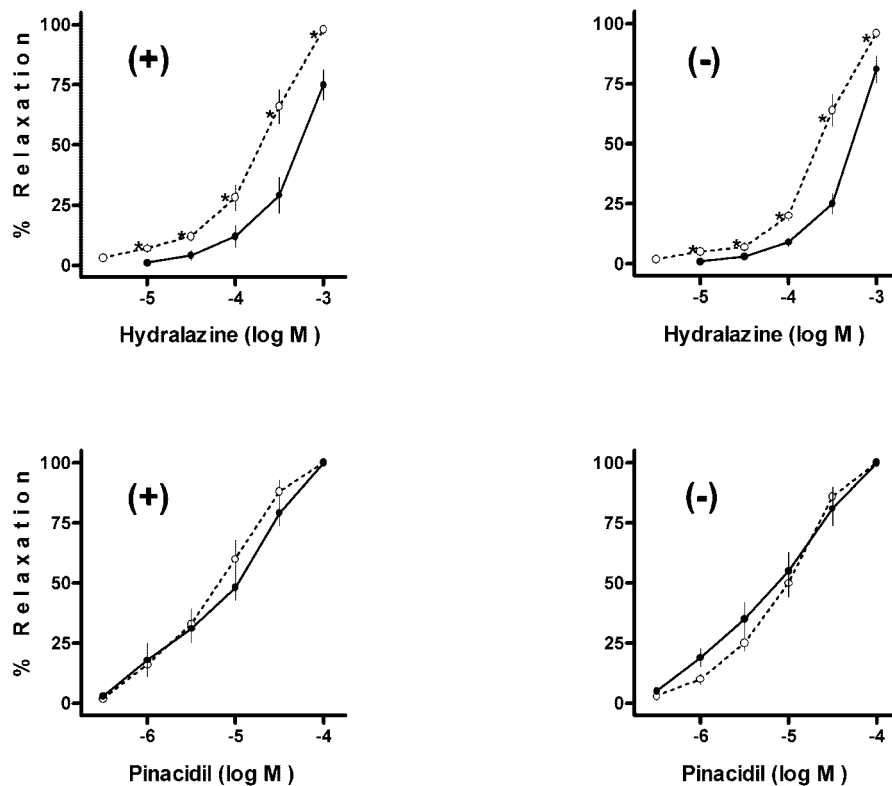


Fig. 5. Concentration-response curves to HYD and pinacidil in norepinephrine-contracted aortic rings obtained from control rats (solid circles) or from rats pretreated 15 h previously with methylamine, 400 mg/kg i.p. (open circles). Functional endothelium was either present (+) or absent (-) in the preparations. Symbols correspond to means of eight or nine rings; vertical lines are S.E.M. Asterisks indicate significant differences from control. Abscissae denote log concentrations of the vasodilators; ordinates, relaxation as percentage of contractile response to norepinephrine.

TABLE 1

pEC₅₀ values for HYD and pinacidil in aortic rings from rats with and without pretreatment with methylamine, 400 mg/kg i.p. 15 h previously

Values are means \pm S.E.M. of 8 or 9 experiments. Rings with (+) and without (-) endothelium.

Group	Control	Methylamine
HYD (+)	3.31 \pm 0.10	3.74 \pm 0.06 ^a
HYD (-)	3.28 \pm 0.05	3.65 \pm 0.05 ^a
Pinacidil (+)	5.12 \pm 0.11	5.22 \pm 0.12
Pinacidil (-)	5.17 \pm 0.13	5.06 \pm 0.07

^a $P < 0.05$ versus control.

of preliminary experiments. The relatively low dose of phenethylamine reflects its limiting central nervous system stimulant activity (Jackson, 1972), whereas the high dose needed for benzylamine is a consequence of its biotransformation by both SSAO and MAO (Blaschko, 1952).

The finding that all three SSAO substrates potentiate hypotension by HYD specifically is compatible with the postulated involvement of SSAO inhibition in pressure responses to the vasodilator. The documentation of potentiation in aortic rings also with or without endothelium further indicates that such involvement occurs in vascular smooth muscle, the site at which both SSAO activity and HYD inhibition thereof are postulated to take place. It is conceivable that preparations obtained from rats pretreated with methylamine contain increased amounts of metabolites of the amine, generated by SSAO and involved in vascular tone regulation; inhibition of the enzyme by HYD therefore results in enhanced relaxation. The higher doses of methylamine and the longer pretreatment interval necessary to demonstrate this effect in aortic rings probably reflects the inevitable partial washout of methylamine and/or its metabolites during tissue

preparation. In addition, HYD hypotension *in vivo* is more directly related to relaxation of resistance vessels, and it is possible that a conductance artery such as the aorta would be less sensitive to changes in reactivity to HYD.

Involvement of a product of SSAO action in HYD potentiation is supported by the experiments with isoniazid. This drug is a potent SSAO inhibitor (Vidrio et al., 2002), and when administered before methylamine, it presumably blocks metabolism of the amine by this enzyme. As a result, accumulation of SSAO products is prevented and potentiation of HYD hypotension is no longer apparent (Fig. 3A). It should be noted that rats pretreated with methylamine and isoniazid have a low baseline blood pressure (80 mm Hg), which contrasts with that of groups receiving methylamine alone (112 and 117 mm Hg). This suggests that the unmetabolized amine exerts a long-lasting hypotensive effect. The products generated by SSAO from benzylamine, phenethylamine, and methylamine are benzaldehyde, phenylacetaldehyde, and formaldehyde, in addition to ammonia and H₂O₂. As a group, these aldehydes are unlikely candidates for mediating increased vascular tone, since benzaldehyde and phenylacetaldehyde lower blood pressure (Wingard et al., 1955), and only formaldehyde is a pressor agent, albeit acting through release of catecholamines (Egle and Hudgins, 1974). At toxic concentrations, ammonia also increases blood pressure (Hindfelt and Plum, 1975), whereas H₂O₂ exerts vasoconstriction (Jin and Rhoades, 1997; Okatani et al., 1997; Rodríguez-Martínez et al., 1998; Yang et al., 1998; Shen et al., 2000). The fact that HYD potentiation by methylamine is prevented by the H₂O₂ scavenger catalase identifies this species as the metabolite involved in the hypotensive effect of HYD. It should be noted that the preventive action of catalase is clearer at the pretreatment interval of 15 min, compared with that of 60 min. This is in keeping with the half-life

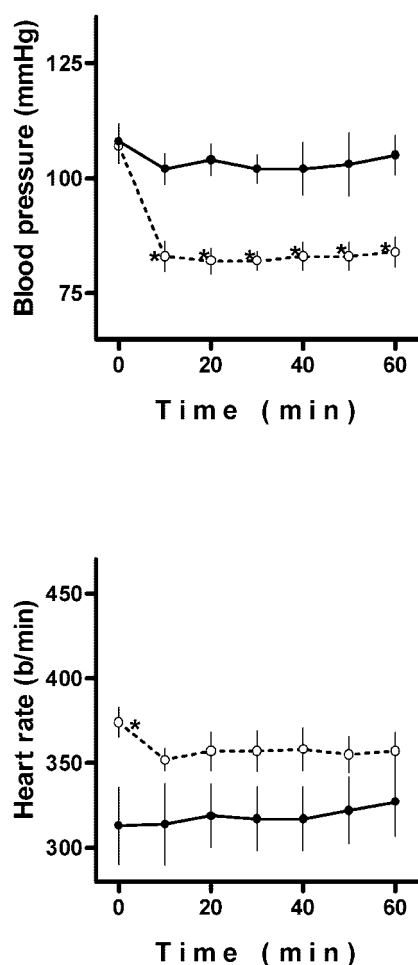


Fig. 6. Mean blood pressure and heart rate after administration of HYD, 0.1 mg/kg i.v. to control rats (solid circles) or to rats pretreated 15 h previously with methylamine, 400 mg/kg i.p. (open circles). Symbols correspond to means of six animals; vertical lines are S.E.M. Asterisks indicate significant differences from control. Abscissae denote time in minutes after HYD injection; ordinates, blood pressure and heart rate in mm Hg and beats/min, respectively.

TABLE 2

Influence of methylamine (200 mg/kg i.p. 2 h previously), HYD (0.1 mg/kg i.v. 15 min previously), a combination of both pretreatments, and catalase (200,000 U/kg i.v. 15 min previously) on plasma H_2O_2 concentration (μM) determined by direct and indirect polarographic methods

Values are means \pm S.E.M. of 12 experiments.

Group	Direct	Indirect
Control	15.25 \pm 0.33	15.32 \pm 0.29
Methylamine	23.19 \pm 0.52 ^a	24.08 \pm 0.37 ^a
HYD	11.28 \pm 0.18 ^a	10.74 \pm 0.19 ^a
Methylamine + HYD	15.28 \pm 0.90	14.75 \pm 0.32
Catalase	8.49 \pm 0.64 ^a	7.34 \pm 0.59 ^a

^a $P < 0.01$ versus control.

of around 20 min reported for the i.v. injected natural enzyme (Turrens et al., 1984).

Additional confirmation of the involvement of H_2O_2 in the methylamine/HYD interaction can be derived from determinations of this oxygen species in plasma. When administered at the schedule used in the blood pressure experiments, methylamine raises H_2O_2 , presumably by increasing availability of SSAO substrate. The opposite effect is apparent after enzyme inhibition with a hypotensive dose of HYD,

when sufficient time (15 min) is allowed for the depressor response to develop. HYD also suppresses the increase in H_2O_2 elicited by methylamine. The experiments with catalase confirm that the dose antagonizing HYD potentiation by methylamine is effective in lowering H_2O_2 levels. No opinion can be formulated as to the adequacy of the H_2O_2 concentration of approximately 15 μM observed in control animals, judging from the fact that reported values for normal rat plasma vary from 2 μM determined by an electrochemical method (Swei et al., 1997) to 45 μM by a radioisotopic procedure (Varma and Devamanoharan, 1991). It should be noted that in view of the unreliability of the methods commonly used to measure H_2O_2 in plasma (Nahum et al., 1988), the specificity of the present procedure was successfully verified as recommended by these authors by adding catalase to the measured samples to suppress the recorded direct signal; adding exogenous H_2O_2 increments the recorded direct signal.

If H_2O_2 generated in vascular smooth muscle contributes to vasoconstrictor tone, increases of this agent after administration of methylamine and, presumably, benzylamine and phenethylamine would be expected to raise blood pressure; however, with the possible exception of benzylamine, no such effect was observed. In fact, methylamine by itself tends to lower blood pressure, and rats pretreated with the presumably unmetabolized amine show a low baseline pressure. These findings imply that the blood pressure effects of methylamine are not due exclusively to generation of H_2O_2 , but that other metabolites as well as the original amine may contribute to the overall response. Such complexity is not apparent with catalase, since its hypotensive effect (Fig. 4B) can be regarded as being related to lowering of H_2O_2 levels. On the other hand, vascular tone regulation involves the local production of relaxing (nitric oxide, prostacyclin) and contracting (endothelins) factors. Normally, the relaxing factors predominate (Lüscher, 1990), so that any enhanced vasoconstrictor influence of H_2O_2 would be efficiently counterbalanced and blood pressure would remain unchanged. Thus, the SSAO substrates would increase only the proportion of vascular tone due to H_2O_2 , and subsequent enzyme inhibition by HYD would result in a greater than normal fall in blood pressure. It should be noted that the potential role of H_2O_2 in the regulation of vascular tone appears to be very complex, and since in some experimental conditions it can also produce vascular smooth muscle relaxation (Burke and Wolin, 1987; Barlow and White, 1998), it can be regarded as both a contracting and a relaxing factor (Wolin, 1991).

Rats pretreated with benzylamine had a baseline blood pressure of 132 mm Hg, higher than any of the other experimental groups. This increased blood pressure is not apparent in Fig. 1 because the corresponding control group was matched in baseline pressure with the pretreated group, as described under *Materials and Methods*. The influence of benzylamine on blood pressure can be ascribed to an action not related to H_2O_2 generation, namely blockade of catecholamine uptake by sympathetic nerve endings (Elliott and Callingham, 1991). This action could also account for the baseline tachycardia after benzylamine and phenethylamine (Fig. 1), although with the latter, its prominent cardiac sympathomimetic effect (Pesce and Adler-Graschinsky, 1983) could also be involved. Although methylamine did not significantly increase resting heart rate except at the higher dose

tested (400 mg/kg), it did uncover a slowly developing tachycardia after administration of HYD or pinacidil (Fig. 2). The present data provide no information as to the nature of this response, although they do suggest that it is partially dependent on increased amine metabolites, since it is considerably diminished by additional isoniazid pretreatment (Fig. 3A). That the metabolite involved is not H_2O_2 is shown by the persistence of tachycardia after catalase pretreatment (Fig. 3, B and C). Unmasking of a reflex reaction to vasodilator-induced hypotension is unlikely because tachycardia occurred indistinctly in the presence of marked (Fig. 2A) or minimal (Fig. 3, B and C) hypotension.

In conclusion, pretreatment of rats with various SSAO substrates enhances the hypotensive effect of HYD, apparently through increased generation of the SSAO vasoconstrictor product H_2O_2 . These results are compatible with the hypothesis that HYD vasodilation involves inhibition of vascular SSAO and decreased production of H_2O_2 . They further suggest that such inhibition could represent a novel mechanism of hypotensive activity, possibly shared by other known antihypertensive drugs (Banchelli et al., 1986).

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