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Mechanisms mediating the vasodilatory effects of *N*-hydroxy-L-arginine in coronary arteries

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Abstract

We assessed the mechanisms of action of N^G-hydroxy-L-arginine in isolated porcine large coronary arterial rings. Increasing (1, 10 and 100 μM) concentrations of N^G-hydroxy-L-arginine evoked endothelium-dependent dilation which was eliminated by 100 μM of N^G-nitro-L-arginine methyl ester, but not affected by a cytochrome P_{450} inhibitors (miconazole or 7-ethoxyresorufin). At a given concentration, the dilatory response to N^G-hydroxy-L-arginine was stronger than that elicited by L-arginine. N^G-Hydroxy-L-arginine (100 μM), but not N^G-hydroxy-D-arginine, potentiated the endothelium-dependent dilation of calcium ionophore A23187 but had no effect on endothelium-independent dilation evoked by an NO donor. NO release by endothelium-intact porcine coronary arterial rings was measured with a chemiluminescence analyser. A23187 (10 μM), N^G-Hydroxy-L-arginine (100 μM), and to a lesser extent N^G-hydroxy-D-arginine (100 μM), significantly increased NO concentration over 15 min observation period. When A23187 and N^G-hydroxy-L-arginine were combined, NO concentration increased in an additive fashion. Enhanced NO release by either A23187, N^G-hydroxy-L-arginine or N^G-hydroxy-D-arginine was attenuated by N^G-nitro-L-arginine methyl ester. We conclude that N^G-hydroxy-L-arginine exerts its effects on the contractility of coronary arteries by acting as a substrate for the endothelial nitric oxide synthase leading to enhanced NO production. Cytochrome P_{450} were not involved the dilatory response to N^G-hydroxy-L-arginine. In this respect, porcine coronary arteries differ significantly from cultured smooth muscle cells in metabolising N^G-hydroxy-L-arginine.

Keywords: Nitric oxide (NO); Vasodilation; Coronary circulation; Nitric oxide (NO) synthase

1. Introduction

The synthesis of nitric oxide (NO) and L-citrulline from L-arginine by a group of hemoproteins, NO synthases, is now recognized as an important function in the physiology of a variety of systems including cardiovascular, nervous and immune systems (Knowles and Moncada, 1994). The exact mechanisms by which NO synthases catalyse the conversion of L-arginine to L-citrulline and NO remain unknown. The only known intermediate in the synthesis of NO by NO synthases is *N*-hydroxy-L-arginine which is formed by the oxidation of L-arginine by the NO synthases (Zembowicz et al., 1991; Klatt et al., 1993; Stuehr et al., 1991). Since that discovery, several investigators have assessed the vascular effects of exogenous *N*-hydroxy-L-arginine. In bioassay system using endothelial cells, *N*-hydroxy-L-arginine induced vascular dilation by enhancing endothelial NO synthase activity, reacting with NO to form a stable adduct and releasing endothelium-derived relaxing factor which is distinct from NO (Zembowicz et al., 1992a,b,c, 1991). It has been proposed recently that *N*-hydroxy-L-arginine is utilized by cytochrome P_{450} to produce NO in smooth muscle cells and hepatic microsomes (Boucher et al., 1992; Schott et al., 1994).

Unlike cultured cell preparations, the effect of exogenous *N*-hydroxy-L-arginine on vascular reactivity is variable. In isolated rabbit aortas, *N*-hydroxy-L-arginine failed to induce dilation and did not potentiate the dilatory response to endothelium-dependent dilators (Zembowicz et al., 1992a). Systemic infusion of *N*-hydroxy-L-arginine in rats, on the other hand, reversed the effects of NO synthase inhibitors on arterial blood pressure and renal blood flow (Walder et al., 1992). In freshly isolated bovine pulmonary arterial rings, *N*-hydroxy-L-arginine elicits a small dilation which is enhanced after the depletion of L-arginine (Wal-

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lace et al., 1991). It has been postulated that this dilatory effect of *N*-hydroxy-L-arginine is due to enhanced NO release by the inducible NO isoform in the vascular smooth muscle cells (Zembowicz et al., 1991) or to the formation of hydroxylamine (DeMaster et al., 1989). Part of the confusion in interpreting these results has been the lack of measurement of NO release in response to *N*-hydroxy-L-arginine. Moreover, the effects of *N*-hydroxy-L-arginine on the contractility of blood vessels have not been compared with the D-isomer (*N*-hydroxy-D-arginine). In the current study, we measured contractility and NO production by in-vitro isolated porcine coronary arteries to assess the following aspects of *N*-hydroxy-L-arginine: (1) potency and endothelium-dependency of the vasoactive effect of *N*-hydroxy-L-arginine; (2) contribution of NO synthase and cytochrome P_{450} enzymes to the dilatory effects of *N*-hydroxy-L-arginine; (3) whether hydroxylamine mediates the dilatory response to *N*-hydroxy-L-arginine; (4) interaction of *N*-hydroxy-L-arginine with endothelium-dependent and endothelium-dependent dilators.

2. Materials and methods

2.1. Materials

Miconazole was obtained from Cedarlane Laboratories (Ontario, Canada). *N*-Hydroxy-L-arginine and 3-morpholino-sydnonimine-hydrochroloride (Sin-1) were obtained from Biomol (Plymouth Meeting, PA, USA). *N*-Hydroxy-D-arginine (purity $\geq 98\%$) was synthesized by Paul E. Feldman, Glaxo Research Institute (Research Triangle Park, NC, USA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation

Pig hearts were obtained locally immediately after slaughter. The hearts were removed and placed in a cold PBS solution previously bubbled with a 95% O_2-5% CO_2 gas and transported to the laboratory. The left anterior descending and circumflex coronary arteries were excised and cleaned of adherent connective tissues. The arteries were cut into rings (4 mm in length) and suspended in 25-ml organ baths filled with a warmed modified Krebs-Ringer bicarbonate solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11.1 glucose, 0.026 calcium-EDTA and 0.01 indomethacin). Arterial rings were suspended by means of two stainless steel stirrups. One of the stirrups was anchored to the bottom of the organ chamber and the other was connected to a force transducer (Grass FT03) to record changes in isometric force. The rings were stretched over 60 min to a baseline tension of 6 g which produces maximum active tension in response to 80 mM KCl stimulation. After 60 min of equilibration, the rings were contracted with prostaglandin $F_{2\alpha}$ (5 μM) to elicit tension equivalent to 60–80% of maximum. To confirm the presence of the endothelium, arterial rings were exposed initially to 0.1 μM bradykinin at the plateau phase of $PGF_{2\alpha}$-induced contractions. At this concentration, bradykinin completely dilates preconstricted coronary arteries. In a few vessels, the endothelium was removed by rubbing with a blunt forceps. Reagents were added directly to the baths. In a series of experiments, cumulative concentration-response curves to A23187 and Sin-1 were produced in the absence or presence of *N*-hydroxy-L-arginine or *N*-hydroxy-D-arginine which were added to the baths 15 min before. Similarly, N^G-nitro-L-arginine methyl ester, miconazole or 7-ethoxyresorufin were added to the bath 15 min before the addition of reagents in a few experiments. Relaxations were expressed as percentage of maximum (elicited by 10 μM of Sin-1).

2.3. NO measurements

NO release in our experiments was measured using modified acid refluxing technique (Archer, 1993). Two to three coronary arterial rings (4 mm in length) were placed into 5-ml airtight glass vials (Sepulco) and bathed with 500 μl modified Krebs-Ringer bicarbonate solution containing 100 U/ml SOD and previously bubbled with 95% O_2-5% CO_2 gas. Temperature was maintained at 37°C. *N*-Hydroxy-L-arginine, *N*-hydroxy-D-arginine or A23187 solutions were added directly to the vials in a volume not exceeding 10 μl. Baseline and peak samples (50 μl) were obtained immediately and 15 min after the addition of these reagents, respectively. We found in preliminary experiments that NO concentration peaks within 15 min of the addition of endothelial NO synthase stimulants with no change thereafter. Samples were injected into a heated (80°C) glass purging chamber containing 5 ml of 1% NaI in glacial acetic acid which is continuously being purged (30–40 ml/min) with argon. The evolved NO was measured with a NO chemiluminescence analyser (Sievers Model 270). The electronic output was acquired with a PC based A/D conversion program (DATAQ, Ohio, USA). Signals were integrated (to calculate the area under the curve. Calibration was performed by injecting a fixed volume (50 μl) of different concentrations of standard sodium nitrite solutions.

2.4. Experimental protocols

Four protocols were conducted in three of which contractility was measured after preconstriction with 5 μM of $PGF_{2\alpha}$. Eight vessels were used in each experiment.

2.4.1. Protocol 1

In separate groups of arteries, endothelium-dependence of the dilatory response to *N*-hydroxy-L-arginine was assessed by measuring changes in tension of endothelium-in-

tact and endothelium-denuded arteries to increasing concentrations of *N*-hydroxy-L-arginine (1, 10 and 100 μM). Each concentration was maintained until tension reached a stable level. Similar protocols were used with *N*-hydroxy-D-arginine and L-arginine.

2.4.2. Protocol 2

The contributions of NO synthase and cytochrome P_{450} enzyme activities to the dilatory response of *N*-hydroxy-L-arginine were assessed by measuring changes in tension of preconstricted endothelium-intact vessels to 100 μM of *N*-hydroxy-L-arginine in the absence and then in the presence of either an NO synthase inhibitor (N^G-nitro-L-arginine methyl ester, 100 μM) or cytochrome P_{450} inhibitors (miconazole or 7-ethoxyresorufin, 100 μM). These inhibitors were added to the bath 30 min before preconstriction with $PGF_{2\alpha}$. Similar protocols were used with *N*-hydroxy-D-arginine (100 μM), L-arginine (100 μM) and hydroxylamine (10 μM).

2.4.3. Protocol 3

In this protocol we assessed the interactions between *N*-hydroxy-L-arginine and endothelium-dependent and endothelium-independent dilators. In separate groups of endothelium-intact arteries, concentration-response curves to calcium ionophore A23187 were constructed either in the absence or the presence of *N*-hydroxy-L-arginine (100 μM) or *N*-hydroxy-D-arginine (100 μM). *N*-hydroxy-L- and D-arginine were added to the bath 20 min before preconstriction with $PGF_{2\alpha}$. Similarly, concentration-response curves were constructed in endothelium-denuded arteries for endothelium-independent dilator, NO donor, (Sin-1), either in the absence or presence of *N*-hydroxy-L-arginine or *N*-hydroxy-D-arginine.

2.4.4. Protocol 4

The release of NO in response to calcium ionophore A23187 (10 μM), L-*N*-hydroxy-L-arginine (100 μM) or *N*-hydroxy-D-arginine (100 μM) was assessed in separate vessels (see Methods). We also measured NO release in the presence of both *N*-hydroxy-L-arginine (100 μM) and A23187 (10 μM). NO concentration of baseline samples (obtained immediately after the addition of agonists) and of peak samples (obtained after 15 min of the addition of these reagents) were measured by injecting 50–100 μl samples into the purging chamber. NO signal was integrated and compared with standard curve constructed by injecting known concentrations of sodium nitrite.

2.5. Statistical analysis

Values are reported as means ± S.E.M. Two-way analysis of variance for repeated measures was used to compare the dilatory response to each concentration of *N*-hydroxy-L-arginine, *N*-hydroxy-D-arginine or L-arginine within a group of vessels. At a given concentration, we also used two-way ANOVA to compare dilations in endothelium-denuded and endothelium-intact vessels. Similar analysis was used in protocols 2, 3 and 4.

3. Results

3.1. Protocol 1

In endothelium-intact coronary arteries, *N*-hydroxy-L-arginine and *N*-hydroxy-D-arginine elicited a significant dose-dependent dilation which reached 85% and 67% of maximum dilation at a concentration of 100 μM, respec-

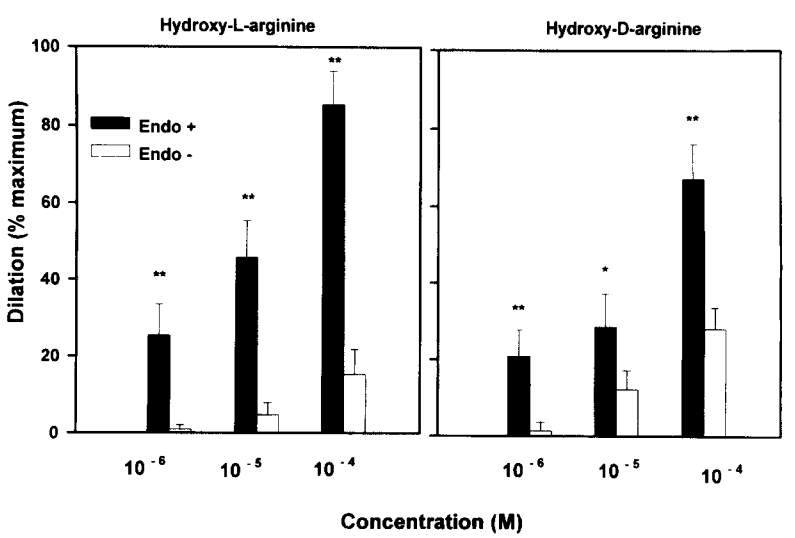

Fig. 1. The degree of dilation (% of maximum) of endothelium-intact (filled columns) and endothelium-denuded (empty columns) porcine coronary arteries in response to increasing concentrations of hydroxy-L- and hydroxy-D-arginine. * $P < 0.05$ and ** $P < 0.01$ compared with endothelium-denuded arteries.

Table 1
The effects of different inhibitors on the dilator responses of endothelium-intact coronary arteries to *N*-hydroxy-L-arginine, *N*-hydroxy-D-arginine, L-arginine and hydroxylamine

Miconazole (100 μM)	0	+	0	0
7-Ethoxyresorufin (100 μM)	0	0	0	+
N^G-Nitro-L-arginine methyl ester (100 μM)	0	0	+	0
N-Hydroxy-L-arginine (100 μM)	85.5 ± 8.5	68.5 ± 16.0	−8.1 ± 6.1 [a]	78.2 ± 7.3
N-Hydroxy-D-arginine (100 μM)	67.0 ± 9.0	65.1 ± 2.1	18.7 ± 13.6 [a]	64.4 ± 4.5
L-Arginine (100 μM)	47.0 ± 4.8	36.5 ± 14.0	2.8 ± 9.6 [a]	42.4 ± 5.2
Hydroxylamine (10 μM)	90.9 ± 2.0	88.0 ± 2.3	94.4 ± 2.1	89.3 ± 4.2

Dilation is expressed as percentage of maximum. Data are means ± S.E.M. [a] $P < 0.05$ compared with values measured without preincubation with N^G-nitro-L-arginine methyl ester.

tively (Fig. 1). By comparison, 1, 10 and 100 μM concentrations of L-arginine produced dilation of about 23, 37 and 47% of maximum, respectively ($P < 0.05$ compared with that elicited by 100 μM *N*-hydroxy-L-arginine). In endothelium-denuded arteries, no significant dilation was evident in response to *N*-hydroxy-L-arginine (Fig. 1). Only 100 μM *N*-hydroxy-D-arginine elicited significant dilation in endothelium-denuded arteries but was much smaller than that seen in endothelium-intact arteries ($P < 0.01$, Fig. 1). L-Arginine had no effect on contractility of endothelium-denuded arteries.

3.2. Protocol 2

Table 1 lists the effects of N^G-nitro-L-arginine methyl ester, miconazole and 7-ethoxyresorufin on the dilatory response to *N*-hydroxy-L-arginine, *N*-hydroxy-D-arginine, L-arginine and hydroxylamine in endothelium-intact arteries. The presence of miconazole or 7-ethoxyresorufin in the bath had no significant effect on the dilatory response to either of these agonists. In contrast, the presence of N^G-nitro-L-arginine methyl ester completely eliminated the dilatory response to *N*-hydroxy-L-arginine and L-arginine and attenuated that of *N*-hydroxy-D-arginine. Hydroxylamine-induced dilation, however, was not affected by N^G-nitro-L-arginine methyl.

3.3. Protocol 3

Fig. 2A shows the concentration-response curves to calcium ionophore A23187 in endothelium-intact arteries in the absence and presence of either *N*-hydroxy-L-arginine (100 μM) or *N*-hydroxy-D-arginine (100 μM). The presence of *N*-hydroxy-L-arginine significantly potentiated the dilatory response to A23187, whereas *N*-hydroxy-D-arginine had no effect. In endothelium-denuded arteries,

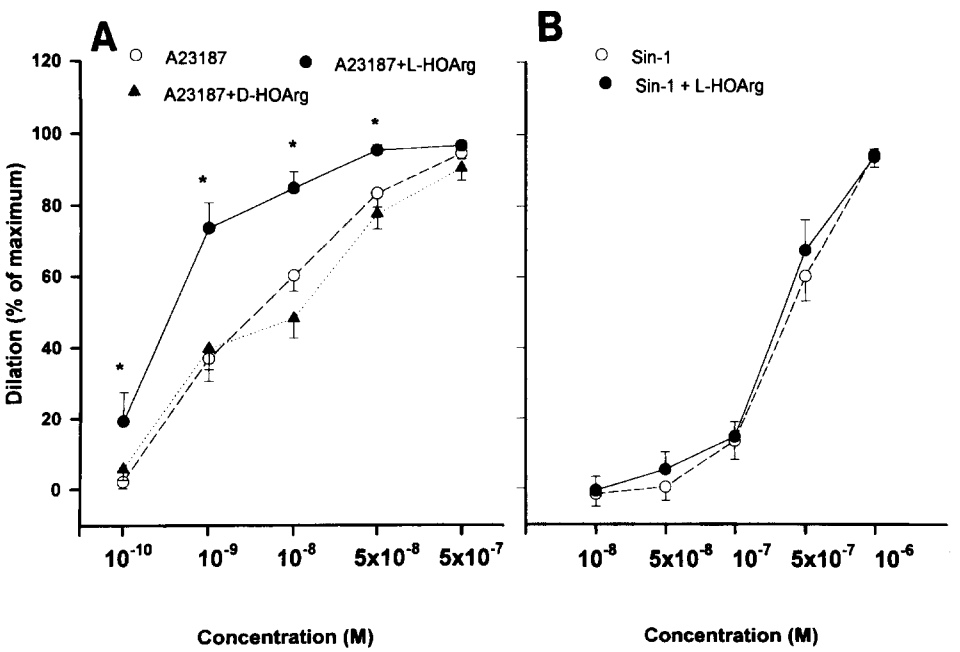

Fig. 2. The effect of *N*-hydroxy-L-arginine (100 μM) and *N*-hydroxy-D-arginine (100 μM) on the dilatory response to increasing concentrations of A23187 in endothelium-intact arteries (left) and to increasing concentrations of Sin-1 in endothelium-denuded arteries (right). * $P < 0.05$ compared with A23187 alone.

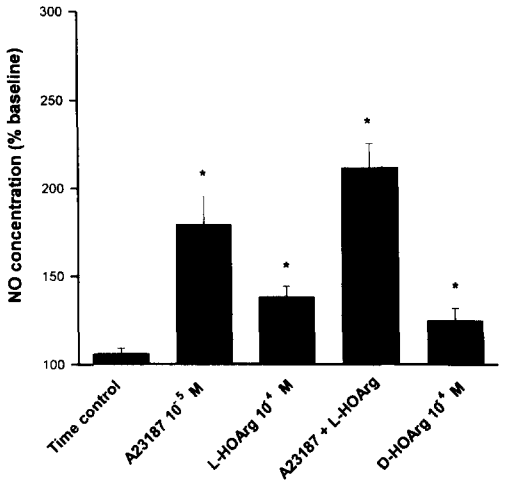

Fig. 3. Peak NO concentrations measured 15 min after the addition of A23187 (10 μM), *N*-hydroxy-L-arginine (100 μM), A23187 + *N*-hydroxy-L-arginine and *N*-hydroxy-D-arginine (100 μM). Values were normalized as percentages of baseline (measured immediately after the addition of reagents). No reagents were added in time control. * $P < 0.05$ compared with baseline values.

the dilatory response to Sin-1 was not influenced by the presence of either *N*-hydroxy-L-arginine (Fig. 2B) or *N*-hydroxy-D-arginine (data not shown).

3.4. Protocol 4

Baseline NO concentration during time-control experiments averaged 0.85 ± 0.10 μM. This values increased by only 6% after 15 min of time control (not significant). A23187 (10 μM) significantly increased NO concentration by about 79% from a mean baseline value of 0.75 μM ($P < 0.05$, Fig. 3). When N^G-nitro-L-arginine methyl ester (100 μM) was combined with A23187, NO concentration increased by only 24% of baseline ($P < 0.05$ compared with that of A23187). *N*-Hydroxy-L-arginine and *N*-hydroxy-D-arginine increased NO concentration by 40% and 25% of baseline values, respectively ($P < 0.05$ compared with baseline values, Fig. 3). In the presence of N^G-nitro-L-arginine methyl ester (100 μM), neither *N*-hydroxy-L-arginine nor *N*-hydroxy-D-arginine was able to increase NO concentration significantly (mean peak values of 105.2 and 107.3% of baseline, respectively). When A23187 was combined with *N*-hydroxy-L-arginine, NO concentration rose in an additive fashion to about 212% of baseline values (Fig. 3).

4. Discussion

The main findings of this study are: (1) *N*-hydroxy-L-arginine elicited strong endothelium-dependent dilation in porcine coronary arteries which was inhibited by N^G-nitro-L-arginine methyl ester but not by miconazole or 7-ethoxyresorufin; (2) *N*-hydroxy-L-arginine potentiated the dilatory response to calcium ionophore A23187 but not to Sin-1; (3) *N*-hydroxy-L-arginine enhanced basal endothelial NO production and potentiated NO release in an additive fashion when combined with A23187.

4.1. Effects of L-arginine

It has been well established that NO synthase isoforms synthesize NO and L-citrulline from L-arginine, although the exact steps required for this reaction remain unknown. Exogenous administration of L-arginine is known to exert variable effects on vessel tension and vascular resistance. In freshly isolated aortas and pulmonary arteries, L-arginine had no significant effect on vessel tension (Rees et al., 1989; Gold et al., 1989). In addition, L-arginine infusion did not influence the vascular resistance of various vascular beds in rats (Gardiner et al., 1990). These results were attributed to the fact that intracellular L-arginine concentration in normal endothelial cells exceeds the K_m value of endothelial NO synthase. Other investigators, however, have demonstrated that exogenous L-arginine induced small, but significant, dilation in a few vessels such as guinea pig pulmonary arteries (Sakuma et al., 1988), isolated guinea pig hearts (Thomas and Ramwell, 1988) and rat cremaster arterioles (Sun et al., 1992). We observed a significant concentration-related and endothelium-dependent dilation in porcine coronary arteries in response to increasing concentrations of L-arginine. We speculate that relatively large L-arginine-induced dilation in our study was due to depletion of endothelial L-arginine stores which rendered endothelial NO synthase activity to be more dependent on exogenous L-arginine. This depletion could have occurred in our preparation because coronary arteries were exposed for about 2 h to L-arginine-free buffer before and during the experimental protocols. Our speculation is based on the observation of Schini and Vanhoutte (1991) that the vasodilatory response to exogenous L-arginine in in-vitro isolated rat aortas increased progressively while increasing the incubation period in L-arginine-free buffer.

One could argue that pH changes is another possible mechanism for L-arginine-induced dilation in our preparation (De Nucci et al., 1988). We think that this was unlikely because we avoided changes in pH by dissolving L-arginine and other compounds in a vehicle of phosphate buffer (pH at 7.4). In addition, we noticed that this vehicle had no significant influence on vascular reactivity. We could, therefore, exclude changes in pH as a mediator of L-arginine-induced dilation.

4.2. Effects of N-hydroxy-L-arginine

It became evident in the past few years that the first step in the synthesis of NO from L-arginine by NO synthases is the formation of *N*-hydroxy-L-arginine (Klatt et al., 1993; Stuehr et al., 1991). Studies using exogenous *N*-hydroxy-L-arginine in cultured endothelial cell prepara-

tions revealed that this intermediate of NO synthesis possess a significant vasodilatory capacity (Zembowicz et al., 1991). Unlike cultured endothelial cell preparations, several investigators reported that exogenous *N*-hydroxy-L-arginine exerted a weak or no dilatory influence on freshly isolated bovine pulmonary arteries (Wallace et al., 1991) and rabbit aortas (Zembowicz et al., 1992a). Only after prolonged incubation in arginine-free buffer did *N*-hydroxy-L-arginine induce strong dilations in endothelium-denuded bovine pulmonary arteries (Wallace et al., 1991). Similarly, relatively high concentrations of *N*-hydroxy-L-arginine elicited strong endothelium-independent dilation in rabbit aortas (Zembowicz et al., 1992a), a response which has been attributed to enhanced activity of the inducible isoform of NO synthase or to the formation of hydroxylamine (DeMaster et al., 1989). We report for the first time that even relatively low concentrations of *N*-hydroxy-L-arginine induce strong endothelium-dependent dilation in isolated porcine coronary arteries. The reason behind the differences between our results and those obtained in other vessels is not clear. We speculate that, in addition to species differences, there could be a fundamental divergence in the ways through which *N*-hydroxy-L-arginine is processed by endothelial and smooth muscle cells in various blood vessels. These ways are summarized below.

Five mechanisms have been proposed so far to explain the vasodilatory response to *N*-hydroxy-L-arginine, three of which require the presence of intact endothelial NO synthase activity.

Firstly: *N*-hydroxy-L-arginine may be utilized as a substrate for endothelial NO synthase resulting in enhanced NO production. We believe that this pathway, which is sensitive to NO synthase inhibitors, is the main cause of *N*-hydroxy-L-arginine-induced dilation in our study and may explain in part the dilatory response to *N*-hydroxy-D-arginine. This assertion is supported by the augmentation of NO release after 15 min of the addition of *N*-hydroxy-L-arginine and *N*-hydroxy-D-arginine (Fig. 3), the elimination of *N*-hydroxy-L-arginine-induced dilation by N^G-nitro-L-arginine methyl ester (Table 1), and the significantly greater dilation in response to *N*-hydroxy-L-arginine compared with that elicited by L-arginine.

Secondly: it has been proposed that *N*-hydroxy-L-arginine induces the generation by the endothelial cells of an endothelium-derived relaxing factor which is distinct from NO (Zembowicz et al., 1991). Although plausible, this mechanism does not explain the rise in NO concentration in response to *N*-hydroxy-L-arginine in protocol 4. Moreover, *N*-hydroxy-L-arginine-induced dilation in our experiments was completely abolished by N^G-nitro-L-arginine methyl ester suggesting that enhanced endothelial NO synthase activity is the main mechanism through which *N*-hydroxy-L-arginine dilates coronary arteries.

Thirdly: using cultured endothelial cells as NO donor, Zembowicz et al. (1992a,b,c) proposed that *N*-hydroxy-L-arginine dilates bioassay arterial rings by reacting with endothelium-derived NO to form a stable adduct. Since this mechanism requires the formation of NO by endothelial NO synthase, stabilization of NO could explain the augmentation of A23187-induced dilation in the presence of *N*-hydroxy-L-arginine and the elimination of the dilatory response to *N*-hydroxy-l-arginine by N^G-nitro-L-arginine methyl ester. However, stabilization of NO by *N*-hydroxy-L-arginine or formation of stable adduct would have resulted in strong potentiation of the dilatory response to Sin-1 (NO donor) (Feelisch and Noack, 1987). That was certainly not the case in our study (Fig. 2). Moreover, we don't believe that the rise in NO concentration in response to *N*-hydroxy-L-arginine was due to stabilization of NO in the media or prevention of NO oxidation because we used adequate concentrations of SOD were used to scavenge O_2^- and stabilizes NO.

Fourthly: it has been proposed recently that, in hepatic microsomes and smooth muscle cells, cytochrome P_{450} may synthesize NO from *N*-hydroxy-L-arginine (Boucher et al., 1992; Schott et al., 1994). We believe that cytochrome P_{450} were not responsible for our results because neither miconazole nor 7-ethoxyresorufin (cytochrome P_{450} inhibitors) had any effect on *N*-hydroxy-L-arginine-induced dilation (Table 1).

Fifthly: Formation of hydroxylamine, a powerful endothelium-independent dilator, has been proposed to be the main mediator of *N*-hydroxy-L-arginine-induced dilation (DeMaster et al., 1989). We speculate that this mechanism was not an important mediator for *N*-hydroxy-L-arginine-induced dilation in our study because the presence of intact endothelium was essential for the action of *N*-hydroxy-L-arginine but not for hydroxylamine (Fig. 1 and Table 1). On the other hand, the endothelium-independent component of *N*-hydroxy-D-arginine-induced dilation (Fig. 1) could be attributed to the formation of hydroxylamine.

In summary, we found in porcine coronary arteries that *N*-hydroxy-L-arginine elicited strong endothelium-dependent dilation, increased NO release and potentiated the dilatory effect of an endothelium-dependent dilator. These effects were inhibited by NO synthase inhibitors but not by cytochrome P_{450} inhibitors.

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