The effects of perfusion rate and N^G-nitro-L-arginine methyl ester on cirazoline- and KCl-induced responses in the perfused mesenteric arterial bed of rats

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1 The purpose of this study was to characterize the effects of N^G-nitro-L-arginine methyl ester (L-NAME) on the perfusion rate/pressure relations, and on the pressor responses induced to cirazoline and KCl in isolated, perfused mesenteric arterial beds from normotensive and spontaneously hypertensive rats.

2 The basal perfusion pressure of arterial beds perfused with either physiological salt solution (PSS) or PSS containing 1% polyvinylpyrrolidone increased as the perfusion rate increased. L-NAME, in concentrations up to 100 µM, failed to alter the basal pressure regardless of the perfusion rate and viscosity; however, at 5 µM, it potentiated cirazoline-induced vasoconstriction at each of the perfusion rates.

3 L-NAME but not D-NAME caused a leftward shift of cirazoline concentration-response curves with a marked increase in the maximal response. The potentiating action of L-NAME was abolished in arterial beds perfused with a Ca²⁺-free physiological salt solution and also in beds denuded of endothelium by an infusion of distilled water for 5 min.

4 In endothelium-intact and -denuded preparations, L-NAME potentiated KCl pressor responses; the endothelium-independent potentiation of KCl pressor activity was stereospecific, time-independent and was not prevented by the presence of dexamethasone (0.5 µM) in the perfusion medium. However, L-NAME failed to potentiate vasoconstriction obtained to KCl in arterial beds denervated by cold storage (4–5°C) for 2 days.

5 The absence of K⁺ in the perfusate did not inhibit the ability of L-NAME to potentiate α-adrenoceptor-mediated pressor responses, and nor did L-NAME inhibit KCl-induced vasodilatation in precontracted arteries. It was thus concluded that L-NAME does not affect Na⁺/K⁺-ATPase activity.

6 No differences in the potentiating ability of L-NAME on either cirazoline- or KCl-mediated pressor responses were apparent between normotensive Sprague Dawley (SD), Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

7 Our data thus provide evidence that: the presence of a vasoconstrictor is required for basal nitric oxide (NO) release in the mesenteric arterial bed from either normotensive or spontaneously hypertensive rats; L-NAME causes potentiation of cirazoline- and KCl-induced vasoconstriction respectively by inhibiting endothelial and neuronal NO synthase(s). Furthermore, our data indicate that NO synthase activity is not impaired in the mesenteric arterial bed of spontaneously hypertensive rats.

Keywords: N^G-nitro-L-arginine methyl ester; nitric oxide; nitric oxide synthase; perfused mesenteric arterial bed; hypertension; α-adrenoceptors; potassium chloride

Introduction

The ability of endothelial cells to modulate vascular smooth muscle tone via the synthesis and release of one or more endothelium-derived relaxing factors (EDRFs) is now well recognized (Furchgott & Zawadzki, 1980; Moncada *et al.*, 1991). Nitric oxide (NO), or a labile nitroso compound, produced from L-arginine has been demonstrated to be an important EDRF in several vascular tissues (Palmer *et al.*, 1988; Ignarro, 1989). Several L-arginine analogues have been developed as nitric oxide synthase inhibitors, for example, N^G-monomethyl-L-arginine (L-NMMA), N-iminoethyl-L-ornithine (L-NIO), and N^G-nitro-L-arginine, or its methyl ester (L-NAME), (Dubbin *et al.*, 1990; Moore *et al.*, 1990; Moncada *et al.*, 1991); all these have been shown to inhibit NO biosynthesis and also endothelium-dependent responses *in vitro* and *in vivo*. In addition to inhibiting endothelium-dependent relaxations, L-arginine analogues can also cause endothelium-dependent contractions of isolated vascular rings (Gold *et al.*, 1990), increases in coronary perfusion pressure in isolated perfused hearts of rabbit (Amezcuca *et al.*,

1989) and guinea-pig (Levi *et al.*, 1990) and marked hypertension and regional vasoconstriction when administered to conscious rats (Gardiner *et al.*, 1990; Moncada *et al.*, 1991). These actions of L-NAME, and the other L-arginine derivatives, have been ascribed to prevention or inhibition of basal or stimulated EDRF (NO)-elicited activation of cytosolic guanylate cyclase.

In the perfused mesenteric arterial bed isolated from either the normotensive (Criscione *et al.*, 1984) or spontaneously hypertensive (Dohi *et al.*, 1990) rats, endothelial denudation augmented α₁-adrenoceptor mediated vasoconstriction; however, neither L-NMMA, methylene blue (Ebeigbe *et al.*, 1990) nor L-NAME (Adeagbo & Triggle, 1992; 1993) caused vasoconstriction as would be expected if basal nitric oxide release, and subsequent elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP), maintains resting tone. In our latter studies, we demonstrated that a putative endothelium-derived hyperpolarizing factor (EDHF), causing vascular smooth muscle hyperpolarization and vasodilatation via the activation of apamin-sensitive K⁺ channels plays a dominant role in the maintenance of vascular tone in this arterial bed (Adeagbo & Triggle, 1992; 1993). However, the possible influence of shear stress induced by the perfusion rate on the

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release of nitric oxide deserves to be addressed. Thus, assuming the failure of L-NAME to induce vasoconstriction of this vascular bed under *in vitro* basal conditions was due to an inadequate shear stress generated in our perfusion system, then, increasing the flow rate and/or viscosity of our perfusion salt solution should result in the activation of the endothelial nitric oxide synthase, and, its subsequent inhibition by L-NAME. Accordingly, the first objective of the present study was to assess the effects of L-, and D-isomers of N^G-nitro-arginine methyl ester on the basal perfusion pressure at different flow rates during perfusion with physiological salt solution (PSS) or PSS plus the plasma protein substitute polyvinylpyrrolidone (PVP), at 1%.

Second, we have also examined the interactions of L-NAME with α -adrenoceptor agonists- and KCl-induced responses with a view to: (a) determining the source (endothelial, neuronal or smooth muscle) of nitric oxide that modulates vasoconstriction to these agonists in this vascular bed; and, (b) comparing the nitric oxide-mediated modulation of vasoconstriction in normotensive Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats with spontaneously hypertensive rats (SHR). There are conflicting reports about the status of endothelial function in resistance vasculature during hypertension. Dohi *et al.* (1990) noted that the release of EDRF(s) in the mesenteric vascular bed is impaired in hypertension, while Randall *et al.* (1991), using an *in situ* blood-perfused mesenteric bed, did not observe an impairment of endothelial function. Thus, if the production of nitric oxide is deficient in the mesenteric bed of hypertensive rats, then the ability of L-NAME to potentiate vasoconstriction should be greater in SD and WKY versus SHR.

Methods

Age-matched (16–20 weeks old) male SD, WKY and SHR rats were anaesthetized by intraperitoneal (i.p.) injections of sodium pentobarbitone (65 mg kg⁻¹) and their systolic blood pressure measured via a cannular (PE 50) inserted into the femoral artery. The cannula, filled with heparinized saline (25 i.u. ml⁻¹), was connected to a Statham pressure transducer and the recordings monitored on a Gould physiopolygraph (model RS 3400). Immediately after blood pressure measurement, the abdominal cavity of individual rats was opened, the mesenteric artery was cannulated through an incision at its confluence with the dorsal aorta and then isolated as previously described by McGregor (1965). The mesenteric bed was flushed with heparinized PSS and subsequently transferred to a warmed chamber and perfused with PSS (maintained at 37°C and gassed with 95% O₂:5% CO₂) at a constant flow rate of 5 ml min⁻¹ using a Microperplex peristaltic pump (LKB Bromma, model 2132). Changes in perfusion pressure were recorded via a Statham pressure transducer coupled to a Grass polygraph recorder (model 7E).

The PSS used had the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 12.5, glucose 11.1 and where applicable, 1% polyvinylpyr-

rolidone (PVP). The PSS routinely contained indomethacin (1 μ M) and where KCl was used as the pressor agent, also contained 0.1 μ M prazosin; the pH of the solution, after saturation with 95% O₂:5% CO₂ gas mixture, was 7.4. Tissues were allowed to equilibrate for 1 h before the start of the experiments. In studies where endothelial denudation was required, this was achieved by an infusion of distilled water for 5 min followed by equilibration in normal PSS for 1 h before challenging with drugs. Endothelial integrity was ascertained functionally with bolus injection of acetylcholine after raising the vascular tone with an infusion of 0.5–1 μ M cirazoline.

Experimental protocol

Series 1 and 2 involved studies, as outlined below on SD rats. Series 3 involved a comparison between SHR and WKY with a protocol identical to that outlined for cirazoline and KCl at the perfusion rate of 5.0 ml min⁻¹, in series 1 and 2.

Series 1: The first series of experiments, using mesenteric vascular beds isolated from SD rats, was performed to determine the effects of changes in perfusion rate and infusion of L-NAME on: (a) the basal perfusion pressure, and (b) the vasoconstrictor responses to cirazoline in vascular beds perfused with PSS at 2.5, 5.0 and 10.0 ml min⁻¹. Arterial beds were usually set-up and equilibrated with PSS at the flow rate of 2.5 ml min⁻¹, this was followed by a bolus injection of the ED₅₀ (determined from preliminary experiments) of cirazoline; subsequently, the flow rate was increased to 5.0 and 10.0 ml min⁻¹ respectively, and, during perfusion at each flow rate, the test dose of cirazoline was injected and the changes in perfusion pressure measured. This procedure was performed in the absence of, and during infusion of L-NAME using either PSS or PSS + 1% PVP to adjust viscosity and maintain osmotic pressure. A separate study of the actions of L- and D-NAME on the dose-response curves of the α -adrenoceptor agonists was then conducted, using PSS and a constant perfusion rate of 5.0 ml min⁻¹.

Series 2: Experiments in this series, also conducted at the perfusion rate of 5.0 ml min⁻¹, were aimed at determining the mode of interactions of L- and D-NAME with KCl vasoconstrictor responses in freshly isolated, and in 48 h cold-stored (4–5°C), vascular beds which were either intact or denuded of endothelium. The protocol, as for cirazoline, involved the establishment of dose-response curves to the agonist in the absence of, and during infusion of a particular concentration of L-, or D-NAME. Loss of vasoconstrictor and vasodilator responses to bolus injections of veratridine and infusion of capsaicin respectively were used to assess the viability or otherwise of periaarterial nerves to the mesenteric beds after cold-storage. Additional experiments were performed to assess the: (a) influence of dexamethasone on L-NAME-KCl interaction; and, (b) contributions of Na⁺/K⁺-ATPase to the action of L-NAME. In (a), the effect of L-NAME on KCl vasoconstriction was determined in PSS containing 0.5 μ M dexamethasone and then compared over a 6 h period with

Table 1 Effects of perfusion rate and inclusion of 1% polyvinylpyrrolidone (PVP) on the basal perfusion pressure (BPP) and the potentiating action of 5 μ M N^G-nitro-L-arginine methyl ester (L-NAME) (measured as potentiating factors, PFs) on cirazoline-induced pressor responses in mesenteric arterial beds of normotensive Sprague-Dawley (SD) rats

Experimental conditions		BPP (mmHg)	PF (ED ₅₀ control:ED ₅₀ L-NAME)
2.5 ml min ⁻¹	PSS	8.8 $\pm$ 0.7 (5)	2.6 $\pm$ 0.3 (6)
	PSS + 1% PVP	*13.2 $\pm$ 0.4 (5)	*1.8 $\pm$ 0.4 (5)
5.0 ml min ⁻¹	PSS	18.5 $\pm$ 1.4 (24)	2.5 $\pm$ 0.3 (6)
	PSS + 1% PVP	*23.6 $\pm$ 1.2 (6)	*1.7 $\pm$ 0.3 (5)
10.0 ml min ⁻¹	PSS	30.2 $\pm$ 1.7 (5)	2.4 $\pm$ 0.2 (6)
	PSS + 1% PVP	*37.9 $\pm$ 2.1 (5)	*1.4 $\pm$ 0.2 (5)

Data represent the means $\pm$ s.e.mean, *n* values in parentheses. Statistically significant difference (*P* < 0.05) from the corresponding values obtained during perfusion with plain physiological salt solution (PSS).

corresponding responses obtained during perfusion with PSS without dexamethasone, and the time control. Experiments ascertaining the role of Na^+/K^+ -ATPase were carried out in mesenteric arterial beds perfused with K^+ -free PSS and involved testing the action of L-NAME against the vasoconstrictor effects of cirazoline, and, in the case of KCl, vasodilator effects after elevating the arterial tone with an infusion of cirazoline ($0.5 \mu\text{M}$).

Series 3: This essentially comprised experiments as outlined for the interaction of L-NAME with the α -adrenoceptor agonists or KCl in series 1 and 2 above but with SHR and WKY. All experiments in this series were performed in vascular beds perfused at a rate of 5.0 ml min^{-1} .

Drugs

The following were used: acetylcholine bromide, dexamethasone, indomethacin, N^G -nitro-L-arginine methyl ester, ouabain, capsaicin (8-methyl-N-vanillyl 6-nonenamide), tetrodotoxin, veratridine and polyvinylpyrrolidone (PVP) purchased from Sigma Chemical Co., MO, U.S.A.; N^G -nitro-D-arginine methyl ester was from Bachem Inc. (Torrance, CA, U.S.A.). Cirazoline and prazosin, as hydrochloride salts, were generous gifts from Synthelabo Laboratories, Paris, France, and Pfizer Inc., Arnprior, Ontario, Canada.

Statistical analysis

Data were analysed by analysis of variance and, for multiple comparison of results, Duncan's Multiple Range test was used to compare group means. In all cases, a probability of error of less than 0.05 was selected as the criterion for statistical significance. Potentiation factors (PF), defined as

Figure 1 Differential effects of N^G -nitro-L-arginine methyl ester (L-NAME) on the basal perfusion pressure and cirazoline-induced vasoconstriction in isolated mesenteric arterial beds from SD rats perfused with either physiological salt solution (PSS, a and c) or PSS + 1% polyvinylpyrrolidone (PVP, b). Arterial beds were perfused at the rates indicated and in all panels the open and hatched columns represent the measured parameters in the absence, and in the presence of $100 \mu\text{M}$ (a and b) and $5 \mu\text{M}$ (c), L-NAME respectively. Data plotted are the means $\pm$ s.e.mean ($n = 6-8$ experiments).

Figure 2 Potentiation by $5 \mu\text{M}$ N^G -nitro-L-arginine methyl ester (L-NAME, a) or endothelium denudation (b) of cirazoline-induced vasoconstrictor responses in mesenteric arterial beds isolated from Sprague Dawley rats. In (a), (○), (●) and (■) represent the responses obtained to cirazoline in the absence, and, in the presence of $5 \mu\text{M}$ L- and D-NAME respectively; while panel (b) compares cirazoline responses in endothelium-intact (○) and endothelium-denuded (▲) arterial beds. Each point on the graphs represents the mean $\pm$ s.e.mean, $n = 5-8$ experiments.

the ratios of ED_{50} in the absence, to that in the presence of L-NAME; ED_{50} denotes the doses of cirazoline or KCl that produced 50% of the maximal vasoconstrictor effect and were obtained from individual dose-response curves under each of the experimental conditions.

Results

Mesenteric basal perfusion pressure: influence of perfusion rate, PVP and L-NAME

The basal perfusion pressure of mesenteric arterial beds perfused with PSS increased significantly with increasing perfusion rates (Table 1) and the same trend was observed in arterial beds perfused with PSS + 1% PVP. However, the basal perfusion pressure values of tissues perfused with PSS + 1% PVP were significantly ($P < 0.05$) greater than the corresponding values in PSS (Table 1). Infusion of L-NAME in concentrations up to $100 \mu\text{M}$ did not alter the basal perfusion pressure at any of the perfusion rates, regardless of whether PVP was present (Figure 1). Conversely, the cirazoline-induced vasoconstrictor response at each of the perfusion rates was significantly potentiated by $5 \mu\text{M}$ L-NAME (see Figure 1). As can be seen in Table 1, the potentiation factors (defined in Methods section) were not significantly different at each of the perfusion rates, but were significantly ($P < 0.05$) greater in PSS versus PSS + 1% PVP; for this reason, subsequent experiments were performed in PSS.

Effects of L-NAME on mesenteric vasoconstrictor responses to cirazoline in normotensive Sprague Dawley rats

In normotensive Sprague Dawley rats, bolus injection of cirazoline (0.01–10.0 nmol) initiated dose-dependent pressor responses with ED_{50} values of 3.1 ± 0.5 ($n = 6$) and 1.8 ± 0.4 ($n = 5$) nmol in endothelium-intact and -denuded preparations respectively; the ED_{50} value is significantly ($P < 0.05$) lower in denuded than in intact preparations. D-NAME (50 μ M), regardless of perfusion rate, was completely devoid of effect on the basal perfusion pressure but L-NAME (5 μ M) caused a leftward shift of the dose-response curves to cirazoline with a marked increase in the maximal effect (Figure 2). However, in arterial bed preparations with intact endothelium but perfused with Ca^{2+} -free PSS, and in those denuded of endothelium by an infusion of distilled water for 5 min, L-NAME failed to potentiate cirazoline-induced vasoconstriction (Figure 3).

Effects of L-NAME on KCl-induced responses

Bolus injections of KCl (1–100 μ mol) initiated pressor responses with ED_{50} values of 1.09 ± 0.10 ($n = 5$) and 1.11 ± 0.15 ($n = 5$) μ mol in endothelium-intact and -denuded arterial preparations respectively, and, regardless of the presence or absence of endothelium, L-NAME potentiated the pressor responses to the same extent (Figure 4). Arterial beds cold-stored (4–5°C) for 2 days responded in a dose-dependent

Figure 3 Lack of potentiation by N^G-nitro-L-arginine methyl ester (L-NAME) of cirazoline responses in isolated arterial beds with intact endothelium and perfused with Ca^{2+} -free PSS (a) or in endothelium-denuded arterial beds (b). In both panels, (○) and (●) represent the control and the responses in the presence of 5 μ M L-NAME respectively. Each point on the graphs represents the mean $\pm$ s.e.mean, $n = 6$ experiments.

Figure 4 The effect of N^G-nitro-L-arginine methyl ester (L-NAME) on KCl dose-response curves in isolated perfused mesenteric arterial beds from SD rats. The data plotted in (a) and (b) were obtained, respectively, from endothelium-intact and denuded freshly isolated arterial beds, while in (c) data presented are from arterial beds denervated by cold storage for 48 h at 4–5°C. In all panels, (○) and (●) represent the responses in the absence and in the presence of 5 μ M L-NAME, respectively. Each point on the graphs represents the mean $\pm$ s.e.mean ($n = 6$), and *denotes statistically significant difference ($P < 0.05$) from corresponding control.

manner to bolus injections of KCl, but the magnitude of such responses were reduced as compared to similar effects in fresh tissues, and, unlike fresh preparations, responses were not potentiated by L-NAME. Furthermore, infusion of 0.1 μ M capsaicin, which caused complete vasodilatation of fresh tissues was ineffective in cold-stored preparations; also, bolus injections of veratridine (1–30 nmol), unlike their effect in fresh preparations, failed to elicit vasoconstrictor action in cold-stored arterial beds (Figure 5).

In endothelium-denuded mesenteric arteries, the pressor responses obtained to bolus injections of 50 μ mol KCl at 2, 4 and 6 hourly periods were not significantly different (107.5 ± 6.9 , 115.3 ± 9.7 and 114.3 ± 7.1 respectively), and L-NAME (5 μ M) potentiated the KCl effects respectively by 40.6 ± 4.3 , 33.7 ± 3.1 and $37.9 \pm 3.9\%$ in the presence of dexamethasone. Potentiation of KCl-induced vasoconstriction by L-NAME in the absence of dexamethasone, over the same observation period was not significantly different from that in its presence. Mesenteric arterial beds, equilibrated with K^+ -free PSS, constricted to cirazoline and such vasoconstrictor responses were potentiated by L-NAME (Figure 6); however, ouabain-sensitive KCl-induced vasodilator responses in arterial beds preconstricted with cirazoline were not inhibited by 1 mM L-NAME (Figure 7).

Figure 5 Representative tracings of the comparative effects of veratridine (a and b) and capsaicin (c and d) in freshly isolated (a and c respectively) versus 48 h cold-stored (4–5°C; b and d respectively) mesenteric arterial beds of Sprague Dawley rats. Veratridine doses (●), given by bolus injections, are in nmol; capsaicin (0.1 μM) was infused into cirazoline (0.3 μM)-precontracted arterial beds and the arrows denote the start of the infusion.

Interactions of L-NAME with cirazoline and KCl in arterial beds isolated from WKY and SHR

WKY rats used in our study had mean systolic/diastolic blood pressures of $95.6 \pm 1.8/51.5 \pm 0.7$ mmHg and a mean heart rate of 311.1 ± 16.3 beats min^{-1} ($n = 10$) while the mean blood pressures for the SHR were $174.6 \pm 7.6/111.5 \pm 4.5$ mmHg and the heart rate was 372.3 ± 8.2 beats min^{-1} ($n = 13$). The systolic and diastolic blood pressures and the heart rates in SHR were significantly ($P < 0.05$) higher than the comparative values for the WKY.

As previously noted for SD rats, L-NAME failed to change the basal perfusion pressure in WKY and SHR, but, at 5 μM, it markedly reduced the ED_{50} values for cirazoline but not KCl, and increased the maximal pressor effects to both compounds in both WKY and SHR (Table 2). Although mesenteric arterial beds from the SHR were more sensitive to cirazoline than those from WKY (ED_{50} : 2.18 ± 0.13 versus 2.94 ± 0.21 nmol respectively), there were no apparent differences in the potentiation, expressed as potentiation factors (PFs), of pressor effects induced by L-NAME in the two groups of rats (Table 2).

Discussion

Arginine derivatives, such as N^G -nitro-L-arginine methyl ester, have been shown to inhibit NO synthesis and endothelium-dependent relaxation and have been used extensively in studies involving the role of NO synthesis in the regulation of vascular tone and blood pressure (Aisaka *et al.*, 1989; Rees *et al.*, 1989; Dohi *et al.*, 1990; Moncada *et al.*, 1991). The present study, with isolated mesenteric arterial beds perfused with PSS, provides evidence that L-NAME, although lacking an effect on the basal tone, caused marked potentiation of α -adrenoceptor-mediated vasoconstriction in an endothelium-dependent manner. KCl-induced vasoconstrictor responses were also enhanced by L-NAME, but, in an endothelium- and time-independent, and dexamethasone-insensitive manner; such enhancement was completely absent in cold-denervated arterial beds. The L-NAME-mediated potentiation of

Figure 6 Effects of N^G -nitro-L-arginine methyl ester (L-NAME) on dose-response curves to cirazoline in mesenteric arterial beds isolated from normotensive Sprague Dawley (SD, a), Wistar Kyoto (WKY, c) and spontaneously hypertensive (SHR, b) rats. In all panels, (○) represents the control responses, while (●) represents the responses during an infusion of 5 μM L-NAME. The arterial beds were perfused with K^+ -free physiological salt solution, and the points on the graphs represent the means $\pm$ s.e.mean, $n = 5$.

Figure 7 Effects of 1 mM N^G -nitro-L-arginine methyl ester (L-NAME) and 100 μM ouabain on KCl-induced vasodilator responses in mesenteric arterial beds perfused with K^+ -free physiological salt solution and precontracted with an infusion of 0.5 μM cirazoline. Arterial beds in (a) were isolated from normotensive Wistar Kyoto (WKY) rats, while those in (b) were from spontaneously hypertensive rats (SHR). Doses of KCl indicated by (●) are in μmol.

Table 2 Effects of N^G-nitro-L-arginine methyl ester (L-NAME, 5 μ M) on cirazoline- and KCl-induced pressor responses in normotensive Sprague Dawley (SD), Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats

Agonists		SD	WKY	*SHR
Cirazoline	PFs	2.95 $\pm$ 0.25	3.55 $\pm$ 0.50	3.51 $\pm$ 0.48
	E _{max} (% increase)	65.4 $\pm$ 7.1	69.4 $\pm$ 8.2	63.7 $\pm$ 9.4
KCl	PFs	1.09 $\pm$ 0.10	1.05 $\pm$ 0.07	1.23 $\pm$ 0.13
	E _{max} (% increase)	48.5 $\pm$ 9.4	57.1 $\pm$ 8.3	43.4 $\pm$ 7.4

Data represent the means $\pm$ s.e.mean ($n = 5-6$), and, *denotes that both the PFs (potentiation factors defined in Methods) and the % increases in the E_{max} obtained in arterial beds from SHR for both agonists did not differ significantly ($P > 0.05$) from those obtained for normotensive SD and WKY. KCl experiments were performed in the presence of 10 nM prazosin added to perfusion medium.

cirazoline and KCl, which occurred in arterial beds isolated from both normotensive rats and SHR, was neither mediated via an action of L-NAME on Na⁺/K⁺-ATPase, nor on the inducible smooth muscle NO synthase, but rather, on endothelial and neuronal NO synthase(s).

Administration of L-NMMA to conscious guinea-pigs (Aisaka *et al.*, 1989), and rabbits (Rees *et al.*, 1989) or to pithed rats (Tabrizchi & Triggle, 1992) and of L-NAME to conscious rats (Gardiner *et al.*, 1990), results in a marked hypertension that has been ascribed to the inhibition of the endogenous synthesis of NO. L-NMMA also causes coronary vasoconstriction in the rabbit isolated perfused heart (Amezcu *et al.*, 1989). In the present study no pressor response was observed during the infusion of L-NAME, and the inclusion of 1% PVP as a plasma protein replacement made no difference. These data suggest that NO may not be spontaneously released under the basal perfusion pressure conditions in the mesenteric vascular bed and/or that circulating vasoconstrictors, or other factors, may be required to trigger release. If the presence of a vasoconstrictor, or active tone, is required as a trigger for NO synthesis and release, conceivably the stimulus is Ca²⁺ entry via the receptor-operated calcium channels (ROCC)/cation channels present in the endothelial cell membrane, and the resultant increase in intracellular levels of Ca²⁺ ions stimulates the *de novo* synthesis of NO.

The endothelium has been postulated to serve as a pressure and a flow sensor (Rubanyi *et al.*, 1990); thus, both of these parameters are critical to the release of endothelium-derived vasoactive factors. However, an increase in the perfusion rate, but not pressure, has been demonstrated to increase the rate of NO release in porcine cultured aortic endothelial cells under basal and stimulated conditions (Kelm *et al.*, 1991). In the present study, L-NAME was still without effect despite the assumed increase in shear stress resulting from an increase in the perfusion rate from 2.5 to 10.0 ml min⁻¹. This finding supports an earlier conclusion that in the rat mesenteric bed endothelium derived NO, (EDNO) is less important than the endothelium-derived hyperpolarizing factor, or EDHF (Adeagbo & Malik, 1990; Adeagbo & Triggle, 1992; 1993). Furthermore, a recent report by Garland & McPherson (1992) also indicates that ACh-mediated hyperpolarization in the rat small mesenteric artery is not mediated by NO.

The present study has demonstrated a stereo-specific enhancement of cirazoline-induced vasoconstriction by nitro-arginine methyl ester. L-NAME but not D-NAME caused a leftward shift, accompanied by a significant increase in the maximal effect, of cirazoline dose-response curves. Since this L-NAME-induced potentiation was abolished in endothelium-denuded mesenteric arterial beds, it can be concluded that the action of L-NAME resulted from an inhibitory action on NO synthesis in endothelial cells. The endothelial NO synthase requires Ca²⁺ and NADPH as co-factors for the conversion of L-arginine to NO and L-citrulline (Myers *et al.*, 1989); thus, the failure of L-NAME to potentiate α -adrenoceptor-mediated pressor responses obtained during perfusion with Ca²⁺-free PSS further supports the conclusion

that endothelial NO synthase is the target of L-NAME action in the present study.

KCl-induced vasoconstrictor responses were also potentiated by L-, but not D-NAME. However, unlike for cirazoline, the ED₅₀s for KCl were not altered by L-NAME, but there were significant increases in the E_{max} in both endothelium-intact and denuded preparations. These results suggest that a component of the effect of L-NAME in mesenteric arterial beds might be independent of the endothelium. The extra-endothelial sites that may contribute to the potentiation induced by L-NAME, include: the inducible smooth muscle, and/or constitutive neuronal NO synthase(s), non-specific interference with the sodium pump, or a direct facilitation of smooth muscle contractile process.

Endotoxin contamination of the mesenteric vasculature may occur during separation of the vascular bed from the bowel and thus, it is conceivable that the inducible smooth muscle NO synthase might serve as an additional target for L-NAME action. If indeed the inducible smooth muscle NO synthase, as described in the rat aortic rings (Schini & Vanhoutte, 1991; Moritoki *et al.*, 1991), is responsible for the non-endothelium-dependent potentiation of KCl pressor responses by L-NAME, then inclusion of a glucocorticoid, such as dexamethasone, in the perfusion medium should counteract the effect of L-NAME. Glucocorticoids have been demonstrated to prevent the induction of smooth muscle NO synthase (Rees *et al.*, 1990). However, the addition of dexamethasone to the perfusing PSS failed to prevent the potentiation by L-NAME of KCl pressor activity and thus our data are incompatible with this hypothesis. This suggests that the mechanism of endothelial-independent potentiation of the KCl effect by L-NAME observed in the present study does not involve the inducible smooth muscle NO synthase. It is also evident from the present study, that the Na⁺/K⁺-ATPase is not involved in the mechanism of action of L-NAME since L-NAME in concentrations up to 1 mM was totally ineffective against the ouabain-sensitive vasodilatation produced by KCl following the infusion of K⁺-depleted PSS, and furthermore, L-NAME still potentiated the cirazoline-induced pressor responses obtained in mesenteric beds perfused with K⁺-depleted PSS.

The rat mesenteric arterial bed has been shown to have dense adrenergic (Furness & Marshall, 1974), peptidergic (Ganz *et al.*, 1986) and capsaicin-sensitive sensory (Wharton *et al.*, 1986; Manzini & Perretti, 1988) innervation. It is conceivable that the release of NO from any of these neuronal sources may contribute to the potentiating effects of L-NAME on KCl vasoconstriction. The neural NO synthase is a homologue of the endothelial enzyme, and shares not only co-factor requirements (Bredt & Snyder, 1990; Crossin, 1991), but, also, sensitivity to nitro-L-arginine; an IC₅₀ of 0.05 μ M has been reported (Lambert *et al.*, 1991). That L-NAME-induced enhancement of KCl vasoconstriction is mediated via inhibition of neuronal NO synthase is evident from the complete loss of potentiation in arterial beds denervated by cold storage (4–5°C) for 2 days. Cold storage is used extensively as a denervation procedure (Akbarali *et al.*, 1987; Jurkiewicz *et al.*, 1992), and the procedure also abol-

ished nerve-mediated vasoconstriction induced by veratridine, and, though less consistently, capsaicin-induced vasodilatation. Veratridine depolarizes nerve terminals via the activation of Na^+ channels (Kirpekar & Prat, 1979). High external K^+ concentrations have previously been demonstrated to stimulate the release of EDRF (presumably NO) in canine femoral arteries (Rubanyi & Vanhoutte, 1988) and to stimulate simultaneously NO release and increase in tissue cyclic GMP levels in cerebellar slices (Dickie *et al.*, 1990).

Another important aspect of the present study pertains to the comparison of L-NAME effects on cirazoline- and KCl-induced pressor activities of Sprague-Dawley, Wistar Kyoto and spontaneously hypertensive rats. The rationale for this comparison is based on the hypothesis that impairment of endothelial function in the hypertensive state reflects a reduced activity/level of the NO synthase, so that a reduced degree of potentiation of pressor activity should be observed. Our data have revealed that although the SHR were significantly more sensitive to cirazoline than SD or WKY rats, the action of L-NAME was qualitatively similar in the three strains of rat *viz* significantly lower ED_{50} s and greater E_{max} values for cirazoline in the presence of L-NAME. In addition, potentiation of pressor responses by L-NAME (measured as PFs) was statistically similar in all strains of rat used. Thus, our data indicates that there is no dysfunction of NO synthase activity in the mesenteric vessels of the SHR. An impairment of endothelium-dependent relaxation has been reported in both isolated thoracic aortic rings from genetically hypertensive rats (Konishi & Su, 1983; Winquist *et al.*, 1984; Lüscher & Vanhoutte, 1986) and small (DeMey & Gray, 1985; Mayhan *et al.*, 1987; Dohi *et al.*, 1990) blood vessels. It is however, worthy of note that an enhancement of endothelial function in hypertension has also been reported (Webb *et al.*, 1987), and also that EDRF release in bioassay experiments does not differ between hypertensive and normotensive control rats (Van de Voorde & Leusen, 1986). Lüscher, in a recent editorial (1990), has concluded that the apparent impairment of endothelial function in the SHR is a result of an increase in the release of a contracting factor(s) rather than decrease in a relaxing factor(s). In a recent study Tabrizchi & Triggle (1991) reported that bolus i.v. injections of L-NAME augmented the increase in blood pressure resulting from stimulation of the sympathetic nerves in the pithed

rat, and that this increase was greater in SHR than in WKY. These authors concluded that the levels of L-arginine-derived NO may be higher in SHR and secondary to the elevated blood pressure of the SHR.

The striking disparity between the enhancement of pressor responses by L-NAME and that due to endothelial denudation brought about by an infusion of distilled water deserves comment. Significantly greater potentiation by L-NAME than endothelial denudation suggests: either (a) that distilled water damaged the underlying smooth muscle cells, or (b) the contribution of a non-cyclo-oxygenase, indomethacin-insensitive endothelium-derived contracting factor(s) which is only unmasked after inhibition of endothelial NO synthase, or, (c) the possibility of L-NAME exhibiting an inhibitory effect on both endothelial and smooth muscle NO synthases. Damage to the smooth muscle cells resulting from the distilled water treatment is an unlikely explanation since sodium nitroprusside-induced vasodilatation was, if anything, potentiated in distilled water-treated versus control, untreated, arterial beds. The enhancement of sodium nitroprusside-induced vasodilatation following endothelial denudation has been previously reported by Ralevic *et al.* (1991). Conceivably the inhibition of NO synthesis promotes the synthesis of endothelial-derived contracting factor(s). Furthermore, a cyclic GMP independent action of NO on Ca^{2+} homeostasis has been reported (Garg & Hassis, 1991) and thus L-NAME may prevent the NO-mediated decrease in cytosolic free Ca^{2+} .

In conclusion, the present study demonstrates that L-NAME potentiated α -adrenoceptor- and KCl-mediated pressor responses in perfused rat mesenteric arterial beds via an inhibitory action on endothelial, and neuronal NO synthase(s). This potentiation involved neither the dexamethasone-sensitive inducible smooth muscle NO synthase, nor Na^+/K^+ -ATPase. Furthermore, since the effects of L-NAME were similar in normotensive SD and WKY compared to SHR, our study indicates that NO synthase activity is not impaired in the mesenteric bed of spontaneously hypertensive rats.

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