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Attenuation of vasoconstriction by endogenous nitric oxide in rat caudal artery

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1 The effects of N^G-nitro-L-arginine (L-NNA), N^G-nitro-L-arginine methyl ester (L-NAME), haemoglobin and methylene blue have been examined on vascular reactivity in the rat isolated caudal artery. The effects of L-NNA and sodium nitroprusside were also investigated on the stimulation-induced (S-I) efflux of noradrenaline in the rat caudal artery.

2 L-NNA (10 μM) and L-NAME (10 μM) significantly attenuated the vasodilator responses to acetylcholine (1 nM–1 μM), but had no effect on vasodilator responses to papaverine (1–100 μM).

3 Vasoconstrictor responses to sympathetic nerve stimulation (3 Hz, 10 s), noradrenaline (0.01–1 μM), methoxamine (1–10 μM), 5-hydroxytryptamine (0.01–0.3 μM), phenylephrine (0.1–10 μM), endothelin-1 (10 nM) and KCl (40 mM) were significantly enhanced by 10 μM L-NNA. L-NAME (10 μM) caused a significant enhancement of vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in endothelium-intact, but not in endothelium-denuded tissues.

4 Haemoglobin and methylene blue (both 10 μM) enhanced the vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline. The enhancements were absent in endothelium-denuded arterial segments.

5 In endothelium-denuded arterial segments precontracted with phenylephrine, the vasodilator responses to the nitric oxide donor, sodium nitroprusside (0.1–300 nM), were decreased by increasing the level of precontraction.

6 L-NNA (10 μM) had no effect on the S-I efflux of radioactivity from arteries in which transmitter stores had been labelled with [^{3}H]-noradrenaline.

7 These results suggest that endothelial nitric oxide attenuates vasoconstrictor responses in the rat caudal artery through activation of soluble guanylate cyclase to decrease smooth muscle contractility. Therefore, the findings provide evidence that nitric oxide acts as a functional antagonist to oppose vasoconstriction.

Keywords: Haemoglobin; methylene blue; nitric oxide; N^G-nitro-L-arginine; N^G-nitro-L-arginine methyl ester; rat caudal artery; sodium nitroprusside; sympathetic nerve stimulation; vasoconstriction; vasodilatation

Introduction

The vascular endothelium synthesizes and releases endothelium-derived relaxing factor (EDRF) (Furchgott, 1984; Moncada *et al.*, 1991a). EDRF mediates the vasodilator effect of acetycholine and other endothelium-dependent vasodilators on blood vessels (Furchgott, 1984) through the activation of soluble guanylate cyclase in the smooth muscle (Waldman & Murad, 1987). Nitric oxide has been reported to account for the biological activity of EDRF (Palmer *et al.*, 1987) and is formed from the conversion of L-arginine to L-citrulline (Palmer *et al.*, 1988). This synthetic pathway is catalysed by the enzyme, nitric oxide synthase, which can be inhibited by the L-arginine analogues, N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME) (Sakuma *et al.*, 1988; Moore *et al.*, 1990; Rees *et al.*, 1990).

Intravenous administration of L-NMMA or L-NAME induces an increase in mean arterial blood pressure and inhibits the hypotension induced by acetylcholine and bradykinin in the anaesthetized rat (Rees *et al.*, 1989; 1990). Similar observations have been reported in man, where infusion of L-NMMA into the brachial artery reduces blood flow in the forearm (Vallance *et al.*, 1989). These observations suggest that nitric oxide is important in the regulation of blood flow and pressure.

It has also been suggested that nitric oxide has a functional role in the modulation of smooth muscle reactivity. Inhibition of nitric oxide synthesis with L-arginine analogues enhances contractile responses in a number of preparations, including the rat anococcygeus (Li & Rand, 1989), the dog mesenteric artery (Toda & Okamura, 1990), and the rat aorta and pulmonary artery (Crawley *et al.*, 1990; Moncada *et al.*, 1991b; Topouzis *et al.*, 1991). We have recently shown that inhibition of nitric oxide synthesis with L-NNA enhances vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the rat caudal artery (Reid *et al.*, 1991; Vo *et al.*, 1991). The enhancement was prevented by L-arginine and was endothelium-dependent.

In the present study, we have extended these observations in the rat caudal artery in order to investigate further the influence of nitric oxide on vascular reactivity. The aims of the study were 3 fold. Firstly, since the specificity of L-arginine analogues as inhibitors of nitric oxide synthesis has been questioned by some investigators (e.g., Thomas *et al.*, 1989; Cocks & Angus, 1991), the effect of a range of inhibitors of nitric oxide-mediated responses has been investigated on vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation, namely: L-NAME, another inhibitor of nitric oxide synthesis; haemoglobin, which inactivates nitric oxide; and methylene blue, an inhibitor of soluble guanylate cyclase, the biological 'receptor' for nitric oxide. Secondly, the effect of L-NNA on vasoconstrictor responses to agonists other than noradrenaline has been examined to determine whether or not nitric oxide-mediated attenuation of vasoconstriction is specific to noradrenaline.

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Thirdly, the effect of L-NNA and the nitric oxide donor sodium nitroprusside has been investigated on the stimulation-induced (S-I) release of noradrenaline from the rat caudal artery, to determine whether nitric oxide-mediated modulation of vasoconstrictor responses to sympathetic nerve stimulation involves prejunctional mechanisms.

Methods

Sprague-Dawley rats (250–350 g) of either sex were pretreated with heparin (1000 u kg^{-1}, i.p.) 30 min before they were killed by stunning with a blow to the head and exsanguination. Two segments of the central caudal artery (2 cm) were carefully dissected free from surrounding connective tissue. Each segment was cannulated at the proximal end with SP 31 polyethylene tubing; the distal end was ligated and an incision was made in the wall of the vessel below the tie. The segment was mounted vertically with the distal end uppermost, under 0.5 g tension. Physiological salt solution (PSS) was perfused through the lumen at 37°C with a Gilson Minipuls 3 peristaltic pump at a rate of 1.5 ml min^{-1} and the perfusate was allowed to superfuse the adventitial surface of the vessel. The PSS was continuously bubbled with 95% O_2/5% CO_2 and had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.03, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14. The perfusion pressure was measured with a Cobe CDX-III pressure transducer connected to a Grass polygraph recorder. Increases in perfusion pressure were taken as indices of vasoconstriction. This method of perfusion/superfusion has been used extensively to assess vascular reactivity (e.g., Medgett & Langer, 1984; 1986; Xiao & Rand, 1989; Vo *et al.*, 1991).

Periarterial nerves were stimulated with 0.1 ms monophasic square pulses at supramaximal voltage (60 V) delivered from a Grass S88 stimulator through circular bipolar platinum electrodes placed around the proximal end of the artery. An equilibration period of 45 min was allowed before experimental observations were made.

Vasoconstrictor responses

Vasoconstrictor responses to sympathetic nerve stimulation, or to cumulative concentrations of noradrenaline, phenylephrine, methoxamine or 5-hydroxytryptamine, or to 40 mM KCl, were obtained before and 30 min after exposure to L-NNA or L-NAME, or 15 min after exposure to haemoglobin or methylene blue. The effects of L-NAME, haemoglobin and methylene blue on vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline were also examined in endothelium-denuded preparations. In these experiments, the vascular endothelium was removed by perfusing arterial segments with a stream of 95% O_2/5% CO_2 at a pressure of 60–70 mmHg for 90 s and then re-perfusing with PSS (Spokas & Folco, 1984; Vo *et al.*, 1991). In time-control experiments, L-NNA, L-NAME, haemoglobin and methylene blue were absent from the perfusate. In experiments where endothelin-1 was used as the vasoconstrictor agent, control responses and responses in the presence of L-NNA were obtained from two different segments of artery taken from the same animal.

Vasodilator responses

In all experiments, the presence of functional endothelium was determined by the vasodilator action of acetylcholine (1 μM) in arteries precontracted with phenylephrine (3 μM); endothelium-intact arterial segments relaxed by at least 50% of the precontraction and endothelium-denuded segments failed to relax in response to acetylcholine. In separate experiments in which the endothelium was intact, cumulative concentration-response curves to acetylcholine and papaverine were obtained in arteries that were precontracted with phenylephrine (3 μM) before and 30 min after exposure to L-NNA or L-NAME. In endothelium-denuded arterial segments, vasodilator responses to sodium nitroprusside were obtained at different levels of precontraction with phenylephrine.

S–I release of noradrenaline

The S–I release of noradrenaline was investigated by the method of Rajanayagam *et al.* (1989). Briefly, segments of rat caudal artery (3 cm in length) were incubated in [^{3}H]-noradrenaline (0.23 μM, 10 μCi ml^{-1} for 30 min) to label transmitter stores. After washing for 90 min, the arteries were given two periods of stimulation ($Stim_1$ and $Stim_2$, 40 min apart) with 0.1 ms monophasic square pulses at supramaximal voltage (60 V) and a frequency of 5–20 Hz for 10 or 60 s. L-NNA or sodium nitroprusside was introduced 30 or 10 min before $Stim_2$, respectively. Idazoxan was added to the perfusate 20 min before either $Stim_1$ or $Stim_2$, as indicated. The vascular endothelium was removed in experiments in which sodium nitroprusside was used.

The perfusate was collected in 2-min samples and analysed for radioactivity by liquid scintillation counting. Counting efficiency, determined by automatic external standardization, was approximately 25%, and all measurements were recorded as disintegrations per min (d.p.m.). The resting efflux of radioactivity was taken as the mean of the radioactive content in the three 2-min samples taken immediately before each stimulation period. The S–I efflux of radioactivity was calculated as the difference between five times the resting efflux and the sum of the radioactive content in the five 2-min samples from the start of stimulation, and was expressed as a fraction (FR) of the radioactivity in the tissue at the commencement of stimulation. The S–I efflux of radioactivity was used as an index of the S–I release of transmitter noradrenaline. The fractional S–I efflux evoked by $Stim_2$ is expressed as a percentage of that evoked by $Stim_1$ (FR_2/FR_1).

Drugs and solutions

The following drugs were used: acetylcholine perchlorate (Sigma), haemoglobin (Sigma), idazoxan hydrochloride (Reckitt & Colman), indomethacin (Merck Sharp & Dohme), methoxamine hydrochloride (Burroughs Wellcome), methylene blue (Sigma), N^G-nitro-L-arginine (L-NNA, Sigma), N^G-nitro-L-arginine methyl ester (L-NAME, Sigma), noradrenaline bitartrate (Sigma), (−)-[ring-2,5,6-^{3}H]-noradrenaline (specific activity 43.7 Ci $mmol^{-1}$; New England Nuclear), papaverine hydrochloride (Sigma), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), sodium nitroprusside (Sigma) and tetrodotoxin (Sigma).

Drugs were dissolved in distilled water to give 10 mM stock solutions, except indomethacin which was dissolved in 0.1 M Na_2CO_3 and prazosin which was dissolved in 2% glycerol and 30% dextrose.

Depolarization-induced contractions to KCl were elicited by replacing normal PSS with a solution in which the equivalent amount of NaCl was replaced by 40 mM KCl.

Statistical analyses

Results are expressed as mean and s.e.mean. Differences between means were assessed by Student's *t* test (one-tailed), or by one- or by two-way analysis of variance (ANOVA), followed by planned comparisons, as indicated. Analyses were carried out with the software package CSS (Statsoft). Values of $P<0.05$ were taken to indicate statistical significance.

Results

Effect of L-NNA and L-NAME on vasoconstrictor responses

Stimulation of sympathetic nerves (3 Hz, 10 s) produced a submaximal increase in perfusion pressure of 41.3 ± 4.5 mmHg ($n = 24$) in the rat caudal artery; the response was consistent throughout the experiment (Table 1) and was abolished by 0.3 μM tetrodotoxin or 10 nM prazosin (data not shown). Exposure of the rat caudal artery to 10 μM L-NNA or 10 μM L-NAME for 30 min had no effect on the resting perfusion pressure of 8.2 ± 0.5 mmHg ($n = 18$), but significantly enhanced the vasoconstrictor response to sympathetic nerve stimulation (Table 1, $P<0.05$, unpaired Student's t test). The enhancement caused by 10 μM L-NAME was absent in endothelium-denuded segments (Table 1). The α_2-adrenoceptor antagonist, idazoxan (0.1 μM) did not affect the enhancing effect of L-NNA on vasoconstrictor responses to sympathetic nerve stimulation ($n = 4$, data not shown).

In time-control experiments, increases in perfusion pressure produced by cumulative concentrations of noradrenaline ($n = 5$), phenylephrine ($n = 4$), methoxamine ($n = 5$) and 5-hydroxytryptamine ($n = 5$) did not alter during the course of the experiment (data not shown). However, 10 μM L-NNA significantly enhanced the vasoconstrictor responses produced by noradrenaline, methoxamine, phenylephrine and 5-hydroxytryptamine (Figures 1 and 2, $P<0.05$, two-way ANOVA). L-NAME (10 μM) caused a significant enhancement of the vasoconstrictor responses to noradrenaline (Figure 3, $P<0.05$, two-way ANOVA) and the enhancement was absent in endothelium-denuded arterial segments (Figure 3).

Endothelin-1 (10 nM) caused slowly developing increases in perfusion pressure in the rat caudal artery. The perfusion pressure returned to baseline 60 min after the removal of endothelin-1, however, subsequent exposure to 10 nM endothelin-1 did not elicit a further response. Therefore, the effect of endothelin-1 in the absence and presence of L-NNA was determined in two different arterial segments taken from the same animal. Pretreatment of arterial segments with 10 μM L-NNA for 30 min produced significantly greater responses ($P<0.05$, unpaired Student's t test) to 10 nM endothelin-1 (21.4 ± 3.2 mmHg, $n = 7$) than in the absence of L-NNA (12.7 ± 2.1 mmHg, $n = 6$). The enhancing effect of L-NNA on responses to endothelin-1 might be underestimated because of the intrinsic property of endothelin-1 of inducing the release of nitric oxide (Warner *et al.*, 1989).

Table 1 The vasoconstrictor responses to nerve stimulation (3 Hz, 10 s) after a 30-min exposure to 10 μM N^G-nitro-L-arginine (L-NNA) or 10 μM N^G-nitro-L-arginine methyl ester (L-NAME), and after a 15-min exposure to 10 μM haemoglobin or 10 μM methylene blue in endothelium-intact or -denuded segments of rat caudal artery

	% of initial response[a]	
Treatment	*Endothelium-intact*	*Endothelium-denuded*
Time-control	107 ± 4% (4)	101 ± 5% (5)
L-NNA	203 ± 9% (6)*	Not done
L-NAME	149 ± 6% (6)*	100 ± 2% (4)
Haemoglobin	140 ± 5% (4)*	102 ± 5% (4)
Methylene blue	149 ± 4% (4)*	104 ± 2% (3)

[a]Results are expressed as a percentage of the initial response before the addition of inhibitors. Values are mean ± s.e.mean from the number of experiments indicated in parentheses.
*Significantly different from the time-control ($P<0.05$, unpaired Student's t test).

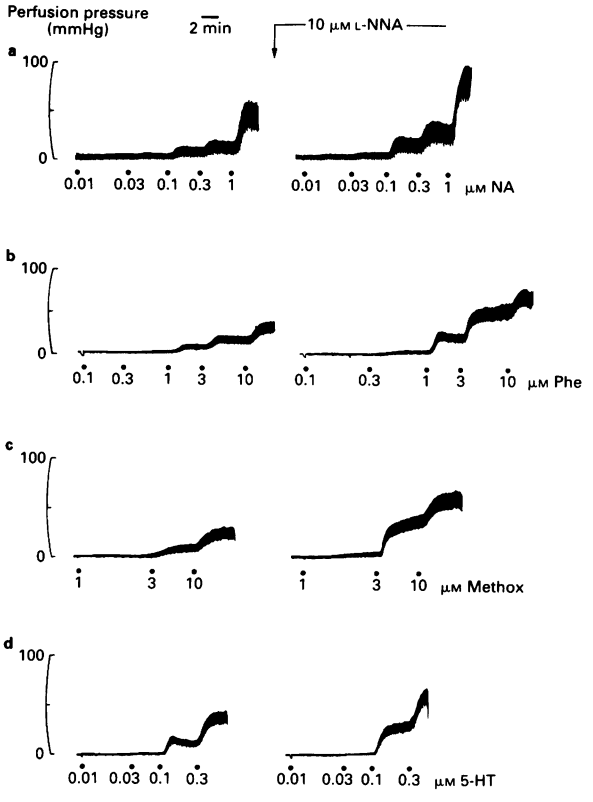

Figure 1 Recordings showing the enhancing effect of 10 μM N^G-nitro-L-arginine (L-NNA) on increases in perfusion pressure produced by cumulative concentrations of (a) noradrenaline (NA), (b) phenylephrine (Phe), (c) methoxamine (Methox) and (d) 5-hydroxytryptamine (5-HT) in endothelium-intact segments of rat caudal artery. L-NNA was added to the perfusate 30 min before the second set of responses was obtained.

The vasoconstrictor response to 40 mM KCl was consistent over a 60-min period (initial response: 79.2 ± 6.0 mmHg; response after 60 min: 84.8 ± 7.2 mmHg; $n = 5$), but was significantly enhanced after a 30-min exposure to 10 μM L-NNA from 74.7 ± 9.3 mmHg to 137 ± 12 mmHg ($P<0.05$, paired Student's t test, $n = 3$).

Effect of haemoglobin and methylene blue on vasoconstrictor responses

Haemoglobin and meythlene blue (both 10 μM) had no effect on the resting perfusion pressure, but caused a significant enhancement of vasoconstrictor responses to sympathetic nerve stimulation (Table 1, $P<0.05$, unpaired Student's t test) and to noradrenaline (Figure 4, $P<0.05$, two-way ANOVA) in the rat caudal artery. The enhancements caused by haemoglobin and methylene blue were absent in endothelium-denuded arterial segments (Table 1, Figure 4).

Effect of L-NNA and L-NAME on vasodilator responses

Acetylcholine and papaverine caused concentration-dependent relaxations of arterial segments precontracted with 3 μM phenylephrine (Figures 5 and 6). L-NNA (10 or 100 μM) and L-NAME (10 μM) had no effect on the resting perfusion pressure, but caused an increase in the sensitivity of the arterial segments to phenylephrine. Therefore, the concentration of phenylephrine was adjusted from 3 μM to 0.3–1 μM in order to achieve an increase in perfusion pressure equivalent to that obtained before the addition of L-NNA or L-NAME. L-NNA (10 or 100 μM) and L-NAME (10 μM) significantly attenuated the vasodilator responses to acetylcholine (Figure

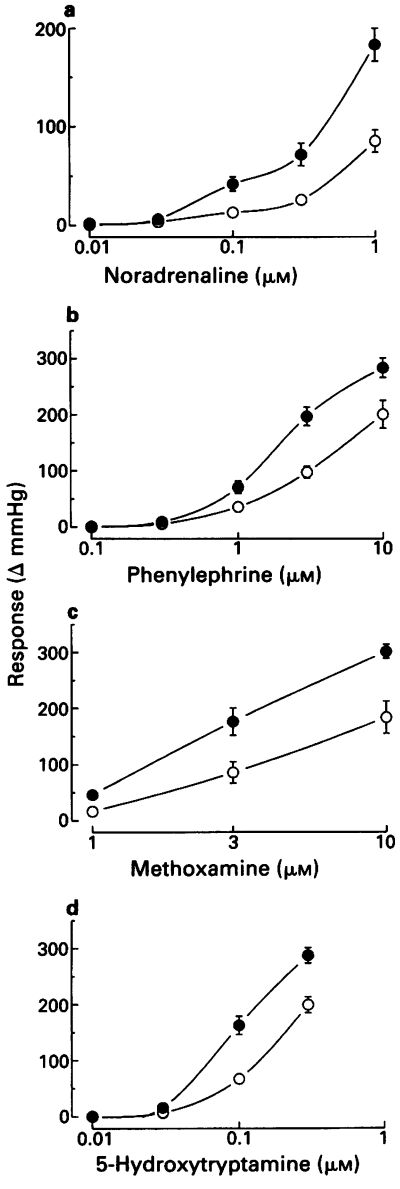

Figure 2 Effect of N^G-nitro-L-arginine (L-NNA) on vasoconstrictor responses to (a) noradrenaline, (b) phenylephrine, (c) methoxamine and (d) 5-hydroxytryptamine in endothelium-intact segments of rat caudal artery. Vasoconstrictor responses before (○) and 30 min after (●) exposure to 10 μM L-NNA are shown. Results are expressed as mean and s.e.mean (vertical bars) from four to six experiments.

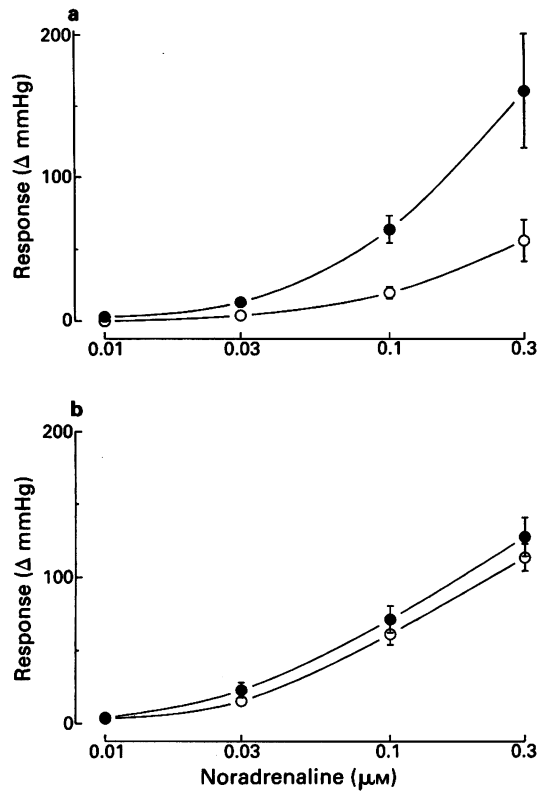

Figure 3 Vasoconstrictor responses to noradrenaline in (a) endothelium-intact and (b) endothelium-denuded segments of the rat caudal artery before (○) and 30 min after (●) exposure to 10 μM N^G-nitro-L-arginine methyl ester. Results are expressed as mean and s.e.mean (vertical bars) from four experiments.

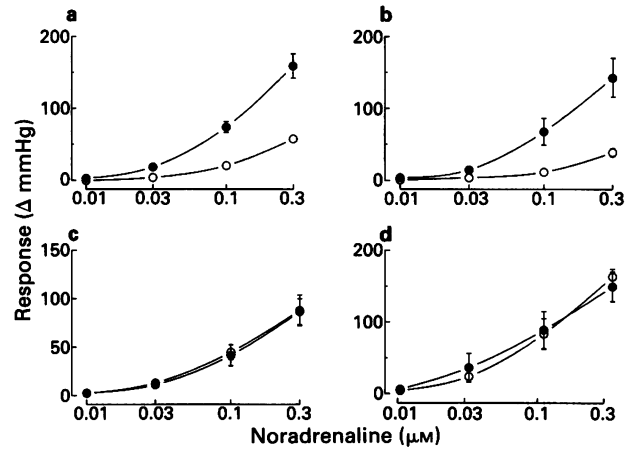

Figure 4 Vasoconstrictor responses to noradrenaline in (a,b) endothelium-intact and (c,d) endothelium-denuded segments of the rat caudal artery. Vasoconstrictor responses before (○) and after (●) 15 min exposure to (a,c) 10 μM methylene blue or (b,d) 10 μM haemoglobin. Results are expressed as mean and s.e.mean (vertical bars) from four to six experiments.

5, $P<0.05$, two-way ANOVA). In contrast, the vasodilator responses to papaverine were not significantly affected by 10 μM L-NNA or 10 μM L-NAME (Figure 6, $P>0.05$, two-way ANOVA). In time-control experiments, vasodilator responses to acetylcholine and papaverine did not change significantly over the time course of the experiment (Figures 5 and 6, $P>0.05$, two-way ANOVA).

Effect of level of precontraction on sensitivity to sodium nitroprusside

To test whether the sensitivity to nitric oxide was changed by the size of the vasoconstrictor response, vasodilator responses to the nitric oxide donor, sodium nitroprusside, were obtained in endothelium-denuded arteries which had been precontracted to different levels. Sodium nitroprusside caused concentration-dependent relaxations of arterial segments precontracted with phenylephrine (1 or 2 μM) by 51 ± 4 mmHg ($n = 5$, Figure 7). At a higher level of precontraction (96 ± 7 mmHg, $n = 5$) obtained with 3 or 10 μM phenylephrine, the relaxant responses to sodium nitroprusside were significantly attenuated (Figure 7, $P>0.05$, two-way ANOVA).

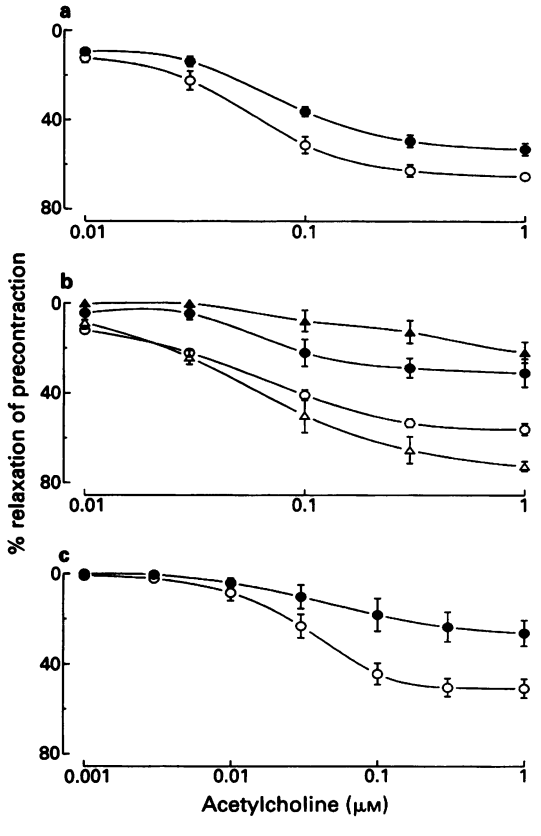

Figure 5 Effect of N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME) on vasodilator responses to acetylcholine in endothelium-intact arteries precontracted with phenylephrine (0.3–3 μM). (a) Time-control experiments in which responses were obtained 30 min apart without the addition of L-NNA or L-NAME (first curve: ○; second curve: ●). (b) Vasodilator responses before (open symbols) and 30 min after (closed symbols) exposure to 10 μM (circles) and 100 μM (triangles) L-NNA. (c) Vasodilator responses before (○) and 30 min after (●) exposure to 10 μM L-NAME. Results are expressed as mean and s.e.mean (vertical bars) from three to five experiments.

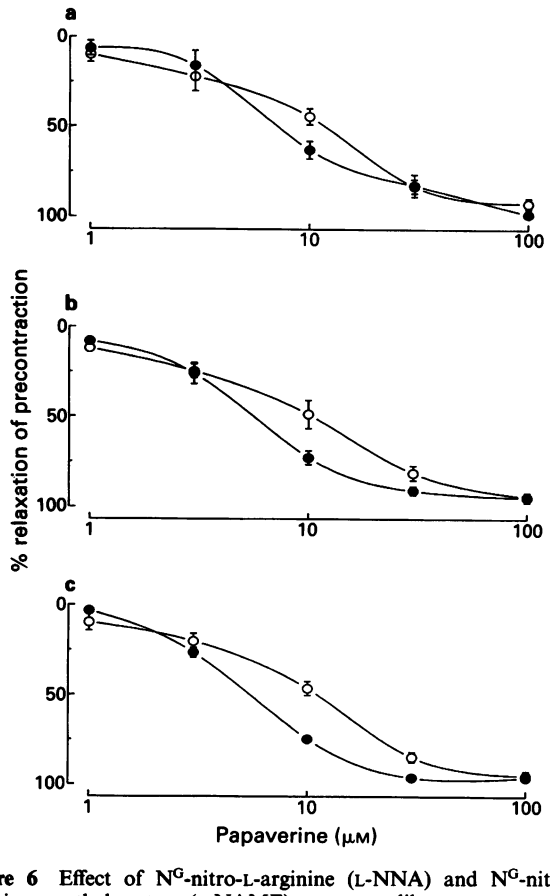

Figure 6 Effect of N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME) on vasodilator responses to papaverine in endothelium-intact arteries precontracted with phenylephrine (0.3–3 μM). (a) Time-control experiments in which responses were obtained 30 min apart without the addition of L-NNA or L-NAME (first curve: ○; second curve: ●). (b) Vasodilator responses before (○) and 30 min after (●) exposure to 10 μM L-NNA. (c) Vasodilator responses before (○) and 30 min after (●) exposure to 10 μM L-NAME. Results are expressed as mean and s.e.mean (vertical bars) from four experiments.

Effect of L-NNA and sodium nitroprusside on S–I efflux of noradrenaline

The resting radioactive efflux from arteries previously incubated in [^{3}H]-noradrenaline fell expontentially for the first 35 min after incubation and after 90 min reached a plateau of 4542 ± 459 d.p.m. ($n = 4$). Electrical stimulation (5 Hz, 60 s, $Stim_1$) caused an increase in the efflux of radioactivity of 6299 ± 303 d.p.m. ($n = 4$) above resting levels. L-NNA (10 and 100 μM) had no effect on the resting efflux of radioactivity. When 10 μM L-NNA was added to the perfusate 30 min before $Stim_2$, the S–I efflux of radioactivity (FR_2/FR_1) was 93 ± 2% ($n = 3$) and was not significantly different from control values (91 ± 2%, $n = 4$).

In order to check that an effect of L-NNA was not being masked by the α_2-adrenoceptor inhibitory feedback system, experiments were carried out in the presence of the α_2-adrenoceptor antagonist, idazoxan. Idazoxan (0.1 μM) introduced 20 min before $Stim_2$ (20 Hz, 10 s), enhanced FR_2/FR_1 from 99 ± 2% ($n = 3$) to 225 ± 13% ($n = 4$). When idazoxan (0.1 μM) was present throughout perfusion, the addition of either 10 or 100 μM L-NNA 30 min before $Stim_2$ caused small and inconsistent changes in the S–I radioactive efflux (Table 2). In addition, with idazoxan (0.1 μM) present throughout the experiment, the nitric oxide donor sodium nitroprusside slightly enhanced the S–I efflux of radioactivity from endothelium-denuded preparations (Table 2).

Discussion

The present study confirms and extends our earlier observations (Reid *et al.*, 1991; Vo *et al.*, 1991) that inhibition of nitric oxide synthesis (with either L-NNA or L-NAME) enhances the vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the rat caudal artery. In addition, suppression of nitric oxide-mediated responses through inhibition of soluble guanylate cyclase with methylene blue, and binding of nitric oxide to the Fe-containing haem group of haemoglobin (Martin *et al.*, 1985; Ignarro, 1989) also caused an enhancement of the vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in this preparation. The enhancements were absent in endothelium-denuded arterial segments. Thus, the effects of the nitric oxide synthase inhibitors, L-NNA and L-NAME, appear to be specific, since similar effects on vasoconstrictor responses were observed when nitric oxide-mediated responses were inhibited by other mechanisms. Previous reports have shown that L-NMMA or L-NNA enhanced vasoconstrictor responses to nerve stimulation and noradrenaline in a number of different vascular preparations, including the rat mesenteric arterial bed (Way *et al.*, 1991) and renal vascular bed (Reid & Rand, 1992), the dog coronary artery (Berkenboom *et al.*, 1991; Toda & Okamura, 1990) and mesenteric artery (Toda & Okamura, 1990), the guinea-pig pulmonary artery (Liu *et al.*, 1991), and the

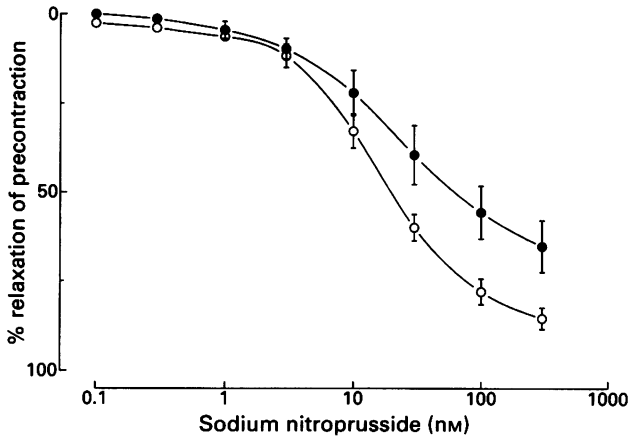

Figure 7 Vasodilator responses to sodium nitroprusside in endothelium-denuded arteries precontracted with 1 or 2 μM (○) and 3 or 10 μM (●) phenylephrine which produced an increase in perfusion pressure of 51 ± 4 mmHg ($n = 5$) and 96 ± 7 mmHg ($n = 5$), respectively. Results are expressed as mean and s.e.mean (vertical bars) from five experiments.

Table 2 The effect of N^G-nitro-L-arginine (L-NNA) and sodium nitroprusside on the stimulation-induced (S–I) efflux of radioactivity from endothelium-intact and endothelium-denuded segments of rat caudal artery, respectively. The arteries were subjected to two periods of stimulation ($Stim_1$ and $Stim_2$) 40 min apart.

	S–I efflux of radioactivity (FR_2/FR_1)[a]	
	10 Hz, 10 s	20 Hz, 10 s
Endothelium-intact arteries		
Control	99.3 ± 2.2% (4)	104.2 ± 5.9% (4)
L-NNA (μM)		
10	115.6 ± 7.2% (3)*	107.4 ± 5.9% (3)
100	95.3 ± 3.3% (4)	85.7 ± 3.4% (4)*
Endothelium-denuded arteries		
Control	88.0 ± 1.0% (4)	
Sodium nitroprusside (μM)		
0.1	101.6 ± 2.9% (4)*	
1	122.9 ± 3.9% (4)*	
10	113.0 ± 5.1% (3)*	

Idazoxan (0.1 μM) was present throughout the experiment and L-NNA or sodium nitroprusside was introduced 30 or 10 min before $Stim_2$ respectively.
[a]The S–I efflux of radioactivity evoked by $Stim_2$ is expressed as a percentage of that evoked by $Stim_1$ (FR_2/FR_1). Values are mean ± s.e.mean from the number of experiments indicated in parentheses. *Significant difference from the appropriate control ($P<0.05$, one-way ANOVA followed by planned comparisons).

human coronary artery (Berkenboom *et al.*, 1991). Methylene blue has also been shown to potentiate noradrenaline-mediated contractions in rat mesenteric and femoral arteries (Urabe *et al.*, 1991), and in dog and human coronary arteries (Berkenboom *et al.*, 1991). Furthermore, haemoglobin has been reported to cause endothelium-dependent potentiation of the contraction to phenylephrine in bovine pulmonary artery and vein (Gold *et al.*, 1990), in accord with the present study. Together these findings suggest that nitric oxide is released from endothelial cells to oppose vasoconstriction, and that the phenomenon is not restricted to either specific vascular beds or species. In addition, the results strengthen the proposal that endothelium-derived nitric oxide is a modulator of smooth muscle contractility.

The inhibitory effect of L-NNA and L-NAME on the vasodilator responses to acetylcholine observed in this study are in agreement with reports by Moore *et al.* (1990) and Rees *et al.* (1990). In contrast, vasodilator responses to the direct smooth muscle relaxant, papaverine, were not affected by either L-NNA or L-NAME. Therefore, the synthesis inhibitors suppressed endothelium-dependent, but not endothelium-independent vasodilatation. These results provide further evidence supporting the specific inhibition of nitric oxide synthesis by L-NNA and L-NAME in the rat caudal artery.

It is possible that the enhancing effect of L-NNA on vasoconstrictor responses to sympathetic nerve stimulation might involve the modulation by L-NNA of the release of noradrenaline from sympathetic nerve terminals. However, this explanation is unlikely because L-NNA (10 μM) had no effect on the S–I efflux of noradrenaline from the rat caudal artery. The absence of an effect of L-NNA on the release of noradrenaline could not be due to a lack of responsiveness of the preparation to prejunctional modulation; the S–I release of noradrenaline could be enhanced by idazoxan, which is consistent with prejunctional modulation by an α_2-adrenoceptor autoinhibitory feedback system in this tissue (Starke, 1987).

The S–I release of radioactivity from endothelium-denuded arterial segments was enhanced by the nitric oxide donor, sodium nitroprusside, but this effect was small and not concentration-dependent. These findings are in contrast to those of Greenberg *et al.* (1990), who reported that sodium nitroprusside decreased the S–I efflux of noradrenaline from segments of dog mesenteric artery, but only at low frequencies (4 Hz) of stimulation. The discrepancy may be partly related to differences between vascular preparations and/or to frequency of stimulation. In our study, frequencies below 5 Hz were not used, because the release of radioactivity was below the limit of detection.

The enhancement of vasoconstriction produced by inhibition of nitric oxide synthesis was not specific to noradrenaline-mediated responses; L-NNA was found to enhance the vasoconstrictor responses induced by methoxamine, 5-hydroxytryptamine, phenylephrine and endothelin-1, as well as depolarization with a high concentration of potassium. Nitric oxide attenuated both receptor- and nonreceptor-mediated vasoconstriction. Other investigators have also reported that nitric oxide synthase inhibitors enhance the contraction in isolated vascular preparations to substances other than noradrenaline, including phenylephrine (Crawley *et al.*, 1990; Gold *et al.*, 1990; Liu *et al.*, 1991), endothelin-1 (Ito *et al.*, 1991) and prostaglandin $F_{2\alpha}$ (Liu *et al.*, 1991).

Although it is apparent that endogenous nitric oxide is released to attenuate contractile responses in the vasculature, the precise mechanism behind the stimulation of nitric oxide release is not clear. Noradrenaline and 5-hydroxytryptamine have been shown to produce endothelium-dependent relaxation through activation of α_2-adrenoceptors and 5-HT receptors, respectively (Cocks & Angus, 1983; Berkenboom *et al.*, 1990). However, it is unlikely that α_2-adrenoceptors are responsible for the stimulation of nitric oxide production in the present study, since the α_2-adrenoceptor antagonist idazoxan did not influence the enhancing effect of L-NNA on vasoconstrictor responses to sympathetic nerve stimulation.

Alternatively, the enhancement of vasoconstrictor responses observed in this study may result from an inhibition of 'basal' release of nitric oxide (for review, see Moncada *et al.*, 1991a) which provides continuous antagonism to contractile responses. This possibility is unlikely in the rat caudal artery since L-NNA, L-NAME, haemoglobin or methylene blue do not affect resting smooth muscle tone. On the other hand, the low resting tone under our experimental conditions might render the caudal artery insensitive to the vasodilator action of 'basal' nitric oxide; the vasodilatation would then

only be detectable after an increase in arterial tone as a result of vasoconstriction. If inhibition of a 'basal' production of nitric oxide could explain the enhancement of vasoconstrictor responses by nitric oxide synthase inhibitors, the same amount of basally released nitric oxide would be available to antagonize vasoconstriction irrespective of the magnitude of contraction. However, this is not the case: there is a positive linear correlation between increases in perfusion pressure caused by sympathetic nerve stimulation and vasoconstrictor agents in the presence of L-NNA and increases obtained in the absence of L-NNA (Figure 8). The regression line has a slope of approximately 2, indicating that the perfusion pressure is doubled when nitric oxide synthesis is inhibited. Furthermore, it was demonstrated that the sensitivity to nitric oxide does not increase with increasing levels of tone, because the vasodilator response to the nitric oxide donor, sodium nitroprusside, did not increase when the level of precontraction was increased; in fact, responses were slightly attenuated by increasing precontraction. Since an altered sensitivity to nitric oxide cannot explain the correlation between level of tone and magnitude of enhancement, the amount of nitric oxide released must be proportional to the level of contraction. Thus a 'basal' release of nitric oxide alone could not fully account for the antagonism of vasoconstriction.

Fluid shear stress produced by a change in perfusate velocity has been demonstrated to stimulate the release of nitric oxide from endothelial cells (Rubanyi *et al.*, 1986; Buga *et al.*, 1991). In the present study, endothelial shear stress caused by increases in vascular tone may provide the stimulus for nitric oxide release. In further support of this suggestion, Lamontagne *et al.* (1992) reported that an increase in shear stress caused by vasoconstriction at constant flow was partially responsible for the release of EDRF from the coronary circulation of the rabbit isolated heart. Additional evidence for a direct relationship between nitric oxide release and tension can be acquired from a study by Ohno *et al.* (1990) using rabbit aorta, where tissue cyclic guanosine monophosphate levels were elevated with increases in resting tension or stretch in endothelium-intact rings but not in endothelium-denuded rings. Alternatively, shear stress may cause the production of a mediator such as endothelin-1, which could stimulate nitric oxide release from endothelial cells (Yanagisawa & Masaki, 1989).

In conclusion, vasoconstrictor responses to sympathetic nerve stimulation and a range of vasoconstrictor agonists were enhanced in the rat isolated caudal artery by nitric oxide synthesis inhibition (with L-NNA and L-NAME), inactivation of nitric oxide (with haemoglobin), and inhibition of guanylate cyclase (with methylene blue). The enhancements were absent in endothelium-denuded arterial segments. These observations indicate that endogenous nitric oxide activates the soluble guanylate cyclase in vascular smooth muscle to induce relaxation, thus opposing vasoconstriction. The release of nitric oxide, probably in response to either shear stress itself or to an intermediate stimulator caused by shear stress, acts as a functional antagonist possibly to limit blood vessel damage during vasoconstriction.

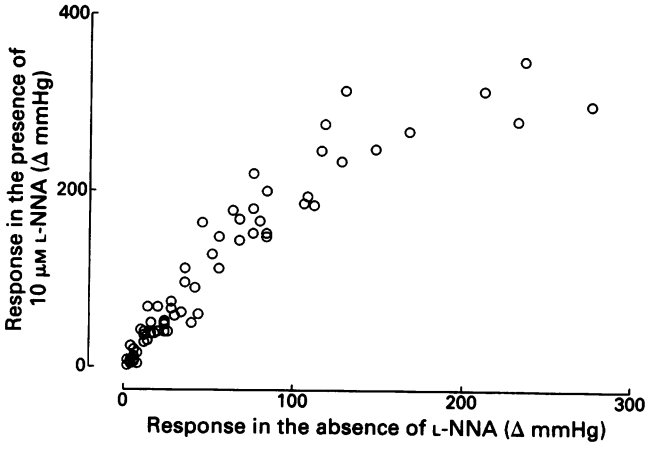

Figure 8 Relationship between the increases in perfusion pressure caused by sympathetic nerve stimulation and vasoconstrictor agonists in the absence and in the presence of 10 μM N^G-nitro-L-arginine (L-NNA). Each point represents the responses from an individual experiment. The points with an abscissa of less than 200 mmHg were fitted to a linear regression, which had a correlation coefficient of 0.955 and a slope of 1.9. The regression line starts to plateau when the abscissa rises above 200 mmHg, probably reflecting the physical limitation of the arterial preparation.

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