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A neuromodulatory role for neuronal nitric oxide in the rabbit renal artery

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1 The effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on vasoconstrictor responses to transmural electrical nerve stimulation and noradrenaline were examined in the rabbit isolated renal artery with and without an intact endothelium. In addition, the effect of removing the endothelium from the renal artery on vasoconstrictor responses to transmural electrical nerve stimulation and noradrenaline was also investigated. Immunohistofluorescence techniques were carried out to determine if there were any nitrergic nerves supplying the renal artery.

2 The vasoconstriction produced in response to transmural electrical nerve stimulation (2–64 Hz) was significantly enhanced in the presence of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M).

3 L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M) did not significantly affect the maximum vasoconstriction produced in response to noradrenaline. However, the noradrenaline dose-response curve was significantly shifted to the left by the addition of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M).

4 The increase in the amplitude of the vasoconstriction, produced in response to transmural electrical nerve stimulation (16 Hz) and noradrenaline (10^{-5} M) in the presence of L-NAME (10^{-5} M) was not observed when L-arginine (10^{-3} M) was added in addition to L-NAME (10^{-5} M).

5 Removing the endothelium did not significantly affect the response to transmural electrical nerve stimulation (1–64 Hz). The maximum vasoconstriction in response to noradrenaline was also unaffected by the removal of the endothelium. The pD_2 value for noradrenaline obtained from vessels with no endothelium was significantly greater than the pD_2 value obtained from vessels with an intact endothelium (5.90 ± 0.11 and 5.16 ± 0.03, respectively).

6 On renal artery segments with no endothelium L-NAME (3×10^{-5} M) significantly enhanced the response to transmural electrical nerve stimulation (2–64 Hz). L-NAME did not affect the maximum response to noradrenaline. However, there was a significant shift to the right of the noradrenaline dose-response curve in the presence of L-NAME (3×10^{-5} M).

7 Both nitric oxide synthase-containing and NADPH-diaphorase stained nerves were located on the adventitial-medial border of the rabbit renal artery.

8 The present study has suggested a presynaptic inhibitory action for nitric oxide (probably derived from identified perivascular nitrergic nerves), on perivascular sympathetic vasoconstrictor nerve mediated responses of the rabbit renal artery. In contrast, the enhancement of the response to noradrenaline by L-NAME can be attributed to inhibition of the synthesis of endothelium-derived nitric oxide.

Keywords: Nitric oxide; rabbit renal artery; neuromodulation; sympathetic neurotransmission

Introduction

Nitric oxide is an important endogenous vasodilator released from vascular endothelial cells (Palmer *et al*., 1987). The synthesis of nitric oxide from L-arginine is catalysed by the enzyme, nitric oxide synthase, which can be inhibited by a variety of L-arginine analogues, including N^G-nitro-L-arginine methyl ester (L-NAME; Rees *et al*., 1990). In blood vessels, besides localization in the endothelium, nitric oxide synthase also occurs in the autonomic nerves in the outer, adventitial layers of various large blood vessels (Bredt *et al*., 1990).

Evidence has been presented which indicate that nitric oxide may be involved in the regulation of sympathetic neurotransmission (Toda & Okamura, 1990; Liu *et al*., 1991; Kasakov *et al*., 1994). L-N^G-monomethyl arginine enhanced vasoconstrictor responses elicited by stimulation of perivascular nerves in perfused segments of dog mesenteric arteries (Toda & Okamura, 1990) and guinea-pig pulmonary artery rings (Liu *et al*., 1991), in which vasoconstrictor responses to noradrenaline, phenylephrine and prostaglandin F_{2d} ($PGF_{2\alpha}$) were also enhanced. N^G-nitro-L-arginine also enhanced vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in the isolated perfused segments of rat tail artery (Reid *et al*., 1991; Vo *et al*., 1991a), rat perfused mesenteric vascular bed (Way *et al*., 1991; Way & Reid, 1991) and rat perfused kidney (Reid & Rand, 1992). This enhancement could be prevented by prior addition of L-arginine to the perfusate (Way & Reid, 1991; Way *et al*., 1991; Vo *et al*., 1991a; Reid & Rand, 1992). Analysis of neuronal nitrergic mechanisms in blood vessels is complicated by the production by endothelial cells of nitric oxide which plays a significant role in the regulation of blood pressure and regional blood flow in animals (Moncada *et al*., 1989; 1991; Gardiner *et al*., 1990). It has previously been shown that removal of the endothelium enhances vasoconstrictor responses to transmural nerve stimulation in the rat tail artery (Hynes *et al*., 1988). In the rat tail artery and mesenteric arterial bed, the enhancement of vasoconstrictor responses to perivascular nerve stimulation by nitric oxide synthase inhibitors was endothelium-dependent (Way & Reid, 1991; Vo *et al*., 1991b). This can be attributed entirely to the loss of the restraint on vasoconstriction normally imposed by endothelium-derived relaxing factor. However, with other vascular preparations a contribution of neuronal nitric oxide has not been completely excluded.

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Renal sympathetic nerve activity is known to be increased at least acutely after administration of nitric oxide synthase inhibitors (Sakuma *et al*., 1992; Togashi *et al*., 1992; Harada *et al*., 1993) and hypertension induced by oral administration of nitric oxide synthase inhibitors has been shown to be renal nerve-dependent (Matsuoka *et al*., 1994). The purpose of this study was to investigate the effects of the nitric oxide synthase inhibitor L-NAME on the vasoconstrictor response to transmural electrical nerve stimulation and noradrenaline in the rabbit isolated renal artery and thus look at the peripheral effects of L-NAME on sympathetic nerve stimulation. In adition, the effects of L-NAME are investigated on vessels with and without an intact endothelium to determine whether endothelial-derived nitric oxide is modulating the vasoconstrictor responses to transmural electrical nerve stimulation and noradrenaline. Immunohistofluorescence techniques are used to determine whether there are nerves within the renal artery capable of producing nitric oxide.

Methods

Male New Zealand white rabbits weighing 2–3.5 kg were killed by a lethal dose of sodium pentobarbitone (Sagatal; 60 mg kg^{-1}), injected into the marginal ear vein, followed by exsanguination. The renal arteries were excised and cleared of surrounding fatty tissue under a dissecting microscope.

Pharmacology

Sections of the isolated artery were cut into rings approximately 5 mm in length and these were mounted horizontally under isometric conditions in 10 ml organ baths by inserting a tungsten wire through the lumen of the vessel ring, taking care not to damage the endothelium. The preparation was then anchored to a stationary support. Another wire similarly inserted was connected to a Grass FT03C force-displacement transducer (Quincy, Massachussetts, U.S.A.). The responses were recorded on a Grass ink-writing polygraph. The arterial ring preparations where then placed under an initial tension of 1 g and allowed to equilibrate for at least 1 h in Bülbring-modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $NaHPO_4$ 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8, $CaCl_2$ 2.52, pH 7.2 (Bülbring, 1953). The solution was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. The endothelium was removed from one pair of adjacent ring segments by drawing a silk thread through the lumen. The mean resting tension of the vessels with and without intact endothelium after the 1 h equlibration period and before the start of the experiment was not significantly different, being 0.33 ± 0.03 ($n=26$) and 0.34 ± 0.03 ($n=28$), respectively. Preliminary pharmacological studies with endothelium-dependent (acetylcholine, substance P) and endothelium-independent (sodium nitroprusside) vasodilators confirmed removal of the endothelium without damage to the smooth muscle (data not shown). In addition, at the end of the experiment, vessel segments were taken at random and scanning electron microscopy was performed to ensure that the endothelium was either present or absent.

Transmural electrical nerve stimulation was delivered to the ring preparation via a pair of platinum electrodes mounted parallel to and on either side of the vessel segment, by a Grass S11 stimulator (Quincy, Massachussetts, U.S.A.). The vessels were stimulated (65 V, 0.1 ms, 1–64 Hz) for 30 s at 4 min intervals. With these parameters the responses were blocked by tetrodotoxin (10^{-6} M). On each preparation frequency-response curves were constructed. L-NAME was then added to the bath and allowed to equilibrate for 20 min before a second frequency-response curve was obtained. The α_1-adrenoceptor antagonist, prazosin (10^{-6} M: Cavero & Roach, 1980), was then added to the bath 20 min before the final frequency-response curve was constructed. Prazosin (10^{-6} M) completely abolished the vasoconstrictor response to transmural electrical nerve stimulation indicating that the response was mediated by noradrenaline acting on α_1-adrenoceptors. In order to establish the specificity of action of L-NAME, additional experiments were carried out in which both L-NAME and L-arginine were added to the organ bath.

On separate vessels, cumulative noradrenaline concentration-response curves (10^{-8}–10^{-2} M), were established on each arterial segment prior to exposure to L-NAME. A single concentration of L-NAME was added to the organ bath 20 min before the final concentration-response curve was constructed. The maximum response and pD_2 values were compared before and after L-NAME. Additional experiments included the addition of L-arginine along with L-NAME.

All these experiments were conducted on vessels with their endothelium intact or removed to distinguish any possible endothelial role for the changes observed. Time control experiments were also carried out and there was no significant difference in the frequency-response and dose-response curves at the different time intervals, in vessels with and without an intact endothelium. A single concentration of KCl (120 mM) was added to the preparation at the end of each experiment. All contractile responses were expressed as a percentage of the contractile response to KCl. Student's *t* tests were used to assess statistical significance between responses obtained before and after antagonist, $P<0.05$ being taken as significant.

L-Arginine, N^G-nitro-L-arginine methyl ester (L-NAME), tetrodotoxin, noradrenaline and prazosin were obtained from Sigma Chemical Company (Poole, U.K.). KCl was obtained from BDH Ltd. Noradrenaline was dissolved in 0.1 mM ascorbic acid. All other compounds were dissolved in distilled water.

Immunohistofluorescence

Specimens of the renal artery were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 2 h and washed thoroughly with 7% sucrose (Analar-Sigma) in PBS containing 0.1% sodium azide (BDH) and stored for at least 24 h at 4°C. An indirect fluorescence technique was used to investigate the presence of nerves showing immunoreactivity to nitric oxide synthase in cryostat sections (10 μm) of the renal artery. The cryostat sections of the tissue were incubated with nitric oxide synthase (Euro-Diagnostica (Ferring Group) Sweden) at a dilution of 1 : 1000 for 18 h at room temperature. The tissues were then incubated at room temperature with a second antibody i.e. biotinylated donkey anti-rabbit IgG (Amersham, U.K.) for 1 h, dilution 1 : 250. The sites of the antigen-antibody binding were revealed by incubating for 1 h at room temperature with streptavidin (Amersham, U.K.) conjugated to fluorescein isothiocyanate (FITC) at a dilution of 1 : 100. The preparations were washed, mounted with citifluor (City University, London U.K.) and viewed with a Zeiss microscope equipped for viewing FITC fluorescence. Selected areas were photographed on Kodak 3200 film. For controls the tissues were incubated with antibody inactivated by the addition of excess antigen (10 nmol of antigen to 1 ml of antisera).

The rabbit renal artery was also studied histochemically for the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase, a marker for nitric oxide-containing nerves (Hope *et al*., 1991). Cryotstat sections were rinsed in 0.1 M Tris HCl, pH 8 and incubated in a freshly prepared solution containing 1.2 mM NADPH, 0.24 mM nitroblue tetrazolium, 15.2 mM L-malic acid and 0.1% Triton in 0.1 M Tris HCl, pH 8 for 20–30 min at 37°C. The reaction was terminated by rinsing in Tris buffer followed by several rinses in distilled water. The sections were mounted with Citifluor, and viewed with a Zeiss photomicroscope. Selected areas were photographed with a TMAX 400 film.

Image analysis was attempted on the preparations but was found to be unsatisfactory because of non-specific background fluorescence. Therefore, a semiquantitative method was used to establish the density of NADPH and nitric oxide synthase in the renal artery. Different categories were used: − absent, + sparse, ++ sparse-moderate, +++ moderate, ++++ moderate–dense.

Results

Pharmacology

Responses to nerve stimulation and noradrenaline Transmural electrical nerve stimulation produced a frequency-dependent vasoconstriction of the rabbit renal artery. Typical frequency responses are illustrated in Figure 1. The frequency-response curve is illustrated in Figure 2a. Cumulative addition of noradrenaline produced a concentration-dependent vasoconstriction. The concentration-response curve for noradrenaline is illustrated in Figure 3b.

Effect of L-NAME and L-arginine The effect of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M) on the vasoconstriction produced in response to transmural electrical nerve stimulation (Figure 1 and 2a) and noradrenaline (Figure 2b) is demonstrated. The vasoconstriction produced in response to transmural electrical nerve stimulation (2–64 Hz) was significantly enhanced in the presence of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M; Figures 1 and 2a). L-NAME (10^{-5} M) induced the maximum increase in the vasoconstriction produced in response to transmural electrical nerve stimulation (Figure 2a).

L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M; Figure 2b) did not significantly affect the maximum vasoconstriction produced in response to noradrenaline in the rabbit renal artery. The noradrenaline dose-response curve was significantly shifted to the left by the addition of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M; Table 1). The pD_2 value for noradrenaline in the rabbit renal artery was 5.16 ± 0.03. This value increased to a maximum of 5.48 ± 0.06 in the presence of L-NAME (10^{-5} M; Table 1). All the pD_2 values for noradrenaline obtained in the presence of L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M; Table 1) were significantly increased.

The increase in the amplitude of the vasoconstriction, produced in response to transmural electrical nerve stimulation (16 Hz) and noradrenaline (10^{-5} M), in the presence of L-NAME (10^{-5} M) was not observed when L-arginine (10^{-3} M; Figure 3a, b) was added in addition to L-NAME.

L-NAME (3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M; data not shown) and L-arginine (10^{-3} M; data not shown) did not significantly affect the basal tone of the preparation.

Effect of removing the endothelium from the renal artery Removing the endothelium did not significantly affect the vasoconstriction in response to transmural electrical nerve stimulation (1–64 Hz; Figures 1b and 4a). The maximum vasoconstriction produced in response to noradrenaline (Figure 4b) in renal arteries denuded of endothelium was not significantly different from the maximum vasoconstriction obtained from vessels with an intact endothelium. The pD_2 value for noradrenaline obtained from vessels denuded of endothelium was significantly greater than the pD_2 value obtained from vessels with an intact endothelium (Table 1), the values being 5.90 ± 0.11 and 5.16 ± 0.03, respectively. This shift to the left of the dose-response curve in vessels in which the endothelium had been removed is clearly demonstrated in Figure 4b.

Effect of L-NAME on vessels in which the endothelium had been removed The effect of L-NAME (3×10^{-5} M) on the vasoconstriction produced in response to transmural electrical nerve stimulation (Figure 5a) and noradrenaline (Figure 5b) in vessels in which the endothelium had previously been removed is demonstrated. The vasoconstrictor response to transmural electrical nerve stimulation (2–64 Hz) was significantly enhanced in the presence of L-NAME (3×10^{-5} M; Figures 1b and 5a). L-NAME (3×10^{-5} M) did not significantly affect the

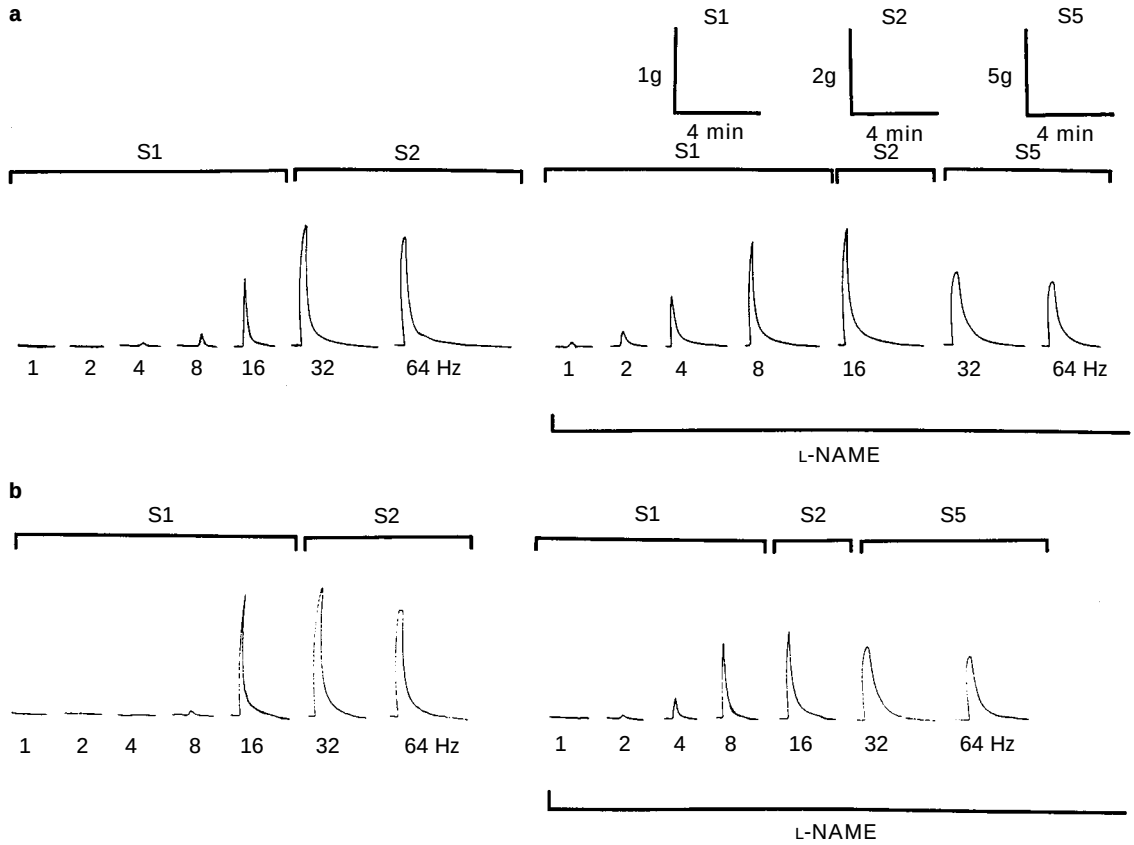

Figure 1 Contractions produced in the isolated renal artery of the rabbit on neurogenic transmural stimulation (0.1 ms: 65 V) for 30 s (a,b) at the frequencies (Hz) indicated. Nerve stimulations were repeated in the presence of L-NAME (3×10^{-5} M) on renal artery segments with (a) and without (b) an intact endothelium, as indicated on the figure by the horizontal bar below each trace. The bars above each response indicate the scale that should be referred to.

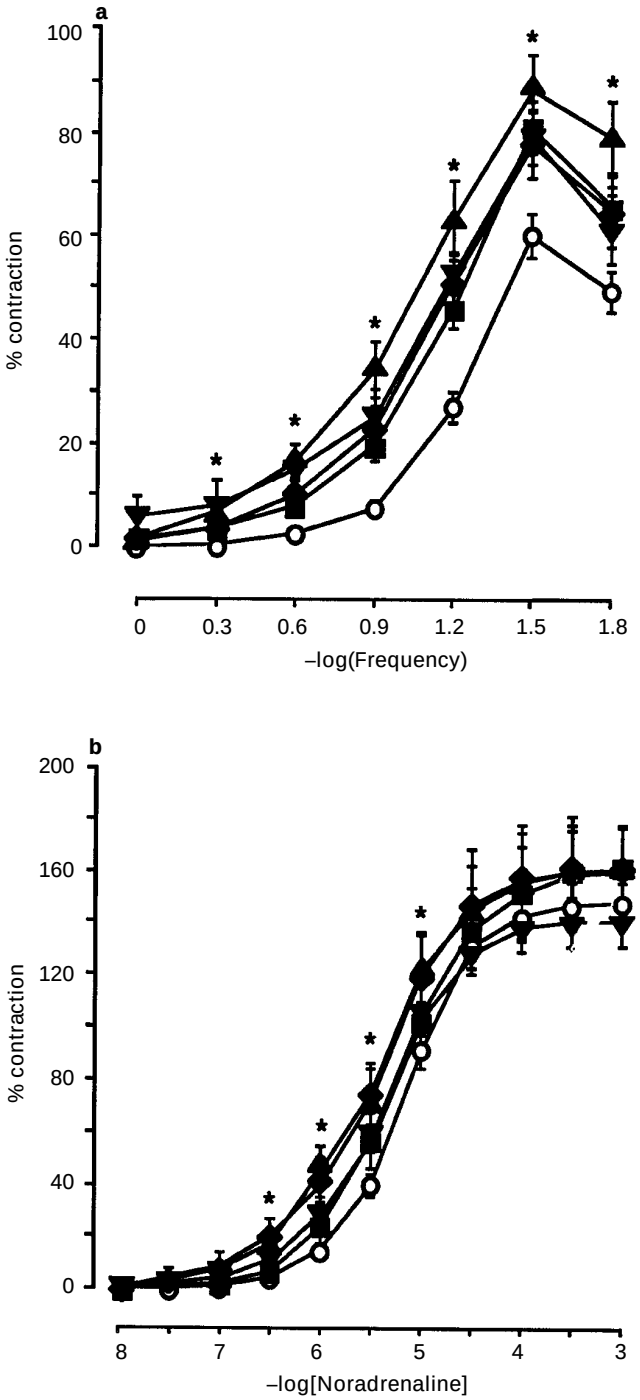

Figure 2 Vasoconstrictor responses of the rabbit renal artery to: (a) transmural electrical nerve stimulation for 30 s (1–64 Hz, 0.1 ms, 65 V) and (b) noradrenaline. The vasoconstriction is expressed as a percentage of the vasoconstriction observed in the presence of KCl (120 mM). The responses observed are in the absence (○) and presence of L-NAME, 3×10^{-6} (■), 10^{-5} (▲), 3×10^{-5} (▼) and 10^{-4} M (◆). The graph shows the mean and vertical lines s.e.mean, $n\geqslant6$. *Indicates that all points directly below it are significantly different from the control (○). $P<0.05$ taken to be significant.

maximum vasoconstriction produced in response to noradrenaline in vessels with no endothelium. The pD_2 values for noradrenaline in vessels without endothelium in the absence and presence of L-NAME (3×10^{-5} M) were 5.90 ± 0.11 and 5.65 ± 0.08, respectively (Table 1). There was a significant shift to the right of the noradrenaline dose-response curve in the presence of L-NAME (3×10^{-5} M) in renal arteries denuded of endothelium (Figure 4b).

Table 1 Effect of L-NAME and the endothelium on the pD_2 value of rabbit isolated renal artery ring preparations in response to noradrenaline

Conditions for NA dose-response	*pD_2 values for NA*
Control	5.16 ± 0.03
Time control	5.20 ± 0.33
L-NAME (3×10^{-6} M)	5.27 ± 0.08*
L-NAME (10^{-5} M)	5.48 ± 0.06*
L-NAME (3×10^{-5} M)	5.42 ± 0.06*
L-NAME (10^{-4} M)	5.46 ± 0.11*
No endothelium	5.90 ± 0.11*
No endothelium + L-NAME (3×10^{-5} M)	5.65 ± 0.08*#

All values are means ± s.e.mean; $n\geqslant6$. Significant differences from control $^{*}P<0.05$. Significant differences from responses obtained to noradrenaline in vessels with no endothelium $^{\#}P<0.05$.

L-NAME (3×10^{-5} M) did not significantly affect the basal tone of the renal arteries in which the endothelium had been removed.

Immunohistofluorescence

Immunohistofluorescence for nitric oxide synthase in the rabbit renal artery is illustrated in Figure 6. In the rabbit renal artery both the nitric oxide synthase-containing (Figure 6a) and NADPH-diaphorase stained nerves were located on the adventitia. The density of both types of nerves was relatively sparse with a score of + +. The nitric oxide synthase-containing nerves were seen 100–150 μm from the medial wall (Figure 6a) and were also found around vasa vasorum (Figure 6b). The NADPH-diaphorase stained nerves were similarly distributed.

Autonomic ganglia containing 2–3 nitric oxide synthase immunoreactive nerve cell bodies and nerve fibres but not NADPH-diaphorase activity, were observed in the adventitia at a distance of 50 μm from the medial wall.

Discussion

The results of this study show that the nitric oxide synthase inhibitor L-NAME (Rees *et al*., 1990) enhances vasoconstrictor responses to transmural electrical field stimulation and noradrenaline. Removal of the endothelium enhances vasoconstrictor responses to noradrenaline but not to transmural electrical nerve stimulation. In rabbit isolated renal ring preparations in which the endothelium had been removed, L-NAME enhanced vasoconstrictor responses to transmural electrical nerve stimulation, but not to noradrenaline.

Recent evidence suggests that nitric oxide is involved in modulation of sympathetic neurotransmitter release, but the results are controversial. It has been shown that nitric oxide, which is released from the endothelium, inhibits the release of noradrenaline from adrenergic sympathetic nerve endings of blood vessels (Cohen & Weisbrod, 1988; Greenberg *et al*., 1989), and attenuates vasoconstrictor responses to nerve stimulation and noradrenaline (Tesfamarian *et al*., 1987). For instance, inhibition of nitric oxide synthesis with N^G-nitro-L-arginine and N^G-monomethyl-L-arginine enhances vasoconstrictor responses to nerve stimulation and noradrenaline in the rat caudal artery (Vo *et al*., 1991a), the rat mesenteric arterial bed (Way *et al*., 1991), the rat renal vascular bed (Reid & Rand, 1992), noradrenaline-induced constriction in dog and human coronary arteries (Berkenboom *et al*., 1991) and constriction to noradrenergic nerve stimulation in the dog mesenteric artery (Toda & Okamura, 1990). Likewise, the nitric oxide synthase inhibitor L-NAME enhanced vasoconstrictor responses to

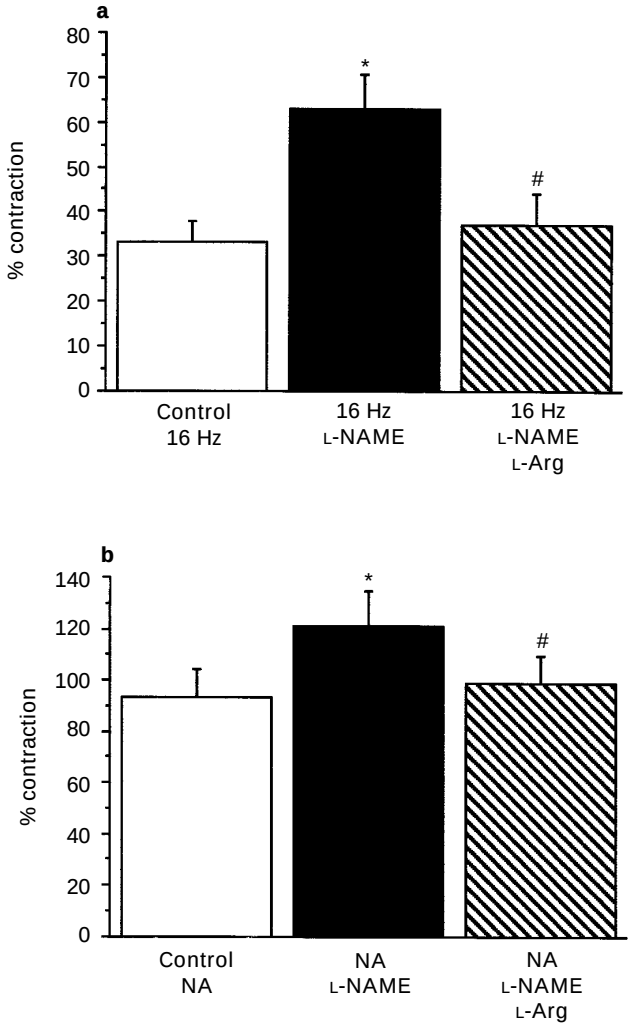

Figure 3 Rabbit renal vasoconstriction in response to (a) transmural electrical nerve stimulation for 30 s (16 Hz, 0.1 ms, 65 V) and (b) noradrenaline (10^{-5} M), expressed as a percentage of the vasoconstriction observed in the presence of KCl (120 mM). The responses are in the absence (open columns) and presence of L-NAME (10^{-5} M; solid columns) and L-NAME (10^{-5} M) with L-arginine (L-Arg) (10^{-3} M; hatched columns). The mean ± s.e.mean are shown, $n \geqslant 6$. *Indicates significant difference from control. #Indicates significant difference from response in presence of L-NAME (solid columns). $P<0.05$ taken to be significant.

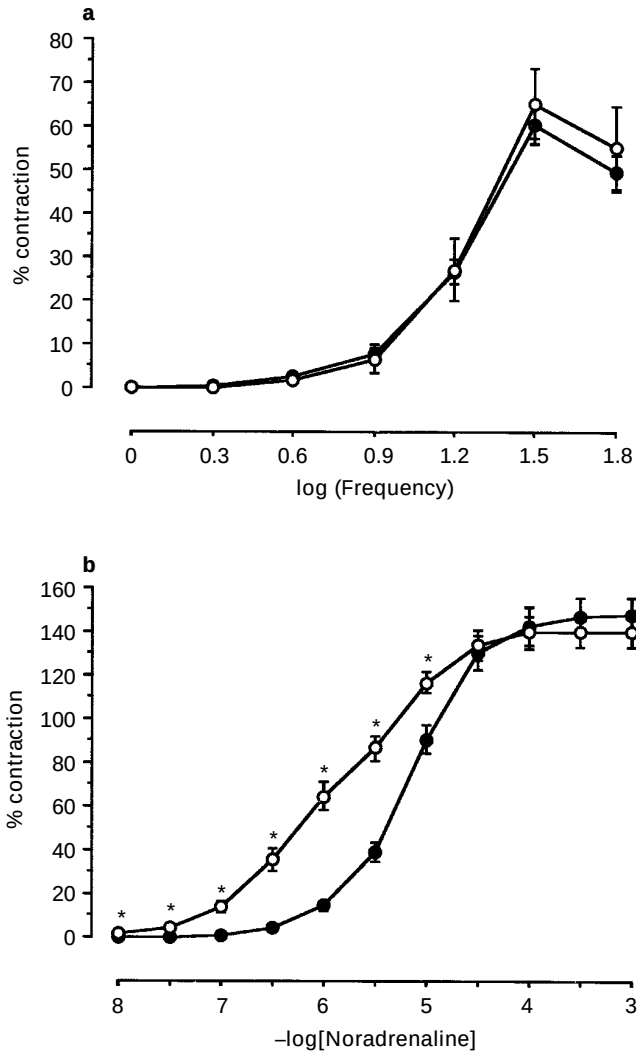

Figure 4 Rabbit renal artery vasoconstriction in response to (a) transmural electrical field stimulation for 30 s (1–64 Hz, 0.1 ms, 65 V) and (b) noradrenaline. The vasoconstriction is expressed as a percentage of the vasoconstriction observed in the presence of KCl (120 mM). The responses are in the absence (○) and presence (●) of an intact endothelium. The graph shows the mean ($n \geqslant 6$) with s.e.mean indicated by vertical lines. *Significantly different from control (●), $P<0.05$.

transmural electrical nerve stimulation and noradrenaline, suggesting that nitric oxide attenuates vasoconstriction in the rabbit renal artery. The enhancement was prevented by prior addition of L-arginine indicating that L-NAME restricts the availability of L-arginine for the synthesis of nitric oxide. In contrast, it has been shown that nitric oxide synthesis inhibitors decrease the release of noradrenaline *in vitro* (Yamamoto *et al.*, 1993) as well as *in vivo* (Halbrugge *et al.*, 1991). In addition guanosine 3′ : 5′-cyclic monophosphate (cyclicGMP) and nitric oxide generators potentiate nicotinic transmission in the rat superior cervical ganglion (Briggs, 1992).

The role of the endothelium as a modulator of sympathetic vasoconstrictor responses depends on the ability of endothelium-derived nitric oxide to reach the perivascular nerves. Endothelium-derived relaxing factor is a diffusable substance with a very short biological half-life (Förstermann *et al.*, 1984; Griffith *et al.*, 1984; Rubanyi *et al.*, 1985; Gryglewski *et al.*, 1986; Angus & Cocks, 1987; Loeb *et al.*, 1987). Therefore, in vascular beds where the distance between the endothelium and perivascular nerves is comparatively small (compared to large blood vessels) endothelium-derived nitric oxide may indeed have a modulatory role on sympathetic vasoconstrictor responses. In contrast, nitric oxide may not be able to reach the perivascular nerves of large blood vessels to have a modulatory effect.

In the rat tail artery and mesenteric arterial bed enhancement of the vasoconstrictor responses to perivascular nerve stimulation by nitric oxide synthase inhibitors was endothelium-dependent (Way & Reid, 1991; Vo *et al.*, 1991b). In the rabbit isolated renal artery the enhancement of the vasoconstrictor response to transmural electrical nerve stimulation by L-NAME was not dependent on an intact endothelium. However, as in the rat tail artery and mesenteric arterial bed (Way & Reid, 1991; Vo *et al.*, 1991b) the enhancement of the vasoconstrictor response to noradrenaline by L-NAME was endothelium-dependent. There are two pieces of information from this study that confirm this. The first is that removal of the endothelium enhanced the vasoconstrictor response to noradrenaline, shifting the dose-response curve for noradrenaline significantly to the left. Secondly, in isolated ring pre-

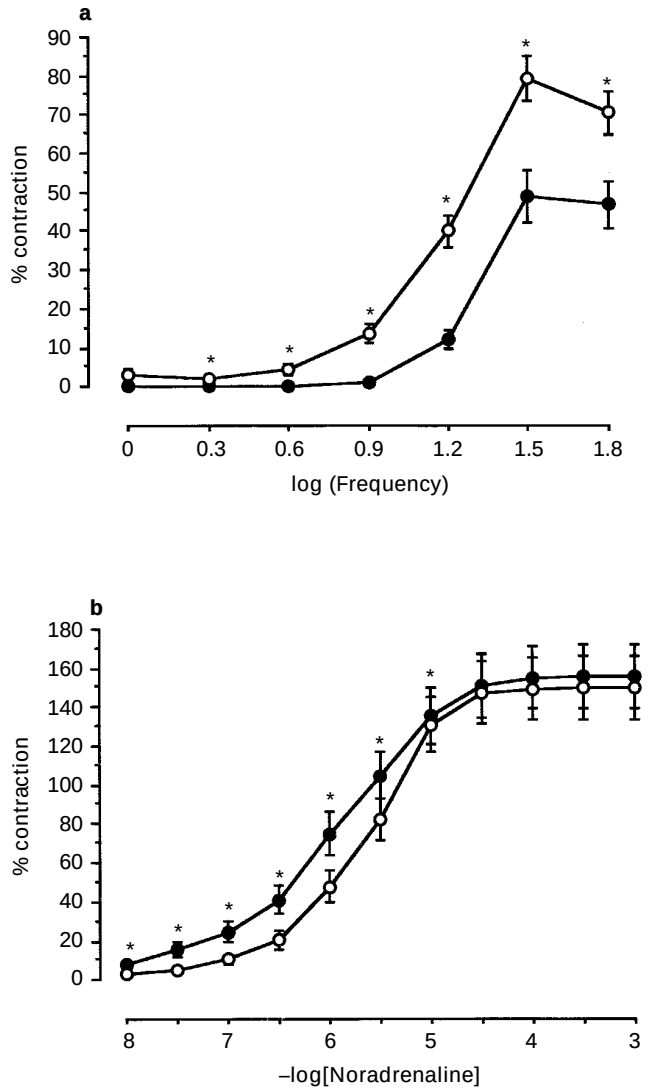

Figure 5 Vasoconstriction of the rabbit renal artery (with the endothelium removed) in response to (a) transmural electrical nerve stimulation for 30 s (1–64 Hz, 0.1 ms, 65 V) and (b) noradrenaline. The vasoconstriction is expressed as a percentage of the vasoconstriction observed in the presence of KCl (120 mM). The responses observed are in the absence (●) and presence (○) of L-NAME (3×10^{-5} M). The graph shows the mean ($n \geqslant 6$) with s.e.mean indicated by vertical lines. *Significantly different from control (●), $P<0.05$.

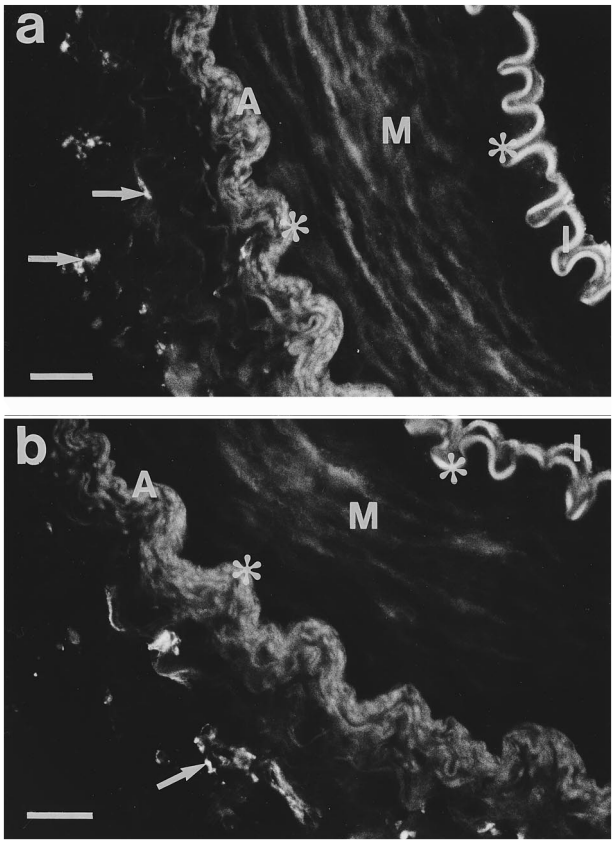

Figure 6 Nitric oxide synthase (NOS)-immunoreactive nerves in the rabbit renal artery. (a) NOS-immunoreactive nerves are seen on the adventitia (A). Note the nerves are located at a distance of approximately 100–150 μm from the medial (M) wall (white arrows). The internal elastic lamina surrounding the intima (I) and the adventitial-medial border are autofluorescent (asterisk). (b) NOS-immunoreactive nerves are seen on the adventitia (A) as in (a). Note the vasa vasorum (white arrows) are also innervated. The internal elastic lamina around the intima (I) and the adventitial-medial border are autofluorescent (asterisk) as in (a). Calibration bar (a) and (b) = 30 μm.

parations in which the endothelium had been removed, L-NAME no longer enhanced the response to noradrenaline, in fact, it significantly shifted the dose-response curve for noradrenaline to the right. Therefore the enhancement of the vasoconstrictor response to noradrenaline by L-NAME can be attributed to the loss of the restraint on vasoconstriction normally imposed by endothelium-derived nitric oxide.

Removal of the endothelium from the isolated renal artery preparation had no effect on the vasoconstrictor response to transmural electrical nerve stimulation. In contrast, it has previously been shown that removal of the endothelium enhances vasoconstrictor responses to tansmural nerve stimulation in the rat tail artery (Hynes *et al*., 1988). There was also an enhancement of the vasoconstrictor response, in the presence of L-NAME, in isolated renal artery preparations in which the endothelium had previously been removed. This suggests another source of nitric oxide other than the endothelium. Nitric oxide synthase and therefore presumably nitric oxide also occurs in the autonomic nerves in the outer, adventitial layers of various blood vessels (Bredt *et al*., 1990). The immunohistofluorescence studies carried out on the rabbit renal artery indicate that both nitric oxide synthase-containing and NADPH-diaphorase stained nerves are located on the adventitial medial border. Thus we can conclude that there are nerves with the ability to produce nitric oxide in the renal artery. This could be the source of the nitric oxide that has the neuromodulatory role on sympathetic neurotransmission in the rabbit renal artery.

The fact that L-NAME enhanced response to transmural electrical nerve stimulation without enhancing renal vasoconstriction to exogenous noradrenaline in arteries denuded of endothelium, suggests that nitric oxide exerts its inhibitory effects via a presynaptic action. In contrast, enhancement of stimulation-induced vasoconstrictor responses in the rat tail artery was not due to an increase in noradrenaline release as this was only slightly affected by the nitric oxide synthase inhibitor N^G-nitro-L-arginine and was actually increased by the nitric oxide donor sodium nitroprusside (Vo *et al*., 1991b). However, in the rat tail artery the enhancement was also endothelium-dependent (Vo *et al*., 1991a). Adrenergic neurotransmission may be associated with increased levels of cyclicGMP within sympathetic neurones (Kalix, 1976). Compounds which increase cyclicGMP in muscle, ganglia and other tissues inhibit the release of acetylcholine, histamine and other substances from neurones, mast cells and neutrophils (Nordstrom & Bartfai, 1981; Barrowman *et al*., 1986) as well as the

release of noradrenaline from adrenergic nerves innervating vascular smooth muscle during transmural nerve stimulation (Itoh *et al*., 1981; Greenberg *et al*., 1987). This is probably how nitric oxide is acting to increase vasoconstrictor responses to transmural electrical nerve stimulation in the rabbit isolated renal artery.

In conclusion, the present study has suggested a presynaptic inhibitory action of nitric oxide in the rabbit renal artery. The source of this nitric oxide is not the endothelium. There is possibly a neural source as nerves with the ability to synthesize nitric oxide were identified in the adventitial layer of the renal artery. The enhancement of the vasoconstrictor response to noradrenaline by L-NAME can be attributed to inhibition of the synthesis of endothelium-derived nitric oxide by L-NAME. Analysis of nitrergic neuromodulation was aided by the removal of the endothelium from the renal artery and thus elimination of the production of endothelium-derived relaxing factor.

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