The role of nitric oxide in the regional vasodilator effects of endothelin-1 in the rat

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1 The role of nitric oxide (NO) derived from L-arginine in the regional vasodilator effects of endothelin-1 has been investigated in anaesthetized, spontaneously hypertensive (SH) rats in which autonomic reflexes were abolished by ganglion blockade. The experimental design incorporated animals infused with phenylephrine to mimic the peripheral vasoconstrictor effects of the NO biosynthesis inhibitors and a single dose per animal paradigm to obviate problems of tachyphylaxis to the vasodilator effects of endothelin-1.

2 Infusion of the inhibitor of NO synthase, N-monomethyl-L-arginine (L-NMMA) at a dose ($5 \text{ mg kg}^{-1} \text{ min}^{-1}$) which maximally raised blood pressure did not influence either the fall in blood pressure or the vasodilator responses induced in the hindquarters and carotid vascular beds by endothelin-1 (1 nmol kg^{-1} , i.v.). The duration (but not the initial magnitude) of the vasodepressor response to endothelin-1 was however significantly attenuated (by 49%) during infusion of the more potent inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (L-NAME), $2 \text{ mg kg}^{-1} \text{ min}^{-1}$.

3 Increasing the dose of L-NAME to 10 and $25 \text{ mg kg}^{-1} \text{ min}^{-1}$ significantly attenuated, but did not abolish, the falls in blood pressure and hindquarters vasodilator responses to acetylcholine, $1 \mu\text{g kg}^{-1}$, and endothelin-1, $1 \text{ nmol kg}^{-1} \text{ min}^{-1}$. The effects were selective in that vasodepressor responses to the endothelium-independent vasodilator, sodium nitroprusside, $1\text{--}10 \mu\text{g kg}^{-1} \text{ min}^{-1}$, were unaltered.

4 The data indicate that NO generated *de novo* from L-arginine mediates a significant component of the vasodilator effect of endothelin-1 in the anaesthetized, ganglion-blocked SH rat. However, a major component of the vasodepressor effects of both endothelin-1 and acetylcholine may occur independently of this mechanism.

Keywords: N^G-monomethyl-L-arginine; nitric oxide; spontaneously hypertensive rats; endothelin-1; regional vasodilatation

Introduction

Prominent regional vasodilator responses to endothelin-1 were described soon after its discovery (Yanagisawa *et al.*, 1988) in anaesthetized rats (Wright & Fozard, 1988) and cats (Lippton *et al.*, 1988). Such responses were subsequently confirmed in rats (see, e.g., Madeddu *et al.*, 1989; Winkquist *et al.*, 1989; Hoffmann *et al.*, 1989; 1990; Gardiner *et al.*, 1990a; Le Monnier de Gouville *et al.*, 1990b; Ohlstein *et al.*, 1990) and cats (Minkes & Kadowitz, 1989; Le Monnier de Gouville *et al.*, 1990a; Minkes *et al.*, 1990) and also demonstrated to occur in dogs (Lippton *et al.*, 1989; Otsuka *et al.*, 1990; Tsuchiya *et al.*, 1990), rabbits (Cocks *et al.*, 1989; Hof *et al.*, 1989; Lippton *et al.*, 1991a), primates (Clozel & Clozel, 1989; Lopez *et al.*, 1990) and man (Clarke *et al.*, 1989; Kiowski *et al.*, 1991). Vasodilator responses to endothelin-1 have equally been demonstrated in a variety of isolated blood vessel preparations (see, e.g., Cocks *et al.*, 1989; Folta *et al.*, 1989; Randall *et al.*, 1989; Warner *et al.*, 1989; Baydoun *et al.*, 1990; Hasunuma *et al.*, 1990; Kitazumi *et al.*, 1990; Perreault & De Marte, 1991).

A number of different mechanisms has been invoked to explain the vasodilator effects of endothelin-1. *In vitro* data suggest the presence of the endothelium is obligatory (Cocks *et al.*, 1989; Folta *et al.*, 1989; Randall *et al.*, 1989; Sakata *et al.*, 1989; Warner *et al.*, 1989; Douglas & Hiley, 1990). The release of vasodilator products of cyclo-oxygenase activity has been implicated (Folta *et al.*, 1989) as has the involvement of ATP-dependent K⁺ channels (Hasunuma *et al.*, 1990). However, data obtained *in vivo* suggest that vasodilator products of cyclo-oxygenase do not mediate the regional vasodilator responses to endothelin-1 in rats (Gardiner *et al.*, 1990b; Ohlstein *et al.*, 1990; Wright & Fozard, 1990), rabbits (Lippton *et al.*, 1991a), cats (Minkes *et al.*, 1990), dogs (Hoffmann *et al.*, 1989) or monkeys (Lopez *et al.*, 1990). Moreover, activation of ATP-dependent K⁺ channels appears to be a mechanism selective for pulmonary vessels (Hasunuma *et al.*,

1990; Lippton *et al.*, 1991b) and is not the mechanism of the systemic vasodilator effects in either rats (Le Monnier de Gouville *et al.*, 1990b) or cats (Lippton *et al.*, 1991b).

The data implicating endothelium derived nitric oxide (NO) in the vasodilator response to endothelin-1 are equivocal. Thus, evidence both for (Warner *et al.*, 1989; Douglas & Hiley, 1990; Kitazumi *et al.*, 1990; Perreault & De Marte, 1991) and against (Baydoun *et al.*, 1990; Folta *et al.*, 1990) the concept has been obtained from *in vitro* experiments. Similarly, whereas Whittle *et al.* (1989) reported significant inhibition of the vasodepressor effects of endothelin-1 in anaesthetized rats by N-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NO formation, Gardiner *et al.* (1989) found no attenuation of either the vasodepressor or the regional vasodilator effects of endothelin-1 following L-NMMA in conscious rats. However, in a subsequent study, also in conscious rats, Gardiner *et al.* (1990c) showed that N^G-nitro-L-arginine methyl ester (L-NAME), a more potent inhibitor of NO biosynthesis than L-NMMA (Moore *et al.*, 1990), attenuated the vasodilator effects of endothelin-1.

The objective of the present study was to explore in detail the effect of inhibition of NO biosynthesis from L-arginine with L-NMMA and L-NAME on the regional vasodilator effects of endothelin-1 *in vivo*. The anaesthetized, spontaneously hypertensive (SH) rat treated with a ganglion blocking agent (Wright & Fozard, 1988; 1990) provides a robust and reproducible model for this purpose. The experimental design incorporated animals infused with phenylephrine to mimic the peripheral vasoconstrictor effects of the NO biosynthesis inhibitors and a single dose per animal paradigm to obviate problems of tachyphylaxis to the vasodilator effects of endothelin-1 (Le Monnier de Gouville *et al.*, 1990a, b).

Methods

In vivo preparations

Male SH rats weighing 339–393 g supplied by Biological Research Laboratories, Füllinsdorf, Switzerland, were used in

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this study. Rats were anaesthetized with Inaktin, 120 mg kg^{-1} , i.p. A tracheotomy was performed and catheters inserted into the right carotid artery for recording blood pressure and heart rate and into the jugular veins for drug administration. In some rats, pulsed Doppler flow probes (Haywood *et al.*, 1981) were placed around the left carotid artery and, through a midline abdominal incision, around the left renal artery, the superior mesenteric artery and the lower abdominal aorta just above the iliac bifurcation (equated to hindquarters blood flow). The incisions were closed and parameters allowed to stabilize for 1 h. Following the stabilization period, the autonomic ganglia were blocked with mecamylamine (0.25 mg kg^{-1} infused intravenously over 15 min).

Experimental design

One hour after the end of the infusion of mecamylamine, rats were infused with either phenylephrine, L-NMMA or L-NAME at doses specified below. The maximum duration of any infusion did not exceed 22 min and in each case the cardiovascular effects were maximal at 4 min and maintained for the duration of the infusion. For this reason the cardiovascular effects during the infusion (see Table 1) are given as the change at 4 min. Three series of experiments were carried out. In the first, involving infusions of phenylephrine, $5\text{--}10 \mu\text{g kg}^{-1} \text{ min}^{-1}$, L-NMMA, $5 \text{ mg kg}^{-1} \text{ min}^{-1}$ or L-NAME, $2 \text{ mg kg}^{-1} \text{ min}^{-1}$, a bolus injection of endothelin-1 1 nmol kg^{-1} , which is just submaximal, was administered 4 min after the start of the infusion. In a second series of experiments involving infusions of phenylephrine, $10 \mu\text{g kg}^{-1} \text{ min}^{-1}$ or L-NAME, $10 \text{ and } 25 \text{ mg kg}^{-1} \text{ min}^{-1}$, a submaximal dose of acetylcholine ($1 \mu\text{g kg}^{-1}$) was given 4 min after the start of the infusion followed by a bolus injection of endothelin, 1 nmol kg^{-1} , 2 min later. A representative tracing

Figure 1 Representative chart record to illustrate the experimental design used to evaluate the effects of N^G-nitro-L-arginine methyl ester (L-NAME) on the responses to acetylcholine (ACh) and endothelin (Et). Since inhibitors of NO synthase induce peripheral vasoconstriction, control animals were infused with phenylephrine, $10 \mu\text{g kg}^{-1} \text{ min}^{-1}$ (illustrated), to increase vascular tone in the preparations to the extent seen with L-NAME (see Table 1). Comparisons could then be made between responses to bolus injections of ACh ($1 \mu\text{g kg}^{-1}$) and Et (1 nmol kg^{-1}) administered during infusions of either phenylephrine or L-NAME. Variables recorded: mean arterial pressure (MAP; mmHg), heart rate (HR; b min^{-1}) and renal (Ren), mesenteric (Mes), carotid (Car) and hindquarters (HQ) blood flows (Q; kHz).

illustrating this latter experimental design is presented in Figure 1. In a third series of experiments carried out in animals without flow probes, the effects of acetylcholine 0.1 , 0.3 and $1 \mu\text{g kg}^{-1}$, sodium nitroprusside, 1 , 3 and $10 \mu\text{g kg}^{-1}$ and endothelin-1 1 nmol kg^{-1} were monitored during infusions of phenylephrine, $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$ or L-NAME, $10 \text{ mg kg}^{-1} \text{ min}^{-1}$. In each experimental series, only one infusion/injection sequence was carried out in each animal.

Drugs

Porcine endothelin-1 (Peptide Institute Inc., Osaka, Japan) was dissolved in acetic acid (10 mM) to 10^{-5} M and stored in aliquots at -20°C . Other drugs were freshly prepared each day and diluted in 0.9% w/v NaCl solution before use. N^G-monomethyl-L-arginine citrate (L-NMMA) was supplied by Ultrafine Chemicals, Salford, UK, N^G-nitro-L-arginine methylester (L-NAME), sodium nitroprusside and acetylcholine chloride were purchased from Sigma. Mecamylamine hydrochloride was the kind gift of Merck, Sharpe and Dohme. Phenylephrine hydrochloride was obtained from Serva.

Statistical analysis

All data are expressed as mean values $\pm$ s.e.mean of n individual determinations. Student's t test was used to evaluate the significance of a difference between group means.

Results

Haemodynamic effects of phenylephrine, L-NMMA and L-NAME: effects on endothelin-1

The data from the first series of experiments in which the effects of endothelin-1 were compared during infusions of phenylephrine, $5\text{--}10 \mu\text{g kg}^{-1} \text{ min}^{-1}$, L-NMMA, $5 \text{ mg kg}^{-1} \text{ min}^{-1}$ or L-NAME, $2 \text{ mg kg}^{-1} \text{ min}^{-1}$ are presented in Figure 2 and Table 1. All the infused drugs gave rapid, marked and sustained rises in mean arterial pressure (MAP) and decreases in conductance in the renal, mesenteric, carotid and hindquarters vascular beds; the effects were maximal 4 min after the start of the infusion, illustrated for phenylephrine in Figure 1. In general, the conductance changes were somewhat less with phenylephrine than with either L-NMMA or L-NAME despite the increases in MAP being similar (Table 1). Heart rate (HR) changed minimally during infusion of L-NMMA and L-NAME (data not illustrated) but increased by approx. 50 b min^{-1} following phenylephrine (evident from the 0 time values in Figure 2).

Endothelin-1, 1 nmol kg^{-1} , injected 4 min after the start of the infusion of phenylephrine induced a pronounced fall in MAP accompanied by increases in conductance in the carotid and hindquarters vascular beds and slight vasoconstrictor responses in the renal and mesenteric vascular beds (Figure 2a). Following L-NMMA, the fall in MAP induced by endothelin-1 was similar to that seen during phenylephrine infusion. The vasodilator responses in the carotid and hindquarters vascular beds were somewhat larger than those seen during phenylephrine infusion and there was a trend towards vasodilatation in the mesenteric artery bed. The renal vasoconstrictor response to endothelin-1 was more marked during infusion of L-NMMA than phenylephrine (Figure 2b). The hypotensive response to endothelin-1 during infusion with L-NAME was of similar magnitude to that seen in the presence of phenylephrine (Figure 2a and c) but of significantly shorter duration (mean time to return 50% towards baseline [$t_{1/2}$] during phenylephrine infusion was $86 \pm 4 \text{ s}$ ($n = 5$) and during L-NAME, $44 \pm 5 \text{ s}$ ($n = 4$) ($P < 0.01$)). The conductance changes in the carotid mesenteric and hindquarters vascular

Figure 2 Circulatory changes following administration of endothelin (Et), 1 nmol kg⁻¹ (i.v. bolus), to anaesthetized, ganglion-blocked SH rats infused with phenylephrine, 5–10 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (a) N-monomethyl-L-arginine (L-NMMA) 5 mg kg⁻¹ min⁻¹ (b) or N^G-nitro-L-arginine methyl ester (L-NAME) 2 mg kg⁻¹ min⁻¹ (c). Variables are mean arterial pressure (MAP, mmHg), heart rate (HR, b min⁻¹) and % change in vascular conductance (C) in renal (●), mesenteric (▲), carotid (◆) and hindquarters (■) beds. The abscissa scale represents time in min after injection of endothelin. Points represent mean values of 5 (MAP, HR) or 3–5 (C) individual values, s.e.mean shown by vertical bars.

beds were similar in the phenylephrine- and L-NAME-infused animals but the renal vasoconstrictor response to endothelin-1 was substantially greater in the latter than the former (Figure 2c).

Figure 3 Circulatory changes following administration of endothelin Et (Et), 1 nmol kg⁻¹ (i.v. bolus), to anaesthetized, ganglion-blocked SH rats infused with phenylephrine, 10 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (a) N^G-nitro-L-arginine methyl ester (L-NAME), 10 mg kg⁻¹ min⁻¹ (b) or N-monomethyl-L-arginine (L-NAME) 25 mg kg⁻¹ min⁻¹ (c). For further details see legend to Figure 2. Symbols as in Figure 2.

Figure 4 Changes in mean arterial pressure (MAP, mmHg) induced by bolus i.v. injections of acetylcholine, 0.1–1 $\mu\text{g kg}^{-1}$, sodium nitroprusside, 1–10 $\mu\text{g kg}^{-1}$ (a) and endothelin, 1 nmol kg⁻¹ (b) during infusion with either phenylephrine, 5 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (open columns (a); ○, (b); $n = 3$ –5 in (a) and (b) or N^G-nitro-L-arginine methyl ester (L-NAME), 10 mg kg⁻¹ min⁻¹ (cross-hatched column (a); ●, (b), $n = 4$ in (a) and (b)). *, ** indicate a significant difference ($P < 0.05$; 0.01, respectively) from the value in the phenylephrine series.

Effects of higher doses of L-NAME

The data from the second series of experiments in which the effects of acetylcholine and endothelin-1 were compared during infusions of phenylephrine, 10 $\mu\text{g kg}^{-1} \text{min}^{-1}$ or L-NAME, 10 and 25 mg kg⁻¹ min⁻¹ are presented in Table 1 and Figures 1 and 3. In this series of experiments, the MAP changes measured 4 min after phenylephrine exceeded those of the first series and were significantly higher than those produced by either dose of L-NAME. Changes in conductance in the renal, mesenteric, carotid and hindquarters vascular beds were similar in the phenylephrine- and L-NAME-infused animals (Table 1). The haemodynamic changes induced by L-NAME were broadly similar at each infusion dose (Table 1).

The hypotensive response to acetylcholine, 1 $\mu\text{g kg}^{-1} \text{min}^{-1}$, elicited 4 min following the start of the infusion of phenylephrine, was 81 ± 2 mmHg, $n = 4$. It was reduced slightly but significantly during infusion with L-NAME 10 mg kg⁻¹ min⁻¹ (to 66 ± 4 mmHg, $n = 4$) and to a similar extent (65 ± 8 mmHg, $n = 3$) during the higher dose. The vasodilator effects of acetylcholine in the mesenteric and carotid vascular beds were unaffected by infusion of L-NAME, 10 or 25 mg kg⁻¹ min⁻¹; in contrast vasodilator responses in the renal and hindquarter vascular beds were significantly attenuated (by 30–40%) and to a similar extent following each dose of L-NAME (data not illustrated).

The changes in MAP and regional conductances induced by endothelin-1 during infusion with phenylephrine were similar to those recorded in the first series except that frank vasodilation was seen initially in the mesenteric artery bed and little further vasoconstriction was induced in the renal vasculature (compare Figures 2a and 3a). Both the hypotension and the peripheral vasodilator responses to endothelin-1 were attenuated during infusion of L-NAME compared to those obtained

Table 1: Haemodynamic effects of phenylephrine, N-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) in anaesthetized SH rats treated with a ganglion blocking agent

	First series				Second series													
	Phenylephrine		L-NMMA		Phenylephrine		L-NMMA											
	Baseline	4'	n	(5 mg kg ⁻¹ min ⁻¹)	Baseline	4'	n	(10 mg kg ⁻¹ min ⁻¹)										
MAP	110 ± 11	167 ± 9	5	121 ± 3	182 ± 6	4	126 ± 8	180 ± 13	4	110 ± 8	208 ± 6	4	114 ± 7	170 ± 8	4	115 ± 8	169 ± 15	3
HR	352 ± 13	388 ± 28	5	337 ± 18	332 ± 16	4	343 ± 15	328 ± 10	3	334 ± 15	369 ± 14	4	361 ± 10	328 ± 8	4	346 ± 18	337 ± 21	3
RenC	2.0 ± 0.9	-40 ± 10	4	3.5 ± 1.1	-57 ± 7	4	3.8 ± 1.6	-60 ± 2	3	1.9 ± 0.3	-58 ± 3	4	1.5 ± 0.4	-53 ± 4	3	6.1 ± 1.0	-67 ± 3	3
MesC	3.3 ± 0.9	-17 ± 14	5	5.1 ± 0.5	-45 ± 3	4	2.4 ± 0.3	-53 ± 3	4	4.5 ± 0.5	-57 ± 6	4	4.7 ± 2.0	-62 ± 4	3	3.7 ± 0.9	-59 ± 2	3
CarC	1.4 ± 0.2	-34 ± 8	5	1.1 ± 0.2	-48 ± 3	4	0.9 ± 0.2	-53 ± 3	4	1.3 ± 0.2	-58 ± 5	4	1.3 ± 0.3	-70 ± 3	4	1.1 ± 0.2	-64 ± 1	3
HQC	1.8 ± 0.1	-23 ± 7	5	1.3 ± 0.1	-42 ± 6	4	1.1 ± 0.1	-35 ± 2	4	1.0 ± 0.1	-40 ± 6	4	1.1 ± 0.2	-38 ± 3	4	1.4 ± 0.2	-40 ± 6	3

MAP = mean arterial pressure in mmHg, HR = heart rate in b min⁻¹; Renal (Ren), mesenteric (Mes), carotid (Car) and hindquarters (HQ) conductances (C) - baseline values in conductance units (100 [kHz mmHg⁻¹]), 4 min values are % changes. Values are means ± s.e.mean of *n* individual observations.

during infusion of phenylephrine (Figure 3). When the dose-response relationship for L-NAME is considered, it is clear that with respect to inhibition of the fall in MAP induced by endothelin-1, the 10 and 25 mg kg⁻¹ min⁻¹ doses of L-NAME were not more effective than the 2 mg kg⁻¹ min⁻¹ dose (compare Figures 3b and c with Figure 2c). The vasodilator effects of endothelin-1 in the carotid and particularly the hindquarters vascular beds were inhibited dose-dependently by L-NAME (Figures 3b and c); however, the change between the 10 and 25 mg kg⁻¹ min⁻¹ was small and clear residual vasodilator responses remained in both beds (Figure 3).

Selectivity of action of L-NAME

To assess the selectivity of action of L-NAME, a third series of experiments were carried out in anaesthetized, ganglion-blocked SHR set up for recording blood pressure and heart rate but without placement of the flow probes. Bolus injections of acetylcholine, 0.1, 0.3 and 1 µg kg⁻¹, sodium nitroprusside, 1, 3 and 10 µg kg⁻¹ and endothelin-1, 1 nmol kg⁻¹, were given sequentially during infusions of phenylephrine, 5 µg kg⁻¹ min⁻¹ or L-NAME, 10 mg kg⁻¹ min⁻¹; which raised MAP from 150 ± 3 to 208 ± 6 (*n* = 5) and from 159 ± 10 to 212 ± 6 (*n* = 4) mmHg, respectively. The results are presented in Figure 4. Responses to acetylcholine were significantly smaller during infusion of L-NAME than during infusion of phenylephrine; in contrast responses to sodium nitroprusside were closely similar in the two experimental situations (Figure 4a). In confirmation of the earlier result (see Figure 3a and b) the magnitude of the depressor response to endothelin-1 was unchanged during perfusion with L-NAME although the response was of significantly shorter duration (Figure 4b).

Discussion

The present experiments were designed specifically to explore the role of NO derived from L-arginine in the vasodilator response to endothelin-1 in the rat by use of two selective inhibitors of NO synthase, L-NMMA and L-NAME (Rees *et al.*, 1989b; Moore *et al.*, 1990). Since both L-NMMA and L-NAME cause marked rises in blood pressure in rats (Gardiner *et al.*, 1989; 1990c; d; Fozard & Part, 1990), presumably a consequence of removing vasodilator NO 'tone', responses to endothelin-1 were compared to those obtained in animals in which vasoconstriction was induced by infusion of phenylephrine. Although there were minor discrepancies, the haemodynamic changes induced by the NO biosynthesis inhibitors and phenylephrine were broadly similar (Table 1).

The first point to be made is that L-NMMA at a dose (5 mg kg⁻¹ min⁻¹) that raises blood pressure to maximum levels in this preparation (see Table 1 and Fozard & Part 1990) caused no inhibition of the vasodepressor responses to endothelin-1 (Figure 2). Since comparisons were made with responses obtained during phenylephrine infusion, the lack of effect cannot be explained by inhibition being offset by the augmenting effects of an elevated vasoconstrictor tone (see for example, Rees *et al.*, 1989b; Gardiner *et al.*, 1990c; van Gelderen *et al.*, 1991). Rather the data would point to the possibility of the vasodilator response to endothelin-1 being independent of NO newly synthesized from L-arginine. However, a discrepancy between the potency of NO biosynthesis inhibitors to increase blood pressure and that to inhibit responses to endothelium-dependent vasodilators such as substance P, bradykinin and acetylcholine has been noted previously (see for example Aisaka *et al.*, 1989; Whittle *et al.*, 1989; Gardiner *et al.*, 1990c; 1991). Thus, an alternative explanation might be that the dose of inhibitor required to block evoked NO release is higher than that needed to block basal release. This would be supported by the fact that the more

potent NO synthase inhibitor, L-NAME, attenuated significantly the duration of the vasodepressor response to endothelin-1 (Figure 2).

To evaluate this hypothesis further, a second series of experiments was carried out in which doses of 10 and 25 mg kg⁻¹ min⁻¹ of L-NAME were infused and in which a comparison with acetylcholine was included. The results were clear-cut in that both the vasodepressor and the hindquarters vasodilator responses to acetylcholine and endothelin-1 were attenuated following L-NAME, 10 mg kg⁻¹ min⁻¹ and that minimal further inhibition occurred with the higher dose (Figure 4). Significantly, the higher doses of L-NAME were no more effective against the vasodepressor effect of endothelin-1 than was the smaller dose of 2 mg kg⁻¹ min⁻¹ (Figure 2c). The data presented in Figure 4 confirm the inhibitory effect of L-NAME 10 mg kg⁻¹ min⁻¹ on endothelin-1 and acetylcholine and show the effect to be selective since responses to the endothelium-independent vasodilator, sodium nitroprusside, remain unchanged. These observations indicate that NO generated by *de novo* synthesis contributes in part to the vasodepressor effects of endothelin-1 and acetylcholine but that a major component of the response in each case may occur independently of this mechanism. In the case of endothelin-1, it is clearly the initial phase of the fall in blood pressure which appears not to involve NO.

Since we have no independent measure of NO generation from L-arginine, it cannot be concluded with certainty that the resistance of the endothelin and acetylcholine vasodilator response to blockade by L-NAME indicates an NO-independent mechanism. However, it might be expected that with the high, supramaximal doses of L-NAME used, NO synthase would be completely blocked. Moreover, with respect to endothelin-1, there are several potential candidates for the mechanism of the component of the vasodilator response resistant to NO biosynthesis inhibition. One possibility is the generation of vasodilator products of cyclo-oxygenase which has been demonstrated to occur *in vitro* following endothelin-1 (De Nucci *et al.*, 1988; Rae *et al.*, 1989; Rakugi *et al.*, 1989). However, this seems not to be important *in vivo* since the vasodepressor and/or regional vasodilator effects of endothelin-1 were resistant to blockade by indomethacin in rats (Gardiner *et al.*, 1990b; Ohlstein *et al.*, 1990; Wright & Fozard, 1990) and other species (Hoffmann *et al.*, 1989; Lopez *et al.*, 1990; Minkes *et al.*, 1990; Lipton *et al.*, 1991a). A second possible mechanism may be advanced based on the observation that pulmonary vasodilatation induced by endothelin-1 in the rat (Hasunuma *et al.*, 1990) and cat (Lipton *et al.*, 1991b) can be inhibited by glibenclamide, an antagonist of ATP-dependent K⁺ channels. The observation would be consistent with the release of endothelium-derived hyperpolarizing factor (EDHF; see Taylor & Weston, 1988), the actions of which are known to be attenuated by ATP-dependent K⁺ channel blockade (Standen *et al.*, 1989; Brayden, 1990). However, whilst the vasodilator effects of endothelin-1 in discrete vascular beds, notably the lung could conceivably be the consequence of such a mechanism, this is unlikely to be a major factor. Thus, the vasodepressor response to endothelin-1 in the anaesthetized rat was totally unaffected by glibenclamide, at a dose (20 mg kg⁻¹) adequate to block the hypotensive response to cromakalim (Le Monnier de Gouville *et al.*, 1990b). A final possibility, a direct hyperpolarizing effect of endothelin-1, has been recorded from smooth muscle cells in culture (Van Renterghem *et al.*, 1988; Hu *et al.*, 1991). Whilst this could conceivably result in direct smooth muscle relaxation, it should be noted that such direct (i.e., endothelium-independent) vasodilator responses have up to the present not been reported.

With respect to acetylcholine, the present data are consistent with a growing number of studies showing a resistance of its vasodilator response *in vivo* to blockade by inhibitors of NO synthase (see for example Aisaka *et al.*, 1989; Gardiner *et al.*, 1990c; Rees *et al.*, 1989a; Ross *et al.*, 1991; Van Gelderen *et al.*, 1991). Our data further indicate the effect to be regional

in that vasodilatation in the hindquarters vascular bed had a clear L-NAME-sensitive component whereas that in the carotid and mesenteric beds had not. A possible explanation for the residual response to acetylcholine is the release of EDHF (Brayden, 1990).

In conclusion, the present data indicate that NO generated *de novo* from L-arginine mediates a significant component of the vasodilator effect of endothelin-1 in the anaesthetized, ganglion-blocked rat. Blockade by the NO biosynthesis inhibitor, L-NAME, is manifested selectively against the later phase

of the vasodepressor response to endothelin-1 and the vasodilator response in the hindquarters vascular bed. The limited blockade of endothelin-1 plus the need for doses higher than those required to raise blood pressure maximally may explain the inconsistencies in the literature with respect to the effects of NO biosynthesis inhibitors on vasodilator responses to endothelin-1 *in vivo*.

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