Br. J. Pharmacol. (1990), **101**, 632–639

Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester

[1]S.M. Gardiner, A.M. Compton, P.A. Kemp & T. Bennett

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Conscious Long Evans rats, chronically instrumented for cardiovascular measurements, were challenged with i.v. bolus doses of glyceryl trinitrate (40 nmol kg^{-1}), acetylcholine (1.2 nmol kg^{-1}), bradykinin (3.2 nmol kg^{-1}), or endothelin-1 (0.25 nmol kg^{-1}). Under control conditions these doses produced similar falls in mean arterial blood pressure (glyceryl trinitrate, -20 ± 3 mmHg; acetylcholine, -24 ± 2 mmHg; bradykinin, -21 ± 3 mmHg; endothelin-1, -25 ± 3 mmHg), associated with renal, mesenteric and hindquarters vasodilatations (except for endothelin-1 which caused mesenteric vasoconstriction).

2 In the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}), a potent inhibitor of nitric oxide biosynthesis and endothelium-dependent vasorelaxation *in vitro*, the hypotensive responses to glyceryl trinitrate, acetylcholine, and endothelin-1 were increased, although that to bradykinin was not. However, comparing the differences between the response to glyceryl trinitrate and that to any other agonist in the absence and presence of L-NAME showed that there were relative attenuations of the hypotensive responses to bradykinin and endothelin-1, but not to acetylcholine, in the presence of L-NAME.

3 This comparative analysis showed that the renal and hindquarters vasodilator responses to bradykinin and endothelin-1 were attenuated in the presence of L-NAME, but the renal, mesenteric and hindquarters vasodilator responses to acetylcholine were not. However, when L-NAME was administered in the presence of pentolinium, captopril and the vasopressin V_1-receptor antagonist, $d(CH_2)_5$[Tyr-(Et)]DAVP, (to abolish baroreflex and neurohumoral mechanisms), there was attenuation of the renal and mesenteric vasodilator effects of acetylcholine relative to those seen with glyceryl trinitrate. Under those conditions only the renal vasodilator effects of bradykinin and endothelin-1 were attenuated.

4 In separate experiments in conscious Long Evans rats, direct measurement of cardiac haemodynamics showed that the hypotensive responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 were entirely attributable to rises in total peripheral conductance since both in the absence and presence of L-NAME there were no reductions in cardiac index in response to these substances.

5 The results indicate that measurement of systemic arterial blood pressure alone in conscious rats does not permit reliable quantitation of the influence of L-NAME on regional vasodilator responses to glyceryl trinitrate, acetylcholine, bradykinin or endothelin-1. Furthermore, these substances exert effects in different vascular beds that may be differentially influenced by baroreflex mechanisms, neurohumoral mechanisms, or both. Moreover, except in the case of the renal vasodilator response to endothelin-1 (which was abolished in the presence of L-NAME), even when L-NAME caused attenuation of the vasodilator effects of acetylcholine or bradykinin (relative to glyceryl trinitrate), substantial responses remained. It is feasible that such responses *in vivo* are nitric oxide-independent.

Introduction

Nitric oxide synthesized from L-arginine appears to be the major mediator of endothelium-dependent vasorelaxation *in vitro* (Palmer *et al.*, 1987; 1988a,b; Moncada *et al.*, 1988; 1989; Rees *et al.*, 1989a,b). The biosynthesis of nitric oxide is antagonised by N^G-monomethyl-L-arginine (L-NMMA; see Moncada *et al.*, 1989) a compound which causes widespread regional vasoconstrictions when administered to conscious rats (Gardiner *et al.*, 1989c,d; 1990c,d,f).

Whittle *et al.* (1989) reported that L-NMMA produced substantial inhibition of the falls in diastolic blood pressure induced by acetylcholine, bradykinin, substance P and endothelin-1 in pentobarbitone-anaesthetized Wistar rats, which is consistent with the hypotension being due to endothelium-derived nitric oxide causing vasodilatation. However, with endothelin-1 we had found, in conscious rats, that the initial hypotensive response was enhanced in the presence of L-NMMA and the associated hindquarters vasodilatation was not attenuated (Gardiner *et al.*, 1989d). In addition, Aisaka *et al.* (1989) reported that L-NMMA did not change the magnitude of the hypotensive response to acetylcholine in pentobarbitone-anaesthetized guinea-pigs.

It is known now that N^G-nitro-L-arginine is a more potent inhibitor than L-NMMA of endothelium-dependent vasorelaxation *in vitro* (Moore *et al.*, 1990), and this effect is associated with marked inhibition of nitric oxide production (Ishii *et al.*, 1990; Mülsch & Busse, 1990). Furthermore, oral or intravenous administration of N^G-nitro-L-arginine methyl ester (L-NAME) in conscious rats causes marked hypertension and regional vasoconstrictions (Gardiner *et al.*, 1990e,f,h), the patterns of which resemble those following L-NMMA, albeit given at a higher dose (Gardiner *et al.*, 1989c,d; 1990c,d,f). Therefore, in the present work we investigated regional haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in the absence and presence of L-NAME. Furthermore, in order to preclude any possible contributions from baroreflex, or neurohumoral mechanisms,

[1] Author for correspondence.

or both, to the responses seen, the same experiments were performed in a second group of animals in the absence and presence of autonomic and neurohumoral blockade (achieved with pentolinium, captopril and an antagonist of the V_1-receptor-mediated actions of vasopressin).

In order to determine the possible contributions of changes in cardiac haemodynamics to the depressor effects of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in the presence and absence of L-NAME, these experiments were also performed in animals chronically instrumented for the measurement of cardiac function.

Methods

The majority of experiments were carried out on male Long Evans rats (3–4 months old; bred in Nottingham). Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p. supplemented as required) and had pulsed Doppler probes (Haywood *et al.*, 1981) sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta below the level of the ileocaecal artery (to monitor flow to the hindquarters) (Gardiner *et al.*, 1990a). Separate animals had electromagnetic flow probes (Skalar, Delft) implanted around the ascending aorta via a transthoracic approach (Smith & Hutchins, 1979; Smits *et al.*, 1982; Gardiner *et al.*, 1990g,h). Animals were given ampicillin (7 mg kg^{-1}, i.m.; Penbritin, Beechams) and left to recover for 7–14 days. Then, under brief anaesthesia (sodium methohexitone, 40 mg kg^{-1}, i.p.), intra-arterial (distal abdominal aorta via the ventral caudal artery) and intravenous (right jugular vein) catheters were implanted. In the case of animals with thoracic aortic flow probes, one of the intravenous catheters was constructed from a 3 cm length of small bore (intravascular) catheter (i.d. 0.28 mm), heat-sealed to a 150 cm length of more rigid, wider bore (i.d. 0.58 mm) nylon tubing. The tip of this catheter was positioned close to the right atrial orifice for recording central venous pressure (Gardiner *et al.*, 1990g,h).

Animals were left to recover in their home cages overnight and experiments were begun the next day, at least 24 h after anaesthesia and catheterization.

Regional haemodynamic effects of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1

In preliminary experiments based on the data of Whittle *et al.* (1989), we determined that glyceryl trinitrate (40 nmol kg^{-1}), acetylcholine (1.2 nmol kg^{-1}), bradykinin (3.2 nmol kg^{-1}) and endothelin-1 (0.25 nmol kg^{-1}) caused comparable falls in mean blood pressure. These doses were then used in the definitive experiments. It should be noted that the results obtained were similar whether measurements were made of changes in diastolic or in mean blood pressures. Animals received glyceryl trinitrate, acetylcholine, and bradykinin in random order, but received endothelin-1 last because of the prolonged secondary pressor effect of this peptide. All substances were administered at least 10 min apart.

In one group of Long Evans rats ($n = 8$) glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 were administered and, at least 90 min after endothelin-1, L-NAME (10 mg kg^{-1}, i.v.) was given. Elsewhere (Gardiner *et al.*, 1990h) we have shown there is a relatively stable haemodynamic profile for at least 60 min after this dose of L-NAME. Therefore, starting 10 min after injection of L-NAME, animals were re-challenged at 10 min intervals with glyceryl trinitrate, acetylcholine, bradykinin (randomized) and, finally, endothelin-1 (i.e. about 40 min after L-NAME injection).

In another group ($n = 8$) of Long Evans rats, glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 were administered in the absence and in the presence of pentolinium (5 mg kg^{-1} bolus followed by 5 mg kg^{-1} h^{-1} infusion), captopril (2 mg kg^{-1} bolus followed by 1 mg kg^{-1} h^{-1} infusion), [(1-β-mercapto-β, β-cyclopentamethylenepropionic acid), 2-(0-ethyl)tyrosine, 8-D-arginine]vasopressin (abbreviated to d(CH$_2$)5[Tyr-(Et)]DAVP; 10 μg kg^{-1} bolus followed by 10 μg kg^{-1} h^{-1} infusion) and L-NAME (10 mg kg^{-1} bolus). Elsewhere we have shown the combination of these doses of pentolinium, captopril and d(CH$_2$)$_5$[Tyr-(Et)]DAVP blocks all endogenous factors contributing to blood pressure recovery in conscious, Long Evans rats (Tomlinson *et al.*, 1990). Hence, these experiments were designed to abolish any possible contributions of baroreflex on neurohumoral mechanisms to the responses evoked by glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1.

In order to simulate the experiments carried out by Whittle *et al.* (1989) we also investigated the effects on blood pressure of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in pentobarbitone-anaesthetized Long Evans ($n = 2$) and Wistar ($n = 3$) rats in the absence and presence of L-NAME (10 mg kg^{-1}) or L-NMMA (50 mg kg^{-1}), respectively.

Cardiac haemodynamic effects of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1

Long Evans rats ($n = 8$) with thoracic aortic flow probes were connected to a Skalar MDL 1401 flowmeter and data were digitized on-line with a haemodynamics microprocessor (Schoemaker, 1989; Gardiner *et al.*, 1990g,h). This system provided values for cardiac index, peak aortic flow, maximum positive slope of aortic flow ($+dF/dt_{max}$), total peripheral conductance, stroke index, mean central venous pressure, heart rate and mean arterial blood pressure. Changes in these variables were measured in response to administration of glyceryl trinitrate, acetylcholine, bradykinin, and endothelin-1 in the absence and in the presence of L-NAME (10 mg kg^{-1}) as above.

Data analysis

The profiles of change in mean blood pressure and regional haemodynamics differed following administration of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1, although the nadirs in blood pressure were similar under control conditions. Furthermore the time courses of the changes in regional flows varied under the different experimental conditions, and so, for each substance in each condition, measurements were made at time points selected to represent the full profile of effect on all variables. Since L-NAME caused substantial haemodynamic changes the responses to glyceryl trinitrate were used as an internal standard and all other responses were compared to them. Thus, for example, the difference between the hypotensive response to glyceryl trinitrate and endothelin-1 in the absence of L-NAME was compared to that in the presence of L-NAME as a means of assessing the extent to which the latter caused a relative attenuation of the hypotensive effect of endothelin-1. In the results the effects of substances relative to glyceryl trinitrate are given also as ratios to facilitate comparisons but statistical procedures were carried out on the raw data. Results were analysed by the Kruskal-Wallis test, Mann-Whitney U test or Wilcoxon's ranks sums test, as appropriate; $P < 0.05$ was taken as indicating statistical significance.

Drugs

Acetylcholine chloride (Sigma), N^G-nitro-L-arginine methyl ester hydrochloride (Sigma), N^G-monomethyl-L-arginine acetate (Wellcome Research Laboratories), glyceryl trinitrate (Tridil; Du Pont, U.K.), pentolinium tartrate (Sigma), captopril (Squibb, U.K.), d(CH$_2$)$_5$[Tyr-(Et)]DAVP (Bachem U.K.), bradykinin (Bachem U.K.) and endothelin-1 (Peptide Institute, Japan) were dissolved in isotonic saline. In the case of the latter peptides, the saline contained 1% bovine serum albumin. All i.v. injections were given as 100 μl boluses which

were flushed in with 100 μl isotonic saline (the dead spaces of the catheters).

Results

Regional haemodynamic effects of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 before and after administration of L-NAME

Blood pressures In the first group of animals ($n = 8$), under control conditions, the falls in arterial blood pressure elicited by glyceryl trinitrate (-20 ± 3 mmHg), acetylcholine (-24 ± 2 mmHg), bradykinin (-21 ± 3 mmHg) and endothelin-1 (25 ± 3 mmHg) were not different. L-NAME increased mean arterial blood pressure from 110 ± 2 to 153 ± 2 mmHg and then the falls in arterial blood pressure elicited by glyceryl trinitrate (-49 ± 3 mmHg), acetylcholine (-48 ± 2 mmHg), and endothelin-1 (-38 ± 2 mmHg) were significantly larger than in the absence of L-NAME, but the response to bradykinin (-29 ± 4 mmHg) was not. When considered relative to glyceryl trinitrate, the depressor effects of bradykinin and endothelin-1 were attenuated in the presence of L-NAME, whereas the hypotensive response to acetylcholine was not (Figure 1 and Table 1).

In the second group of animals ($n = 8$), the depressor responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 under control conditions were similar to those seen in the first group (Figure 1 and Table 2). During combined administration of pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP, mean arterial blood pressure was 47 ± 3 mmHg; 10 min after additional administration of L-NAME blood pressure had risen to 84 ± 6 mmHg. Under those circumstances the absolute hypotensive responses to glyceryl trinitrate (-29 ± 4 mmHg), and acetylcholine (-28 ± 5 mmHg), were not different from those under control conditions. However, the depressor effect of bradykinin (-39 ± 4 mmHg) was enhanced, whereas the response to endothelin-1 (-19 ± 2 mmHg) was reduced. Thus, when considered relative to glyceryl trinitrate, the depressor effects of acetylcholine and endothelin-1 were attenuated whereas the response to bradykinin was augmented (Figure 1 and Table 2). In addition, the later pressor effect of endothelin-1 was enhanced although the absolute pressure level reached was not increased (Figure 1).

In anaesthetized, Long Evans rats ($n = 2$) or anaesthetized, Wistar rats ($n = 3$) treatment with L-NAME (10 mg kg^{-1}) or L-NMMA (50 mg kg^{-1}), respectively, augmented hypotensive responses. For example in anaesthetized Wistar rats in the absence of L-NMMA the falls in mean arterial blood pressure evoked by glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 were -14 ± 2, -25 ± 1, -11 ± 3 and -16 ± 3 mmHg, respectively. The corresponding values in the presence of L-NMMA were -47 ± 10, -49 ± 8, -27 ± 5 and -46 ± 12 mmHg, respectively (Figure 2).

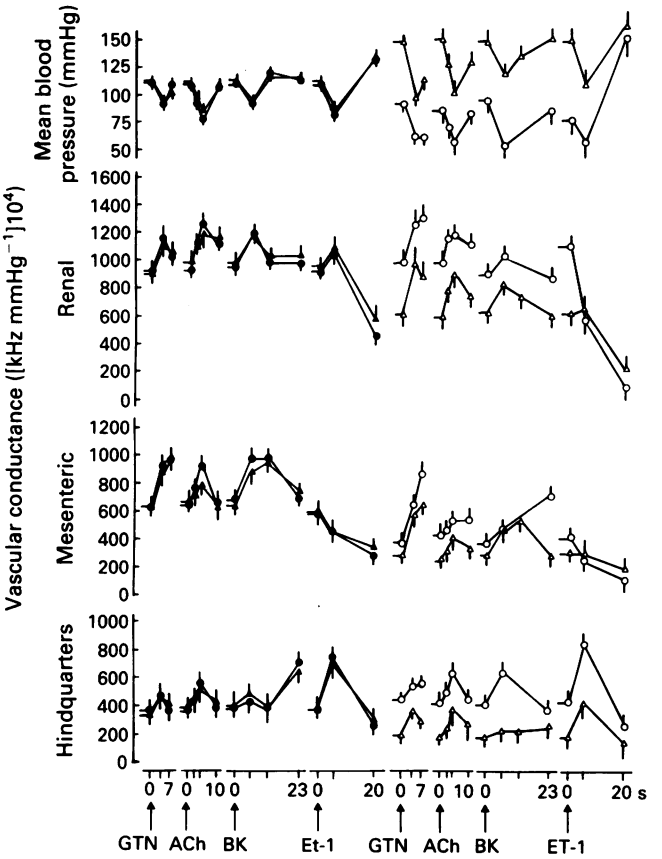

Figure 1 Regional haemodynamic responses to i.v. bolus doses of glyceryl trinitrate (GTN, 40 nmol kg^{-1}), acetylcholine (ACh, 1.2 nmol kg^{-1}), bradykinin (BK, 3.2 nmol kg^{-1}) or endothelin-1 (ET-1, 0.25 nmol kg^{-1}) in conscious Long Evans rats. Left hand panels show responses in two separate groups (●, ▲), $n = 8$ in each) under control conditions. Right hand panels show responses in the same animals either in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}) alone (△) or after administration of L-NAME in the presence of pentolinium (5 mg kg^{-1} bolus, 5 mg kg^{-1} h^{-1} infusion), captopril (2 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion) and $d(CH_2)_5$[Tyr-(Et)]DAVP (10 μg kg^{-1} bolus, 10 μg kg^{-1} h^{-1} infusion) (○). Values are the mean and the vertical lines show the s.e.mean.

Renal vascular conductances In the first group of animals, under control conditions, all 4 substances caused initial increases in renal vascular conductance (Figure 1 and Table 1). L-NAME (10 mg kg^{-1}) decreased renal vascular conductance from 952 ± 62 to 583 ± 77 units. Following administration of L-NAME, the increases in renal vascular conductance elicited by glyceryl trinitrate (352 ± 24 units) and acetylcholine (300 ± 18 units) were significantly larger than in the absence of L-NAME (188 ± 34 and 208 ± 21 units, respectively), but the renal vasodilator response to bradykinin

Table 1 Ratios between the depressor and the vasodilator responses to acetylcholine, to bradykinin or to endothelin-1 and those to glyceryl trinitrate in the absence of N^G-nitro-L-arginine methyl ester (control) or in its presence (+ L-NAME)

		Acetylcholine	*Bradykinin*	*Endothelin-1*
Mean blood pressure	Control	1.20	1.05	1.25
	+ L-NAME	0.98	0.59	0.78
Renal conductance	Control	1.11	1.21	0.77
	+ L-NAME	0.85	0.57	0.10
Mesenteric conductance	Control	0.49	0.96	*
	+ L-NAME	0.51	0.86	*
Hindquarters conductance	Control	1.17	2.08	2.48
	+ L-NAME	1.08	0.43	1.43

* Endothelin-1 caused mesenteric vasoconstriction only.
Doses as in Figure 1; ratios were derived from the results shown in Figure 1.

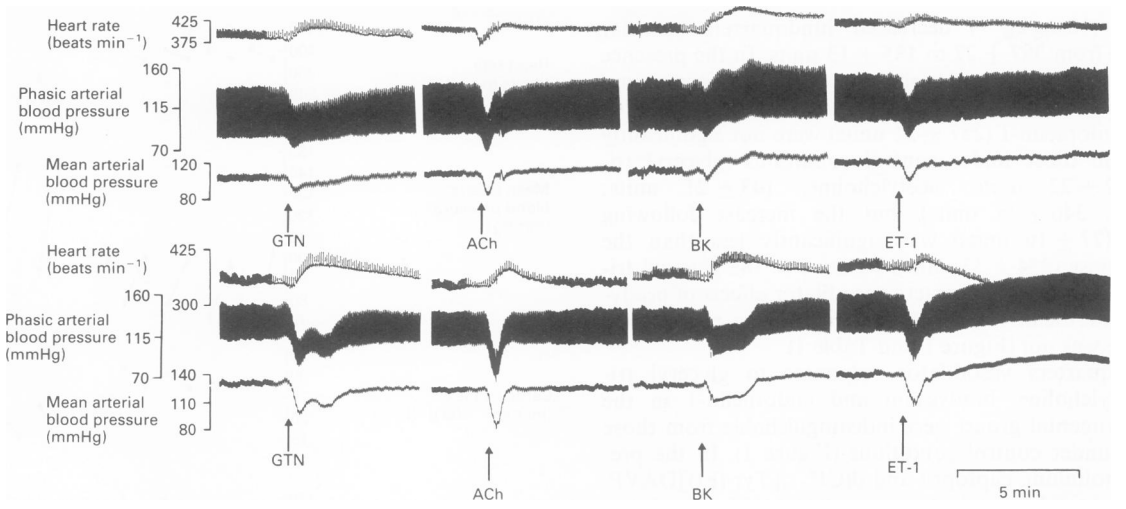

Figure 2 Recordings of blood pressure and heart rate in a Wistar rat anaesthetized with sodium pentobarbitone. The upper traces show the responses to glyceryl trinitrate (GTN, 40 nmol kg^{-1}), acetylcholine (ACh, 1.2 nmol kg^{-1}), bradykinin (BK, 3.2 nmol kg^{-1}) and endothelin-1 (ET-1, 0.25 nmol kg^{-1}). The lower traces show responses to the same 4 substances in the same animal following injection of N^G-monomethyl-L-arginine (50 mg kg^{-1}).

(control, 227 ± 31; plus L-NAME, 199 ± 31 units) was not; there was no significant renal vasodilatation following endothelin-1 in the presence of L-NAME (Figure 1). When considered relative to glyceryl trinitrate, the renal vasodilator effects of bradykinin and endothelin-1 were attenuated in the presence of L-NAME, whereas the response to acetylcholine was not (Figure 1 and Table 1).

In the second group of animals, under control conditions, all 4 substances caused initial increases in renal vascular conductance, similar to those seen in the first group (Figure 1 and Table 2). During combined administration of pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP, renal vascular conductance was 1618 ± 190 units but 10 min after additional administration of L-NAME, renal vascular conductance was 990 ± 16 units. Under these conditions the increases in renal vascular conductance in response to glyceryl trinitrate (327 ± 63 units) and bradykinin (135 ± 84 units) were not significantly different from the responses under control conditions (glyceryl trinitrate, 225 ± 8; bradykinin, 243 ± 29 units). However, the increase in renal vascular conductance in response to acetylcholine (189 ± 68 units) was attenuated (control, 342 ± 55 units) and the renal vasodilatation following endothelin-1 was abolished (Figure 1). Relative to the response to glyceryl trinitrate, the renal vasodilator responses to acetylcholine and bradykinin were both attenuated (Figure 1 and Table 2).

Mesenteric vascular conductances In the first group of animals under control conditions, glyceryl trinitrate, acetylcholine and bradykinin increased mesenteric vascular conductance, whereas endothelin-1 caused mesenteric vasoconstriction (Figure 1).

L-NAME (10 mg kg^{-1}) decreased mesenteric vascular conductance (from 615 ± 76 to 233 ± 35 units) but in the presence of L-NAME, the increases in response to glyceryl trinitrate (309 ± 32 units), acetylcholine (152 ± 17 units) and bradykinin (258 ± 34 units) were not significantly different from those under control conditions (glyceryl trinitrate, 261 ± 63; acetylcholine, 127 ± 51; bradykinin, 250 ± 82 units; Figure 1). Furthermore, relative to glyceryl trinitrate, there was no change in the mesenteric vasodilator responses to acetylcholine or bradykinin (Figure 1 and Table 1).

In the second group of rats the patterns of mesenteric vasodilator responses to glyceryl trinitrate, acetylcholine and bradykinin were similar to those in the first group under control conditions (Figure 1 and Table 2).

During combined administration of pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP, mesenteric vascular conductance was 1222 ± 199 units but, 10 min after the additional administration of L-NAME, mesenteric conductance was 431 ± 68 units (Figure 1). Under those conditions the increases in conductance in response to glyceryl trinitrate (472 ± 98 units) and bradykinin (357 ± 86 units) were not significantly different from those under control conditions (glyceryl trinitrate, 341 ± 32; bradykinin, 285 ± 65 units), but the response to acetylcholine (101 ± 49 units) was reduced (control, 285 ± 57 units). Relative to glyceryl trinitrate, only the response to acetylcholine was attenuated (Figure 1 and Table 2).

Hindquarters vascular conductances In the first group of animals under control conditions all 4 substances caused increases in vascular conductance, although the patterns of change differed, with bradykinin causing a delayed vasodilatation (Figure 1).

Table 2 Ratios between the depressor and the vasodilator responses to acetylcholine, to bradykinin or to endothelin-1 and those to glyceryl trinitrate in the absence of N^G-nitro-L-arginine methyl ester (control) or in the presence of pentolinium, captopril, $d(CH_2)_5$[Tyr-(Et)]DAVP and N^G-nitro-L-arginine (+ PCA, + L-NAME)

		Acetylcholine	*Bradykinin*	*Endothelin-1*
Mean blood pressure	Control	1.52	0.86	1.33
	+ PCA + L-NAME	0.97	1.34	0.66
Renal conductance	Control	1.52	1.08	0.55
	+ PCA + L-NAME	0.58	0.41	*
Mesenteric conductance	Control	0.83	0.84	*
	+ PCA + L-NAME	0.21	0.76	*
Hindquarters conductance	Control	1.61	3.31	3.72
	+ PCA + L-NAME	1.77	2.87	3.57

* No vasodilator responses to endothelin-1.

L-NAME (10 mg kg^{-1}) decreased hindquarters vascular conductance from 387 ± 27 to 185 ± 13 units. In the presence of L-NAME the increases in vascular conductance in response to glyceryl trinitrate (180 ± 30 units), acetylcholine (195 ± 19 units) and endothelin-1 (257 ± 21 units) were not significantly different from those under control conditions (glyceryl trinitrate, 122 ± 22 units; acetylcholine, 143 ± 21 units; endothelin-1, 346 ± 38 units), but the increase following bradykinin (77 ± 16 units) was significantly less than the control response (254 ± 33 units). Relative to the glyceryl trinitrate response, the hindquarters vasodilator effects of bradykinin and endothelin-1 were attenuated but the response to acetylcholine was not (Figure 1 and Table 1).

The hindquarters vasodilator responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in the second experimental group were indistinguishable from those in the first, under control conditions (Figure 1). In the presence of pentolinium, captopril and d(CH$_2$)$_5$[Tyr-(Et)]DAVP, hindquarters vascular conductance was 843 ± 126 units, but it fell to 438 ± 67 units 10 min after the additional administration of L-NAME. Under those conditions the increases in hindquarters vascular conductance to glyceryl trinitrate (116 ± 39 units), acetylcholine (205 ± 57 units), bradykinin (333 ± 71 units) and endothelin-1 (414 ± 67 units) were not different from those under control conditions (100 ± 28, 161 ± 39, 331 ± 61, 372 ± 49 units respectively), although the peak response to bradykinin occurred sooner (Figure 1). There were no significant changes in the hindquarters vasodilator responses to acetylcholine, bradykinin or endothelin-1 relative to glyceryl trinitrate (Figure 1 and Table 2).

Cardiac haemodynamic effects of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 before and after administration of L-NAME

Under control conditions the hypotensive responses to glyceryl trinitrate (−23 ± 3 mmHg), acetylcholine (−23 ± 3 mmHg), bradykinin (−21 ± 2 mmHg) and endothelin-1 (−21 ± 3 mmHg) were due entirely to reductions in total peripheral conductance since cardiac index did not fall (Figure 3). Indeed, there were significant rises in cardiac index following administration of bradykinin and endothelin-1 (Figure 3). In the presence of L-NAME there were marked reductions in all indices of cardiac function in association with a substantial increase in mean blood pressure and a fall in total peripheral conductance (Figure 3). Under these conditions the hypotensive responses to glyceryl trinitrate (−48 ± 3 mmHg), acetylcholine (−45 ± 4 mmHg), bradykinin (−35 ± 3 mmHg) and endothelin-1 (−37 ± 3 mmHg) were enhanced compared to the responses in the absence of L-NAME (Figure 3). However, relative to the response to glyceryl trinitrate the hypotensive effect of bradykinin was attenuated, but those of acetylcholine and endothelin-1 were not (the latter was on the borderline of significance (Figure 3 and Table 3)).

The rises in total peripheral conductance elicited by bradykinin and endothelin-1 (relative to glyceryl trinitrate) were reduced in the presence of L-NAME, but the relative vasodilator effect of acetylcholine was not (Figure 3 and Table 3).

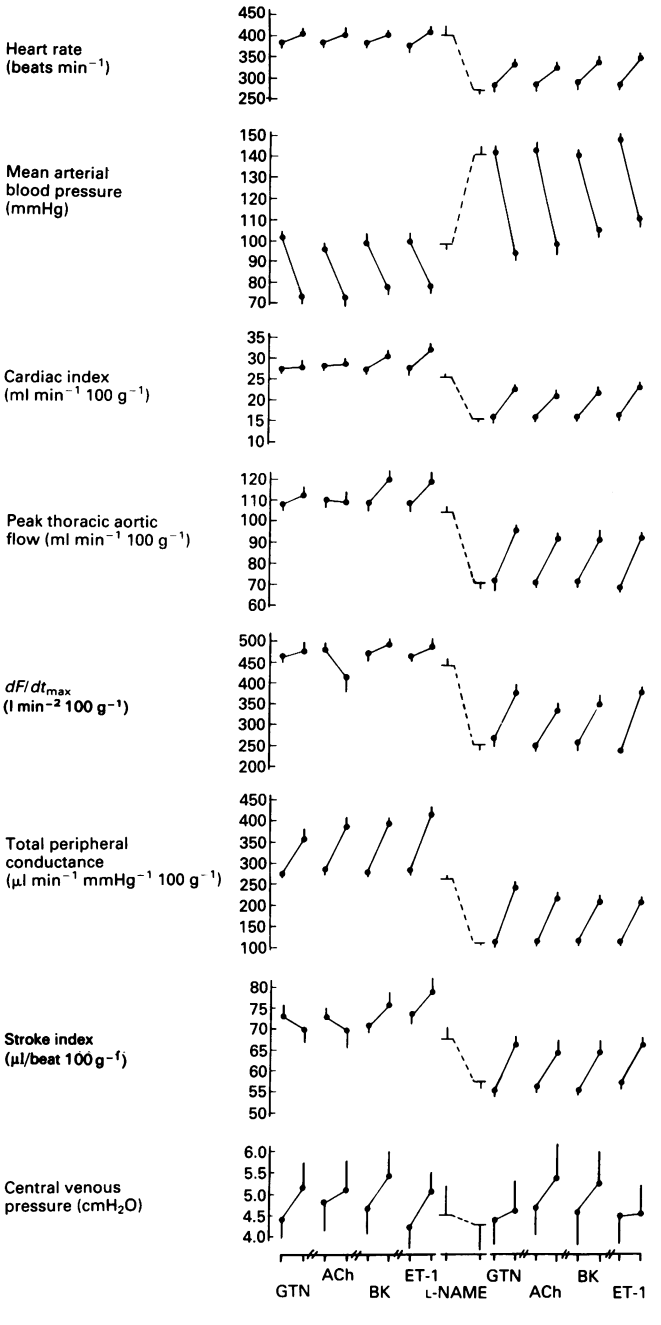

Figure 3 Cardiac haemodynamic responses to i.v. bolus doses of glyceryl trinitrate (GTN, 40 nmol kg^{-1}), acetylcholine (ACh, 1.2 nmol kg^{-1}), bradykinin (BK, 3.2 nmol kg^{-1}) or endothelin-1 (ET-1, 0.25 nmol kg^{-1}) in conscious Long Evans rats (n = 8) in the absence (left hand panels) or in the presence (right hand panels) of N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}). The steady state changes in cardiovascular variables elicited by L-NAME are shown by the dotted lines in the middle of the figure. The points plotted represent those at the nadir of the hypotensive responses. Values are the mean and the vertical lines show the s.e.mean.

Table 3 Ratios between the depressor and the vasodilator responses to acetylcholine, to bradykinin or to endothelin-1 and those to glyceryl trinitrate in the absence of N^G-nitro-L-arginine methyl ester (control) or in its presence (+ L-NAME)

		Acetylcholine	*Bradykinin*	*Endothelin-1*
Mean blood pressure	Control	1.0	0.91	0.91
	+ L-NAME	0.94	0.73	0.77
Total peripheral conductance	Control	1.21	1.40	1.60
	+ L-NAME	0.82	0.73	0.76

Doses as in Figure 1; ratios were derived from the results shown in Figure 3.

Discussion

The primary objective of the present work was to delineate the effects of L-NAME on the hypotensive and vasodilator effects of acetylcholine, bradykinin and endothelin-1 in conscious rats. Since acetylcholine and bradykinin are considered to be 'classical' endothelium-dependent vasodilators (Furchgott, 1983), and since nitric oxide produced by endothelial cells mediates vasodilatation (Palmer *et al.*, 1987; Moncada *et al.*, 1989; Rees *et al.*, 1989a,b), we reasoned that inhibition of nitric oxide biosynthesis with L-NAME (Moore *et al.*, 1990) ought to affect responses to acetylcholine and bradykinin and, possibly, those to endothelin-1 (Whittle *et al.*, 1989). However, administration of L-NAME had substantial effects on cardiovascular status (see also Gardiner *et al.*, 1990h), so it was necessary to assess the responses to acetylcholine, bradykinin and endothelin-1 relative to a stimulus that acted via the same final common pathway (i.e. cyclic GMP), but not through the production of nitric oxide from L-arginine (Moncada *et al.*, 1988). We chose glyceryl trinitrate for this purpose, matched to give the same hypotensive effects as acetylcholine, bradykinin and endothelin-1 under control conditions in conscious Long Evans rats.

The hypotensive effect of the dose of glyceryl trinitrate used was associated with renal, mesenteric and hindquarters vasodilatations (the effect being most marked in the mesenteric vascular bed). L-NAME caused hypertension and regional vasoconstrictions (Gardiner *et al.*, 1990h) and, under these circumstances, the hypotensive and renal vasodilator effects of glyceryl trinitrate were augmented but the mesenteric and hindquarters vasodilator responses were not changed significantly. In experiments in which L-NAME was administered in the presence of pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP, animals were not hypertensive, and the hypotensive and regional vasodilator effects of glyceryl trinitrate were not augmented, although they were prolonged (Figure 1). These findings indicate that baroreflex and neurohumoral mechanisms may oppose the cardiovascular effects of glyceryl trinitrate under normal conditions. From these results it was clear that the responses to acetylcholine, bradykinin and endothelin-1 could only be considered relative to those of glyceryl trinitrate.

Responses to acetylcholine

Only in the presence of pentolinium, captopril, $d(CH_2)_5$[Tyr-(Et)]DAVP and L-NAME was there any attenuation of the hypotensive and vasodilator (renal and mesenteric) responses to acetylcholine relative to the effects of glyceryl trinitrate. While these results indicate a component of the control response to acetylcholine was probably due to nitric oxide-mediated mechanisms, substantial responses remained under these conditions, and, in particular, the hindquarters vasodilator response was not different from normal. There are several possible explanations for these findings, including: (1) the responses were due to a mechanism not involving nitric oxide (see Long & Berkowitz, 1989); (2) the responses were due to release of nitric oxide from a preformed pool (see Aisaka *et al.*, 1989); (3) the responses were due to nitric oxide, but relatively unaffected by L-NAME due to efficient receptor-effector coupling (Giles *et al.*, 1990). Considering this last possibility we have carried out preliminary experiments to assess the effect of atropine on the sensitivity of acetylcholine-induced vasodilator responses to L-NAME (Giles *et al.*, 1990). However, we have not been able to render the hypotensive or regional vasodilator responses to acetylcholine more sensitive to L-NAME by this intervention (Gardiner, Compton & Bennett unpublished observations).

As indicated above, our present findings do not preclude the possibility that preformed nitric oxide was responsible for the effects of acetylcholine, but they do demonstrate marked differences between *in vitro* (e.g. Moore *et al.*, 1990) and *in vivo* findings. Furthermore, they corroborate previous observations (Gardiner *et al.*, 1989d) showing a basic difference between our results and those of Whittle *et al.* (1989). Thus, under no conditions did we find that the absolute falls in mean (or diastolic) arterial blood pressure elicited by acetylcholine were attenuated in the presence of L-NAME. This result was not peculiar to our main experimental protocol since we obtained similar effects both in anaesthetized rats (Long Evans and Wistar) and when we used L-NMMA rather than L-NAME. It has been suggested (Aisaka *et al.*, 1989) that it is the duration rather than magnitude of hypotensive response to acetylcholine which is affected by inhibiting nitric oxide synthesis, but that did not apply to our results since there was an increase in both the magnitude and duration of the fall in blood pressure following acetylcholine in the presence of L-NAME (Figures 1 and 3).

Initially we thought that the hypotensive responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 might have been contributed to by differential changes in cardiac function under the different experimental conditions. However, direct measurement showed there was an enhanced increase in cardiac index following administration of all four substances in the presence of L-NAME (Figure 3), and for acetylcholine there was no significant attenuation of the rise in total peripheral conductance (relative to that seen with glyceryl trinitrate) under these conditions. Whatever the explanation of the present findings it is clear that administration of L-NAME (or L-NMMA) *in vivo* does not provide a simple means of quantifying the involvement of nitric oxide in the cardiovascular responses to acetylcholine.

Responses to bradykinin

The comments made above about acetylcholine-mediated responses also pertain, in some respects, to bradykinin, although the detailed picture differed. Thus, there was a relative attenuation of the hypotensive effects of bradykinin in the presence of L-NAME alone. This was associated with renal and hindquarters vasodilatations that were reduced relative to the responses to glyceryl trinitrate, as was the rise in total peripheral conductance in the animals instrumented for measurement of cardiac haemodynamics. However, when L-NAME was administered in the presence of pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP, the hypotensive response to bradykinin was enhanced relative to glyceryl trinitrate and there was no attenuation of the hindquarters vasodilator responses to bradykinin under these conditions (probably due to inhibition of bradykinin catabolism by captopril). These findings indicate that baroreflex or neurohumoral mechanisms, or both, could influence the hindquarters vasodilator responses to bradykinin selectively, since the renal vasodilator effects of bradykinin (relative to glyceryl trinitrate) were attenuated under all experimental conditions, while the peak mesenteric vasodilator effects of bradykinin were not reduced under any conditions (although the profile of change was affected). These results also raise the possibility that nitric oxide-dependent and nitric oxide-independent vasodilator responses to agonists such as bradykinin may be differentially expressed in different vascular beds *in vivo*.

Responses to endothelin-1

The hypotensive effect of endothelin-1 relative to glyceryl trinitrate was attenuated in the presence of L-NAME and the early renal vasodilator effects of endothelin-1 were abolished. In animals in which cardiac haemodynamics were measured there was attenuation of the rise in total peripheral conductance elicted by endothelin-1 relative to glyceryl trinitrate under these conditions. However, the attenuation of the relative hindquarters vasodilator effect of endothelin-1 seen in the presence of L-NAME was not apparent when pentolinium, captopril and $d(CH_2)_5$[Tyr-(Et)]DAVP were also administered, indicating that in the presence of L-NAME alone the hindquarters vasodilator effect of endothelin-1 was probably offset by either baroreflex or neurohumoral mechanisms, or

both. Using a different experimental protocol with a lower dose of endothelin-1, we concluded previously that L-NMMA did not attenuate the hindquarters vasodilator response to this peptide (Gardiner *et al.*, 1989d).

It is of interest that under no conditions did endothelin-1 cause mesenteric vasodilatation, in spite of the finding that release of nitric oxide and cyclo-oxygenase products can cause mesenteric vasodilatation in response to endothelins *in vitro* (De Nucci *et al.*, 1988; Warner *et al.*, 1989a,b; Randall *et al.*, 1989). The present results indicate that our previous observations on the mesenteric haemodynamic effects of endothelin-1 *in vivo* (Gardiner *et al.*, 1989a,d; 1990a,b) were not due to a selective effect in this vascular bed of whatever mechanisms (baroreflex, neurohumoral, or both) activated by the fall in arterial blood pressure.

Conclusions

It appears that in *in vivo* experiments involving L-NAME (or L-NMMA) to determine the possible involvement of nitric oxide in the hypotensive and vasodilator responses to acetylcholine, bradykinin and endothelin-1 an internal standard is required, such as the responses to glyceryl trinitrate, in order to control for the change in cardiovascular variables. Furthermore, it seems that even under those conditions the results obtained may be influenced by baroreflex or neurohumoral mechanisms, or both, in different ways in different vascular beds. In addition, it is likely that the vasodilator responses are contributed to by autoregulatory mechanisms to different extents in different vascular beds, judging by the differential flow patterns seen (Gardiner *et al.*, 1989b; Bennett *et al.*, 1989). One intriguing possibility that arises from the present work is that, *in vivo*, substantial components of some of the active vasodilator responses to acetylcholine, bradykinin and endothelin-1 are nitric oxide-independent, and that this phenomenon is less apparent in studies *in vitro*.

We are grateful to Drs Salvador Moncada and Richard Palmer for useful discussion and suggestions during this study, and also for gifts of L-NMMA.

References

AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989). L-Arginine availability determines the duration of acetylcholine-induced systemic vasodilatation *in vitro*. *Biochem. Biophys. Res. Commun.*, **163**, 710–717.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1989). Regional haemodynamic effects of acetylcholine (ACh) or sodium nitroprusside (SNP) in conscious Wistar rats. *J. Physiol.*, **410**, 51P.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797–9800.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989a). Regional hemodynamic effects of endothelin-1 in conscious, unrestrained Wistar rats. *J. Cardiovasc. Pharmacol.*, **12**, suppl. 5, S202–S204.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989b). Regional haemodynamic effects of bradykinin or substance P in conscious, Long Evans rats. *Br. J. Pharmacol.*, **96**, 119P.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1989c). Haemodynamic effects of N^G-monomethyl-L-arginine (L-NMMA) in conscious Long Evans rats. *Br. J. Pharmacol.*, **98**, 623P.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1989d). N^G-monomethyl-L-arginine does not inhibit the hindquarters vasodilator action of endothelin-1 in conscious rats. *Eur. J. Pharmacol.*, **171**, 237–240.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990a). Regional haemodynamic effects of endothelin-1 and endothelin-3 in conscious Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **99**, 107–112.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990b). Effects of indomethacin on the regional haemodynamic responses to low doses of endothelins and sarafotoxin. *Br. J. Pharmacol.*, **100**, 158–162.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990c). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension*, **15**, 486–492.

GARDINER, A.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990d). Regional haemodynamic effects of inhibiting endothelial cell production of nitric oxide with N^G-monomethyl-L-arginine in conscious rats. In *Nitric Oxide from L-Arginine: A Bioregulatory System*. ed. Moncada, S. & Higgs, E.A. Amsterdam: Elsevier (in press).

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990e). Regional haemodynamic changes in Brattleboro rats during oral ingestion of L-nitroarginine methyl ester (L-NAME). *J. Physiol.*, (in press).

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990f). Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester in conscious Brattleboro rats. *Br. J. Pharmacol.*, (in press).

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990g). Cardiac output effects of endothelin-1, -2 and -3 and sarafotoxin S6b in conscious rats. *J. Auton. Nerv. Syst.*, **30**, 143–148.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990h). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.*, (in press).

GILES, H., BOLOFO, M.L. & MARTIN, G.R. (1990). Agonist- and tissue-dependence of susceptibility of endothelium-dependent relaxations to L-NAME. *Br. J. Pharmacol.* Proc. Suppl., (in press).

HAYWOOD, J.R., SHAFFER, R., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am. J. Physiol.*, **241**, H273–H278.

ISHII, K., CHANG, B., KERWIN, J.F., HUANG, Z.J. & MURAD, F. (1990). N-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur. J. Pharmacol.*, **179**, 219–223.

LONG, C.J. & BERKOWITZ, B.A. (1989). What is the relationship between endothelium derived relaxant factor and nitric oxide? *Life Sci.*, **45**, 1–14.

MONCADA, S., PALMER, R.M.J. & HIGGS, A.E. (1988). The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension*, **12**, 365–372.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem. Pharmacol.*, **38**, 1709–1715.

MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitroarginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.*, **99**, 408–412.

MÜLSCH, A. & BUSSE, R. (1990). N^G-nitro-L-arginine (N^5-[imino-(nitroamino)-methyl]-L-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine. *Naunyn Schmiedebergs Arch. Pharmacol.*, **341**, 143–147.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988a). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.

PALMER, R.M.J., REES, D.D., ASHTON, D.S. & MONCADA, S. (1988b). L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.*, **153**, 1251–1256.

RANDALL, M.D., DOUGLAS, S.A. & HILEY, C.R. (1989). Vascular activities of endothelin-1 and some alanyl substituted analogues in resistance beds of the rat. *Br. J. Pharmacol.*, **98**, 685–699.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989a). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.

REES, D.D., PALMER, R.M.J. & MONCADA, S. (1989b). The role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci., U.S.A.*, **86**, 3375–3378.

SCHOEMAKER, R. (1989). Experimental heart failure in rats. *Ph. D. Thesis, University of Limburg*, Netherlands.

SMITH, T.L. & HUTCHINS, P.M. (1979). Central hemodynamics in the developmental stage of spontaneous hypertension in the unanesthetized rat. *Hypertension*, **1**, 508–517.

SMITS, J.F.M., COLEMAN, T.G., SMITH, T.L., KASBERGEN, C.M., VAN ESSEN, H. & STRUYKER-BOUDIER, H.A.J. (1982). Antihypertensive effect of propranolol in conscious spontaneously hypertensive rats: central hemodynamics, plasma volume, and renal function during β-blockade with propranolol. *J. Cardiovasc. Pharmacol.*, **4**, 903–914.

TOMLINSON, K.C., GARDINER, S.M. & BENNETT, T. (1990). Blood pressure in streptozotocin-treated Brattleboro and Long Evans rats. *Am. J. Physiol.*, **258**, R852–R859.

WARNER, T.D., DE NUCCI, G. & VANE, J.R. (1989a). Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur. J. Pharmacol.*, **159**, 325–326.

WARNER, T.D., MITCHELL, J.A., DE NUCCI, G. & VANE, J.R. (1989b). Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. *J. Cardiovasc. Pharmacol.*, **13**, (suppl. 5), S85–S88.

WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989). Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.

(Received May 17, 1990
Revised June 27, 1990
Accepted July 10, 1990)