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Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats

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1 Regional haemodynamic responses to i.v. bolus doses (0.1–10.0 mg kg^{-1}) of N^G-nitro-L-arginine methyl ester (L-NAME) were measured in conscious, Long Evans rats ($n = 8$) chronically instrumented with renal, mesenteric and hindquarters pulsed Doppler flow probes and intravascular catheters.

2 L-NAME caused dose-dependent pressor effects associated with renal, mesenteric and hindquarters vasoconstrictions. The mesenteric vascular bed showed earlier onset with more rapid, and greater, maximum vasoconstrictions than the renal or hindquarters vascular beds; however, the hindquarters vasoconstriction was more persistent. D-NAME was without significant effects ($n = 2$).

3 Primed infusion of L-arginine (100 mg kg^{-1} bolus followed by 100 mg kg^{-1} h^{-1} infusion), starting 10 min after an i.v. bolus injection of L-NAME (10 mg kg^{-1}), caused significant reversal of the pressor responses, and renal and mesenteric vasoconstrictions, but not of the hindquarters vasoconstriction. Primed infusions of L-arginine (100 mg kg^{-1}, 100 mg kg^{-1} h^{-1}) starting 5 min after L-NAME (1 mg kg^{-1}) additionally caused some reversal of the hindquarters vasoconstriction, but this effect was transient.

4 Primed infusion of L-arginine (100 mg kg^{-1}, 100 mg kg^{-1} h^{-1}) starting 30 min before i.v. bolus injection of L-NAME (10 mg kg^{-1}) caused significant attenuation of the pressor effects and the renal and mesenteric vasoconstrictions but not of the hindquarters vasoconstriction.

5 In a separate group of rats ($n = 8$) chronically instrumented with thoracic aortic electromagnetic flow probes for the measurement of cardiac haemodynamics, i.v. bolus injection of L-NAME (10 mg kg^{-1}) produced significant reductions in total peripheral conductance, cardiac output, stroke volume, peak thoracic aortic flow and the maximum rate of rise of aortic flow; these were coincident with the maximum pressor and vasoconstrictor effects.

6 These results, collectively, are consistent with L-NAME interfering with L-arginine-nitric oxide pathways that have important influences on regional vascular conductances *in vivo*. The pressor effect resulting from L-NAME-induced vasoconstrictions is offset by a substantial reduction in cardiac function that may depend on direct and/or indirect effects of L-NAME on the heart.

Introduction

Nitric oxide is the major endothelium-derived relaxing factor (see Moncada *et al.*, 1989). Inhibition of nitric oxide biosynthesis *in vivo* by intravenous (i.v.) administration of N^G-monomethyl-L-arginine (L-NMMA) causes increases in blood pressure in anaesthetized rabbits (Rees *et al.*, 1989) and guinea-pigs (Aisaka *et al.*, 1989). In conscious rats, i.v. administration of L-NMMA causes dose-dependent hypertension and widespread vasoconstrictions (renal, superior mesenteric, hindquarters and internal carotid vascular beds), although the patterns of change in vascular conductances are different in the different regions (Gardiner *et al.*, 1989b; 1990d,e,g; Bennett *et al.*, 1990). The *in vivo* vasoconstrictor effects seen following administration of L-NMMA are not observed after injection of D-NMMA, and the effects of L-NMMA are partially reversed by L-arginine but not by D-arginine (Bennett *et al.*, 1990; Gardiner *et al.*, 1990e). These results are consistent with nitric oxide generated from L-arginine being involved in the control of regional vascular conductances under basal conditions in conscious animals.

It is now known that analogues of arginine other than L-NMMA can influence endothelium-dependent vasorelaxation *in vitro*. Indeed, Moore *et al.* (1990) have found that N^G-nitro-L-arginine inhibits more potently the vasodilator effects of acetylcholine in the perfused, pre-constricted superior mesenteric vascular bed from the rat than does L-NMMA. However, there are no published data on the *in vivo* cardiovascular effects of N^G-nitro-L-arginine, but obviously such information is important for our understanding of the involvement of the L-arginine-nitric oxide system in cardiovascular regulation. Since N^G-nitro-L-arginine is a relatively insoluble compound, we investigated the effects of the highly soluble N^G-nitro-L-arginine methyl ester (L-NAME; Moore *et al.*, 1990) in conscious rats instrumented for monitoring regional or cardiac haemodynamics. Some of the results have been presented to the Physiological Society (Gardiner *et al.*, 1990f).

Methods

All studies were carried out on male, Long Evans rats (350–450 g) bred in the Animal Unit at Nottingham.

Regional haemodynamic measurements

Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p., supplemented as required) and, through a mid-line incision, miniaturized pulsed Doppler probes (Haywood *et al.*, 1981) were 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). The probe wires ran from the abdominal cavity, through a small incision in the left flank, and then subcutaneously to emerge at the back of the neck where they were anchored to the skin by a suture. Following closure of the incisions all animals received an intramuscular injection of ampicillin (Penbritin, Beecham, 7 mg kg^{-1}) and were returned to their home cages for 7–14 days with free access to food and water. After this time they were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) and had intravenous (left jugular vein) and intra-arterial (distal abdominal aorta via ventral caudal artery) catheters implanted. The catheters were then run subcutaneously to emerge at the back of the neck at the same point as the probe wires. The latter were soldered

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into a 6-way micro-connector (Microtech Inc., Boothwyn, U.S.A.) that was clamped into a home-made harness worn by the rat. The catheters ran through a flexible spring connected to the harness, and a connector for the lead to the pulsed Doppler flowmeter ((Crystal Biotech, Massachussetts) modified to a pulse repetition frequency of 125 kHz (Gardiner et al., 1990c)) was taped to the spring. The latter was supported by a counterbalanced, universally-jointed lever, thereby giving the animal unrestrained movement in its home cage. Free access to water and food was allowed throughout the studies which began the following day when animals were fully conscious.

Baseline measurements were made for at least 30 min before any intervention was started. Continuous recordings (on a Gould ES 1000 recorder) of phasic arterial blood pressure, mean blood pressure (electronically derived from the phasic signal), instantaneous heart rate, phasic Doppler shift signals and mean Doppler shift signals (electronically derived from the former) were made before, during and after all interventions. At selected time points, percentage changes in mean Doppler shift signals were calculated to give an index of blood flow change relative to baseline (Haywood et al., 1981) and at those times percentage changes in regional vascular conductances were calculated from the mean Doppler shift signals and mean blood pressure (i.e. mean Doppler shift/mean blood pressure).

Cardiac output measurements

Animals were anaesthetized as above and an electromagnetic flow probe (Skalar, Delft, Netherlands) was implanted around the ascending thoracic aorta by the technique of Smith & Hutchins (1979) as modified by Smits et al. (1982). The probe lead was run subcutaneously and the plug was securely positioned at the back of the neck. Animals were given an intramuscular injection of ampicillin (Penbritin, 7 mg kg^{-1}) and returned to their home cages with free access to food and water for at least 7 days. After this time animals were briefly re-anaesthetized (as above) and had intravenous (left jugular vein) and intra-arterial (distal abdominal aorta via ventral caudal artery) catheters implanted. One intravenous catheter (i.d. 0.28 mm) was advanced until the tip lay close to the junction of the superior vena cava with the right atrium (as judged by the pressure waveform). This catheter was used for monitoring central venous pressure and, in order to ensure the quality of the recording was not impaired by the formation of blood clots around its tip, a continuous infusion (0.3 ml h^{-1}) of sterile saline (154 mM NaCl) was given through it via a fluid-filled swivel until the experiment started the next day.

Continuous recordings (on a Gould ES 1000) of phasic arterial blood pressure, mean central venous pressure, phasic and mean thoracic aortic blood flow (Skalar MDL 1401 flowmeter) and instantaneous heart rate were made. These signals were also fed into a microprocessor (designed and built in the Departments of Pharmacology and Instrument Services, University of Limburg, Netherlands) interfaced with a microcomputer (Tandon 386). This system digitized and averaged data over 2 s epochs and also derived values for mean blood pressure, stroke volume, total peripheral conductance, maximum rate of rise of thoracic aortic flow ($+dF/dt_{max}$) and peak aortic flow; the latter two variables are indices of ventricular contractility (Schoemaker, 1989). All data were stored on disc for subsequent review and analysis. The values given in the text represent means of up to 20 observations (i.e. over a 40 s measurement period). As with the regional haemodynamic studies baseline recordings were made for at least 30 min to ensure animals were in a steady state before any interventions were carried out.

Experimental protocols

Experiment 1: Regional and cardiac haemodynamic effects of L-*NAME* Rats ($n = 8$) instrumented for recording renal, mesenteric and hindquarters blood flows were given i.v. bolus (0.1 ml) injections of L-NAME (0.1, 1.0 and 10 mg kg^{-1}). The doses were given in ascending order with at least 60 min between the first and second dose and at least 90 min between the second and third. An additional two animals were given D-NAME (10 mg kg^{-1}).

A separate group of rats ($n = 8$) instrumented for measurement of cardiac haemodynamics was given L-NAME at a dose of 10 mg kg^{-1}.

Experiment 2: Effects of post-treatment with L-*arginine on the regional haemodynamic responses to* L-*NAME* One group of animals ($n = 8$) instrumented for recording renal, mesenteric and hindquarters blood flows was given a bolus injection of L-NAME (10 mg kg^{-1}) and, starting 10 min later, a primed infusion of L-arginine hydrochloride (100 mg kg^{-1} bolus followed by 100 mg kg^{-1} h^{-1} infusion).

A separate group of animals ($n = 8$) was given a bolus injection of L-NAME (1 mg kg^{-1}) followed 5 min later by a primed infusion of L-arginine hydrochloride (100 mg kg^{-1}, 100 mg kg^{-1} h^{-1}).

Experiment 3: Effects of pretreatment with L-*arginine on the regional haemodynamic responses to* L-*NAME* A further group of animals ($n = 9$) instrumented for recording renal, mesenteric and hindquarters blood flows was given L-arginine hydrochloride by primed infusion (100 mg kg^{-1} bolus, 100 mg kg h^{-1} infusion) started 30 min before administration of L-NAME (10 mg kg^{-1}).

Drugs

All substances used were dissolved in sterile saline and injected in volumes of 0.1 ml, flushed in with 0.1 ml (this volume being the dead space of the i.v. catheter); infusions were given at a rate of 0.3 ml h^{-1}. Administration of saline alone in these volumes had no consistent, sustained cardiovascular effects. L-NAME hydrochloride and L-arginine hydrochloride were obtained from Sigma Chemical Co. and were dissolved in sterile saline (154 mM NaCl). D-NAME acetate was synthesized at Wellcome Research Laboratories (Beckenham, Kent).

Data analysis

Within-group analysis was done by Friedman's test (Theordorsson-Norheim, 1987) and between-group comparisons were made with the Mann-Whitney U test applied to areas under or over curves. These areas were quantified for each individual animal on the basis of the change relative to baseline and the time interval (using a Fortran programme written in our Department). A P value of <0.05 was taken as significant.

Results

Regional haemodynamic effects of L-*NAME*

Bolus injection of L-NAME at a dose of 0.1 mg kg^{-1} had no significant effect on mean blood pressure, but there was a slight bradycardia (Figure 1). There were no significant changes in renal or hindquarters haemodynamics (Figures 1 and 2), although mesenteric flow (Figure 1) and vascular conductance (Figure 2) showed small decreases.

Over the range 1.0 to 10 mg kg^{-1}, L-NAME caused pressor effects, the magnitudes and durations of which were dose-related (Figures 1 and 3), although following L-NAME at 10 mg kg^{-1}, mean blood pressure was still significantly elevated ($+16 \pm 2$ mmHg) after 2 h. The bradycardias after L-NAME were less clearly dose-dependent than the pressor effects (Figure 1), and it is notable that 2 h after L-NAME at a dose of 10 mg kg^{-1} heart rate was not significantly different from baseline (-6 ± 11 beats min^{-1}) in spite of the elevation in mean blood pressure (see above).

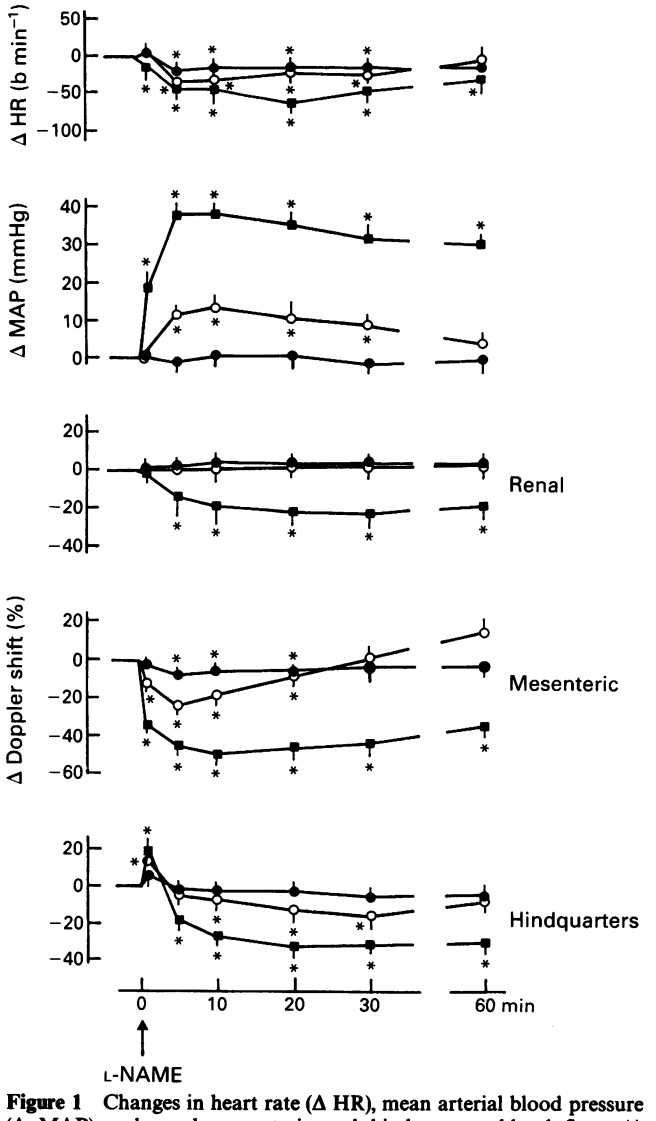

Figure 1 Changes in heart rate (Δ HR), mean arterial blood pressure (Δ MAP) and renal, mesenteric and hindquarters blood flows (Δ Doppler shift) following i.v. bolus doses of N^G-nitro-L-arginine methyl ester (L-NAME) at 0.1 mg kg^{-1} (●), 1.0 mg kg^{-1} (○) and 10.0 mg kg^{-1} (■) in conscious, Long Evans rats ($n = 8$). Values are mean with s.e.mean shown by vertical lines; * $P < 0.05$ versus baseline (Friedman's test).

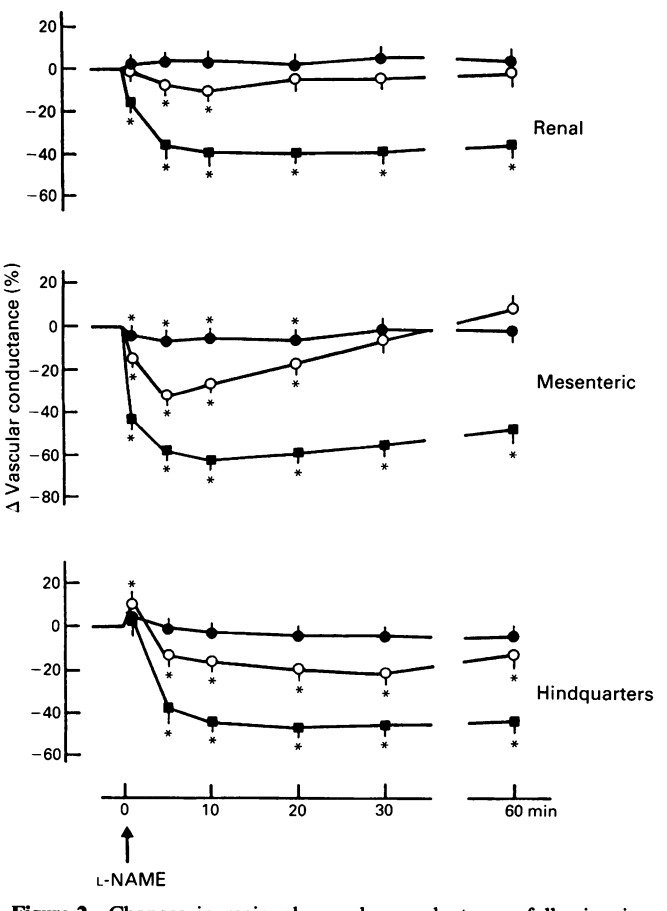

Figure 2 Changes in regional vascular conductances following i.v. bolus doses of N^G-nitro-L-arginine methyl ester (L-NAME) at 0.1 mg kg^{-1} (●), 1.0 mg kg^{-1} (○) and 10.0 mg kg^{-1} (■). Data derived from those in Figure 1. Values are mean with s.e.mean shown by vertical lines; * $P < 0.05$ versus baseline (Friedman's test).

While mesenteric blood flow (Figures 1 and 3) and vascular conductance (Figure 2) showed dose-related reductions, renal blood flow was reduced only after the 10 mg kg^{-1} dose of L-NAME (Figures 1 and 3). However, renal vasoconstriction occurred following L-NAME at 1.0 and 10 mg kg^{-1} (Figure 2). Hindquarters blood flow showed initial increases following L-NAME (1.0 and 10 mg kg^{-1}) but thereafter flow fell (Figures 1 and 3). There was an initial rise in hindquarters vascular conductance following L-NAME at 1.0 mg kg^{-1} only (Figure 2); the subsequent hindquarters vasoconstrictions were dose-dependent (Figure 2).

Following L-NAME at a dose of 10 mg kg^{-1} the mesenteric vasoconstriction occurred earlier and was more rapid in onset. Also, the nadir of mesenteric vascular conductance was lower than those seen in the renal and hindquarters vascular beds (Figures 1–3). Two h after this dose of L-NAME, renal and mesenteric blood flows were not significantly different from baseline (-5 ± 5 and -7 ± 7%, respectively), but hindquarters blood flow was reduced (-28 ± 5%). Since mean blood pressure was still elevated (see above), there were vasoconstrictions in all 3 vascular beds (renal, -16 ± 5%; mesenteric -18 ± 7%; hindquarters -36 ± 4% change in vascular conductance), although that in the hindquarters was greatest.

D-NAME at a dose of 10 mg kg^{-1} was without pressor or vasoconstrictor effects (Figure 3). There was a transient tachycardia (Figure 3) following D-NAME, but a similar effect was also seen following L-NAME (Figure 3) or saline injection, and was thus not due to the drugs administered.

Cardiac haemodynamic effects of L-*NAME*

On the basis of the changes described above, the effects of L-NAME on cardiac haemodynamics were assessed 10 min after a bolus dose of 10 mg kg^{-1}. Table 1 shows that L-NAME caused substantial bradycardia and hypertension, accompanied by reductions in cardiac output, stroke volume, $+dF/dt_{max}$, peak thoracic aortic flow and total peripheral conductance. However, central venous pressure did not change significantly. It is notable that although the increases in mean blood pressure following L-NAME (10 mg kg^{-1}) were very similar in animals in which regional haemodynamics were studied (Figure 1) and those in which cardiac haemodynamics were measured (Table 1), the bradycardia in the latter was significantly greater than that in the former. However, the resting heart rates in the 2 groups were significantly different (cardiac haemodynamics group = 378 ± 7 beats min^{-1}; regional haemodynamics group = 323 ± 10 beats min^{-1}) and hence the nadirs in heart rate were similar (272 ± 7 and 278 ± 14 beats min^{-1}, respectively).

Effects of post-treatment with L-*arginine on the regional haemodynamic effects of* L-*NAME*

The regional haemodynamic and pressor effects of L-NAME (10 mg kg^{-1}) were not significantly different in the two

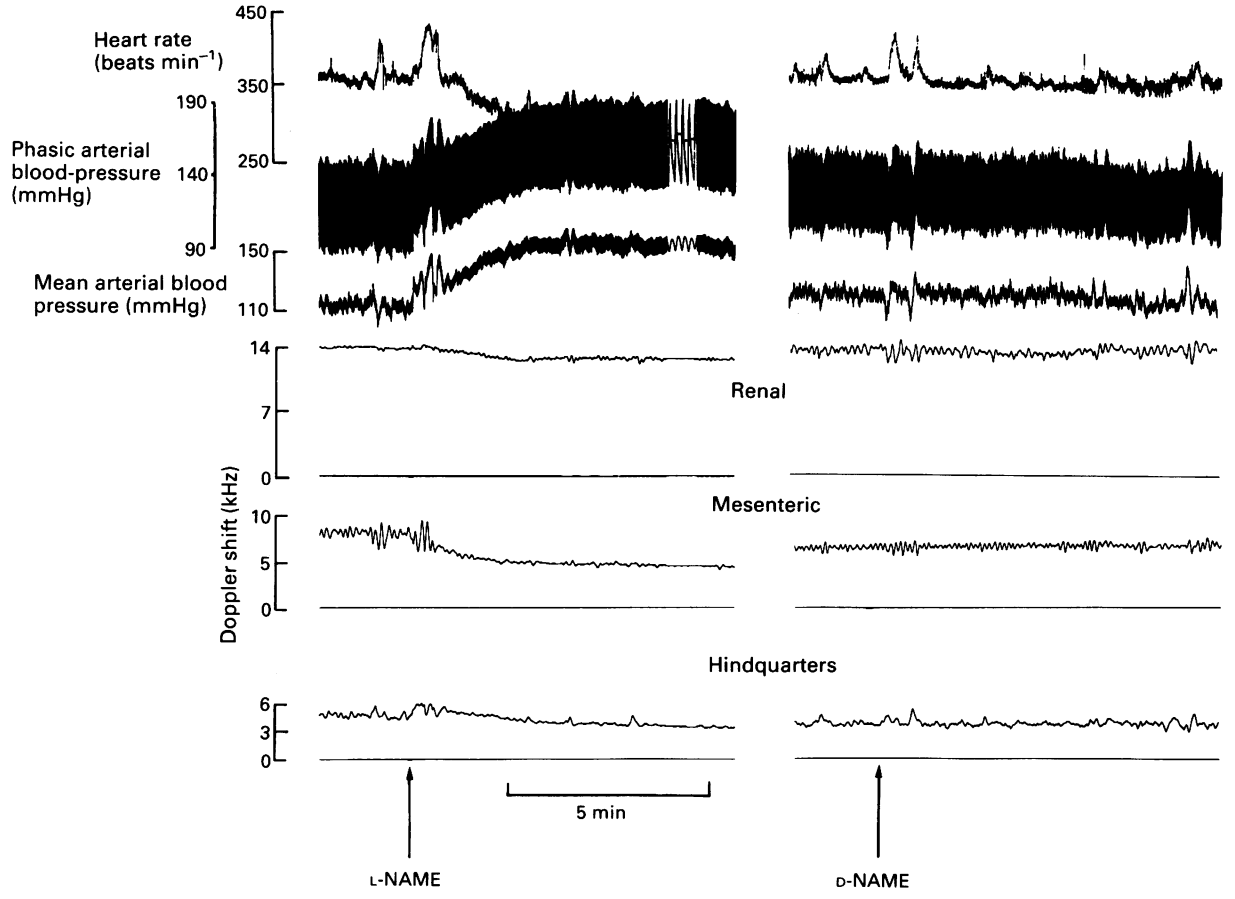

Figure 3 Original recordings of cardiovascular responses to an i.v. bolus injection (10 mg kg^{-1}) of N^{G}-nitro-L-arginine methyl ester (L-NAME) or N^{G}-nitro-D-arginine methyl ester (D-NAME) in 2 different, conscious, Long Evans rats.

separate groups of animals receiving this treatment (Figures 4 and 5). Infusion of L-arginine 10 min after bolus injection of L-NAME caused significant attenuation of the pressor effect ($P < 0.041$) and of the reductions in renal ($P = 0.014$) and mesenteric ($P < 0.019$), but not hindquarters, blood flows following L-NAME (Figure 4). Thus L-arginine caused significant reversals of the renal ($P < 0.019$) and mesenteric ($P < 0.014$), but not of the hindquarters, vasoconstrictions following L-NAME (Figure 5).

In animals receiving L-arginine (100 mg kg^{-1} bolus and 100 mg kg^{-1} h^{-1} infusion) starting 5 min after L-NAME at a dose of 1 mg kg^{-1}, renal vasoconstriction was reversed and mesenteric vasoconstriction was substantially reduced. In addition, there was a significant, albeit transient, attenuation of the hindquarters vasoconstriction (Figure 6).

Effects of pretreatment with L-*arginine on the haemodynamic effects of* L-*NAME*

Infusion of L-arginine had no consistent, sustained effect on any variable (Figures 4, 5 and 7). However, in the presence of L-arginine the pressor effect of L-NAME was significantly ($P = 0.019$) attenuated, as were the reductions in mesenteric ($P = 0.019$) and hindquarters ($P < 0.019$) blood flow (Figure 4). Although the changes in renal blood flow following L-NAME were not significantly different in the absence and presence of L-arginine, there was no significant reduction in renal blood flow following L-NAME administration in the presence of L-arginine (Figure 4). L-Arginine pretreatment caused significant attenuation of the renal ($P = 0.041$) and mesenteric ($P = 0.032$), but not of the hindquarters, vasoconstrictor responses to L-NAME (Figure 5).

Discussion

The present findings, which indicate that L-NAME has potent pressor effects associated with regional vasoconstrictions *in vivo* in conscious rats, are consistent with our previous observations on the effects of L-NMMA (Gardiner *et al.*, 1989b; 1990d,e,g) at higher doses. The findings that D-NAME was without such effects, and that some of the actions of L-NAME could either be attenuated by pretreatment with L-arginine, or completely or partially reversed by post-treatment with L-

Table 1 Cardiac haemodynamic variables before and 10 min after an i.v. bolus injection of N^{G}-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}) in conscious, Long Evans rats

	Before L-*NAME*	*After* L-*NAME*
Heart rate (beats min^{-1})	378 ± 7	272 ± 7*
Mean blood pressure (mmHg)	99 ± 2	142 ± 3*
Cardiac output (ml min^{-1})	100 ± 3	61 ± 1*
Stroke volume (μl/beat)	264 ± 12	226 ± 6*
$+dF/dt$ max (1 min^{-1} min^{-1})	1732 ± 60	1000 ± 30*
Peak thoracic flow (ml min^{-1})	408 ± 13	281 ± 8*
Total peripheral conductance (μl min^{-1} mmHg^{-1})	1014 ± 25	431 ± 13*
Central venous pressure (cmH_2O)	4.54 ± 0.73	4.33 ± 0.68

Values are mean ± s.e.mean; $n = 8$; *$P < 0.05$ (Wilcoxon's test).

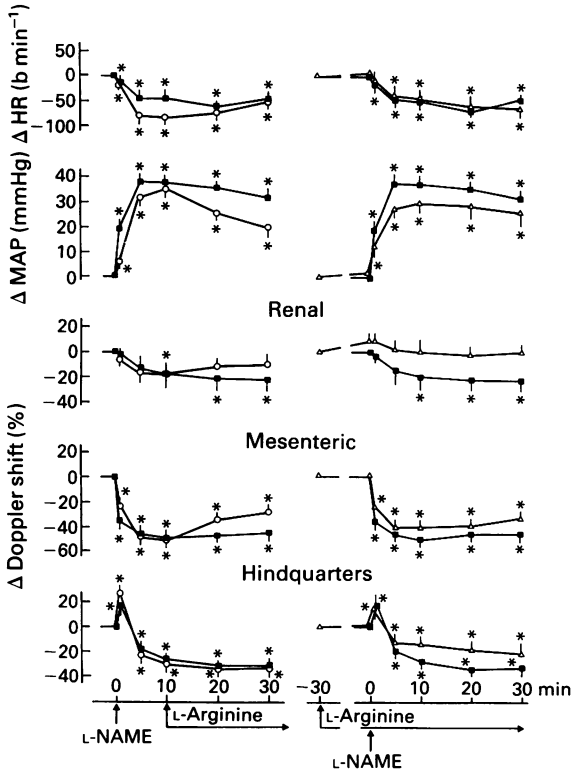

Figure 4 Left-hand panels show changes in heart rate (Δ HR), mean arterial blood pressure (Δ MAP) and renal, mesenteric and hindquarters blood flows (Δ Doppler shift) following an i.v. bolus dose of N^G-nitro-L-arginine methyl ester (L-NAME) at 10.0 mg kg^{-1} (■) in a group of conscious, Long Evans rats (n = 8). A separate group (n = 8; ○) of animals also received an i.v. bolus injection of L-NAME (10.0 mg kg^{-1}) but followed 10 min later by a primed infusion (100 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} infusion) of L-arginine. Right-hand panels show the data in the group receiving L-NAME alone (■) compared to a separate group (n = 8; Δ) receiving a primed infusion of L-arginine starting 30 min before administration of L-NAME. Values are mean with s.e.mean shown by vertical lines; * $P < 0.05$ versus baseline (Friedman's test). Statistics for the differences between groups (as judged from areas under or over curves) are given in the results.

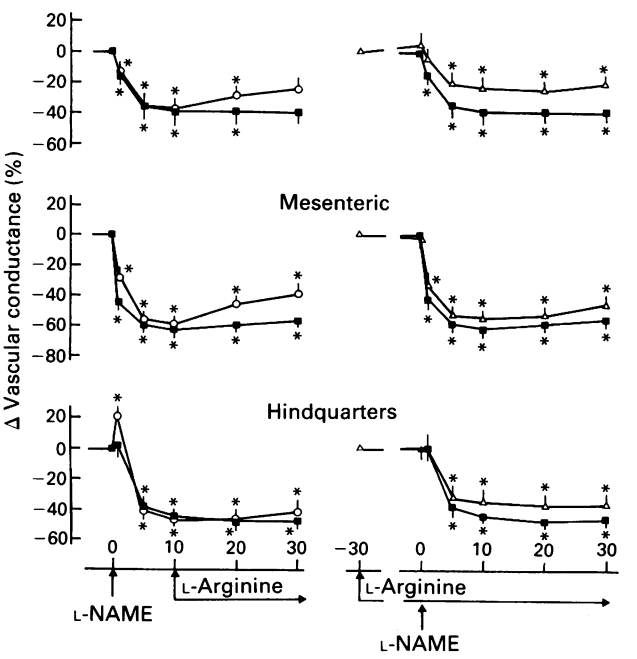

Figure 5 Changes in regional vascular conductances derived from the data in Figure 4. Animals receiving N^G-nitro-L-arginine methyl ester (L-NAME) alone (■); animals receiving L-NAME followed by L-arginine (○); animals receiving L-arginine followed by L-NAME (Δ). Values are mean with s.e.mean shown by vertical lines; * $P < 0.05$ versus baseline. Statistics for the differences between groups (as judged from areas under or over curves) are given in the results.

arginine, are further evidence for a major role of the L-arginine-nitric oxide pathway in the control of regional vascular conductances *in vivo* (Gardiner *et al.*, 1990e).

While a substantial contribution of endothelial cell nitric oxide production to this regulatory system is likely (see Moncada *et al.*, 1989), an additional influence of the L-arginine-nitric oxide pathway at the level of the efferent control of the circulation, for example, should not be dismissed. Thus, the differential profiles of effect of L-NAME in the renal, mesenteric and hindquarters vascular beds could be due to different degrees of involvement of neural mechanisms in the control of conductances in the different vascular beds. However, it is also possible, and perhaps more likely, that these differences represent different dynamics of the interactions between L-NAME and the L-arginine-nitric oxide pathway in the different vascular beds. In this regard it is noteworthy that L-NMMA also has differential effects on regional vascular conductances (Gardiner *et al.*, 1989b; 1990d,e,g) and, furthermore, the profile of haemodynamic effects of L-NMMA is similar to that seen here following administration of L-NAME. In addition, a relative resistance of the hindquarters vasoconstrictor response to reversal by L-arginine is also seen following L-NMMA (Gardiner *et al.*, 1990e; Bennett *et al.*, 1990). However, in confirmation of the *in vitro* data (Moore *et al.*, 1990), L-NAME appears to be about 10 fold more potent than L-NMMA (Figure 8), and its effect more long-lasting and more resistant to reversal by L-arginine. As yet no information is available regarding the details of the interactions between L-NAME or L-NMMA and the L-arginine-nitric oxide pathway in different vascular beds at the level of the biochemistry of the synthetic machinery.

A recent publication from Boulanger & Lüscher (1990) provides evidence that endothelial cell nitric oxide might act to inhibit endothelin release. Hence the vasoconstriction resulting from inhibition of nitric oxide synthesis could be due to loss of nitric oxide-mediated vasodilator tone together with vasoconstriction due to increased endothelin release. Elsewhere we have pointed out that the vascular beds responding most markedly to L-NMMA are those that show pronounced vasoconstriction to exogenous endothelins (Gardiner *et al.*, 1990g). In addition, it is of interest that the hindquarters vascular bed also shows an initial vasodilator response to bolus injections of endothelins (Gardiner *et al.*, 1989a; 1990a,b) sarafotoxin S6b (Gardiner *et al.*, 1990b), L-NMMA (Gardiner *et al.*, 1989b; 1990d,e,g) and L-NAME (this study).

In spite of the apparent differences in potency of L-NAME and L-NMMA, acute i.v. administration of supramaximal doses of either compound fails to increase mean blood pressure much beyond the levels reported here (S.M. Gardiner, A.M. Compton & T. Bennett unpublished results). This does not seem to be due to desensitization to repeated doses since in the present study the pressor response to L-NAME at 10 mg kg^{-1} was very similar in animals exposed to L-NAME on a single occasion and in those receiving incremental doses of the compound. Thus, the maximum pressor effect of L-NAME may represent the result of the loss of vasodilator tone (i.e. unopposed vasoconstriction) and of factors that act to buffer the resulting rise in blood pressure. As mentioned in the results, although the pressor responses to L-NAME were very consistent the associated bradycardias varied in direct relation to the resting heart rates. Furthermore, 2 h after 10 mg kg^{-1} L-NAME there was no bradycardia, although mean blood pressure was still elevated. Hence, it is unlikely that reflex slowing of the heart in itself is a major factor acting to oppose pressor changes following L-NAME. However, there were substantial reductions in cardiac output that would certainly have buffered the hypertensive effects of the

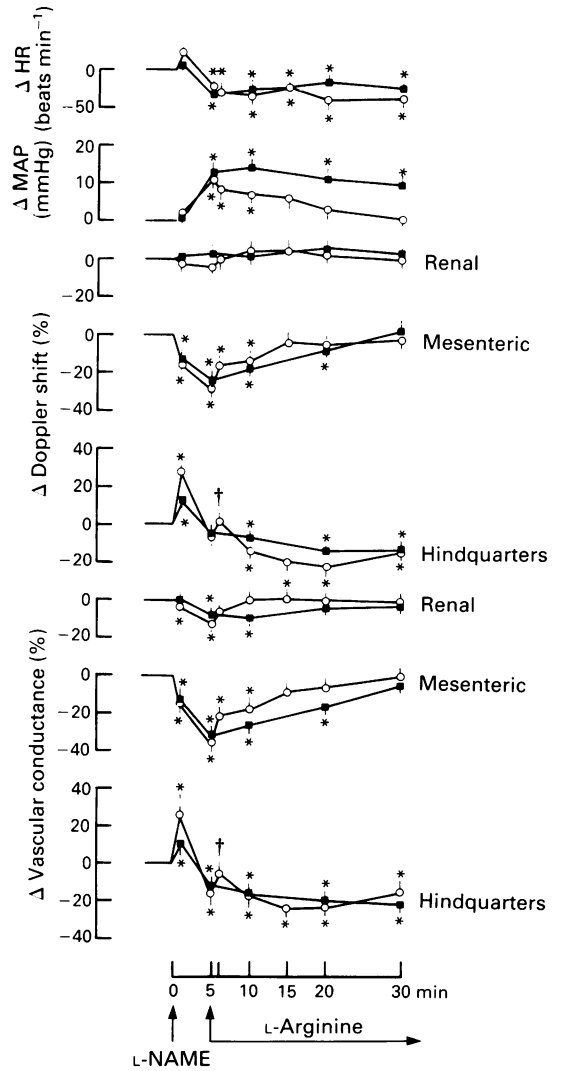

Figure 6 Changes in heart rate (ΔHR), mean arterial blood pressure (ΔMAP) and renal, mesenteric and hindquarters blood flows (Δ Doppler shift) and vascular conductances following an i.v. bolus dose of N^G-nitro-L-arginine methyl ester (L-NAME) at 1.0 mg kg^{-1} (■) in a group of conscious, Long Evans rats ($n = 8$). A separate group ($n = 8$; ○) of animals also received an i.v. bolus injection of L-NAME (1.0 mg kg^{-1}) followed by a primed infusion (100 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} infusion) of L-arginine. Values are mean with s.e.mean shown by vertical lines; $*P < 0.05$ versus baseline (Friedman's test), $\dagger P < 0.05$ versus pre-L-arginine value (Friedman's test). Following L-arginine administration there were significant attenuations of the pressor and renal and mesenteric vasoconstrictor effects, but only a transient reduction of the hindquarters vasoconstrictor effect of L-NAME.

L-NAME-induced vasoconstriction. While it is feasible that the reduction in cardiac output was a direct consequence of the increase in afterload (i.e. decreased total peripheral conductance), the marked reductions in stroke volume, $+dF/dt_{max}$ and peak thoracic aortic flow are all consistent with negative inotropic changes following administration of L-NAME. It is possible such effects resulted from L-NAME causing coronary vasoconstriction (Amezcua *et al.*, 1988) or from a direct myocardial action or both.

Central venous pressure did not show any consistent changes following L-NAME. However, because of the substantial increase in afterload, central venous pressure may not give a reliable index of venous tone and, hence, the present results cannot be taken to indicate different degrees of involvement of the L-arginine-nitric oxide pathway in control of the arterial and venous sides of the circulation *in vivo*.

In conclusion, the present results are consistent with L-arginine-nitric oxide pathways acting tonically *in vivo* to

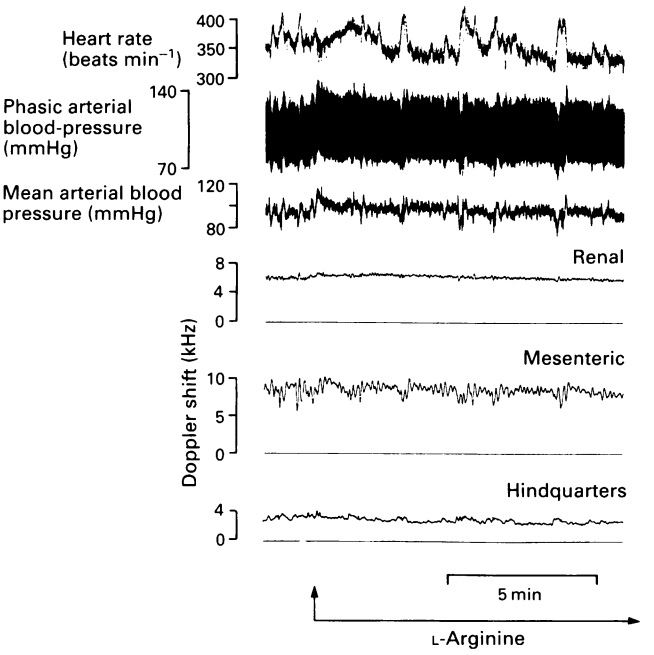

Figure 7 Original recording from a conscious, Long Evans rat. At the arrow a primed infusion (100 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} infusion) of L-arginine was begun, but with little effect on haemodynamic status.

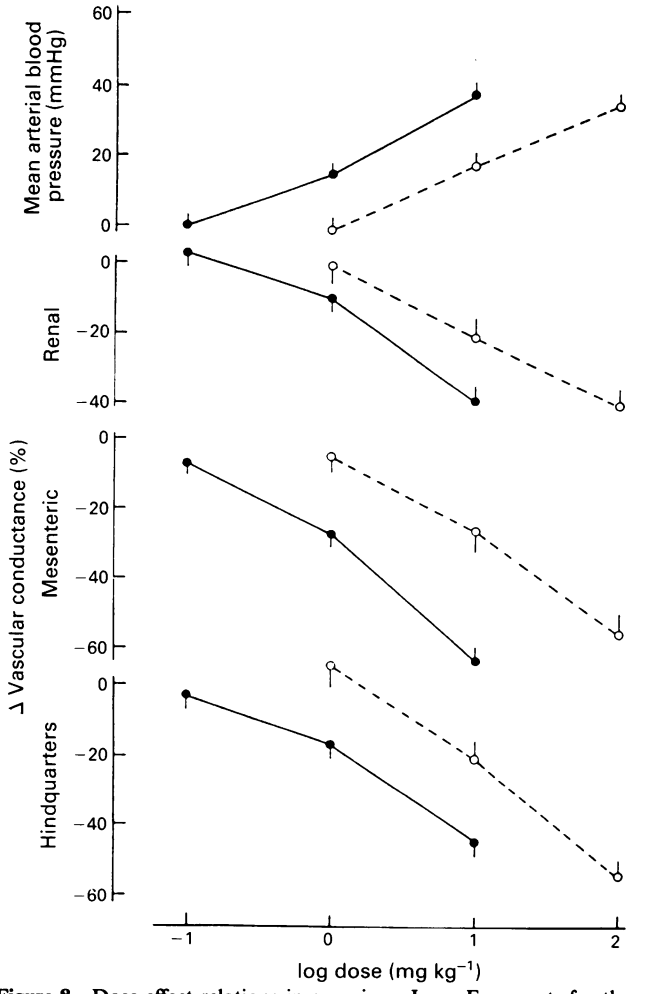

Figure 8 Dose-effect relations in conscious, Long Evans rats for the haemodynamic actions of N^G-nitro-L-arginine methyl ester (L-NAME, ●) and N^G-monomethyl-L-arginine (○). The data for the latter compound were obtained from the study of Gardiner *et al.* (1990e), whereas those for L-NAME are from the present study; measurements were made 10 min after i.v. bolus injections. Values are mean and vertical lines show s.e.mean ($n = 8$ in both groups).

oppose vasoconstrictor influences and thereby maintain regional vascular conductances appropriate for the required perfusion of the peripheral tissues. Treatment with L-NAME results in regional vasoconstrictions of variable magnitudes but which, collectively, produce a hypertension that is opposed by marked reductions in cardiac function.

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