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# Regional and cardiac haemodynamic effects of $N^G$, $N^G$,dimethyl-L-arginine and their reversibility by vasodilators in conscious rats

[1]S.M. Gardiner, P.A. Kemp, T. Bennett, *R.M.J. Palmer & *S. Moncada

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

**1** A series of experiments was carried out on 3 separate groups of male Long Evans rats, chronically instrumented for the measurement of regional haemodynamics, to compare the effects of $N^G$,$N^G$, dimethyl-L-arginine (ADMA) and $N^G$-monomethyl-L-arginine (L-NMMA), and their reversibility by the nitric oxide donors, S-nitroso-*N*-acetyl-penicillamine (SNAP), S-nitroso-glutathione (SNOG), sodium nitroprusside (SNP), and the vasodilator, hydralazine.

**2** As previously reported for L-NMMA, ADMA (1–100 mg $kg^{-1}$) caused dose-dependent pressor and bradycardic effects, accompanied by renal, mesenteric and hindquarters vasoconstrictions. The magnitude and duration of these effects were similar for ADMA and L-NMMA, consistent with their being equipotent inhibitors of nitric oxide synthase.

**3** Infusion of SNAP or SNOG (300 $\mu g\ kg^{-1}\ h^{-1}$) after injection of ADMA or L-NMMA (100 mg $kg^{-1}$) reversed the pressor but did not abolish the vasoconstrictor, effects of ADMA or L-NMMA. However, a higher dose of SNAP (3 mg $kg^{-1}\ h^{-1}$) caused complete reversal of the pressor and mesenteric haemodynamic effects of ADMA (100 mg $kg^{-1}$), although its renal and hindquarters vasoconstrictor effects were not abolished.

**4** Infusion of SNP (300 $\mu g\ kg^{-1}\ h^{-1}$) after administration of L-NMMA (100 mg $kg^{-1}$), caused complete reversal of its pressor and mesenteric and hindquarters haemodynamic effects, and reduced substantially its renal vasoconstrictor action; hydralazine (7.5 mg $kg^{-1}\ h^{-1}$) was almost as effective as SNP in reversing all these variables.

**5** In animals chronically instrumented for the measurement of cardiac haemodynamics, ADMA (100 mg $kg^{-1}$) caused a pressor effect accompanied by a rise in central venous pressure, and reductions in heart rate, cardiac index, stroke index, peak aortic flow, maximum rate of rise of aortic flow and total peripheral conductance. The reversal of the pressor effect of ADMA by SNAP (300 $\mu g\ kg^{-1}\ h^{-1}$) was accompanied by a reduction of central venous pressure below resting levels and a further diminution of stroke index; all other variables showed an increase, but they still remained below resting levels (with the exception of heart rate).

**6** Thus, following inhibition of NO synthesis, pharmacological intervention with NO donors, or other vasodilators, may cause normalisation of the mean arterial pressure without necessarily returning all associated cardiovascular variables to normal.

**Keywords:** $N^G$,$N^G$-dimethyl-L-arginine; S-nitroso-*N*-acetyl-penicillamine; S-nitroso-glutathione; sodium nitroprusside; hydralazine; haemodynamics

## Introduction

It was reported recently, that $N^G$,$N^G$-dimethyl-L-arginine (asymmetrical dimethyl-L-arginine, ADMA), an inhibitor of nitric oxide (NO) synthase, is a constituent of normal human plasma, being present in a concentration about 10 fold greater than $N^G$-monomethyl-L-arginine (L-NMMA) (Vallance *et al.*, 1992). L-NMMA is also an inhibitor of NO synthase (see Moncada *et al.*, 1991), which, on acute i.v. administration to conscious rats, causes a hypertensive response accompanied by widespread regional vasoconstriction (Gardiner *et al.*, 1990b). Vallance *et al.* (1992) reported that i.v. injection of ADMA in anaesthetized guinea-pigs induced dose-dependent pressor effects. However, it is not known whether the profiles of effect of ADMA and L-NMMA, on regional haemodynamics, are similar. Therefore, our first objective was to compare the regional haemodynamic effects of ADMA and L-NMMA in the same conscious rats.

At present, it is not known whether NO donors, or other vasodilators, are able to reverse the hypertensive and haemodynamic effects which result from systemic inhibition of NO in conscious rats. Therefore, our second objective was to assess the influence of the NO donors, S-nitroso-*N*-acetyl penicillamine (SNAP) (Ignarro *et al.*, 1981; Boughton-Smith *et al.*, 1990; Lopez-Belmonte *et al.*, 1993), S-nitroso-glutathione (SNOG) (Kowaluk & Fung, 1990; Radomski *et al.*, 1992), and sodium nitroprusside (SNP) on the pressor and regional haemodynamic effects of ADMA and/or L-NMMA and to determine if hydralazine, a vasodilator which is not an NO donor, was also able to influence the effects of NO synthase inhibition in a similar fashion.

Finally, since we found that the vasodilators studied could reverse the pressor effects of the NO synthase inhibitors without necessarily abolishing their vasoconstrictor effects, we measured the cardiac haemodynamic responses to ADMA, and the influence of SNAP upon them, to determine the extent to which changes in cardiac function contributed to the ability of SNAP to reverse the hypertensive effects of ADMA. Some of these results have been presented to the British Pharmacological Society (Bennett *et al.*, 1993; Gardiner *et al.*, 1993).

[1] Author for correspondence.

## Methods

All experiments were carried out on male, Long Evans rats (350–450 g), bred in the Animal Unit at Nottingham. All surgery was carried out under sodium methohexitone anaesthesia (40–60 mg $kg^{-1}$, i.p., supplemented as required).

### *Measurement of regional haemodynamics*

Miniaturised pulsed Doppler probes (Haywood *et al.*, 1981) were sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor flow to the hindquarters). Probe wires were led subcutaneously to emerge at the back of the neck where their ends were fixed to the skin by a suture. Animals were given ampicillin (Penbritin, Beecham; 7 mg $kg^{-1}$, i.m.) and returned to their home cages with free access to food and water. One to 2 weeks later, animals were anaesthetized again (sodium methohexitone, 40 mg $kg^{-1}$, i.p.) and the signals from the flow probes were checked. Animals with acceptable signals from all 3 probes had intravascular catheters implanted (1 in the distal abdominal aorta via the ventral caudal artery and 3 in the right jugular vein). The catheters were led subcutaneously to emerge at the same point as the probe wires. The latter were soldered into a 6-way microconnector (Microtech Inc., Boothwyn, U.S.A.) that was clamped into a harness, and the catheters ran through the spring for protection. An extension of the lead to the Doppler flowmeter (a modified (Gardiner *et al.*, 1990a) Crystal Biotech VF-1 mainframe) was taped to the spring and the whole assembly was supported by a counterbalanced lever system to allow the animal free movement. Rats were housed in their home cages with free access to food and water throughout the experiment protocols, which began at least 1 day after catheter implantation.

Throughout the experiments, continuous recordings were made of phasic and mean arterial blood pressure, instantaneous heart rate and phasic and mean Doppler shift signals from the renal, mesenteric and hindquarters probes. Percentage changes in the mean Doppler shift signals were taken as an index of flow changes (Haywood *et al.*, 1981) and percentage changes in vascular conductance were calculated from the mean Doppler shift and mean arterial blood pressure signals (Gardiner *et al.*, 1990d).

### *Measurement of cardiac haemodynamics*

An electromagnetic flow probe (Skalar, Delft) was implanted around the ascending thoracic aorta via a transthoracic approach (Smith & Hutchins 1979; Smits *et al.*, 1982; Gardiner *et al.*, 1990a,c,d). Animals were given ampicillin (as above) and left to recover for at least 7 days. At that time, they were anaesthetized (sodium methohexitone 40 mg $kg^{-1}$, i.p.) and had intra-arterial and intravenous catheters implanted; one of the latter was designed and positioned so as to permit recording of central venous pressure (Gardiner *et al.*, 1990c,d). At least 1 day after catheter implantation, when animals were fully conscious, the output from the thoracic aortic probe was connected to a Skalar MDL 1401 flowmeter. The output from this device, together with pressure signals were digitised on-line using a custom-built microprocessor (University of Limburg, Department of Instrument Services; Schoemaker, 1989; Gardiner *et al.*, 1990c,d). The microprocessor provided values for mean systemic arterial blood pressure and central venous pressure; from the aortic flow signal it determined the integrated aortic flow, peak aortic flow and maximum rate of rise of aortic flow ($dF/dt_{max}$). The integrated aortic flow (which represents cardiac output minus coronary flow) was divided by heart rate to give an estimate of stroke volume, and the microprocessor also calculated total peripheral conductance by dividing integrated aortic flow by mean systemic arterial blood pressure. The microprocessor was set to perform these procedures for two consecutive cardiac cycles every 2 s, and then to average the data before storing them on disk. Off-line, data were retrieved and averaged (using the microprocessor system) over 40–50 s across the times of interest. These data for each animal were then entered into a spread-sheet that calculated cardiac index (i.e. integrated aortic flow 100 $g^{-1}$ body weight), stroke index (stroke volume 100 $g^{-1}$ body weight) and also factored peak aortic flow, $dF/dt_{max}$ and total peripheral conductance by body weight before calculating means and s.e.mean for all variables, including mean systemic arterial blood pressure. While $dF/dt_{max}$ is a useful index of contractility (de Wildt & Sangster, 1983) it is not independent of preload or afterload.

Experiments were performed, in random order, over several days. The treatment groups were as follows:-

*Group 1 Haemodynamic interactions between ADMA or L-NMMA and SNAP or SNOG* In 8 conscious Long Evans rats incremental bolus doses (1–100 mg $kg^{-1}$, i.v.) of ADMA or L-NMMA were given at intervals of at least 60 min to allow all variables to return to baseline before administration of the next dose. Five min after administration of the highest dose of ADMA or L-NMAA, an isotonic saline (154 nmol $l^{-1}$ NaCl) infusion was begun and continued for 10 min as a control.

The same group of animals, on separate occasions were given continuous infusions of SNAP or SNOG (300 μg $kg^{-1}$ $h^{-1}$) for 10 min.

Again, using the same group of animals on other occasions, bolus injections of ADMA or L-NMMA (100 mg $kg^{-1}$) were administered. Five min later, an infusion of SNAP or SNOG (300 μg $kg^{-1}$ $h^{-1}$) was begun and was continued for 10 min. The changes observed were compared to those seen when saline was administered following the injection of ADMA or L-NMMA (see earlier).

*Group2 Haemodynamic interactions between ADMA and a high concentration of SNAP* In a separate group of animals ($n = 7$) SNAP was administered at a 10 fold higher dose (i.e. 3 mg $kg^{-1}$ $h^{-1}$) for 10 min. On a separate occasion these animals received a bolus injection of ADMA (100 mg $kg^{-1}$) followed 5 min later by SNAP at a dose of 3 mg $kg^{-1}$ $h^{-1}$ for 10 min.

*Group 3 Haemodynamic interactions between L-NMMA and SNP or hydralazine* A third group of animals ($n = 8$) were given 10 min infusions of SNP (300 μg $kg^{-1}$ $h^{-1}$) or hydralazine (7.5 mg $kg^{-1}$ $h^{-1}$).

On separate experimental days, the animals in this group were given 10 min infusions of SNP or hydralazine beginning 5 min after bolus injection of L-NMMA (100 mg $kg^{-1}$).

*Group 4 Cardiac haemodynamic responses to ADMA and SNAP* A fourth group of animals ($n = 8$), that had previously had an electromagnetic flow probe implanted around the ascending thoracic aorta, received a 10 min infusion of SNAP (300 μg $kg^{-1}$ $h^{-1}$).

At least 60 min later these animals were given ADMA (100 mg $kg^{-1}$) followed 5 min later by a 10 min infusion of SNAP.

*Group 5 Haemodynamic responses to SDMA* The final group of animals ($n = 5$) received bolus injections (100 mg $kg^{-1}$, i.v.) of $N^G,N^G$-dimethyl-L-arginine (symmetrical dimethyl-L-arginine; SDMA).

### *Plasma levels of ADMA*

In order to determine resting plasma levels of endogenous ADMA and the effects of i.v. bolus injection of exogenous ADMA, Long Evans rats ($n = 2$) were anaesthetized (sodium methohexitone 40 mg $kg^{-1}$, i.p.) and had catheters implanted in the distal abdominal aorta and right jugular vein. At least 24 h later, an arterial blood sample (500 μl) was taken 30 min prior to i.v. injection of ADMA (100 mg $kg^{-1}$), and blood

**Table 1** Resting cardiovascular variables in 3 of the groups of animals in which regional haemodynamics were studied (see Methods)

| | *Group 1* ($n = 8$) | *Group 2* ($n = 7$) | *Group 3* ($n = 8$) |
|---|---|---|---|
| Heart rate (beats $min^{-1}$) | 312 ± 10 | 343 ± 11 | 356 ± 12 |
| Mean arterial blood pressure (mmHg) | 103 ± 2 | 101 ± 2 | 98 ± 2 |
| Renal flow (kHz) | 8.8 ± 0.5 | 7.6 ± 0.7 | 7.4 ± 0.6 |
| Mesenteric flow (kHz) | 5.7 ± 0.2 | 7.2 ± 0.6 | 6.9 ± 0.6 |
| Hindquarters flow (kHz) | 3.5 ± 0.1 | 5.5 ± 0.5 | 4.9 ± 0.2 |
| Renal conductance ([kHz $mmHg^{-1}$]$10^3$) | 85 ± 5 | 75 ± 8 | 76 ± 6 |
| Mesenteric conductance ([kHz $mmHg^{-1}$]$10^3$) | 56 ± 3 | 71 ± 6 | 71 ± 7 |
| Hindquarters conductance ([kHz $mmHg^{-1}$]$10^3$) | 35 ± 2 | 54 ± 5 | 50 ± 3 |

Values are mean ± s.e.mean.

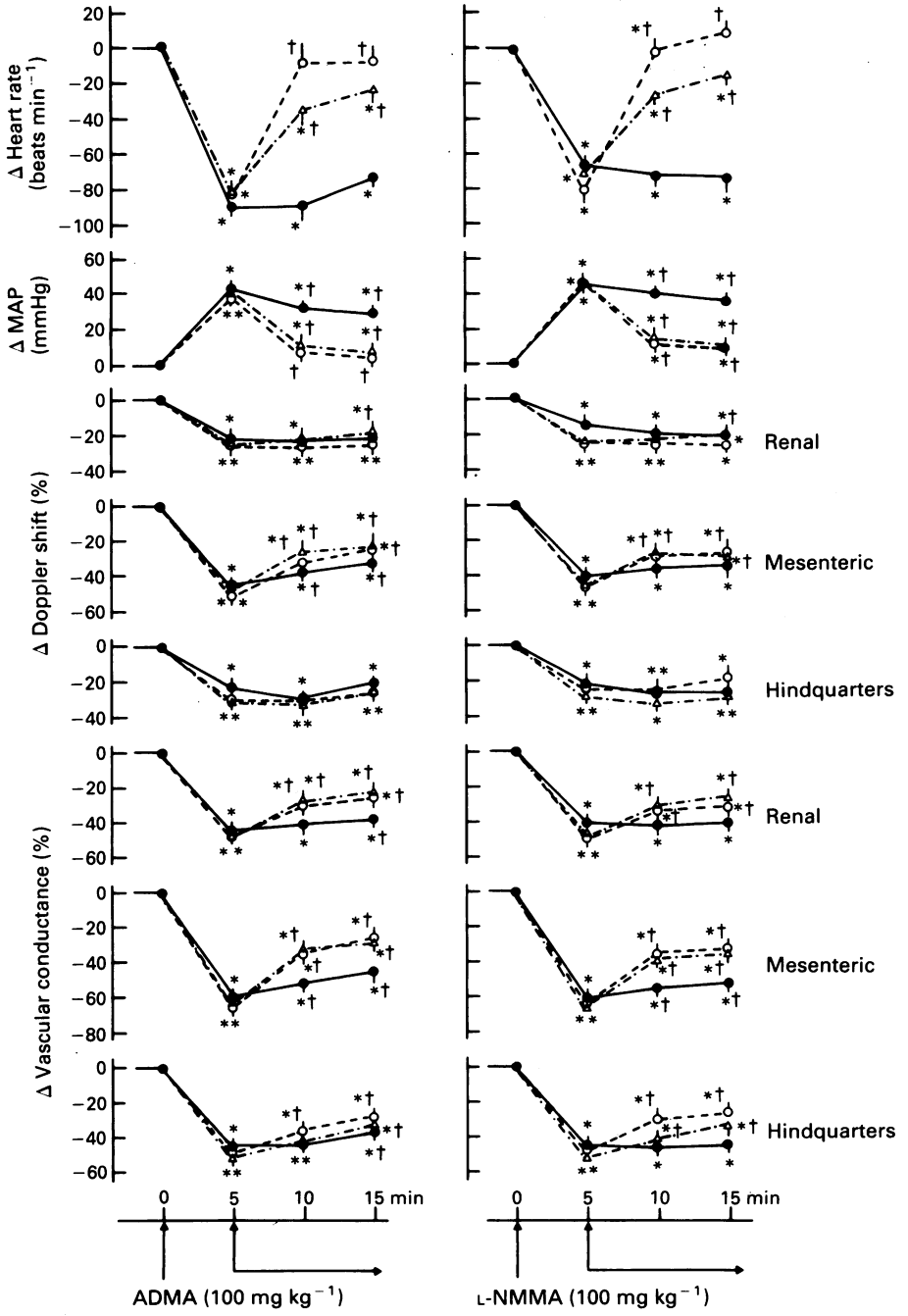


**Figure 1** Cardiovascular responses to $N^G$,$N^G$-dimethyl-L-arginine (ADMA, 100 mg $kg^{-1}$, left-hand panels) or $N^G$-monomethyl-L-arginine (L-NMMA, 100 mg $kg^{-1}$, right-hand panels) followed after 5 min by infusion of saline (●) or S-nitroso-*N*-acetyl-penicillamine (SNAP, 300 μg $kg^{-1}$ $h^{-1}$; ○) or S-nitroso-glutathione (SNOG, 300 μg $kg^{-1}$ $h^{-1}$; Δ) in conscious, Long Evans rats (Group 1, $n = 8$). Values are mean ± s.e.mean; $*P < 0.05$ versus baseline; $\dagger P < 0.05$ versus value at 5 min.

samples were taken at 5, 15 and 60 min after ADMA injection. Plasma ADMA concentrations were measured with the technique described by Vallance et al. (1992), relative to a spiked standard.

### Data analysis

Responses to the incremental bolus doses of ADMA or L-NMMA were assessed from the maximum changes, which occurred about 5 min after the injection.

The responses to SNAP, SNOG, SNP or hydralazine were estimated from the average values recorded across the 5 and 10 min time points during the infusions.

In order to assess the effects of SNAP or SNOG on responses to ADMA or L-NMMA, measurements were made around 5, 10 and 15 min following injection of the 100 mg $kg^{-1}$ dose of ADMA or L-NMMA in the presence of saline, SNAP or SNOG. The effects of SNP or hydralazine on responses to L-NMMA were assessed in the same way.

All within-group responses were analysed by Friedman's test applied to changes relative to baseline. A $P$ value $<0.05$ was taken as significant.

### Drugs

With the exception of SNP and hydralazine (Sigma, UK), all compounds were synthesized by Dr Harold Hodson (Wellcome Research Laboratories). They were dissolved in sterile, isotonic saline (154 nmol $l^{-1}$ NaCl); bolus injections were given in a volume of 100 µl and flushed in with 100 µl saline while infusions were given at a rate of 0.3 ml $h^{-1}$. Administration of vehicle alone in these volumes had no consistent cardiovascular effects.

## Results

Resting values for cardiovascular variables in the three major groups of animals studied in the experiments involving measurement of regional haemodynamics are shown in Table 1.

### Regional haemodynamic responses to ADMA or L-NMMA

ADMA and L-NMMA caused dose-dependent rises in mean arterial blood pressure and reductions in heart rate and renal, mesenteric and hindquarters flow and vascular conductance (Figure 1). There were no significant differences between the responses to ADMA and L-NMMA (Figures 1 and 2).

SDMA had no significant cardiovascular effects (data not shown).

In the two separate animals in which plasma ADMA levels were measured, resting values were 3 and 4 µmol $l^{-1}$. Five min after injection of ADMA plasma levels had increased to 1800 and 1500 µmol $l^{-1}$, respectively; at 15 min they were 800 and 740 µmol $l^{-1}$, respectively, and by 60 min they had fallen to 290 and 140 µmol $l^{-1}$, respectively.

### Regional haemodynamic responses to SNAP or SNOG

The 10 min infusions of 300 µg $kg^{-1}$ $h^{-1}$ of SNAP or SNOG, alone, caused slight hypotension and tachycardia (Table 2). There were small and variable reductions in regional blood flows; only during SNAP infusion was there a slight increase in mesenteric vascular conductance (Table 2).

During the 10 min infusion of SNAP at a dose of 3 mg $kg^{-1}$ $h^{-1}$ there was a fall in mean arterial blood pressure, similar to that seen during infusion of the lower dose of SNAP, but the tachycardia was greater (Tables 2 and 3). Moreover, the higher dose of SNAP caused significant reductions in renal, mesenteric and hindquarters flows, although these changes were not accompanied by significant changes in vascular conductances (Table 3).

### Effects of SNAP or SNOG on regional haemodynamic responses to ADMA or L-NMMA

The pressor and bradycardic effects of ADMA and L-NMMA were almost completely reversed by SNOG and, particularly, SNAP (Figures 1 and 3). However, the pressure changes were accompanied by relatively small effects on the reductions in flows and vascular conductances elicited by the NO synthase inhibitors (Figures 1 and 3).

Infusion of the higher dose of SNAP following ADMA reversed the hypertensive and bradycardic effects of the latter and caused slight hypotension and tachycardia (Table 3, Figure 4). In addition, the reduction in mesenteric flow caused by ADMA was almost abolished, and the accompanying reduction in mesenteric vascular conductance was completely inhibited (Table 3, Figure 4). In contrast, SNAP had no significant effect on the reductions in renal and hindquarters flow caused by ADMA, and the diminutions in conductances in these vascular beds were not reversed completely (Table 3, Figure 4).

### Regional haemodynamic responses to SNP or hydralazine

The 10 min infusion of SNP alone caused tachycardia and a modest hypotension accompanied by a progressive fall in renal flow and a transient increase in mesenteric flow; the latter was associated with a significant rise in vascular conductance (Table 4).

Infusion of hydralazine alone caused a tachycardia and a

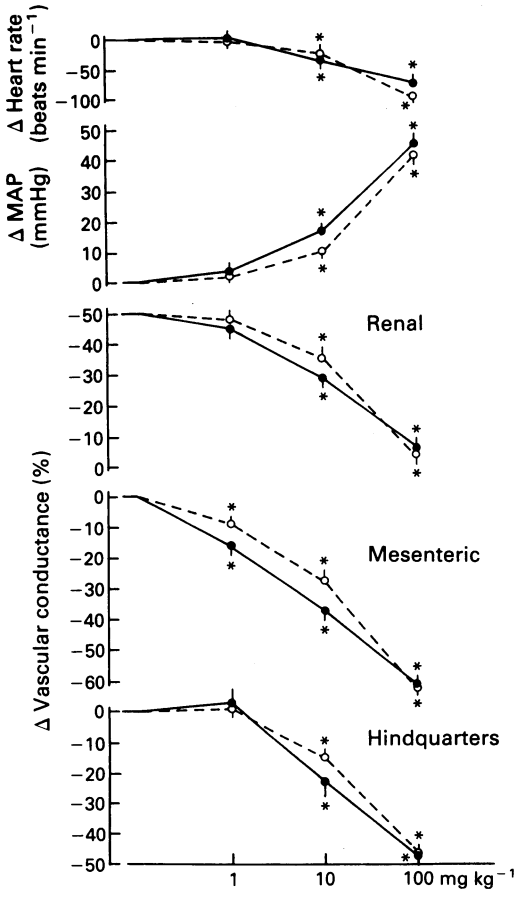


**Figure 2** Maximum changes in heart rate, mean arterial blood pressure and renal mesenteric and hindquarters vascular conductances in the same conscious Long Evans rats (Group 1, $n = 8$) after i.v. injection of increasing bolus doses of $N^G$-monomethyl-L-arginine (L-NMMA, ●) or $N^G$,$N^G$,dimethyl-L-arginine (ADMA, ○). Values are mean ± s.e.mean; $*P<0.05$ versus baseline.

**Table 2** Cardiovascular changes during 10 min infusions of S-nitroso-*N*-acetyl-penicillamine (SNAP; 300 μg kg$^{-1}$ h$^{-1}$) or S-nitroso-glutathione (SNOG; 300 μg kg$^{-1}$ h$^{-1}$) in conscious, Long Evans rats (Group 1, $n = 8$)

| | *SNAP* | | *SNOG* | |
|---|---|---|---|---|
| *Time* | 5 min | 10 min | 5 min | 10 min |
| Δ Heart rate (beats min$^{-1}$) | 31 ± 11* | 39 ± 12* | 15 ± 5* | 16 ± 6* |
| Δ Mean blood pressure (mmHg) | −10 ± 1* | −9 ± 1* | −5 ± 1* | −4 ± 2* |
| Δ Renal flow (%) | −10 ± 3* | −8 ± 4 | −6 ± 2* | −7 ± 2* |
| Δ Mesenteric flow (%) | −3 ± 2 | −3 ± 3 | −2 ± 3 | −8 ± 2* |
| Δ Hindquarters flow (%) | −11 ± 2* | −10 ± 3* | −3 ± 2 | −10 ± 4 |
| Δ Renal conductance (%) | 0 ± 3 | 0 ± 4 | −2 ± 2 | −3 ± 2 |
| Δ Mesenteric conductance (%) | 8 ± 2* | 6 ± 4 | 2 ± 3 | −5 ± 2 |
| Δ Hindquarters conductance (%) | −1 ± 2 | −2 ± 3 | 2 ± 3 | −6 ± 4 |

Values are mean ± s.e.mean; *$P < 0.05$ for change.

**Table 3** Cardiovascular changes during 10 min infusions of S-nitroso-*N*-acetyl-penicillamine (SNAP, 3 mg kg$^{-1}$ h$^{-1}$) alone or starting 5 min after administration of $N^G$,$N^G$,dimethyl-L-arginine (ADMA 100 mg kg$^{-1}$) in conscious Long Evans rats (Group 2, $n = 7$)

| | *SNAP* | | *ADMA* | *ADMA + SNAP* | |
|---|---|---|---|---|---|
| *Time* | 5 min | 10 min | 5 min | 5 min | 10 min |
| Δ Heart rate (beats min$^{-1}$) | 93 ± 12* | 89 ± 17* | −92 ± 12* | 72 ± 16*† | 85 ± 19*† |
| Δ Mean blood pressure (mmHg) | −10 ± 2* | −10 ± 1* | 41 ± 5* | −8 ± 2*† | −10 ± 2*† |
| Δ Renal flow (%) | −23 ± 4* | −21 ± 6* | −24 ± 3* | −23 ± 3* | −26 ± 3* |
| Δ Mesenteric flow (%) | −12 ± 3* | −11 ± 4* | −44 ± 2* | −12 ± 2*† | −12 ± 4*† |
| Δ Hindquarters flow (%) | −17 ± 3* | −12 ± 5 | −41 ± 3* | −34 ± 6* | −32 ± 6* |
| Δ Renal conductance (%) | −13 ± 5 | −13 ± 6 | −45 ± 3* | −15 ± 4*† | −18 ± 3*† |
| Δ Mesenteric conductance (%) | −2 ± 5 | −1 ± 5 | −60 ± 2* | −4 ± 2† | −2 ± 5† |
| Δ Hindquarters conductance (%) | −8 ± 3 | −2 ± 5 | −58 ± 3* | −27 ± 6*† | −25 ± 7*† |

Values are mean ± s.e.mean; *$P < 0.05$ for change relative to baseline; †$P < 0.05$ versus ADMA alone.

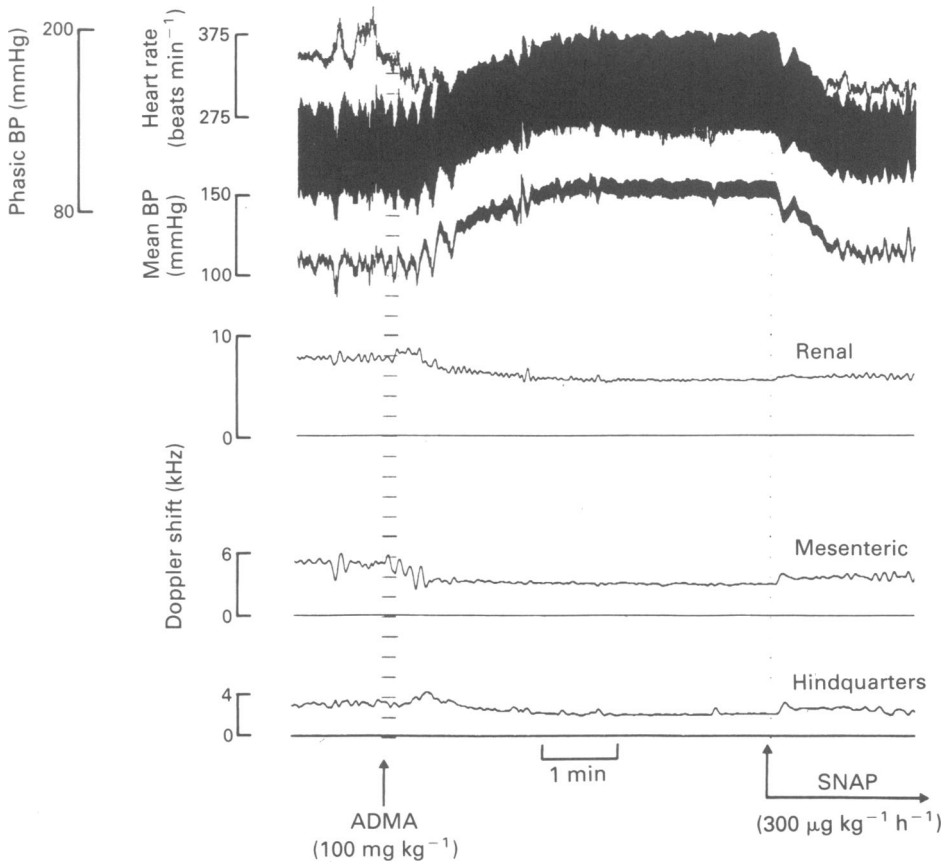


**Figure 3** Cardiovascular responses at the onset of S-nitroso-*N*-acetyl-penicillamine (SNAP) infusion (300 μg kg$^{-1}$ h$^{-1}$) 5 min after i.v. bolus injection of $N^G$,$N^G$,dimethyl-L-arginine (ADMA, 100 mg kg$^{-1}$) in a conscious, Long Evans rat.

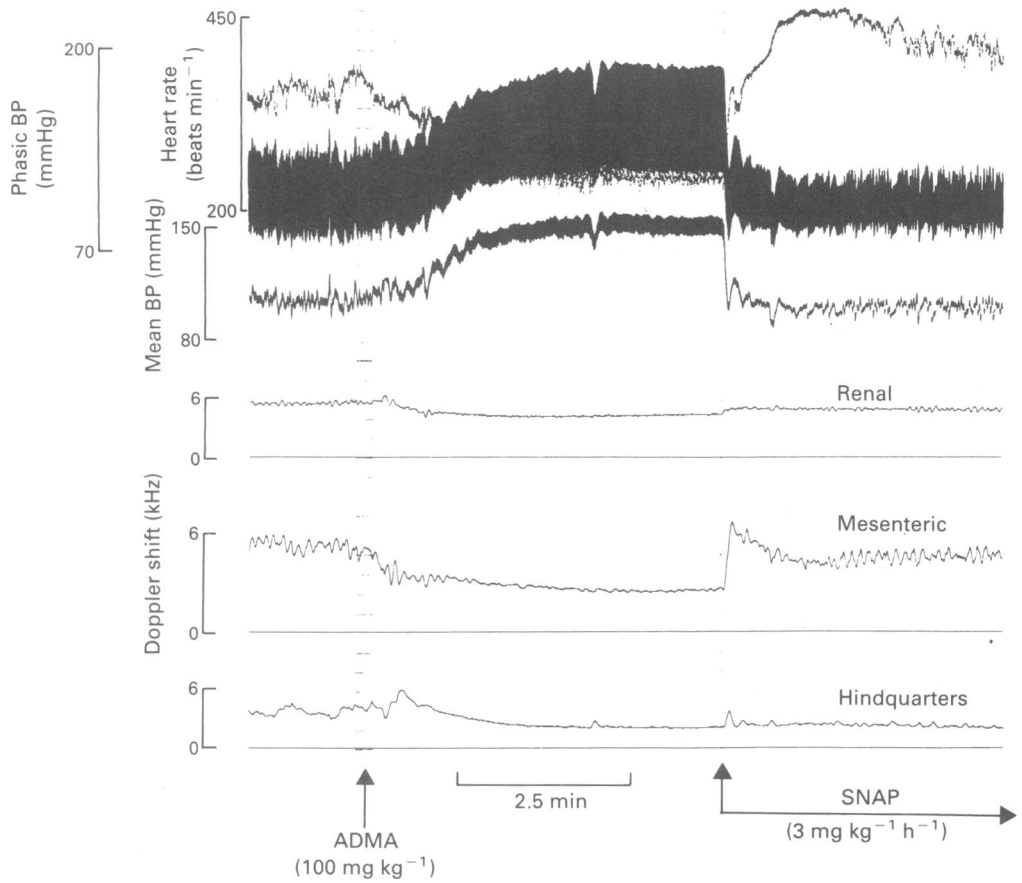


**Figure 4** Cardiovascular responses at the onset of S-nitroso-*N*-acetyl-penicillamine (SNAP) infusion (3 mg kg$^{-1}$ h$^{-1}$) 5 min after administration of N$^G$,N$^G$,dimethyl-L-arginine (ADMA, 100 mg kg$^{-1}$) in a conscious, Long Evans rat.

**Table 4** Cardiovascular changes during 10 min infusion of sodium nitroprusside (SNP, 300 μg kg$^{-1}$ h$^{-1}$) or hydralazine (7.5 mg kg$^{-1}$ h$^{-1}$) in the same conscious Long Evans rats ($n = 8$, Group 3)

| | *SNP* | | *Hydralazine* | |
|---|---|---|---|---|
| *Time* | 5 min | 10 min | 5 min | 10 min |
| Δ Heart rate (beats min$^{-1}$) | 69 ± 14* | 56 ± 12* | 95 ± 16* | 122 ± 16* |
| Δ Mean arterial blood pressure (mmHg) | −12 ± 2* | −9 ± 3* | −9 ± 2* | −15 ± 3* |
| Δ Renal flow (%) | −8 ± 4 | −14 ± 4* | 8 ± 3* | 4 ± 6 |
| Δ Mesenteric flow (%) | 12 ± 2* | 6 ± 3 | 33 ± 6 | 41 ± 7* |
| Δ Hindquarters flow (%) | −4 ± 5 | −3 ± 8 | 24 ± 4* | 28 ± 7* |
| Δ Renal conductance (%) | 5 ± 5 | −5 ± 6 | 19 ± 3* | 22 ± 6* |
| Δ Mesenteric conductance (%) | 28 ± 5* | 16 ± 6* | 46 ± 8* | 66 ± 10* |
| Δ Hindquarters conductance (%) | 10 ± 7 | 7 ± 9 | 35 ± 5* | 51 ± 10* |

Values are mean ± s.e.mean; *$P < 0.05$ for change.

**Table 5** Cardiovascular variables under resting conditions and 5 and 10 min after onset of infusion of S-nitroso-*N*-acetyl-penicillamine (SNAP, 300 μg kg$^{-1}$ h$^{-1}$) alone or SNAP starting 5 min after administration of N$^G$,N$^G$-dimethyl-L-arginine (ADMA, 100 mg kg$^{-1}$) in conscious, Long Evans rats (Group 4, $n = 8$)

| | | *SNAP* | | *ADMA + SNAP* | | |
|---|---|---|---|---|---|---|
| | Resting | 5 min | 10 min | ADMA | 5 min | 10 min |
| Heart rate (beats min$^{-1}$) | 364 ± 8 | 369 ± 10* | 389 ± 8* | 285 ± 9* | 355 ± 15† | 367 ± 13† |
| Mean arterial blood pressure (mmHg) | 102 ± 2 | 95 ± 3* | 97 ± 2 | 148 ± 9* | 113 ± 2*† | 110 ± 1*† |
| Central venous pressure (cm$H_2O$) | 3.6 ± 0.2 | 3.0 ± 0.2* | 2.9 ± 0.2* | 4.7 ± 0.5* | 2.7 ± 0.3*† | 2.5 ± 0.3*† |
| Cardiac index (ml min$^{-1}$ 100 g$^{-1}$) | 25.8 ± 0.9 | 24.5 ± 0.8 | 25.2 ± 0.7 | 17.6 ± 0.7* | 19.4 ± 0.9* | 20.7 ± 0.9*† |
| Peak thoracic aortic flow (ml min$^{-1}$ 100 g$^{-1}$) | 104 ± 3 | 106 ± 3 | 106 ± 2 | 76 ± 3* | 88 ± 3*† | 92 ± 3*† |
| Maximum rate of rise of aortic flow (1 min$^{-2}$ 100 g$^{-1}$) | 439 ± 10 | 458 ± 13 | 452 ± 10 | 276 ± 10* | 364 ± 16*† | 384 ± 17*† |
| Stroke index (μl beat$^{-1}$ 100 g$^{-1}$) | 71 ± 3 | 62 ± 3* | 65 ± 3* | 62 ± 2* | 55 ± 3*† | 57 ± 3*† |
| Total peripheral conductance (μl min$^{-1}$ mmHg$^{-1}$ 100 g$^{-1}$) | 253 ± 72 | 260 ± 15 | 260 ± 9 | 120 ± 6* | 173 ± 10*† | 189 ± 9*† |

Values are mean ± s.e.mean; *$P < 0.05$ versus resting; †$P < 0.05$ versus ADMA alone.

progressive hypotension; there was a transient increase in renal flow, but an incremental increase in mesenteric flow and a sustained increase in hindquarters flow (Table 4). There were significant increases in conductance in renal, hindquarters and, particularly, mesenteric vascular beds (Table 4).

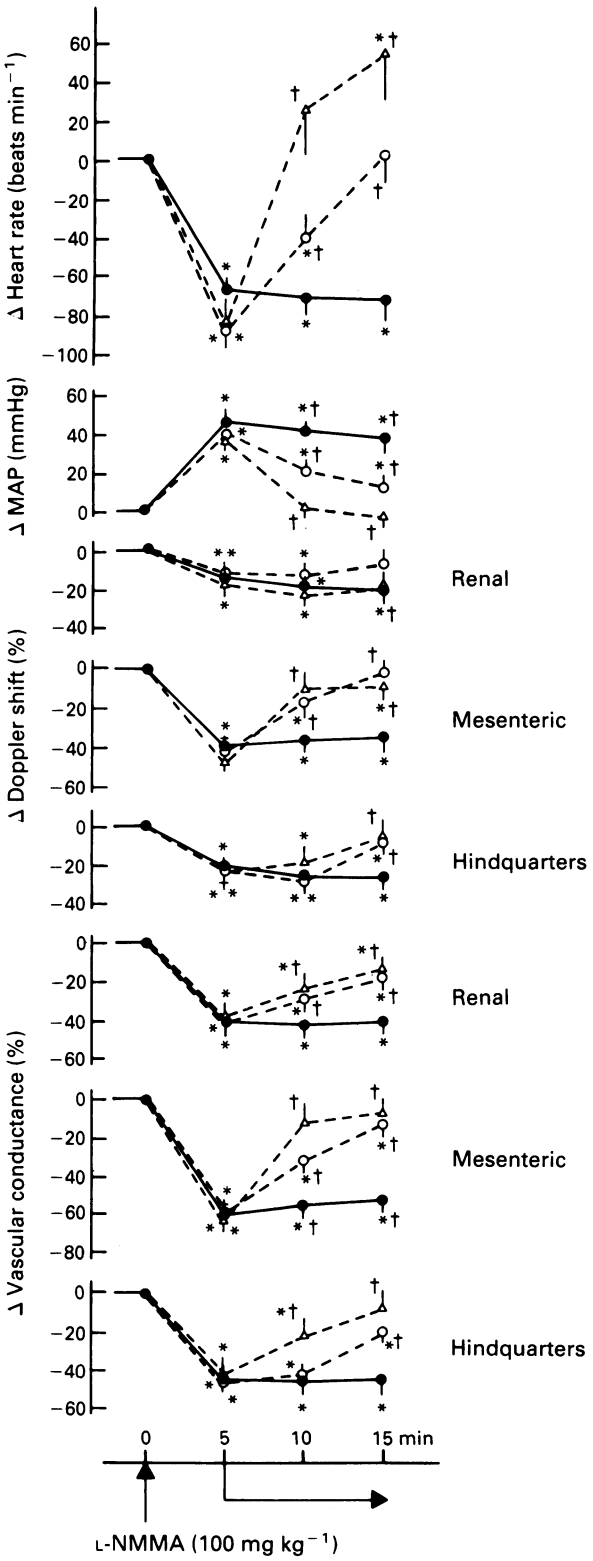


**Figure 5** Cardiovascular responses to $N^G$-monomethyl-L-arginine (L-NMMA 100 mg $kg^{-1}$) followed 5 min later by a 10 min infusion of saline (●—●) or (SNP, 300 μg $kg^{-1}$ $h^{-1}$; Δ–Δ) or hydralazine (7.5 mg $kg^{-1}$ $h^{-1}$; O—O). The results for L-NMMA followed by saline are those shown in Figure 1; those for L-NMMA followed by SNP or hydralazine were obtained in a separate group of conscious Long Evans rats (Group 3, $n = 8$). Values are mean ± s.e.mean; *$P < 0.05$ versus baseline; †$P < 0.05$ versus value at 5 min.

### *Effects of SNP or hydralazine on regional haemodynamic responses to L-NMMA*

SNP abolished the pressor effect of L-NMMA and converted the bradycardia to a tachycardia (Figure 5). SNP also reversed completely the L-NMMA-induced reductions in flow and conductance in the mesenteric and hindquarters, but not in the renal, vascular beds (Figure 5).

In spite of the fact that hydralazine alone had a more marked hypotensive and tachycardic effect than SNP (Table 4), hydralazine did not reverse completely the pressor effect of L-NMMA, although it abolished the bradycardic action (Figure 5). Hydralazine caused some reversal of the reductions in flow and conductance in the renal, mesenteric and hindquarters vascular beds caused by L-NMMA (Figure 5).

### *Cardiac haemodynamic responses to SNAP*

During infusion of SNAP there was a tachycardia and slight transient hypotension (Table 5), similar to the changes seen in animals instrumented for measurement of regional haemodynamics (Table 2). These changes were accompanied by significant reductions only in central venous pressure and stroke index (Table 5).

### *Effects of SNAP on cardiac haemodynamic responses to ADMA*

The pressor and bradycardic effects of ADMA were accompanied by an increase in central venous pressure, but reductions in all other variables (Table 5). In the presence of ADMA, SNAP caused marked inhibition of the pressor effect and complete reversal of the bradycardia (Table 5), and central venous pressure was reduced below resting levels (Table 5). Although SNAP caused some reversal of the ADMA-induced reductions in all other variables, they still remained significantly below the resting values (Table 5).

## Discussion

The three major objectives of this study were:- (1) to delineate the regional haemodynamic effects of ADMA, and to compare them with those of L-NMMA in the same conscious rats; (2) to determine the extent to which the NO donors, SNAP, SNOG and SNP, and the NO-independent vasodilator, hydralazine, were capable of reversing the effects of ADMA or L-NMMA; and (3) to quantitate the cardiac haemodynamic effects of ADMA, and to assess how cardiac variables changed when the pressor effects of ADMA were reversed by SNAP.

The present results, showing dose-dependent pressor, bradycardic and regional vasoconstrictor effects of L-NMMA in conscious rats, are consistent with our earlier findings (Gardiner *et al.*, 1990b) and were included in the current protocols to represent a proper comparison for the effects of ADMA. It is clear from our results that the pressor responses to ADMA were due to haemodynamic changes similar to those seen with L-NMMA, and most likely attributable to inhibition of synthesis of NO by endothelial cells (Gardiner *et al.*, 1990b). The lack of effects of SDMA, and the potent actions of ADMA, corroborate the observations of Vallance *et al.* (1992).

Bolus i.v. injection of ADMA (100 mg $kg^{-1}$) increased resting plasma levels from 3–4 μmol $l^{-1}$ to a peak of 1500–1800 μmol $l^{-1}$. It could be claimed that such plasma levels of endogenous ADMA would never be encountered, even in pathological conditions. However, the recent finding that endothelial cells produce ADMA (Fickling *et al.*, 1993),

raises the possibility that inhibition of NO synthase by elevation of local levels of ADMA could occur in the absence of any notable change in plasma levels of ADMA. Thus, injection of exogenous ADMA might be much less effective at inhibiting NO synthase than would local production of endogenous ADMA.

Although all the vasodilator compounds tested were capable of abolishing the mean arterial pressor effects of ADMA or L-NMMA, this action was accompanied by varying degrees of restitution of the normal regional haemodynamic profile. In all cases, the vasodilators were most effective in normalising mesenteric haemodynamics. In the presence of ADMA or L-NMMA, the more marked vasodilator effects of SNAP and SNOG on the mesenteric, than on the renal or hindquarters, vascular bed could have been due to a greater sensitivity of this vascular bed to the vasodilator effects of exogenous NO in the presence of NO synthase inhibitors. However, we found that SNP or hydralazine could reverse almost all regional haemodynamic effects of L-NMMA. Thus, although the doses of the vasodilators were chosen for their equi-antihypertensive effects they were clearly not matched for their regional vasodilator actions. It is feasible that the differential regional vasodilator responses were due to activation of the renin-angiotensin system, for example, opposing renal vasodilatation. However, we (S.M. Gardiner, P.A. Kemp & T. Bennett, unpublished observations) have found that the $AT_1$-receptor antagonist, losartan, does not render the renal vascular bed any more susceptible to the vasodilator effects of SNP in the presence of L-NMMA.

It is clear from the present findings that substantial changes in cardiac haemodynamics must have been occurring in the various experimental conditions studied. This was confirmed by direct measurement, showing that the mean arterial pressure effects of ADMA were accompanied by an increase in preload (i.e. central venous pressure), but reductions in all other variables. It is likely that the marked reductions in cardiac index, peak aortic flow and $dF/dt_{max}$ in the presence of ADMA were probably due to the in-increase in afterload, rather than any direct negative inotropic effects (De Wildt & Sangster, 1983). Likewise, the partial reversal of the cardiac haemodynamic actions of ADMA by SNAP were probably due to the (incomplete) reduction in afterload. The failure of cardiac output to return to baseline levels under this condition could be attributed to the persistent increase in afterload. However, it is notable that, in the presence of ADMA, SNAP caused a significant reduction in preload (i.e., central venous pressure), indicating that venodilatation may have prevented cardiac output from rising further.

As pointed out elsewhere, the magnitude of the vasoconstrictor effect due to inhibition of NO synthase is underestimated on account of the concurrent reduction in cardiac function (Gardiner *et al.*, 1990d). Here, we show that the extent of reversal of the regional vasoconstrictor effects of ADMA by SNAP are overestimated by using normalisation of mean arterial pressure as an index of this effect, because the 'normal' arterial pressure is accompanied by a total peripheral conductance which is still below control levels, together with a reduced cardiac output.

In conclusion, it is clear that normalisation of blood pressure by NO donors, or by hydralazine, in the presence of NO synthase inhibitors, can occur in spite of persistent vasoconstriction and decreased cardiac function. This observation may have important clinical implications, for it indicates that organ perfusion, in conditions in which NO synthase is inhibited, may not necessarily be improved by administration of NO donors, or other vasodilators.

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