Br. J. Pharmacol. (1991), **103**, 1725–1732

Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5′-N-ethylcarboxamidoadenosine or salbutamol in conscious rats

[1]Sheila M. Gardiner, Philip A. Kemp & Terence Bennett

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

1 Conscious, Long Evans rats ($n = 16$), chronically instrumented for the measurement of regional haemodynamics were given 3 min, randomized infusions of two doses of sodium nitroprusside (1.5 and 15 $\mu g\,min^{-1}$), acetylcholine (0.4 and 4 $\mu g\,min^{-1}$), 5′-N-ethylcarboxamidoadenosine (NECA; 45 and 450 $ng\,min^{-1}$), and salbutamol (24 and 240 $ng\,min^{-1}$) in the absence and in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 1 $mg\,kg^{-1}\,h^{-1}$), a potent inhibitor of nitric oxide biosynthesis.

2 Sodium nitroprusside caused hyperaemic vasodilatation in the mesenteric, and common carotid vascular beds. These effects were enhanced in the presence of L-NAME, as was the hypotension.

3 Acetylcholine caused hyperaemic vasodilatation in the renal, internal carotid and common carotid vascular beds. These effects were attenuated in the presence of L-NAME, but the hypotension was unaffected.

4 NECA caused hyperaemic vasodilatation in the renal, mesenteric, hindquarters, internal carotid and common carotid vascular beds. However, only the hindquarters and internal carotid responses were diminished in the presence of L-NAME and the hypotension was unchanged.

5 Salbutamol caused hyperaemic vasodilatation in the hindquarters vascular bed only. This effect was reduced in the presence of L-NAME, but the hypotension was unchanged.

6 The results indicate marked regional variations in the sensitivity of vasodilator responses to L-NAME that can depend on the vasodilator agent chosen and the dose employed. It is clear from these findings also that measurement of mean arterial blood pressure alone cannot provide adequate information on which to judge the involvement of L-NAME-sensitive mechanisms in vasodilator responses *in vivo*.

Keywords: N^G-nitro-L-arginine methyl ester; acetylcholine; 5′-N-ethylcarboxamidoadenosine; salbutamol; regional blood flow

Introduction

On the basis of many *in vitro* experiments, there is now persuasive evidence that the major endothelium-derived relaxing factor is nitric oxide (see Moncada *et al.*, 1989; Moncada & Higgs, 1990). Considering the ability of inhibitors of nitric oxide synthesis to abolish endothelium-dependent vasorelaxation *in vitro*, it is notable that, although these substances cause cardiovascular changes consistent with inhibition of an important vasodilator tone *in vivo* (Rees *et al.*, 1989; Aisaka *et al.*, 1989a; Gardiner *et al.*, 1990c,d,e), their ability to antagonize regional haemodynamic responses to classical 'endothelium-dependent' vasodilators in this setting is less impressive (see Gardiner *et al.*, 1990e). These observations led us (Gardiner *et al.*, 1990e) to suggest that, *in vivo*, different vasodilator agonists might depend, to varying extents, on nitric oxide for their effects. In addition, we proposed that nitric oxide-dependent and nitric oxide-independent responses to any given vasodilator agonist might be involved to different degrees in different vascular beds (Gardiner *et al.*, 1990e). If this is the case it means that susceptibility of any particular regional vasodilator response to antagonism by, for example, N^G-nitro-L-arginine methyl ester (L-NAME, Moore *et al.*, 1990; Rees *et al.*, 1990) is a good indication that nitric oxide is involved in that response. However, resistance of the response to inhibition by L-NAME does not necessarily mean nitric oxide is not involved (Giles *et al.*, 1990), or that the response is endothelium-independent. Despite this caveat, it would be of interest to know whether or not one could clearly demonstrate regional selectivity in the inhibitory effects of L-NAME on vasodilator responses to a range of agonists, and, particularly, if this selective antagonism differed depending on the agonist.

In our previous studies (Gardiner *et al.*, 1990e) the vasodilator challenges used were single bolus doses of glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1, given before and after a bolus dose of L-NAME (10 $mg\,kg^{-1}$), which we had previously shown to provide a stable haemodynamic profile for up to 60 min (Gardiner *et al.*, 1990d). However, it could be argued that apparent resistance to L-NAME of the responses to bolus injections of vasodilators was due to their dependence only on release of nitric oxide from a pre-formed pool that was not influenced by inhibition of nitric oxide synthesis (Aisaka *et al.*, 1989b). Therefore, we adopted a different strategy in the present study and examined vasodilator responses to infusions of depressor agents at two different dose levels, since infusion of a vasodilator to cause sustained effects ought to be more dependent on *de novo* synthesis of nitric oxide, if nitric oxide were involved in the response (see Mustafa *et al.*, 1990; Tolins *et al.*, 1990; Walder *et al.*, 1991).

In order to assess fully the haemodynamic responses to infusions of vasodilator agents in the presence of L-NAME, the haemodynamic effects of the latter needed to be established and relatively stable for longer than 60 min. Therefore, we administered L-NAME by continuous infusion. Furthermore, it was necessary to use vasodilator agents other than endothelin-1, glyceryl trinitrate and bradykinin since, in conscious rats, infusion of endothelin-1 does not cause vasodilatation (Gardiner *et al.*, 1989; 1990a), glyceryl trinitrate does not cause sustained hypotension, and bradykinin does not produce reproducible responses (unpublished observations). However, because our objective was to determine whether or not L-NAME could be shown to inhibit, selectively, different regional vasodilator responses to different agonists, it was

[1] Author for correspondence.

necessary only to use agents that had clear-cut and wide-ranging effects on regional blood flow. In considering possible candidates we asked ourselves, why choose only vasodiltor agents that are said to be 'endothelium-dependent' (see Furchgott & Vanhoutte, 1989) from the findings of *in vitro* studies? On the basis of this heresy, and some pilot experiments, we therefore proceeded to investigate the regional haemodynamic effects of infusions of two doses of sodium nitroprusside, acetylcholine, 5'-N-ethylcarboxamidoadenosine (NECA; Rose'Meyer & Hope, 1990) and salbutamol, in the absence and in the presence of L-NAME.

Methods

Male, Long Evans rats (350–450 g) were used in all experiments. Under sodium methohexitone anaesthesia (60 mg kg^{-1}, i.p., supplemented as required) 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 measure hindquarters blood flow) (Gardiner *et al.*, 1990c,d,e). In other animals probes were sutured around both common carotid arteries after the external carotid artery had been ligated on the left side. This arrangement permitted measurement of changes in common and internal carotid blood flow simultaneously (Gardiner *et al.*, 1990f). At least 7 days after probe implantation, animals were briefly re-anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.) for the placement of intravascular catheters in the abdominal aorta (via the caudal artery) for monitoring systemic arterial blood pressure and in the right jugular vein for drug administration. Animals were left to recover for at least 24 h before experiments were begun, and the protocols ran over the following 1–2 days. There were 8 animals with renal, mesenteric and hindquarters probes and 8 with internal and common carotid probes.

In random order, 3 min infusions (at 0.15 ml min^{-1}) of sodium nitroprusside (1.5 and 15 μg min^{-1}), acetylcholine (0.4 and 4 μg min^{-1}), NECA (45 and 450 ng min^{-1}) and salbutamol (24 and 240 ng min^{-1}) were given separated by at least 20 min; in all cases the low doses were given before the high doses. The lower dose of each agent was selected on the basis of it causing clear haemodynamic effects with minimum change in mean arterial blood pressure, whereas the higher dose was selected because it caused clear hypotension. On the second experimental day L-NAME was infused (0.3 ml h^{-1}, 1 mg kg^{-1} h^{-1}) continuously starting 60 min before infusion of the vasodilators. Throughout the experiments continuous recordings were made of mean arterial blood pressure and regional Doppler shift signals, both phasic and mean (using a modified Crystal Biotech VF-1 system; Gardiner *et al.*, 1990b). Since all comparisons of the effects of vasodilator agents in the absence and presence of L-NAME were made in the same animals, absolute Doppler shift values were taken as indices of flow and regional vascular conductances were calculated by dividing mean Doppler shift by mean blood pressure (Gardiner *et al.*, 1990c,d,e).

Data analysis

Within any experimental run, changes relative to baseline were analysed by Friedman's test (Theodorsson-Norheim, 1987). Comparisons of resting cardiovascular status in the absence and presence of L-NAME were made by Wilcoxon's ranks sums test, and the same test was used when responses to any given vasodilator agent were considered in the absence and presence of L-NAME. In that case, areas under or over curves, derived by computer analysis (Gardiner *et al.*, 1990d,e), were compared. A P value <0.05 was taken as significant.

Drugs

Sodium nitroprusside (Sigma), acetylcholine chloride (Sigma), NECA (Sigma), salbutamol (Sigma) and L-NAME hydrochloride (Sigma) were all dissolved in isotonic saline. Administration of isotonic saline alone at the volumes employed in the present study had no systemic effects.

Results

Effects of sodium nitroprusside in the absence and in the presence of L-NAME

Mean arterial blood pressure Sodium nitroprusside caused dose-dependent hypotension in the absence of L-NAME (Figures 1 and 2). In the presence of L-NAME, mean arterial blood pressure was increased, but under these conditions only the overall hypotensive effect of the higher dose of sodium nitroprusside was enhanced (Figures 1 and 2).

Renal haemodynamics In the absence of L-NAME, the lower dose of sodium nitroprusside caused a small fall in renal blood flow but no significant change in vascular conductance, whereas the higher dose caused renal vasodilatation unaccompanied by a significant change in renal blood flow (Figure 1). L-NAME did not affect renal blood flow or vascular conductance significantly and under those conditions the haemo-

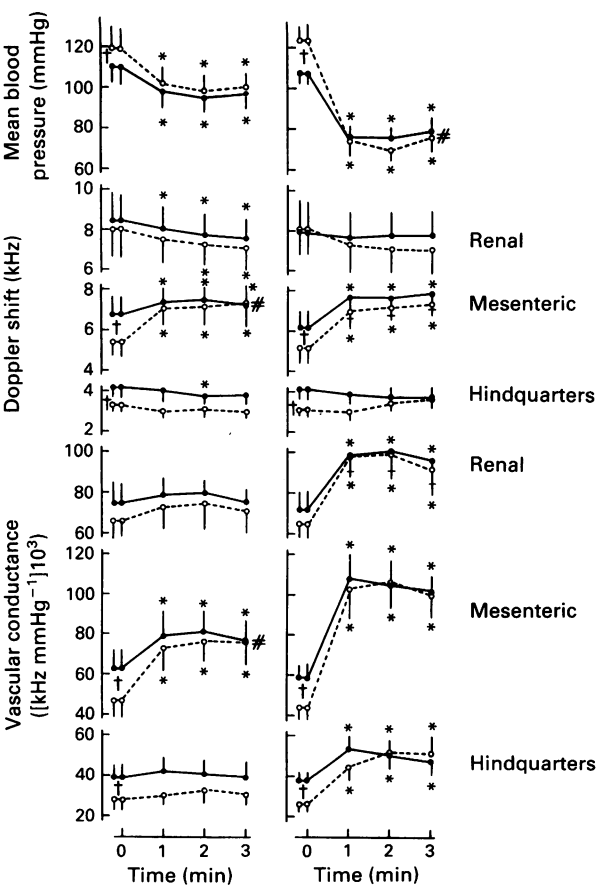

Figure 1 Changes in cardiovascular variables during 3 min infusions of sodium nitroprusside (left-hand panels, responses to sodium nitroprusside at 1.5 μg min^{-1}; right-hand panels, sodium nitroprusside at 15 μg min^{-1}) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 mg kg^{-1} h^{-1}). Values are mean and bars show s.e.mean. *$P < 0.05$ vs. baseline; †$P < 0.05$ for resting values in the absence and presence of L-NAME; #$P < 0.05$ for areas under or over curves in the absence and in the presence of L-NAME.

dynamic response to sodium nitropursside in the renal vascular bed was unchanged (Figure 1).

Mesenteric haemodynamics Sodium nitroprusside, in the absence of L-NAME, caused dose-dependent increases in mesenteric blood flow and vascular conductance (Figure 1). L-NAME caused a reduction in mesenteric blood flow and vascular conductance (Figure 1), but under those conditions only the haemodynamic response to the lower dose of sodium nitroprusside in the mesenteric vascular bed was enhanced (Figure 1).

Hindquarters haemodynamics In the absence of L-NAME, the lower dose of sodium nitroprusside caused a small fall in hindquarters blood flow but no change in vascular conductance, whereas the higher dose caused vasodilatation unaccompanied by a change in blood flow (Figure 1). In the presence of L-NAME, there was a reduction in hindquarters blood flow and a vasoconstriction, but under those conditions the haemodynamic effect of sodium nitroprusside in the hindquarters was unchanged (Figure 1).

Internal carotid haemodynamics Neither dose of sodium nitroprusside affected internal carotid blood flow, in the absence of L-NAME, but there were dose-dependent increases in vascular conductance (Figure 2). L-NAME caused a fall in blood flow and vascular conductance and under those conditions both doses of sodium nitroprusside increased internal carotid blood flow and these effects were greater than in the absence of L-NAME (Figure 2). However, only the rise in vascular conductance with the higher dose of sodium nitroprusside was enhanced (Figure 2).

Common carotid haemodynamics Common carotid blood flow was increased only by the higher dose of sodium nitroprusside, in the absence of L-NAME, but there was a dose-dependent vasodilatation (Figure 2). L-NAME reduced common carotid blood flow and vascular conductance, and under these conditions there was a dose-dependent increase in common carotid blood flow in response to sodium nitroprusside. The increases in blood flow were greater than those seen in the absence of L-NAME (Figure 2). However, only the rise in vascular conductance with the higher dose of sodium nitroprusside was enhanced (Figure 2).

Effects of acetylcholine in the absence and in the presence of L-NAME

Mean arterial blood pressure In the absence of L-NAME, the lower dose of acetylcholine had no significant effect on mean arterial blood pressure, whereas the higher dose caused modest hypotension (Figures 3 and 4). L-NAME increased mean arterial blood pressure but did not affect the responses to acetylcholine (Figures 3 and 4).

Renal haemodynamics Acetylcholine caused dose-dependent increases in both renal blood flow and vascular conductance, in the absence of L-NAME (Figure 3). Although L-NAME did not cause a significant reduction in renal blood flow, there was a significant renal vasoconstriction (Figure 3). Under those conditions, the renal haemodynamic response to the lower dose of acetylcholine was unchanged, but the renal

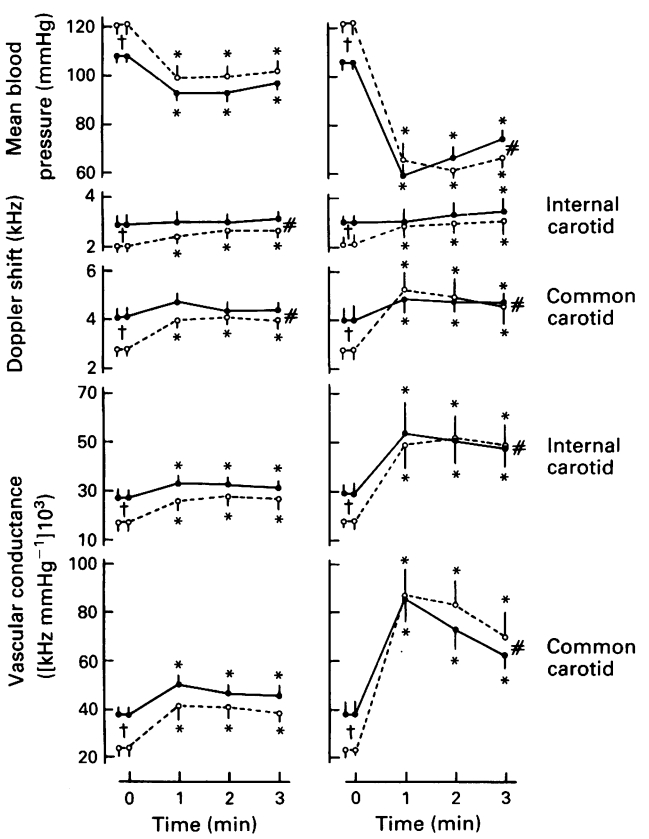

Figure 2 Changes in cardiovascular variables during 3 min infusions of sodium nitroprusside (left-hand panels, responses to sodium nitroprusside at 1.5 $\mu g\,min^{-1}$; right-hand panels, sodium nitroprusside at 15 $\mu g\,min^{-1}$) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 $mg\,kg^{-1}\,h^{-1}$). Values are mean and bars show s.e.mean. *$P < 0.05$ vs. baseline; †$P < 0.05$ for resting values in the absence and presence of L-NAME; #$P < 0.05$ for areas under or over curves in the absence and in the presence of L-NAME.

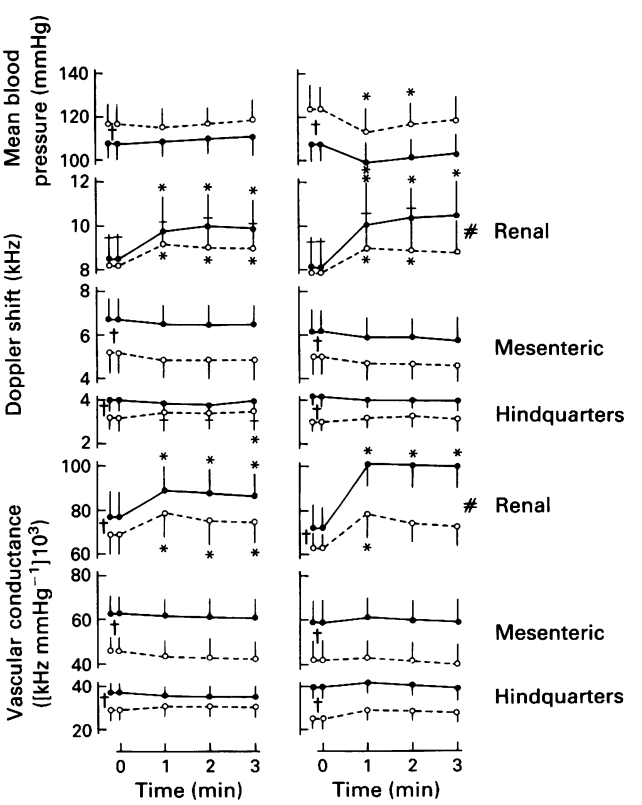

Figure 3 Changes in cardiovascular variables during 3 min infusions of acetylcholine (left-hand panels, responses to acetylcholine at 0.4 $\mu g\,min^{-1}$; right-hand panels, acetylcholine at 4 $\mu g\,min^{-1}$) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 $mg\,kg^{-1}\,h^{-1}$). Values are mean and bars show s.e.mean. *$P < 0.05$ vs. baseline; †$P < 0.05$ for resting values in the absence and presence of L-NAME; #$P < 0.05$ for areas under curves in the absence and in the presence of L-NAME.

hyperaemic and vasodilator responses to the higher dose of acetylcholine were reduced (Figure 3).

Mesenteric haemodynamics In the absence and in the presence of L-NAME, acetylcholine had no effect on mesenteric blood flow or vascular conductance (Figure 3).

Hindquarters haemodynamics There were no responses to either dose of acetylcholine in the absence or in the presence of L-NAME (Figure 3).

Internal carotid haemodynamics In the absence of L-NAME there were increases in both blood flow and dose-dependent rises in vascular conductance in response to acetylcholine (Figure 3). In the presence of L-NAME the responses to the lower dose of acetylcholine were attenuated, whereas those to the higher dose were not (Figure 4).

Common carotid haemodynamics There was a dose-dependent increase in both blood flow and vascular conductance in response to acetylcholine in the absence of L-NAME (Figure 4). In the presence of L-NAME the rise in blood flow in response to the lower dose of acetylcholine was attenuated, whereas that to the higher dose was not (Figure 4). However, the increase in vascular conductance with both doses of acetylcholine was reduced (Figure 4).

Effects of NECA in the absence and in the presence of L-NAME

Mean arterial blood pressure In the absence of L-NAME, there was a fall in mean arterial blood pressure only in response to the higher dose of NECA (Figures 5 and 6). The overall pattern of change in mean arterial blood pressure in response to NECA was unaffected in the presence of L-NAME, although the slight hypotension, following administration of the lower dose of NECA in the animals with carotid probes was significant under these conditions (Figure 6).

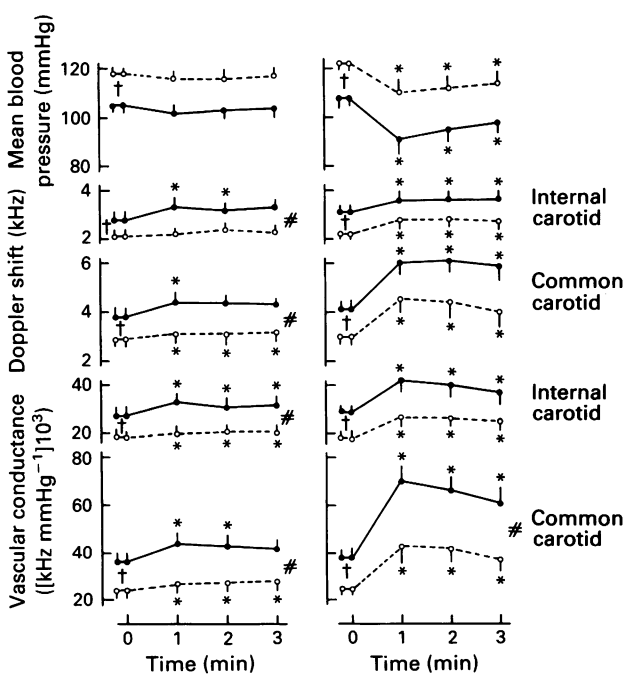

Figure 4 Changes in cardiovascular variables during 3 min infusions of acetylcholine (left-hand panels, responses to acetylcholine at $0.4\,\mu g\,min^{-1}$; right-hand panels, acetylcholine at $4\,\mu g\,min^{-1}$) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at $1\,mg\,kg^{-1}\,h^{-1}$). Values are mean and bars show s.e.mean. $*P < 0.05$ vs. baseline; $\dagger P < 0.05$ for resting values in the absence and presence of L-NAME; $\#P < 0.05$ for areas under curves in the absence and in the presence of L-NAME.

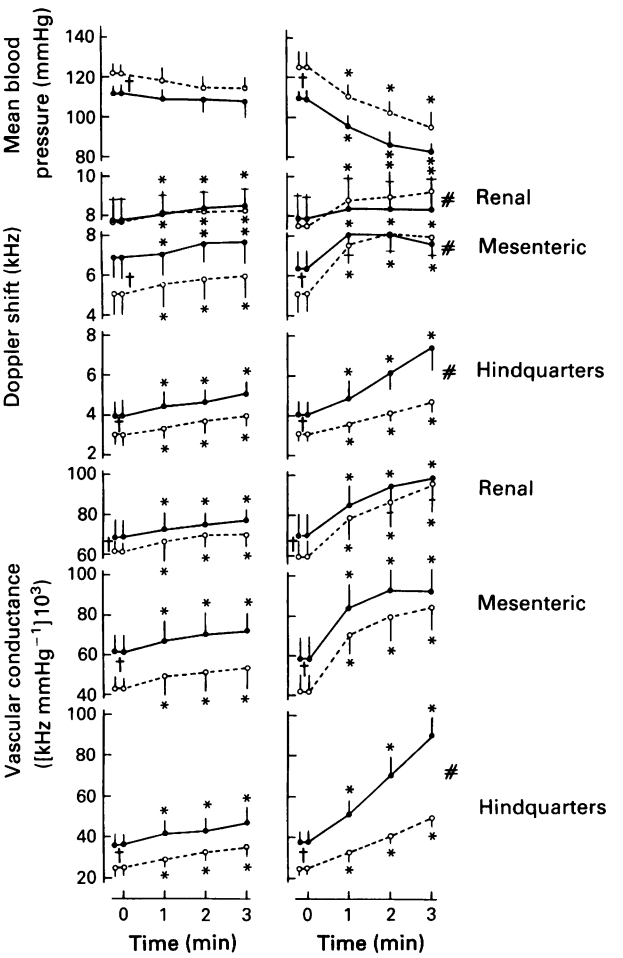

Figure 5 Changes in cardiovascular variables during 3 min infusions of 5'-N-ethylcarboxamidoadenosine (NECA) (left-hand panels, responses to NECA at $45\,\mu g\,min^{-1}$; right-hand panels, NECA at $450\,\mu g\,min^{-1}$) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at $1\,mg\,kg^{-1}\,h^{-1}$). Values are mean and bars show s.e.mean. $*P < 0.05$ vs. baseline; $\dagger P < 0.05$ for resting values in the absence and presence of L-NAME; $\#P < 0.05$ for areas under curves in the absence and in the presence of L-NAME.

Renal haemodynamics The lower dose of NECA caused a slight increase in renal blood flow, whereas the higher dose had no significant effect in the absence of L-NAME (Figure 5); however, there was a dose-dependent vasodilatation in the renal vascular bed (Figure 5). In the presence of L-NAME, there was a significant enhancement of the renal hyperaemic response to the higher dose of NECA (Figure 5), but there were no other significant changes in the responses to NECA (Figure 5).

Mesenteric haemodynamics In the absence of L-NAME, NECA caused a dose-dependent increase in both mesenteric blood flow and vascular conductance (Figure 5). In the presence of L-NAME, there was a significant augmentation of the hyperaemic response to the higher dose of NECA, but no other significant changes in the responses to NECA (Figure 5).

Hindquarters haemodynamics There was a slowly-developing, dose-dependent increase in both blood flow and vascular conductance in response to NECA in the absence of L-NAME. In the presence of L-NAME, there were significant attenuations of the responses to the higher dose, but not to the lower dose, of NECA (Figure 5).

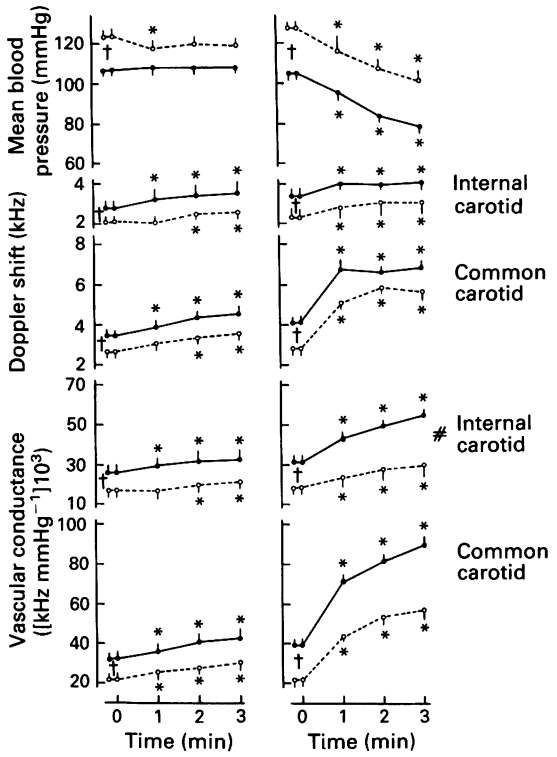

Figure 6 Changes in cardiovascular variables during 3 min infusions of 5'-N-ethylcarboxamidoadenosine (NECA) (left-hand panels, responses to NECA at 45 μg min^{-1}; right-hand panels, NECA at 450 μg min^{-1}) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 mg kg^{-1} h^{-1}). Values are mean and bars show s.e.mean. *$P < 0.05$ vs. baseline; †$P < 0.05$ for resting values in the absence and presence of L-NAME; #$P < 0.05$ for areas under curves in the absence and in the presence of L-NAME.

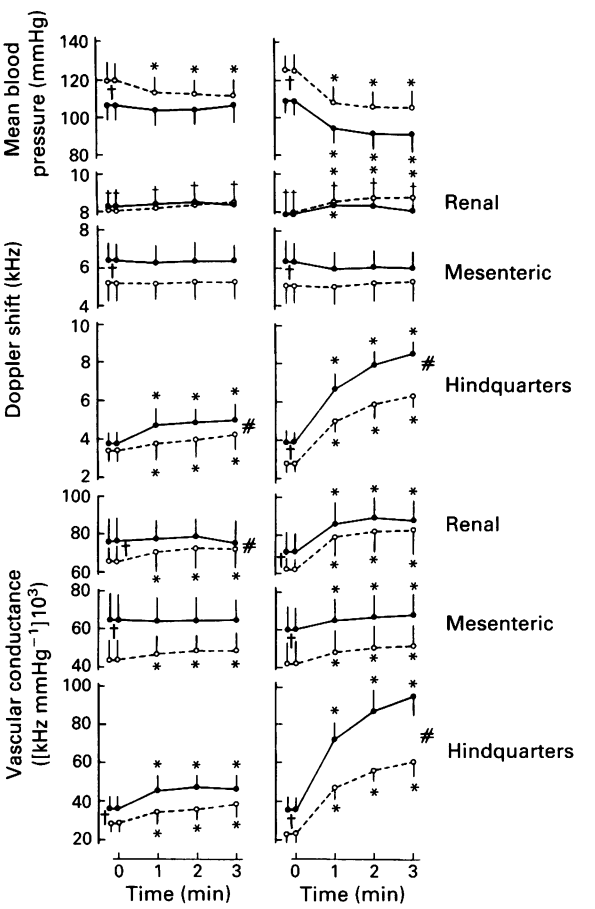

Figure 7 Changes in cardiovascular variables during 3 min infusions of salbutamol (left-hand panels, responses to salbutamol at 24 μg min^{-1}; right-hand panels, salbutamol at 240 μg min^{-1}) in the same conscious, Long Evans rats ($n = 8$) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 mg kg^{-1} h^{-1}). Values are mean and bars show s.e.mean. *$P < 0.05$ vs. baseline; †$P < 0.05$ for resting values in the absence and presence of L-NAME; #$P < 0.05$ for areas under curves in the absence and in the presence of L-NAME.

Internal carotid haemodynamics In the absence of L-NAME, NECA caused a dose-dependent rise in both internal carotid blood flow and vascular conductance (Figure 6). L-NAME attenuated the rise in vascular conductance in response to the higher dose of NECA, but had no other significant effect on the responses to NECA (Figure 6).

Common carotid haemodynamics There was a dose-dependent increase in both common carotid blood flow and vascular conductance in response to NECA and these were unaffected by L-NAME (Figure 6).

Effects of salbutamol in the absence and presence of L-NAME

Mean arterial blood pressure In the absence of L-NAME, only the higher dose of salbutamol caused a reduction in mean arterial blood pressure (Figures 7 and 8). However, in the presence of L-NAME, both doses of salbutamol induced hypotension, although the responses were not different from those seen in the absence of L-NAME (Figures 7 and 8).

Renal haemodynamics Only the higher dose of salbutamol caused a small increase in renal blood flow together with a vasodilatation in the absence of L-NAME (Figure 7). In the presence of L-NAME, these responses were unchanged, but there was also a rise in renal vascular conductance in response to the lower dose of salbutamol that was significantly greater than in the absence of L-NAME (Figure 7).

Mesenteric haemodynamics In the absence of L-NAME, salbutamol had no effect on mesenteric blood flow, although the higher dose caused vasodilatation (Figure 7). In the presence of L-NAME, there were no significant changes in mesenteric blood flow in response to salbutamol but there were vasodilatations with both doses (Figure 7).

Hindquarters haemodynamics There was a dose-dependent increase in both blood flow and vascular conductance in response to salbutamol in the absence of L-NAME (Figure 7). In the presence of L-NAME, the hyperaemias were attenuated, but only the rise in vascular conductance in response to the higher dose of salbutamol was reduced (Figure 7).

Internal carotid haemodynamics In the absence of L-NAME, the lower dose of salbutamol had no effect on internal carotid blood flow, and the higher dose caused a fall, although there was a slight rise in vascular conductance after administration of both doses (Figure 8). In the presence of L-NAME, there was no change in blood flow in response to either dose of salbutamol, and hence there was a significant difference between the effects of the higher dose of salbutamol in the presence and in the absence of L-NAME (Figure 8). However, L-NAME did not change the slight vasodilator effect of salbutamol (Figure 8).

Common carotid haemodynamics In the absence of L-NAME, there was a slight increase in both common carotid blood flow

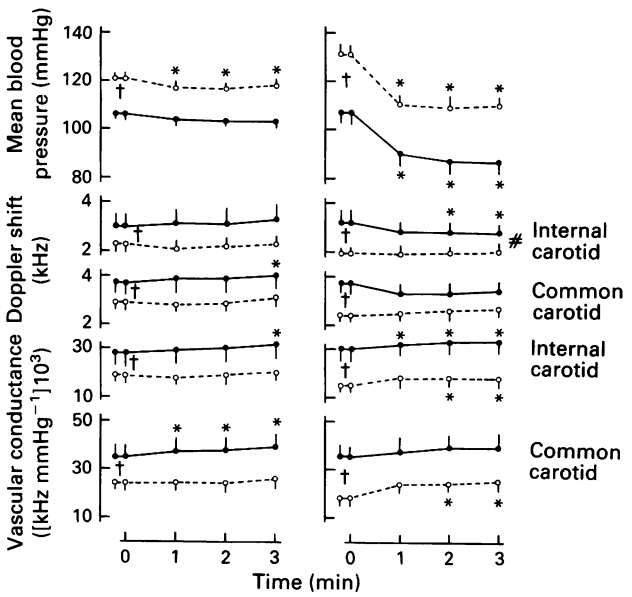

Figure 8 Changes in cardiovascular variables during 3 min infusions of salbutamol (left-hand panels, responses to salbutamol at 24 μg min^{-1}; right-hand panels, salbutamol at 240 μg min^{-1}) in the same conscious, Long Evans rats (n = 8) in the absence (●) and in the presence (○) of N^G-nitro-L-arginine methyl ester (L-NAME) (infused at 1 mg kg^{-1} h^{-1}). Values are mean and bars show s.e.mean. *P < 0.05 vs. baseline; †P < 0.05 for resting values in the absence and presence of L-NAME; #P < 0.05 for areas under curves in the absence and in the presence of L-NAME.

and vascular conductance in response to the lower dose of salbutamol, but no significant effects of the higher dose (Figure 8). In the presence of L-NAME, there was no significant change in the blood flow response to salbutamol, although the higher dose caused a slight vasodilatation (Figure 8).

Discussion

In the present work, we compared a range of vasodilators with differing regional haemodynamic profiles in the absence and in the presence of L-NAME, a potent inhibitor of nitric oxide synthesis (Moore *et al.*, 1990; Rees *et al.*, 1990). Our primary objective was to identify and quantify any differential regional haemodynamic effects that were selectively inhibited by L-NAME.

Administration of L-NAME caused hypertension associated with reductions in mesenteric, hindquarters and internal and common carotid blood flows and decreases in renal, mesenteric, hindquarters and internal and common carotid vascular conductances (Gardiner *et al.*, 1990d,e). These changes, themselves, might have been expected to influence subsequent responses to vasodilator agents. In other studies, the hypotensive response to a vasodilator has been expressed as a percentage of the immediately preceding baseline level, in order to allow for any augmentation of the response due to the hypertension caused by inhibition of nitric oxide production (e.g. Walder *et al.*, 1991). However, this approach presents a problem in the case of vascular conductance since inhibition of nitric oxide production causes a reduction in conductance and expressing any rise in conductance in response to a vasodilator agent as a percentage of the reduced baseline level could give the impression that the response was unaffected when, in absolute terms, it was reduced. Furthermore, we know of no evidence that elevation of mean arterial blood pressure would, necessarily, enhance the effect of all depressor agents. For these reasons, in the present work, we analysed all data in their raw forms and compared responses in different conditions by considering areas under or over curves (expressed as absolute units).

Responses to sodium nitroprusside

Although sodium nitroprusside is well-known as a vasodilator agent, the lower dose caused an increase only in mesenteric blood flow, while the higher dose caused mesenteric and common carotid hyperaemia. Hence, the renal, internal carotid and hindquarters vasodilatation seen under the latter circumstance could have been entirely autoregulatory. Those vascular beds (mesenteric and common carotid) that had shown hyperaemic vasodilatation in response to sodium nitroprusside alone exhibited enhanced responses in the presence of L-NAME, to either the low or the high dose of sodium nitroprusside.

Several questions arise from these observations. Firstly, why are some vascular beds more susceptible than others to the vasodilator effects of sodium nitroprusside? It is interesting to note that a similar regional haemodynamic profile is seen with glyceryl trinitrate (Gardiner *et al.*, 1990e and unpublished observations) and yet the two compounds release nitric oxide by different mechanisms (Feng & Hedner, 1990). Indeed, sodium nitroprusside releases nitric oxide spontaneously (Feng & Hedner, 1990), so it is unlikely that regional differences in its pharmacokinetics could account for the differences seen in its regional vasodilator effects. One possibility is that the vascular beds that were apparently insensitive to sodium nitroprusside simply had more effective counterregulatory mechanisms (but see below for acetylcholine).

The second question arising from our findings is why did the vascular beds that were the most sensitive to the vasodilator effect of sodium nitroprusside show enhanced responses in the presence of L-NAME? It seems that the vasoconstriction induced by L-NAME could not, itself, account for this because the hindquarters vascular bed was constricted by L-NAME but its vasodilator response to sodium nitroprusside was not changed significantly (although there was a trend towards potentiation). There is some evidence from *in vitro* experiments which indicates that in the absence of a functional endothelium, or in the presence of the L-arginine analogue, N^G-monomethyl-L-arginine (to inhibit endogeneous nitric oxide production), vasorelaxant responses to sodium nitroprusside (Shirasaki & Su, 1985), SIN-1 (Lüscher *et al.*, 1989; Busse *et al.*, 1989; Flavahan & Vanhoutte, 1989) and glyceryl trinitrate (Forster *et al.*, 1990) are enhanced. It is feasible that the present findings are an *in vivo* corollary of those *in vitro* observations, and possibly due to increased sensitivity of guanylate cyclase to exogenous nitric oxide when production of endogenous nitric oxide is reduced. If, in the presence of L-NAME, there is enhancement of the effects of sodium nitroprusside, use of this compound as an internal control, as advocated for glyceryl trinitrate (Gardiner *et al.*, 1990e), could be misleading. However, in the case of glyceryl trinitrate we could detect no evidence of sensitization in the presence of L-NAME if neurohumoral mechanisms were also blocked (Gardiner *et al.*, 1990e).

Responses to acetylcholine

At the doses used it was possible to demonstrate regionally-selective hyperaemic vasodilatation (renal, internal carotid and common carotid) in response to acetylcholine that was attenuated in the presence of L-NAME. This suggests that at least a component of the vasodilatation was due to acetylcholine-induced release of endothelium-derived nitric oxide, consistent with *in vitro* observations (Moore *et al.*, 1990; Rees *et al.*, 1990). Previously, it was not possible for us straightforwardly to demonstrate inhibition by L-NAME of

any regional haemodynamic effects of acetylcholine (Gardiner *et al.*, 1990e). However, a major difference between our two studies is that in this one we used an infusion of acetylcholine, whereas previously we gave rapid bolus injections. Thus, it is feasible that the responses elicited by infusion of acetylcholine depend, to some extent, on newly-formed nitric oxide, the production of which is inhibited by L-NAME. However, other factors must be involved because in the present work L-NAME attenuated the internal carotid vasodilator response to infusion of the lower, but not of the higher dose, of acetylcholine. Hence, in different vascular beds there may be subtle differences in the interactions between L-NAME and the L-arginine/nitric oxide pathway, and these may be more or less obvious, depending on the dose of vasodilator agonist used.

Even in those cases where vasodilator responses to acetylcholine were attenuated by L-NAME, there were still significant increases in blood flow in response to acetylcholine in the presence of L-NAME (but see Tolins *et al.*, 1990). Possible explanations for the inability of L-NAME to obliterate totally the effects of acetylcholine are: (1) insufficient L-NAME was given: (2) the residual responses were mediated by a source of nitric oxide not blocked by L-NAME; (3) nitric oxide was not the mediator of the vasodilatation (Long & Berkowitz, 1990), and (4) the hyperaemia that was resistant to L-NAME was not endothelium-dependent, but was due to acetylcholine exerting a pre-junctional inhibitory action on noradrenergic vasoconstrictor tone (Vanhoutte & Levy, 1980). This latter proposition is consistent with the finding that, in the presence of ganglion blockade the renal vasodilator responses to bolus doses of acetylcholine showed increased sensitivity to L-NAME (Gardiner *et al.*, 1990e). However, this is not likely to be a complete explanation since in the present experiments acetylcholine infusions did not cause hyperaemia in the hindquarters in spite of the fact that this vascular bed has a high degree of noradrenergic tone *in vivo* (Gardiner & Bennett, 1988).

It is particularly noteworthy that the vascular beds that dilated most to acetylcholine (renal, internal and common carotid) were not identical to those which responded most markedly to sodium nitroprusside (mesenteric and common carotid). Thus, neither regional differences in sensitivity to nitric oxide nor differential effectiveness of counterregulatory mechanisms can be the sole explanation of these findings. It is feasible that for acetylcholine there are regional differences in pre- and post-junctional muscarinic receptor distribution (Thomas *et al.*, 1988), and/or post-receptor coupling mechanisms.

Responses to NECA

The regional haemodynamic profile of NECA was interesting inasmuch as all vascular beds showed hyperaemic vasodilatation, but the time-course and extent of the changes varied between regions. In the mesenteric and, to a lesser extent, the common carotid vascular beds, the vasodilatation was relatively prompt in onset, whereas the vasodilatation in the internal carotid and particularly the hindquarters vascular bed, developed more slowly; the latter but not the former was attenuated by L-NAME. Differential involvement of L-NAME-susceptible vasodilator responses to NECA in different vascular beds is consistent with *in vitro* data showing that vasorelaxant effects of NECA may involve at least two different mechanisms (Rose'Meyer & Hope, 1990). From the present work it appears that the hindquarters and internal carotid vascular beds show vasodilator responses to NECA that are particularly sensitive to L-NAME.

The fact that the higher dose of NECA caused substantial hyperaemic vasodilatation in all vascular beds, in spite of marked hypotension is consistent with the proposition that the failure of all vascular beds to dilate in response to sodium nitroprusside or acetylcholine was not due to recruitment of baroreflex-mediated mechanisms (see above). Indeed, in the presence of L-NAME there were significant increases in the hyperaemic responses to the higher dose of NECA in the renal and mesenteric vascular beds, indicating sensitization to this agonist under those conditions. This effect, at least in the renal vascular bed, was not associated with a reduction in blood flow by L-NAME; the mechanism involved remains to be elucidated.

Responses to salbutamol

Under all conditions salbutamol caused hindquarters hyperaemia, consistent with the presence of β_2-adrenoceptor-mediated vasodilator mechanisms in that vascular bed (Gardiner & Bennett, 1988). However, although it is generally considered that β_2-adrenoceptor-mediated vasodilatation is endothelium-independent (Furchgott & Vanhoutte, 1989), in the present work the hindquarters hyperaemic vasodilator effect of salbutamol was reduced significantly in the presence of L-NAME. These results are in agreement with some *in vitro* data showing that β-adrenoceptor-mediated vasorelaxant effects may involve the endothelium (Rubanyi & Vanhoutte, 1985; Kamata *et al.*, 1989; Gray & Marshall, 1991). Nonetheless, the substantial residual hindquarters vasodilator response to salbutamol in the presence of L-NAME is consistent with additional involvement of nitric oxide-independent mechanisms.

Conclusions

Depending on the vasodilator agent used and the doses employed it is possible to demonstrate L-NAME-sensitive increases in blood flow and vascular conductance in most peripheral vascular beds *in vivo*. However, it seems such effects are not necessarily more apparent with a higher dose of the vasodilator agent. Furthermore, although sodium nitroprusside and NECA can cause potent mesenteric hyperaemic vasodilatations we have yet clearly to demonstrate L-NAME-sensitive vasodilatation to any agent in this vascular bed *in vivo*. Finally, it is apparent that proper assessment of the involvement of L-NAME-sensitive mechanisms in the responses to agents such as acetylcholine, NECA and salbutamol must involve measurement of regional haemodynamics, since monitoring mean arterial blood pressure alone would give misleading results.

References

AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989a). N^G-methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea-pig: does nitric oxide regulate blood pressure *in vivo*? *Biochem. Biophys. Res. Commun.*, **160**, 881–886.

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

BUSSE, R., POHL, U., MÜLSCH, A. & BASSENGE, E. (1989). Modulation of the vasodilator action of SIN-1 by the endothelium. *J. Cardiovasc. Pharmacol.*, **14**, Suppl. 11, S81–S85.

FENG, Q. & HEDNER, T. (1990). Endothelium-derived relaxing factor (EDRF) and nitric oxide (NO). *Clin. Physiol.*, **10**, 503–526.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1989). Mechanisms underlying the inhibitory interaction between the nitrovasodilator SIN-1 and the endothelium. *J. Cardiovasc. Pharmacol.*, **14**, Suppl. 11, S86–S90.

FORSTER, C., MAIN, J.S. & ARMSTRONG, P.W. (1990). Endothelium modulation of the effects of nitroglycerin on blood vessels from dogs with pacing-induced heart failure. *Br. J. Pharmacol.*, **101**, 109–114.

FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.*, **3**, 2007–2018.

GARDINER, S.M. & BENNETT, T. (1988). Regional haemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.*, **255**, H813–H824.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989). 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. (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. & HARTLEY, C.J. (1990b). Can pulsed Doppler technique measure changes in aortic flow in conscious rats? *Am. J. Physiol.*, **259**, H448–H456.

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, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990d). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.*, **101**, 625–631.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990e). 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. *Br. J. Pharmacol.*, **101**, 632–649.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., KEMP, P.A. & NEY, U. (1990f). Synergistic internal carotid vasodilator effects of human α-calcitonin gene-related peptide and nimodipine in conscious rats. *Br. J. Pharmacol.*, **99**, 830–834.

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.*, **100**, 452P.

GRAY, D.W. & MARSHALL, I. (1991). Isoprenaline relaxation of rat thoracic aorta is endothelium-dependent, releases nitric oxide and raises cyclic GMP and cyclic AMP. *Br. J. Pharmacol.*, Proc. Suppl. **102**, 125P.

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.

KAMATA, K., MIYATA, N. & KASUYA, Y. (1989). Involvement of endothelial cells in relaxation and contraction responses of the aorta to isoproterenol in naive and streptozotocin-induced diabetic rats. *J. Pharmacol. Exp. Ther.*, **249**, 890–894.

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

LÜSCHER, T.F., RICHARD, V. & YANG, Z. (1989). Interaction between endothelium-derived nitric oxide and SIN-1 in human and porcine blood vessels. *J. Cardiovasc. Pharmacol.*, **14**, Suppl. 11, S76–S80.

MONCADA, S. & HIGGS, E.A. (1990). *Nitric Oxide from L-Arginine: A Bioregulatory System.* Amsterdam: Excerpta Medica.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension*, **12**, 365–372.

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

MUSTAFA, M., MESTER, P.A., THIEMERMANN, C., HECKER, M. & VANE, J.R. (1990). N^{ω}-nitro-L-arginine (NO_2Arg) and NO_2Arg-L-phenylalanine inhibit endothelium-dependent vasodilatations *in vitro* in rabbits and *in vivo* in the anaesthetized rat. *J. Physiol.*, **429**, 72P.

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

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

ROSE'MEYER, R.B. & HOPE, W. (1990). Evidence that A_2 purinoceptors are involved in endothelium-dependent relaxation of the rat thoracic aorta. *Br. J. Pharmacol.*, **100**, 576–580.

RUBANYI, G.M. & VANHOUTTE, P.M. (1985). Endothelium removal decreases relaxations of canine coronary arteries caused by beta-adrenergic agonists and adenosine. *J. Cardiovasc. Pharmacol.*, **7**, 139–144.

SHIRASAKI, Y. & SU, C. (1985). Endothelium removal augments vasodilation by sodium nitroprusside and sodium nitrite. *Eur. J. Pharmacol.*, **114**, 93–96.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.*, **17**, 85–99.

THOMAS, G.R., THIEMERMANN, C., WALDER, C. & VANE, J.R. (1988). The effects of endothelium-dependent vasodilators on cardiac output and their distribution in the anaesthetized rat: a comparison with sodium nitroprusside. *Br. J. Pharmacol.*, **95**, 986–992.

TOLINS, J.P., PALMER, R.M.J., MONCADA, S. & RAIJ, L. (1990). Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *Am. J. Physiol.*, **258**, H655–H662.

VANHOUTTE, P.M. & LEVY, M.N. (1980). Prejunctional cholinergic modulation of adrenergic neurotransmission in the cardiovascular system. *Am. J. Physiol.*, **238**, H275–H281.

WALDER, C., THIEMERMANN, C. & VANE, J.R. (1991). The involvement of endothelium-derived relaxing factor in the regulation of renal cortical blood flow in the rat. *Br. J. Pharmacol.*, **102**, 967–973.

(Received January 4, 1991
Revised February 28, 1991
Accepted March 20, 1991)