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Influence of aminoguanidine and the endothelin antagonist, SB 209670, on the regional haemodynamic effects of endotoxaemia in conscious rats

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1 We compared the regional haemodynamic responses to lipopolysaccharide (LPS; 150 $\mu g\ kg^{-1}\ h^{-1}$, i.v.) in the presence of saline, aminoguanidine (AG; 45 mg kg^{-1} bolus, 45 mg $kg^{-1}\ h^{-1}$ infusion), or AG and the non-selective endothelin receptor antagonist, SB 209670 (600 $\mu g\ kg^{-1}\ h^{-1}$), in conscious, chronically instrumented, Long Evans rats (350-450 g; $n=8$ in all groups). We used AG because there is evidence that it is a selective inhibitor of inducible nitric oxide synthase (iNOS), although recently it has been claimed AG also inhibits constitutive NOS.

2 Infusion of LPS in the presence of saline caused an early, transient hypotension (1-2 h) and a renal vasodilatation, with a secondary, delayed fall in mean arterial blood pressure (MAP), progressive tachycardia, and renal and hindquarters vasodilatation.

3 AG alone caused a rapid (within 30 s) transient rise in MAP (Δ 27±3 mmHg), accompanied by tachycardia and regional vasoconstrictions, but no reduction in regional flows, indicating the pressor effect of AG was, probably, largely due to an increase in cardiac output. These effects are not consistent with AG inhibiting constitutive NOS. In the presence of AG, LPS still caused an early, transient fall in MAP accompanied by a renal vasodilatation, but thereafter there was a significant rise in MAP (17±3 mmHg, 3 h after onset of LPS infusion) accompanied by bradycardia and marked mesenteric and hindquarters vasoconstrictions. However, 23 h after the onset of co-infusion of AG and LPS all variables were not different from baseline, except heart rate and renal vascular conductance, which were increased.

4 In the presence of AG and SB 209670, LPS caused progressive hypotension and increases in renal, mesenteric and hindquarters vascular conductances. Hence, SB 209670 prevented the rise in MAP and the regional vasoconstrictions seen with AG and LPS, indicating an involvement of endothelin in these events.

5 In the presence of AG and SB 209670, 23 h after the onset of LPS infusion, the AT_1-receptor antagonist, losartan (10 mg kg^{-1}), and the V_1-receptor antagonist, $d(CH_2)_5$-0-Me-Tyr-AVP (10 $\mu g\ kg^{-1}$, 10 $\mu g\ kg^{-1}\ h^{-1}$) caused additional incremental falls in MAP and increases in renal, mesenteric and hindquarters vascular conductances. Under these circumstances, MAP was lower and regional vascular conductances higher than in the other experiments following administration of losartan and $d(CH_2)_5$-0-Me-Tyr-AVP. Thus, although the findings are consistent with AG inhibiting iNOS, thereby revealing the pressor and vasoconstrictor actions of endothelin released by LPS, it is clear that LPS activates a very powerful hypotensive/vasodilator mechanism(s) which is resistant to AG, and whose full influence is only unmasked when the actions of endothelin, angiotensin II and vasopressin are inhibited.

Keywords: Lipopolysaccharide; aminoguanidine; SB 209670; endothelin; angiotensin II; vasopressin

Introduction

Recently, we reported preliminary observations that, during infusion of aminoguanidine (AG) and lipopolysaccharide (LPS) in conscious rats, there was a rise in mean arterial blood pressure accompanied by constriction in renal, mesenteric and hindquarters vascular beds (Waller *et al.*, 1994b). A possible explanation of these findings is that AG is not a selective inhibitor of inducible nitric oxide synthase (iNOS) (Laszlo *et al.*, 1995; Lopez-Belmonte & Whittle, 1995), and hence the pressor and vasoconstrictor effects were due to AG inhibiting constitutive NOS. However, this assertion goes against a substantial body of evidence indicating that AG is a selective inhibitor of iNOS (e.g. Corbett *et al.*, 1992; Misko *et al.*, 1993; Griffiths *et al.*, 1993; 1995; Joly *et al.*, 1994; Wu *et al.*, 1995a; Nakane *et al.*, 1995), and our own observations, in conscious rats, showing that, in the absence of LPS, AG has little pressor action, or effect on vasodilator responses to acetylcholine (Waller *et al.*, 1994b; 1995), unlike compounds which inhibit constitutive NOS (Gardiner *et al.*, 1990a, b, c; 1993). Thus, a more likely explanation of the rise in pressure and regional vasoconstrictions seen during infusion of AG and LPS is that suppression of iNOS activity by AG reveals the effects of endogenous vasoconstrictor mechanisms activated by LPS.

We have found that the non-selective endothelin antagonist, SB 209670 (Ohlstein *et al.*, 1994; Douglas *et al.*, 1995a, b), markedly enhances the hypotensive and vasodilator effects of LPS in conscious rats (Gardiner *et al.*, 1995a). Therefore, endothelin is a likely endogenous vasoactive agent, released during endotoxaemia (Sugiura *et al.*, 1989; Vemulapalli *et al.*, 1991), with vasoconstrictor effects that could be unmasked by AG. If that were the case, then the rise in mean arterial blood pressure and the regional vasoconstrictions seen during infusion of AG and LPS ought to be inhibited by SB 209670. In the present work we assessed that possibility.

Methods

All experiments were carried out on male, Long Evans rats (350-450 g) bred in the Biomedical Services Unit in Not-

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tingham. Under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1} i.p., supplemented as required) miniaturised pulsed Doppler probes (Haywood *et al.*, 1981) were placed around the left renal and superior mesenteric arteries, and the distal abdominal aorta. The probe wires ran through a small incision in the right flank and then subcutaneously to the back of the neck, where their free ends were taped together and anchored by a suture. Following surgery, animals were given free access to food and water, but were kept individually to recover. At least 7 days after probe implantation, rats were anaesthetized (see above) and had a catheter positioned in the distal abdominal aorta (via the ventral caudal artery) to record systemic arterial blood pressure and heart rate, and 3 catheters placed in the right jugular vein for the i.v. administration of substances. Experiments were begun the following day with animals unrestrained in their home cages with free access to food and water (Gardiner *et al.*, 1990a, b, c; 1991).
The following experimental protocols were run:

Effects of saline and LPS co-infusion (n = 8)

At 07h 00 min, an i.v. infusion of isotonic saline (154 mmol l^{-1} NaCl, 0.4 ml h^{-1}) was begun and continued for 24 h. One hour after the onset of saline administration, an infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$, 0.4 ml h^{-1}; Waller *et al.*, 1994a; Gardiner *et al.*, 1995b) was begun and continued for 23 h.

Effects of AG and LPS co-infusion (n = 8)

At 07h 00 min, a primed infusion of AG (45 mg kg^{-1} bolus, 45 mg $kg^{-1}\ h^{-1}$ infusion, 0.4 ml h^{-1}) (Waller *et al.*, 1994b) was begun, followed 1 h later by LPS infusion (as above).

Effects of AG, SB 209670 and LPS co-infusion (n = 8)

At 07h 00 min, a bolus injection of AG (45 mg kg^{-1}) was given immediately followed by an infusion (0.4 ml h^{-1}) of a mixture of AG (as above) and SB 209670 (600 $\mu g\ kg^{-1}\ h^{-1}$; Gardiner *et al.*, 1995a); 1 h later an infusion of LPS (as above) was begun.

In all protocols, 24 h after the beginning of the experiment, the AT_1-receptor antagonist, losartan, was administered (10 mg kg^{-1} i.v.; Batin *et al.*, 1991), followed 15 min later by the V_1-receptor antagonist, $d(CH_2)_5$-0-Me-Tyr-vasopressin (abbreviated to AVPX) (10 $\mu g\ kg^{-1}$ bolus, 10 $\mu g\ kg^{-1}\ h^{-1}$; Gardiner *et al.*, 1989). This approach allowed assessment of the possible involvement of angiotensin II (AII) and arginine vasopressin (AVP) in the maintenance of cardiovascular status (Gardiner *et al.*, 1996).

Data analysis

Continuous recordings were made of phasic and mean arterial blood pressures, instantaneous heart rate, and phasic and mean Doppler shift signals. Vascular conductances were calculated from mean arterial blood pressure and mean Doppler shift signals (Gardiner *et al.*, 1990a, b, c; 1995b; Waller *et al.*, 1994a). Within-group analysis was by Friedman's test; between-group analysis was by the Kruskal-Wallis test, applied either to the integrated responses (i.e., areas under or over curves), or to values at selected time points. A P value <0.05 was taken as significant.

Materials

LPS (*E. coli* serotype 0127 B8) was obtained from Sigma (U.K.); AG hydrochloride was obtained from Aldrich (U.K.); $d(CH_2)_5$-0-Me-Tyr-AVP was obtained from Bachem (U.K.). Losartan was a gift from Dr R.D. Smith (DuPont, U.S.A.) and SB 209670 ([(±)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy) indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.).

Results

Resting cardiovascular variables in the 3 groups of rats studied are shown in Table 1. There were no significant differences between the groups.

Effects of saline and LPS co-infusion

As previously reported (Waller *et al.*, 1994a; Gardiner *et al.*, 1995b), during co-infusion of saline and LPS there was a modest, biphasic fall in mean arterial blood pressure (MAP), and a progressive tachycardia and renal and hindquarters vasodilatation (Figure 1).

Effects of AG and LPS co-infusion

The bolus injection of AG at the onset of the primed infusion of AG evoked a rapid rise in MAP (peak increase 27±3 mmHg at 30 s (Figure 2)), tachycardia, increases in renal and mesenteric flows, but decreases in renal, mesenteric and hindquarters vascular conductance (Figure 2). By 1 h after the start of AG infusion there was a small, but significant, elevation in MAP, and there were slight reductions in heart rate and in hindquarters flow and vascular conductance (Figures 1 and 2).

During co-infusion of AG and LPS, the initial fall in MAP (at 1 h after LPS) was similar to that seen during co-infusion of

Table 1 Resting cardiovascular variables in conscious, Long Evans rats

	Saline + LPS (n = 8)	*Aminoguanidine + LPS (n = 8)*	*Aminoguanidine + SB 209670 + LPS (n = 8)*
Heart rate (beats min^{-1})	331±4	321±8	342±6
Mean arterial blood pressure (mmHg)	103±1	106±1	106±2
Renal Doppler shift (kHz)	6.2±0.9	6.6±0.8	7.0±0.7
Mesenteric Doppler shift (kHz)	7.4±0.8	6.8±0.8	6.7±0.5
Hindquarters Doppler shift (kHz)	4.0±0.5	4.4±0.2	4.6±0.3
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	60±9	62±8	66±7
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	72±8	64±8	63±5
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	39±4	42±2	44±3

Values are mean±s.e.mean.

saline and LPS, but by 2 h the hypotension had waned in the former group, and thereafter there was a significant rise in MAP (4–6 h) (Figure 1). By 23 h after the start of AG and LPS co-infusion, MAP was not different from baseline, in contrast to the hypotension seen in the saline and LPS group (Figure 1).

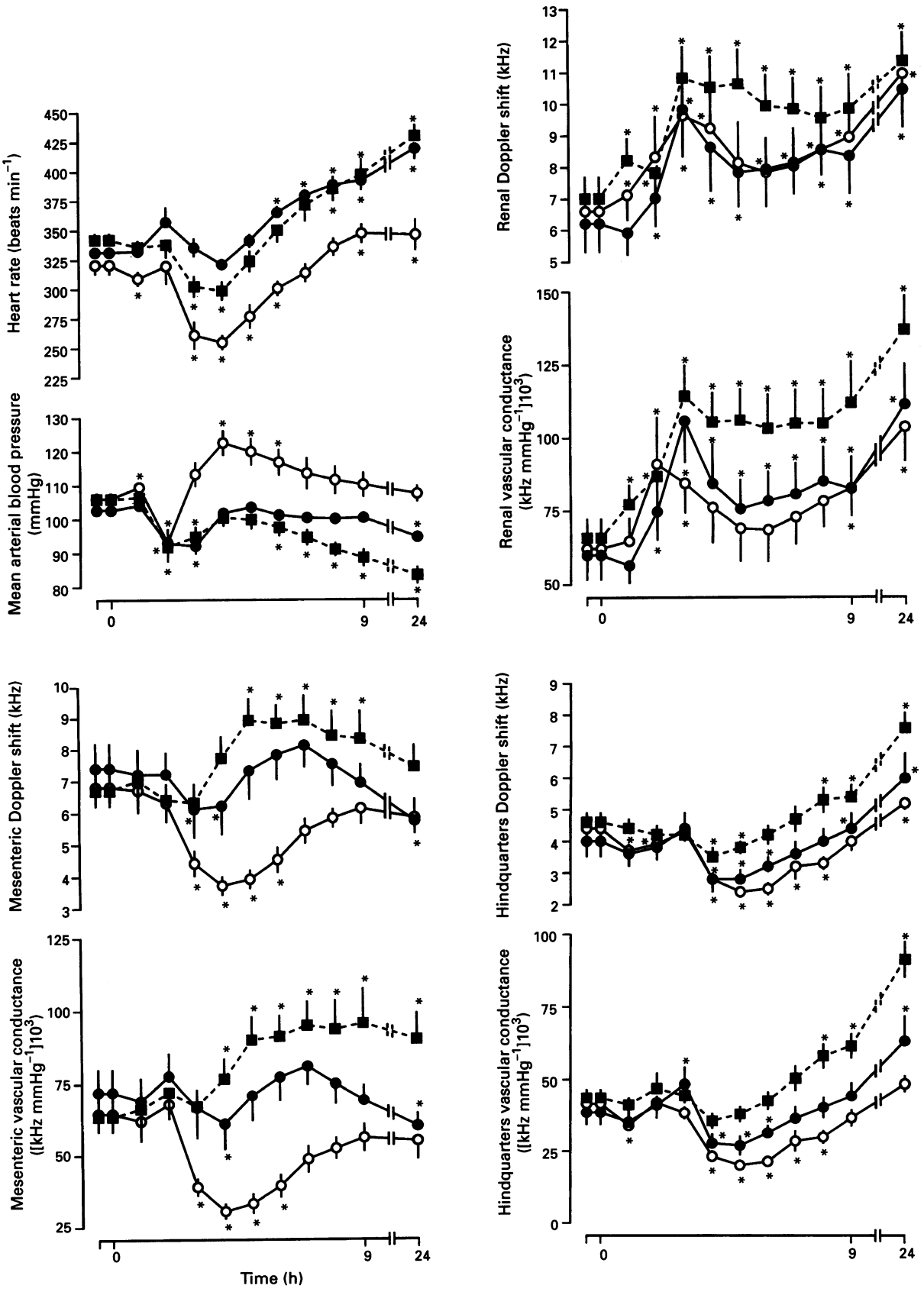

Figure 1 Resting cardiovascular variables and responses to saline infusion (beginning at time = 0 h) followed 1 h later by co-infusion of LPS (150 μg kg^{-1} h^{-1}; ●, $n=8$), or aminoguanidine (45 mg kg^{-1}, 45 mg kg^{-1} h^{-1}) followed 1 h later by LPS (○, $n=8$) or aminoguanidine, and SB 209670 (600 μg kg^{-1} h^{-1}) followed 1 h later by LPS (■, $n=8$). Values are mean with s.e.mean; *$P<0.05$ versus baseline. Intergroup differences are given in the text.

The rise in MAP during co-infusion of AG and LPS was accompanied by a significant bradycardia and the subsequent tachycardia was significantly attenuated relative to the response in the group receiving saline and LPS; this difference was still present 23 h after the start of LPS infusion (Figure 1).

The changes in renal haemodynamics during co-infusion of AG and LPS were not different from those during co-infusion of saline and LPS (Figure 1). However, there were transient reductions in mesenteric flow and vascular conductance in the former group that were significantly greater than the changes seen in the latter group, although 23 h after the onset of LPS infusion these variables were not different in the two groups (Figure 1). The initial reductions and subsequent increases in hindquarters flow were similar during co-infusion of AG and LPS, and saline and LPS (Figure 1). However, after 23 h of AG and LPS co-infusion, hindquarters vascular conductance was not different from baseline, in contrast to the vasodilatation seen in the saline and LPS-treated group (Figure 1). The initial mesenteric and hindquarters vasoconstrictions coincided with the rise in MAP (Figure 1).

Effects of AG, SB 209670 and LPS co-infusion

The bolus injection of AG evoked responses as described above. During co-infusion of AG and SB 209670, prior to administration of LPS, there were significant increases in renal flow and vascular conductance (Figure 1). There was no change in MAP, in contrast to the small increase seen in the group receiving AG without SB 209670 (Figure 1).

By 1 h after the start of LPS infusion, MAP had fallen to the same extent as in the presence of saline and LPS, but from 5 h onwards there was a progressive hypotension in the presence of AG, SB 209670 and LPS that was significantly greater than in the other experiments (Figure 1); this difference persisted 23 h after the onset of LPS infusion. The tachycardia with AG, SB 209670 and LPS was not different from that during co-infusion of saline and LPS, but it was greater than in the presence of AG and LPS (Figure 1).

During co-infusion of AG, SB 209670 and LPS, the renal vasodilatation was greater than during co-infusion of AG and LPS, but not significantly different from that during infusion of saline and LPS (Figure 1).

There were significant increases in mesenteric flow and mesenteric and hindquarters vascular conductances during co-infusion of AG, SB 209670 and LPS that were significantly different from the responses in the other two experiments (Figure 1); these inter-group differences persisted 23 h after the onset of LPS infusion.

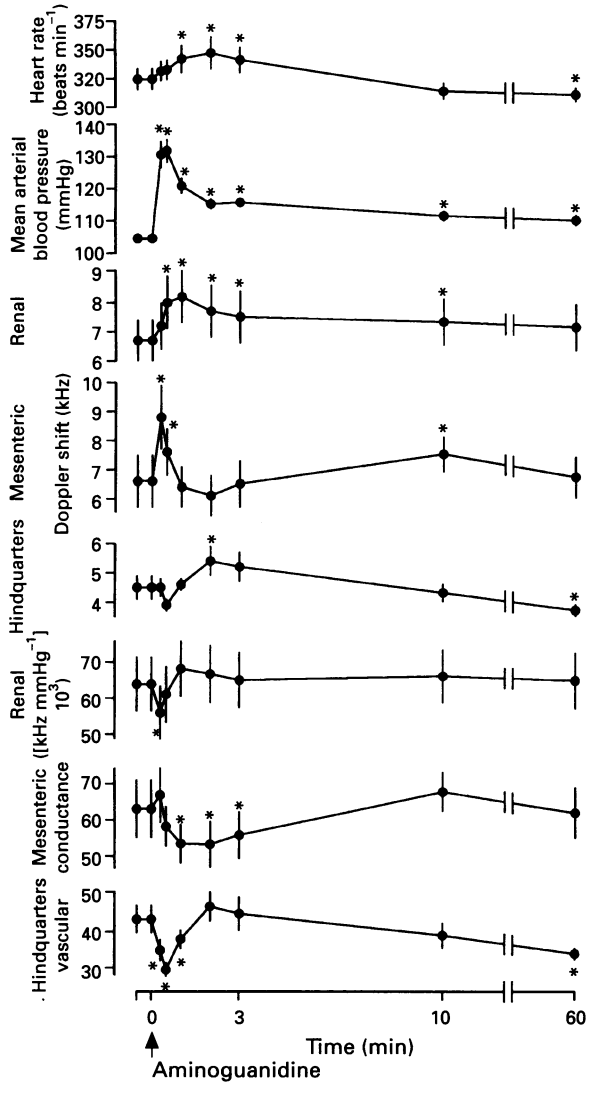

Figure 2 Resting cardiovascular variables and changes following i.v. injection of aminoguanidine (45 mg kg^{-1}, 45 mg kg^{-1} h^{-1}) in conscious rats. Values are mean with s.e.mean, $n=8$; $^*P<0.05$ versus baseline.

Effects of losartan and d(CH$_2$)$_5$-0-Me-Tyr-AVP (V_1-receptor antagonist)

In all three experiments, losartan caused similar, significant falls in MAP and increases in heart rate (Figure 3). Following losartan, the vasopressin antagonist (abbreviated to AVPX) evoked additional, similar falls in MAP in all three protocols, but the further tachycardia with AVPX was significantly greater in the presence of AG and LPS than in the presence of saline and LPS, or AG, SB 209670 and LPS (Figure 3). Fifteen min after the onset of AVPX infusion, MAP was lower in the animals that had received AG, SB 209670, LPS and losartan than in the other two groups (which were similar), but heart rates were not different (Figure 3).

In all three protocols, losartan caused similar increases in renal flow, but the renal vasodilatation was greater in the presence of AG, SB 209670 and LPS than in the other two conditions (Figure 3). AVPX had no additional effects on renal flow, but it caused a similar vasodilatation in the presence of AG and LPS, and in the presence of AG, SB 209670 and LPS; this effect was not apparent in the presence of saline and LPS (Figure 3). At the end of the experiment, renal vascular conductance was higher in the group receiving AG, SB 209670, LPS, losartan and AVPX than in the other two groups (Figure 3).

In the presence of AG, SB 209670 and LPS, losartan caused increases in mesenteric flow and vascular conductance that were greater than in the other two experimental groups (Figure 3). AVPX caused additional increases in mesenteric flow and vascular conductance in the presence of AG and LPS, and in the presence of AG, SB 209670 and LPS, but in mesenteric vascular conductance only in the presence of saline and LPS (Figure 3). In the latter group, the increase in mesenteric vascular conductance was less than in the other two experiments (Figure 3). At the end of the experiment, mesenteric vascular conductance was highest in the group receiving AG, SB 209670, LPS, losartan and AVPX (Figure 3).

Losartan had no effect on hindquarters flow in any group, but caused vasodilatation in all (Figure 3). The vasodilatation was significantly greater in the presence of AG, SB 209670 and LPS than in the presence of saline and LPS, but there were no other significant inter-group differences.

AVPX caused a slight increase in hindquarters flow only in the group receiving AG and LPS, but the hindquarters vasodilator effect was similar in all three groups (Figure 3). At the end of the experiment, hindquarters flow and vascular conductance were highest in the group receiving AG, SB 209670, LPS, losartan and AVPX (Figure 3).

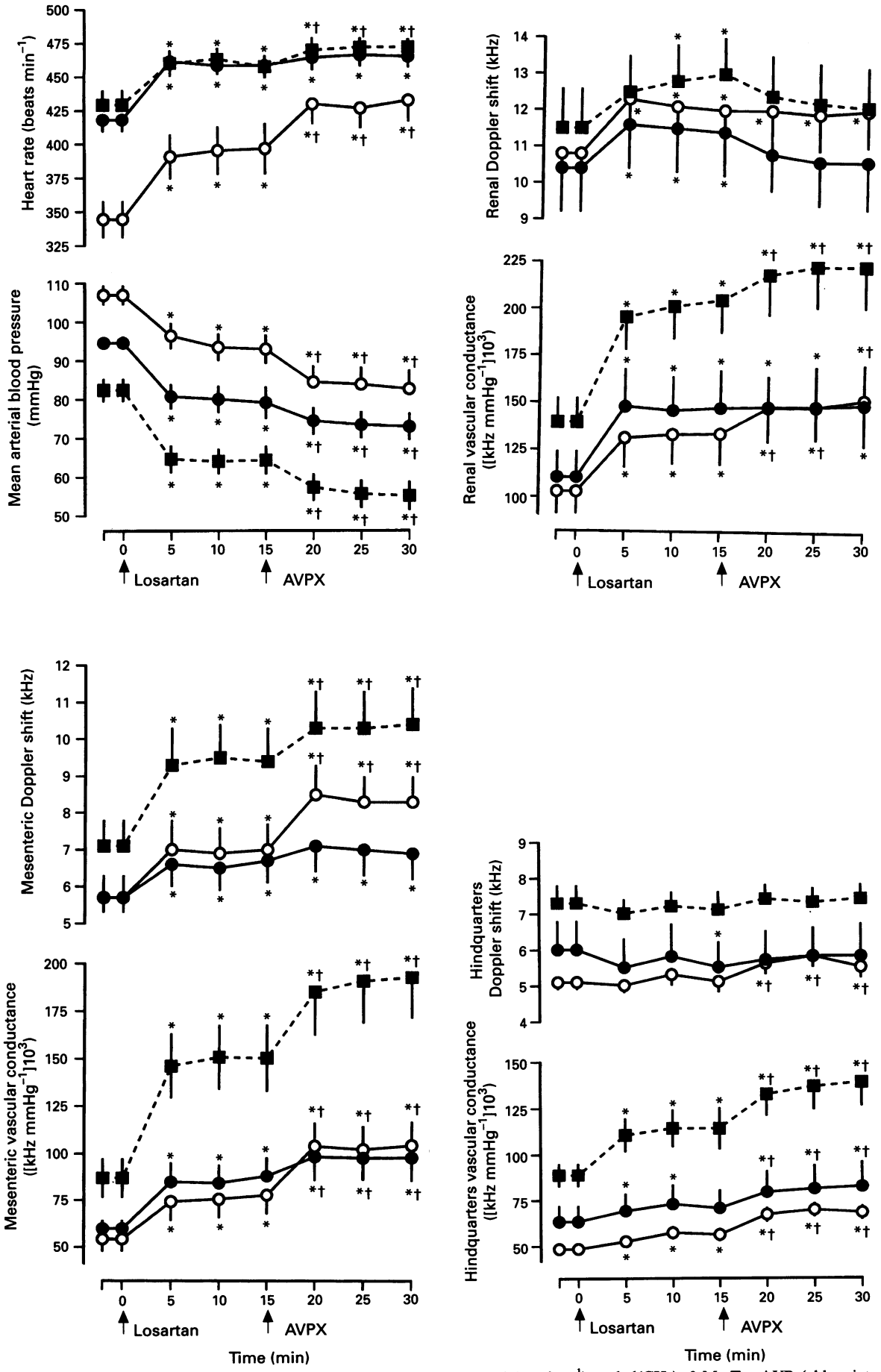

Figure 3 Resting cardiovascular variables and responses to losartan (10 mg kg^{-1}) and d(CH$_2$)$_5$-0-Me-Tyr-AVP (abbreviated to AVPX, 10 μg kg^{-1}, 10 μg kg^{-1} h^{-1}), 23 h after start of saline and LPS co-infusion (●, $n=8$) or aminoguanidine and LPS co-infusion (○, $n=8$) or aminoguanidine, SB 209670 and LPS co-infusion (■, $n=8$). Values are mean with s.e.mean; *$P<0.05$ versus baseline, †$P<0.05$ versus value immediately before AVPX. Intergroup differences are given in the text.

Discussion

In the present work we tested the hypothesis that the pressor and vasoconstrictor effects seen during infusion of AG and LPS were due to the former unmasking the actions of endothelin released by the latter. Our results are more consistent with this hypothesis than with the proposal that the pressor and vasoconstrictor effects of AG are attributable to AG inhibiting constitutive NOS (Laszlo *et al.*, 1995).

Recently, Laszlo *et al.* (1995) reported that AG showed only a slight (about 2 fold) selectivity for iNOS, and observed that AG injected s.c. (50 mg kg^{-1}) caused a substantial pressor effect (maximum Δ 33 $\pm$ 5 mmHg) in pentobarbitone-anaesthetized, control rats, consistent with inhibition of constitutive NOS. These findings appeared to be corroborated by Lopez-Belmonte & Whittle (1995) who reported that AG injected i.v. (at doses between 25 and 100 mg kg^{-1}) caused substantial pressor effects (maximum $\Delta = 35 \pm 2$ mmHg) in pentobarbitone-anaesthetized rats. However, Lopez-Belmonte & Whittle (1995) found the pressor effect of AG peaked at 1 min and was gone by 60 min, whereas Laszlo *et al.* (1995) reported that the pressor effect of AG developed progressively over the 60 min following injection. Although these disparate findings were commented upon by Lopez-Belmonte & Whittle (1995), they offered no explanation for them, and it seems unlikely they could have been due solely to the different routes of administration of AG.

Here we show that, although a primed infusion of AG had a similar maximal pressor effect to that described by Lopez-Belmonte & Whittle (1995), this effect was very transient and was not accompanied by any significant reduction in regional blood flows or heart rate. Hence, it is likely that the pressor action of AG was largely due to an increase in cardiac output. Such an influence is quite different from that of established inhibitors of constitutive NOS, the pressor effects of which are accompanied by reductions in regional blood flows and cardiac output (Gardiner *et al.*, 1990a, b, c). Moreover, these effects of inhibitors of constitutive NOS are well-maintained for over 60 min, whereas the rapid-onset cardiovascular effects of AG were poorly maintained, in spite of its being infused. Thus, our results fit with the finding that the selectivity of AG for iNOS (Wu *et al.*, 1995a; Nakane *et al.*, 1995) is similar to that of the more recently described agents, such as S-ethylisothiourea (Nakane *et al.*, 1995; Southan *et al.*, 1995). Of course, this does not mean that additional properties of AG (Seiler *et al.*, 1985; Kumari *et al.*, 1991; Ohrui *et al.*, 1992; Ou & Wolff, 1993) can be ignored. Indeed, they may account for its acute, rapid-onset cardiovascular effects described here, the underlying mechanism for which is unknown.

Whatever the explanation of the initial cardiovascular actions of AG, it is clear that in its presence, the early (within 1 h) hypotensive and haemodynamic responses to LPS were unaffected, consistent with these being due to factors other than increased iNOS activity (Gardiner *et al.*, 1995b). Subsequently, in the presence of AG and LPS, there was a significant rise in MAP, accompanied by reductions in mesenteric and hindquarters flows and vascular conductances. These effects were absent in the additional presence of SB 209670, which fits with endothelin being responsible for them.

Twenty three hours after the start of infusion of AG, SB 209670 and LPS, MAP was lower and renal, mesenteric and hindquarters vascular conductances were higher than in the other conditions in the present study. The finding that the haemodynamic profile at this juncture was not significantly different from that in animals treated only with SB 209670 and LPS for 23 h (Gardiner *et al.*, 1995a), indicates that endothelin normally opposes the actions of a vasodilator agent(s) other than NO, consistent with iNOS activity not being increased at this stage (Gardiner *et al.*, 1995b).

In the combined presence of dexamethasone, SB 209670 and LPS, there is an enhanced, but transient hypotension and renal and mesenteric vasodilatation (Gardiner *et al.*, 1996). In contrast, in the combined presence of AG, SB 209670 and LPS, the fall in MAP and regional vasodilatations are persistent (present study). One possible explanation of the delayed AG-insensitive, dexamethasone-suppressible events is that they are due to LPS-induced expression of cyclo-oxygenase-2 (Kujubu & Herschman, 1992; Wu *et al.*, 1995b). However, we cannot discount the possibility that prevention of vascular hyporeactivity by dexamethasone (Paya *et al.*, 1993; Szabo *et al.*, 1993; Wu *et al.*, 1995b) contributes to its ability to suppress the later hypotensive and vasodilator responses to LPS, which are resistant to AG. It is feasible such an action of dexamethasone is a consequence of suppression of iNOS induction, but, if so, a similar effect ought to have been seen with AG.

In this context, the relative enhancement of the vasodilator responses to losartan and AVPX in the presence of AG, SB 209670 and LPS (compared to the other conditions) is notable. One interpretation of these findings could otherwise have been increased vascular sensitivity to AII and AVP, due to inhibition of iNOS by AG. An alternative explanation, then, is that the relative hypotension in the presence of AG, SB 209670 and LPS was a greater stimulus for activation of the renin-angiotensin system and vasopressin release, and/or that SB 209670 antagonized inhibitory effects of endothelin on release of renin and vasopressin, although such effects are contentious (see Rubanyi & Polokoff, 1994).

In the presence of AG, SB 209670, LPS, losartan, and AVPX, MAP was low, regional blood flows elevated, and regional vascular conductances were higher than we have found in any other hypotensive state. Thus, whatever the vasodilator mechanism(s) activated under these conditions, they are remarkably powerful.

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