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Temporal differences between the involvement of angiotensin II and endothelin in the cardiovascular responses to endotoxaemia in conscious rats

[1]S.M. Gardiner, P.A. Kemp, J.E. March & T. Bennett

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

1 Male, Long Evans rats were instrumented chronically with pulsed Doppler probes and intravascular catheters to allow assessment of regional haemodynamic changes during i.v. infusion of lipopolysaccharide (LPS, 150 $\mu g\ kg^{-1}\ h^{-1}$).

2 In the presence of the AT_1-receptor antagonist, losartan (10 mg kg^{-1} + 10 mg $kg^{-1}\ h^{-1}$), the initial (1-2 h) hypotensive and renal, mesenteric and hindquarters vasodilator responses to LPS were enhanced significantly. Thereafter these effects waned, but between 8-23 h after the onset of LPS infusion, a further fall in mean arterial blood pressure (MAP) and increases in renal and hindquarters flows and conductances occurred. All these changes were significantly greater than seen with losartan or LPS alone, and exceeded the sum of their effects.

3 In the presence of captopril (2 mg kg^{-1} + 2 mg $kg^{-1}\ h^{-1}$), the initial hypotensive and renal vasodilator responses to LPS were enhanced, but less so than in the presence of losartan. However, the effects of LPS in the presence of losartan and captopril together were not different from those in the presence of losartan alone. These observations indicate that the ability of captopril to inhibit the degradation of bradykinin had no additional influence, and the differences between the effect of captopril and losartan on the initial effects of LPS were probably due to more effective suppression of the action of angiotensin II by losartan.

4 In the absence of LPS, co-infusion of losartan and the non-selective endothelin antagonist, SB 209670 (600 $\mu g\ kg^{-1}$ + 600 $\mu g\ kg^{-1}\ h^{-1}$), caused a substantial, progressive hypotension (-25 ± 2 mmHg at 24 h) accompanied by increases in renal, mesenteric and hindquarters vascular conductances (31 ± 13, 44 ± 9 and 45 ± 12%, respectively), indicating an involvement of angiotensin II and endothelin in the maintenance of normal cardiovascular status in conscious, Long Evans rats.

5 In the presence of losartan and SB 209670, the initial, LPS-induced fall in MAP (-42 ± 2 mmHg) was not different from that in the presence of losartan (-39 ± 4 mmHg), and the increases in renal, in mesenteric, and in hindquarters vascular conductances were similar in the two conditions. However, there was no recovery in MAP, and there were persistent renal, mesenteric and hindquarter vasodilatations.

6 In all experiments involving LPS, administration of the V_1-receptor antagonist, $d(CH_2)_5$-O-Me-Tyr-AVP (10 $\mu g\ kg^{-1}$), 23 h after the start of LPS infusion caused additional hypotension and mesenteric vasodilatation, particularly. This effect was most marked in animals pretreated with losartan and SB 209670.

7 The results indicate that the initial (1-2 h) depressor and dilator effects of LPS infusion in conscious Long Evans rats are opposed by the actions of angiotensin II, rather than endothelin. However, between 2-8 h after the onset of LPS infusion the involvement of endothelin develops and that of angiotensin II fades. By 24 h after the start of infusion of LPS, the pressor and vasoconstrictor actions of endothelin wane, and a role of vasopressin is apparent. At no stage is there clear evidence for an involvement of bradykinin in the haemodynamic sequelae of endotoxaemia in this model.

Keywords: Lipopolysaccharide; angiotensin II; endothelin; vasopressin; losartan; SB 209670; captopril; $d(CH_2)_5$-O-Me-Tyr-AVP

Introduction

It has been known for some time that vasoconstrictor and vasodilator mechanisms are activated in endotoxaemia (e.g. Schaller *et al.*, 1985; see Bone, 1991). Recently, the emphasis has been on vasodilator mechanisms, in part due to the predominance of research on nitric oxide, but also, probably, on account of the widespread reports that responses to exogenous vasoconstrictor agents are blunted in endotoxaemia (Parratt, 1973; Schaller *et al.*, 1985; Guc *et al.*, 1990; Szabo *et al.*, 1993; Waller *et al.*, 1994). Hence, even if circulating levels of catecholamines, angiotensin II, vasopressin and endothelin are elevated in this condition (Schaller *et al.*, 1985; Sugiura *et al.*, 1989; Vemulapalli *et al.*, 1991), it does not follow that these agents will be exerting significant vasoconstrictor influences (see Schaller *et al.*, 1985). However, we have found (Gardiner *et al.*, 1995a) that pretreatment with the non-selective endothelin antagonist, SB 209670 (Ohlstein *et al.*, 1994; Douglas *et al.*, 1995a,b), markedly enhances the hypotensive and mesenteric and hindquarters vasodilator effects of lipopolysaccharide (LPS) infusion in conscious rats, indicating an important role for this peptide in opposing the vasodilator mechanisms activated by endotoxaemia. While it could be that endothelin is unique in this respect, we subsequently observed (Gardiner *et al.*, 1996a, b) that the AT_1-receptor antagonist, losartan, caused modest, but significant, hypotensive and vasodilator effects, 24 h after the onset of LPS infusion, indicating that angiotensin II was contributing to the maintenance of cardiovascular status, at least at that stage.

[1] Author for correspondence.

These observations raised the possibility that angiotensin II could have a more important role at earlier time points during endotoxaemia, so the first objective of the present work was to delineate regional haemodynamic changes during co-infusion of the AT_1-receptor antagonist, losartan, and LPS. To permit a full assessment of the effects of losartan (or the other drugs used, see below) on the cardiovascular responses to LPS, we included a group of animals that received a continuous co-infusion of saline and LPS.

In this first experiment, we chose to use an AT_1-receptor antagonist, rather than an angiotensin-converting enzyme (ACE) inhibitor, because there is evidence for some effects of ACE inhibitors being due to inhibition of bradykinin degradation (see Linz *et al.*, 1995 for review), and for an involvement of bradykinin in the cardiovascular sequelae of endotoxaemia (Katori *et al.*, 1989; see Mombouli & Vanhoutte, 1995). Thus, one would expect significant differences between the effects of an AT_1-receptor antagonist and an ACE inhibitor on the cardiovascular responses to LPS. Because this is an important point, our second objective was to assess cardiovascular responses to LPS in the presence of the ACE inhibitor, captopril, or captopril and losartan, to determine if captopril had additional properties that could be attributed to inhibition of bradykinin degradation.

In the absence of LPS, we have found that SB 209670 and losartan have more than an additive hypotensive effect in conscious rats (Gardiner *et al.*, 1995c), consistent with a positive interaction between endothelin and angiotensin II (Yoshida *et al.*, 1991; 1992). Since LPS stimulates the production of both these peptides (see above), it is feasible their combined involvement in the support of cardiovascular status would be enhanced in that circumstance. Therefore, our third objective was to compare the cardiovascular effects of co-infusion of SB 209670 and losartan, together with saline or LPS.

Since in previous experiments we obtained evidence for an involvement of vasopressin in maintaining cardiovascular status after a 24 h infusion of LPS (Gardiner *et al.*, 1996a,b), we assessed responses to the V_1-receptor antagonist, $d(CH_2)_5$-O-Me-Tyr-AVP, at the end of all experiments in the present study, to determine whether vasopressin was involved to different extents, when other systems were antagonized.

Methods

All experiments were performed on male, Long Evans rats (350–450 g), bred in Nottingham. The surgical procedures for implanting pulsed Doppler probes (renal, mesenteric and hindquarters) and intravascular catheters were carried out under anaesthesia (sodium methohexitone (Brietal, Lilly), 40–60 mg kg^{-1} i.p., supplemented as required), as described in detail previously (Gardiner *et al.*, 1989).

At least 24 h after the last surgical intervention (catheter implantation), with animals conscious, unrestrained, and with free access to food and water in their home cages, the following randomized experimental protocols were run:-

Experiment 1: Cardiovascular changes during infusion of saline and LPS

Saline (154 mmol l^{-1} NaCl, 0.4 ml h^{-1}) administration was begun 1 h before co-infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$ in saline, infused at 0.4 ml h^{-1}; Group 1, $n=9$) for 23 h; at that juncture, losartan (10 mg kg^{-1}; Batin *et al.*, 1991) and the vasopressin, V_1-receptor antagonist, $d(CH_2)_5$-O-Me-Tyr-AVP (abbreviated to AVPX) (10 $\mu g\ kg^{-1}$, Gardiner *et al.*, 1989) were administered.

Experiment 2: Cardiovascular changes during infusion of losartan together with saline or LPS

Losartan (10 mg kg^{-1} bolus + 10 mg $kg^{-1}\ h^{-1}$ infusion, 0.4 ml h^{-1}) administration was begun 1 h before co-infusion of saline (0.4 ml h^{-1}; Group 2, $n=8$) or LPS (150 $\mu g\ kg^{-1}\ h^{-1}$ in saline, infused at 0.4 ml h^{-1}; Group 3, $n=9$) for 23 h; at that juncture, the AVPX (as above) was administered.

Experiment 3: Cardiovascular changes during infusion of captopril and LPS, or co-infusion of captopril and losartan, and LPS

Captopril (2 mg kg^{-1} bolus + 2 mg $kg^{-1}\ h^{-1}$ infusion, 0.4 ml h^{-1}, Group 4, $n=11$), or captopril and losartan (Group 5, $n=8$) administration was begun, 1 h before co-infusion of LPS for 23 h; at that juncture, AVPX was administered (as above).

Experiment 4: Cardiovascular changes during co-infusion of losartan and SB 209670, and saline or LPS

Losartan and SB 209670 (600 $\mu g\ kg^{-1}$ bolus + mg $kg^{-1}\ h^{-1}$ infusion at 0.4 ml h^{-1}) administration was begun 1 h before co-infusion of saline (Group 6, $n=8$) or LPS (Group 7, $n=8$) for 23 h, after which AVPX was given (as above).

Data analysis

Recordings were made of phasic and mean arterial blood pressures and instantaneous heart rate, together with phasic and mean Doppler shift signals from the renal, mesenteric and hindquarters probes. Percentage changes in mean Doppler shift were taken as indices of changes in flow, and percentage changes in vascular conductance were calculated

Table 1 Resting cardiovascular variables in the 7 groups (see Methods) of conscious, Long Evans rats studied

	Experiment 1	*Experiment 2*		*Experiment 3*		*Experiment 4*	
	Group 1 (n=9)	*Group 2* (n=8)	*Group 3* (n=9)	*Group 4* (n=11)	*Group 5* (n=8)	*Group 6* (n=8)	*Group 7* (n=8)
Heart rate (beats min^{-1})	321±5	326±9	336±7	332±5	328±8	342±11	348±5
Mean arterial blood pressure (mmHg)	104±1	101±2	103±1	103±2	102±1	102±2	102±1
Renal Doppler shift (kHz)	6.4±0.2	6.4±0.5	6.6±0.6	6.6±0.8	5.8±0.3	6.9±0.8	5.5±0.3
Mesenteric Doppler shift (kHz)	6.3±0.6	6.1±0.6	6.8±0.6	5.9±0.6	5.8±0.5	6.8±0.5	5.6±0.3
Hindquarters Doppler shift (kHz)	4.1±0.2	3.7±0.3	4.3±0.3	5.0±0.3	5.7±0.4	4.6±0.2	4.3±0.2
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	61±2	63±5	64±6	65±7	57±3	68±8	54±3
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	61±6	60±5	66±6	57±5	57±5	68±5	55±3
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	40±2	36±3	42±3	49±4	55±4	46±2	42±2

Values are mean±s.e.mean.

from mean Doppler shift divided by mean arterial blood pressure (Gardiner *et al.*, 1996a).

Between-group comparisons were made with the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Within-group comparisons were made with Friedman's test; $P<0.05$ was taken as significant.

Materials

LPS (*E coli* serotype 0127 B8) and captopril were obtained from Sigma (U.K.). Losartan was a gift from Dr R.D. Smith (Du Pont, U.S.A.) and SB 209670 ([(±)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-

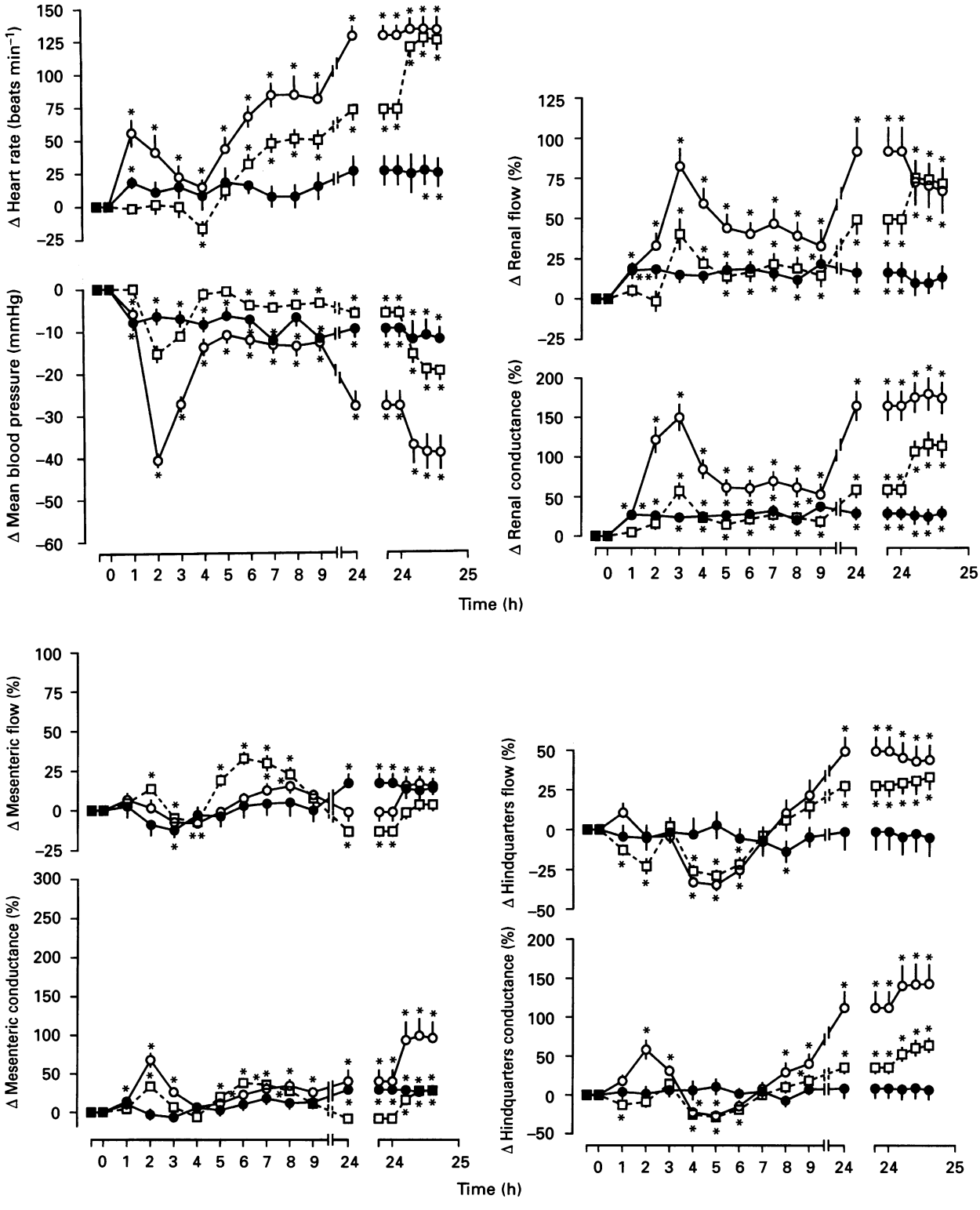

Figure 1 Cardiovascular changes in conscious Long Evans rats receiving saline infusion for 1 h before co-infusion of LPS (150 μg kg^{-1} h^{-1}) for 23 h (□, $n=9$, Group 1), or losartan (10 mg kg^{-1}, 10 mg kg^{-1} h^{-1}) for 1 h before co-infusion of saline for 23 h (●, $n=8$, Group 2), or losartan for 1 h before co-infusion of LPS for 23 h (○, $n=9$, Group 3). Twenty four hours after the beginning of the experiments, the vasopressin antagonist, d(CH$_2$)$_5$-O-Me-Tyr-AVP (10 μg kg^{-1}) was administered alone (Groups 2 and 3) or together with losartan (10 mg kg^{-1}; Group 1); responses to these interventions are indicated in the text, as are between-group differences. Note the change in time scale between 24 and 25 h. Values are mean with s.e.mean; *$P<0.05$ versus original baseline.

(prop-1-yloxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.). Captopril was buffered to pH 7.4 with Na_2CO_3 (0.5%). d(CH_2)$_5$-O-Me-Tyr-AVP was purchased from Bachem (UK) Ltd.

Results

Resting cardiovascular variables are shown in Table 1.

Cardiovascular changes during infusion of saline and LPS (Group 1)

During the first hour, when saline was infused alone, there were slight reductions in hindquarters flow and vascular conductance only (Figure 1). Over the first 2 h of the onset of co-infusion of LPS, there was a fall in mean arterial blood pressure (MAP) and increases in renal and mesenteric flows and vascular conductances, but slight decreases in the hindquarters flow (Figure 1). Thereafter, MAP recovered as the renal and mesenteric vasodilatation waned, and hindquarters vasoconstriction occurred (Figure 1). The subsequent redevelopment of a slight fall in MAP (5–8 h) was accompanied by sustained tachycardia and a transient mesenteric vasodilatation, with a more persistent elevation in renal vascular conductance and a progressive increase in hindquarters conductance (Figure 1). Twenty four hours after the beginning of the experiment, administration of losartan and the AVPX caused a significant further fall in MAP and tachycardia, accompanied by increases in renal and mesenteric flows and renal, mesenteric and hindquarters vascular conductances (Figure 1).

Cardiovascular changes during infusion of losartan and saline (Group 2)

Over the first hour, when losartan was administered alone, there was a small, but significant fall in MAP and a tachycardia, accompanied by increases in renal flow and vascular conductance (Figure 1); these effects were significantly more than those during infusion of saline. Over the following 2 h, when saline was co-infused with losartan, the tachycardia was inconsistent, but the fall in MAP and renal hyperaemia and vasodilatation persisted. Changes in mesenteric flow were variable, but by 23 h after the onset of co-infusion of losartan and saline, mesenteric flow and vascular conductance were increased (Figure 1). Twenty four hours after the beginning of the experiment, administration of the AVPX caused a slight reduction in renal flow only (Figure 1).

Cardiovascular changes during infusion of losartan and LPS (Group 3)

Prior to the onset of LPS infusion, losartan caused a slight fall in MAP together with tachycardia and increases in renal flow and vascular conductance (Figure 1). These changes were qualitatively similar to those seen in Group 2 during the first hour after the start of losartan administration, although the magnitude of the tachycardia was larger in Group 3 than Group 2; we have no explanation for this. Between 1 and 2 h after the onset of co-infusion of losartan and LPS, there was a marked, additional fall in MAP, and further increases in renal flow and vascular conductance, together with vasodilatation in the mesenteric and hindquarters vascular beds. All these effects were significantly different from the changes, or the sum of the changes, seen in groups 1 and 2 (see above), although, at that stage, the tachycardia was not increased (Figure 1). However, heart rate increased thereafter, and MAP showed a marked recovery, although it remained significantly below baseline levels (Figure 1, Table 2). The recovery in MAP was accompanied by a waning of the substantial increases in renal flow and vascular conductance, and of the mesenteric vasodilatation; also there were reductions in hindquarters flow and vascular conductance (Figure 1, Table 2). Between 9 and 24 h after the beginning of the experiment, there was further hypotension and tachycardia, accompanied by marked increases in renal and hindquarters flows and vascular conductances (Figure 1, Table 2). These changes were significantly different from those seen in groups 1 and 2 and exceeded the sum of their effects (Figure 1). At 24 h, the AVPX caused a marked further fall in MAP, similar to that seen in animals given losartan and the AVPX in the presence of saline and LPS, although there was no additional tachycardia (Figure 1). Renal flow fell, although renal vascular conductance rose slightly, but less than in animals given losartan and the AVPX in the presence of saline and LPS (Figure 1). There were significant increases in mesenteric flow and vascular conductance, and a slight increase in hindquarters vascular conductance (Figure 1). The mesenteric vasodilatation was significantly greater than that seen in groups 1 and 2 (Figure 1).

Table 2 Cardiovascular changes in conscious, Long Evans rats during infusion of LPS, in the presence of losartan (n=9, Group 3), in the presence of losartan and captopril (n=8, Group 5), or in the presence of losartan and SB 209670 (n=8, group 7)

		Time after onset of LPS function		
		1–2 h	*8 h*	*23 h*
Δ Heart rate (beats min^{-1})	Group 3	41±14	80±12	127±8
	Group 5	43±9	61±10	118±9
	Group 7	25±10	96±8†	86±22
Δ Mean arterial blood pressure (mmHg)	Group 3	−41±2	−13±2	−28±3
	Group 5	−39±4	−13±3	−27±4
	Group 7	−42±2	−42±1*†	−41±4*†
Δ Renal vascular conductance (%)	Group 3	150±17	53±13	164±19
	Group 5	172±21	59±11	181±24
	Group 7	158±12	123±7*†	148±13
Δ Mesenteric vascular conductance (%)	Group 3	68±9	26±5	40±15
	Group 5	87±17	46±11	26±13
	Group 7	87±9	234±10*†	151±18*†
Δ Hindquarters vascular conductance (%)	Group 3	58±12	40±13	111±24
	Group 5	41±16	19±14	75±23
	Group 7	38±6	99±8*†	81±8

At the earliest time point shown, all changes are at 1 h, except for renal vascular conductance which peaked at 2 h. Values are mean±s.e. mean; with the exception of the change in hindquarters vascular conductance at 8 h in Group 5, all changes were significant ($P<0.05$, Friedman's test), *$P<0.05$ versus group 3, †$P<0.05$ versus Group 5 (Kruskal Wallis test).

Cardiovascular changes during infusion of captopril, losartan and LPS, or captopril and LPS (Groups 4 and 5)

During infusion of losartan, captopril and LPS (Figure 2), the cardiovascular changes were not different from those in animals receiving losartan and LPS (Figure 1, Table 2). In animals receiving captopril and LPS, the early fall in MAP (1 h after onset of LPS infusion) and the increases in renal and mesenteric vascular conductance were significantly less than those seen in animals given losartan, captopril and LPS (Figure 2). Subsequently, the cardiovascular changes were not

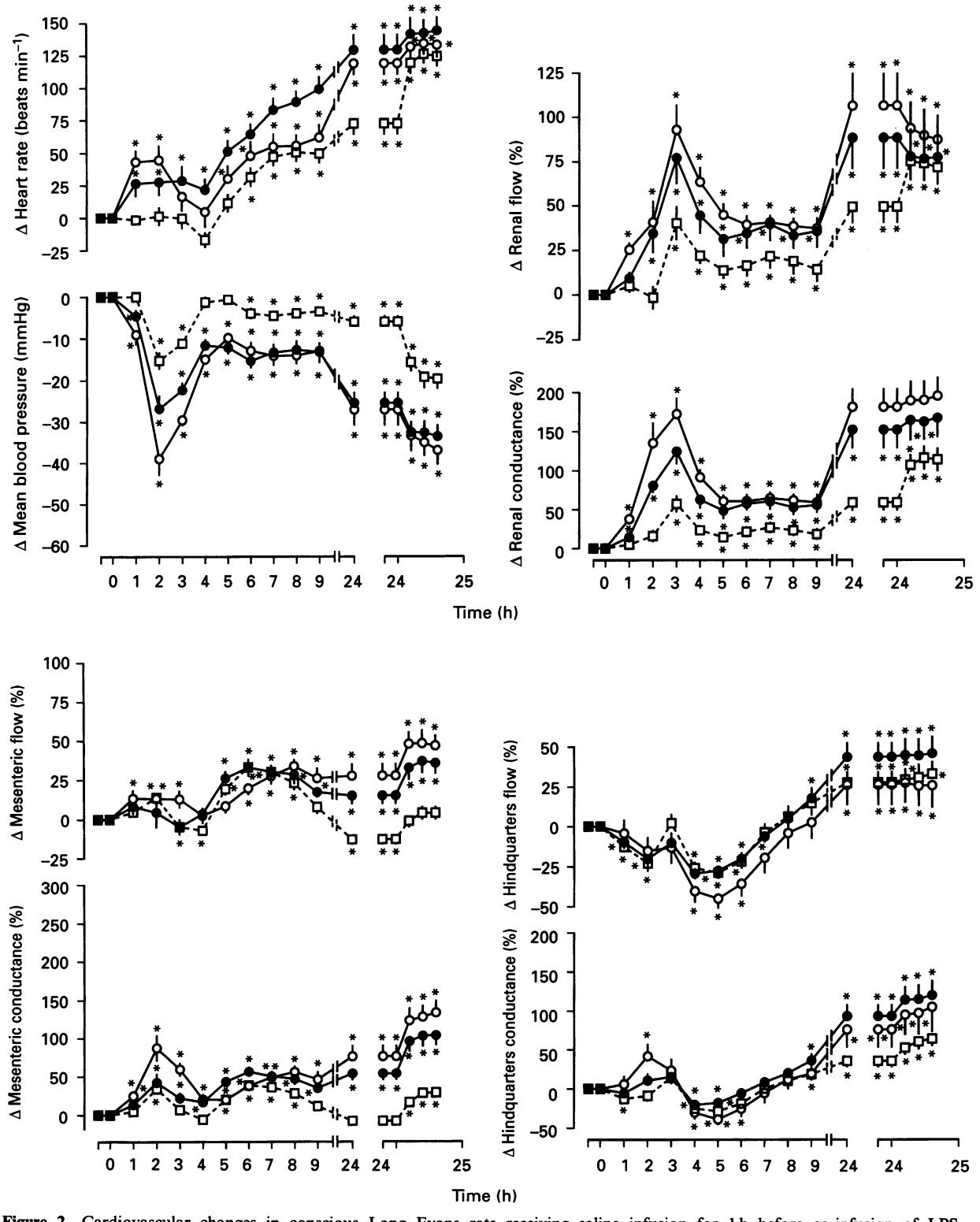

Figure 2 Cardiovascular changes in conscious Long Evans rats receiving saline infusion for 1 h before co-infusion of LPS (150 μg kg^{-1} h^{-1}) for 23 h (□, $n=9$, Group 1), or captopril (2 mg kg^{-1}, 2 mg kg^{-1} h^{-1}) for 1 h before co-infusion of LPS for 23 h (●, $n=11$, Group 4), or losartan (10 mg kg^{-1} 10 mg kg^{-1} h^{-1}) and captopril for 1 h before co-infusion of LPS for 23 h (○, $n=8$, Group 5). Twenty four hours after the beginning of the experiments, the vasopressin antagonist, d(CH$_2$)$_5$-O-Me-Tyr-AVP (10 μg kg^{-1}) was administered alone (Groups 4 and 5) or together with losartan (10 mg kg^{-1}; Group 1); responses to these interventions are indicated in the text, as are between-group differences. Note the change in time scales between 24 and 25 h. Values are mean with s.e.mean; *$P<0.05$ versus original baseline.

different in animals receiving losartan, captopril and LPS and those receiving captopril and LPS (Figure 2), and the responses to the AVPX in the two groups were similar (Figure 2).

Cardiovascular changes during infusion of losartan, SB 209670 and saline (Group 6)

Infusion of losartan and SB 209670 for 1 h caused a significant fall in MAP, and increases in heart rate, renal flow, and renal

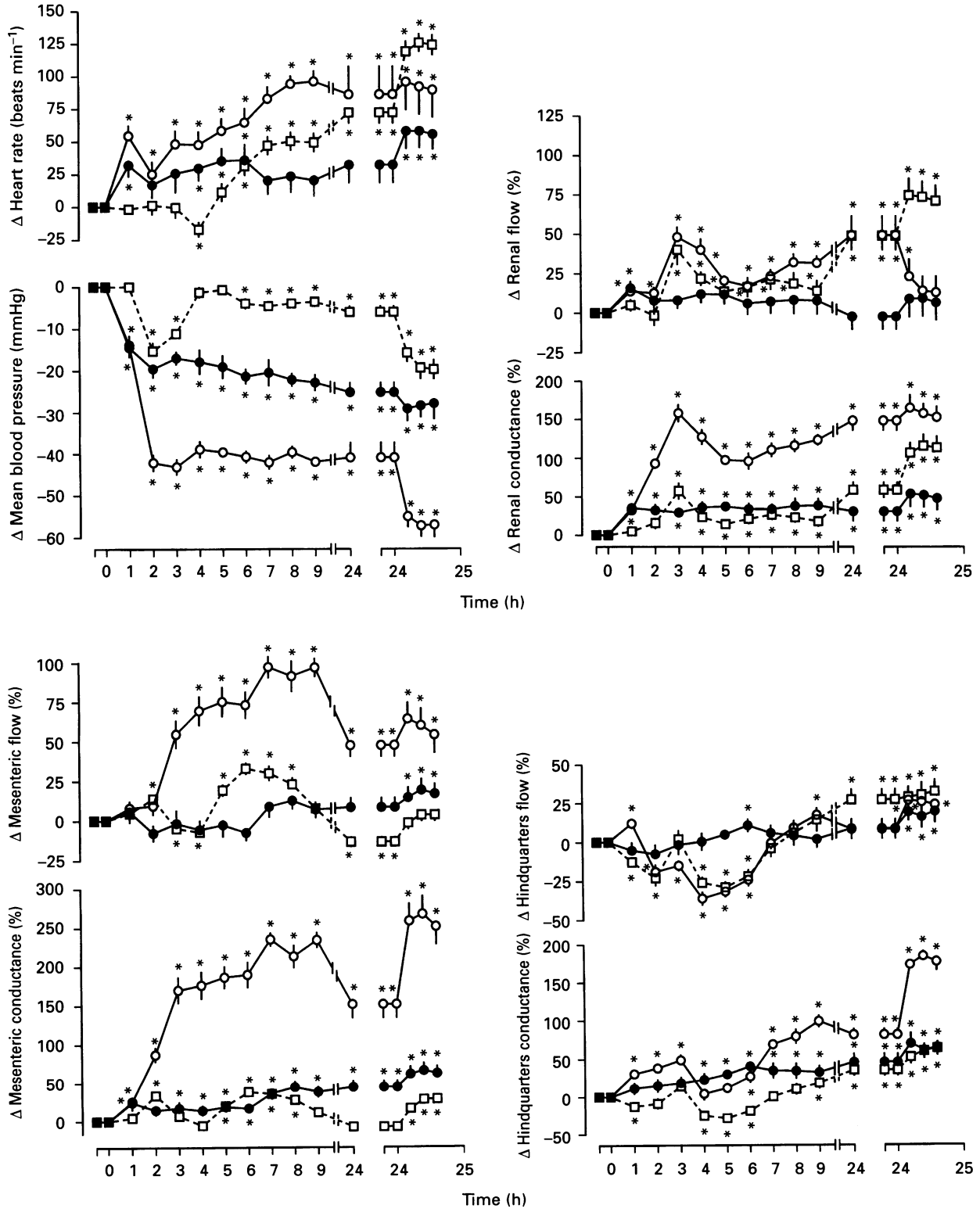

Figure 3 Cardiovascular changes in conscious Long Evans rats receiving saline infusion for 1 h before co-infusion of LPS (150 μg kg^{-1} h^{-1}) for 23 h (□, $n=9$, Group 1), or losartan (10 mg kg^{-1}, 10 mg kg^{-1} h^{-1}) and SB 209670 (600 μg kg^{-1} h^{-1}) for 1 h before co-infusion of saline for 23 h (●, $n=8$, Group 6), or losartan and SB 209670 for 1 h before co-infusion of LPS for 23 h (○, $n=8$, Group 7). Twenty four hours after the beginning of the experiments, the vasopressin antagonist, d(CH$_2$)$_5$-O-Me-Tyr-AVP (10 μg kg^{-1}) was administered alone (Groups 6 and 7) or together with losartan (10 mg kg^{-1}; Group 1); responses to these interventions are indicated in the text, as are between-group differences. Note the change to time scale between 24 and 25 h. Values are mean, and vertical bars show s.e.mean; *$P<0.05$ versus original baseline.

and mesenteric vascular conductances (Figure 3). During the subsequent 23 h, when saline was co-infused, there was a further, progressive fall in MAP. The tachycardia was variable, but there were sustained, albeit modest, increases in renal, mesenteric and hindquarters vascular conductances (Figure 3). Twenty four hours after the beginning of the experiment, the AVPX caused a slight, additional fall in MAP, a tachycardia and increases in renal, mesenteric, and hindquarters vascular conductance (Figure 3).

Cardiovascular changes during infusion of losartan, SB 209670 and LPS (Group 7)

In the presence of losartan and SB 209670, the nadir in MAP, 1–2 h after the onset of LPS infusion (Figure 3, Table 2), was similar to that seen in the presence of losartan (Figure 1, Table 2), or losartan and captopril (Figure 2, Table 2), but was lower than in the other experiments (Figures 1–3). However, there was no subsequent recovery in MAP, and during this time there were larger increases in mesenteric flow and vascular conductance than seen under any other condition (Figures 1–3, Table 2). Furthermore, although the initial peak (at 3 h) in renal vascular conductance (Figure 3) was similar to that seen during co-infusion of losartan and LPS (Figure 1, Table 2) or losartan, captopril and LPS (Figure 2, Table 2), this effect was more sustained than in the other conditions (Figures 1–3). There was also a significantly greater overall hindquarters vasodilatation than in the other experiments (Figures 1–3). Twenty three hours after the onset of co-infusion of losartan, SB 209670 and LPS, animals were markedly hypotensive and tachycardic, with significant increases in renal, mesenteric and hindquarters flows and vascular conductances (Figure 3). Administration of the AVPX at that juncture caused a substantial further fall in MAP, but no additional tachycardia (Figure 3); the level of MAP was significantly lower than under any other condition (Figures 1–3). Renal flow fell and there was no increase in renal vascular conductance, in contrast to the mesenteric and hindquarters beds where there were significant, albeit small, increases in flow, and substantial increases in vascular conductance (Figure 3). The mesenteric and hindquarters vasodilatations were significantly greater than in the other experiments (Figures 1–3).

Discussion

We have previously shown that pretreatment with SB 209670 has a more marked effect on the later (6–8 h) hypotensive and vasodilator responses to LPS infusion than on the initial changes (within 1–2 h) (Gardiner *et al.*, 1995a). The present work has shown that the initial hypotensive and vasodilator responses to LPS infusion are enhanced as much by losartan alone as they are by losartan together with SB 209670. Moreover, the changes seen in the presence of losartan and LPS are greater than the sum of those with losartan and with LPS alone. These findings indicate that angiotensin II, rather than endothelin, plays a major role in opposing the early hypotensive and vasodilator effects of LPS infused continuously at a low dose in conscious rats, and that this involvement is triggered by LPS administration. However, the onset and extent of involvement of endothelin in supporting MAP probably depends on the model of endotoxaemia used. For example, Ruetten *et al.* (1996) have reported that within 10 min of a bolus injection of LPS (10 mg kg^{-1}, i.v.) in anaesthetized rats, MAP was reduced by about −40 mmHg, compared to about −60 mmHg in the presence of SB 209670. The pattern of change in heart rate in our experiments indicate the involvement of baroreflex mechanisms together with the effects of increased circulating catecholamines (Schaller *et al.*, 1985).

Elsewhere, we have reported that the initial hypotensive and vasodilator effects of LPS are relatively unaffected by dexamethasone (Gardiner *et al.*, 1996a) or aminoguanidine (Gardiner *et al.*, 1996b), indicating that inducible nitric oxide synthase is not involved (see also Gardiner *et al.*, 1995b). Bradykinin is a possible candidate as a vasodilator peptide whose generation is stimulated by LPS (Katori *et al.*, 1989; see Mombouli & Vanhoutte, 1995), although in the study of Paya & Stoclet (1995) the bradykinin, B_2-receptor antagonist, Hoe 140, was without effect on the initial hypotensive response to LPS. However, it is feasible that a putative hypotensive action of bradykinin, or a metabolite, is mediated through B_1-receptors, induced by LPS (see Regoli & Barabé, 1980; Campos *et al.*, 1996). But, from our results, it appears unlikely that bradykinin contributed to the early depressor and/or dilator responses to LPS, because these were less enhanced by captopril (which inhibits bradykinin degradation, in addition to angiotensin II formation) than by the AT_1-receptor antagonist, losartan. It could be suggested this difference was due to bradykinin exerting pressor and vasoconstrictor effects under these conditions (Fasciolo *et al.*, 1990; Gardiner & Bennett, 1992). However, this seems unlikely because the effects of LPS in the presence of losartan were the same as those in the combined presence of losartan and captopril, indicating that the ability of the latter to inhibit the degradation of bradykinin had no additional effect. Thus, the disparity between the early responses to LPS in the presence of losartan, compared to captopril, were probably due to a more effective suppression of the actions of angiotensin II by the former. Although we have shown the dose of captopril used is supramaximal for suppressing the effects of exogenous angiotensin I under other conditions (Muller *et al.*, 1990), that may not have been the case with the marked stimulation of renin release caused by LPS (Schaller *et al.*, 1985), and the possible involvement of local renin-angiotensin systems.

Our findings appear to differ substantially from those of Schaller *et al.* (1985), who found little evidence for the involvement of angiotensin II in the maintenance of MAP in endotoxaemic rats. However, their experiments involved assessment of the responses to captopril 90 min after a bolus injection of LPS, and hence they could have missed an earlier involvement of angiotensin II. An indication of a waning involvement of angiotensin II in the maintenance of MAP, even during infusion of LPS, comes from the observation that, in the presence of SB 209670, there is a progressive hypotensive response to LPS (Gardiner *et al.*, 1995a), although this could have been due, in part, to loss of a synergistic interaction between endothelin and angiotensin II (Yoshida *et al.*, 1991; 1992).

When the cardiovascular actions of angiotensin II were suppressed, in the presence of losartan and/or captopril, there was still substantial recovery from the initial hypotensive and vasodilator responses 2–8 h after the onset of LPS infusion. In contrast, in the presence of losartan and SB 209670, there was no recovery in MAP over the same period, and the persistent hypotension was accompanied by marked increases in mesenteric flow and conductance, a persistent renal hyperaemia and vasodilatation, and an unmasking of a modest hindquarters vasodilatation. These results are in line with an important vasoconstrictor action of endothelin, particularly in the mesenteric vascular bed, acting to oppose the powerful depressor and dilator mechanisms activated by LPS (Waller *et al.*, 1994; Gardiner *et al.*, 1995a,b; 1996a,b). The fact that regional flows were well-maintained, or markedly increased, when MAP was reduced so dramatically in the presence of losartan, SB 209670 and LPS, does not fit comfortably with the proposal that an SB 209670-induced increase in organ dysfunction in endotoxaemic rats is secondary to its hypotensive action (Ruetten *et al.*, 1996).

The similar recovery in MAP, following the initial hypotensive response to LPS in the presence of losartan or captopril, indicates that the ability of the latter to inhibit endothelin secretion (Momose *et al.*, 1993) was not apparent from our measurements. Moreover, the clear enhancement of LPS-induced renal vasodilatation in the presence of losartan and SB 209670 suggests that the failure of the latter to augment the

renal vasodilator action of LPS (Gardiner *et al.*, 1995a) was due to a persisting renal vasoconstrictor effect of angiotensin II. Even in that circumstance, renal perfusion was not compromised, hence the renal dysfunction induced by SB 209670 in anaesthetized rats given a large i.v. bolus dose of LPS (Ruetten *et al.*, 1996), may be peculiar to that experimental model, as indicated by the observations of Wellings *et al.* (1995), showing that SB 209670 had no effect on renal function, 6 h after i.p. injection of LPS.

In the presence of losartan, SB 209670 and LPS, MAP remained low and stable between 9–24 h, and there was no additional vasodilatation in any vascular bed. However, in the presence of losartan and LPS, or captopril and LPS, or losartan and captopril and LPS, over the same time there was a marked reversal of the recovery in MAP (seen from 2–8 h), accompanied by renal and hindquarters vasodilatation. These changes could be explained by the actions of endothelin diminishing between 8–23 h after the onset of LPS infusion. If that was the case, the delayed hypotensive and renal and hindquarters vasodilator effects of LPS do not require one to postulate the involvement of additional vasodilator mechanisms. Our previous studies are consistent with a substantial, but transient, involvement of endothelin in the haemodynamic effects of LPS infusion (Gardiner *et al.*, 1995a; 1996a,b). But they also indicate that both dexamethasone-sensitive and dexamethasone-resistant vasodilator mechanisms may be expressed transiently during LPS infusion (Gardiner *et al.*, 1995a; 1996a,b). The transience of these vasodilator mechanisms might account for the fading of the mesenteric hyperaemia and vasodilatation, 8–23 h after the onset of LPS infusion in the presence of losartan and SB 209670 (Figure 3). The fact that this change in mesenteric haemodynamics was not accompanied by an increase in MAP could be explained by the rise in afterload causing a fall in cardiac output. Another factor that could have contributed to this rise in afterload is the mesenteric vasoconstrictor action of vasopressin (Gardiner *et al.*, 1988).

An involvement of vasopressin is indicated by the finding that, in the presence of losartan, SB 209670 and LPS, the AVPX caused substantial, additional hypotension and mesenteric and hindquarters vasodilatation. It could be suggested this was due simply to the pre-existing hypotension, acting as an increased stimulus for vasopressin release. However, this seems unlikely, since animals given losartan and SB 209670, but not LPS, were more hypotensive than those given losartan and LPS, and yet the latter showed more marked hypotensive and vasodilator responses to the AVPX. Clearly, administration of losartan in the absence of LPS did not render cardiovascular status dependent on vasopressin, since under this circumstance the AVPX only had a trivial effect. Hence, some additional stimulus for vasopressin release must be present in endotoxaemia (Kasting *et al.*, 1985). Overall, the findings indicate an important, albeit variable, contribution from vasopressin to the maintenance of cardiovascular status in endotoxaemia, and hence differ from those of Schaller *et al.* (1985). As with angiotensin II, we suggest their results were a reflection of the experimental model they used, but a similar caveat applies to our studies. Thus, in future experiments, we need to delineate the involvement of vasopressin at earlier stages during infusion of LPS. In addition, it is clear that in the different experimental groups a number of other variables could be altered to differing extents prior to administration of the AVPX (e.g. blood volume). Furthermore, the pattern of change in heart rate in our experiments indicate some differences in the involvement of baroreflex mechanisms. Thus, without further experimentation it is difficult to reach clear conclusions regarding the involvement of vasopressin.

One notable finding in the present work, but not related to endotoxaemia, was the striking haemodynamic effect of administering losartan together with SB 209670 in conscious, Long Evans rats. In a previous study, involving Sprague Dawley rats, infusion of losartan, or losartan and SB 209670, for 8 h caused falls in MAP of about −3 and −14 mmHg, respectively (Gardiner *et al.*, 1995c). Here, the corresponding values in Long Evans rats were about −7 and −22 mmHg, respectively, and these effects were rapid in onset (−8 and −15 mmHg, respectively, at 1 h). These findings indicate a variable synergistic contribution from angiotensin II and endothelin to the normal maintenance of MAP in different strains of rat. In the light of these observations, and the clear evidence for substantial, but temporally disparate, involvement of both angiotensin II and endothelin in haemodynamic changes during LPS infusion, it is feasible that interstrain differences could be important when considering the factors influencing cardiovascular responses to endotoxaemia in conscious rats. Finally, in spite of evidence for reduced pressor responses to exogenous angiotensin II, endothelin and vasopressin in endotoxaemia (e.g. Schaller *et al.*, 1985; Hauser *et al.*, 1995), it is clear that, in conscious, Long Evans rats infused with LPS, endogenous angiotensin II, endothelin, and vasopressin can exert important haemodynamic influences.

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