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Effects of dexamethasone and SB 209670 on the regional haemodynamic responses to lipopolysaccharide 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 (350-450 g) Long Evans rats were chronically instrumented to permit regional haemodynamics to be monitored in the conscious state. In the first experiment, either saline (0.4 ml h^{-1}) or dexamethasone (3 mg kg^{-1}, 125 $\mu g\ kg^{-1}\ h^{-1}$) was infused continuously for 24 h, before co-infusion of lipopolysaccharide of (LPS, 150 $\mu g\ kg^{-1}\ h^{-1}$) for 24 h. Dexamethasone prevented the delayed (5-24 h) fall in mean arterial blood pressure (MAP) and the renal and hindquarters vasodilatation seen with LPS infusion alone, but not the initial (about 2 h) fall in MAP or renal vasodilatation. However, at this dose, dexamethasone itself caused a significant rise in MAP and regional vasoconstrictions.

2 In the second experiment, dexamethasone at a lower dose (12.5 $\mu g\ kg^{-1}\ h^{-1}$) had only slight pressor and vasoconstrictor effects. However, in its presence, infusion of LPS caused a substantial and progressive rise in MAP (maximum at 8 h, $+32 \pm 3$ mmHg) together with persistent mesenteric and hindquarters vasoconstriction and a transient renal vasodilatation.

3 In the third experiment, the non-selective endothelin antagonist, SB 209670 (600 $\mu g\ kg^{-1}\ h^{-1}$), blocked the slight pressor and regional vasoconstrictor effects of the lower dose of dexamethasone. Furthermore, in the presence of dexamethasone and SB 209670, infusion of LPS caused marked, but transient hypotension (nadir at 5 h, -24 ± 2 mmHg) and renal and mesenteric vasodilatation.

4 At the end of all experimental protocols, sequential administration of the AT_1-receptor antagonist, losartan, followed by the V_1-receptor antagonist, $(+)$-$(CH_2)_5$-O-Me-Tyr, vasopressin, caused effects indicating a variable involvement of angiotensin and vasopressin in the maintenance of cardiovascular status.

5 Collectively, the results indicate that, in the conscious rat, dexamethasone interacts with vasoconstrictor and vasodilator mechanisms, and hence its influence on the haemodynamic responses to LPS cannot be attributed, simply, to inhibition of the activity of inducible nitric oxide synthase and/or cyclo-oxygenase-2.

Keywords: Dexamethasone; lipopolysaccharide; endothelin; SB 209670

Introduction

Pretreatment with the synthetic glucocorticoid, dexamethasone, prevents some of the haemodynamic sequelae of lipopolysaccharide (LPS) administration (e.g. Wright *et al.*, 1992; Paya *et al.*, 1993; Szabo *et al.*, 1993; Wu *et al.*, 1995), consistent with its ability to inhibit activity of inducible nitric oxide synthase (iNOS; Radomski *et al.*, 1990; Szabo *et al.*, 1993; Wu *et al.*, 1995) and cyclo-oxygenase-2 (COX-2) (Kujubu & Herselman, 1992; Masferrer & Seibert, 1994; Wu *et al.*, 1995). However, LPS administration in rats not only causes activation of vasodilator systems, but also release of vasoconstrictor agents, such as catecholamines, angiotensin II (AII) and vasopressin (AVP) (e.g. Schaller *et al.*, 1985), and endothelin (ET) (Sugiura *et al.*, 1989; Vemulapalli *et al.*, 1991). LPS causes hyporesponsiveness to these agonists (Paya *et al.*, 1993; Szabo *et al.*, 1993; Wu *et al.*, 1995; Hauser *et al.*, 1995), and hence the ability of dexamethasone to oppose some of the haemodynamic effects of LPS could be due to its ability to reverse this hyporesponsiveness (Paya *et al.*, 1993; Szabo *et al.*, 1993; Wu *et al.*, 1995). However, the picture is complicated by the fact that glucocorticoids inhibit release of AVP (see Raff, 1987, for review) while stimulating renin release (see Drayer *et al.*, 1984); interactions between glucocorticoids and endogenous ET have not been described (Walker & Williams, 1992).

The major objective of the present work was to delineate the regional haemodynamic effects of dexamethasone in the absence and presence of LPS in conscious rats, and to assess the influence of the ET_A-, ET_B-receptor antagonist, SB 209670 (Ohlstein *et al.*, 1994). In addition, at the end of each experimental protocol, we determined the effect of sequential administration of the AT_1-receptor antagonist, losartan, and the V_1-receptor antagonist, $(+)$- $(CH_2)_5$-O-Me-Tyr, AVP (abbreviated to AVPX). A preliminary account of some of this work has been given to the British Pharmacological Society (Gardiner *et al.*, 1995a).

Methods

Experiments were carried out on male, Long Evans rats (350-450 g) bred in the Biomedical Services Unit in Nottingham. All surgery was performed under sodium methohexitone anaesthesia (Brietal, Lilly; 40-60 mg kg^{-1}, i.p., supplemented as required). The details of the procedures for implanting pulsed Doppler probes and intravascular catheters have been published (Gardiner *et al.*, 1990a,b; Waller *et al.*, 1994). All experiments were carried out in conscious unrestrained rats, at least 24 h after catheterization. The following experimental protocols were run:-

Effects of saline, and co-infusion of saline and LPS

Animals ($n=8$, Group 1), were given an i.v. infusion of saline (0.4 ml h^{-1}) for 24 h before co-infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$; Waller *et al.*, 1994; Gardiner *et al.*, 1995c) for 24 h. A bolus dose of losartan (10 mg kg^{-1}; Batin *et al.*,

[1] Author for correspondence.

1991) was then given followed 15 min later by the AVPX (10 $\mu g\ kg^{-1}$; 10 $\mu g\ kg^{-1}\ h^{-1}$; Gardiner *et al.*, 1989).

Effects of dexamethasone, and co-infusion of dexamethasone and LPS

Animals ($n=9$, Group 2) were given a primed i.v. infusion of dexamethasone (3 $mg\ kg^{-1}$, 125 $\mu g\ kg^{-1}\ h^{-1}$); in previous studies the bolus dose of dexamethasone has been shown to inhibit substantially LPS-induced vascular hyporesponsiveness and iNOS activity (Paya *et al.*, 1993; Szabo *et al.*, 1993). Dexamethasone was given continuously for 24 h before co-infusion of LPS for 24 h (as above); thereafter losartan and AVPX were given (as above).

From this experiment it became clear that dexamethasone itself caused significant haemodynamic changes (see Results), therefore, in subsequent experiments we used a 10 fold lower dose with a shorter (i.e., 1 h) pretreatment period.

Effects of saline and LPS co-infusion

Animals ($n=2$) were given saline for 1 h prior to co-infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$) for 23 h, followed by losartan and AVPX. Haemodynamic responses in these animals were indistinguishable from those in the 8 animals infused with saline for 24 h prior to LPS (Group 1). Therefore, the results from all these rats ($n=10$) were pooled as control data for the following experiments.

Effects of dexamethasone and saline co-infusion

Animals ($n=7$, Group 3) were given an infusion of dexamethasone (12.5 $\mu g\ kg^{-1}\ h^{-1}$, with no bolus dose) for 1 h before co-infusion of saline for 23 h, followed by losartan and AVPX (as above).

Effects of dexamethasone and LPS co-infusion

Animals ($n=8$, Group 4) were given dexamethasone (12.5 $\mu g\ kg^{-1}\ h^{-1}$) for 1 h before co-infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$) for 23 h, followed by losartan and AVPX (as above).

Effects of SB 209670 and saline co-infusion

Elsewhere (Gardiner *et al.*, 1995e) we have shown that the non-selective endothelin antagonist, SB 209670 (Ohlstein *et al.*, 1994; Douglas *et al.*, 1995a,b) has little haemodynamic effect in normotensive Sprague-Dawley rats when infused for 8 h. In this experiment we infused SB 209670 (600 $\mu g\ kg^{-1}\ h^{-1}$ dissolved in saline; $n=8$, Group 5) for 1 h before co-infusion of saline for 23 h, to determine its effects when infused for 24 h in Long Evans rats. At that stage animals were given losartan and AVPX (as above).

Effects of dexamethasone, SB 209670 and saline co-infusion

Animals ($n=7$, Group 6), were infused with a mixture of dexamethasone (12.5 $\mu g\ kg^{-1}\ h^{-1}$) and SB 209670 (600 $\mu g\ kg^{-1}\ h^{-1}$) for 1 h, prior to co-infusion of saline for 23 h; losartan and AVPX were then given, as above.

Effects of dexamethasone, SB 209670 and LPS co-infusion

Animals ($n=8$, Group 7) were infused with a mixture of dexamethasone and SB 209670 (as above) for 1 h before co-infusion of LPS for 23 h; losartan and AVPX were then given (as above).

Data analysis

Measurements were made as described previously (Gardiner *et al.*, 1990a,b; Waller *et al.*, 1994). Within group analysis was by Friedman's test; between group analysis was by the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. A P value <0.05 was taken as significant.

Materials

LPS (*E.coli* serotype 0127 B8) and dexamethasone (water soluble) were obtained from Sigma (U.K.); (+)-$(CH_2)_5$-O-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.). Infusions were given at 0.4 ml h^{-1}; bolus injections were given in 0.1 ml, flushed in with 0.1 ml.

Results

Resting, pretreatment cardiovascular variables are shown in Table 1.

Effects of saline, and co-infusion of saline and LPS

During infusion of saline for 24 h, there were slight and variable increases in MAP and reductions in renal and mesenteric haemodynamics, with more marked reductions in the hindquarters (Figure 1).

During the subsequent co-infusion of saline and LPS, there was a biphasic fall in MAP and tachycardia, accompanied by early onset, sustained increases in renal flow and vascular conductance, reductions in mesenteric flow and vascular conductance over the first 8 h of infusion, and initial (up to 6 h)

Table 1 Resting, pretreatment cardiovascular variables in the 7 groups of rats studied

	Group 1 (n=8)	*Group 2 (n=9)*	*Group 3 (n=7)*	*Group 4 (n=8)*	*Group 5 (n=8)*	*Group 6 (n=7)*	*Group 7 (n=8)*
Heart rate (beats min^{-1})	331±8	326±6	333±6	330±12	336±7	321±11	327±8
Mean blood pressure (mmHg)	100±2	100±1	104±2	105±2	100±1	103±2	105±2
Renal Doppler shift (kHz)	7.6±0.8	6.3±0.5	6.7±0.6	6.1±0.7	6.7±0.4	6.3±0.8	6.8±0.7
Mesenteric Doppler shift (kHz)	6.9±0.7	8.2±0.4	5.8±0.3	6.0±0.4	5.8±0.6	7.4±0.5	6.4±0.5
Hindquarters Doppler shift (kHz)	4.3±0.3	3.7±0.3	4.0±0.3	4.2±0.4	4.6±0.2	4.1±0.2	3.8±0.5
Renal vascular conductance ([kHz $mmHg^{-1}$] 10^3)	76±8	63±5	66±6	59±7	68±5	61±7	66±8
Mesenteric vascular conductance ([kHz $mmHg^{-1}$] 10^3)	70±7	82±5	56±3	57±4	58±6	72±5	63±6
Hindquarters vascular conductance ([kHz $mmHg^{-1}$] 10^3)	43±3	37±3	38±3	41±5	46±2	40±2	37±6

The groups are defined in Methods; values are mean±s.e.mean.

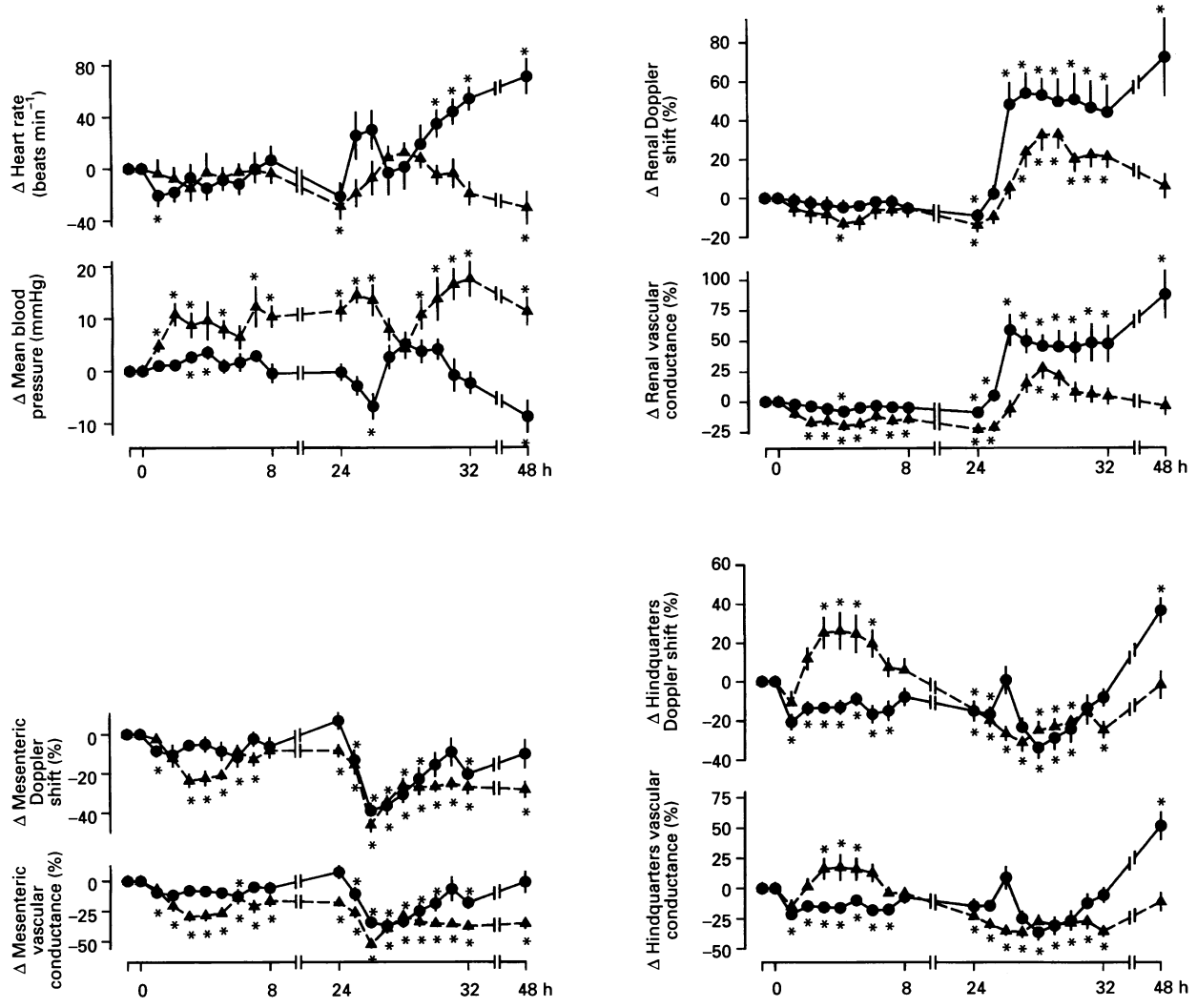

Figure 1 Cardiovascular changes in conscious rats during infusion of saline for 24 h followed by co-infusion of LPS ($150\,\mu g\,kg^{-1}\,h^{-1}$) for 24 h (●, $n=8$), or during infusion of dexamethasone ($3\,mg\,kg^{-1}$, $125\,\mu g\,kg^{-1}\,h^{-1}$) for 24 h followed by co-infusion of LPS for 24 h (▲, $n=9$). LPS infusion started at the 24 h time point. Values are mean with s.e.mean. $^{*}P<0.05$ versus original baseline.

reductions in hindquarters flow and vascular conductance, followed by a developing hyperaemic vasodilatation (Figure 1).

Effects of dexamethasone, and co-infusion and dexamethasone and LPS

During primed infusion of dexamethasone ($3\,mg\,kg^{-1}$, $125\,\mu g\,kg^{-1}\,h^{-1}$) for 24 h there was a significant and sustained increase in MAP (within 2 h), but the change in heart rate was not different from that during saline infusion (i.e., there was no reflex bradycardia) (Figure 1). There were significant reductions in renal and mesenteric flows and vascular conductances, with the vasoconstrictions being greater than during saline infusion (Figure 1). In contrast, there were significant, but transient, increases in hindquarters flow and vascular conductance, rather than the decreases seen during saline infusion (Figure 1).

During co-infusion of dexamethasone and LPS, the initial fall in MAP was delayed, and did not go below baseline; at 24 h MAP was still above baseline, and there was a bradycardia, rather than a tachycardia (Figure 1). Although there were increases in renal flow and vascular conductance, these changes were slower in onset than during saline and LPS co-infusion, and were not sustained (Figure 1). Moreover, the reductions in mesenteric flow and vascular conductance were persistent, and there was no delayed hyperaemic vasodilatation in the hindquarters (Figure 1).

Effects of dexamethasone and saline co-infusion

During infusion of the lower dose of dexamethasone ($12.5\,\mu g\,kg^{-1}\,h^{-1}$) and saline there was a modest, but persistent, increase in MAP and reduction in heart rate (Figure 2). These effects were accompanied by moderate reductions in renal and mesenteric flow and vascular conductance, and variable reductions in the hindquarters (Figure 2).

Effects of dexamethasone and LPS co-infusion

During co-infusion of the lower dose of dexamethasone and LPS, there was a marked and progressive increase in MAP over the first 8 h, but a return to baseline by 24 h, with no significant change in heart rate (Figure 2). The increase in MAP was significantly greater than that during co-infusion of LPS and the higher dose of dexamethasone (Figure 1). There were only transient, early increases in renal flow and vascular conductance, but persistent decreases in mesenteric flow and

vascular conductance (Figure 2). Hindquarters flow and vascular conductance progressively fell up to 8 h after the onset of LPS infusion, and there was no subsequent hyperaemic vasodilatation (Figure 2).

Effects of SB 209670 and saline co-infusion

During co-infusion of SB 209670 and saline, there was a progressive, albeit small, fall in MAP, and a slight, transient bradycardia (Figure 3). These changes were accompanied by modest reductions in renal flow and vascular conductance; mesenteric flow and vascular conductance showed a small increase after 24 h, but there were no changes in hindquarters haemodynamics (Figure 3).

Effects of dexamethasone, SB 209670 and saline co-infusion

During co-infusion of dexamethasone, SB 209670 and saline, the fall in MAP, and the changes in renal and mesenteric haemodynamics were not different from those seen during co-infusion of SB 209670 and saline, but there was no significant

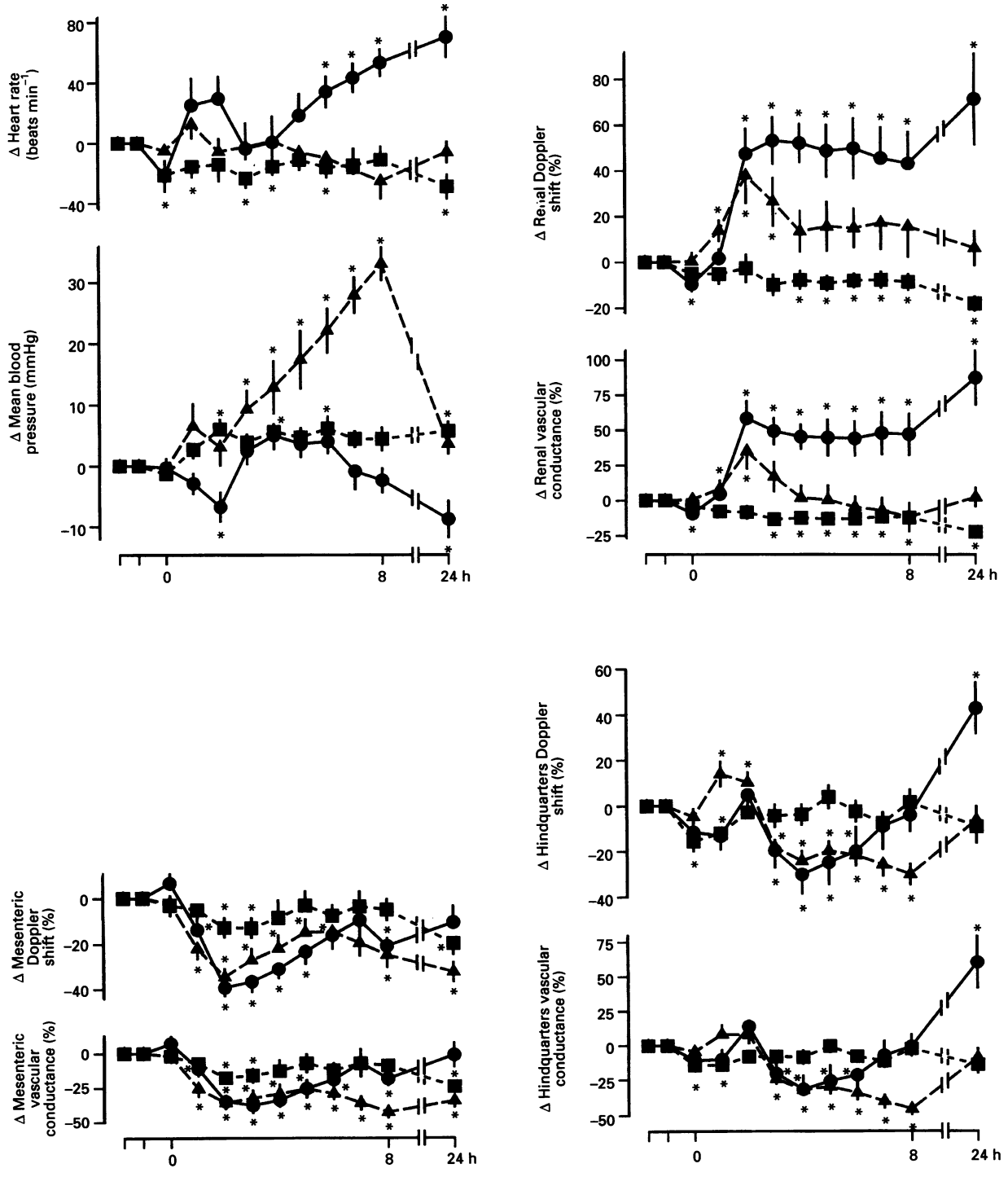

Figure 2 Cardiovascular changes in conscious rats during co-infusion of saline and LPS ($150\,\mu g\,kg^{-1}\,h^{-1}$) (●, $n=10$) or dexamethasone ($12.5\,\mu g\,kg^{-1}\,h^{-1}$) and saline (■, $n=7$), or dexamethasone and LPS (▲, $n=8$). LPS infusion was begun at the 0 h time point; values are mean with s.e.mean. $^*P<0.05$ versus original baseline.

bradycardia (Figure 3). There were clear increases in hindquarters flow and conductance that were significantly different from the lack of change during co-infusion of SB 209670 and saline (Figure 3). Hence, in the presence of SB 209670, the slight pressor, bradycardic, and vasoconstrictor effects of dexamethasone were abolished (compare Figures 2 and 3).

Effects of dexamethasone, SB 209670 and LPS co-infusion

During infusion of LPS in the presence of dexamethasone and SB 209670, there was a marked hypotension and tachycardia which peaked at 5 h and 7 h, respectively; by 24 h the change in MAP and heart rate were not different from those in the absence of LPS (Figure 3). There were increases in renal flow and conductance, in contrast to the changes seen in the absence of LPS, but these were not sustained (Figure 3). In the mesenteric vascular bed, flow and vascular conductance showed substantial increases that were significantly different from the changes seen in the absence of LPS, but these peaked at 4 h and had waned by 8 h (Figure 3). However, the increases in flow and conductance in the hindquarters vascular bed were sustained, and the vasodilatation was significantly greater than that seen during co-infusion of dexamethasone, SB 209670 and saline (Figure 3).

Effects of losartan and the AVPX after co-infusion of saline and LPS

Twenty-four hours after saline and LPS co-infusion (i.e. at a time when the animals were slightly hypotensive, with a tachycardia and marked hyperaemic renal and hindquarters vasodilatations; see Figures 1 and 2), losartan caused further hypotension, tachycardia, and clear increases in renal flow and vascular conductance (Figure 4). Mesenteric flow and vascular conductance showed slight increases, but hindquarters flow was not increased and vascular conductance rose only slightly (Figure 4).

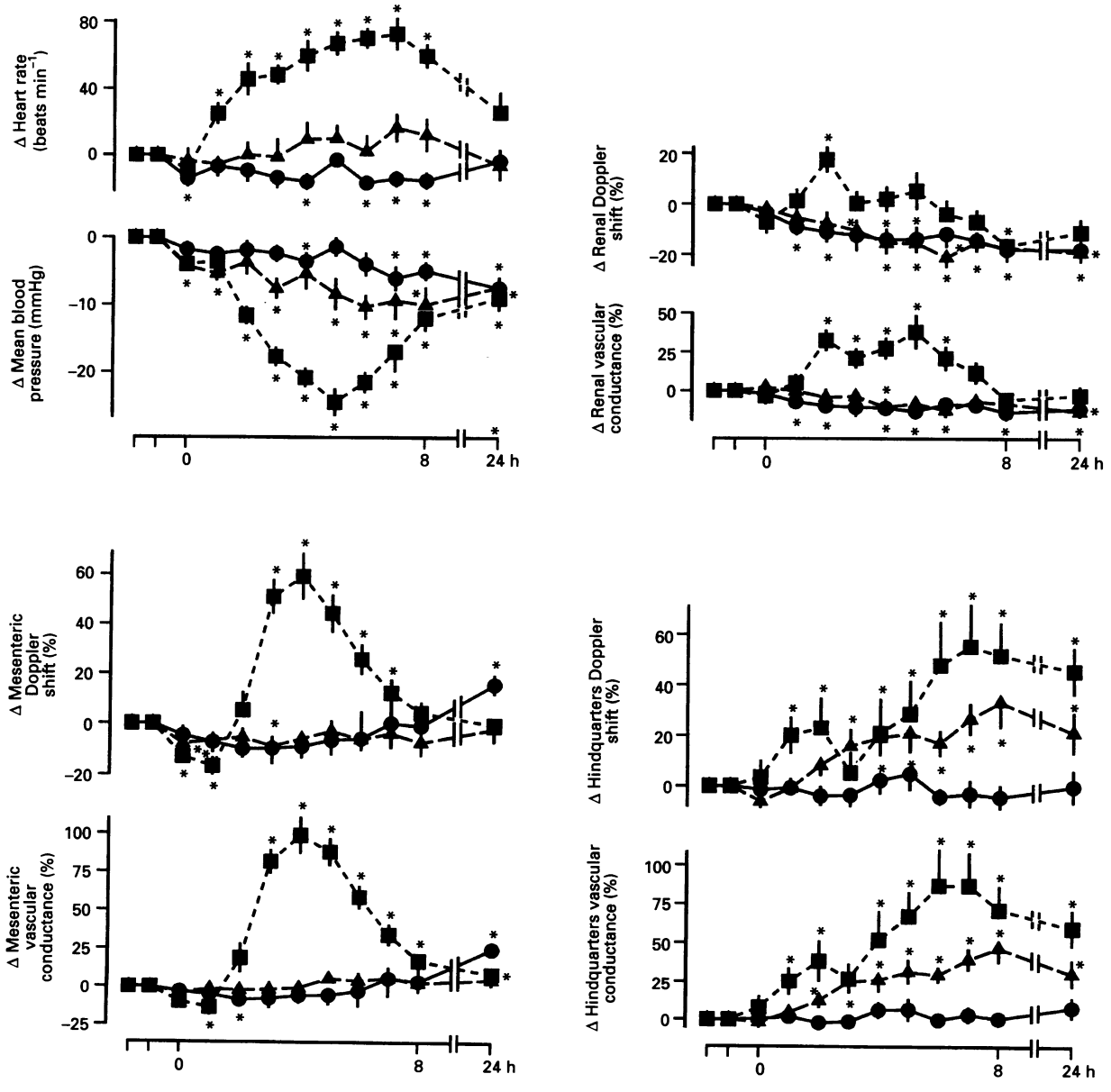

Figure 3 Cardiovascular changes in conscious rats during co-infusion of SB 209670 ($600\,\mu g\,kg^{-1}\,h^{-1}$) and saline (●, $n=8$), or co-infusion of dexamethasone ($12.5\,\mu g\,kg^{-1}\,h^{-1}$), SB 209670 and saline (▲, $n=7$), or co-infusion of dexamethasone, SB 209670 and LPS ($150\,\mu g\,kg^{-1}\,h^{-1}$) (■, $n=8$). LPS infusion was begun at the 0 h time point; values are mean with s.e.mean. $^{*}P<0.05$ versus original baseline.

Subsequent administration of the AVPX caused an additional fall in MAP, but no further tachycardia (Figure 4), although heart rate was not maximal. There were no significant further increases in any regional flow, but slight, significant, additional dilatations in renal, mesenteric and hindquarters vascular beds (Figure 4).

Effects of losartan and the AVPX after co-infusion of dexamethasone and LPS

Twenty-four hours after the onset of co-infusion of LPS with the higher dose of dexamethasone (i.e., when MAP was above the original baseline and there was bradycardia and mesenteric

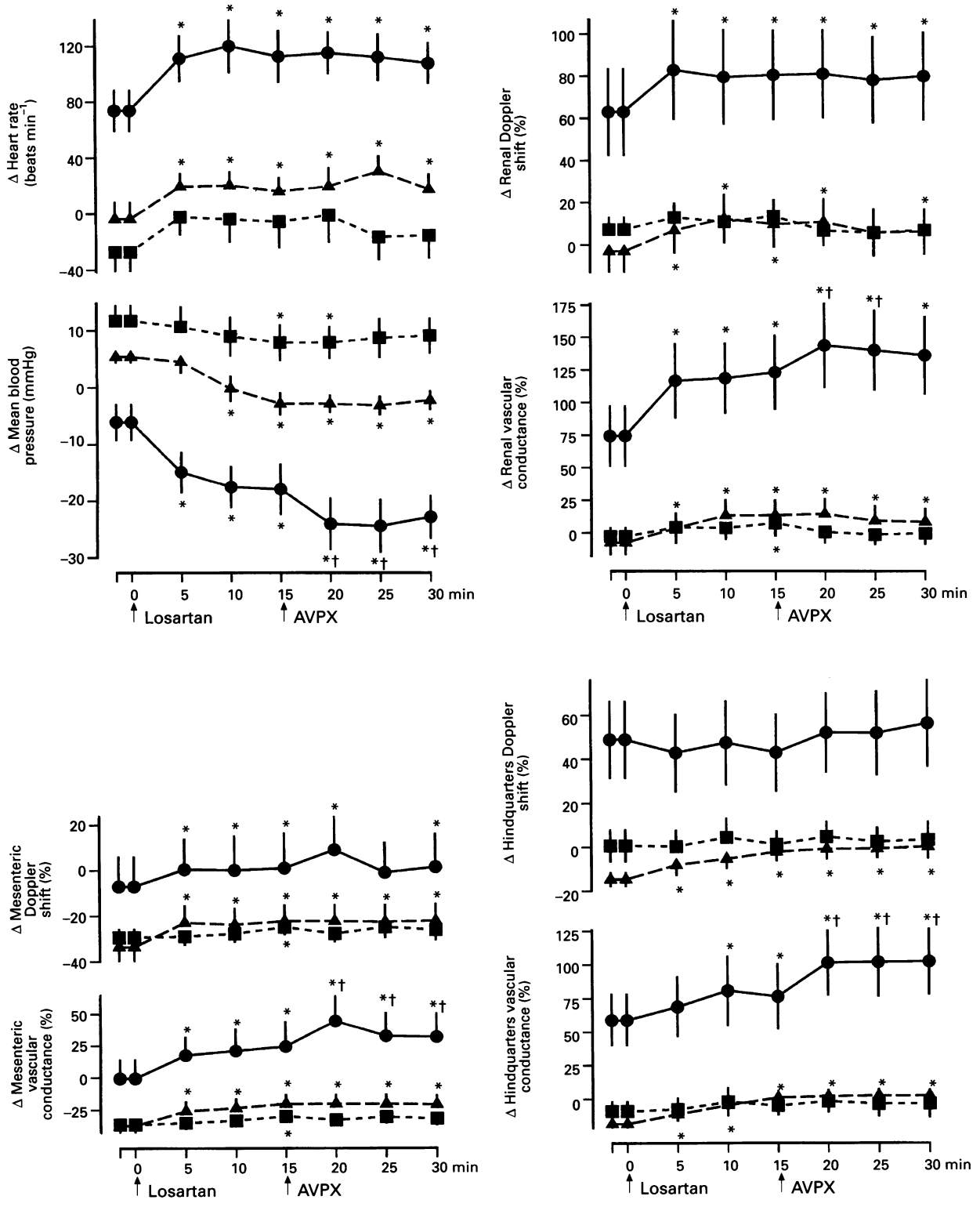

Figure 4 Cardiovascular changes in conscious rats given losartan (10 mg kg^{-1}) and (+)-$(CH_2)_5$-O-Me-Tyr, vasopressin (AVPX, 10 μg kg^{-1}, 10 μg kg^{-1} h^{-1}) following co-infusion of saline and LPS (150 μg kg^{-1} h^{-1}) for 24 h (●, $n=10$), or dexamethasone (3 mg kg^{-1}, 125 μg kg^{-1} h^{-1}) and LPS for 24 h (■, $n=9$), or dexamethasone (12.5 μg kg^{-1} h^{-1}) and LPS for 23 h (▲, $n=8$). Values are mean with s.e. mean; $^{*}P<0.05$ *versus* 0 time point, $^{\dagger}P<0.05$ *versus* 15 min time point.

vasoconstriction), losartan caused only a slight fall in MAP, no significant tachycardia, and very little change in regional haemodynamics (Figure 4). Administration of AVPX had no additional effects (Figure 4).

Twenty-three hours after the onset of LPS infusion with the lower dose dexamethasone (when MAP was slightly elevated and there was vasoconstriction in the mesenteric vascular bed), losartan caused a fall in MAP that was greater than that in the presence of the higher dose of dexamethasone and LPS, but less than that in the presence of saline and LPS (Figure 4). There was a significant tachycardia, but this was not different from the response seen in the presence of the higher dose of dexamethasone (Figure 4). Likewise, although there were significant increases in mesenteric and hindquarters flows and renal, mesenteric and hindquarters vascular conductances, these were not different from the changes in the presence of the higher dose of dexamethasone. In the renal vascular bed the increased in flow and conductance were significantly less than those seen after co-infusion of saline and LPS (Figure 4). Administration of AVPX had no additional effects (Figure 4).

Effects of losartan and AVPX after co-infusion of SB 209670 and saline

Twenty-three hours after co-infusion of SB 209670 and saline (when MAP was slightly reduced and there was a mesenteric vasodilatation) losartan caused a fall in MAP, tachycardia, and increases in renal, mesenteric and hindquarters flows and vascular conductances (Figure 5). AVPX caused only slight additional mesenteric and hindquarters vasodilatation.

Effects of losartan and AVPX after co-infusion of dexamethasone, SB 209670 and saline

Twenty-three hours after co-infusion of dexamethasone, SB 209670 and saline (when MAP was slightly reduced), losartan caused a clear fall in MAP, together with increases in heart rate and renal, mesenteric and hindquarters flows and vascular conductances (Figure 5). The AVPX caused a slight, additional fall in MAP and hindquarters vasodilatation.

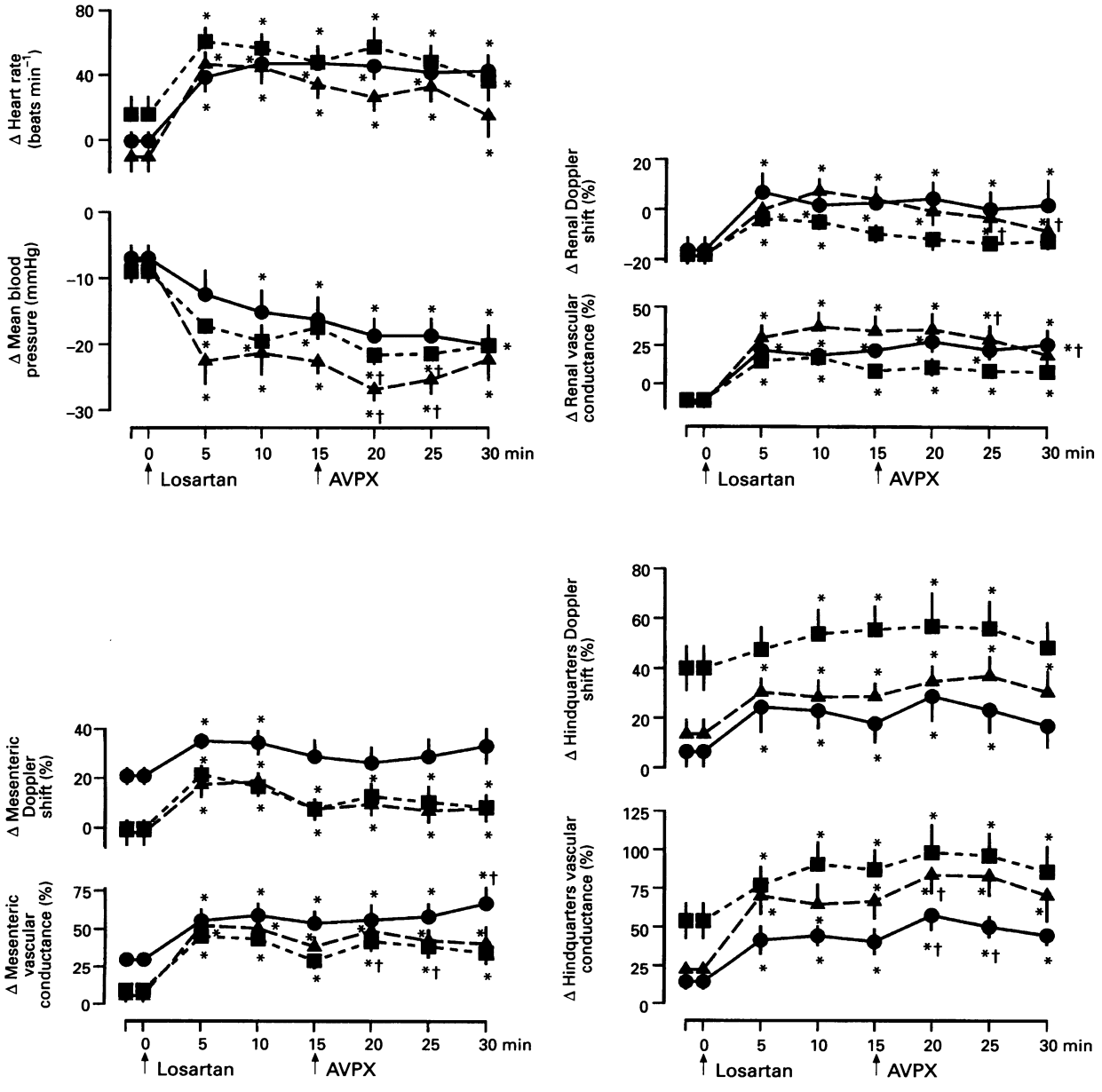

Figure 5 Cardiovascular changes in conscious rats given losartan (10 mg kg^{-1}) and (+)-$(CH_2)_5$-O-Me-Tyr, vasopressin (AVPX, 10 μg kg^{-1}, 10 μg kg^{-1} h^{-1}) following co-infusion of SB 209670 (600 μg kg^{-1} h^{-1}) and saline for 23 h (●, $n=8$), dexamethasone (12.5 μg kg^{-1} h^{-1}), SB 209670 and saline for 23 h (▲, $n=7$), or dexamethasone, SB 209670 and LPS (150 μg kg^{-1} h^{-1}) for 23 h (■, $n=8$). Values are mean with s.e.mean; $^{*}P<0.05$ versus 0 time point, $^{\dagger}P<0.05$ versus 15 min time point.

Effects of losartan and AVPX after co-infusion of dexamethasone, SB 209670 and LPS

Twenty-three hours after co-infusion of dexamethasone, SB 209670 and LPS (when MAP was slightly reduced and there was a hyperaemic hindquarters vasodilatation), losartan caused a fall in MAP accompanied by a tachycardia and increases in renal, mesenteric and hindquarters flows and vascular conductances (Figure 5); these changes were significantly greater than those in animals given losartan after co-infusion of saline and LPS or dexamethasone and LPS (Figure 4). AVPX had no additional effect.

Discussion

In the present work, the haemodynamic changes during saline infusion for 24 h, and those during LPS infusion for 24 h, were generally as we have described previously (Waller *et al.*, 1994; Gardiner *et al.*, 1995c). Thus, with LPS infusion at a rate of 150 $\mu g\ kg^{-1}\ h^{-1}$, there is a reproducible biphasic, but modest fall in MAP and tachycardia, with an early onset and progressive hyperaemic renal vasodilatation, a transient mesenteric vasoconstriction, and a hindquarters vasoconstriction followed by a delayed hyperaemic vasodilatation.

In the presence of the higher dose of dexamethasone, the hypotension and tachycardia at 24 h were prevented, as were the renal and hindquarters vasodilatations, while the mesenteric vasoconstriction persisted. Dexamethasone inhibits the activity of iNOS and COX-2 (see Introduction), hence it is feasible that their products are involved in triggering the later haemodynamic sequelae of endotoxaemia, despite iNOS activity being normal at the stage (Gardiner *et al.*, 1995c). But the higher dose of dexamethasone did not prevent the early fall in MAP (see also Paya *et al.*, 1993; Szabo *et al.*, 1993) or the hyperaemic renal vasodilatation, so it seems other factors were responsible for these phenomena. However, it is notable that, in the presence of the higher dose of dexamethasone, the initial fall in MAP and the renal vasodilatation during LPS infusion were delayed, possibly because the mechanisms responsible were different from those involved in the absence of dexamethasone (see later).

A difficulty with this first experiment was that dexamethasone itself caused a clear increase in MAP, accompanied by regional vasoconstrictions, thereby complicating the interpretation of the subsequent effects of LPS. To some extent we were able to avoid this problem by giving a lower dose of dexamethasone which had a significantly smaller pressor effect and caused less renal, mesenteric and hindquarters vasoconstriction. In this experiment during LPS infusion the lower dose of dexamethasone, like the higher dose, prevented the late fall in MAP and renal and hindquarters vasodilatation and the waning of the mesenteric vasoconstriction, consistent with inhibition of the actions of iNOS (Radomski *et al.*, 1990; Szabo *et al.*, 1993; Wu *et al.*, 1995) and/or COX-2 (Kujubu & Herselman, 1992; Masferrer & Seibert, 1994; Wu *et al.*, 1995). As with the higher dose of dexamethasone, the initial fall in MAP and the hyperaemic renal vasodilatation during LPS infusion were not prevented. However, in this case these events were not delayed, and hence it could be the lower dose of dexamethasone was insufficient to inhibit completely iNOS and/or COX-2 activity. But this is not a likely explanation, because the early haemodynamic effect of LPS precede the increase in iNOS activity (Szabo *et al.*, 1993; Gardiner 1995c). Moreover, the lower dose of dexamethasone has a clear functional effect since, in its presence, infusion of LPS caused a substantial rise in MAP which was incremental up to 8 h. This effect was not seen in the presence of the higher dose of dexamethasone, but in that experiment the latter was infused for 24 h before LPS, making comparison difficult.

We considered it most likely that the marked rise in MAP in the presence of the lower dose of dexamethasone and LPS was due to inhibition of iNOS and/or COX-2 activity unmasking the cardiovascular effects of ET because, in a recent study we had found that the non-selective ET antagonist, SB 209670, markedly augmented the hypotensive and mesenteric and hindquarters vasodilator responses to LPS (Gardiner *et al*, 1995d). Therefore, we hypothesized that, during co-infusion of SB 209670, dexamethasone and LPS, when the actions of ET, iNOS and COX-2 would be minimized, there should be little hypotension or vasodilatation. However, rather than the marked, transient rise in MAP seen during co-infusion of dexamethasone and LPS in the absence of SB 209670, there was a substantial, albeit, transient, fall in MAP, accompanied by renal and mesenteric vasodilatation, and an augmented increase in hindquarters vascular conductance. While we cannot exclude the possibility that inhibiting the actions of ET revealed the effects of residual iNOS and/or COX-2 activity (but see later), an interesting alternative is that additional vasodilator mechanism were unmasked in this experiment. In this context a notable candidate is adrenomedullin, since this peptide causes marked vasodilatation in conscious rats (Gardiner *et al.*, 1995b) and its production is stimulated by LPS (Sugo *et al.*, 1995), and by dexamethasone (Minamino *et al.*, 1995).

The finding that the hypotensive and vasodilator effects of LPS are sustained in the presence of SB 209670 (Gardiner *et al.*, 1995d), but not in the presence of SB 209670 and dexamethasone, could be explained by the influence of the latter to inhibit iNOS and COX-2 expression. However, it is feasible also that dexamethasone opposed hypotension and vasodilatation by augmenting sensitivity to endogenous vasoconstrictors (Paya *et al.*, 1993; Szabo *et al.*, 1993, such as AII and/or AVP.

Twenty-four hours after the onset of LPS infusion, there were significant hypotensive and regional vasodilator responses to losartan and the AVPX, indicating an involvement of AII and AVP in the maintenance of haemodynamic status (Schaller *et al.*, 1985). However, in the presence of either dose of dexamethasone and LPS, the hypotensive and haemodynamic effects of losartan and the AVPX were diminished, possibly because MAP was not reduced, and hence activation of these systems was less. But, in the presence of dexamethasone and SB 209670, 23 h after the onset of LPS infusion, MAP was reduced by the same amount as in the presence of LPS alone, yet the mesenteric and hindquarters vasodilator effects of losartan were augmented in the former condition (compare Figures 4 and 5). This observation is consistent with dexamethasone enhancing vascular sensitivity, at least to AII. However, interpretation of these experiments is complicated by the fact that glucocorticoids can stimulate renin release (see Drayer *et al.*, 1984), and inhibit AVP release (see Raff, 1987), while ET can stimulate or inhibit renin release, and stimulate AVP release (see Rubanyi & Polokoff, 1994).

A particularly striking finding in the present work was that the pressor and renal mesenteric and hindquarters vasoconstrictor effects of dexamethasone were abolished in the presence of SB 209670. Indeed, in this circumstance, dexamethasone caused a progressive and persistent increase in hindquarters flow and vascular conductance, indicating activation of regionally selective vasodilatation. It is notable that the higher dose of dexamethasone, alone, also caused selective hyperaemic vasodilatation in the hindquarters, indicating that, in this vascular bed, dexamethasone may interact with ET and with vasodilator mechanisms. Although ET itself can elicit hindquarters vasodilatation in conscious rats (Gardiner *et al.*, 1990a), it is not likely that ET was responsible for the hindquarters vasodilator effect of dexamethasone because, as mentioned above, this effect was enhanced in the presence of SB 209670. Since it has been reported that dexamethasone augments the production of adrenomedullin by vascular smooth muscle cells (Minamino *et al.*, 1995), and elsewhere we have shown this peptide causes hindquarters vasodilatation in conscious rats (Gardiner *et al.*, 1995b), it is possible that the hindquarters vasodilator effect of dexamethasone was mediated by adrenomedullin. However, this does not explain the

absence of renal or mesenteric vasodilator responses to the higher dose of dexamethasone alone, or to the lower dose of dexamethasone in the absence or presence of SB 209670, because adrenomedullin also elicits marked dilatation in these vascular beds (Gardiner *et al.*, 1995b). It is feasible that, in the latter, other direct and/or indirect vasoconstrictor effects of dexamethasone predominate (see Drayer *et al.*, 1984; Grünfeld & Eloy, 1987).

In summary, the present work has shown a complex interplay between dexamethasone and vasoconstrictor and vasodilator mechanisms, in the absence and presence of LPS, in conscious rats. Hence, any therapeutic effect of dexamethasone in endotoxaemia is not likely to be due, straightforwardly, to its ability to inhibit iNOS and/or COX-2 activity. Furthermore, it is apparent that pretreatment with dexamethasone can cause marked enhancement of the pressor and regional vasoconstrictor responses to LPS infusion in conscious rats.

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