Regional and temporal changes in cardiovascular responses to norepinephrine and vasopressin during continuous infusion of lipopolysaccharide in conscious rats

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Background. Reduced pressor responsiveness to norepinephrine (NE) in sepsis is well documented but the associated regional haemodynamic changes are less well characterized, and there are varying reports of changes in haemodynamic responses to arginine vasopressin (AVP). We compared changes in regional haemodynamic responsiveness to AVP and NE during a 24 h continuous infusion of lipopolysaccharide (LPS) in conscious rats.

Methods. Conscious, male Sprague–Dawley rats were infused with saline (0.4 ml h⁻¹) or LPS (150 μg kg⁻¹ h⁻¹). Renal, mesenteric, and hindquarter haemodynamic responses to 3 min infusions of AVP (0.25, 0.625, and 1.25 ng kg⁻¹ min⁻¹) or NE (75, 250, and 750 ng kg⁻¹ min⁻¹) were assessed 2, 6, and 24 h after the onset of LPS or saline.

Results. Two and six hours after the onset of LPS, all haemodynamic effects of NE were markedly reduced, but by 24 h, there was some recovery in the vasoconstrictor actions of NE although the pressor and bradycardic effects were still depressed. Two hours after the onset of LPS, the cardiovascular effects of AVP were depressed but there was some recovery in vascular responsiveness at 6 h. By 24 h, only the mesenteric vasoconstrictor effect of AVP was consistently reduced.

Conclusions. During low dose LPS infusion, there are differential changes in haemodynamic responsiveness to AVP and NE, which show different temporal and regional profiles of recovery with time. Furthermore, reduced pressor responsiveness to NE is not necessarily accompanied by a reduced capacity of vessels for vasoconstriction.

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There is burgeoning interest in the possibility that vasopressin (AVP) may have beneficial haemodynamic effects in catecholamine-resistant, septic shock states. Previous studies have shown that plasma AVP increases but then decreases to inappropriately low levels during septic shock. In addition, septic shock patients may be hypersensitive to the pressor effects of low-dose AVP. Recently, co-administration of AVP or its analogues has been shown to reduce the requirements for norepinephrine (NE), along with certain clinical benefits, such as reduction in the incidence of tachyarrhythmias, and improvements in cardiac output and mean arterial pressure. "A recent abstract" indicated differential changes in the pressor responsiveness to NE and AVP in anaesthetized, endotoxic rats, and a more recent paper has shown differential effects of NE and AVP on organ perfusion in anaesthetized dogs with septic shock, but we are unaware of any published study in which comparative assessments of the regional haemodynamic effects of AVP and NE have been carried out in conscious animals in the normal state, and in the endotoxic condition, across a 24 h time-period. This experimental approach is important as it is clear there are temporally related changes in haemodynamic status during developing endotoxaemia and there may be dissociation between
the pressor and regional vasoconstrictor effects of agonists in sepsis.\textsuperscript{12,13}

Therefore, the aim of the present work was to compare changes in cardiovascular responsiveness to NE and AVP in conscious, chronically instrumented rats utilizing a model of endotoxaemia, which involves infusion of lipopolysaccharide (LPS) for 24 h.\textsuperscript{12–14} Some of these results have been presented to the British Pharmacological Society.\textsuperscript{15,15}

Materials and methods
Male, Sprague–Dawley rats (400–450 g) were anaesthetized (fentanyl and medetomidine, 300 $\mu$g kg\textsuperscript{-1} of each i.p.) and had miniaturized pulsed Doppler flow probes sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta below the level of the ileocecal artery (to monitor changes in flow to the hindquarters). Anaesthesia was reversed, and analgesia provided, by atipamezole and nalbuphine (1 mg kg\textsuperscript{-1} of each s.c.). Animals were housed singly, with free access to food and water for at least 14 days before they were anaesthetized (as above) and had intra-arterial (distal abdominal aorta via ventral caudal artery) and i.v. (right jugular vein) catheters implanted. A counterbalanced tether system connected to a harness fitted to the rat and i.v. (right jugular vein) catheters carried all the catheters and flow probe connectors, and allowed relatively unrestricted movement when the rat was housed in its home cage for the duration of the experiment (with free access to food and water). Double channel swivel systems\textsuperscript{16} were used to allow overnight i.v. administration of saline or LPS (see below) and ensure maintenance of the patency of the arterial catheter by infusion of heparinized (15 $\mu$ml\textsuperscript{-1}) saline.

Four groups of rats were studied; two groups were infused with sterile saline (0.4 ml h\textsuperscript{-1}) for 25 h, and two groups received a continuous infusion of LPS (150 $\mu$g kg\textsuperscript{-1} h\textsuperscript{-1}) for 25 h. One of the saline-infused groups was challenged with NE and the other with AVP, and likewise for the LPS-infused groups. Studies in vitro have shown that the mode of agonist administration can determine whether or not vascular hyporeactivity is detected in tissues taken from endotoxic animals, for example hyporesponsiveness to methoxamine is seen with infusions, but not with bolus doses.\textsuperscript{17} Thus, in contrast to an earlier study in which we compared bolus doses of AII and AVP,\textsuperscript{13} here we chose to administer NE and AVP as infusions. As NE and AVP are both fast-acting, steady-state was achieved with 3 min infusions. NE was given at three incremental doses (75, 250, and 750 ng kg\textsuperscript{-1} min\textsuperscript{-1}), each infused at a rate of 0.15 ml min\textsuperscript{-1} for 3 min, separated by at least 10 min to allow variables to return to baseline levels. A similar protocol was followed with AVP (0.25, 0.625, and 1.25 ng kg\textsuperscript{-1} min\textsuperscript{-1}). The infusions of NE or AVP were begun at 2, 6, and 24 h after the onset of infusion of saline or LPS. Cardiovascular variables were monitored continuously for the first 7 h of the experiment and again between 24 and 25 h, to allow changes during saline or LPS infusion and the responses to NE or AVP to be quantified. Data were digitized using a custom-designed haemodynamics data acquisition system (HDAS; built by the Bioinstrumentation Laboratories at the University of Maastricht) and stored to disk to allow offline analysis.

For each animal in each experiment, values for heart rate, mean arterial arterial pressure, and renal, mesenteric, and hindquarters Doppler shift were averaged electronically over selected time periods. Doppler shift was divided by mean arterial pressure to give an index of vascular conductance, and per cent changes in the latter were analysed as indices of vasodilatation (i.e. increase in vascular conductance) and vasoconstriction (i.e. decrease in vascular conductance).\textsuperscript{12–14}

Data analysis
Within-group analysis was by Friedman’s test,\textsuperscript{18} to determine changes relative to original baseline (i.e. during infusion of saline or LPS). Responses to NE or AVP were assessed from the dose-related integrated responses (areas under or over curves) during the 3 min infusion. Because resting cardiovascular variables changed during LPS infusion (see Results) integrated responses were expressed as both absolute and as per cent changes (i.e. % of the pre-dose value). Comparison between groups (saline/LPS, NE/AVP) was by Kruskal–Wallis test; $P<0.05$ was taken as significant.

Drugs
LPS (Escherichia coli serotype 0127: B8) (Sigma, UK), NE bitartrate (Sigma, UK) and AVP (Bachem, UK) were dissolved in sterile, isotonic saline.

Anaesthetic and reversing agents were as follows: fentanyl citrate (Janssen-Cilag, UK), medetomidine hydrochloride ((Domitor) Pfizer, UK), atipamezole hydrochloride ((Anti-sedan) Pfizer, UK), nalbuphine hydrochloride ((Nubain) Bristol-Myers Squibb, UK).

Results
Resting variables in the four groups of rats studied are shown in Table 1. During infusion of saline there were no consistent
cardiovascular changes (Fig. 1). However, during infusion of LPS there was tachycardia, early, short-lived hypotension, renal vasodilatation, biphasic hindquarters vasodilatation, and a delayed mesenteric vasodilatation (Fig. 1). The vasodilatations were associated with increases in flow in the renal (significant from 30 min onwards), mesenteric (significant from 5 h onwards), and hindquarters (significant between 45 min and 2 h and again at 24 h) vascular beds (data not shown).

Responses to NE

Saline-infused rats. Two, six, and twenty-four hours after the onset of saline infusion, NE caused dose-dependent pressor effects together with reductions in heart rate and in renal, mesenteric and hindquarters vascular conductances, in that rank order (Fig. 2). The increase in systolic arterial pressure was greater than that in diastolic arterial pressure (i.e. there was an increase in pulse pressure), consistent with a positive inotropic effect of NE (Table 2). The pressor effects of NE were accompanied by reductions in flow (Doppler shift) in the renal and mesenteric, but not in the hindquarters vascular beds (Table 3).

LPS-infused rats. Two hours after the onset of LPS infusion, all the effects of NE were significantly reduced however expressed (Fig. 2), and the modest vasoconstrictions were not associated with reductions in flow in any vascular bed (Table 3). This situation was relatively unchanged 6 h after onset of infusion of LPS (Fig. 2). However, by 24 h after the onset of LPS infusion there was some recovery of the regional vascular actions of NE, although its pressor and bradycardic effects remained significantly depressed (Fig. 2). As in the saline-infused rats, the NE-induced increase in systolic arterial pressure in LPS-infused rats was greater than that in diastolic arterial pressure, but the difference was less marked (Table 2).

Responses to AVP

Saline-infused rats. Two, six, and twenty-four hours after the onset of saline infusion, AVP caused dose-dependent pressor and bradycardic effects accompanied by reductions in mesenteric, renal, and hindquarters vascular conductances, in that rank order (Fig. 3). AVP induced reductions in flow in all three vascular beds, but the effect was most marked in the mesenteric bed (Table 4). Although AVP increased systolic arterial pressure more than diastolic arterial pressure, the difference was less than that seen with NE (Table 2).

LPS-infused rats. There was a more variable reduction in the effects of AVP 2 h after the onset of LPS infusion when expressed in absolute terms than when expressed in per cent terms (Fig. 3). However expressed, the pressor action of AVP was reduced at that stage (Fig. 2), and there was no difference between the AVP-induced changes in systolic and diastolic arterial pressure (Table 2). Mesenteric and hindquarters vasoconstrictor effects of AVP were generally reduced 2 h after the onset of LPS infusion but the renal vasoconstrictor effect was only reduced when expressed as per cent change (Fig. 3).

Fig 1 Changes in cardiovascular variables during a 24-h infusion of saline or LPS in rats challenged with NE or AVP at 2, 6, and 24 h after the onset of infusion. Values are mean (SEM); n=7 or 8 animals/group. *P<0.05 vs baseline (Friedman’s test).
Whilst most of the effects of AVP showed some recovery by 6 h after the onset of LPS infusion, there was still a slight reduction in its pressor action (Fig. 3). However, by 24 h after the onset of LPS infusion, the pressor and bradycardic effects of AVP were not consistently reduced, and renal and hindquarters vasoconstrictor effects were enhanced in absolute terms, presumably as a result of the underlying vasodilatation (Fig. 3). Only the mesenteric vasoconstrictor actions of AVP were consistently reduced 24 h after the onset of LPS infusion (i.e. in absolute and per cent terms). There was no recovery in the ability of AVP to increase pulse pressure (Table 2).

### Comparison of NE and AVP effects

**Saline-infused rats.** At doses of NE and AVP, which caused comparable pressor effects (250 and 1.25 ng kg\(^{-1}\) min\(^{-1}\), respectively), NE caused similar bradycardic, renal, and hindquarters vasoconstrictor effects to AVP, but the latter had a more marked mesenteric vasoconstrictor action (Figs 2 and 3; Tables 3 and 4).

**LPS-infused rats.** Figure 4 shows integrated responses to NE (250 ng kg\(^{-1}\) min\(^{-1}\)) and AVP (1.25 ng kg\(^{-1}\) min\(^{-1}\)) in LPS-infused rats expressed as per cent of the corresponding responses in saline-infused rats at the different time points. Two hours after the onset of LPS infusion, the pressor and bradycardic effects of both NE and AVP were reduced although constrictor responses to NE were more consistently affected (Figs 2–4) and NE caused no reduction in mesenteric flow, but the pattern of the decrease in mesenteric flow caused by AVP was unchanged (Tables 3 and 4).

By 6 h into the LPS infusion, there was some recovery of the vasoconstrictor effects of NE (Fig. 2), but this was less than seen with AVP (Figs 3 and 4), and this difference was
Table 3  Arterial pressures (BP) and regional blood flows (Doppler shift, DS) before (0) and at the end of 3-min infusions of NE. Values are mean (SEM). R: renal, M: mesenteric, H: hindquarters. *P<0.05 vs pre-infusion (baseline) value at time 0 (Friedman’s test).

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<tr>
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<td>7.9 (0.7)*</td>
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<tr>
<td>HDS (kHz)</td>
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<td>3.5 (0.3)</td>
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<tr>
<td>BP (mm Hg)</td>
<td>107 (3)</td>
<td>115 (2)*</td>
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<tr>
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<td>8.5 (0.8)</td>
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<td>HDS (kHz)</td>
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Fig 3 Integrated (0–3 min areas under or over curves) cardiovascular responses to AVP (triangles) at 2, 6, and 24 h after the onset of infusion of saline (closed symbols) or LPS (open symbols) infusion. Data (mean (SEM)) are expressed as absolute changes (left hand panels) or percentage changes (right hand panels) for low dose (L), middle dose (M), and high dose (H) of AVP (0.25, 0.625 and 1.25 ng kg⁻¹ min⁻¹, respectively). *A significant difference (P<0.05, Kruskal–Wallis test) between saline and LPS-infused groups at specific time-points.
In this endotoxaemic model, the mechanisms underlying continued LPS infusion. We have shown previously that vasoconstrictor effects of NE that we observed during perhaps more interesting, however, is the recovery of the responsiveness to the earlier study in which we reported diminished vascular vasodilation, were fully recovered. In contrast, cardiovascular responsiveness to AVP was better preserved and, at 24–25 h after the start of some recovery in the vasoconstrictor and pressor effects of NE were markedly reduced. Furthermore, the latters to cause reductions in renal and, especially, mesenteric flow (Tables 3 and 4). Twenty-four hours after the onset of LPS infusion, there was recovery in the ability of NE to cause regional vasoconstriction, together with reductions in renal and mesenteric flows, although its pressor and bradycardic actions remained generally depressed, in contrast to those of AVP (Figs 2–4).

Discussion

The aim of the present study was to characterize and compare changes in cardiovascular responsiveness to NE and AVP across a 24 h period of LPS or saline infusion in conscious rats. The results show that, 2 h into the LPS infusion, the pressor, bradycardic and all measured regional vasoconstrictor effects of NE were markedly reduced. Furthermore, with continued LPS infusion (up to 25 h), whilst there was some recovery in the vasoconstrictor and pressor effects of NE, the accompanying bradycardia remained markedly reduced. In contrast, cardiovascular responsiveness to AVP was better preserved and, at 24–25 h after the start of the LPS infusion, all effects, except AVP-induced mesenteric vasoconstriction, were fully recovered.

The present findings of diminished vasoconstrictor effects of NE in LPS-infused rats are broadly consistent with an earlier study in which we reported diminished vascular responsiveness to the α-adrenoceptor agonist, methoxamine, in this endotoxaemic model.12 The mechanisms underlying the early (at 2 h) loss of vasoconstrictor responsiveness to NE cannot be determined from this study, although others have suggested a variety of possible contributing factors.19–21 Perhaps more interesting, however, is the recovery of the vasoconstrictor effects of NE that we observed during continued LPS infusion. We have shown previously that iNOS expression is maximal 6 h after the onset of LPS infusion,14 so if overproduction of NO under the influence of the inducible enzyme was responsible for the effects seen, we would have expected that NE responses would be maximally reduced at 6 h. But the vascular hyporeactivity to NE was most apparent at 2 h, indicating that factors other than NO produced by iNOS were more important in this regard. Two hours after the start of LPS infusion, the animals had undergone a period of transient hypotension accompanied by vasodilation, and arterial pressure had recovered to near normal levels. The mediators responsible for the recovery in arterial pressure likely include angiotensin and/or endothelin and/or AVP,22 but sympathoadrenal activation may also contribute,23 and the latter would be expected to diminish the effects of exogenous NE. However, the sustained tachycardia seen throughout the 24 h LPS infusion is indicative of persistent sympathoadrenal activation. Thus, if the early loss of vascular responsiveness to exogenous NE was associated with receptor down-regulation and/or receptor occupancy,19,21 our findings of a recovery in NE responsiveness in the face of signs of persistent sympathoadrenal activation might indicate that α-adrenoceptors up-regulate to counteract the early loss of effect. The residual reduction in pressor response to NE seen 24–25 h after the start of the LPS infusion was not associated with a reduced vascular response, but the inferred cardiac component (i.e. increase in pulse pressure) was still diminished, perhaps indicating that cardiac β-adrenoceptors are less able to adapt to persistent sympathetic activation than are vascular α-adrenoceptors.24

The persistent lack of bradycardic response to NE in LPS-infused rats is intriguing. It is likely that the heart rate response to NE was a combination of a positive chronotropic effect of NE and reflex vagal activation, with the latter being the prevailing effect in normal animals. The loss of bradycardic response to NE in the LPS-infused rats, against the background of an underlying tachycardia, and at a time when

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<td></td>
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| Table 4 | Arterial pressures (BP) and regional blood flows (Doppler shift, DS) before (0) and at the end of 3 min infusions of AVP. Values are mean (SEM). R: renal, M: mesenteric, H: hindquarters. *P<0.05 vs pre-infusion (baseline) value at time 0 (Friedman’s test) |
there was some pressor effect of the catecholamine (24 h) most likely indicates a failure in the baroreflex response, but whether this was at the afferent, central, or efferent limb of the process is unknown. Notably, however, sustained loss of bradycardia was not seen when AVP was used to increase arterial pressure (see below).

The extent and pattern of changes in responses to AVP during LPS infusion differed from those to NE, possibly supporting the suggestion that NE responses were influenced by underlying sympathoadrenal activation. Thus, although we observed some loss of responsiveness to AVP 2 h after the onset of LPS infusion, this effect occurred under conditions in which the ability of AVP to reduce mesenteric flow was reasonably well maintained, whereas the effect of NE on mesenteric flow was lost. Moreover, we observed a recovery of responses to AVP during LPS infusion at a time when those to NE remained depressed (i.e. at 6 h). This is consistent with AVP being more effective than NE in patients with hypotension as a result of vasodilatation (see Introduction). However, the marked renal and mesenteric vasoconstrictor effects of AVP during LPS infusion indicate that this agent may have the potential to cause ischaemia. Indeed, in a recent study in endotoxaemic pigs, it was shown that AVP caused a reduction in cardiac output and gut blood flow sufficient to produce jejunal luminal lactate release and signs of visceral dysoxia. But, in the present study, although AVP was able to cause marked reductions in the elevated renal and mesenteric flows seen, particularly after 6 h of LPS infusion, the effect was largely a result of inhibition of the LPS-induced hyperaemia. Hence, the ability of AVP to suppress pathological hyperaemia could result in redistribution of blood to the brain and heart. A beneficial effect of terlipressin has been reported recently in endotoxaemic rats undergoing concurrent volume expansion. As our model of endotoxaemia produces a hyperdynamic circulation, that is increased cardiac output and total peripheral vascular conductance, it may be that deleterious effects of AVP are characteristic of the hypodynamic state.

Cytokine-mediated down-regulation of vasopressin V1A receptors has been reported, which could explain the early, transient, loss of AVP-induced vasoconstrictor responsiveness observed here, as cytokine levels are maximally elevated 1–2 h after the onset of LPS infusion in our model, and then wane. However, down-regulation of α-adrenoceptors has also been attributed to a cytokine-mediated event, but here we found different patterns of recovery in response to NE and AVP. Thus, it is unlikely that a common underlying mechanism was responsible for the changes seen. It is notable that we observed no signs of the increased sensitivity to the pressor actions of AVP reported by others, indicating that such a phenomenon may depend on the clinical condition or experimental model used. As the pressor response to AVP recovered during LPS infusion, so did the accompanying bradycardia, which contrasts with the changes seen in response to NE. Although there is evidence for unique interactions between AVP and the baroreflex control of heart rate, there are several studies failing to show such effects.

In conclusion, we have identified some differences in temporal and regional changes in the haemodynamic effects of NE and AVP in a conscious rat model of sepsis in which the circulation is in a hyperdynamic state. Although the exact mechanisms are not clear at this stage, the important findings in clinical terms are that the reduced pressor response to NE in sepsis is not necessarily accompanied by reduced capacity of vessels to constrict, and that responsiveness to AVP is less affected than that to NE. The logical extension of this work is to investigate the effects of co-administration of AVP and NE to determine if the effects of the latter are improved by the former, and to elucidate the underlying mechanisms.

Acknowledgement
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References


