British Journal of Pharmacology (1998) 123, 1367–1374

Differential effects of endotoxaemia on pressor and vasoconstrictor actions of angiotensin II and arginine vasopressin in conscious rats

Siobhán B. Tarpey, Terence Bennett, Michael D. Randall & [1]Sheila M. Gardiner

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

1 Regional haemodynamic responses to arginine vasopressin (AVP; 0.5, 1.0, 5.0 pmol i.v.) and angiotensin II (AII; 5.0, 10.0, 50.0 pmol i.v.) were measured in conscious Long Evans rats at various times (0, 2, 6 and 24 h) during infusion of lipopolysaccharide (LPS, 150 μg kg^{-1} h^{-1}, i.v., $n=9$) or saline ($n=9$). Additional experiments were performed in vasopressin-deficient (Brattleboro) rats infused with LPS ($n=7$) or saline ($n=8$) to determine whether or not, in the absence of circulating vasopressin, responses to the exogenous peptides differed from those in Long Evans rats.

2 In the Long Evans rats, during the 24 h infusion of LPS, there was a changing haemodynamic profile with renal vasodilatation from 2 h onwards, additional mesenteric vasodilatation at 6 h, and a modest hypotension (reduction in mean arterial blood pressure (MAP) from 103 ± 1 to 98 ± 2 mmHg) associated with renal and hindquarters vasodilatation at 24 h.

3 In the Brattleboro rats, the changes in regional haemodynamics during LPS infusion were more profound than in the Long Evans rats. At 2 h and 6 h, there was a marked fall in MAP (from 103 ± 3 mmHg; to 65 ± 3 mmHg at 2 h, and to 82 ± 4 mmHg at 6 h) associated with vasodilatation in all three vascular beds. After 24 h infusion of LPS, the hypotension was less although still significant (from 103 ± 3 mmHg; to 93 ± 4 mmHg, a change of 10 ± 4 mmHg), and there was renal and hindquarters vasodilatation, but mesenteric vasoconstriction.

4 During infusion of LPS, at each time point studied, and in both strains of rat, pressor responses to AII and AVP were reduced, but the changes were less marked at 6 h than at 2 h or 24 h. The reduced pressor responses were not accompanied by generalized reductions in the regional vasoconstrictor responses. Thus, in the Long Evans rats, the renal vasoconstrictor responses to both peptides were enhanced (at 6 h and 24 h for AVP; at all times for AII), whereas the mesenteric vasoconstrictor response to AVP was unchanged at 2 h, enhanced at 6 h and reduced at 24 h. The mesenteric vasoconstrictor response to AII was reduced at 2 h, normal at 6 h and reduced at 24 h. The small hindquarters vasoconstrictor responses to both peptides were reduced at 2 h and 6 h, but normal at 24 h.

5 In the Brattleboro rats, the renal vasoconstrictor responses to both peptides were reduced at 2 h and enhanced at 6 h and 24 h, whereas the mesenteric vasoconstrictor response to AVP was normal at 2 h and 6 h, and reduced at 24 h. The response to AII was reduced at 2 h, normal at 6 h and reduced again at 24 h. There were no reproducible hindquarters vasoconstrictions to AVP in the Brattleboro rats. The small hindquarters vasoconstrictor responses to AII were unchanged at 2 h and enhanced at 6 h and 24 h.

6 In isolated perfused mesenteric vascular beds, removed after 24 h of LPS infusion *in vivo*, there was an increase in the potency of AVP in both strains (Long Evans, ED_{50} saline: 56.9 ± 15.0 pmol, ED_{50} LPS: 20.4 ± 4.8 pmol, Brattleboro, ED_{50} saline: 38.6 ± 4.2, ED_{50} LPS: 19.6 ± 2.9 pmol), but no change in the responses to AII.

7 These findings indicate that a reduced pressor response to a vasoconstrictor challenge during LPS infusion is not necessarily associated with a reduced regional vasoconstriction. The data obtained in the Brattleboro rats indicate a potentially important role for vasopressin in maintaining haemodynamic status during LPS infusion in Long Evans rats. However, it is unlikely that the responses to exogenous AVP (or AII) are influenced by changes in the background level of endogenous vasopressin, since the patterns of change were similar in Long Evans and Brattleboro rats.

8 The results obtained in isolated perfused mesenteric vascular beds differed from those *in vivo*, possibly due to the conditions pertaining with *in vitro* perfusion.

Keywords: Regional haemodynamics; Long Evans rats; Brattleboro rats; endotoxaemia; lipopolysaccharide; arginine vasopressin; angiotensin II; mesenteric arterial bed; vasopressin-deficient

Introduction

There are many accounts in the literature of reduced pressor responses to administration of vasoconstrictor substances in a variety of animal models of sepsis (e.g. Guc *et al*., 1990; Joulou-Schaeffer *et al*., 1990; Thiemermann & Vane, 1990; Gray *et al*., 1991; Paya *et al*., 1993a,b), and in septic patients (Chernow *et al*., 1986). In addition, reduced responsiveness of aortic strip preparations, particularly to noradrenaline, is a common finding in experimental endotoxaemia (Beasley *et al*., 1990; Joulou-Schaeffer *et al*., 1990; Schott *et al*., 1993).

Although it might be assumed that the reduced pressor responsiveness is due to the vascular hyporeactivity, there are now several findings which suggest that, in endotoxaemia, the responsiveness of the resistance vasculature may not change in the same way as that of the aorta (e.g. Li *et al*., 1992; Mitchell

[1] Author for correspondence.

et al., 1993; Fatehi-Hassanabad *et al*., 1995). Indeed, there is one study which shows a markedly reduced pressor response to systemic administration of angiotensin II (AII) associated with an augmented vasoconstrictor response to AII in cremaster muscle arterioles, following *in vivo* administration of lipopolysaccharide (LPS) (Baker *et al*., 1992). Therefore, we hypothesized that the reduced pressor responses to vasoconstrictor challenges found in experimental endotoxaemia and in clinical sepsis (see above) are not associated with a uniform reduction in the accompanying regional vasoconstrictions.

To test this hypothesis, it would be necessary to measure, concurrently, the blood pressure and regional vasoconstrictor effects of vasoconstrictor agents in a well defined paradigm of experimental endotoxaemia (Waller *et al*., 1994; Gardiner *et al*., 1995). In addition, the choice of vasoconstrictor needs consideration. In the present study we elected to use AII and vasopressin (AVP). We know from previous studies that both peptides are involved in maintaining cardiovascular status in our model of endotoxaemia (Gardiner *et al*., 1996), and it is feasible that changes in the background levels of endogenous peptides, such as AII and AVP, could influence the responsiveness to the exogenous substances. Rats congenitally deficient in vasopressin (i.e. Brattleboro rats), in which plasma vasopressin levels are not detectable (Valtin & Schroeder, 1965), provide an interesting model in which changes in the response to exogenous AVP can be assessed in the absence of a changing background (plasma) level of the endogenous peptide. Such a model is not available for AII, but responses to AII can be influenced by AVP (see Baker *et al*., 1992).

Therefore, we assessed the changes in pressor and regional vasoconstrictor responses to AII and AVP in normal (Long Evans) rats and in Brattleboro rats, at various stages during continuous infusion of LPS for 24 h.

Although there are several previous studies on the effects of LPS on reactivity in perfused vascular beds (e.g. Li *et al*., 1992; Mitchell *et al*., 1993; Mitola-Chieppa *et al*., 1996; Martinez *et al*., 1996), none have used the model of endotoxaemia employed by us (24 h infusion of a relatively low dose of LPS), and none included *in vivo* data on vascular responsiveness, with which to compare the *ex vivo* results. So, an additional aim of this study was to assess responses to AII and AVP in perfused mesenteric vascular beds taken from the animals in which responses to AII and AVP had been measured *in vivo*.

Methods

Male, Long Evans and homozygous Brattleboro rats (350–450 g), bred in the Biomedical Services Unit, University of Nottingham, were prepared as described in detail previously (Gardiner *et al*., 1993; Waller *et al*., 1994). Briefly, under anaesthesia (sodium methohexitone, 40–60 mg kg^{-1} i.p.), pulsed Doppler probes were placed around the left renal and superior mesenteric arteries and distal abdominal aorta to monitor blood flow through the renal, mesenteric and hindquarters vascular beds. After at least 10 days recovery, the rats were anaesthetized (sodium methohexitone, as before) and catheters were inserted; 3 in the left jugular vein, for infusion of saline or LPS and for peptide administration, and a fourth catheter in the tail artery to measure mean arterial blood pressure (MAP) and heart rate (HR). Double channel swivels (Blair *et al*., 1980) were used to allow overnight, intravenous and intra-arterial infusion of heparin-treated saline (15 u ml^{-1}, 0.4 ml h^{-1}), to maintain catheter patency. Twenty four hours later, experimentation began.

Experimental protocol

Regional haemodynamic studies Rats were infused i.v. with either saline (0.4 ml h^{-1}, Long Evans, $n=9$; Brattleboro, $n=8$) or LPS (150 μg kg^{-1} h^{-1}; *E coli* serotype 0127:B8, Long Evans, $n=9$; Brattleboro, $n=7$) for 24 h. Before and starting at 2, 6 and 24 h into the infusion, each animal was given i.v. bolus doses of AII (5, 10 and 50 pmol) and AVP (0.5, 1, 5 pmol), alternately, at 15 min intervals. The Brattleboro rats received an additional low dose of AII (1 pmol), since the pressor response to the 5 pmol dose was more marked than in the Long Evans rats. For the duration of the experiment, recordings were made of MAP, HR and the renal, mesenteric and hindquarters Doppler shift signals, by use of a modified pulsed Doppler system (Gardiner *et al*., 1990a) (Crystal Biotech, Holliston, U.S.A.). Regional vascular conductances for the three vascular beds were calculated from mean Doppler shift and MAP (Gardiner *et al*., 1990b). The rats were infused with either saline or LPS for a further 60 min following the last bolus dose of either AII or AVP, after which they were anaesthetized (sodium pentobarbitone, 44 mg kg^{-1}, i.v.) for removal of the mesenteric arterial bed.

Preparation of the isolated buffer-perfused mesenteric arterial bed Following a midline laparotomy, the superior mesenteric artery was cannulated under anaesthesia and the mesenteric arterial bed removed as described by Randall *et al*. (1988) and perfused at 5 ml min^{-1} with Krebs-Henseleit solution containing (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.0 and glucose 10.0. The Krebs-Henseleit solution was gassed with 5% CO_2 and 95% O_2 and maintained at 37 ± 1°C. Perfusion pressure was continuously monitored by a pressure transducer placed close to the inflow cannula. The flow rate was constant, therefore changes in perfusion pressure reflect changes in vascular conductance. Following 30 min equilibration with normal buffer solution, a dose-response curve for AVP (0.3 pmol–1.0 nmol, 12 min apart) was obtained. In the case of AII (0.1, 1.0 and 10 nmol, 1 h apart), it was found necessary to increase the extracellular K^+ in the buffer to 25 mM to obtain reproducible results; this resulted in a rise of about 5 mmHg in perfusion pressure.

Data analysis

Haemodynamic responses to the peptides were assessed, at the time of peak change in MAP for the groups (30 s for AVP; 15 s for AII), as the peak changes from baseline. Significance was calculated by the Friedman's test (Theodorsson-Norheim, 1987) for changes from baseline within a group, and the Mann-Whitney U test for changes between the saline and LPS groups *in vivo*. All data are presented as mean$\pm$s.e.mean and $P<0.05$ was considered significant.

The data for the effects of AVP *in vitro*, which are presented as mean$\pm$s.e.mean, were compared by analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. ED_{50} values for vasoconstrictor responses were obtained from individual dose-response curves as the dose at which the half-maximal pressor response occurred. These variables were determined by fitting the data to the following logistic equation:

$$R = \frac{R_{max} \cdot A^{n_H}}{ED_{50}^{n_H} + A^{n_H}}$$

where R is the increase in perfusion pressure, A the dose of the vasoconstrictor, R_{max} the maximum response, n_H the slope function and ED_{50} the dose of vasoconstrictor giving half the maximal response (Randall *et al*., 1988). The curve fitting was

carried out by use of KaleidaGraph Software (Synergy, Reading, PA, U.S.A.) running on a Macintosh LCII computer. The EC_{50} values were converted to logarithmic (pD_2) values for statistical analysis. ANOVA with Bonferroni's *post-hoc* test was also used to test for significance of the AII data. $P<0.05$ was considered significant.

Drugs

LPS (*E. Coli* serotype 0127: B8) was obtained from Sigma (U.K.). AII and AVP were purchased from Bachem (U.K.) Ltd. All drugs were dissolved in saline.

Results

Baseline haemodynamic variables in vivo

Long Evans rats (Figure 1) There were no significant differences in the baseline haemodynamic variables between the two groups of Long Evans rats except for the heart rate which was significantly higher in the LPS group before infusion. During the 24 h period of saline infusion, the haemodynamic variables did not differ significantly from baseline. However, in the rats receiving LPS there was a biphasic hypotension and tachycardia; renal vascular conductance was markedly and persistently elevated, whereas in the mesenteric and hindquarters vascular beds the changes in vascular conductance were relatively small.

Brattleboro rats (Figure 2) There were no significant differences in the baseline haemodynamic variables between the two groups of Brattleboro rats before the infusion of saline or LPS. After 1 h of LPS infusion, there was a substantial hypotension associated with tachycardia and vasodilatation in all 3 vascular beds and these changes persisted for the following 5 h of LPS infusion. Twenty four hours after the start of LPS infusion, there was hypotension and vasodilatation in the renal and hindquarters vascular beds.

Haemodynamic responses to AVP in Long Evans and Brattleboro rats before and during saline or LPS infusion (Figures 3 and 4)

In both strains of rat, before the start of saline infusion, AVP caused a dose-related increase in MAP accompanied by a bradycardia and falls in vascular conductance in the renal and mesenteric vascular beds; a vasoconstrictor response in the hindquarters was only significant at the highest dose of AVP. Before the start of LPS infusion, in both strains, the responses to AVP were similar to those described above.

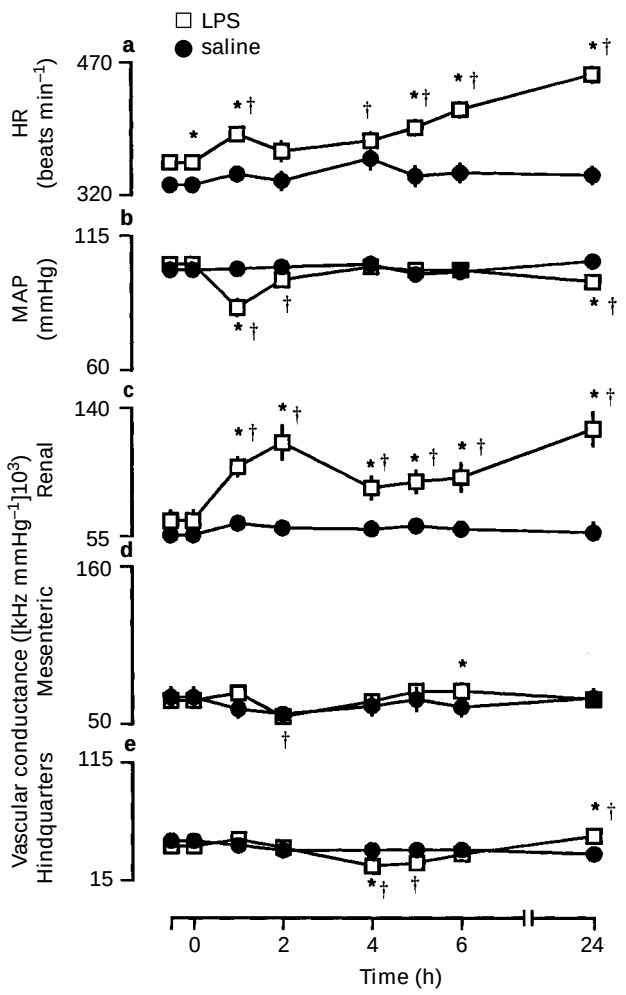

Figure 1 Blood pressure (b), heart rate (a) and regional haemodynamics (c, d, e) in conscious chronically instrumented Long Evans rats, during a 24 h infusion of saline (0.4 ml h^{-1}; $n=9$) or LPS (150 μg kg^{-1} h^{-1}; $n=9$). *$P<0.05$ versus saline group (Mann-Whitney U test). †$P<0.05$ versus baseline (Friedman's test).

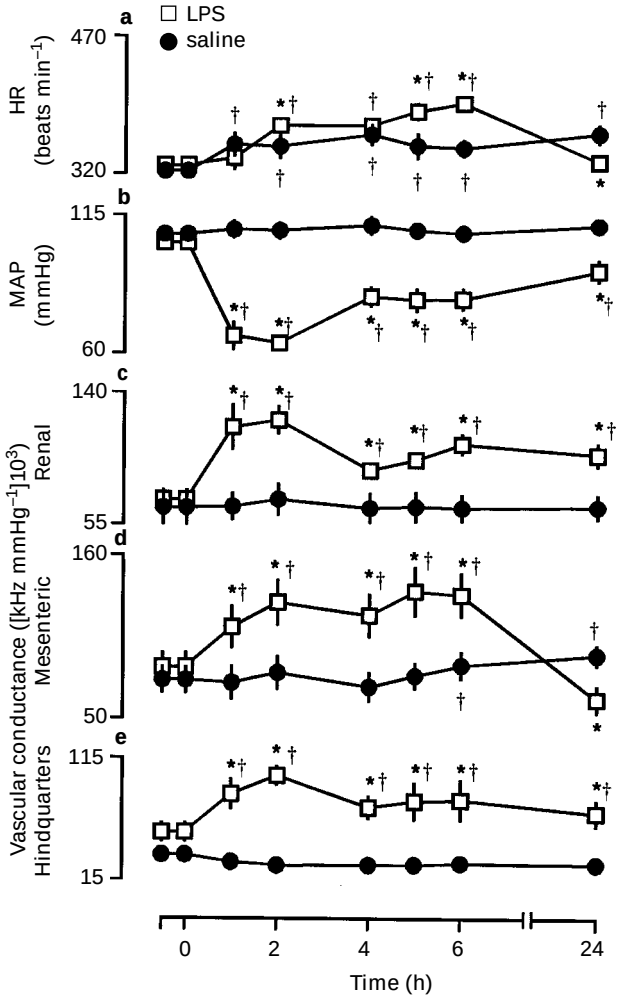

Figure 2 Blood pressure (b), heart rate (a) and regional haemodynamics (c, d, e) in conscious chronically instrumented Brattleboro rats, during a 24 h infusion of saline (0.4 ml h^{-1}; $n=8$) or LPS (150 μg kg^{-1} h^{-1}; $n=7$). *$P<0.05$ versus saline group (Mann-Whitney U test). †$P<0.05$ versus baseline (Friedman's test).

After 2 h infusion of LPS, responses to AVP in both strains differed from those in the corresponding saline-infused groups in several respects. The pressor responses were blunted (at 0.5 and 5 pmol in Long Evans and at 1.0 and 5.0 pmol in Brattleboro rats), there was a smaller bradycardia (at 1 and 5 pmol in both strains), and the mesenteric vasoconstrictor response to the lowest dose was abolished in Long Evans rats and significantly reduced (at 0.5 and 1.0 pmol) in Brattleboro rats. In addition, in the Long Evans rats, the hindquarters vasoconstrictor response to the highest dose was substantially reduced, whereas in the Brattleboro rats, neither group showed any hindquarters vasoconstrictor response to AVP at this juncture.

After 6 h infusion of LPS there were several differences in response to AVP between the saline and LPS groups. The pressor response to the highest dose (5 pmol) was depressed in both strains, and in Long Evans rats only, this was accompanied by a smaller bradycardia at the highest dose (5 pmol), although at 1.0 pmol there was a significantly larger bradycardia. The renal vasoconstrictor response to the highest dose was significantly enhanced in both strains; the mesenteric vasoconstrictor response was also enhanced, but not significantly in the Brattleboro rats, and there was a smaller vasoconstrictor response in the hindquarters in Long Evans rats only.

After 24 h infusion of LPS, there was still a significantly blunted pressor response to AVP (at 1 and 5 pmol in both strains). The vasoconstrictor response to all doses of AVP in the renal vascular bed was significantly greater than in the corresponding saline group in both strains of rat, and in the mesenteric vascular bed there was a significantly smaller vasoconstrictor response (at the 1 pmol dose in Long Evans rats, at 0.5 and 1 pmol doses in Brattleboro rats).

Haemodynamic responses to AII in Long Evans and Brattleboro rats before and during saline or LPS infusion (Figures 5 and 6)

In both strains of rat, before the start of saline infusion, AII caused a dose-related increase in MAP accompanied by bradycardia and vasoconstriction in all three vascular beds, although the response in the hindquarters was smaller and less consistent. Before the start of the LPS infusion both strains showed responses to AII that were similar to those described above.

After 2 h of LPS infusion, the pressor and bradycardic effects of all doses of AII were significantly smaller than in the corresponding saline infused groups, and there were smaller falls in renal vascular conductance (at 5 pmol in Long Evans rats; at 1, 5 and 10 pmol in Brattleboro rats), and mesenteric vascular conductance (at 5, 10 and 50 pmol in Long Evans rats; at 1 and 5 pmol in Brattleboro rats). In the Long Evans rats, the renal vasoconstrictor response to the highest dose of AII was enhanced. In the hindquarters vascular bed, there was a small but significant vasodilator response to AII (at the 5 and 10 pmol doses in Long Evans

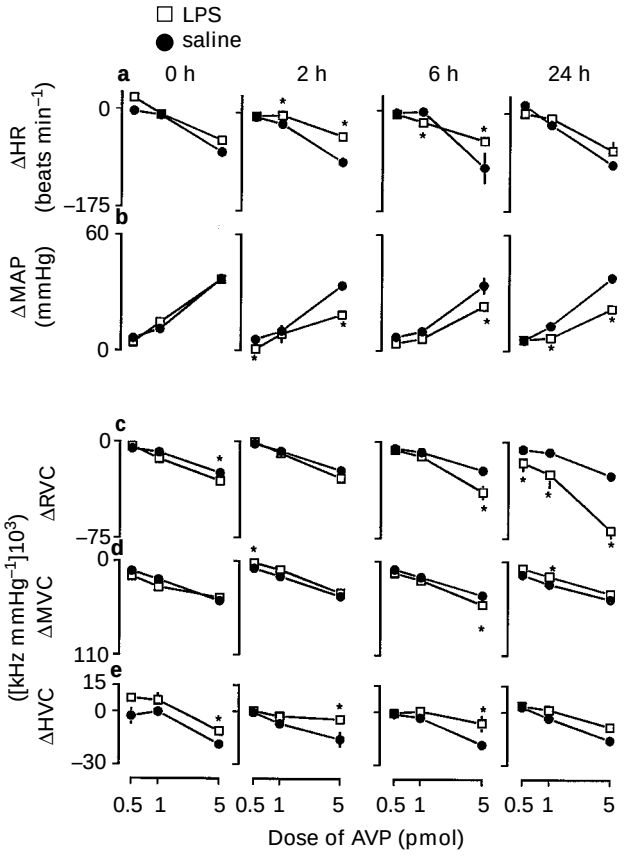

Figure 3 Pressor (b), heart rate (a) and peripheral vascular responses (c, d, e) to AVP (0.5, 1.0 and 5.0 pmol), in chronically instrumented Long Evans rats, before and during (at 2, 6 and 24 h) saline (0.4 ml h^{-1}; $n=9$) or LPS (150 μg kg^{-1} h^{-1}; $n=9$) infusion. ΔHR = change in heart rate; ΔMAP = change in mean arterial blood pressure; ΔRVC, ΔMVC, ΔHVC = change in renal, mesenteric and hindquarters vascular conductance, respectively. *$P<0.05$ versus saline group (Mann-Whitney U test).

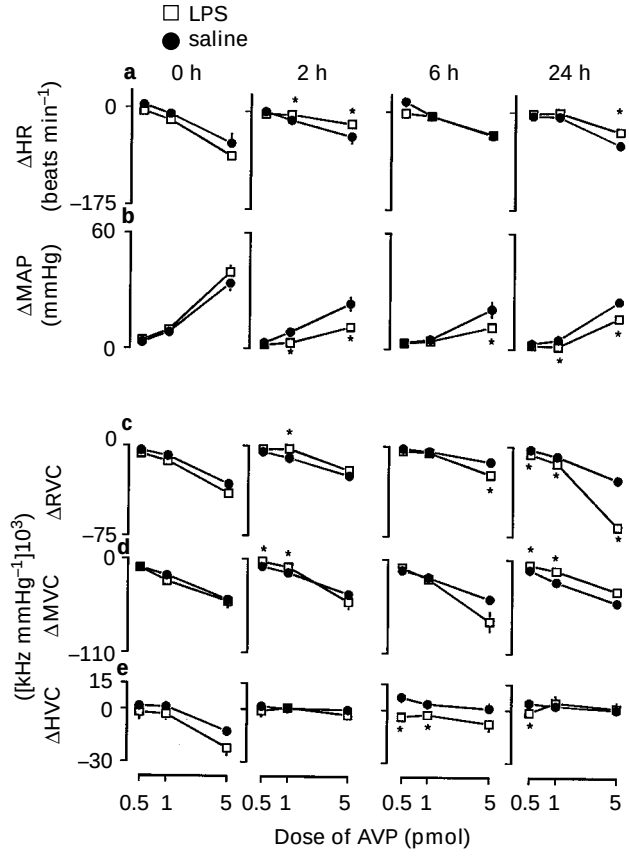

Figure 4 Pressor (b), heart rate (a) and peripheral vascular responses (c, d, e) to AVP (0.5, 1.0 and 5.0 pmol), in chronically instrumented Brattleboro rats, before and during (at 2, 6 and 24 h) saline (0.4 ml h^{-1}; $n=8$) or LPS (150 μg kg^{-1} h^{-1}; $n=7$) infusion. ΔHR = change in heart rate; ΔMAP = change in mean arterial blood pressure; ΔRVC, ΔMVC, ΔHVC = change in renal, mesenteric and hindquarters vascular conductance, respectively. *$P<0.05$ versus saline group (Mann-Whitney U test).

rats; at the 1 and 10 pmol doses in Brattleboro rats), and in the Long Evans rats, the vasoconstrictor response to the highest dose was reduced.

After 6 h infusion of LPS in Long Evans rats, there was a reduced pressor response accompanied by a smaller bradycardia at the 5 and 10 pmol doses of AII. In the Brattleboro rats the pressor response was reduced at the 5 pmol dose, but the bradycardia was unaffected. In the renal vascular bed there was a significantly greater vasoconstrictor response (at 10 and 50 pmol in Long Evans rats, at 50 pmol in Brattleboro rats). The Brattleboro rats also showed enhanced constrictor responses to the highest dose of AII in the mesenteric and hindquarters vascular beds, whereas in Long Evans rats in the hindquarters there was only a vasodilator response to the 5 pmol dose of AII, and no change in vascular conductance at the 10 pmol dose.

At all doses of AII in Long Evans rats, there was a significantly reduced pressor response after 24 h infusion of LPS. In the Brattleboro rats the pressor responses to the 5 and 10 pmol doses were reduced. There was an increased vasoconstrictor response in the renal vascular bed (at 10 and 50 pmol in Long Evans rats, at 50 pmol in Brattleboro rats), and in the mesenteric vascular bed a depressed vasoconstrictor response to the lower doses of AII (5 pmol in Long Evans rats, 1, 5 and 10 pmol in Brattleboro rats). The vasoconstrictor response in the hindquarters was significantly increased at the highest dose (50 pmol) in the Brattleboro rats.

Isolated perfused mesenteric arterial bed

Basal perfusion pressures The basal perfusion pressures of isolated mesenteric arterial beds for the saline and LPS treated groups of Long Evans rats were comparable with values of 14.4 ± 2.0 mmHg ($n=9$) and 15.2 ± 1.3 mmHg ($n=9$), respectively. Similarly, in the isolated mesenteric arterial beds from the Brattleboro rats, the basal perfusion pressure of the saline group (15.2 ± 2.0 mmHg, $n=8$) was comparable to the LPS treated group (17.2 ± 3.5 mmHg, $n=7$).

Responses to AVP AVP caused dose-dependent increases in perfusion pressure in preparations from both groups of Long Evans rats. AVP (0.3 pmol – 1.0 nmol) was significantly ($P<0.05$) more potent in the preparations from LPS-treated rats compared to the saline-treated preparations (ED_{50} saline: 56.9 ± 15.0 pmol, ED_{50} LPS: 20.4 ± 4.8 pmol), but there was no difference in the maximum response (R_{max}) between the groups (R_{max} saline: 153 ± 9 mmHg, R_{max} LPS: 164 ± 9 mmHg).

AVP caused dose-dependent increases in perfusion pressure in the isolated mesenteric vascular beds from both the saline- and LPS-treated Brattleboro rats. However, in the preparations from Brattleboro rats treated with LPS, the R_{max} was significantly ($P=<0.05$) increased (R_{max} saline: 110 ± 14 mmHg, R_{max} LPS: 187 ± 15 mmHg) and the ED_{50} significantly ($P<0.05$) reduced (ED_{50} saline: 38.6 ± 4.2 pmol, ED_{50} LPS:

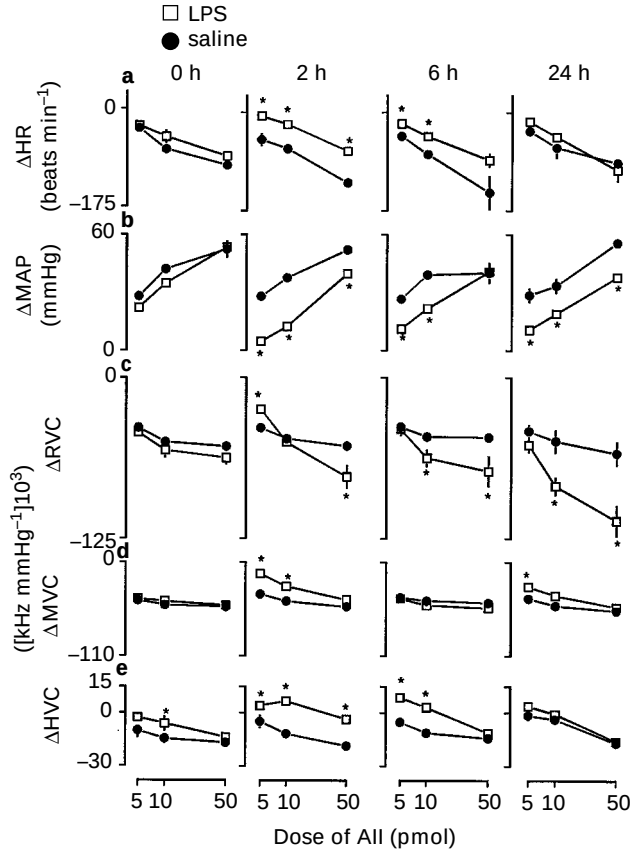

Figure 5 Pressor (b), heart rate (a) and peripheral vascular responses (c, d, e) to AII (5.0, 10.0 and 50.0 pmol), in chronically instrumented Long Evans rats, before and during (at 2, 6 and 24 h) saline (0.4 ml h^{-1}; $n=9$) or LPS (150 μg kg^{-1} h^{-1}; $n=9$) infusion. ΔHR = change in heart rate; ΔMAP = change in mean arterial blood pressure; ΔRVC, ΔMVC, ΔHVC = change in renal, mesenteric and hindquarters vascular conductance, respectively. $^*P<0.05$ versus saline group (Mann-Whitney U test).

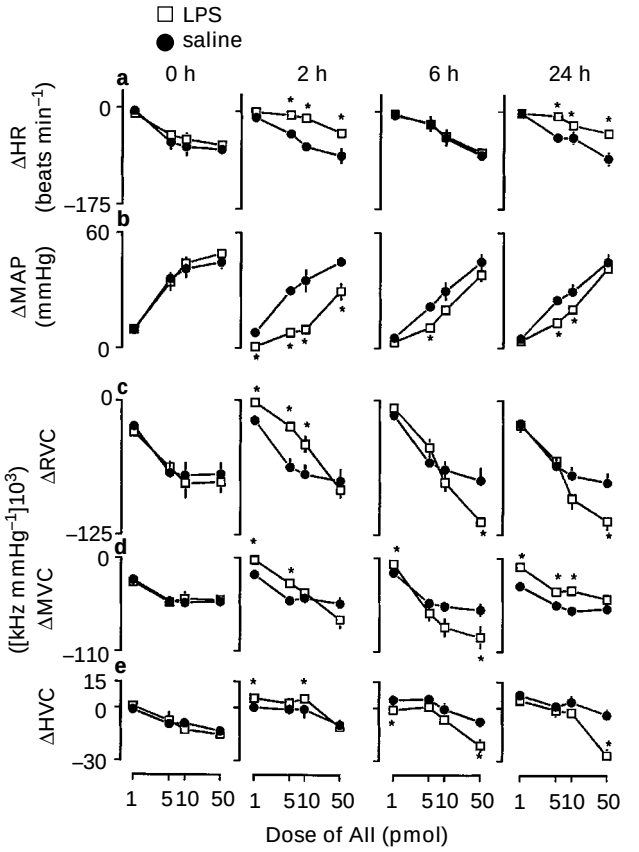

Figure 6 Pressor (b), heart rate (a) and peripheral vascular responses (c, d, e) to AVP (0.5, 1.0 and 5.0 pmol), in chronically instrumented Brattleboro rats, before and during (at 2, 6 and 24 h) saline (0.4 ml h^{-1}; $n=8$) or LPS (150 μg kg^{-1} h^{-1}; $n=7$) infusion. ΔHR = change in heart rate; ΔMAP = change in mean arterial blood pressure; ΔRVC, ΔMVC, ΔHVC = change in renal, mesenteric and hindquarters vascular conductance, respectively. $^*P<0.05$ versus saline group (Mann-Whitney U test).

19.6±2.9 pmol) compared with preparations from rats treated with saline.

Responses to AII Increasing the K^+ concentration in the buffer increased the basal perfusion pressure of the isolated mesenteric vascular beds from Long Evans rats slightly, but not significantly, (from 16.1±2.1 mmHg to 19.3±2.3 mmHg ($n=7$) in the saline group, and from 13.9±1.2 mmHg to 16.4±1.6 mmHg ($n=7$) in the LPS group). However in the preparations from Brattleboro rats these changes were significant with increases in basal perfusion pressure from 15.2±1.3 to 18.7±2.0 mmHg ($n=8$) in the saline group, and from 17.2±3.5 to 23.0±2.9 mmHg ($n=7$) in the LPS group. Under these conditions, AII (0.1 to 10 pmol) caused dose-dependent increases in perfusion pressure in both the saline and LPS groups of both strains, which were not significantly different (responses to AII 10.0 nmol, Long Evans; saline: 38.2±13.9 mmHg, LPS: 18.3±5.2 mmHg, Brattleboro; saline: 12.2±2.1 mmHg, LPS: 14.1±2.9 mmHg).

Discussion

Our unifying hypothesis was that the reduced blood pressure responses to vasoconstrictor challenges in endotoxaemia are not associated with a widespread reduction in the accompanying regional vasoconstrictions. To test this, we quantified the pressor responses to AII or AVP at selected time points during a 24 h infusion of LPS, and measured the accompanying regional vasoconstrictor responses to these agonists. Groups of vasopressin-deficient (Brattleboro) rats were included to determine whether or not a change in the plasma level of vasopressin, which would be predicted to occur in Long Evans rats (Kasting *et al*., 1985), could explain any change in responsiveness to the administration of exogenous peptides. Overall, our results show that our hypothesis cannot be rejected, since there were reduced pressor responses to both peptides in both strains of rat, but these were not always accompanied by reduced regional vasoconstrictions.

The changes in pressor and regional vasoconstrictor responses to AII and to AVP during LPS infusion in the Brattleboro rats, were remarkably similar to the changes which occurred in the Long Evans rats. This could indicate that a reduced responsiveness to AVP, for example, in the Long Evans rats, was not secondary to LPS-induced AVP release (Kasting *et al*., 1985) causing an increase in its plasma level resulting in prior occupancy of the receptors, since in the Brattleboro rats, there would be no endogenous peptide released.

One notable finding with the Brattleboro rats in this study was the marked hypotension evoked by the LPS infusion, particularly at 2 and 6 h. This contrasts with some of our earlier, preliminary observations in which we found that Brattleboro rats responded similarly to Long Evans rats when infused with LPS (Bennett *et al*., 1996). In the light of the current findings, we have since confirmed that the baseline haemodynamic responses to LPS are not influenced by the intervening AII and AVP challenges, since a separate group of Brattleboro rats given LPS without peptide administrations showed similar marked hypotension (Tarpey *et al*., unpublished data). We have no explanation for the different results. Variation between batches of LPS obtained from the supplier is a possibility although, in our experience over several years, we have never observed such differences between groups of normal rats. Alternatively, it might be that the Brattleboro rats used previously had some minor (subclinical) endotoxaemia which caused them to be more resistant to the LPS challenge. Whatever the explanation, one interpretation of the present findings could be that AVP plays an important role in maintaining blood pressure in endotoxaemia (see Reid, 1997), but more detailed experimentation would be required before reaching such a conclusion. It is interesting to note that in a very recent study published by Kusano *et al*. (1997), it was shown that AVP inhibited interleukin-1β-induced NO production. Thus, it is feasible that, in the absence of an inhibitory effect of AVP on interleukin-1β-induced NO production in the Brattleboro rats, NO production was greater than in the Long Evans rats and contributed to the more profound hypotension and vasodilatation in the former strain.

The results clearly show pressor responses to AII and AVP in both strains of rat were reduced as early as 2 h after the onset of the LPS infusion. At that stage, the extent of the reductions in pressor effects were very similar to the findings of Schaller *et al*. (1985), who measured responses to AII and lysine vasopressin, 90 min after a bolus injection of LPS. However, in that study, measurements were limited to MAP, so the novelty of our results is the finding that there was not an accompanying, generalized, reduction in the regional vasoconstrictor responses to AII or AVP.

In the control animals of both strains, AVP evoked a predominant mesenteric vasoconstriction, as shown previously (Gardiner *et al*., 1988). Two hours after the onset of LPS infusion, the peak BP responses to the high dose of AVP was reduced by about 50%, and yet the marked mesenteric vasoconstriction was unaffected. The only constrictor effect of AVP that was reduced was the relatively small hindquarters response, which is unlikely to have accounted for the diminished pressor response. Since the pressor activity of AVP is normally accompanied by a reduction in cardiac output (Gardiner *et al*., 1991), the most likely explanation for its diminished pressor action 2 h after the onset of LPS infusion is that its overall effects on vascular conductance were accompanied by an enhanced fall in cardiac output (see below).

In response to AII, the control animals of both strains showed marked, and similar, vasoconstrictions in the renal and mesenteric vascular beds, with a smaller response in the hindquarters (Gardiner *et al*., 1988). Two hours after the onset of LPS infusion, the pressor responses to all doses of AII were substantially reduced, but the accompanying vasoconstrictions were not uniformly diminished. In the mesenteric and hindquarters vascular beds, the constrictor responses to lower doses of AII were generally reduced; in fact, in the hindquarters there was a small vasodilator response instead. However, in the renal vascular bed, the constrictor response to AII was less than that in the corresponding control animals at the lower doses, not different (Long Evans) or reduced (Brattleboro) at the 10 pmol dose, and greater than (Long Evans) or similar to (Brattleboro) the corresponding controls at the 50 pmol dose. It is feasible that the renal vasodilatation caused by the LPS infusion augmented the vasoconstrictor effects of AII but, if so, it is unclear why such an effect was not apparent at all doses, and did not occur in the Brattleboro rats at any dose. Alternatively, it is possible that, with the different doses of AII, there was a different involvement of prostanoids, for example. Vicaut *et al*. (1996) showed that acute exposure of vascular preparations to LPS resulted in enhanced AII-induced contractions, as a result of a change in the balance between vasodilator and vasoconstrictor prostanoids in favour of the vasoconstrictor, thromboxane A_2. It is possible such an effect was only apparent in the Long Evans rats with

the high dose of AII used here, but it is not clear why it should have been localized to the renal vasculature. Obviously, further studies are required to examine the possible involvement of prostanoids in the different regional vascular effects of AII, and how they are influenced by LPS. In addition, as for AVP (see above), a possible influence of differential changes in cardiac output in response to AII would need to be considered.

Six hours after the onset of LPS infusion, the reduced pressor response to the high dose of AVP was accompanied by enhanced constrictions in both the renal and mesenteric vascular beds. Since, at that juncture, baseline vascular conductance was increased in both regions, then the underlying vasodilatation could explain the enhanced capacity to constrict in response to AVP. However, it is notable that, in the Brattleboro rats, the underlying vasodilatation in the mesenteric vascular bed was more marked than in the Long Evans rats, yet in the former strain the enhancement in the mesenteric vasoconstrictor effect of AVP failed to reach significance. Interestingly, in the Long Evans rats under the same conditions, the renal vasoconstriction, but not the mesenteric vasoconstriction, to AII was enhanced, whereas in the Brattleboro rats both the renal and mesenteric vasoconstrictor effects of AII were augmented.

Baker *et al.* (1990) showed increased contractile responses to topical application of AVP in cremaster arterioles of endotoxaemic rats, and suggested their findings could be explained by an interaction between endogenous AII (or noradrenaline) and exogenous AVP (Baker *et al.*, 1992). However, in our endotoxaemic model, we have shown that endogenous AII may be more important in supporting the circulation at 2 h, than at 6 h, into the LPS infusion (Gardiner *et al.*, 1996), and yet the vasoconstrictor effects of AVP were more enhanced at 6 h than at 2 h. Hence, these observations do not fit easily with a simple synergistic interaction between endogenous AII and exogenous AVP. Since, in normal rats, indomethacin enhances renal vasoconstrictor responses to AVP (Gardiner *et al.*, 1991), it is feasible that LPS-induced changes in responses to AVP involve modulation of prostanoid-mediated mechanisms, but this needs further investigation.

There was a reduction in the pressor response to AII at 6 h which was not as marked as that seen at 2 h, and generally not significant in the Brattleboro rats. Moreover, although the renal vasoconstrictor response to AII was still enhanced, its mesenteric vasoconstrictor effect in the Long Evans rats was normal. Several possible explanations for an enhanced renal vasoconstrictor response to AII have already been discussed above. The finding that, overall, the responses to exogenous AII were less affected at 6 h than at 2 h is interesting against the background of our earlier investigations, indicating that the cardiovascular influence of endogenous AII was less at 6 h than at 2 h after the onset of LPS infusion (Gardiner *et al.*, 1996). One possible explanation of these earlier findings was that the vascular sensitivity to AII diminished with time during LPS infusion, but the present results indicate that this may not have been the case. It is feasible that the reduced sensitivity to exogenous AII, seen 2 h after the onset of LPS infusion, was associated with increased activity of the endogenous renin-angiotensin system at that stage, resulting in elevated circulating levels of AII, thereby causing desensitization to exogenous AII. However, it could be argued also that prior occupancy of some receptors by endogenous AII would render the system more sensitive to exogenous AII (Baker *et al.*, 1992). A further complication is the possibility of LPS-induced changes in AII-receptor number, with both increases (Burnier *et al.*, 1995) and decreases (Cahill *et al.*, 1995) being obtained. However, those studies both involved aortic vascular smooth muscle cells in culture, with substantial effects being observed only after 18–24 h incubation with LPS, and so are not immediately relevant to the present work.

After 24 h of LPS infusion, the pressor responses to both peptides were markedly reduced, whereas the renal vasoconstrictor responses were either enhanced or unchanged. As discussed above, this could, at least in part, be explained by the marked underlying renal vasodilatation, although it is notable that similar results were not obtained when methoxamine was used as the vasoconstrictor challenge, albeit at a single dose (Waller *et al.*, 1994). In the mesenteric vascular bed, there was some attenuation of the vasoconstrictor responses to AII and AVP, particularly at the lower doses of the peptides, and more consistently in the Brattleboro rats.

Interestingly, when studied *ex vivo*, vascular sensitivity to AVP was enhanced in the perfused mesenteric vascular beds taken from LPS-infused compared to saline-infused rats, and the difference was particularly striking in the Brattleboro rats. These alterations in responses were specific for AVP, as endotoxaemia had no effect on the responses to AII, or on the small effect on tone of the modest elevation of K^+. This might indicate a selective up-regulation of the mechanisms responsible for AVP-induced vasoconstriction possibly at the receptor level, although Burnier *et al.* (1995) found that AII, but not AVP, receptor numbers increased in cultured aortic endothelial cells exposed to LPS. The fact that the findings *in vivo* were not directly mirrored by the *ex vivo* results may have been due to factors such as the availability of L-arginine (Schott *et al.*, 1993; Schnieder *et al.*, 1994), or some other factor present in blood but not in the Krebs-Henseleit solution. However, it is interesting that in a recent study it was shown that the mesenteric vascular hyporeactivity which occurs in a model of portal hypertension is equally demonstratable under *in vitro* and *in situ* conditions (Heinemann *et al.*, 1997).

Whatever the explanation for our overall findings, it is clear that a reduced pressor response to AII or AVP *in vivo* is not necessarily associated with uniformly reduced regional vasoconstrictions. The regions of the vasculature covered by the present observations (renal, superior mesenteric, hindquarters) comprise a major part of the overall vascular tree. Thus, to explain our findings of a smaller rise in blood pressure in response to AII or AVP in the LPS-treated rats, without, overall, a smaller vasoconstriction, there would either have to have been vasodilatation in a region (or regions) not measured, or a greater reduction in cardiac output. Interestingly, the latter proposition is consistent with the finding by Saetre *et al.* (1996) showing that administration of a NO synthase inhibitor to pigs receiving a continuous infusion of LPS, caused pronounced vasoconstriction, but no change in MAP, since there was also a large fall in cardiac output.

Since reduced pressor responsiveness to vasoconstrictor agents is not a phenomenon confined to experimental endotoxaemia but is also a clinical problem (Chernow *et al.*, 1986) the present findings indicate the need for caution in the clinical setting when treatment is aimed at normalizing blood pressure and regional perfusion is not measured. For instance, if an underlying vasoconstriction in the mesenteric vascular bed is enhanced even though the blood pressure is not increased, then localized splanchnic perfusion could be, unknowingly, seriously compromised.

S.B.T. holds a Sir Francis Hill studentship.

References

BAKER, C.H., SUTTON, E.T., ZHOU, Z. & DIETZ, J.R. (1990). Microvascular vasopressin effects during endotoxin shock in the rat. *Circ. Shock,* **30,** 81–95.

BAKER, C.H., SUTTON, E.T. & DIETZ, J.R. (1992). Endotoxin alteration of muscle microvascular renin-angiotensin responses. *Circ. Shock,* **36,** 224–230.

BEASLEY, D., COHEN, R.A. & LEVINSKY, N.G. (1990). Endotoxin inhibits contraction of vascular smooth muscle in vitro. *Am. J. Physiol.,* **258,** H1187–H1192.

BENNETT, T., KEMP, P.A., MARCH, J.E. & GARDINER, S.M. (1996). Haemodynamic effects of lipopolysaccharide (LPS) infusion in conscious, vasopressin-deficient Brattleboro rats. *Br. J. Pharmacol.,* **117,** 228P.

BLAIR, R., FISHMAN, B., AMIT, Z. & WEEKS, J.R. (1980). A simple double channel swivel for infusions of fluids into unrestrained animals. *Pharmacol. Biochem., Behav.,* **12,** 463–466.

BURNIER, M., CENTENO, G., WAEBER, G., CENTENO, C. & BÜRKI, E. (1995). Effect of endotoxin on the angiotensin II receptor in cultured vascular smooth muscle cells. *Br. J. Pharmacol.,* **116,** 2524–2530.

CAHILL, P.A., REDMOND, E.M., FOSTER, C. & SITZMANN, J.V. (1995). Nitric oxide regulates angiotensin II receptors in vascular smooth muscle cells. *Eur. J. Pharmacol.,* **288,** 219–229.

CHERNOW, B., RAINEY, T.L. & LAKE, C.R. (1986). Endogenous and exogenous catecholamines in critical care medicine. *Crit. Care Med.,* **10,** 409–416.

FATEHI-HASSANABAD, Z., FURMAN, B.L. & PARRATT, J.R. (1995). The effect of endotoxin on sympathetic responses in the rat isolated perfused mesenteric bed; involvement of nitric oxide and cyclo-oxygenase products. *Br. J. Pharmacol.,* **116,** 3316–3322.

GARDINER, S.M., BENNETT, T. & COMPTON, A. (1988). Regional haemodynamic effects of neuropeptide Y, vasopressin and angiotensin II in conscious, unrestrained, Long Evans and Brattleboro rats. *J. Auton. Nerv. Syst.,* **24,** 15–27.

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

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

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1991). Effects of N^G-nitro-L-arginine methyl ester or indomethacin on differential regional and cardiac haemodynamic actions of arginine vasopressin and lysine vasopressin in conscious rats. *Br. J. Pharmacol.,* **102,** 65–72.

GARDINER, S.M., KEMP, P.A., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1993). Regional and cardic haemodynamic effects of N^G, N^G, dimethyl-L-arginine and their reversibility by vasodilators in conscious rats. *Br. J. Pharmacol.,* **110,** 1457–1464.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995). Cardiac and regional haemodynamics, inducible nitric oxide synthase (NOS) activity, and the effects of NOS inhibitors in conscious, endotoxaemic rats. *Br. J. Pharmacol.,* **116,** 2005–2016.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996). Temporal differences between the involvement of angiotensin II and endothelin in the cardiovascular responses to endotoxaemia in conscious rats. *Br. J. Pharmacol.,* **119,** 1619–1627.

GRAY, G.A., SCHOTT, C., JOULOU-SCHAEFFER, G., FLEMING, I.G., PARRATT, J.R. & STOCLET, J.-C. (1991). The effect of inhibitors of the L-arginine/nitric oxide pathway on endotoxin-induced loss of vascular responsiveness in anaesthetized rats. *Br. J. Pharmacol.,* **103,** 1218–1224.

GUC, M.O., FURMAN, B.L. & PARRATT, J.R. (1990). Endotoxin-induced impairment of vasopressor and vasodepressor responses in the pithed rat. *Br. J. Pharmacol.,* **101,** 913–919.

HEINEMANN, A., WACHTER, C.H., HOLZER, P., FICKERT, P. & STAUBER, R.E. (1997). Nitric oxide-dependent and -independent vascular hyporeactivity in mesenteric arteries of portal hypertensive rats. *Br. J. Pharmacol.,* **121,** 1031–1037.

JOULOU-SCHAEFFER, G., GRAY, G.A., FLEMING, I., SCHOTT, C., PARRATT, J.R. & STOCLET, J.-C. (1990). Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. *Am. J. Physiol.,* **259,** H1038–H1043.

KASTING, N.W., MAZUREK, M.F. & MARTIN, J.B. (1985). Endotoxin increases vasopressin release independently of known physical stimuli. *Am. J. Physiol.,* **248,** E420-E424.

KUSANO, E., TIAN, S., UMINO, T., TETSUKA, T., ANDO, Y. & ASANO, Y. (1997). Arginine vasopressin inhibits interleukin-1β-stimulated nitric oxide and cyclic guanosine monophosphate production via the V1 receptor in cultured rat vascular smooth muscle cells. *J. Hypertension,* **15,** 627–632.

LI, T., CROCE, K. & WINQUIST, R.J. (1992). Regional differences in the effects of septic shock on vascular reactivity in the rabbit. *J. Pharmacol. Exp. Ther.,* **261,** 959–963.

MARTÍNEZ, M.C., MULLER, B., STOCLET, J.-C. & ANDRIANTSITOHAINA, R. (1996). Alteration by lipopolysaccharide of the relationship between intracellular calcium levels and contraction in rat mesenteric artery. *Br. J. Pharmacol.,* **118,** 1218–1222.

MITCHELL, J.A., KOHLHAAS, K.L., SORRENTINO, R., WARNER, T.D., MURAD, F. & VANE, J.R. (1993). Induction by endotoxin of nitric oxide synthase in the rat mesentery: lack of effect on vasoconstrictors. *Br. J. Pharmacol.,* **109,** 265–270.

MITOLA-CHIEPPA, D., SERIO, M., POTENZA, M.A., MONTAGNANI, M., MANSI, G., PECE, G., JIRILLO, E. & STOCLET, J.-C. (1996). Hyporeactivity of mesenteric vascular bed in endotoxin-treated rats. *Eur. J. Pharmacol.,* **309,** 175–182.

PAYA, D., GRAY, G.A., FLEMING, I. & STOCLET, J.-C. (1993a). Effect of dexamethasone on the onset and persistence of vascular hyporeactivity induced by *E. coli* lipopolysaccharide in rats. *Circ. Shock,* **41,** 103–112.

PAYA, D., GRAY, G.A. & STOCLET, J.-C. (1993b). Effects of methylene blue on blood pressure and reactivity to norepinephrine in endotoxaemic rats. *J. Cardiovasc. Pharmacol.,* **21,** 926–930.

RANDALL, M.D., KAY, A.P. & HILEY, C.R. (1988). Endothelium-dependent modulation of the pressor activity of arginine vasopressin in the isolated superior mesenteric arterial bed of the rat. *Br. J. Pharmacol.,* **95,** 646–652.

REID, I.A. (1997). Role of vasopressin deficiency in the vasodilatation of septic shock. *Circulation,* **95,** 1108–1110.

SAETRE, T., SMISETH, D.A., SCHOLZ, T., CARLSEN, H., NORDSLETTEN, L., FAHLSTROM, E. & AASEN, A.O. (1996). Nitric oxide synthase inhibition reduces venous return in porcine endotoxaemia. *Am. J. Physiol.,* **271,** H1325–H1332.

SCHALLER, M.D., WAEBER, B., NUSSBERGER, J. & BRUNNER, H.R. (1985). Angiotensin II, vasopressin, and sympathetic activity in conscious rats with endotoxaemia. *Am. J. Physiol.,* **249,** H1086–H1092.

SCHNEIDER, F., BUCHER, B., SCHOTT, C., ANDRE, A., JOULOU-SCHAEFFER, G. & STOCLET, J.-C. (1994). Effect of bacterial lipopolysaccharide on function of rat small femoral arteries. *Am. J. Physiol.,* **266,** H191–H198.

SCHOTT, C., GRAY, G.A. & STOCLET, J.-C. (1993). Dependence of endotoxin-induced vascular hyporeactivity on extracellular L-arginine. *Br. J. Pharmacol.,* **108,** 38–43.

THIEMERMANN, C. & VANE, J. (1990). Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. *Eur. J. Pharmacol.,* **182,** 591–595.

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

VALTIN, H. & SCHROEDER, H.A. (1965). Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). *Am. J. Physiol.,* **206,** 425–430.

VICAUT, E., RASETTI, C. & BAUDRY, N. (1996). Effects of tumour necrosis factor and interleukin-1 on the constriction induced by angiotensin II in rat aorta. *J. Appl. Physiol.,* **80,** 1891–1897.

WALLER, J., GARDINER, S.M. & BENNETT, T. (1994). Regional haemodynamic responses to acetylcholine, methoxamine, salbutamol and bradykinin during lipopolysaccharide infusion in conscious rats. *Br. J. Pharmacol.,* **112,** 1057–1064.

(Received July 10, 1997
Revised November 20, 1997
Accepted December 23, 1997)