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Effects of the novel selective endothelin ET_A receptor antagonist, SB 234551, on the cardiovascular responses to endotoxaemia in conscious rats

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1 In conscious, freely moving, male, Long Evans rats, regional haemodynamic responses to exogenous endothelin-1 (ET-1; 25, 50 and 250 pmol kg^{-1} i.v.) were assessed in the presence of vehicle, or the selective ET_A-receptor antagonist, SB 234551. On the following day, the effects of SB 234551 on the haemodynamic responses to lipopolysaccharide (LPS) infusion (150 μg kg^{-1} h^{-1}, i.v.) were determined.

2 When SB 234551 was given i.v. by primed infusion at a dose of 0.3 mg kg^{-1} bolus, 0.3 mg kg^{-1} h^{-1} infusion, it caused selective inhibition of the vasoconstrictor effects of exogenous endothelin-1, whereas at a dose of 1 mg kg^{-1}, 1 mg kg^{-1} h^{-1}, SB 234551 also inhibited some of the vasodilator effects of endothelin-1.

3 Infusion of LPS, in the presence of vehicle, caused a short-lived (1–2 h) hypotension, tachycardia, and vasodilatation in renal, superior mesenteric and hindquarters vascular beds. Thereafter, blood pressure, heart rate and mesenteric vascular conductance returned to baseline values, but renal vasodilatation persisted, and there was vasoconstriction in the hindquarters.

4 In the presence of SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}), the early (1–2 h) cardiovascular responses to LPS infusion were unaffected, but the subsequent recovery of mean arterial blood pressure was impaired, due to developing vasodilatation in the mesenteric and, to a lesser extent, hindquarters, vascular beds. SB 234551 had no effect on the renal haemodynamic responses to LPS infusion.

5 The results confirm an important, regionally-selective, vasoconstrictor role for endogenous endothelin in this model of endotoxaemia.

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Abbreviations: ET, endothelin; LPS, lipopolysaccharide; NO, nitric oxide

Introduction

The cardiovascular sequelae of the systemic inflammatory response syndrome (SIRS), sepsis, and septic shock are complex, being influenced by many different vasoactive mediators, particularly those derived from the endothelium (see Beishuizen *et al*., 1999, for review). Although systemic vasodilatation, possibly due to nitric oxide (NO), is a characteristic of SIRS (Thiemermann, 1997), there is evidence for an important interplay between vasodilator and vasoconstrictor systems in certain vascular beds. For example, in animal models of endotoxaemia (e.g., Gardiner *et al*., 1996a, b; Iskit *et al*., 1999; Heyman *et al*., 2000) and in human septic shock (Avontuur *et al*., 1999), NO may interact with endothelin in the control of vascular tone, consistent with the substantial evidence for activation of the endothelin system in experimental endotoxaemia (e.g., Kaddoura *et al*., 1996; Curzen *et al*., 1997), and in clinical sepsis (Pittet *et al*., 1991). Furthermore, it appears that endothelin may play an important role in the pathophysiology of septic and endotoxic shock in a number of different ways, in addition to its effects on vascular tone (see Wanecek *et al*., 2000, for review).

Endothelin causes vasoconstriction, mainly *via* ET_A-receptors, but also *via* a subtype of ET_B-receptor, and vasodilatation, *via* ET_B-receptors (Haynes & Webb, 1998). In normal man, the predominant vascular effect of endothelin is believed to be constriction (Haynes & Webb, 1998), but there is some evidence that ET_B-receptor-mediated vasodilatation may contribute to normal cardiovascular status in rats (Gellai *et al*., 1996). Moreover, ET_B-receptor function may change in experimental endotoxaemia (Curzen *et al*., 1997).

We have described the regional haemodynamic changes in a model of endotoxaemia, achieved by continuous infusion of lipopolysaccharide (LPS) in conscious rats (Gardiner *et al*., 1995a), and have shown an involvement of endothelin in the cardiovascular changes in that model, using the non-peptide, non-selective, endothelin receptor antagonist, SB 209670 (Gardiner *et al*., 1995b; 1996a, b, c). Those studies consistently revealed an effect of endothelin in supporting blood pressure and restraining vasodilatation in the mesenteric and hindquarters vascular beds, but surprisingly, not in the kidney. One possible explanation for those

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findings was that the apparent lack of effect of SB 209670 on the renal haemodynamic changes associated with endotoxaemia, was due to concurrent antagonism of ET_A- and ET_B-receptor-mediated vasoconstriction, and of ET_B-receptor-mediated vasodilatation. Thus, the aim of the present study was to test the hypothesis that treatment with the ET_A-receptor-selective antagonist, SB 234551 (Ohlstein *et al*., 1998), would enhance the renal vasodilator effects of LPS infusion in conscious rats, by leaving unopposed the ET_B-receptor-mediated vasodilator actions of endothelin.

Although the effects of SB 234551 on blood pressure responses to exogenous endothelin in rats (Ohlstein *et al*., 1998; Gardiner *et al*., 2000a), and its influence on the renal haemodynamic effects of endothelin in dogs (Brooks *et al*., 1998) have been described, we are unaware of any studies in rats in which the effects of SB 234551 on the regional vascular actions of exogenous endothelin have been characterized. Therefore, to establish the dose of SB 234551 to be used in the experiments involving LPS, we first assessed the effects of SB 234551 on the regional haemodynamic responses to a range of doses of endothelin in conscious rats.

Some of these results have been presented to the British Pharmacological Society (Gardiner *et al*., 2000b).

Methods

Experiments were carried out on male, Long Evans rats (350–450 g), bred in the Biomedical Services Unit, University of Nottingham. Animals were housed under a 12 h light/dark cycle, (with lights on from 06.00 to 18.00 h), and had free access to food and water throughout. The Home Office project licence, under which the experiments were performed, was approved by the University of Nottingham Ethical Review Committee.

Under anaesthesia (Hypnorm, Janssen (0.126 mg kg^{-1} fentanyl citrate, 4 mg kg^{-1} fluanisone i.p.) and midazolam (Antigen Pharmaceuticals, 5 mg kg^{-1} i.p.), miniaturized pulsed Doppler flow probes were implanted around the left renal artery, the superior mesenteric artery and the distal aorta (hindquarters). After surgery, the anaesthesia was reversed with naloxone (Narcan, DuPont, 0.1 mg kg^{-1} i.p.), and the animals were given post-operative analgesia (buprenorphine hydrochloride, Vetergesic, Reckitt & Colman, 10 mg kg^{-1} i.m.). Subsequently, and at least 12 days after the probe placement, the animals were anaesthetized again (as above), and catheters were implanted in the distal abdominal aorta (*via* the caudal artery) to monitor arterial blood pressure and heart rate, and in the right jugular vein for drug administrations. Experiments began 24 h after catheter placement, when the animals were fully conscious and freely-moving in their home cages.

Continuous recordings of cardiovascular variables were made using a customized, computer-based system (Haemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht), which sampled the raw data every 2 ms, averaged every cardiac cycle, and stored to disc at 5 s intervals. Data were analysed offline using software (Datview, University of Limburg, Maastricht) which interfaced with HDAS.

Effects of SB 234551 on the regional haemodynamic responses to exogenous endothelin

On the first experimental day, animals were given three, i.v. doses of endothelin-1 (25, 50 and 250 pmol kg^{-1}) in ascending order, 60 min apart, starting 2 h after the onset of infusion of SB 234551 (for doses see below), or vehicle (0.5% Na_2CO_3 diluted 1 : 4 with 5% dextrose, $n=8$). In a previous study (Gardiner *et al*., 2000a), we used a dose of SB 234551 (1 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion) which, unlike SB 209670, did not affect the depressor effects of a high bolus dose of endothelin after a 2 h infusion, although selectivity was lost with the passage of time (Gardiner *et al*., 2000a). So, in the present study, in the first experiments ($n=4$) we used that dose of SB 234551 (i.e., 1 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion), but it became clear that the hindquarters vasodilator effect of endothelin was inhibited (see Results). Therefore, the effects of a lower dose of SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}) on responses to endothelin were assessed in another group of animals ($n=8$).

Effects of SB 234551 on the regional haemodynamic responses to LPS infusion

On the second experimental day, animals that had received vehicle or SB 234551 on day 1 were given the same treatment, starting 1 h before, and continuing throughout, a 6 h infusion of LPS (*E. coli* serotype 0127 B8, 150 μg kg^{-1} h^{-1}), and cardiovascular variables were recorded continuously.

Data analysis

Within-group analysis of data was by Friedman's test and between-group comparisons were made using the Mann–Whitney *U*-test applied to integrated responses (areas under or over curves, 0–10 min for responses to endothelin, 0–6 h for responses to LPS). A P value <0.05 was taken as significant; values given are mean $\pm$ s.e.mean.

Materials

Endothelin-1 (human) was obtained from Bachem (U.K.). A stock solution (20 nmol ml^{-1}) was prepared in sterile water, and subsequent dilutions were made using sterile saline. SB 234551 ([(E)-alpha-[[1-butyl-5-[2-[2-carboxyphenyl)methoxyl]-4-methoxyphenyl]-1H-pyrazol-4-yl]methylene]-6-methoxyl-1,3-benzodioxole-5-propionic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.). LPS (*E. coli* serotype 0127 B8) was purchased from Sigma (U.K.) and dissolved in sterile isotonic saline. Bolus injections were given in a volume of 0.1 ml and infusions were at a rate of 0.4 ml h^{-1}. All substances were administered by i.v. injection and/or infusion.

Results

Effects of SB 234551 on the regional haemodynamic responses to exogenous endothelin-1

At the start of the first experimental day, resting haemodynamic variables in the three groups of animals, prior to the

onset of infusion of vehicle ($n=8$), or SB 234551 (1 mg kg^{-1}, 1 mg kg^{-1} h^{-1} ($n=4$)), or SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1} ($n=8$)), were similar (heart rate, 362 ± 10, 327 ± 7, 342 ± 12 beats min^{-1}; mean arterial blood pressure, 105 ± 2, 108 ± 5, 110 ± 3 mmHg; renal vascular conductance, 85 ± 9, 94 ± 14, 94 ± 10 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 85 ± 8, 76 ± 4, 69 ± 2 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 44 ± 6, 49 ± 7, 41 ± 3 (kHz mmHg^{-1})10^3, respectively.

During the 2 h infusion period, there was a small fall in mesenteric vascular conductance in all groups, but no other haemodynamic changes. Thus, immediately before the first dose of endothelin-1, there were no differences between the haemodynamic variables in the rats receiving vehicle, or SB 234551 (1 mg kg^{-1}, 1 mg kg^{-1} h^{-1}), or SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}) (heart rate, 329 ± 8, 328 ± 13, 327 ± 16 beats min^{-1}; mean arterial blood pressure, 109 ± 2, 108 ± 4, 109 ± 4 mmHg; renal vascular conductance, 78 ± 11, 91 ± 16, 83 ± 10 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 66 ± 7, 65 ± 5, 50 ± 6 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 36 ± 5, 44 ± 9, 42 ± 3 (kHz mmHg^{-1})10^3, respectively).

In vehicle-infused rats, endothelin-1 (25 and 50 pmol kg^{-1}) caused a dose-dependent pressor effect, accompanied by an initial tachycardia and subsequent bradycardia (Figure 1a,b). The highest dose of endothelin-1 (250 pmol kg^{-1}) caused an initial, transient depressor effect, followed by a rise in mean arterial blood pressure (Figure 1c); these changes were associated with substantial tachycardia and bradycardia, respectively (Figure 1c). Accompanying these responses, endothelin-1 caused dose-dependent, renal and mesenteric vasoconstrictor effects (Figure 1a–c). In the hindquarters vascular bed there was initial, dose-dependent vasodilatation, followed by vasoconstriction. At the highest dose of endothelin-1, the maximum fall in mesenteric vascular conductance was delayed (Figure 1c).

In rats receiving SB 234551 at the higher dose (1 mg kg^{-1}, 1 mg kg^{-1} h^{-1}; data not illustrated), the increase in hindquarters vascular conductance following administration of endothelin-1 was smaller than in the rats receiving vehicle only (significant for 50 pmol kg^{-1}; $+14\pm12\%$ *vs* $+58\pm11\%$ at 15 s, respectively). Furthermore, the rate of development of the mesenteric vasoconstrictor effect of the highest dose of endothelin-1 (250 pmol kg^{-1}) was more rapid ($-56\pm6\%$ *vs* $-25\pm6\%$ at 15 s, respectively).

In rats receiving SB 234551 at the lower dose (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}; Figure 1a–c), the integrated pressor and renal and mesenteric vasoconstrictor effects of the two lower doses of endothelin-1 were significantly less than in the vehicle-infused rats, but the hindquarters vascular responses were not different (Figure 1a,b). Only the renal vasoconstrictor effect of the highest dose of endothelin-1 was significantly antagonized by SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}) (Figure 1c).

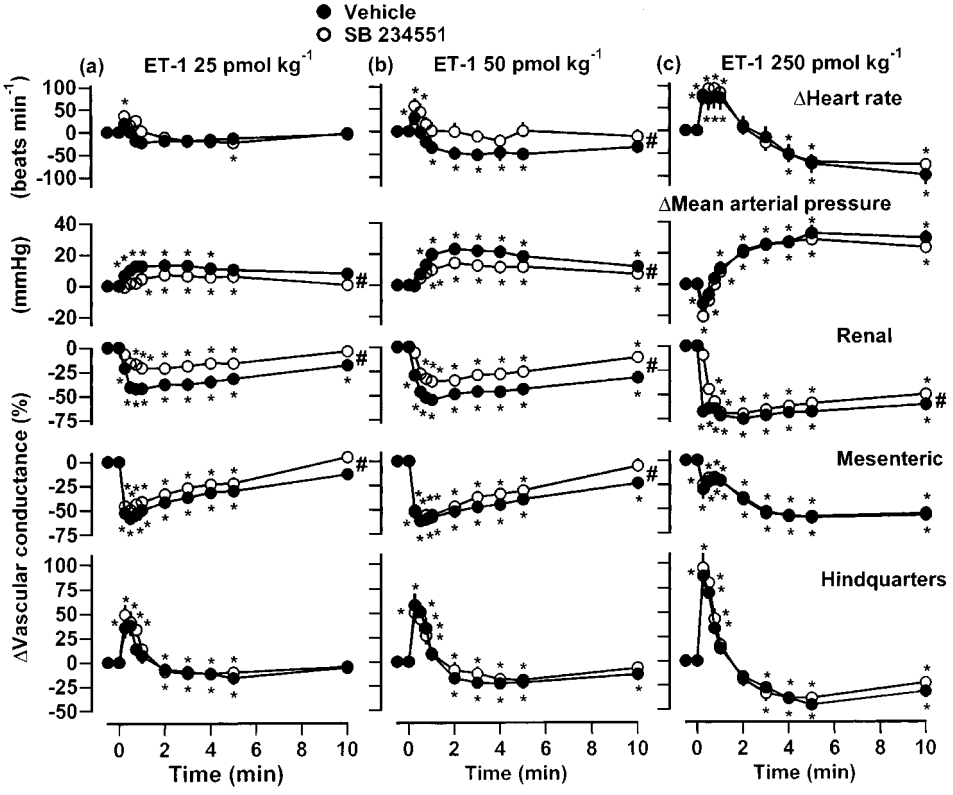

Figure 1 Changes in cardiovascular variables in response to endothelin-1 (ET-1) in conscious rats receiving vehicle ($n=8$) or SB 234551 (0.3 mg kg^{-1}; 0.3 mg kg^{-1} h^{-1} ($n=8$)). Values are mean and vertical bars show s.e.mean. *$P<0.05$ *vs* baseline (Friedman's test); #$P<0.05$ between the integrated responses (Mann–Whitney *U*-test).

Effects of SB 234551 on the regional haemodynamic responses to LPS infusion

At the start of the second experimental day, resting haemodynamic variables in the three groups of animals, prior to the onset of infusion of vehicle ($n=8$), or SB 234551 (1 mg kg^{-1}, 1 mg kg^{-1} h^{-1} ($n=4$)), or SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1} ($n=8$)) were similar (heart rate, 341±10, 324±12, 318±9 beats min^{-1}; mean arterial blood pressure, 105±2, 108±6, 105±3 mmHg; renal vascular conductance, 76±10, 99±19, 92±11 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 75±5, 75±9, 67±6 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 37±5, 43±9, 42±4 (kHz mmHg^{-1})10^3, respectively). Furthermore, infusion of vehicle or SB 234551, at either dose, for 1 h, had no significant effects and, hence, immediately before the onset of infusion of LPS, there were no differences between the haemodynamic variables in the rats receiving vehicle, or SB 234551 (1 mg kg^{-1}, 1 mg kg^{-1} h^{-1}), or SB 234551 (0.3 mg kg^{-1}, 0.3 mg kg^{-1} h^{-1}) (heart rate, 344±10, 346±12, 310±10 beats min^{-1}; mean arterial blood pressure, 106±3, 105±5, 105±3 mmHg; renal vascular conductance, 78±10, 90±18, 89±10 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 73±4, 75±9, 63±5 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 38±4, 43±9, 39±3 (kHz mmHg^{-1})10^3, respectively).

In vehicle-infused rats, during the first 2 h of infusion of LPS, there was a transient fall in arterial blood pressure, a tachycardia, and vasodilatation in renal, mesenteric and hindquarters vascular beds (Figure 2). Thereafter, blood pressure and mesenteric vascular conductance returned to the baseline values, and there was a transient fall in heart rate; renal vasodilatation waned, although renal vascular conductance remained significantly above baseline, and there was vasoconstriction (at 4 h) in the hindquarters (Figure 2).

In rats receiving the higher dose of SB 234551, the pattern of haemodynamic changes during LPS infusion was similar to that seen in the vehicle-infused rats, except that, in the mesenteric vascular bed, vasodilatation did not occur during the early (1–2 h) phase, but developed progressively thereafter (Figure 2). However, the integrated (0–6 h) responses to LPS in vehicle-infused and SB 234551-infused rats were not different.

In rats receiving the lower dose of SB 234551, the early (0–2 h) haemodynamic responses to LPS infusion were similar to those seen in vehicle-infused rats (Figure 3). However, between 2 and 6 h after the onset of LPS infusion, blood pressure failed to return to baseline values, and there was progressive vasodilatation in the mesenteric, and, to a lesser extent, hindquarters vascular beds; heart rate generally remained elevated in rats receiving SB 234551. Thus, the integrated (0–6 h) changes in heart rate, blood pressure, and mesenteric and hindquarters vascular conductances, were all significantly greater than seen in the vehicle-infused animals (Figure 3). Notably, however, changes in renal vascular conductance were not different in the two groups (Figures 2 and 3).

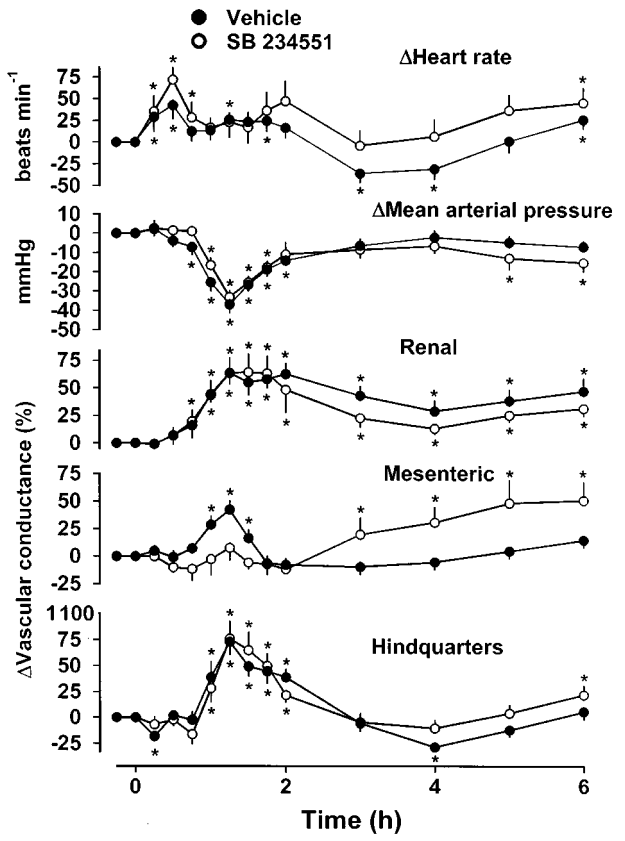

Figure 2 Changes in cardiovascular variables in rats infused with LPS (150 μg kg^{-1} h^{-1} starting at time 0 h) in the presence of vehicle ($n=8$) or SB 234551 (1 mg kg^{-1}; 1 mg kg^{-1} h^{-1} ($n=4$)). Values are mean and vertical bars show s.e.mean. *$P<0.05$ *vs* baseline (Friedman's test).

Discussion

The main aim of the present study was to test the hypothesis that treatment with an endothelin receptor antagonist, which shows selectivity for the ET_A-receptor-mediated effects of the peptide (i.e., SB 234551 (Ohlstein *et al*., 1998)), would enhance the renal vasodilator effects of LPS infusion in conscious rats. The results clearly show that neither dose of SB 234551 used in the experiments influenced the renal haemodynamic response to LPS infusion. We have discussed previously (Gardiner *et al*., 2000a) the problems associated with demonstrating effective endothelin receptor antagonism *in vivo*. In the present study, it was clear that the higher dose of SB 234551 (1 mg kg^{-1} bolus; 1 mg kg^{-1} h^{-1} infusion) was not selective for the vasoconstrictor effects of exogenous endothelin, and hence we used a lower dose. However, it must be acknowledged that, at the chosen dose (0.3 mg kg^{-1} bolus; 0.3 mg kg^{-1} h^{-1} infusion), the degree of antagonism of the renal vasoconstrictor effects of exogenous endothelin was no more than 50%. Perhaps it could be argued that we should have reduced the dose of SB 234551 even further, but that is not consistent with the dose-response effects reported *in vivo* by Ohlstein *et al*. (1998). Finally, it should be noted that the vascular bed in which the antagonism was most apparent was the kidney. Collectively, on the basis of these observations, we believe our hypothesis must be rejected, and thus it would appear that the previously reported failure of the non-selective endothelin antagonist, SB 209670, to influence the renal haemodynamic effects of LPS in our model (Gardiner *et al*., 1995b) was not due to concurrent

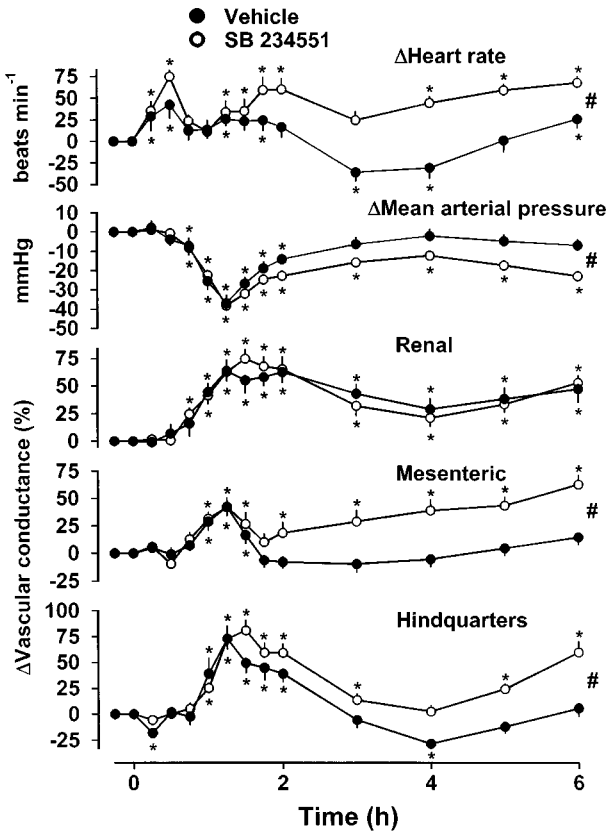

Figure 3 Changes in cardiovascular variables in rats infused with LPS (150 μg kg^{-1} h^{-1} start at time 0 h) in the presence of vehicle ($n=8$) or SB 234551 (0.3 mg kg^{-1}; mg kg^{-1} h^{-1} ($n=8$)). Values are mean and vertical bars show s.e.mean. $*P<0.05$ *vs* baseline (Friedman's test); $\#P<0.05$ between the integrated responses (Mann–Whitney test).

antagonism of renal vasodilator and vasoconstrictor effects of endogenous endothelin.

In another rat model of endotoxaemia which shares some characteristics with the model used by us (i.e., little or no fall in arterial blood pressure at 6 h, Kaddoura *et al*., 1996; Curzen *et al*., 1997), it has been shown that there were no significant changes in kidney or skeletal muscle endothelin-1 mRNA, in spite of clear changes in other tissues. Although this does not preclude endothelin acting as a circulating factor to influence renal haemodynamics, perhaps the most straightforward explanation for our findings is that increased local release of endothelin influences the mesenteric and hindquarters haemodynamic changes during LPS infusion, but such a local effect does not occur in the kidney. Interestingly, several other groups have failed to show an effect of endothelin blockade, in different models of endotoxaemia, when measuring other indices of renal blood flow and function (e.g., Oldner *et al*., 1998; Filep, 2000; Pham *et al*., 2000); in contrast, some have been able to demonstrate effects (Morise *et al*., 1994; Ruetten *et al*., 1996; Mitaka *et al*., 1999; Heyman *et al*., 2000). It is likely that factors, such as the severity of the endotoxic challenge, and ensuing cardiovascular changes, influence the results obtained in different experimental paradigms. While the findings of an increase in renal blood flow during endotoxaemia is not consistent across different experimental models, it is a routine observation in the model used here (see Gardiner *et al*., 1994b; 1995a, b, 1996a, b, c). The underlying mechanisms are largely uncharacterized but they are influenced by pretreatment with dexamethasone (Gardiner *et al*., 1996a). In the present work we did not make measurements of renal function during LPS infusion, but after LPS infusion for 24 h, when renal blood flow is still elevated, there is a significant increase in plasma creatinine concentration, indicative of renal dysfunction (Gardiner *et al*., 1999).

The present findings with SB 234551 are broadly similar to our previous findings with SB 209670 (Gardiner *et al*., 1995b), namely, that during the LPS infusion, in the presence of the endothelin receptor antagonist, there is a late-onset fall in blood pressure with vasodilatation in the mesenteric and, to a lesser extent, the hindquarters vascular beds. Several other investigations have compared mixed endothelin antagonists with ET_A selective antagonists in different models of endotoxaemia, with variable results (reviewed by Wanecek *et al*., 2000). In the majority of experiments, there appear to be no major differences between the effects of antagonists, although a notable exception to this is the work of Oldner *et al*. (1999), who found that, in a porcine endotoxaemic model, only mixed endothelin receptor antagonism was able to improve splanchnic haemodynamics; ET_A-receptor antagonism was ineffective, whilst ET_B-receptor antagonism was lethal (Oldner *et al*., 1999; Wanecek *et al*., 1999).

The influence of SB 234551, on the mesenteric haemodynamic changes during LPS infusion, are consistent with endothelin-1 being a potent vasoconstrictor in that vascular bed in conscious rats (e.g., Gardiner *et al*., 1990; 1994a, b; see also present study), and indicate that, in this model of endotoxaemia, as in several others (reviewed in Wanecek *et al*., 2000), there is an important vasoconstrictor influence of endothelin in the splanchnic circulation. Although the predominant effect of exogenous endothelin-1 in the mesenteric circulation is vasoconstriction, there is evidence to suggest that, at high doses, there may be covert vasodilatation (Bigaud & Pelton, 1992; Gardiner *et al*., 1994a, b), manifest as a delay to reach the peak vasoconstrictor effect. Interestingly, such a phenomenon was also apparent in the present study, and revealed when the higher dose of SB 234551 was used. Under those conditions, there was no delay in the onset of the mesenteric vasoconstrictor effects of the highest dose of exogenous endothelin-1, suggesting that the delay, in the vehicle-infused animals, was due to concurrent vasodilatation and vasoconstriction.

The higher dose of SB 234551 also had an interesting effect on mesenteric haemodynamics in the LPS-infused animals, where it inhibited an early (1–2 h) vasodilatation. It is possible that this effect was mediated *via* ET_B receptors, since the higher dose of SB 234551 was found to inhibit some vasodilator effects of exogenous endothelin-1. Others (Curzen *et al*., 1997) have reported a down-regulation of vasodilator ET_B receptors, 4 h after the onset of endotoxaemia in rats, and this, together with recruitment of other vasoconstrictor systems, such as angiotensin (Gardiner *et al*., 1996c), could explain the transient nature of the inhibitory effect of SB 234551 on mesenteric vasodilator responses to LPS. However, our previous studies, using this model of endotoxaemia, have not consistently shown a mesenteric vasodilatation at

this early stage (Gardiner *et al*., 1995a, b; 1996a, b; but see Gardiner *et al*., 1996c). There are two possible explanations for this apparent difference. Firstly, the anaesthetic used here was different to that used in our previous studies, due to the unavailability of sodium methohexitone. We feel, however, that this is an unlikely explanation, given that LPS infusion was not begun until 48 h after anaesthesia for catheterization. A more likely explanation for the apparent difference is the nature of the recording system now employed. Previously, using an analogue system, we made recordings for 10 min periods around each hour of the LPS infusion. Now, using a digital, computer-based system, recordings are made continuously. The short-lived, early, mesenteric vasodilatation occurred between the first and second hour of infusion, and would, therefore, have been missed from our previous recordings.

The ability of SB 234551 to augment the hindquarters vasodilator effects of LPS infusion deserves comment, against the background of the failure of SB 234551 to inhibit the vasoconstrictor effects of exogenous endothelin-1 in that vascular bed, and the absence of increases in endothelin-1 mRNA in skeletal muscle in endotoxaemia (see above). One interpretation of our findings is that the effects of SB 234551 were not directly due to antagonism of a vasoconstrictor action of locally-produced endothelin-1 in that vascular bed, but were due to an indirect effect, perhaps involving angiotensin (Rossi *et al*., 1999). Alternatively, it is feasible that changes in endothelin receptor populations during the LPS infusion (Curzen *et al*., 1997), occurred, such that the vasoconstrictor effects of endothelin in the hindquarters predominated, and were inhibited by SB 234551. Others have shown inhibition of the depressor effects of exogenous endothelin-1, but preservation of its pressor effects, in endotoxaemic rats (Guc *et al*., 1990), which is consistent with this proposal. Clearly, in future studies it would be interesting to assess the effects of exogenous endothelin during the course of the LPS infusion, and to evaluate the influence of SB 234551 thereupon.

In conclusion, the present results confirm an important vasoconstrictor role for endogenous endothelin-1 in endotoxaemia in conscious rats. However, it appears that the previously reported failure of the non-selective endothelin antagonist, SB 209670, to influence the renal haemodynamic sequelae of endotoxaemia was not due to concurrent antagonism of vasoconstrictor and vasodilator actions of endothelin-1 in the kidney, since selective ET_A-receptor antagonism with SB 234551 is also without influence on renal haemodynamics.

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