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SPECIAL REPORT

Enhancement of the hypotensive and vasodilator effects of endotoxaemia in conscious rats by the endothelin antagonist, SB 209670

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In conscious, chronically-instrumented rats, the non-selective endothelin antagonist, SB 209670 (10 μg kg^{-1} min^{-1}), caused marked enhancement of the fall in mean arterial blood pressure during infusion of lipopolysaccharide (LPS) for 24 h (LPS alone = −6 ± 3 mmHg; LPS + SB 209670 = −30 ± 2 mmHg). This effect was accompanied by a conversion of the mesenteric vasoconstriction to a substantial mesenteric vasodilatation and an augmentation of the hindquarters vasodilatation, seen with LPS alone. Notably, the marked renal hyperaemic vasodilatation during LPS infusion was not affected significantly by SB 209670. These results indicate that endothelin, directly and/or indirectly, plays a pivotal role in the cardiovascular sequelae of endotoxaemia in conscious rats, and prevents marked hypotension, particularly by opposing mesenteric vasodilatation.

Keywords: Endothelin; lipopolysaccharide; haemodynamics; SB 209670

Introduction The regional haemodynamic changes during prolonged infusion of lipopolysaccharide (LPS) in conscious rats vary with time, probably reflecting the complex interplay between vasodilator and vasoconstrictor mechanisms (Waller *et al.*, 1994a; Gardiner *et al.*, 1995). It is known that LPS elevates plasma endothelin (ET) levels in rats (Sugiura *et al.*, 1989; Vemulapalli *et al.*, 1991), but the contribution of ET to the regional haemodynamic changes during LPS infusion in conscious rats has not been determined. Therefore, we compared responses to LPS infusion in conscious rats receiving saline or the ET_A-, ET_B-receptor antagonist, SB 209670 (Ohlstein *et al.*, 1994; Douglas *et al.*, 1995).

Methods Experiments were carried out in male Long Evans rats (350–450g). All surgery was carried out under anaesthesia with sodium methohexitone (Brietal, Lilly, 40–60 mg kg^{-1}, i.p.); the procedures for implanting pulsed Doppler flow probes and intravascular catheters have been described in detail previously (Waller *et al.*, 1994a).

The main experiment involved two groups of rats ($n = 8$ in each); one group was given a continuous infusion of sterile isotonic saline (154 mmol l^{-1} NaCl; 0.4 ml h^{-1}) beginning 1 h before a continuous infusion of LPS (150 μg kg^{-1} h^{-1} in sterile saline at 0.4 ml h^{-1} for 24 h). The other group was given SB 209670 in sterile saline (10 μg kg^{-1} min^{-1}; Douglas *et al.*, 1995) beginning 1 h before co-infusion of LPS for 24 h.

To determine the effects of SB 209670 alone, a separate group of animals ($n = 3$) was infused with this drug (dose as above) for 24 h.

Recordings were made as described previously (Waller *et al.*, 1994a) and data were analysed with Friedman's test (within group) or the Mann-Whitney U test as appropriate; a P value < 0.05 was taken as significant.

SB 209670 ([(±)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy) indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.). LPS (*E. coli* serotype 0127 B8) was obtained from Sigma (UK).

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Results In the presence of saline, infusion of LPS caused cardiovascular changes similar to those described previously (Waller *et al.*, 1994a), namely, a biphasic fall in mean arterial blood pressure and tachycardia, an early and sustained renal hyperaemia and vasodilatation, early reductions in mesenteric flow and vascular conductance as seen in saline-infused control animals (Waller *et al.*, 1994a), and initial falls and delayed increases in hindquarters flow and vascular conductance (Figure 1).

Infusion of SB 209670 over the 1 h preceding LPS had no significant cardiovascular effects and hence at the onset of LPS infusion there were no significant differences between the group receiving saline and that receiving SB 209670 (Figure 1). However, in the presence of SB 209670, and within 1 h of the start of LPS infusion, there was a significant hypotension which developed progressively over the following 6 h and was still present at 24 h (Figure 1); the hypotension was accompanied by a substantial incremental tachycardia. Renal flow and vascular conductance showed early increases and remained elevated throughout the LPS infusion, mesenteric flow and vascular conductance increased with maxima at 6 h after the onset of LPS infusion, and hindquarters flow and vascular conductance showed early increases (2 h) and progressively rose from 5 h onwards (Figure 1).

Comparison of the integrated responses (areas under or over curves from 0–24 h) during LPS infusion showed significant differences between the saline-infused and SB 209670-infused groups for all variables except renal flow and vascular conductance (Figure 1).

In animals receiving SB 209670 alone for 24 h there was a small reduction in mean arterial blood pressure (−7 ± 2 mm Hg at 24 h), but this was not accompanied by increased flow or vascular conductance in any region monitored (data not shown).

Discussion The present results indicate that pretreatment with the non-selective ET-receptor antagonist, SB 209670, causes marked enhancement of the hypotensive response to LPS infusion in conscious rats. This effect is associated with an unmasking of a substantial mesenteric vasodilator response and augmentation of the hindquarters vasodilatation usually seen during LPS infusion. It is notable that SB 209670 did not cause significant enhancement of the renal hyperaemic vasodilatation associated with LPS infusion, co-

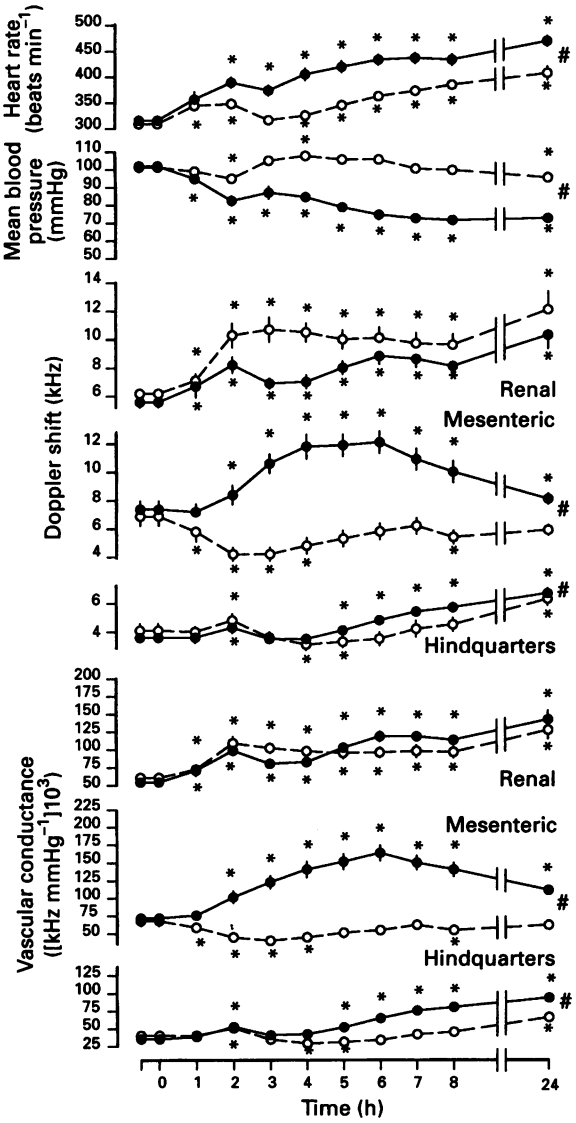

Figure 1 Cardiovascular variables in conscious, Long Evans rats before and during infusion of saline and LPS ($150\,\mu g\,kg^{-1}\,h^{-1}$; ○, $n=8$) or SB 209670 ($10\,\mu g\,kg^{-1}\,min^{-1}$) and LPS (●, $n=8$). Values are mean with s.e. mean; $^{*}P<0.05$ versus baseline (Friedman's test); $^{\#}P<0.05$ for differences between integrated responses in the two groups (Mann Whitney U test).

nsidering the marked renal vasoconstrictor action of exogenous ET (Gardiner *et al.*, 1994). One interpretation of this observation is that locally released ET is responsible for regionally differentiated haemodynamic changes during endotoxaemia. However, ET modulates the renin-angiotensin system and vasopressin release (see Rubanyi & Polokoff, 1994, for review), and the marked hypotension in the presence of SB 209670 and LPS would be expected to activate those systems. Thus, the differential effects of SB 209670 might reflect regional variations in the overall constrictor influences of circulating ET, angiotensin II and vasopressin (Gardiner *et al.*, 1995). While it could be argued that SB 209670 should block the cardiac inotropic effects of ET (see Rubanyi & Polokoff, 1994, for review), and so the hypotension could be due to compromised cardiac output, this was not apparent because all flows increased in the presence of SB 209670.

Elsewhere (Waller *et al.*, 1994b) we have suggested that treatment of conscious, endotoxaemic rats with inhibitors of inducible nitric oxide (NO) synthase could be detrimental because loss of the vasodilator effects of NO unmasks the actions of vasoconstrictor mechanisms (Gardiner *et al.*, 1995). The present results indicate that ET is particularly important in that regard, and raise the possibility that a combination of an inducible NO synthase inhibitor and an ET antagonist could offer a new therapeutic strategy in the treatment of the cardiovascular sequelae of endotoxaemia. However, we have not demonstrated that SB 209670 only has effects on endothelin receptors in our experimental model. Thus, since endotoxaemia can stimulate many mediators with cardiovascular actions, we cannot exclude the possibility that the influence of SB 209670 is due to direct and/or indirect interactions with such systems.

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