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Enhanced involvement of endothelin in the haemodynamic sequelae of endotoxaemia in conscious, hypertensive, transgenic ((mRen-2)27) rats

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1 Age-matched (3–4 months old) male, heterozygous, hypertensive, transgenic ((mRen-2)27) rats (abbreviated to TG rats) and the normotensive control animals (homozygous, Hannover Sprague-Dawley rats (abbreviated to SD rats), were chronically instrumented for the assessment of regional haemodynamic responses to continuous lipopolysaccharide (LPS) infusion (150 μg kg^{-1} h^{-1}, i.v.)

2 The early (1–2 h) hypotension in SD rats (-11 ± 3 mmHg; $n=7$) was significantly less than that in TG rats (-35 ± 3 mmHg; $n=8$), but by 24 h mean arterial blood pressure (MAP) in both strains of rat was not different from the pre-LPS value (SD rats: baseline, 108 ± 3 mmHg; 24 h LPS, 112 ± 4 mmHg; TG rats: baseline, 171 ± 2 mmHg; 24 h LPS, 169 ± 3 mmHg). At this stage in the SD rats there was a renal vasodilatation (Δ vascular conductance, 29 ± 10 [kHz mmHg^{-1}]10^3) but not in TG rats (Δ vascular conductance 2 ± 3[kHz mmHg^{-1}]10^3).

3 Co-infusion of LPS and the non-selective endothelin receptor antagonist, SB 209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1}) between 24 and 31 h in SD rats caused a fall in MAP of 16 ± 2 mmHg accompanied by hindquarters vasodilatation (Δ vascular conductance 11 ± 3 (kHz mmHg^{-1})10^3). In TG rats, under the same conditions, the fall in MAP was -60 ± 6 mmHg, and there were renal, mesenteric and hindquarters vasodilatations (Δ vascular conductance, 23 ± 5, 32 ± 7, and 14 ± 4 (kHz mmHg^{-1})10^3, respectively). All effects, except the hindquarters vasodilatation, were greater in TG than in SD rats.

4 In TG rats infused with LPS alone for 31 h, between 24 and 31 h the fall in MAP was -17 ± 4 mmHg, and the changes in renal, mesenteric and hindquarters vascular conductances were 5 ± 3, -4 ± 5, and 12 ± 4 (kHz mmHg^{-1})10^3, respectively.

5 Administration of the angiotensin (AT_1)-receptor antagonist, losartan (10 mg kg^{-1}, i.v.) following co-infusion of LPS and SB 209670 between 24 and 31 h caused similar falls in MAP in SD and TG rats (-12 ± 3 and -14 ± 4 mmHg, respectively).

6 These results, together with previous findings, are consistent with a relative enhancement of the contribution of endothelin to the maintenance of cardiovascular status in endotoxaemic TG rats, particularly through a mesenteric vasoconstrictor action.

Keywords: Transgenic rats; endotoxaemia; endothelin

Introduction

Under normal conditions, in normal rats, endothelin plays a relatively minor role in the control of haemodynamic status (Nishikibe *et al*., 1993; Ohlstein *et al*., 1993; Douglas *et al*., 1994; 1995a; Gardiner *et al*., 1995c). However, in endotoxaemia, plasma levels of endothelin are elevated markedly (Sugiura *et al*., 1989; Vemulapalli *et al*., 1991), and we have shown that the non-selective endothelin (ET)-receptor antagonist, SB 209670 (Ohlstein *et al*., 1994; Douglas *et al*., 1995a,b) causes marked augmentation of the hypotension and peripheral vasodilatation seen in conscious Long Evans rats in this condition (Gardiner *et al*., 1995a). These findings indicate that endothelin may exert overt cardiovascular effects in endotoxaemia, and others have reached a similar conclusion with regard to the involvement of endothelin in congestive heart failure (CHF; Kiowski *et al*., 1995; see Love & McMurray, 1996).

Both endotoxaemia and CHF are conditions in which plasma endothelin levels are elevated. In contrast, hypertensive transgenic ((mRen-2)27; abbreviated to TG) rats do not have elevated plasma endothelin levels, yet they show significant hypotension and peripheral vasodilatation in response to SB 209670 (Gardiner *et al*., 1995c). This is not a non-specific effect of SB 209670, since the compound is without significant cardiovascular action in another genetic model of hypertension (Gardiner *et al*., 1996d). On the basis of these findings we concluded that the involvement of endothelin in the hypertension seen in TG rats might be due to an influence of tissue renin-angiotensin activity (see Lee *et al*., 1996, for review) on local endothelin synthesis (Gardiner *et al*., 1995c).

In the light of these various findings we hypothesized that the prior involvement of endothelin in the maintenance of cardiovascular status in hypertensive TG rats would produce a relative reduction of its influence in endotoxaemia, and in the present work we tested this hypothesis. Some of the results have been presented to the British Pharmacological Society (Bennett *et al*., 1997).

Methods

Male, heterozygous TG rats (3–4 months old), and homozygous age-matched, male Sprague-Dawley (SD) rats of the strain from which the TG rats were originally derived (Mullins *et al*., 1990), were bred in the Biomedical Services Unit in Nottingham from animals originally supplied by Dr J.J. Mullins (Genome Research Centre, Edinburgh University). Homozygous TG rats in the breeding colony were given captopril in their drinking water (50 mg l^{-1}; Mullins *et al*.,

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1990), but the heterozygous TG animals studied were untreated.

At least 14 days before the experiments, animals were anaesthetized (sodium methohexitone, Brietal Lilly, 40–60 mg kg^{-1}, i.p., supplemented as required), and had miniaturized pulsed Doppler probes implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor hindquarters flow) (Gardiner *et al*., 1995b; 1996a). Thereafter, animals were kept in separate home cages with free access to food and water. The day before experimentation, rats were anaesthetized (as above) and had an intra-arterial catheter implanted in the distal abdominal aorta (via the caudal artery), for recording arterial blood pressure and heart rate, and catheters implanted in the right jugular vein for the infusion of lipopolysaccharide (LPS) and the administration of drugs (Gardiner *et al*., 1995b; 1996a). In conscious, freely-moving rats the following protocols were performed.

Effects of LPS followed by SB 209670 and the AT_1-receptor antagonist, losartan

In 7 SD and 8 TG rats a continuous infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$) was given for 32 h. Starting 24 h after the onset of LPS infusion, a primed infusion (600 $\mu g\ kg^{-1}$ bolus, 600 $\mu g\ kg^{-1}\ h^{-1}$ infusion; Gardiner *et al*., 1996b,c) of SB 209670 was begun, and at 31 h a bolus injection of losartan (10 mg kg^{-1}; Gardiner *et al*., 1996b,c) was given.

Effects of LPS followed by saline

The above experiment revealed such substantial hypotensive effects of SB 209670 in TG rats (see Results), it raised the possibility that, in the absence of SB 209670, the continued infusion of LPS alone would cause marked hypotension between 24 and 31 h. Therefore, 6 TG rats were infused continuously with LPS (as above) for 31 h, with a co-infusion (0.4 ml h^{-1}) of saline between 24 and 31 h. For comparison, 2 SD rats were put through the same protocol.

Data analysis

Within-group analysis of data was by Friedman's test (Theodorsson-Norheim, 1987). Between-group comparisons were made by Mann-Whitney U tests applied to integrated responses (areas under or over curves); P value <0.05 was taken as significant.

Drugs

SB 209670 ([(±)-(1**S**, 2**R**, 3**S**)-3-(2-carboxy-methoxy-4-methoxyphenyl)-1-(3,4-methylene-dioxyphenyl)-5-(-prop-1-yloxy) indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SmithKline Beecham, U.S.A.), and losartan potassium was a gift from Dr R.D. Smith (Du Pont, U.S.A.). LPS (*E. coli*, serotype 0127: B8) and captopril were obtained from Sigma (U.K.). All substances were dissolved in sterile saline.

Results

Resting cardiovascular variables in all the SD and TG rats studied are summarized in Table 1. As shown previously (Gardiner *et al*., 1995c), the hypertension in TG rats was accompanied by significant, and similar, reductions in conductances in renal, mesenteric and hindquarters vascular beds (Table 1).

During the 24 h infusion of LPS, SD rats showed tachycardia, and an initial, slight hypotension, with a significant, albeit modest, rise in mean arterial blood pressure

Table 1 Resting cardiovascular variables in control Sprague Dawley (SD) rats and hypertensive transgenic (TG) rats

	SD ($n=9$)	*TG* ($n=14$)
Heart rate (beats min^{-1})	356±6	319±7
Mean blood pressure (mmHg)	108±2*	168±2
Renal Doppler shift (kHz)	7.0±0.7	7.2±0.4
Mesenteric Doppler shift (kHz)	8.0±0.6*	5.8±0.3
Hindquarters Doppler shift (kHz)	5.7±0.7	4.5±0.3
Renal vasular conductance ([kHz $mmHg^{-1}$]10^3)	65±7*	43±2
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	75±6*	35±2
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	53±6*	27±2

*$P<0.05$ (Mann-Whitney U test).

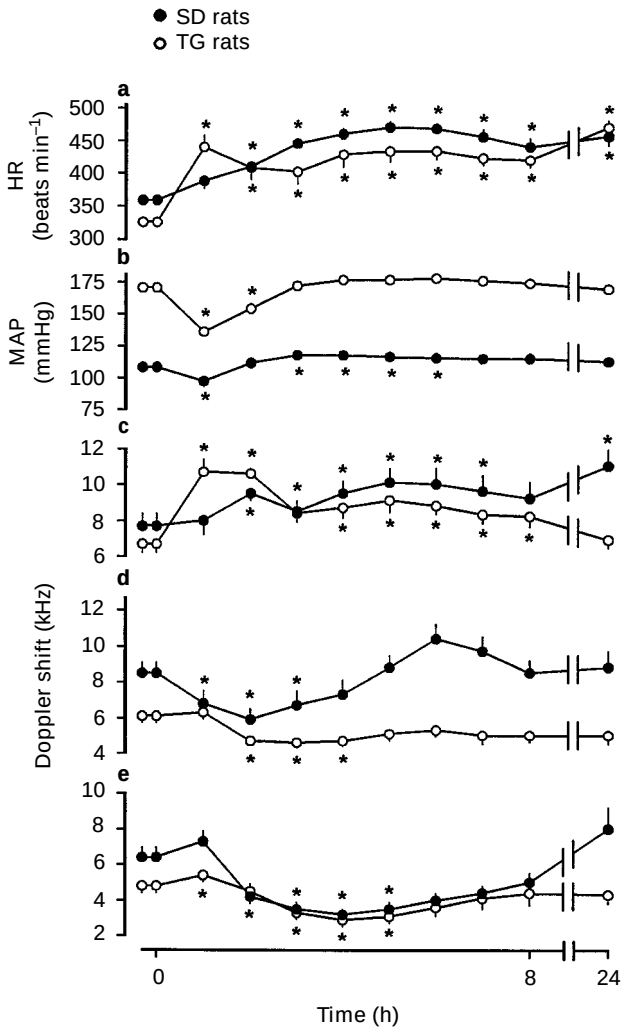

Figure 1 Cardiovascular changes ((a) heart rate, (b) mean arterial pressure, (c) renal, (d) mesenteric and (e) hindquarters flow) in conscious Hannover Sprague-Dawley (SD) rats ($n=7$) and hypertensive, transgenic ((mRen-2)27) rats ($n=8$) during continuous infusion of lipopolysaccharide (150 $\mu g\ kg^{-1}\ h^{-1}$). Values are mean and vertical lines show s.e.mean. *$P<0.05$ versus original baseline (Friedman's test).

(MAP) thereafter (Figure 1. The latter was accompanied by mesenteric and hindquarters vasoconstriction, but increases in renal flow and conductance (Figures 1 and 2). By 24 h, MAP was not different from baseline, but flow and conductance were increased in the renal vascular bed (Figures 1 and 2).

In TG rats, the initial hypotension (at 1 h) during LPS infusion (-35 ± 3 mmHg) was more marked ($P<0.05$) than in SD rats (-11 ± 3 mmHg), and there was a renal vasodilatation in the former, but not in the latter strain (Δ vascular conductance 40 ± 2 and 11 ± 6 [kHz mmHg^{-1}] 10^3, respectively) (Figure 1). Although the general pattern of change in regional haemodynamics was similar to that described above for the SD rats, by 24 h after the onset of LPS infusion, MAP, and flows and conductances in the renal, mesenteric and hindquarters vascular beds were not different from baseline, but there was a tachycardia (Figures 1 and 2).

During co-infusion of LPS and SB 209670 in SD rats, there were slight reductions in MAP (-12 ± 3 mmHg at 31 h) and renal flow, and an increase in hindquarters vascular conductance (Figures 3 and 4). In the same experimental protocol, the fall in MAP in the TG rats was substantial (-62 ± 3 mmHg at 31 h), and accompanied by renal, mesenteric and hindquarters vasodilatations (Figures 3 and 4). With the exception of the hindquarters vasodilatation, all the changes were significantly greater than in SD rats (Figure 4).

After 7 h co-infusion of LPS and SB 209670, all cardiovascular variables except heart rate, still differed significantly in SD and TG rats (Figures 3 and 4). Following administration of losartan in this circumstance, there were similar, further falls in MAP in the two groups of rats (30 min after losartan:- SD rats, MAP down to 84 ± 4 mmHg from 96 ± 3 mmHg; TG rats, MAP down to 95 ± 2 mmHg from 109 ± 4 mmHg), accompanied by renal, mesenteric and hindquarters vasodilatations, although the rise in mesenteric vascular conductance was more marked than any other change in the SD rats (SD rats, vascular conductances ([kHz mmHg^{-1}]10^3) renal, up to 117 ± 12 from 91 ± 6; mesenteric, up to 135 ± 15 from 93 ± 13; hindquarters, up to 95 ± 19 from 85 ± 14: TG rats, renal up to 80 ± 4 from 64 ± 4; mesenteric, up to 79 ± 5 from 62 ± 7; hindquarters, up to 43 ± 4 from 39 ± 3).

In TG rats, the hypotension and renal and mesenteric vasodilatations seen during co-infusion of LPS and SB 209670 between 24 and 31 h were greater than the changes, over the same time, in animals receiving LPS alone (Figure 5).

In the 2 SD rats given LPS alone for 31 h, there were no consistent cardiovascular changes between 24 and 31 h (data not shown).

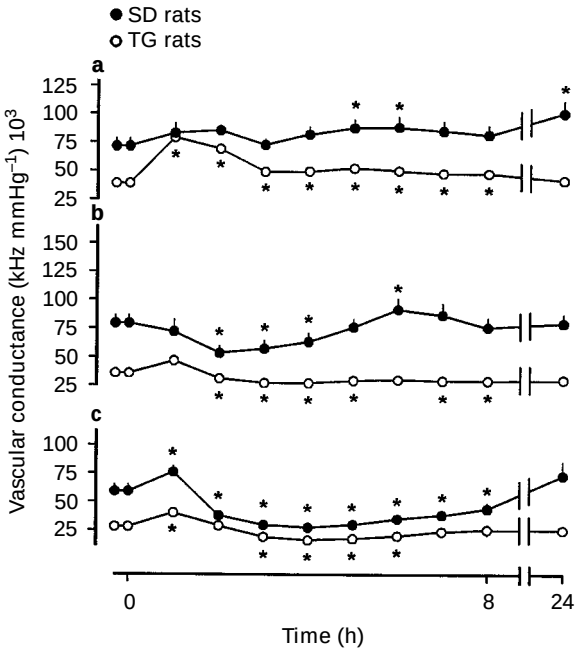

Figure 2 Cardiovascular changes ((a) renal, (b) mesenteric and (c) hindquarters vascular conductance) in conscious Hannover Sprague-Dawley (SD) rats ($n=7$) and hypertensive, transgenic ((mRen-2)27) rats ($n=8$) during continuous infusion of lipopolysaccharide (150 μg kg^{-1} h^{-1}). Values are mean and vertical lines show s.e.mean. *$P<0.05$ versus original baseline (Friedman's test).

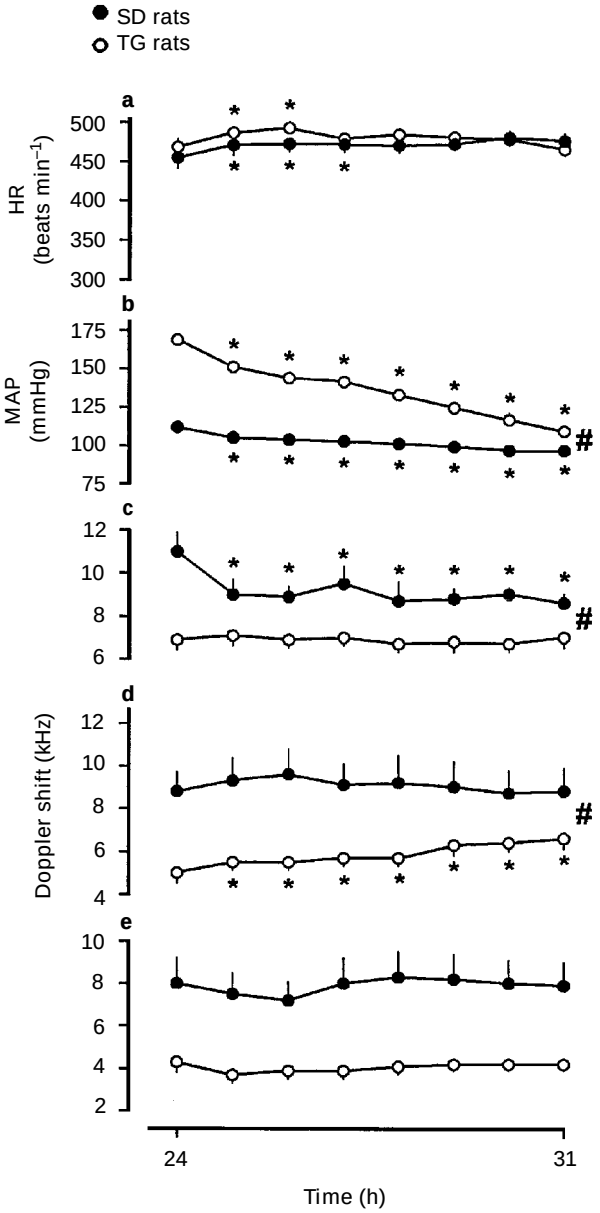

Figure 3 Cardiovascular changes ((a) heart rate, (b) mean arterial pressure, (c) renal, (d) mesenteric and (e) hindquarters flow) in conscious Hannover Sprague-Dawley (SD) rats ($n=7$) and hypertensive, transgenic ((mRen-2)27) rats ($n=8$) during continuous co-infusion of lipopolysaccharide and SB 209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1}) beginning 24 h after the onset of lipopolysaccharide infusion, and continuing for 7 h. Values are mean and vertical lines show s.e.mean. *$P<0.05$ versus 24 h baseline (Friedman's test); #$P<0.05$ for difference between integrated responses in the two groups (Mann-Whitney U test).

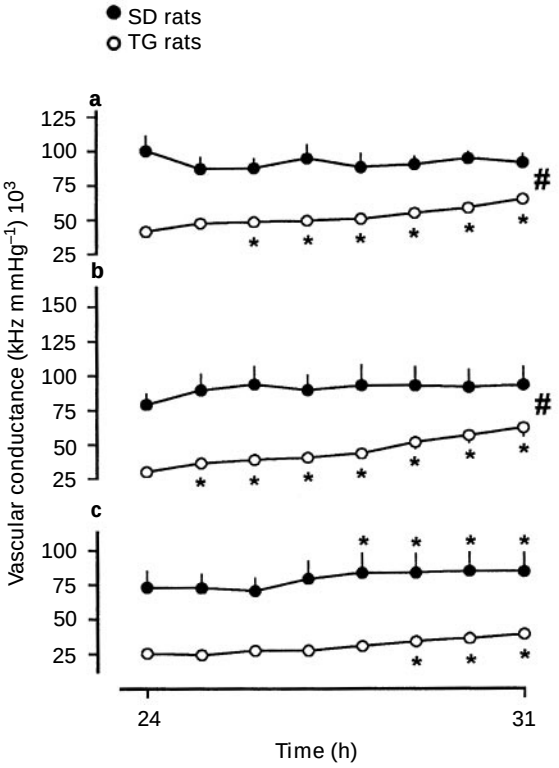

Figure 4 Cardiovascular changes ((a) renal, (b) mesenteric and (c) hindquarters) in conscious Hannover Sprague-Dawley (SD) rats ($n=7$) and hypertensive, transgenic ((mRen-2)27) rats ($n=8$) during continuous co-infusion of lipopolysaccharide and SB 209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1}) beginning 24 h after the onset of lipopolysaccharide infusion, and continuing for 7 h. Values are mean and vertical lines show s.e.mean. $^*P<0.05$ versus 24 h baseline (Friedman's test); $\#P<0.05$ for difference between integrated responses in the two groups (Mann-Whitney U test).

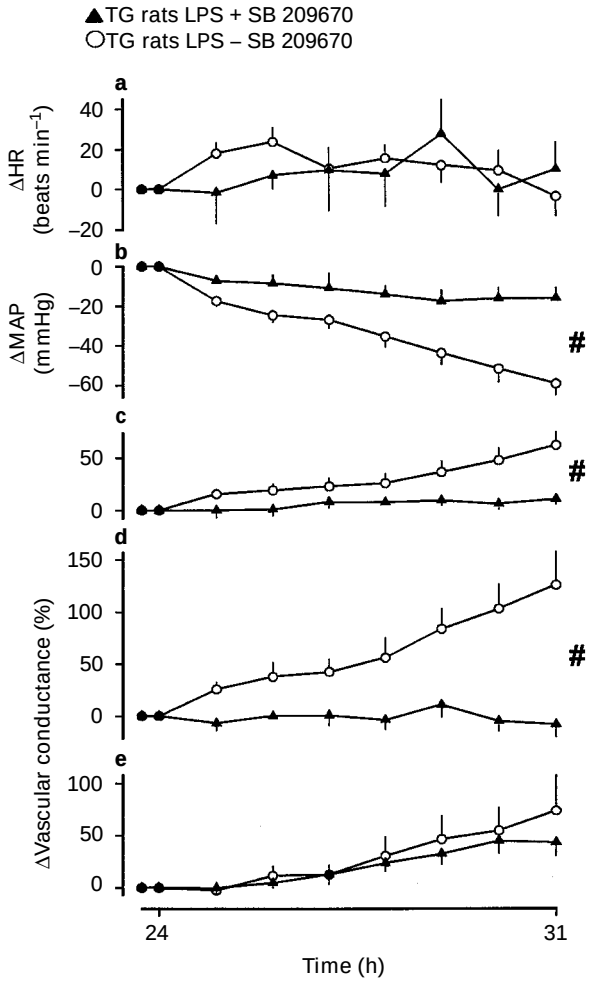

Figure 5 Cardiovascular changes ((a) heart rate, (b) mean arterial pressure, (c) mesenteric and (d) hindquarters vascular conductance) in conscious hypertensive transgenic ((mRen-2)27) rats ($n=6$) between 24 and 31 h after the onset of lipopolysaccharide infusion for 31 h compared to transgenic rats ($n=8$) receiving SB 209670 together with lipopolysaccharide between 24 and 31 h. Values are mean and vertical lines show s.e.mean. $\#P<0.05$ for difference between integrated responses in the two groups (Mann-Whitney U test).

Discussion

On the basis of the present findings we can reject our hypothesis that the prior involvement of endothelin in the maintenance of hypertension in TG rats (Gardiner *et al*., 1995c) would produce a relative reduction in its contribution to cardiovascular status in endotoxaemia. Indeed, it appears that the opposite may be true, inasmuch as the hypotensive and vasodilator effects of the non-selective ET-receptor antagonist, SB 209670, were enhanced in endotoxaemic TG rats, compared to endotoxaemic SD rats.

The cardiovascular responses to LPS infusion in the SD rats differed from those previously observed in Long Evans rats (Gardiner *et al*., 1995c), in that the initial fall in MAP and the accompanying vasodilatations were less marked in SD rats. Furthermore, 24 h after onset of LPS infusions, there was no hypotension, and only modest renal and hindquarters vasodilatation in SD rats, whereas there was reproducible hypotension and marked renal and hindquarters vasodilatation in Long Evans rats under these conditions (Gardiner *et al*., 1995b).

In Long Evans rats, the initial hypotensive and vasodilator responses which occur 1–2 h after the onset of LPS infusion are not affected by non-selective nitric oxide synthase (NOS) inhibitors, such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME) (Gardiner *et al*., 1995b), or by the more selective inducible NOS (iNOS) inhibitor, aminoguanidine (Gardiner *et al.,* 1996a), or by dexamethasone (Gardiner *et al*., 1996b). However, the initial hypotensive, but not renal or hindquarters vasodilator, responses to LPS are inhibited by the sulphonylurea, glibenclamide (Gardiner *et al*., 1997), consistent with an involvement of ATP-sensitive K^+ channels in the fall in MAP during LPS infusion in Long Evans rats. Furthermore, the early hypotension and vasodilatation are offset by activation of the renin-angiotensin system (Gardiner *et al*., 1996c). Therefore, the less marked changes observed in the SD rats in this study could be due to lesser activation of vasodilator mechanism(s), and/or more effective recruitment of vasoconstrictor systems, such as the renin-angiotensin system, during LPS infusion.

Similarly, after 24 h of LPS infusion in Long Evans, iNOS activity and plasma tumour necrosis factor-α (TNF-α) levels are back to normal (Gardiner *et al*., 1995b; Waller *et al*., 1995), and we have concluded that some other factors, possibly triggered by the earlier increase in iNOS activity, must underlie the delayed cardiovascular changes (Gardiner *et al*., 1995b). Moreover, at that later stage, endothelin and, to a lesser extent,

angiotensin are involved in supporting blood pressure in Long Evans rats (Gardiner *et al*., 1996c). Thus, it is again feasible that the differences between the Long Evans and SD rats could be due to differential activation of vasoconstrictor and/or vasodilator mechanisms. Others have observed strain differences in the effects of LPS on, for example, cytokine production (Perretti *et al*., 1993) and cytochrome P450 expression (Sewer *et al*., 1996). Although the present study was not designed to compare formally Long Evans and SD rats, or explore possible underlying mechanisms to explain the differences, the results obtained signal a need for caution when extrapolating information obtained from one strain of 'normal' rat to another.

In the TG hypertensive rats, the early (1 – 2 h) fall in blood pressure and the renal vasodilatation during LPS infusion were greater than in SD rats. Wu *et al*. (1996) observed enhanced hypotensive effects of LPS in anaesthetized spontaneously hypertensive rats (SHR) over a 3 h period following a bolus dose of LPS. In that work (Wu *et al*., 1996) and a subsequent, related study (Yen *et al*., 1997), it was shown that administration of LPS resulted in a greater increase in NO production in the SHR than in the normotensive control strain. Since SHRs have been found to exhibit augmented vasodilator responses to exogenous nitrovasodilators (Papapetropoulos *et al*., 1994), it was concluded the more severe hypotensive effect of LPS in SHR was related to increased production of and, to some extent, greater vasodilator responsiveness to, NO (Wu *et al*., 1996).

Little is known of the functioning of cardiovascular control mechanisms in hypertensive TG rats (for review, see Lee *et al*., 1996). However, in our experience, vasodilator responses to sodium nitroprusside are, if anything, reduced, although the associated hypotension may be enhanced (March *et al*., 1996). This could be explained by a failure in the counter-regulatory baroreflex mechanism in TG rats, and a similar explanation could apply to the present results with LPS. Thus, an inability to recruit effectively the renal renin-angiotensin system could explain the more marked early fall in blood pressure in the TG rats during LPS infusion, consistent with the finding that, when the influence of the renin-angiotensin system is suppressed in Long Evans rats, they show a substantial enhancement of the initial hypotensive and renal vasodilator responses to LPS infusion (Gardiner *et al*., 1996c). It is known that renal renin activity and angiotensin II content are reduced in heterozygous TG rats (see Lee *et al*., 1996), thus it is likely the renal-angiotensin system is less able to be activated to maintain cardiovascular status in these animals. This problem could be exacerbated by the occurrence of downregulation of AT_1-receptors recently described in TG rats (Nickenig *et al*., 1997). Although the latter phenomenon is not apparent from the considerable hypotensive and vasodilator effects of losartan in normal TG rats (Gardiner *et al*., 1995b), it is consistent with the modest responses to losartan in TG rats given LPS and SB 209670 in the present work.

After 24 h of LPS infusion, cardiovascular status appeared to be relatively normal in the TG rats, the only feature being a significant tachycardia. Superficially, this profile could be interpreted as due to a lesser activation of vasodilator mechanisms in these animals or, indeed, a lack of effect of the LPS. However, administration of SB 209670 at this juncture revealed a major influence of ET in supporting blood pressure under these conditions. Thus, in TG rats, SB 209670, in the presence of LPS, caused a fall in MAP of -62 ± 3 mmHg, whereas in our earlier studies (Gardiner *et al*., 1995c), SB 209670 administration to TG rats in the absence of LPS caused a fall in MAP of 32 ± 2 mmHg. The vasodilator effects of SB 209670 in the presence of LPS were apparent in the renal and mesenteric vascular beds, and in our previous study we found that SB 209670 in TG rats, in the absence of LPS, caused vasodilatation in renal, mesenteric and hindquarters vascular beds (Gardiner *et al*., 1995b). Comparing those results with the present findings, it appears that in endotoxaemia in TG rats, the enhanced hypotensive effect of SB 209670 is preferentially attributable to a mesenteric vasodilator action, consistent with a particularly important vasoconstrictor action of endogenous endothelin in this vascular bed in endotoxaemia (Gardiner *et al*., 1995a).

References

BENNETT, T., KEMP, P.A., MARCH, J.E. & GARDINER, S.M. (1997). Haemodynamic responses to chronic lipopolysaccharide (LPS) infusion in conscious, hypertensive transgenic ((mRen-2)27) rats: involvement of endothelin. *Br. J. Pharmacol.,* **122,** 231P.

DOUGLAS, S.A., EDWARDS, R.M., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995a). *In vivo* pharmacological characterization of the non-peptide endothelin receptor antagonist SB 209670. *Br. J. Pharmacol.,* **114,** 405 – 413.

DOUGLAS, S.A., GELLAI, M., EZEKIEL, M., FEUERSTEIN, G.Z., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995b). Antihypertensive actions of the novel nonpeptide endothelin receptor antagonist SB 209670. *Hypertension,* **25,** 818 – 822.

DOUGLAS, S.A., GELLAI, M., EZEKIEL, M. & OHLSTEIN, E.H. (1994). BQ-123, a selective endothelin subtype A-receptor antagonist, lowers blood pressure in different rat models of hypertension. *J. Hypertens.,* **12,** 561 – 567.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995a). Enhancement of the hypotensive and vasodilator effects of endotoxaemia in conscious rats by the endothelin antagonist, SB 209670. *Br. J. Pharmacol.,* **116,** 1718 – 1719.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995b). 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. (1996a). Effects of dexamethasone and SB 209670 on the regional haemodynamic responses to lipopolysaccharide in conscious rats. *Br. J. Pharmacol.,* **118,** 141 – 149.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996b). Influence of aminoguanidine and the endothelin antagonist, SB 209670, on the regional haemodynamic effects of endotoxaemia in conscious rats. *Br. J. Pharmacol.,* **118,** 1822 – 1828.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996c). 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.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996d). Regional haemodynamic effects of antagonists of angiotensin II, endothelin and adrenoceptors in conscious, vasopressin-deficient, genetically hypertensive rats. *Br. J. Pharmacol.,* **118,** 325 – 334.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1997). Effects of glibenclamide on regional haemodynamic responses to lipopolysaccharide (LPS) in conscious rats. *Br. J. Pharmacol.,* **122,** 392P.

GARDINER, S.M., MARCH, J.E., KEMP, P.A., MULLINS, J.J. & BENNETT, T. (1995c). Haemodynamic effects of losartan and the endothelin antagonists, SB 209670, in conscious, transgenic ((mRen-2)27), hypertensive rats. *Br. J. Pharmacol.,* **116,** 2237 – 2244.

KIOWSKI, W., SUTSCH, G., HUNZIKER, P., MULLER, P., KIM, J., OECHSLIN, E., SCHMITT, R., JONES, R & BERTEL, O. (1995). Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet,* **346,** 732 – 736.

LEE, M.A., BOHM, M., PAUL, M., BADER, M., GANTEN, U. & GANTEN, D. (1996). Physiological characterization of the hypertensive transgenic rat TGR(mREN2)27. *Am. J. Physiol.,* **270,** E919 – E929.

LOVE, M.P. & MCMURRAY, J.J.V. (1996). Endothelin in chronic heart failure: current position and future prospects. *Cardiovasc. Res.,* **31,** 665 – 674.

MARCH, J.E., GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1996). Regional haemodynamic responses to vasodilators in conscious, transgenic [(mRen-2)27], hypertensive rats. *Br. J. Pharmacol.,* **117,** 227P.

MULLINS, J.J., PETERS, J. & GANTEN, D. (1990). Fulminant hypertension in transgenic rats harbouring the mouse *Ren-2* gene. *Nature,* **344,** 541 – 544.

NICKENIG, G., LAUFS, U., SCHNABEL, P., KNORR, A., PAUL, M. & BOHM, M. (1997). Down-regulation of aortic and cardiac AT_1 receptor gene expression in transgenic (mRen-2) 27 rats. *Br. J. Pharmacol.,* **121,** 134 – 140.

NISHIKIBE, M., TSUCHIDA, S., OKADA, M., FUKURODA, T., SHIMAMOTO, K., YANO, M., ISHIKAWA, K. & IKEMOTO, F. (1993). Antihypertensive effect of a newly synthesized endothelin antagonist, BQ-123, in a genetic hypertensive model. *Life Sci.,* **52,** 717 – 724.

OHLSTEIN, E.H., DOUGLAS, S.A., EZEKIEL, M. & GELLAI, M. (1993). Antihypertensive effects of the endothelin receptor antagonist BQ-123 in conscious spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.,* **22, (Suppl. 8),** S321 – S324.

OHLSTEIN, E.H., NAMBI, P., DOUGLAS, S.A., EDWARDS, R.M., GELLAI, M., LAGO, A., LEBER, J.D., COUSINS, R.D., GAO, A., FRAZEE, J.S., PEISHOFF, C.E., BEAN, J.W., EGGLESTON, D.S., ELSHOURBAGY, N.A., KUMAR, C., LEE, J.A., YUE, T.-L., LOUDEN, C., BROOKS, D.P., WEINSTOCK, J., FEUERSTEIN, G., POSTE, G., RUFFOLO, R.R., GLEASON, J.G. & ELLIOTT, J.D. (1994). SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 8052 – 8056.

PAPAPETROPOULOS, A., MARCZIN, N., SNEAD, M.D., CHENG, C., MILICI, A. & CATRAVAS, J.D. (1994). Smooth muscle cell responsiveness to nitrovasodilators in hypertensive and normotensive rats. *Hypertension,* **23,** 476 – 484.

PERRETTI, M., DUNCAN, G.S., FLOWER, R.J. & PEERS, S.H. (1993). Serum corticosterone, interleukin-1 and tumour necrosis factor in rat experimental endotoxaemia: comparison between Lewis and Wistar strains. *Br. J. Pharmacol.,* **110,** 868 – 874.

SEWER, M.B., KOOP, D.R. & MORGAN, E.T. (1996). Endotoxaemia in rats is associated with induction of the P4504A subfamily and suppression of several other forms of cytochrome P450. *Drug Metab. Dispos.,* **24,** 401 – 407.

SUGIURA, M., INAGAMI, T. & KON, V. (1989). Endotoxin stimulates endothelin release *in vivo* and *in vitro* as determined by radioimmunoassay. *Biochem. Biophys. Res. Commun.,* **161,** 1220 – 1227.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade test: 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.

VEMULAPALLI, S., CHIU, P.J.S., RIVELLI, M., FOSTER, C.J. & SYBERTZ, E.J. (1991). Modulation of circulating endothelin levels in hypertension and endotoxaemia in rats. *J. Cardiovasc. Pharmacol.,* **18,** 895 – 903.

WALLER, J., GARDINER, S.M., JOSE, J. & BENNETT, T. (1995). Lack of effect of TNF antibodies on the cardiovascular sequelae of lipopolysaccharide infusion in conscious rats. *Br. J. Pharmacol.,* **116,** 2487 – 2495.

WU, C.-C., HONG, H.-J., CHOU, T.-C., DING, Y.-A. & YEN, M.-S. (1996). Evidence for inducible nitric oxide synthase in spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.,* **228,** 459 – 466.

YEN, M.-H., LIU, Y.-C., HONG, H.-J., SHEU, J.-R. & WU, C.-C. (1997). Role of nitric oxide in lipopolysaccharide-induced mortality from spontaneously hypertensive rats. *Life Sci.,* **60,** 1223 – 1230.

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