Haemodynamic effects of losartan and the endothelin antagonist, SB 209670, in conscious, transgenic ((mRen-2)27), hypertensive rats

[1]S.M. Gardiner, J.E. March, P.A. Kemp, *J.J. Mullins & T. Bennett

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Centre for Genome Research, University of Edinburgh

1   Hypertensive transgenic (TGR(mRen-2)27) (abbreviated to TG) rats ($n=6$) and their normotensive Sprague-Dawley (SD) control strain ($n=7$) were chronically instrumented for the measurement of cardiac haemodynamics. The hypertension in TG rats (mean blood pressure 181 ± 9 mmHg) was entirely attributable to a reduction in total peripheral conductance (TG rats $=169\pm7$, SD rats $=292\pm15$ μl min^{-1} mmHg^{-1} 100g^{-1}) since cardiac index was not different in the two strains (TG rats $=30.5\pm1.2$, SD rats $= 29.5\pm1.6$ ml min^{-1} 100g^{-1}).

2   In other animals instrumented for the assessment of regional haemodynamics, the extent of peripheral vasoconstriction was similar in renal, mesenteric and hindquarters vascular beds in the TG rats (reduction in vascular conductance relative to SD rats $=42\%$, 46% and 49%, respectively).

3   During an 8 h observation period with saline infusion, or following injection of losartan (10 mg kg^{-1}) in SD rats there was no hypotension or regional vasodilatation. With infusion of the endothelin antagonist, SB 209670 (10 μg kg^{-1} min^{-1}), there was a slight hypotension, but no significant vasodilatation; co-administration of losartan and SB 209670 caused a similar profile of effect, although the hypotension was increased

4   With the same experimental protocol in TG rats, losartan caused a biphasic, progressive fall in mean arterial blood pressure accompanied by renal, mesenteric and hindquarters vasodilatation. Although the response to SB 209670 was not biphasic, its hypotensive and vasodilator effects were not different from those of losartan after 8 h. In the combined presence of losartan and SB 209670, mean arterial blood pressure (116 ± 5 mmHg) was significantly lower than with SB 209670 (132 ± 4 mmHg) or losartan (136 ± 6 mmHg) alone, and renal, mesenteric and hindquarters vascular conductances (61 ± 3, 90 ± 14 and 52 ± 4 [kHz mmHg^{-1}]10^3, respectively) were higher than the corresponding values following either SB 209670 (49 ± 4, 52 ± 4 and 34 ± 3 [kHz mmHg^{-1}]10^3, respectively) or losartan (43 ± 5, 59 ± 13 and 35 ± 4 [kHz mmHg^{-1}]10^3, respectively) alone. These results indicate the maintenance of hypertension in TG rats is dependent upon renal, mesenteric and hindquarters vasoconstriction, mediated by angiotensin II (AII) and endothelin (ET). Since we found that plasma ET-1 levels in TG rats (12.06 ± 2.87 pmol l^{-1}) were lower than in SD rats (21.53 ± 3.94 pmol l^{-1}), then it is possible that locally-generated, rather than circulating ET-1 contributes to the widespread vasoconstriction in TG rats.

Keywords: Transgenic rats; losartan; endothelin; SB 209670; vasoconstriction

Introduction

Introduction of the mouse Ren-2 renin gene (encoding sub-mandibular gland renin) into the rat genome produces transgenic (TG) animals with fulminant hypertension (Mullins et al., 1990) but it is unknown if TG rats have peripheral vasoconstriction and/or an elevated cardiac output. Therefore, the first aim of the present study was to compare resting systemic haemodynamics in male, untreated, heterozygous TG rats and the normotensive control strain. Heterozygous animals were used in preference to homozygotes since the latter develop such severe hypertension they do not survive well, unless treated with antihypertensive drugs (Mullins et al., 1990).

TG rats respond to acute or chronic administration of angiotensin converting enzyme (ACE) inhibitors or angiotensin II (AT$_1$)-receptor antagonists with marked falls in blood pressure (Bader et al., 1992; Hirth-Dietrich et al., 1994; Moriguchi et al., 1994). However, it is not known if administration of an AT$_1$-receptor antagonist exerts differential regional haemodynamic effects, since studies to date have measured only blood pressure and heart rate responses to chronic oral administration of losartan. Therefore, the second objective of our work was to assess regional haemodynamic responses to i.v. losartan in TG rats, and their normotensive controls.

In vitro evidence indicates that angiotensin II (AII) can stimulate the synthesis and release of endothelin-1 (ET-1) (Emori et al., 1991; Imai et al., 1992), so it is feasible that the latter contributes to the maintenance of hypertension in TG rats. Hence, the third objective of this work was to measure plasma ET-1 levels, and to determine the influence of a non-selective, ET$_A$-, ET$_B$-receptor antagonist, SB 209670 (Ohlstein et al., 1994; Douglas et al., 1995a, b) on regional haemodynamics in control and TG rats. For completeness, we also determined responses to co-administration of losartan and SB 209670, in both strains.

Methods

Animals either were obtained from the Centre for Genome Research, Edinburgh, or were bred from animals supplied from that source. Male, heterozygous TG rats (3-4 months old) and age-matched, normotensive Sprague-Dawley (SD) rats (originally obtained from the Zentralinstitut fur Versuchstierkunde, Hannover, Germany) were studied. The latter

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[1] Author for correspondence.

strain of rat was that used originally in the production of the TGR (mRen-2)27 strain (Mullins et al., 1990). TG rats were bred in Nottingham by crossing male, homozygous TG rats with the control Sprague-Dawley rats. The former were kept on chronic captopril treatment by adding it to the drinking water (50 mg l^{-1}) (Mullins et al., 1990). Animals were anaesthetized with sodium methohexitone (40–60 mg kg^{-1}, i.p.) and had either pulsed Doppler probes implanted to monitor regional haemodynamics, or an ascending thoracic aortic electromagnetic flow probe implanted to monitor cardiac haemodynamics. After a recovery period (4–7 days for animals with electromagnetic flow probes; 7–14 days for animals with pulsed Doppler probes), all animals were again anaesthetized for the implantation of catheters, for the measurement of arterial blood pressure and heart rate, and for the i.v. administration of drugs. All procedures have been described in detail previously (Gardiner et al., 1990a, b; 1991).

Resting cardiac haemodynamics

In 7 SD and 6 TG rats, resting cardiac haemodynamics were recorded between 07 h 00 min and 08 h 00 min, and analysed as described previously (Gardiner et al., 1990b; 1991). Subsequently, blood samples were obtained from some of these animals for the determination of plasma levels of ET-1 and big ET-1 (see below).

Regional haemodynamic changes during infusion of saline

Beginning at 07 h 00 min, continuous recordings were made of mean arterial blood pressure, heart rate, and Doppler shift signals from renal, mesenteric and hindquarters probes. Thirty min later, an infusion of saline (0.4 ml h^{-1}, 154 mmol l^{-1}-NaCl) was begun and continued for 8 h. Cardiovascular variables were averaged over 5 min at each hour; vascular conductance was calculated by dividing mean Doppler shift by mean arterial blood pressure; this protocol was carried out in 8 SD and 8 TG rats.

Regional haemodynamic changes following administration of losartan

The time course of this experiment was as above, but 30 min after recordings began an i.v. injection of losartan (10 mg kg^{-1}) was given. Elsewhere (Batin et al., 1991) we have shown this dose of losartan inhibits responses to exogenous AII (50 pmol) for at least 9 h; this protocol was run in 5 SD and 7 TG rats.

Regional haemodynamic changes during infusion of SB 209670

The procedure in this experiment was as with saline infusion, except that the saline contained SB 209670 (10 μg kg^{-1} min^{-1}). (In pilot experiments we found this dose of SB 209670 completely reversed the haemodynamic effects of an infusion of exogenous ET-1 (120 pmol h^{-1}) which increased mean arterial blood pressure by 30–40 mmHg; see also Douglas et al., 1995b). This experiment was run in 8 SD and 7 TG rats; all of the former, and 3 of the latter, had been in the group infused with saline on the previous day.

Regional haemodynamic changes following administration of losartan and SB 209670

The time course of this experiment was as above, except that 30 min after recordings began losartan (10 mg kg^{-1} bolus) and SB 209670 (10 μg kg^{-1} min^{-1}) were administered. This experiment was run in 5 SD and 7 TG rats. All the SD rats had received losartan on the previous day; of the TG rats, 3 had received SB 209670 and 4 had received losartan, 24 h previously.

Radioimmunoassay of plasma ET-1 and big ET-1

Blood samples (1 ml) were obtained from the conscious animals instrumented for measurement of cardiac haemodynamics (above), after haemodynamic measurements had been made. Solid phase extraction of ET-1 and big ET-1 was carried out as described by Davenport et al. (1990), and peptides were measured by the radioimmunoassay described by Plumpton et al. (1993). The measurements of ET-1 and big ET-1 were carried out by Dr C. Plumpton and Dr A.P. Davenport (Clinical Pharmacology Unit, University of Cambridge).

Data analysis

Within-group comparisons were made using Friedman's test (Theodorsson-Norheim, 1978); between-group comparisons were made with the Mann-Whitney U test, or the Kruskal-Wallis test, as appropriate. A P value <0.05 was taken as significant.

Drugs

Losartan potassium was a gift from Dr R.D. Smith (Du Pont, U.S.A.) and SB 209670 ([(±)-(1S, 2R, 3S)-3-(2-carboxy-methoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(-prop-1-yloxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SmithKline Beecham, U.S.A.).

Results

Resting haemodynamics in TG and SD rats

Table 1 shows that the hypertension in TG rats was not associated with a significant change in any of the measured cardiac variables, but there was marked peripheral vasoconstriction. Measurements of regional haemodynamics showed that the Doppler shift signals and calculated vascular conductances were significantly lower in renal, mesenteric and hindquarters vascular beds in the TG rats than in the normotensive controls (Table 2). The proportional reductions in total peripheral conductance (42%), and in renal (48%), mesenteric (46%) and hindquarters (49%) vascular conductances were similar (Tables 1 and 2).

Regional haemodynamic changes during infusion of saline, or with administration of losartan, or SB 209670, or losartan together with SB 209670 in SD rats

During infusion of saline there were no significant changes in heart rate, mean arterial pressure or renal haemodynamics in SD rats (Figure 1). There were slight and variable decreases in mesenteric flow and vascular conductance, but more consistent reductions in hindquarters flow and vascular conductance (Figure 1).

Table 1 Resting cardiac haemodynamic variables in conscious rats

	SD rats (n = 7)		TG rats (n = 6)
Heart rate (beats min^{-1})	378 ± 11		354 ± 18
Mean blood pressure (mmHg)	101 ± 2	*	181 ± 9
Cardiac index (ml min^{-1} 100 g^{-1})	29.5 ± 1.6		30.5 ± 1.2
Stroke index (μl beat^{-1} 100 g^{-1})	78 ± 3		87 ± 5
Peak aortic flow (ml min^{-1} 100 g^{-1})	114 ± 6		109 ± 3
dF/dt_{max} (l min^{-2} 100 g^{-1})	479 ± 31		452 ± 16
Total peripheral conductance (μl min^{-1} mmHg^{-1} 100 g^{-1})	292 ± 15	*	169 ± 7
Central venous pressure (cmH$_2$O)	5.42 ± 15		6.04 ± 0.48

g refers to body weight. Values are mean ± s.e.mean.
*$P < 0.05$ (Mann-Whitney U test)

Table 2   Resting regional haemodynamic variables prior to any intervention in conscious rats

	SD rats ($n = 13$)		TG rats ($n = 17$)
Heart rate (beats min^{-1})	365 ± 6		345 ± 7
Mean blood pressure (mmHg)	108 ± 2		169 ± 7
Renal Doppler shift (kHz)	6.9 ± 0.5	*	5.3 ± 0.3
Mesenteric Doppler shift (kHz)	8.4 ± 0.5	*	7.0 ± 0.8
Hindquarters Doppler shift (kHz)	5.2 ± 0.3	*	4.1 ± 0.3
Renal vascular conductance ([kHz mmHg^{-1}]10^3)	64 ± 5	*	33 ± 3
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3)	79 ± 4	*	43 ± 6
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3)	49 ± 3	*	25 ± 3

Values are mean ± s.e.mean.
*$P < 0.05$ (Mann-Whitney U test)

Following administration of losartan, there was an early slight tachycardia (37 ± 15 beats min^{-1} at 15 min), but this had waned by 1 h, and thereafter the slight change in heart rate was

not different from that seen during saline infusion, and there was no hypotension; likewise, there were no changes in renal haemodynamics (Figure 1). There were reductions in mesenteric flow and vascular conductance, but these were not significantly different from those during saline infusion, and the same picture was seen in the hindquarters (Figure 1).

During administration of SB 209670 there was a slight hypotension, but no tachycardia, or changes in renal haemodynamics (Figure 1). The fall in mean arterial blood pressure was significantly greater than that seen during infusion of saline (AOC$_{0-8 h}$ 43 ± 6 vs 19 ± 9 mmHg h, respectively; Figure 1).There were no reductions in mesenteric haemodynamics, in significant contrast to the changes seen during saline infusion, or following losartan administration, and similar, albeit smaller, differences were apparent in the hindquarters (Figure 1).

Prior to administration of losartan and SB 209670 together, resting haemodynamics in the SD rats were not different from the values 24 h previously, i.e., just before administration of losartan (Figure 1). Administration of losartan and SB 209670 together caused hypotension, but no tachycardia (Figure 1). The hypotension was greater than that seen with the other interventions (AOC$_{0-8 h}$ 86 ± 20 mmHg h, Figure 1). There
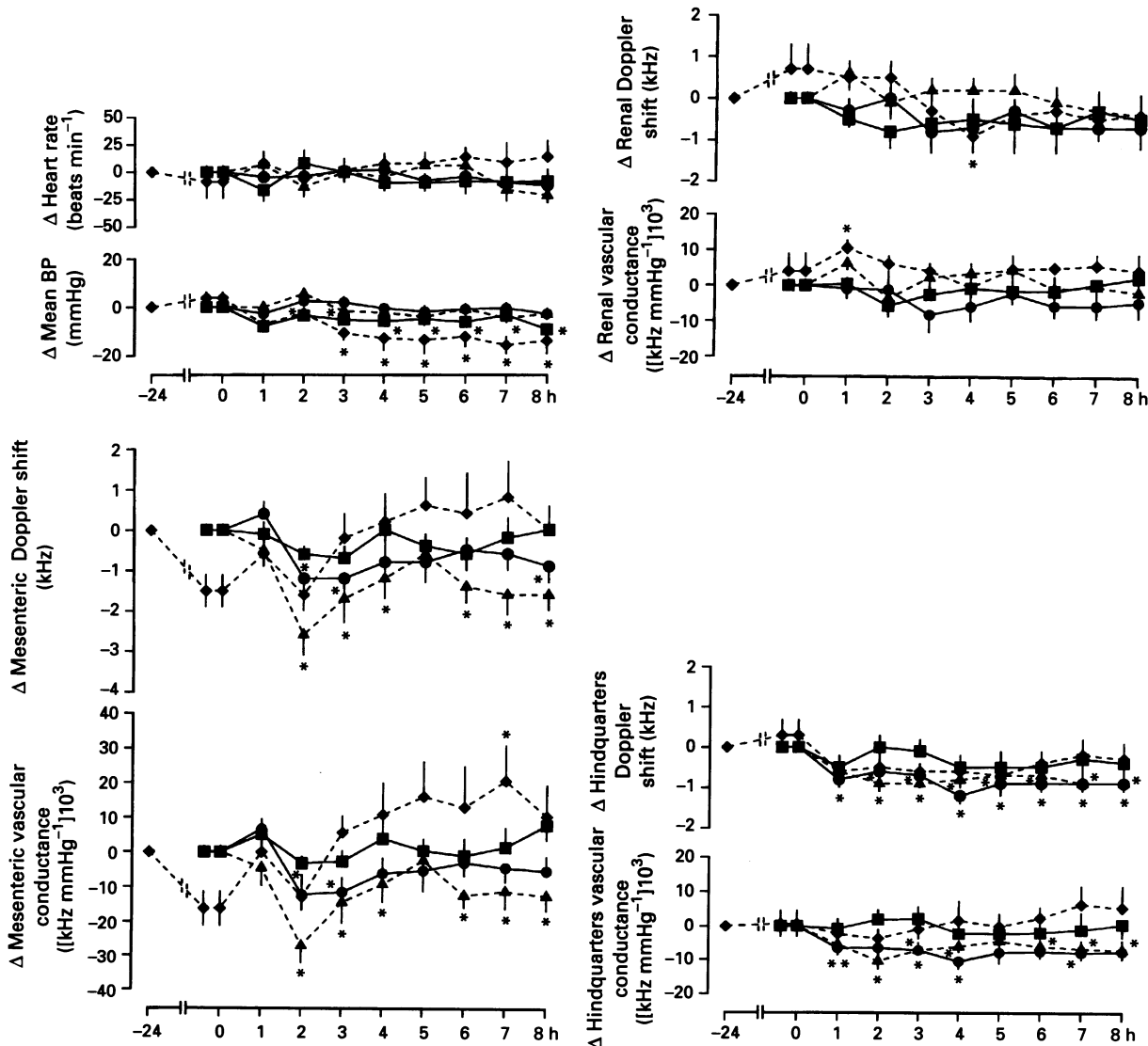

Figure 1   Cardiovascular changes in SD rats during infusion of saline (0–8 h, ●; $n = 8$) or SB 209670 (10 μg kg^{-1} min^{-1}, 0–8 h, ■; $n = 8$), or following injection of losartan (10 mg kg^{-1} at $t = 0$h, ▲; $n = 5$), or following combined administration of losartan with SB 209670 (10 mg kg^{-1} at $t = 0$h and 10 μg kg^{-1} h^{-1}, 0–8 h respectively, ◆; $n = 5$). Values are mean ± s.e.mean; *$P < 0.05$ versus baseline (i.e. $t = 0$h or $t = 0$h and $t = -24$h in the case of the group receiving both antagonists together, since these animals had received losartan on the previous day) (Friedman's test).

were variable changes in renal flow, but a tendency towards renal vasodilation ($AUC_{0-8\,h}$ 47±10 [kHz mmHg^{-1}]10^3 h; Figure 2) which was not different from that seen with losartan ($AUC_{0-8\,h}$ 28±14 [kHz mmHg^{-1}]10^3 h; Figure 1).

The changes in mesenteric and hindquarters haemodynamics were not different from those seen with administration of SB 209670 alone (Figure 1).

Regional haemodynamic changes during infusion of saline, or with administration of losartan, or SB 209670, or losartan together with SB 209670 in TG rats

During infusion of saline, there were no significant haemodynamic changes in TG rats (Figure 2; Table 3).

Following administration of losartan, there was a rapid-onset hypotension and tachycardia (−17±4 mmHg and +51±12 beats min^{-1} at 15 min). The latter had disappeared by 1 h, although the hypotension increased progressively over that time. However, between 1 and 2 h after administration of losartan, blood pressure showed some recovery, but thereafter fell progressively (Figures 2 and 3). Similar biphasic changes in renal and mesenteric haemodynamics were apparent (Δ renal vascular conductance, 12±2, Δ mesenteric vascular conductance, 14±3 [kHz mmHg^{-1}]10^3 at 15 min), but such a pattern of change was less obvious in the hindquarters vascular bed (Δ hindquarters vascular conductance, 6±2 [kHz mmHg^{-1}]10^3 at 15 min) (Figure 2). All the haemodynamic changes after losartan administration were significantly different from those during saline infusion (Table 3).

During infusion of SB 209670 there was no tachycardia, but a slow-onset, progressive hypotension, accompanied by increases in renal, mesenteric and hindquarters flows and vascular conductances (Figures 2 and 4). All these changes were significantly different from those during saline infusion, but from 1 h onwards, not different from those after losartan administration (Figure 2, Table 3).

Prior to administration of losartan and SB 209670 together, resting blood pressure (156±5 mmHg) was significantly lower than at the start of the previous day (181±7 mmHg, Figure 2) i.e., just before administration of losartan ($n=4$) or SB 209670 ($n=3$). However, there were no significant differences between regional blood flows or vascular conductances on the two occasions (Figure 2).
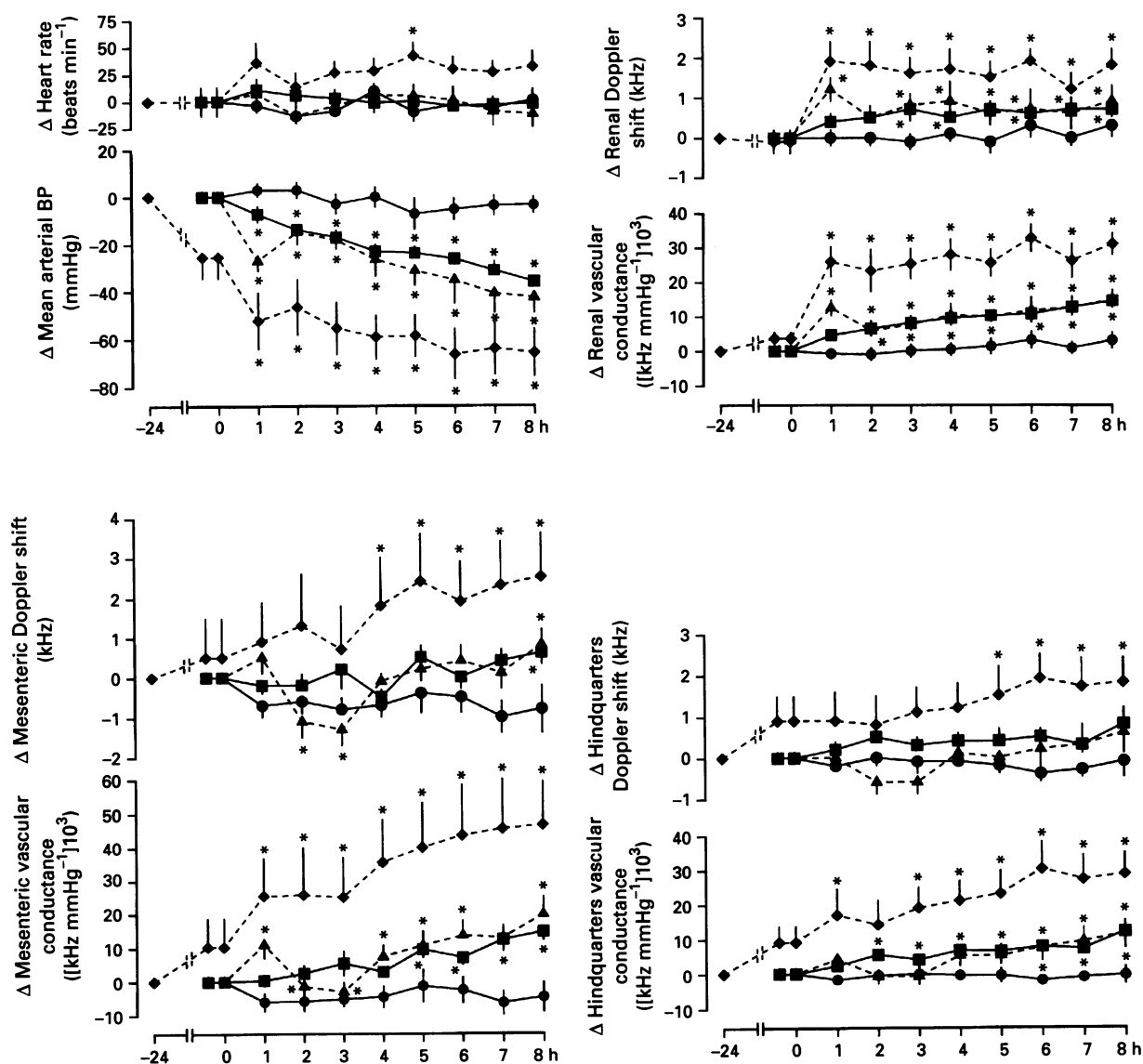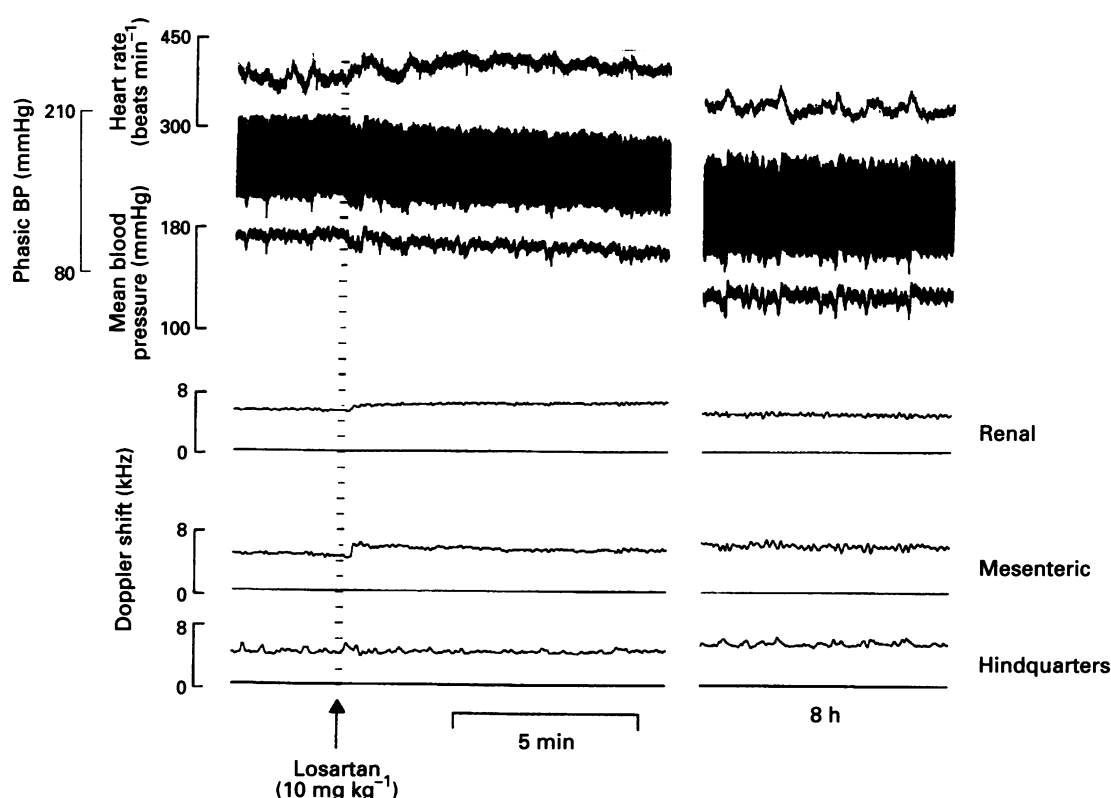

Figure 2   Cardiovascular changes in TG rats during infusion of saline (0–8 h, ●; $n=8$) or SB 209670 (10 μg kg^{-1} min^{-1}, 0–8 h, ■; $n=7$), or following injection of losartan (10 mg kg^{-1} at $t=$Oh, ▲; $n=7$), or following combined administration of losartan with SB 209670 (10 mg kg^{-1} at $t=$Oh and 10 μg kg^{-1} h^{-1}, 0–8 h respectively, ◆; $n=7$). Values are mean±s.e. mean; *$P<0.05$ versus baseline (i.e. $t=$Oh or $t=$Oh and $t=$ −24 h in the case of the group receiving both antagonists together, since these animals had received either losartan ($n=4$) or SB 209670 ($n=3$) on the previous day) (Friedman's test).

Table 3 Integrated (AUC, AOC_{0-8h}) cardiovascular changes during saline infusion, or with administration of losartan, or SB 209670, or losartan and SB 209670, in conscious, transgenic rats

	(a) Saline (n = 8)	(b) Losartan (n = 7)	(c) SB 209670 (n = 7)	(d) Losartan + SB209670 (n = 7)
Δ Heart rate (beats 10^{-3})	-4.74 ± 1.5	5.28 ± 1.92	3.96 ± 1.62^{ab}	14.52 ± 3.96^{abc}
Δ Mean blood pressure (mmHg h)	-43 ± 21	-217 ± 38^{a}	-163 ± 14^{a}	-435 ± 78^{abc}
Δ Renal flow (kHz h)	2.3 ± 0.6	6.4 ± 2.1^{a}	5.0 ± 1.1^{a}	12.9 ± 2.9^{abc}
Δ Mesenteric flow (kHz h)	-6.0 ± 2.0	3.0 ± 0.7^{a}	2.9 ± 1.0^{a}	14.9 ± 7.5^{abc}
Δ Hindquarters flow (kHz h)	-2.2 ± 0.8	2.8 ± 1.4^{a}	3.8 ± 0.9^{a}	11.7 ± 3.9^{abc}
Δ Renal vascular conductance ([kHz mmHg^{-1}]10^3 h)	22 ± 7	80 ± 17^{a}	70 ± 12^{a}	203 ± 32^{abc}
Δ Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3 h)	47 ± 18	71 ± 19^{a}	53 ± 14^{a}	268 ± 97^{abc}
Δ Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3 h)	-17 ± 6	45 ± 15^{a}	48 ± 7^{a}	169 ± 49^{abc}

For the group given losartan and SB 209670, the integrated responses were calculated relative to the pre-drug baseline on the previous day (see Methods). Values are mean $\pm$ s.e.mean.
Superscripts $= P < 0.05$ versus corresponding column (Kruskal-Wallis test).

Figure 3 Cardiovascular changes following bolus injection of losartan in a conscious TG rat.

With administration of losartan and SB 209670 together there was a variable tachycardia, but a prompt, and sustained hypotension, accompanied by substantial increases in renal, mesenteric, and hindquarters flows and vascular conductances (Figure 2). All these changes were significantly different from those in the other experiments (Table 3).

At the end of the 8 h period, in the presence of losartan and SB 209670, mean arterial blood pressure (116 ± 5 mmHg) was significantly lower than in the presence of either SB 209670 (132 ± 4 mmHg) or losartan (136 ± 6 mmHg) alone. Similarly, renal, mesenteric and hindquarters vascular conductances 8 h after administration of losartan and SB 209670 together (61 ± 3, 90 ± 14 and 52 ± 4 [kHz mmHg^{-1}]10^3, respectively) were higher than the corresponding values following either SB 209670 (49 ± 4, 52 ± 4 and 34 ± 3 [kHz mmHg^{-1}]10^3) or losartan (43 ± 5, 59 ± 13 and 35 ± 4 [kHz mmHg^{-1}]10^3) given alone.

Plasma levels of ET-1 and big ET-1

In SD rats the plasma level of ET-1 (21.53 ± 3.94 pmol 1^{-1}, $n = 6$) was significantly higher than in TG rats (12.06 ± 2.87 pmol 1^{-1}, $n = 5$). However, there was no significant difference between the plasma levels of big ET-1 in the two strains (SD = 14.88 ± 2.05, TG = 12.48 ± 0.65 pmol 1^{-1}).

Discussion

The present work has shown that the marked hypertension in male, heterozygous (mRen2)-27 TG rats is not due to an elevated cardiac output, but to a decreased peripheral vascular conductance; the latter was due to similar degrees of vasoconstriction in renal, mesenteric and hindquarters vascular
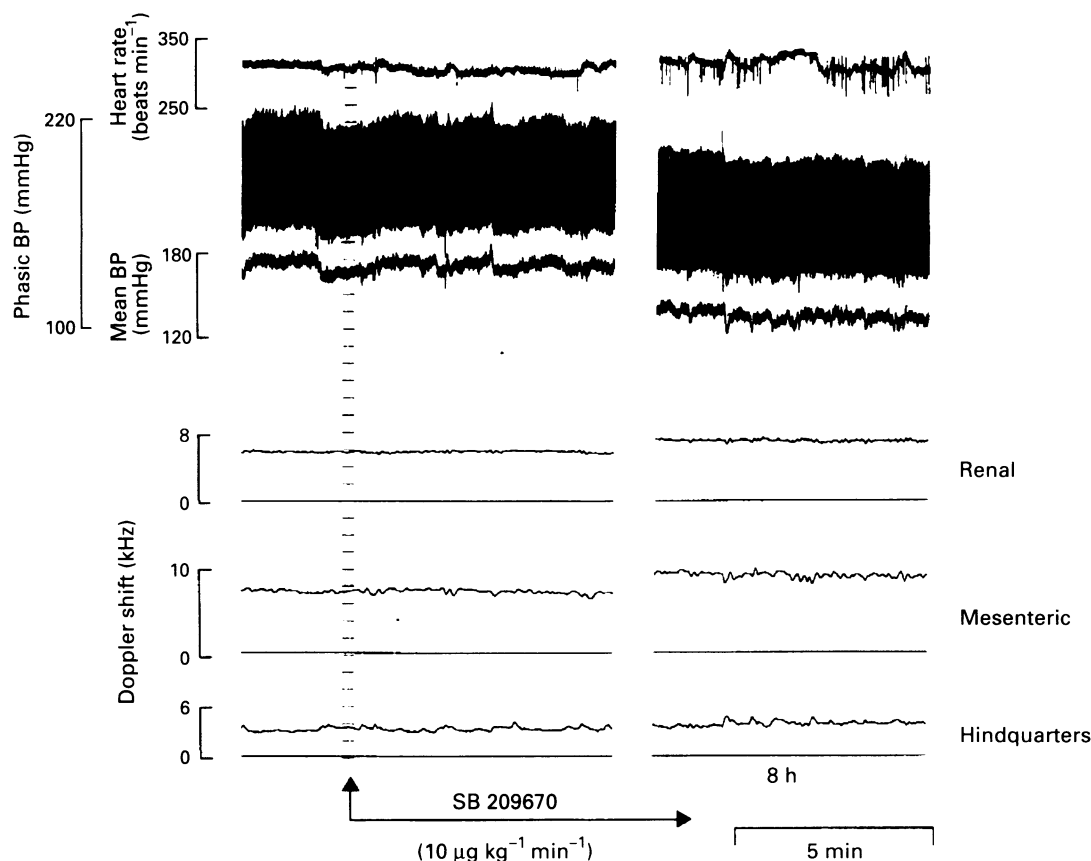
Figure 4 Cardiovascular changes during infusion of SB 209670 $(10\,\mu g\,kg^{-1}\,min^{-1})$ in a conscious TG rat.

beds. The finding that TG rats showed no signs of cardiac depression is surprising in light of the evidence for heterologous desensitization of adenylyl cyclase, downregulation of β_1-adrenoceptors, and reduced positive inotropic responses to isoprenaline in hearts from these animals (Böhm et al., 1994), and the marked increase in cardiac afterload shown here. However, we measured cardiac haemodynamics under resting conditions only, so cannot exclude the existence of abnormal responses to increased myocardial workload without further experiments.

Further support for the proposal that the hypertension in TG rats is due to peripheral vasoconstriction comes from the finding that, 8 h after co-treatment with losartan and SB 209670, renal, mesenteric and hindquarters vascular conductances in TG rats $(61\pm3, 90\pm14$ and 52 ± 4 [kHz $mmHg^{-1}]10^3$, respectively) were not different from those in SD rats under the same conditions $(70\pm3, 81\pm6,$ and 54 ± 10 [kHz $mmHg^{-1}]10^3$, respectively).

In the normotensive, SD rats, losartan, SB 209670, and the combination thereof, had relatively slight hypotensive and haemodynamic effects. These findings are consistent with a lack of involvement of the RAS and/or ET in the maintenance of cardiovascular status in normal, conscious rats (e.g. Batin et al., 1991; Nishikibe et al., 1993; Ohlstein et al., 1993, Douglas et al., 1995b), and indicate that the substantial effects of these drugs in TG rats were not due to non-specific vasodilator actions.

During the infusion of saline in TG rats, there were no significant changes in mean arterial blood pressure between 07 h 00 min and 15 h 00 min. This finding is in contrast to the observations of Lemmer et al. (1993) who reported that, in TG rats, blood pressure values were maximal around noon (when the light cycle was from 07 h 00 min to 19 h 00 min). Lemmer et al. (1993) monitored blood pressure by telemetry, and it may be this approach preserves abnormal circadian variation in blood pressure in TG rats; however, it does not allow monitoring of regional or cardiac haemodynamic variables.

Considering that the TG rats were produced by insertion of the mouse Ren-2 gene into their genome (Mullins et al., 1990), the marked cardiovascular effects of losartan were not unexpected, and consistent with earlier findings, albeit with oral or chronic administration of ACE inhibitors or losartan (see Introduction). Nonetheless, certain features of our findings are novel. For example, although previous authors have described falls in blood pressure following these interventions in TG rats, we are the first to show that the hypotension is associated with peripheral vasodilatation. Furthermore, it was notable that within the first 1 h following i.v. injection of losartan there was a substantial hypotension and renal and mesenteric vasodilatation, but these effects waned between 1 and 2 h, and then redeveloped thereafter, together with a hindquarters vasodilatation. In the water-deprived Brattleboro (i.e., vasopressin-deficient) rat there is marked activation of the renal RAS, and in these animals losartan causes an initial hypotension and renal and mesenteric vasodilatation which is maximal within 5 min (Widdop et al., 1993). Hence the relatively slow-onset of the initial hypotensive and vasodilator effects of losartan in TG rats may indicate a greater contribution from local RASs, than from the renal RAS, to the hypertension and peripheral vasoconstriction (see Mullins et al., 1990), and suggest that any such local effects may be expressed similarly in renal, mesenteric and hindquarters vascular beds.

Eight h after i.v. injection of losartan, mean arterial blood pressure was reduced by 43 ± 7 mmHg, while renal, mesenteric and hindquarters vascular conductances were increased by $54\pm11, 54\pm15,$ and $64\pm30\%$, respectively. This hypotensive effect is similar to that reported by Moriguchi et al. (1994), following treatment of TG rats with losartan for 7 days. Hence it seems likely this influence of losartan is due to interference with the vasoconstrictor, rather than trophic, actions of AII.

Although we considered it possible that ET-1 might contribute to the maintenance of the hypertension in TG rats (see Introduction), we were surprised by the magnitude of the hy-

potensive and vasodilator effects of the ET_A-, ET_B-receptor antagonist in these animals. Thus, after 8 h infusion of SB 209670, mean arterial blood pressure was reduced by 36 ± 2 mmHg, and renal, mesenteric and hindquarters vascular conductances were increased by 43 ± 6, 40 ± 6, and $61 \pm 17\%$, respectively. From the present experiments, we cannot determine the extent to which the effects of SB 209670 were due to inhibition of the synergism between ET-1 and AII (Yoshida et al., 1991; 1992), or how far inhibition of AII-mediated ET-1 release (Emori et al., 1991; Imai et al., 1992) contributed to the haemodynamic effects of losartan. Moreover, we cannot exclude the possibility that the vasculature of TG rats is more sensitive to the constrictor actions of AII and ET-1 than is the vasculature of the normotensive control rats.

Following the onset of infusion of SB 209670, its hypotensive and vasodilator effects developed slowly and progressively over the 8 h period of observation, and this profile of effect could explain why there was no reflex tachycardia. However, it may be that SB 209670 has actions in the central nervous system, interfering with baroreflex mechanisms. We know from our pilot experiments (unpublished data) that, in animals made hypertensive by continuous infusion of ET-1 $(120 \text{ pmol h}^{-1}$; Gardiner et al., 1990a), administration of SB 209670, at the dose used here, does not have any immediate effects, but causes a gradual fall in blood pressure, such that 1 h after the onset of SB 209670 infusion, the ET-1-mediated pressor response $(40 \pm 1$ mmHg; $n = 3)$ is inhibited by 74%, and after 2 h, blood pressure is normal. One possibility is that the slow onset of effect is due to the pharmacokinetic profile of the compound. Alternatively, since SB 209670 is an antagonist at both ET_A- and ET_B-receptors, with a greater potency at the latter in vivo (Douglas et al., 1995a), it is feasible that the profile of haemodynamic change represented an amalgam of the suppression of the vasodilator (ET_B-mediated) effects of ET, together with developing antagonism of its vasoconstrictor (ET_A- and ET_B-mediated) actions (see Warner et al., 1994). Such a proposition is consistent with the rapid-onset of hypotensive effect of the ET_A-receptor antagonist BQ-123, in rats with renal hypertension (Douglas et al., 1994), and of FR 139317 in rats with DOCA/salt-induced hypertension (Fujita et al., 1995). However, it is apparent that administration of ET_A-receptor antagonists does not always produce rapid-onset effects, because infusion of BQ-123 in spontaneously hypertensive rats produced a fall in mean arterial blood pressure and increase in total peripheral conductance that developed progressively over 6 h (Douglas et al., 1994).

Since ET antagonists have been found to lower arterial blood pressure in several models of experimental hypertension (e.g. Nishikibe et al., 1993; Ohlstein et al., 1993; Okada et al., 1994; Douglas et al., 1994; 1995b), it is feasible that hypertension per se is responsible for stimulating increased synthesis and release of ET. However, Larivière et al. (1993) found that the ET-1 content of the aorta and mesenteric vascular bed was increased in rats with DOCA/salt-induced hypertension, but not in rats with spontaneous hypertension. Thus, factors other than elevated blood pressure may be responsible for the involvement of ET in hypertension. AII is a likely candidate (see Introduction), but it is notable that Villarreal et al. (1995) have described increased levels of mRNA for transforming growth factor-β_1(TGF-β_1) in the heart of TG rats, since TGF-β_1 is a potent stimulus for ET synthesis and release in vitro (e.g., Kurihara et al., 1989; Kanse et al., 1991; Endo et al., 1992), and the in vivo haemodynamic effects of TGF-β_1 are inhibited by SB 209670 (S.M. Gardiner et al., unpublished observations). However, it should be noted that plasma ET-1 levels were lower in TG, than in SD, rats, indicating the involvement of ET-1 may be as a local, rather than systemic, vasoconstrictor.

In conclusion, the present work provides evidence that hypertension in male, heterozygous TG rats is due to vasoconstriction in renal, mesenteric, and hindquarters vascular beds (at least), mediated by AII and ET. Since losartan and SB 209670 together caused blood pressure to fall to a lower level, and conductances to rise to higher levels, than either antagonist alone, then it seems that ET and AII have, to some extent, independent roles in the maintenance of the hypertensive state.

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