British Journal of Pharmacology (1999) 128, 1795–1803
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Cardiovascular responses to angiotensins I and II in normotensive and hypertensive rats; effects of NO synthase inhibition or ET receptor antagonism

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1 We compared the cardiovascular responses to angiotensins (I and II), and any possible modulatory influences thereupon of nitric oxide (NO) or endothelin (ET) in conscious male, normotensive, Hannover Sprague-Dawley (SD) rats, and hypertensive, heterozygous ((mRen-2)27), transgenic (TG) rats.

2 The pressor effects of angiotensin I or of angiotensin II were not consistently different in SD and TG rats. The accompanying absolute reductions in renal and mesenteric vascular conductances were smaller in TG rats, but probably due to the baseline vasoconstriction in those animals.

3 Inhibition of NO synthase with L-NAME had no significant effects on the pressor responses to angiotensin I or angiotensin II in either SD or TG rats. L-NAME reduced the absolute, but not percentage, reductions in renal and mesenteric vascular conductances in response to angiotensin I and angiotensin II. L-NAME abolished the hindquarters vasodilator effects of angiotensin I and angiotensin II in both strains of rat.

4 ET receptor antagonism (with SB209670) had no significant influence on the pressor or renal or mesenteric vasoconstrictor effects of angiotensin II in SD rats. In TG rats, the pressor responses to angiotensin II were unaffected by SB209670; the accompanying falls in renal and mesenteric vascular conductances were enhanced in absolute, but not in percentage terms.

5 These results provide no evidence for a buffering action of NO, or a modulatory influence of ET, on the pressor or vasoconstrictor effects of angiotensin I and/or angiotensin II in SD rats. Furthermore, there is no evidence for an altered sensitivity to angiotensin I or angiotensin II, and no evidence for a differential modulatory influence of either NO or ET in TG, compared to SD, rats.

Keywords: Angiotensin; N^G-nitro-L-arginine methyl ester; transgenic rats; endothelin

Abbreviations: L-NAME, N^G-nitro-L-arginine methyl ester; SD rats, Hannover Sprague-Dawley rats; TG rats, ((mRen-2)27) transgenic rats

Introduction

The vascular actions of angiotensin are complex, with evidence for an interactive influence of a variety of endothelium-derived vasodilator and vasoconstrictor substances, including nitric oxide (NO) and endothelin (ET) (for review see Vanhoutte *et al*., 1993). Furthermore, the extent to which the endothelium modulates responses to angiotensin II *in vitro* depends on the vascular bed studied (Chen *et al*., 1995).

We (Gardiner *et al*., 1988) and others (e.g., Corder *et al*., 1986; Li & Zimmerman, 1990; Heinemann *et al*., 1997) have shown that, *in vivo*, acute systemic administration of angiotensin II is associated with regionally-selective effects, i.e., vasoconstriction in the renal and mesenteric vascular beds, but a tendency towards vasodilatation in the hindquarters (Gardiner *et al*., 1988). However, relatively few studies have explored the possible involvement of NO and ET in the cardiovascular effects of acute systemic administration of angiotensin II. Regarding ET, Balakrishnan *et al*. (1996) showed that ET receptor antagonism with bosentan reduced the effects of angiotensin II on blood pressure and total peripheral conductance, and those effects were more pronounced in spontaneously hypertensive rats (SHR) than in normotensive control rats; the regional vascular changes were not assessed. Regarding NO, Heinemann *et al*. (1997) showed that NO synthase inhibition could abolish the femoral vasodilator effects of angiotensin II. That study also reported a slight inhibitory effect of bosentan on the pressor response to angiotensin II, but no effect on the femoral vasodilatation; mesenteric haemodynamics were measured but not reported (Heinemann *et al*., 1997).

Therefore, one aim of the present study was to determine the effects of NO synthase inhibition (with N^G-nitro-L-arginine methyl ester (L-NAME)) and ET receptor antagonism (with SB209670, (Douglas *et al*., 1995)) on the regional haemodynamic responses to acute systemic i.v. administration of angiotensin II in conscious rats. Since vasodilator responses to angiotensin II have been reported to be more pronounced following bolus injections rather than infusions (Heinemann *et al*., 1997), whereas any involvement of ET has been shown using angiotensin II infusion (Balakrishnan *et al*., 1996), we examined the effects of L-NAME on responses to bolus doses of angiotensin II, and effects of SB209670 on responses to short-term infusions of angiotensin II.

In hypertensive rats, produced by insertion of the mouse Ren-2 gene into the rat genome (abbreviated to TG rats) (Mullins *et al*., 1990), vascular production of angiotensin II is increased (Hilgers *et al*., 1992; 1994), there may be up-regulation of the ET system (Gardiner *et al*., 1995; Cargnelli *et*

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al., 1998; but see also Whitworth *et al*., 1995), and endothelial NO production may be reduced (e.g., Pinto *et al*., 1997), normal (Gardiner *et al*., 1998), or enhanced (Moriguchi *et al*., 1994). However, the impact of these changes on vascular sensitivity to angiotensin I or angiotensin II has been little studied *in vivo*. In fact, the only publication we have found on this topic appeared while the present paper was under review, and reported a selective enhancement of the pressor responses to i.v. angiotensin II in pentobarbital-anaesthetized, heterozygous, female TG rats (Jacinto *et al*., 1999). The few *in vitro* studies that have been performed to assess angiotensin I and/or angiotensin II sensitivity have yielded conflicting results, inasmuch as some have shown an increase in contractile responsiveness to angiotensin II in aortic (Arribas *et al*., 1994) and to angiotensin I and angiotensin II in mesenteric vascular (Noll *et al*., 1997) preparations from TG rats, whereas others (Nickenig *et al*., 1997) have reported reduced aortic contractile responses to angiotensin II together with down-regulation of AT_1-receptor gene expression.

Therefore, a further aim of the present study was to compare the regional vascular effects of angiotensin II in conscious TG rats with those of the normotensive control strain (i.e., Hannover Sprague-Dawley (SD) rats). In addition, any possible modulatory influence of NO and ET in TG rats was examined using L-NAME and SB209670, respectively (as above).

Since responses to angiotensin I are due to its conversion to angiotensin II, any enhancement of this process *in vivo* ought to be manifest as an increased response to angiotensin I. Therefore, in some experiments we compared responses to angiotensin I and angiotensin II.

Methods

As described previously (Gardiner *et al*., 1995; 1998) all animals were bred in the Biomedical Services Unit from stock supplied by Dr J.J. Mullins (Genome Research Centre, University of Edinburgh). Male, heterozygous TG rats and the control, male normotensive inbred Hannover SD rats, were studied at 3–4 months of age.

All surgery was carried out under anaesthesia (sodium methohexitone, Brietal, Lilly; 40–60 mg kg^{-1} i.p., supplemented as required). At least 7 days before experiments, pulsed Doppler probes were implanted around the left renal and superior mesenteric arteries, and around the distal abdominal aorta (to monitor hindquarters flow). The day before experiments were to run, intravascular catheters were implanted, and on the day of experiments recordings were made of arterial blood pressures, heart rate and renal, mesenteric and hindquarters Doppler shift signals (Gardiner *et al*., 1995; 1998).

Effects of L-NAME on responses to bolus doses of angiotensin I and angiotensin II in SD and TG rats

TG rats ($n=10$) and SD rats ($n=9$) were given three i.v. bolus doses (0.1 ml) of angiotensin II (1.25, 12.5 and 125 pmol kg^{-1}) and three doses of angiotensin I (2.5, 25 and 250 pmol kg^{-1}). Animals were randomized to receive angiotensin I or angiotensin II first, but lower doses of the peptides were always given before higher doses. This protocol was organized such that challenges with angiotensin I and angiotensin II were given over a 15 s period and separated by at least 10 min to allow variables to return to baseline before the next challenge. Subsequently, an infusion of L-NAME (3 mg kg^{-1} h^{-1}) was begun and, 90–120 min after the onset of this infusion, angiotensin II and angiotensin I were administered, as before, except the highest doses of angiotensin II and angiotensin I were omitted (in pilot experiments these doses of the peptides in the presence of L-NAME caused cardiovascular deterioration).

From this experiment it was clear that L-NAME did not enhance the pressor effects of angiotensin I or angiotensin II (see Results). However, Conrad & Whittemore (1992) described enhancement of the pressor effects of angiotensin II in the presence of low doses ($\sim$0.5–1.5 mg kg^{-1} h^{-1}) but not a high dose ($\sim$10 mg kg^{-1} h^{-1}) of L-NAME. Therefore, in SD and TG rats ($n=2$ for each) we assessed pressor responses only to angiotensin I and angiotensin II in the absence and presence of L-NAME at a dose of 1 mg kg^{-1} h^{-1} (protocol as above).

Effects of SB209670 on responses to infusions of angiotensin II in SD and TG rats

TG rats ($n=8$) and SD rats ($n=7$) were given four i.v. infusions (0.15 ml min^{-1}) of angiotensin II (3, 9, 30 and 90 pmol kg^{-1} min^{-1}) for 3 min in ascending order, with at least 10 min between each dose, to allow variables to return to baseline before the next challenge. Subsequently, SB209670 was administered (600 μg kg^{-1} bolus; 600 μg kg^{-1} h^{-1} infusion; Gardiner *et al*., 1995) and the angiotensin II infusions were repeated, starting 1 and 6 h after the onset of SB209670 administration.

Data analysis

Continuous recordings of cardiovascular variables were made, but for simplification, only values measured at the peak of the mean blood pressure (BP) response to bolus administration (0.5 min) and at the steady-state during the infusions (3 min) are presented. As noted previously (Gardiner *et al*., 1995; 1998), the elevated mean arterial blood pressure in TG rats was accompanied by reductions in renal, mesenteric and hindquarters vascular conductances, and L-NAME and SB209670 influenced cardiovascular status (see Results). These differences in baseline conditions could, themselves, account for any differences in subsequent responses to vasoconstrictor agents, and the best way of describing such responses has been much debated (e.g., Stark, 1968; Rodbard, 1971; Folkow, 1982; Lautt, 1989; Korner *et al*., 1989; O'Leary, 1991; Fozard & Part, 1992; Fitzgerald *et al*., 1995). In our opinion, to date, O'Leary (1991) has produced the most persuasive arguments for the use of vascular conductance, rather than resistance, as the better measure of the importance of the response in pressure regulation, whilst acknowledging that further understanding would be achieved if cardiac output was also known. In that report (O'Leary, 1991), there is also a persuasive argument for expressing data as absolute changes, whereas others (e.g., Folkow, 1982) assert such variables should be considered in the form of per cent changes. For these reasons we chose to express our data in terms of both absolute and per cent changes in vascular conductance, derived from the Doppler shift signals and mean BP.

Within-group comparisons of variables or changes were made with Friedman's test (Theodorsson-Norheim, 1987), or the Wilcoxon test, as appropriate. Between-group comparisons were made using the Mann-Whitney *U*-test or Kruskal-Wallis test, as appropriate. A P value <0.05 was taken as significant.

In pilot experiments ($n=3$ for each strain in each experimental protocol) we confirmed that over the time course

of the experiments, responses to angiotensin II and angiotensin I were reproducible.

Materials

Angiotensin II and angiotensin I were obtained from Bachem (U.K.) and L-NAME was from Sigma (U.K.). Angiotensin II and angiotensin I were dissolved in sterile saline (154 mmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma). SB209670 ([(±)-(1**S**, 2**R**, 3**S**)-3-(2-carboxymethoxy-4-methoxyphenyl)- 1 -13,4 - methylenedioxyphenyl)-5-(prop-1-yl-oxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.).

Results

Cardiovascular responses to bolus doses of angiotensin II and angiotensin I in SD and TG rats

Table 1 shows the resting cardiovascular variables at the beginning of the experiment. As described previously (Gardiner *et al*., 1995; 1998) the hypertension in TG rats was associated with reduced renal, mesenteric and hindquarters vascular conductances.

In both strains of rat, angiotensin II and angiotensin I had dose-dependent pressor, and renal and mesenteric vasoconstrictor actions (Figures 1 and 2). In SD rats there was only vasodilatation in the hindquarters (significant at 12.5 pmol kg^{-1} angiotensin II and 25 pmol kg^{-1} angiotensin I) whereas in TG rats the lower doses of angiotensin II and angiotensin I tended to cause vasodilatation, whereas the highest doses caused clear-cut vasoconstriction (Figures 1 and 2). The lowest doses of angiotensin II and angiotensin I caused slight tachycardia, but the higher doses caused dose-dependent bradycardia.

The pressor responses to angiotensin II and angiotensin I in the two strains were generally similar; in both cases the absolute rises in mean BP in response to the middle doses were smaller in SD than in TG rats, whereas the percentage change in response to the highest doses were greater in the former. With the exception of the renal vascular response to the highest doses of angiotensin I and angiotensin II, the smaller absolute reductions in renal and mesenteric vascular conductances in TG rats were not seen when the data were expressed as per cent changes. The hindquarters vascular responses to angiotensin II and angiotensin I differed between the strains, both in absolute and in per cent terms (Figures 1 and 2).

Effects of L-NAME on responses to bolus doses of angiotensin II and angiotensin I in SD and TG rats

In both SD and TG rats, L-NAME (3 mg kg^{-1} h^{-1}) caused an increase in mean BP, bradycardia, and reductions in renal, mesenteric and hindquarters Doppler shifts and vascular conductances (Table 1). The absolute reduction in mesenteric vascular conductance was less in TG than in SD rats, but the other changes were not different in the two strains (Table 1).

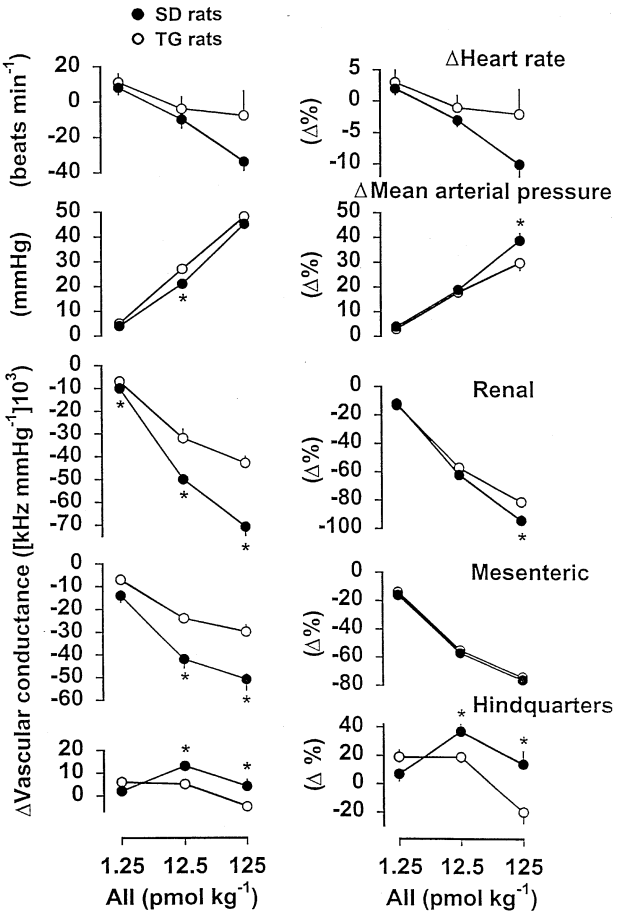

Figure 1 Cardiovascular responses in Sprague Dawley (SD) rats ($n=9$) and transgenic (TG) rats ($n=10$) to bolus i.v. doses of angiotensin II (AII). Values were measured 0.5 min after injection and represent the mean; vertical bars show s.e.mean. Data are expressed as absolute changes (left hand panel) or percentage changes (right hand panel). *$P<0.05$ for a difference between SD and TG rats (Kruskal-Wallis test).

Table 1 Cardiovascular variables in conscious rats

		Heart rate (beats min^{-1})	*Mean Pressure* (mmHg)	*Doppler shift* *Renal* (kHz)	*Mesenteric* (kHz)	*Hindquarters* (kHz)	*Vascular conductance* *Renal*	*Mesenteric*	*Hindquarters* ([kHz $mmHg^{-1}$]10^3)
Sprague-Dawley rats ($n=9$)	Control	351±6	112±2	9.0±0.4	9.0±0.4	4.8±0.4	80±3	80±8	40±4
	+L-NAME	281±7*	153±3*	6.2±0.6*	4.6±0.4*	2.5±0.3*	40±3*	30±3*	17±2*
Transgenic rats ($n=10$)	Control	343±7	156±5†	8.3±0.5	7.3±0.4	4.6±0.4	58±4†	47±3†	30±3†
	+L-NAME	295±8*	201±4*	4.5±0.3*	3.3±0.2*	2.2±0.3*	23±2*	17±1*	10±1*

Control values were recorded under baseline conditions, and L-NAME values were recorded 90–120 min after the onset of infusion of L-NAME (3 mg kg^{-1} h^{-1}). Values are mean±s.e.mean; *$P<0.05$ versus corresponding control value; †$P<0.05$ versus control value in Sprague-Dawley rats.

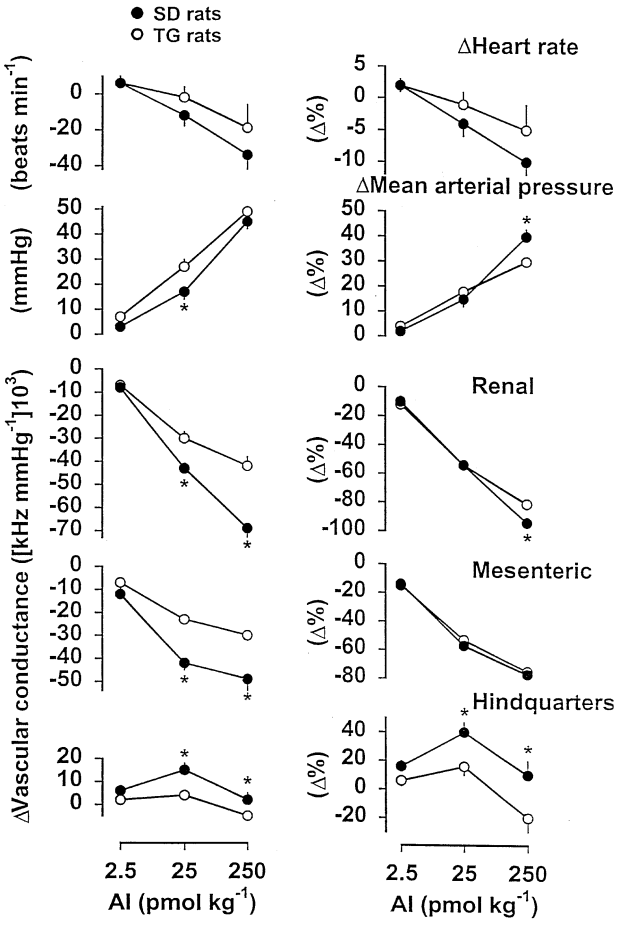

Figure 2 Cardiovascular responses in Sprague Dawley (SD) rats ($n=9$) and transgenic (TG) rats ($n=10$) to bolus i.v. doses of angiotensin I (AI). Values were measured 0.5 min after injection and represent the mean; vertical bars show s.e.mean. Data are expressed as absolute changes (left hand panel) or percentage changes (right hand panel). $^*P<0.05$ for a difference between SD and TG rats (Kruskal-Wallis test).

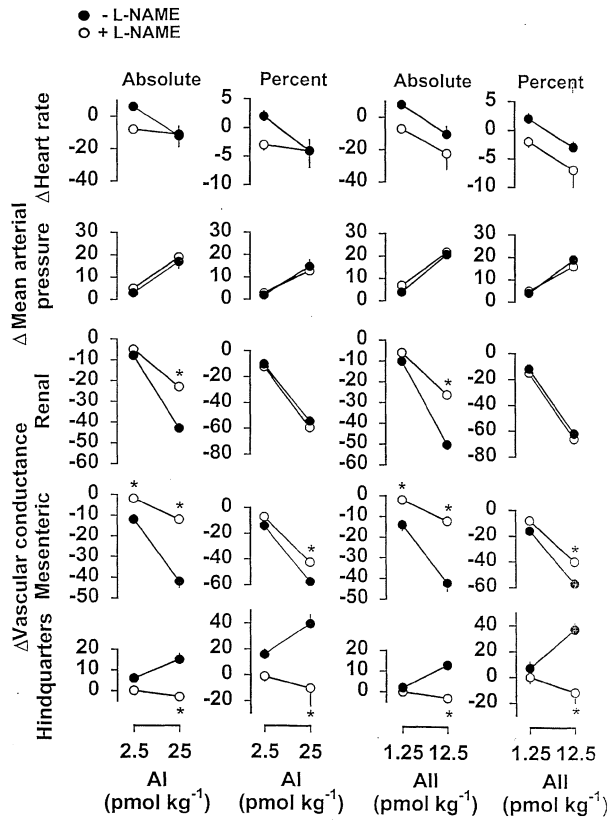

Figure 3 Cardiovascular responses in Sprague Dawley (SD) rats ($n=9$) to bolus doses of angiotensin I (AI) and angiotensin II (AII) in the absence and presence of L-NAME (3 mg kg^{-1} h^{-1}). Data are expressed as absolute (units as in Figure 1) and percentage changes. Values were measured 0.5 min after injection of angiotensin I or angiotensin II and represent the mean; vertical bars show s.e.mean. $^*P<0.05$ for a difference in the presence of L-NAME (Wilcoxon test).

In SD rats, L-NAME had no effect on the pressor response to angiotensin II or angiotensin I (Figure 3), whereas in TG rats, L-NAME reduced the pressor response to angiotensin I (25 pmol kg^{-1}) and angiotensin II (12.5 pmol kg^{-1}) in both absolute and per cent terms (Figure 4). In absolute terms, the renal and mesenteric vasoconstrictor responses to angiotensin I and angiotensin II in both strains were reduced by L-NAME but, with the exception of the mesenteric vascular responses to the higher doses, this difference was not seen when the data were expressed in percentage terms (Figures 3 and 4). Any hindquarters vasodilator effects of angiotensin I and angiotensin II were abolished by L-NAME (Figures 3 and 4).

L-NAME at a dose of 1 mg kg^{-1} h^{-1} increased mean BP by $\sim$5 mmHg and $\sim$9 mmHg in SD and TG rats, respectively. In the presence of L-NAME (1 mg kg^{-1} h^{-1}) the pressor effects of angiotensin I or angiotensin II were not consistently enhanced either in SD rats (e.g., angiotensin II (1.25 pmol kg^{-1}) before L-NAME = +7 mmHg, during L-NAME = +7 mmHg; angiotensin II (12.5 pmol kg^{-1}) before L-NAME = +15 mmHg, during L-NAME = +17 mmHg) or in TG rats (e.g., angiotensin II (1.25 pmol kg^{-1}) before L-NAME = +6 mmHg, during L-NAME = +5 mmHg; angiotensin II (12.5 pmol kg^{-1}) before L-NAME = +22 mmHg, during L-NAME = +14 mmHg).

Cardiovascular responses to infusions of angiotensin II in SD and TG rats

Table 2 shows the resting cardiovascular variables at the beginning of the experiment. The differences between SD and TG rats were as described above (compare Tables 1 and 2). In both strains of rat, the pressor and renal and mesenteric vasoconstrictor responses to angiotensin II infusions were dose-dependent, but there were no significant changes in the hindquarters vascular conductances (Figure 5). The pressor responses to angiotensin II infusions in the two strains were generally similar (Figure 5). In absolute, but not in percentage terms the falls in renal and mesenteric vascular conductances in TG rats were smaller than in SD rats (Figure 5); the exception to this was the renal vascular response to the highest dose of angiotensin II, which was smaller in TG rats, however expressed.

Effects of SB209670 on responses to infusions of angiotensin II in SD and TG rats

During infusion of SB209670 in SD rats there was a modest fall in mean BP, but no other significant changes (Table 2). In TG rats, SB209670 caused a marked fall in mean BP associated with vasodilatation in all three vascular beds (Table 2).

In SD rats, SB209670 had no significant effect on the pressor, or renal, or mesenteric vasoconstrictor effects of angiotensin II (Figure 6). In the presence of SB209670, at both

1 and 6 h, there was a hindquarters vasoconstrictor response to angiotensin II which was not seen in its absence (Figure 5).

In TG rats, SB209670 had no significant effect on the pressor effects of angiotensin II (Figure 7). There was some augmentation of the renal and mesenteric vasoconstrictor responses to angiotensin II, but only when the data were expressed in absolute terms (Figure 7). In the presence of SB209670 (at 1 h only), there was a hindquarters vasoconstrictor response to angiotensin II (30 pmol kg^{-1} min^{-1}) which was not seen in its absence (Figure 7).

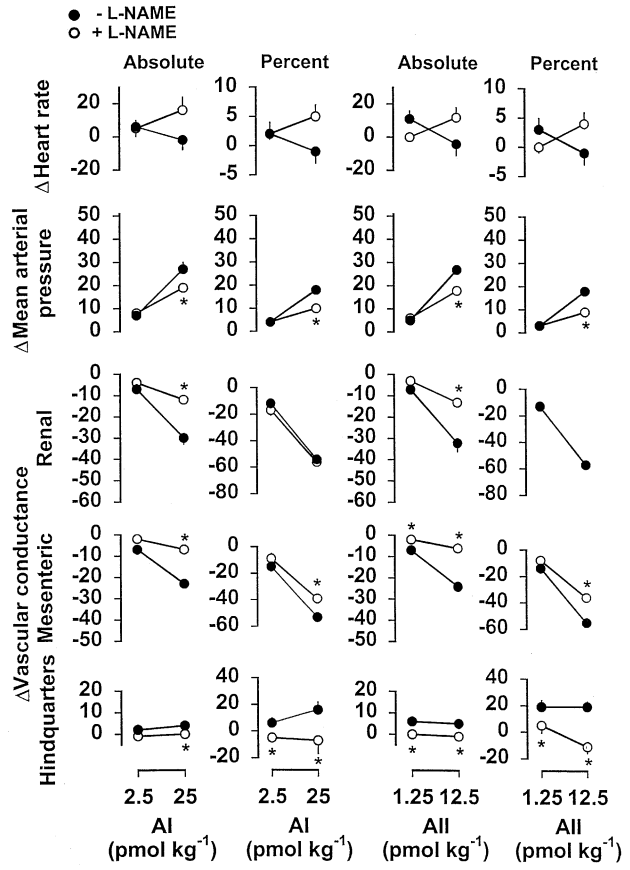

Figure 4 Cardiovascular responses in transgenic (TG) rats ($n=11$) to bolus doses of angiotensin I (AI) and angiotensin II (AII) in the absence and presence of L-NAME (3 mg kg^{-1} h^{-1}). Data are expressed as absolute (units as in Figure 1) and percentage changes. Values were measured 0.5 min after injection of angiotensin I or angiotensin II and represent the mean; vertical bars show s.e.mean. *$P<0.05$ for a difference in the presence of L-NAME (Wilcoxon test).

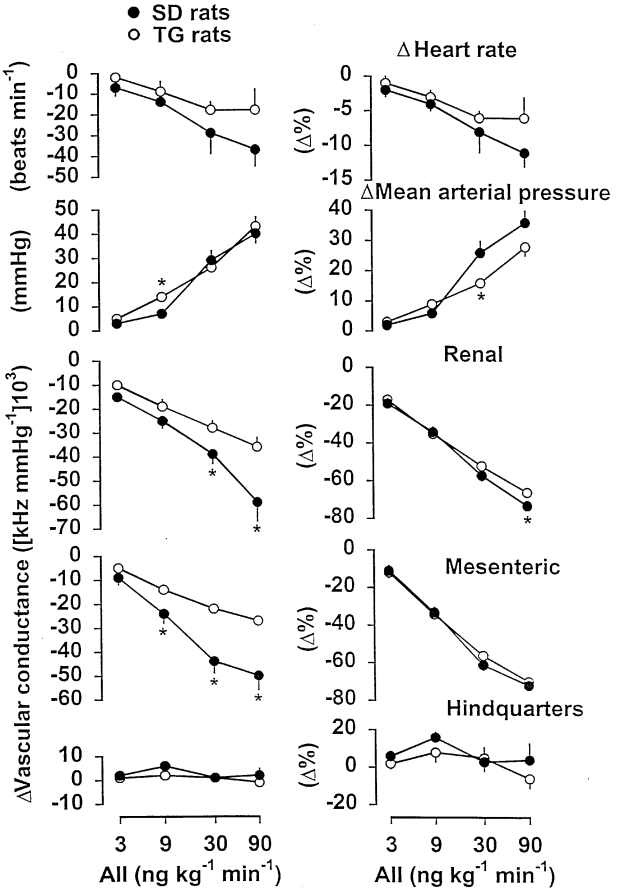

Figure 5 Cardiovascular responses in Sprague Dawley (SD) rats ($n=7$) and transgenic (TG) rats ($n=8$) to i.v. infusions of angiotensin II (AII). Values were measured in the steady state, 3 min after the start of the infusion and represent the mean; vertical bars show s.e.mean. Data are expressed as absolute changes (left hand panel) or percentage changes (right hand panel). *$P<0.05$ for a difference between SD and TG rats (Kruskal-Wallis test).

Table 2 Cardiovascular variables in conscious rats

		Heart rate (beats min^{-1})	*Mean Pressure* (mmHg)	*Doppler shift* *Renal* (kHz)	*Mesenteric* (kHz)	*Hindquarters* (kHz)	*Vascular conductance* *Renal*	*Mesenteric*	*Hindquarters* ([kHz $mmHg^{-1}$]10^3)
Sprague-Dawley rats ($n=7$)	Control	341±5	111±2	8.6±0.8	7.9±1.1	4.1±0.2	78±9	70±9	37±2
	SB209670 (1 h)	354±6	106±2*	8.4±0.9	8.3±1.1	4.5±0.2	80±10	78±10	43±2
	SB209670 (6 h)	337±6	101±3*	7.4±0.6	8.3±0.9	4.3±0.2	74±7	82±8	43±3
Transgenic rats ($n=9$)	Control	323±5	155±5†	8.7±0.8	6.1±0.3	3.8±0.2	57±7†	40±2†	25±2†
	SB209670 (1 h)	332±7	142±4*	8.5±0.6	5.6±0.3	4.2±0.3	61±6*	39±1	30±3*
	SB209670 (6 h)	338±7	124±3*	8.7±0.6	6.4±0.3	4.2±0.3	71±6*	52±2*	34±3*

Control values (baseline) and values recorded 1 h and 6 h after the onset of infusion of SB209670 (600 μg kg^{-1} bolus, 600 μg kg^{-1} h^{-1}) in the same animals. Values are mean±s.e.mean; *$P<0.05$ versus corresponding control value; †$P<0.05$ versus control value in Sprague-Dawley rats.

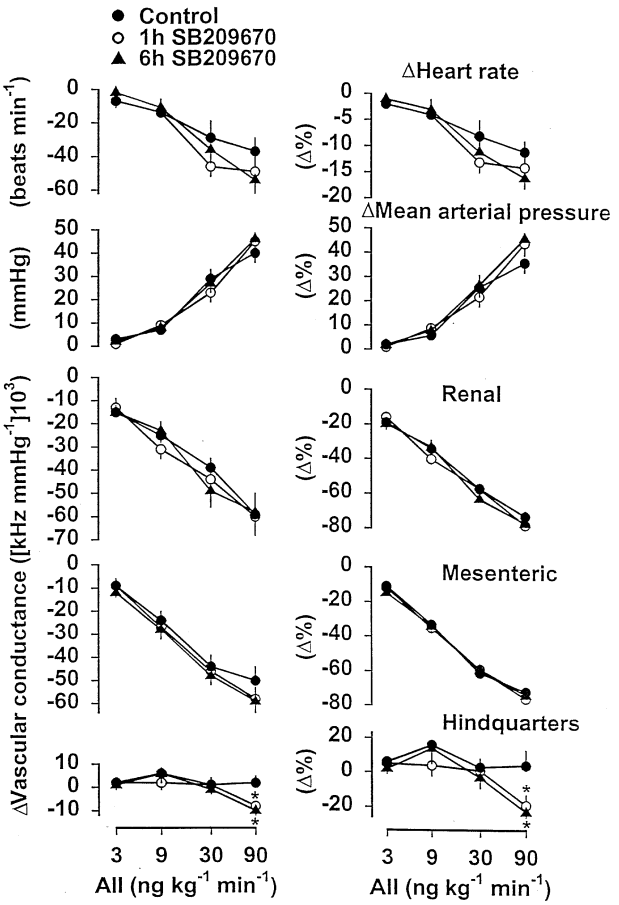

Figure 6 Cardiovascular responses in Sprague Dawley (SD) rats ($n=7$) to i.v. infusions of angiotensin II (AII) before, and 1 and 6 h after the onset of infusion of SB209670 (600 μg kg^{-1} h^{-1}). Values were measured in the steady-state, 3 min after the start of angiotensin II infusion, and represent the mean; vertical bars show s.e.mean. Data are expressed as absolute changes (left hand panel) and percentage changes (right hand panel). *$P<0.05$ for a difference between the absence and presence of SB209670 (Friedman's test).

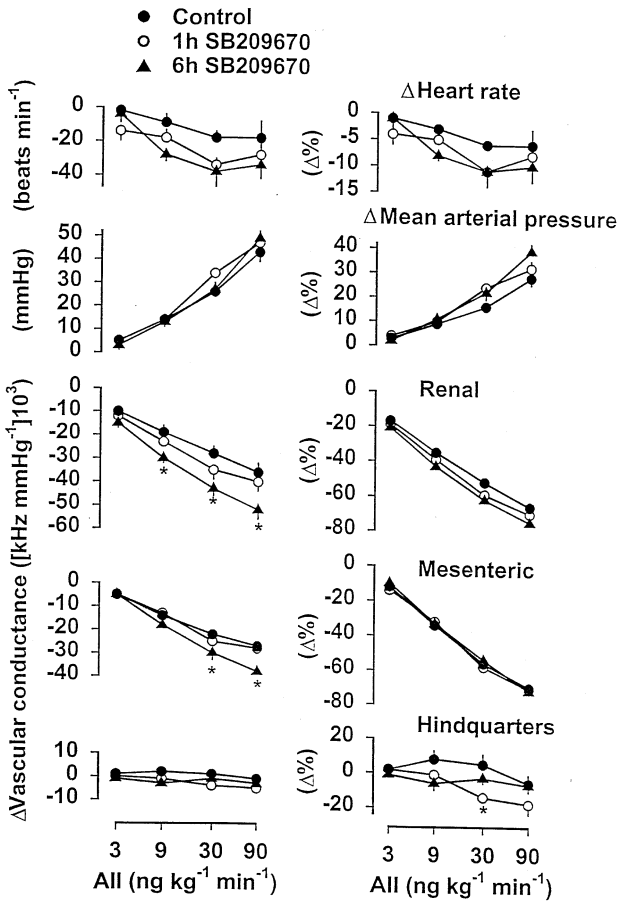

Figure 7 Cardiovascular responses in transgenic (TG) rats ($n=8$) to i.v. infusions of angiotensin II (AII) before, and 1 and 6 h after the onset of infusion of SB209670 (600 μg kg^{-1} h^{-1}). Values were measured in the steady-state, 3 min after the start of angiotensin II infusion, and represent the mean; vertical bars show s.e.mean. Data are expressed as absolute changes (left hand panel) and percentage changes (right hand panel). *$P<0.05$ for a difference between the absence and presence of SB209670 (Friedman's test).

Discussion

The aims of the present work were:- (1) to assess the contribution from NO and ET to the regional vascular effects of acute systemic angiotensin I and/or angiotensin II administration in conscious, normotensive rats; (2) to compare the cardiovascular responses to angiotensin I and to angiotensin II in normotensive (SD) rats with those in a model of hypertension induced by inserting the mouse ren-2 gene into the rat genome (TG rats); and (3) to evaluate any differential contribution from either NO or ET to the cardiovascular effects of angiotensin I and/or angiotensin II in TG compared to SD rats.

The following discussion will deal, firstly, with our findings in the normotensive (SD) rats and secondly, with the comparisons to TG rats.

Effects of NO synthase inhibition or ET receptor antagonism on the cardiovascular responses to angiotensin I and/or angiotensin II in SD rats

Although there is good evidence that NO synthase inhibition can potentiate the renal vascular effects of angiotensin II (see review Navar, 1996), previous experiments have generally been performed in isolated kidney preparations, or with intra-renal administration of angiotensin II in anaesthetized animals. Since the interaction between NO and angiotensin II is enhanced by anaesthesia (Sigmon & Beierwaltes, 1993), those findings are not strictly comparable with ours. The effects of acute NO synthase inhibition on the cardiovascular effects of acute systemic administration of angiotensin II in conscious rats were first reported by Conrad & Whittemore (1992) and Molnár & Hertelendy (1992). In both studies it was observed that the pressor response to angiotensin II was enhanced in the presence of NO synthase inhibition, contrary to our findings. Conrad & Whittemore (1992) used infusions of L-NAME of 2, 5 and 50 μg min^{-1} which raised BP by 6 ± 2, 15 ± 3 and 40 ± 3 mmHg, respectively. The highest dose of L-NAME did not enhance the pressor effects of angiotensin, whereas the two lower doses did. While it could be argued that the marked pressor effect of the highest dose of L-NAME masked any possible enhancement of the pressor action of angiotensin II, it is notable that Molnár & Hertelendy (1992) administered N^{G}-nitro-L-arginine at 10 mg kg^{-1} h^{-1}, which increased mean BP by about 40 mmHg, and yet they saw a potentiation of responses to angiotensin II under those conditions. Nonetheless, we could not dismiss the possibility that, in our hands, the pressor effect of angiotensin I and/or angiotensin II would be enhanced by a lower dose of L-NAME, as seen by Conrad & Whittemore (1992). Therefore, we examined pressor responses for angiotensin I and angiotensin II, in the absence

and presence of L-NAME at a dose of 1 mg kg^{-1} h^{-1} in SD and TG rats ($n=2$ in each group). Even under these conditions there were no signs of augmentation of responses to angiotensin I or angiotensin II.

Thus, it seems likely that something other than the change in mean BP in response to L-NAME must account for the differences between the findings. It is notable that Conrad & Whittemore (1992) studied female Long Evans rats, and Molnár & Hertelendy (1992) used female Wistar rats, while we investigated male Long Evans rats. This raises the possibility that gender and/or strain differences contributed to the disparate results. However, in a very recent study, Symons *et al*. (1999) claimed to show that, in male Wistar rats, angiotensin II caused greater pressor and vasoconstrictor effects in the presence of L-NAME than in its absence. While these findings may again indicate strain differences, close inspection of the data in the study of Symons *et al*. (1999) highlights a problem of interpretation. For example, the assertion that L-NAME enhanced the pressor effect of angiotensin II was based on the finding that mean BP in the presence of L-NAME and angiotensin II was higher than in the absence of L-NAME. Viewed in that way, our data would be consistent with theirs. However, consideration of the change in mean BP caused by angiotensin II in the different conditions shows that in the experiments of Symons *et al*. (1999) the pressor action of angiotensin II was reduced by L-NAME, consistent with our findings in TG rats.

In the present study, although NO synthase inhibition did not augment the pressor or vasoconstrictor actions of angiotensin II, there was a blunting of the hindquarters vasodilator effects of the latter. This finding is consistent with that of Heinemann *et al*. (1997). It was notable that the hindquarters vasodilator effects of angiotensin II (or angiotensin I) were not dose-dependent in our study, whereas Heinemann *et al*. (1997) showed dose-dependent femoral vasodilator responses to angiotensin II. It is possible that these different results were due to the fact that, in our system, we measured flow to the entire hindquarters (rather than one femoral vascular bed) and, perhaps, there were greater contributions from vasoconstriction in the skin at the highest dose of angiotensin I and angiotensin II, in our experiments.

In the experiments in which we assessed the effects of ET receptor antagonism, we used the dose-range of angiotensin II reported in the study of Balakrishnan *et al*. (1996), in which it was shown that bosentan caused a modest reduction in the pressor and systemic vasoconstrictor effects of the lower doses of angiotensin II in normotensive (WKY) rats. Our findings of a lack of effect of SB209670, therefore, are at variance with those of Balakrishnan *et al*. (1996). Whilst it could be argued that the choice of ET receptor antagonist was responsible for this difference, others have also failed to show any marked effect of acute ET receptor antagonism on response to acute angiotensin II administration (Heinemann *et al*., 1997; Muller *et al*., 1998), and in those cases bosentan was used. (Interestingly, in the study describing the characterization of SB209670 as an ET-receptor antagonist (Douglas *et al*., 1995), it was shown that, in SD rats, angiotensin II-mediated responses were unaffected by SB209670, although those data were not discussed in the current context). Thus, it is feasible that the inhibitory effects of bosentan on responses to angiotensin II described by Balakrishnan *et al*. (1996), which were greater in SHR than in WKY rats, are unique to those strains of rat. Since SB209670 antagonizes both ET_A- and ET_B-receptors, it could be suggested that our findings were due to concurrent inhibition of vasoconstrictor and vasodilator effects of ET released by angiotensin II. However, a similar effect would be expected with bosentan. The only influence of SB209670 on the haemodynamic responses to angiotensin II we observed was that it converted a lack of hindquarters vascular response into a vasoconstriction at the highest dose, but we have no ready explanation for this effect.

Comparisons between the cardiovascular effects of angiotensin I and angiotensin II in TG and SD rats

As alluded to in the data analysis section, the interpretation of our (and others') results is complicated by differences in resting haemodynamic status in normotensive, SD and hypertensive TG rats, and for that reason we chose to express the data both in absolute and in percentage terms. As far as the pressor responses to angiotensin II (and angiotensin I) were concerned, expressed either way, there were no consistent differences between the TG and SD rats. Furthermore, most of the apparent differences in vasoconstrictor effects of angiotensin II were presumably a reflection of the different starting values, since they were not seen when the data were expressed as percentages. The exception to this was the renal vasoconstrictor effect of the highest doses of angiotensin I and angiotensin II which were smaller in the TG rats, irrespective of the way in which the data were expressed. Collectively, therefore, our findings do not corroborate the *in vitro* data showing increased contractile responses to angiotensin II in vascular preparations from TG rats (Arribas *et al*., 1994; Noll *et al*., 1997). Furthermore, our observations are at odds with those of Jacinto *et al*. (1999) who reported enhanced pressor and renal vasocontrictor effects of angiotensin II in pentobarbital-anaesthetized, female, heterozygous TG rats. As discussed above, it is feasible gender difference contributed to the disparate findings in this study and that of Jacinto *et al*. (1999), although, as acknowledged by the latter authors, the presence of pentobarbital anaesthesia may have influenced their results.

We compared the responses to angiotensin I and angiotensin II to determine whether or not there was any functional evidence for increased conversion of angiotensin I to angiotensin II in TG rats. In man, systemically administered angiotensin I is partly converted to angiotensin II at a local (vascular) level (Krekels *et al*., 1998), and, in rats, there is incomplete conversion of intravenously administered angiotensin I to angiotensin II during first pass through the lungs (Heller & Mohrman, 1998). Thus, we reasoned that, if there was increased vascular conversion of angiotensin I to angiotensin II in TG rats (Hilgers *et al*., 1992; 1994), it should be apparent as a relative increase in vascular responsiveness to angiotensin I in TG compared to SD rats. However, we could find no evidence for this, since the pressor and regional vasoconstrictor effects of angiotensin I and angiotensin II, at the doses chosen, were very similar in the two strains.

Effects of NO synthase inhibition or ET receptor antagonism on the cardiovascular responses to angiotensin I and/or angiotensin II in TG rats

In a previous study (Gardiner *et al*., 1998) we found the absolute pressor effect of L-NAME (at the dose used here) was significantly greater in TG than in SD rats, although the per cent changes in blood pressure were not different. In the present study the pressor effects of L-NAME, expressed either way, were not different. However, the regional haemodynamic responses to L-NAME in the two studies were very similar; therefore, we suggest the present results corroborate our previous conclusion, namely, that there is no evidence for an

increased buffering capacity of NO in TG rats (*c.f.* Moriguchi *et al.*, 1994). Furthermore, as in SD rats, we saw no enhancement by L-NAME of the pressor or renal or mesenteric vasoconstrictor actions of angiotensin II (or angiotensin I) in TG rats, suggesting that NO was not acting as a buffer in those circumstances. The hindquarters vasodilator effects of angiotensin II (and angiotensin I) were inhibited by L-NAME, consistent with NO mediating that effect in TG rats, as in SD rats (see above).

The involvement of ET in baseline cardiovascular status in adult, heterozygous TG rats has been shown by us previously (Gardiner *et al.*, 1995), and the present results with SB209670 support those earlier findings. However, we were unable to demonstrate an involvement of endogenous ET in the responses to acute systemic administration of angiotensin II in TG rats. Any changes seen during the infusion of SB209670 were a reflection of the changing baseline status, and, thus, were not apparent when the data were expressed as percentages. Hence, the enhanced involvement of ET in responses to angiotensin II, as shown by Balakrishnan *et al.* (1996) in SHR may be unique to that model of hypertension.

In conclusion, our results can be summarized as showing little or no involvement of either NO or ET in the overall cardiovascular effects of angiotensin I or angiotensin II in SD rats, no major differences between the cardiovascular effects of angiotensin I or angiotensin II in TG compared to SD rats, and no greater or lesser contribution from either NO or ET to the cardiovascular effects of angiotensin I and/or angiotensin II in TG rats compared to SD rats.

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(Received June 29, 1999
Revised September 24, 1999
Accepted October 1, 1999)