British Journal of Pharmacology (1998) 124, 299–306
http://www.stockton-press.co.uk/bjp

The contribution of nitric oxide to cardiovascular status and responses to vasodilators in conscious, hypertensive, transgenic ((mRen-2)27) rats

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

School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 The aim of the study was to measure the regional haemodynamic responses to vasodilators, and the effects of nitric oxide (NO) synthase inhibition, in conscious, hypertensive, transgenic ((mRen-2)27) rats (TG rats) and normotensive, Hannover Sprague-Dawley (SD) rats.

2 The hypotensive response to acetylcholine was greater in TG than in SD rats, but the renal vasodilator responses were not different.

3 The responses to bradykinin were similar in the two strains, except that hindquarters vasodilatation occurred only in SD rats.

4 Salbutamol caused smaller renal and hindquarters vasodilatation in TG rats than in SD rats, and there was mesenteric vasodilatation only in the latter strain.

5 The hypotensive response to sodium nitroprusside was smaller, but the accompanying mesenteric vasodilatation was greater, in SD than in TG rats.

6 The contribution of NO to the vasodilator responses was taken as the difference between the responses in the presence of the NO synthase inhibitor, N^G-nitro-L-arginine methylester (L-NAME), compared to those in the presence of a co-infusion of angiotensin II and vasopressin (to match the haemodynamic effects of L-NAME).

7 In TG rats, L-NAME caused a greater absolute pressor effect, but a smaller mesenteric vasoconstriction, than in SD rats.

8 L-NAME affected the vasodilator responses to all the challenges similarly in the two strains.

9 Collectively, the results provide no direct evidence for impaired NO-mediated vasodilator mechanisms in TG rats. It is feasible that the reduced hindquarters response to bradykinin and the reduced renal and hindquarters responses to salbutamol, in TG rats are due to abnormal β_2-adrenoceptor-mediated processes.

Keywords: Transgenic rats; vasodilator responses; nitric oxide

Introduction

With the increasing availability of different experimental models of hypertension, it is becoming apparent that subtle variations exist, indicating that elevation of blood pressure, alone, cannot account for the observed differences in changes in vascular and cardiac function (e.g., Mulvany & Korsgaard, 1983; Mulvany, 1993; Arnal *et al*., 1993; Gardiner *et al*., 1994; Sawada *et al*., 1994; Koller & Huang, 1994; Dunn & Gardiner, 1995a,b; 1997; Schiffrin, 1995; 1996; Wu *et al*., 1996; Vanhoutte, 1996; Matrougui *et al*., 1997). Clearly, the more detailed information available on cardiovascular function in as wide a range of hypertensive models as possible, the more likely it is that an explanation of the aetiology of any cardiovascular dysfunction will be forthcoming.

There are numerous studies concerned with assessing vasodilator mechanisms in hypertensive man, and in animal models of hypertension, in particular with regard to endothelial function and the involvement of nitric oxide (NO) (see Shore & Tooke, 1994; Vanhoutte, 1996; Ferro & Webb, 1997, for reviews). However, whether or not any impairment in endothelial function exists is contentious; moreover, even when there is a concensus that endothelial function is abnormal, whether or not it is a cause or a consequence of the hypertensive state, is age-dependent, is regionally-selective, is agonist-specific, and/or is confined to basal versus agonist-stimulated NO release, are all unresolved issues (e.g., Koller & Huang, 1994; Huang & Koller, 1996; Wirth *et al*., 1996; Bennett *et al*., 1996; Gil-Longo *et al*., 1996; White *et al*., 1996; Rodrigo *et al*., 1997). Most of the animal studies on these topics have been performed in spontaneously hypertensive rats and, perhaps, one of the reasons for the discrepancies in results obtained is the lack of a proper control for these animals (see Louis & Howes, 1990).

The recent development (Mullins *et al*., 1990) of a transgenic rat, expressing the mouse Ren-2 gene (abbreviated to TG rat), has provided an interesting model of experimental hypertension, which has the advantage that any changes can, in theory, be attributed to the single genetic alteration (Lee *et al*., 1996), and, in addition, the correct control rats are available. Studies on vasodilator function in TG rats are limited, although there are reasons for expecting a change. For example, vascular smooth muscle structure is altered in a variety of ways (Peiro *et al*., 1992; Thybo *et al*., 1992; Struyker-Boudier *et al*., 1996; Dunn & Gardiner, 1997). Furthermore, there is histological evidence for changes in markers of endothelial injury and turnover (Strawn *et al*., 1997). *In vitro* studies have demonstrated reduced endothelium-dependent relaxation in aortic ring preparations from male (Pinto *et al*., 1997) and ovariectomized female (Li *et al*., 1997) TG rats, whereas in isolated coronary vessels, basal,

[1] Author for correspondence.

but not acetylcholine-stimulated, NO release is impaired (Tschudi *et al*., 1994).

To our knowledge there have been no systematic *in vivo* studies of regional vasodilator responses in conscious TG rats. Therefore, the first aim of this study was to measure the haemodynamic responses to a variety of vasodilator substances in conscious TG and control (Hannover Sprague-Dawley; SD) rats. The choice of vasodilators was based on our previous studies (Gardiner *et al*., 1990c; 1991b; 1994) in which we have demonstrated NO-dependent components to the responses obtained.

There is increasing evidence that the vasodilator effects of, for example, acetylcholine and bradykinin, may involve an endothelium-derived factor which is not a prostanoid, and not NO (see Félétou & Vanhoutte, 1996; Mombouli & Vanhoutte, 1997, for reviews). Thus 'normal' responses to such a vasodilator, would not necessarily indicate a 'normal' involvement of NO, but could be due to an increased contribution from an NO-independent component. So, our second aim was to evaluate the involvement of NO by assessing the influence of NO synthase (NOS) inhibition (by use of N^G nitro-L-arginine methylester (L-NAME)) on the responses to the vasodilator challenges in TG and SD rats. However, since NOS inhibitors cause substantial changes in haemodynamics in conscious rats (Gardiner *et al*., 1990a,b), the assessment of their influence on responses to vasodilators is complicated. In many studies, the pressor effects of NOS inhibitors have been controlled for by giving equipressor doses of, for example, phenylephrine or noradrenaline (e.g., Wang *et al*., 1993a,b; Zanchi *et al*., 1995) with the tacit assumption this matches the haemodynamic effects of the NOS inhibitor. But, in our experience, α-adrenoceptor agonists, such as phenylephrine, do not simulate the regional haemodynamic effects of L-NAME (unpublished data (from our laboratory)). However, in pilot experiments, we have found that co-infusion of angiotensin II (AII) and arginine vasopressin (AVP), at appropriate doses, can mimic, to a reasonable extent, the pressor and regional haemodynamic effects of L-NAME in SD and TG rats (see Results). Therefore, to fulfil our second objective, we compared responses to vasodilators in the two strains, in the presence of L-NAME, and in the presence of AII and AVP, under conditions in which haemodynamic variables were reasonably matched before administration of the vasodilators. Some of this work has been presented to the British Pharmacological Society (March *et al*., 1996; 1997).

Methods

Animals were bred (see Gardiner *et al*., 1995) in the Biomedical Services Unit in Nottingham from stock supplied by Dr J.J. Mullins at the Genome Research Centre, Edinburgh University. The TG rats were produced by crossing male, homozygous TG rats with female Hannover Sprague Dawley rats. The offspring were, therefore, 100% heterozygous F1s and all such TG animals were hypertensive. Male, heterozygous, TG rats (3–4 months old) and age-matched, male, inbred, SD rats were used in all experiments. The SD rats were originally obtained from the Zentralinstitut fur Versuchstierkunde (Hannover, Germany); this colony of animals was that used in the original production of the TG rats (Mullins *et al*., 1990).

Under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1}, i.p., supplemented as required), miniature, pulsed Doppler probes were implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor hindquarters flow changes). Seven to 14 days later, animals were again anaesthetized, and had venous and arterial catheters implanted (Gardiner *et al*., 1995); experiments were begun the following day. In both SD and TG rats, 3 different, randomized protocols were run in 3 different groups.

Vasodilator challenges before and after saline

SD ($n=13$) and TG ($n=11$) rats had baseline cardiovascular variables and responses to (randomized) 3 min infusions of acetylcholine (ACh; 10 μg $kg^{-1}min^{-1}$), bradykinin (BK; 38 μg $kg^{-1}min^{-1}$), salbutamol (Salb; 600 ng $kg^{-1}min^{-1}$) and sodium nitroprusside (SNP; 19 μg kg^{-1} min^{-1}) measured before, and starting 90 min and 270 min after the onset of saline infusion (0.3 ml h^{-1}) to control for the other treatments (see below). Vasodilator challenges were separated by intervals of at least 10 min. Since there were no differences between the effects of the vasodilator challenges on the 3 occasions, the data presented in the Results section are those obtained starting 270 min after the onset of saline infusion.

Vasodilator challenges before and after co-infusion of AII and AVP or L-NAME

In preliminary experiments we found that the absolute pressor effect of L-NAME (3 mg kg^{-1} h^{-1}) was greater in TG than in SD rats, whereas the pressor effects of a co-infusion of AII and AVP were similar in the two strains. Hence, to match the pressor effects of L-NAME required different co-infusions of AII and AVP in the two strains. Therefore, in both strains, in separate groups, we assessed responses to vasodilators before and after co-infusion of AII (2 μg kg^{-1} h^{-1} or 1 μg kg^{-1} h^{-1}) and AVP (0.2 μg kg^{-1} h^{-1} or 0.1 μg kg^{-1} h^{-1}) for 90 min (to match the time course of the saline experiment, above). The co-infusion of AII and AVP was continued whilst the vasodilators were being given but then stopped for 90 min, to allow variables to return to baseline levels. Thereafter, L-NAME was infused for 90 min (at 3 mg kg^{-1} h^{-1}) before responses to the vasodilator challenges were assessed again; the L-NAME infusion was continued during the vasodilator tests. The data presented in the Results section are those obtained during the AII plus AVP infusion which matched the effects of L-NAME, i.e., AII (2 μg kg^{-1} h^{-1}) plus AVP (0.2 μg kg^{-1} h^{-1}) in TG rats ($n=12$), and AII (1 μg kg^{-1} h^{-1}) plus AVP (0.1 μg kg^{-1} h^{-1}) in SD rats ($n=11$).

For technical reasons data were not available for all vasodilator responses in all animals. In SD rats, the results in the Figures were obtained from at least 10 animals; for TG rats the results were obtained from at least 9 animals.

Data analysis

Within-group analysis of data was by the Wilcoxon test, and between-group analysis was by the Mann–Whitney U test; $P<0.05$ was taken as significant.

Drugs

Acetylcholine chloride, salbutamol hemisulphate, sodium nitroprusside and L-NAME hydrochloride were obtained from Sigma (Poole, Dorset; U.K.); bradykinin, angiotensin II and arginine vasopressin were obtained from Bachem UK (Saffron Walden, U.K.). Peptides were dissolved in saline

containing 1% bovine serum albumin (Sigma; U.K.). Sodium methohexitone (Brietal) was obtained from Eli Lilly (Basingstoke; U.K.).

Results

Resting cardiovascular variables and responses to vasodilators in SD and TG rats in the presence of saline

As shown previously (Gardiner *et al*., 1995), TG rats showed hypertension accompanied by significant reductions in renal, mesenteric and hindquarters vascular conductances, but no bradycardia (Table 1).

Responses to ACh In SD rats, a 3 min infusion of ACh evoked a fall in mean arterial blood pressure (area over curve (AOC) 15±2 mmHg min) and tachycardia (area under curve (AUC) 238±18 beats), together with renal vasodilatation and mesenteric and hindquarters vasoconstriction (Figure 1). In TG rats the tachycardia (AUC 322±28 beats), hypotension (AOC 29±4 mmHg min) and hindquarters vasoconstriction were greater, and the mesenteric vasoconstriction smaller (Figure 1), than in SD rats.

Responses to BK In SD rats, a 3 min infusion of BK had no significant effect on mean blood pressure, but increased heart rate (AUC 282±31 beats) and mesenteric and hindquarters vascular conductances (Figure 1). These effects were not different from those in TG rats (AUC for heart rate, 322±31 beats), with the exception of the increase in hindquarters vascular conductance, which did not occur in the TG rats (Figure 1).

Responses to salbutamol In SD and TG rats, a 3 min infusion of Salb evoked similar hypotension (AOC 22±3 and 16±3 mmHg min, respectively), and tachycardia (AUC 145±17 and 170±17 beats, respectively) (Figure 1). In the SD rats, there were increases in renal, mesenteric and hindquarters vascular conductances (AUC 29±4; 18±4; 87±9 (kHz mmHg^{-1}) 10^3 min, respectively). However, in the TG rats, only the renal and hindquarters vascular beds showed significant increases in conductance (AUC 9±1; 36±5 (kHz mmHg^{-1}) 10^3, respectively), and these were smaller than the corresponding changes in the SD rats (Figure 1).

Responses to SNP A 3 min infusion of SNP evoked a smaller fall in mean blood pressure in SD rats (AOC 43±7 mmHg min) than in TG rats (AOC 66±7 mmHg min), in spite of TG rats showing a smaller

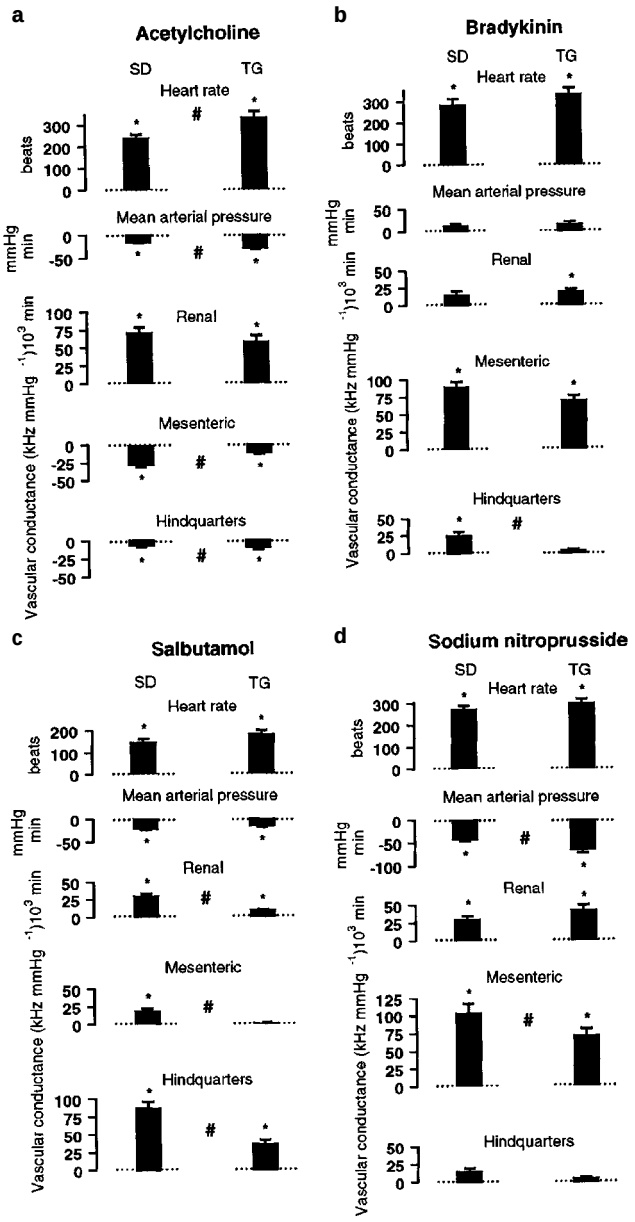

Figure 1 Integrated (areas under or over curves) cardiovascular responses during a 3 min infusion of: (a) acetylcholine (10 μg kg^{-1} min^{-1}), (b) bradykinin (38 μg kg^{-1} min^{-1}), (c) salbutamol (600 ng kg^{-1} min^{-1}), or (d) sodium nitroprusside (19 μg kg^{-1} min^{-1}) in conscious, normotensive Hannover Sprague-Dawley (SD) or hypertensive, transgenic ((mRen-2)27) (TG) rats, after administration of saline (0.3 ml h^{-1} for at least 270 min; see Methods). Values are means and vertical lines show s.e.means; $^*P<0.05$ for response (Wilcoxon test). $^{\#}P<0.05$ for difference between responses in SD and TG rats (Mann–Whitney U test).

Table 1 Resting cardiovascular variables and changes after a 90 min infusion of L-NAME (3 mg kg^{-1} h^{-1}).

	Resting values			*Changes after L-NAME*		
	SD rats ($n=13$)		*TG rats* ($n=11$)	*SD rats* ($n=11$)		*TG rats* ($n=12$)
Heart rate (beats min$^{-1)}$	355±11		331±11	−66±16	*	−20±10
Mean blood pressure (mmHg)	111±2	*	164±5	29±4	*	43±4
Renal vascular conductance ([kHz mmHg^{-1}]10^3)	75±6	*	38±3	−31±3		−23±2
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3)	68±5	*	36±4	−34±3	*	−19±3
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3)	44±2	*	29±3	−15±3		−13±1

The resting values represent the baselines after saline infusion for 270 min, i.e., immediately before vasodilator challenges (see Methods). Values are mean±s.e.mean. $^*P<0.05$ (Mann–Whitney U test).

increase in mesenteric vascular conductance (Figure 1). The accompanying increases in heart rate and renal, and hindquarters vascular conductances were not different in the two strains (Figure 1).

Responses to vasodilators in SD and TG rats in the presence of L-NAME or AII and AVP

L-NAME caused a greater absolute rise in mean blood pressure in TG rats (43 ± 4 mmHg) than in SD rats (29 ± 4 mmHg), although SD rats showed greater absolute reductions in mesenteric vascular conductance (Table 1). However, % changes in mean arterial blood pressure were the same (SD rats $+29\pm4\%$; TG rats $+27\pm3\%$). In addition, the L-NAME-induced rise in mean blood pressure was accompanied by a significant bradycardia in SD (-66 ± 16 beats min^{-1}) but not in TG (-20 ± 10 beats min^{-1}) rats (Table 1). As described in the Methods, the pressor effect of co-infusion of AII and AVP was the same in TG and SD rats. Thus, twice the dose of AII and AVP was required to match the haemodynamic effects of L-NAME in TG rats, compared to SD rats (Table 2). At the chosen doses, the increase in mean blood pressure in TG rats (40 ± 5 mmHg) was greater than in SD rats (26 ± 6 mmHg), whereas there was only a significant bradycardia (-38 ± 12 beats min^{-1}) in SD rats.

Before the administration of the vasodilator challenges, cardiovascular variables, for each strain of rat, were closely matched in the presence of AII and AVP, compared to L-NAME (Table 2).

Responses to ACh In SD and in TG rats, the renal vasodilator response to ACh (measured at 1, 2 and 3 min during infusion) was abolished in the presence of L-NAME, but not in the presence of AII and AVP (Figure 2), although the hypotensive effects of ACh were not affected differentially. Only in the TG rats did L-NAME cause a relative inhibition of the tachycardic effects of ACh (Figure 2).

Responses to BK In SD rats, in the presence of L-NAME, the tachycardic, and renal and mesenteric vasodilator responses to BK were significantly smaller than in the presence of AII and AVP, but the hypotension and the hindquarters vasodilatation were not different (Figure 3). In the TG rats, the hypotension and renal and mesenteric vasodilatator responses to BK were smaller in the presence of L-NAME than in the presence of AII and AVP (Figure 3).

Responses to salbutamol In SD and TG rats the haemodynamic responses to Salb in the presence of AII and AVP were similar to those seen in the presence of L-NAME, with the exception of the hindquarters vasodilatation, which was significantly smaller in the presence of L-NAME in both strains (Figure 4).

Responses to SNP In SD and in TG rats, the hypotensive and renal, mesenteric and hindquarters vasodilator effects of SNP were significantly greater in the presence of L-NAME, than in the presence of AII and AVP (Figure 5).

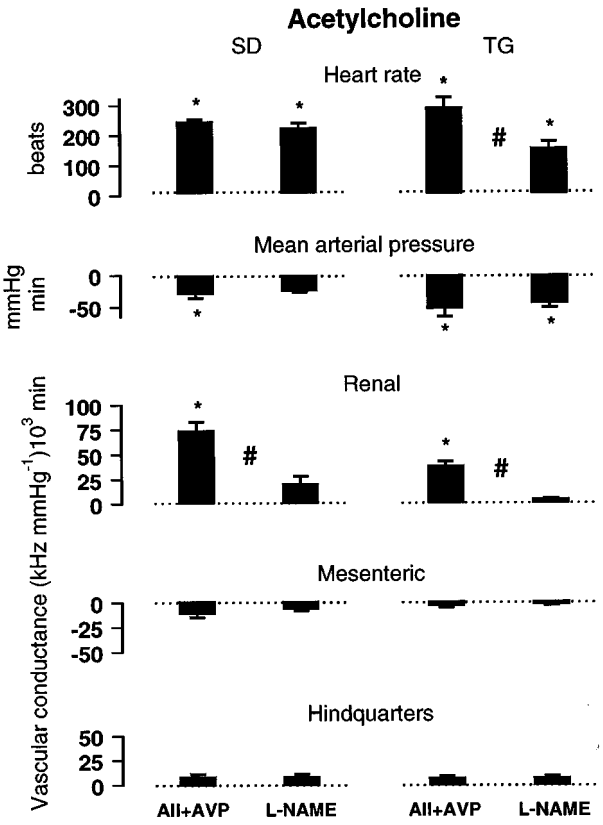

Figure 2 Integrated cardiovascular responses during a 3 min infusion of acetylcholine (10 $\mu g\ kg^{-1}\ min^{-1}$) in conscious, normotensive Hannover Sprague-Dawley (SD) or hypertensive, transgenic ((mRen-2)27) (TG) rats, after co-administration of AII and AVP or L-NAME (see Methods). Values are means and vertical lines s.e.means; $^{*}P<0.05$ for response (Wilcoxon test), $^{\#}P<0.05$ for difference between responses in SD and TG rats (Mann–Whitney U test).

Table 2 Cardiovascular variables prior to the administration of the vasodilator challenges, after a 90 min infusion of either AII (2 $\mu g\ kg^{-1}\ h^{-1}$ in TG rats; 1 $\mu g\ kg^{-1}\ h^{-1}$ in SD rats) and AVP (0.2 $\mu g\ kg^{-1}\ h^{-1}$ in TG rats; 0.1 $\mu g\ kg^{-1}\ h^{-1}$ in SD rats) or L-NAME (3 mg $kg^{-1}\ h^{-1}$ in both strains)

	SD rats		*TG rats*	
	AII+AVP ($n=11$)	*L-NAME* ($n=11$)	*AII+AVP* ($n=10$)	*L-NAME* ($n=12$)
Heart rate (beats min^{-1})	315 ± 12	279 ± 11	321 ± 6	306 ± 13
Mean blood pressure (mmHg)	136 ± 32	130 ± 4	206 ± 5	202 ± 5
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	49 ± 7	44 ± 6	22 ± 2	17 ± 2*
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	48 ± 5	38 ± 3	13 ± 1	13 ± 1
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	29 ± 2	22 ± 2*	15 ± 1	12 ± 2

For technical reasons, data from 2 of the TG rats receiving AII and AVP were not obtained. Values are mean$\pm$s.e. mean. $^{*}P<0.05$ (Mann–Whitney U test) compared to the same strain receiving AII plus AVP.

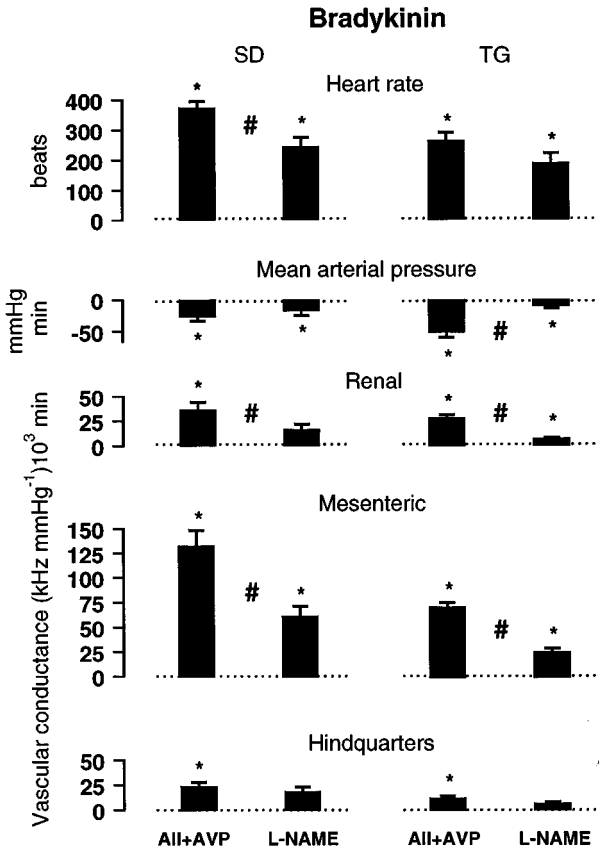

Figure 3 Integrated cardiovascular responses during a 3 min infusion of bradykinin in conscious, normotensive Hannover Sprague-Dawley (SD) or hypertensive, transgenic ((mRen-2)27) (TG) rats, after co-administration of AII and AVP or L-NAME (see Methods). Values are means and vertical lines show s.e.means; $^*P<0.05$ for response (Wilcoxon test), $^{\#}P<0.05$ for difference between responses in SD and TG rats (Mann–Whitney U test).

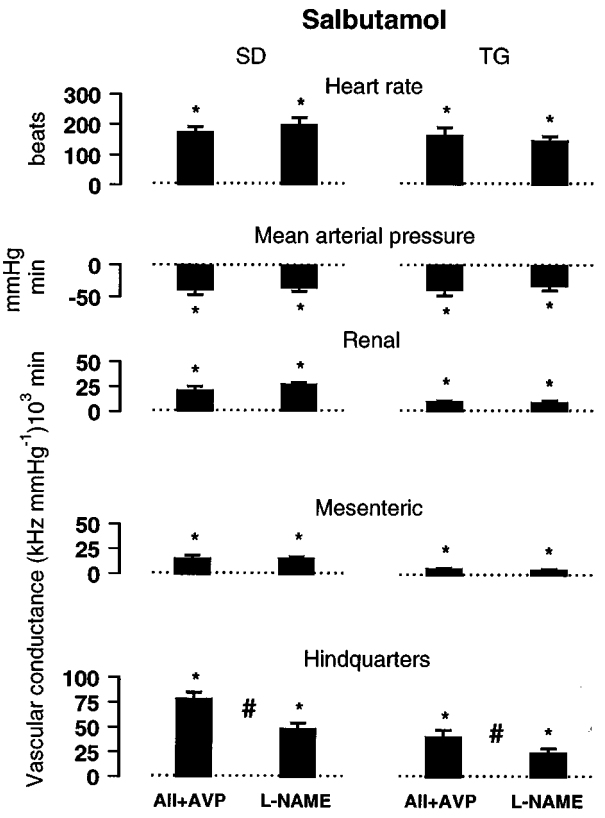

Figure 4 Integrated cardiovascular responses during a 3 min infusion of salbutamol in conscious, normotensive Hannover Sprague-Dawley (SD) or hypertensive, transgenic ((mRen-2)27) (TG) rats, after co-administration of AII and AVP or L-NAME (see Methods). Values are means and vertical lines show s.e.means; $^*P<0.05$ for response (Wilcoxon test), $^{\#}P<0.05$ for difference between responses in SD and TG rats (Mann–Whitney U test).

Discussion

The present results confirm our previous observations in hypertensive TG rats (Gardiner *et al*., 1995) which showed that the elevation in blood pressure was associated with peripheral vasoconstriction. In that study, as in the present one, the percentage reduction in conductance was similar in renal, mesenteric and hindquarters vascular beds. Using techniques different to those used by us, Flaim & Minteer (1980) showed that the vascular territories included in our measurements receive about 75% of the cardiac output at rest in rats (kidney, 19%, gastrointestinal system, 21%, skeletal muscle, 33%). It would be expected, therefore, that all 3 regions could importantly influence the control of arterial blood pressure with, possibly, skeletal muscle providing the greatest contribution.

From the present work it is clear that the hypertension in TG rats is increased by inhibition of NO production, and the absolute increase in mean arterial blood pressure is greater than in SD rats (see also Moriguchi *et al*., 1994). One interpretation of this observation is that NO plays a greater tonic vasodilator role in TG than in SD rats. However, in the TG rats, the greater rise in blood pressure caused by L-NAME was accompanied by a smaller reduction in mesenteric vascular conductance, and no significant bradycardia. Elsewhere we have shown that the pressor influence of L-NAME is due to regional vasoconstrictions, the effects of which are offset by a reduction in cardiac output (Gardiner *et al*., 1990b; Widdop *et al*., 1992). So, without knowing the concurrent changes in cardiac output, and what the absolute changes in regional conductance represent in terms of cardiac afterload, it is not possible to make detailed comparative statements about SD and TG rats, other than to say the effects of L-NAME provide no evidence for a diminished role of NO in buffering any further rise in MAP in the TG rats. Although our findings are consistent with those of Moriguchi *et al*. (1994), who described an enhanced pressor response to L-NMMA in female TG rats, for the reasons given above, we feel it is incautious to conclude, as they did, that 'vasoconstriction in this TG line is in part buffered by an enhanced action of EDRF'. As discussed by Fozard & Part (1991), any greater pressor or vasoconstrictor effects of NOS inhibitors in hypertensive, compared to normotensive, rats, may be a reflection of changes in cardiovascular structure, rather than an indication of differential involvement of NO in cardiovascular regulation. However, if cardiovascular remodelling was responsible for the difference between the absolute pressor effects of L-NAME in TG and SD rats, then the similar haemodynamic responses to the co-infusion of AII and AVP in the two strains is not easy to explain.

In response to the various vasodilator challenges, we could find no evidence for an up-regulation of agonist-stimulated NO release in TG rats. Thus, although their hypotensive response to ACh was greater than that in SD rats, the accompanying renal vasodilatation was not. In addition,

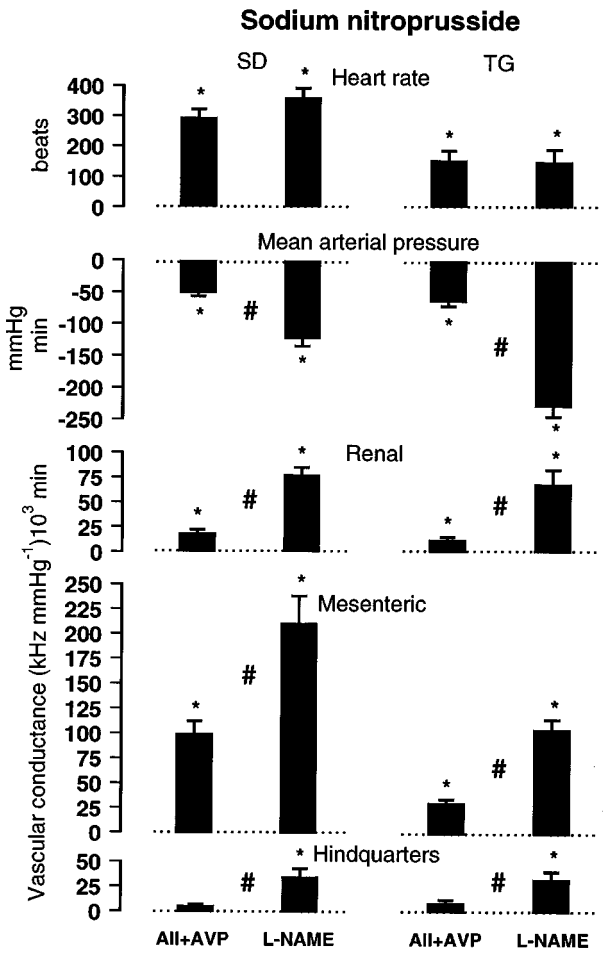

Figure 5 Integrated cardiovascular responses during a 3 min infusion of sodium nitroprusside in conscious, normotensive Hannover Sprague-Dawley (SD) or hypertensive, transgenic ((mRen-2)27) (TG) rats, after co-administration of AII and AVP or L-NAME (see Methods). Values are means and vertical lines show s.e.means; $^*P<0.05$ for response (Wilcoxon test), $^{\#}P<0.05$ for difference between responses in SD and TG rats (Mann–Whitney U test).

there were clear reductions in the hindquarters vasodilator effects of BK and of Salb in TG rats, possibly indicating impairment of β_2-adrenoceptor-mediated mechanisms (Gardiner *et al*., 1991b; 1992), and/or the involvement of NO in the responses to BK and Salb (Gardiner *et al*., 1991b; 1992, see below).

It was notable that under basal conditions, neither strain of rat showed an hypotensive response to BK. As discussed previously (Gardiner *et al*., 1992), the cardiovascular effects of BK are complex, with reflex, and/or direct sympathoadrenal activation contributing to the overall cardiovascular response. In another strain of normotensive rat (Long Evans) we have shown that the dose of BK used here caused qualitatively similar vasodilator (particularly hindquarters) changes, but the responses were larger and there was a reduction in the blood pressure (Waller *et al*., 1994). The difference between the strains perhaps reflects varying degrees of sympathoadrenal activation evoked by BK.

In order to delineate more clearly any putative differential involvement of NO in vasodilator responses in SD and TG rats, we compared the effects of ACh, BK, Salb and SNP in the two strains, when the effects of L-NAME on baseline cardiovascular status were matched by a co-infusion of AII and AVP. Under these conditions it appeared that NO was responsible, entirely, for the sustained renal vasodilator effects of ACh (measured at 1, 2 and 3 min during infusion) in SD and TG rats, with no evidence of a contribution from other mechanisms (see Introduction). While the apparent inhibitory effect of L-NAME on the tachycardic response to ACh in TG rats could be an indication of muscarinic antagonism (Buxton *et al*., 1993), we have seen no evidence of this in previous studies (Gardiner *et al*., 1991a; Widdop *et al*., 1992), and it was not observed in SD rats challenged with ACh in the present work. ACh caused mesenteric vasoconstriction in TG rats, but this effect was less than that in SD rats, in contrast to the *in vitro* findings of Noll *et al*. (1997). Moreover, L-NAME did not enhance mesenteric vasoconstrictor responses to ACh in either strain of rat, whereas Noll *et al*. (1997) observed a marked augmentation in TG rats, but not in SD rats.

Although the TG rats showed an enhanced depressor response to SNP under normal conditions, this was accompanied by a smaller mesenteric vasodilatation, possibly due to baroreflex activation of counter-regulatory mechanisms (Phillips *et al*., 1991). Since the hypotensive response to nitrovasodilators in conscious rats is associated with a reduction in stroke index (Gardiner *et al*., 1993b), it is feasible that a greater reduction in venous return, and hence cardiac output, contributed to the hypotensive response to SNP observed in the TG rats. The lesser mesenteric vasodilator effect of SNP in TG rats was still apparent in the presence of L-NAME, when there was clear sensitization to the depressor, and renal, mesenteric and hindquarters vasodilator effect of SNP in both strains of rat. If the increase in the haemodynamic effects of SNP in the presence of L-NAME can be attributed to upregulation of the sensitivity of guanylyl cyclase to exogenous NO, when endogenous NO production is suppressed (Moncada *et al*., 1991; Gardiner *et al*., 1993a), then our results provide no evidence of an impairment of this process in TG rats.

In some studies in which impaired endothelium-mediated vasodilator responses have been detected, normal responses to SNP have been taken as evidence that vascular smooth muscle function is intact (e.g., Johnstone *et al*., 1993; Cardillo *et al*., 1997). We would argue that an apparently normal response to SNP, together with impaired endothelium-mediated vasodilator responses, might indicate vascular smooth muscle dysfunction, although it could be that, for example, a combination of reduced endothelial receptor sensitivity to ACh, with normal basal production of NO and normal vascular smooth muscle function, would give rise to the situation of reduced responses to ACh, but normal responses to SNP. (We are grateful to one of the Referees for suggesting this scenario).

In both SD and TG rats, renal and mesenteric vasodilator responses to BK, and the hindquarters vasodilator response to salbutamol, were similarly reduced by L-NAME. These results are consistent with earlier findings in Long Evans rats (Gardiner *et al*., 1992), indicating that vasodilator responses to BK may involve adrenal medullary-mediated, β_2-adrenoceptor activation, and this effect, and that of salbutamol, may utilize NO (Gardiner *et al*., 1991b; 1992).

Collectively, our findings of a reduced hindquarters vasodilator response to BK, and lesser vasodilator responses to salbutamol, in TG compared to SD rats under normal conditions, are best explained by downregulation of β_2-adrenoceptors in the former strain, particularly as there is already evidence for downregulation of cardiac β_1-adrenoceptors in TG rats (Böhm *et al*., 1994). Since the apparent involvement of NO in β_2-adrenoceptor-mediated vasodilata-

tion may be due to synergism between adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (Delpy *et al*., 1996) and since the results with SNP (above) showed no evidence of impairment of cyclic GMP-mediated vasodilatation, then the dysfunction in TG rats may be due to abnormalities in β_2-adrenoceptors, and/or their coupling to cyclic AMP generation.

Without additional experiments it is not possible to comment on NO-independent vasodilator mechanisms in TG rats, compared to SD rats, except to say there were no signs of upregulation of such mechanisms in the former strain.

Abbreviations

ACh, acetylcholine; AII, angiotensin II; AVP, arginine vasopressin; BK, bradykinin; L-NAME, N^G-nitro-L-arginine methylester; NO, nitric oxide; Salb, salbutamol; SD, Sprague Dawley; SNP, sodium nitroprusside; TG rats, transgenic ((mRen-2)27) rats

References

ARNAL, J.F., AL AMRANI, A-I., CHATELLIER, G., MÉNARD, J. & MICHEL, J-B. (1993). Cardiac weight in hypertension induced by nitric oxide synthase blockade. *Hypertension,* **22,** 380–387.

BENNETT, M.A., HILLIER, C. & THURSTON, H. (1996). Endothelium-dependent relaxation in resistance arteries from spontaneously hypertensive rats: effect of long-term treatment with perindopril, quinapril, hydralazine or amlodipine. *J. Hypertens.,* **14,** 389–397.

BÖHM, M., MOLL, M., SCHMID, B., PAUL, M., GANTEN, D., CASTELLANO, M. & ERDMANN, E. (1994). β-Adrenergic neuroeffector mechanisms in cardiac hypertrophy of renin transgenic rats. *Hypertension,* **24,** 653–662.

BUXTON, I.L.O., CHEEK, D.J., ECKMAN, D., WESTFALL, D.P., SANDERS, K.M. & KEEF, K.D. (1993). N^G-nitro L-arginine methylester and other alkyl esters of arginine are muscarinic receptor antagonists. *Circ. Res.,* **72,** 387–395.

CARDILLO, C., KILCOYNE, C.M., QUYYUMI, A.A., CANNON, R.O. & PANZA, J.A. (1997). Decreased vasodilator response to isoproterenol during nitric oxide inhibition in humans. *Hypertension,* **30,** 918–921.

DELPY, E., COSTE, H. & LE MONNIER DE GOUVILLE, A-C. (1996). Effects of cyclic GMP elevation on isoprenaline-induced increase in cyclic AMP and relaxation in rat aortic smooth muscle: role of phosphodiesterase 3. *Br. J. Pharmacol.,* **119,** 471–478.

DUNN, W.R. & GARDINER, S.M. (1995a). Structural and functional properties of isolated, pressurized, mesenteric resistance arteries from a vasopressin-deficient rat model of genetic hypertension. *Hypertension,* **26,** 390–396.

DUNN, W.R. & GARDINER, S.M. (1995b). Vascular structure in hypertension induced by N^G-nitro-L-arginine methyl ester: methodological considerations for studies of small arteries in hypertension – reply. *J. Hypertens.,* **13,** 1217–1221.

DUNN, W.R. & GARDINER, S.M. (1997). Differential alteration in vascular structure of resistance arteries isolated from the cerebral and mesenteric vascular beds of transgenic [(mRen-2)27], hypertensive rats. *Hypertension,* **29,** 1140–1147.

FÉLÉTOU, M. & VANHOUTTE, P.M. (1996). Endothelium-derived hyperpolarizing factor. *Clin. Exp. Pharmacol. Physiol.,* **23,** 1082–1090.

FERRO, C.J. & WEBB, D.J. (1997). Endothelial dysfunction and hypertension. *Drugs,* **53,** (Suppl. 1), 30–41.

FLAIM, S.F. & MINTEER, W.J. (1980). Ventricular volume overload alters cardiac output distribution in rats during exercise. *J. Appl. Physiol.,* **49,** 482–490.

FOZARD, J.R. & PART, M-L. (1991). Haemodynamic responses to N^G-monomethyl-L-arginine in spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br. J. Pharmacol.,* **102,** 823–826.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990a). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension,* **15,** 486–492.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990b). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.,* **101,** 625–631.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990c). Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.,* **101,** 632–639.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1991a). Hemodynamic effects of N^G-monomethyl-L-arginine and N^G-nitro-L-arginine methyl ester in conscious, unrestrained rats. *J. Cardiovasc. Pharmacol.,* **17** (Suppl. 3), S173–S181.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991b). Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5′-N-ethylcarboxamidoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.,* **103,** 1725–1732.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1993a). Effects of chronic treatment with nitric oxide synthase inhibitors on regional haemodynamic responses to vasodilators in conscious Brattleboro rats. *Br. J. Pharmacol.,* **109,** 222–228.

GARDINER, S.M., KEMP, P.A., BENNETT, T., BOSE, C., FOULKES, R. & HUGHES, B. (1992). Involvement of β_2-adrenoceptors in the regional haemodynamic responses to bradykinin in conscious rats. *Br. J. Pharmacol.,* **105,** 839–848.

GARDINER, S.M., KEMP, P.A., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1993b). Regional and cardiac haemodynamic effects of N^G, N^G,dimethyl-L-arginine and their reversibility by vasodilators in conscious rats. *Br. J. Pharmacol.,* **110,** 1457–1464.

GARDINER, S.M., MARCH, J.E., KEMP, P.A. & BENNETT, T. (1994). Resting cardiovascular status and vasodilator function in a vasopressin-deficient, hypertensive strain of rat. *J. Hypertens.,* **12,** 1217–1224.

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

GIL-LONGO, J., FDEZ-GRANDAL, D., ALVAREZ, M., SIEIRA, M. & ORALLO, F. (1996). Study of *in vivo* and *in vitro* resting vasodilator nitric oxide tone in normotensive and genetically hypertensive rats. *Eur. J. Pharmacol.,* **310,** 175–183.

HUANG, A. & KOLLER, A. (1996). Both nitric oxide and prostaglandin-mediated responses are impaired in skeletal muscle arterioles of hypertensive rats. *J. Hypertens.,* **14,** 887–895.

JOHNSTONE, M.T., CREAGER, S.J., SCALES, K.M., CUSCO, J.A., LEE, B.K. & CREAGER, M.A. (1993). Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation,* **88,** 2510–2516.

KOLLER, A. & HUANG, A. (1994). Impaired nitric oxide – mediated flow-induced dilation in arterioles of spontaneously hypertensive rats. *Circ. Res.,* **74,** 416–421.

LEE, M.A., BÖHM, 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.

LI, P., FERRARIO, C.M., GANTEN, D. & BROSNIHAN, K.B. (1997). Chronic estrogen treatment in female transgenic (mRen2)27 hypertensive rats augments endothelium-derived nitric oxide release. *Am. J. Hypertens.,* **10,** 662–670.

LOUIS, W.J. & HOWES, L.G. (1990). Genealogy of the spontaneously hypertensive rat and Wistar-Kyoto rat strains: implications for studies of inherited hypertension. *J. Cardiovasc. Pharmacol.,* **16** (Suppl. 7), S1–S5.

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.

MARCH, J.E., GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1997). Cardiovascular responses to N^G-nitro-L-arginine methyl ester (L-NAME) in conscious, hypertensive transgenic [(mRen-2)27] rats. *Br. J. Pharmacol.,* **122,** 116P.

MATROUGUI, K., MACLOUF, J., LEVY, B.I. & HENRION, D. (1997). Impaired nitric oxide- and prostaglandin-mediated responses to flow in resistance arteries of hypertensive rats. *Hypertension,* **30,** 942 – 947.

MOMBOULI, J-V. & VANHOUTTE, P.M. (1997). Endothelium-derived hyperpolarizing factor(s): updating the unknown. *Trends Pharmacol. Sci.,* **18,** 252 – 256.

MONCADA, S., REES, D.D. SCHULZ, R. & PALMER, R.M.J. (1991). Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 2166 – 2170.

MORIGUCHI, A., BROSNIHAN, K.B., KUMACAI, H., GANTEN, D. & FERRARIO, C.M. (1994). Mechanisms of hypertension in transgenic rats expressing the mouse *Ren-2* gene. *Am. J. Physiol.,* **266,** R1273 – R1279.

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

MULVANY, M.J. (1993). Resistance vessel structure and the pathogenesis of hypertension. *J. Hypertens.,* **11** (Suppl. 5), S7 – S12.

MULVANY, M.J. & KORSGAARD, N. (1983). Correlations and otherwise between blood pressure, cardiac mass and resistance vessel characteristics in hypertensive, normotensive and hypertensive/normotensive hybrid rats. *J. Hypertens.,* **1,** 235 – 244.

NOLL, G., LANG, M.G., TSCHUDI, M.R., GANTEN, D. & LÜSCHER, T.F. (1997). Endothelial vasoconstrictor prostanoids modulate contractions to acetylcholine and ANG II in Ren-2 rats. *Am. J. Physiol.,* **272,** H493 – H500.

PEIRO, C., ROSA DE SAGARRA, M., REDONDO, J., SANCHEZ-FERRER, C.F. & MARÍN, J. (1992). Vascular smooth muscle proliferation in hypertensive transgenic rats. *J. Cardiovasc. Pharmacol.,* **20** (Suppl. 12), S128 – S131.

PHILLIPS, K., GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991). Factors affecting the regional haemodynamic responses to glyceryl trinitrate and molsidomine in conscious rats. *Br. J. Pharmacol.,* **104,** 151 – 158.

PINTO, Y.M., BUIKEMA, H., VAN GILST, W.H., SCHOLTENS, E., VAN GEEL, P-P., DE GRAEFF, P.A., WAGNER, J. & PAUL, M. (1997). Cardiovascular end-organ damage in Ren-2 transgenic rats compared to spontaneously hypertensive rats. *J. Mol. Med.,* **75,** 371 – 377.

RODRIGO, E., MAESO, R., MUÑOZ-GARCÍA, R., NAVARRO-CID, J., RUILOPE, L.M., CACHOFEIRO, V. & LAHERA, V. (1997). Endothelial dysfunction in spontaneously hypertensive rats: consequences of chronic treatment with losartan or captopril. *J. Hypertens.,* **15,** 613 – 618.

SAWADA, Y., SAKAMAKI, T., NAKAMURA, T., SATO, K., ONO, Z. & MURATA, K. (1994). Release of nitric oxide in response to acetylcholine is unaltered in spontaneously hypertensive rats. *J. Hypertens.,* **12,** 745 – 750.

SCHIFFRIN, E.L. (1995). Vascular structure in N^G-nitro-L-arginine methyl ester-induced hypertension: methodological considerations for studies of small arteries in hypertension. *J. Hypertens.,* **13,** 817 – 821.

SCHIFFRIN, E.L. (1996). The endothelium of resistance arteries: physiology and role in hypertension. *Prost. Leuk. Essent. Fatty Acids,* **54,** 17 – 25.

SHORE, A.C. & TOOKE, J.E. (1994). Microvascular function in human essential hypertension. *J. Hypertens.,* **12,** 717 – 728.

STRAWN, W.B., GALLAGHER, P., DEAN, R.H., GANTEN, D. & FERRARIO, C.M. (1997). Endothelial injury in transgenic (mRen-2)27 hypertensive rats. *Am. J. Hypertens.,* **10,** 51 – 57.

STRUIJKER-BOUDIER, H.A.J., VAN ESSEN, H., FAZZI, G., DE MEY, J.G.R., QIU, H.Y. & LÉVY, B.I. (1996). Disproportional arterial hypertrophy in hypertensive mRen-2 transgenic rats. *Hypertension,* **28,** 779 – 784.

THYBO, N.K., KORSGAARD, N. & MULVANY, M.J. (1992). Morphology and function of mesenteric resistance arteries in transgenic rats with low-renin hypertension. *J. Hypertens,* **10,** 1191 – 1196.

TSCHUDI, M.R., NOLL. G., ARNET, U., NOVOSEL, D., GANTEN, D. & LÜSCHER, T.F. (1994). Alterations in coronary artery vascular reactivity of hypertensive Ren-2 transgenic rats. *Circulation,* **89,** 2780 – 2786.

VANHOUTTE, P.M. (1996). Endothelial dysfunction in hypertension. *J. Hypertens.,* **14,** (Suppl. 5), S83 – S93.

WALLER, J., GARDINER, S.M. & BENNETT, T. (1994). Regional haemodynamic responses to acetylcholine, methoxamine, salbutamol and bradykinin during lipopolysaccharide infusion in conscious rats. *Br. J. Pharmacol.,* **112,** 1057 – 1064.

WANG, Y-X., POON, C.I. & PANG, C.C.Y. (1993). Vascular pharmacodynamics of N^G-nitro-L-arginine methyl ester *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.,* **267,** 1091 – 1099.

WANG, Y-X., POON, K.S., RANDALL, D.J. & PANG, C.C.Y. (1993). Endothelium-derived nitric oxide partially mediates salbutamol-induced vasodilatations. *Eur. J. Pharmacol.,* **250,** 335 – 340.

WHITE, R.M., RIVERA, C.O. & DAVISON, C.B. (1996). Differential contribution of endothelial function to vascular reactivity in conduit and resistance arteries from deoxycorticosterone-salt hypertensive rats. *Hypertension,* **27,** 1245 – 1253.

WIDDOP, R.E., GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1992). The influence of atropine and atenolol on the cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.,* **105,** 653 – 656.

WIRTH, K.J., LINZ, W., WIEMER, G. & SCHOLKENS, B.A. (1996). Differences in acetylcholine and bradykinin induced vasorelaxation of the mesenteric vascular bed in spontaneously hypertensive rats of different ages. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **354,** 38 – 43.

WU, X., MÄKYNEN, M., ARVOLA, P. & PÖRSTI, I. (1996). Mesenteric arterial function *in vitro* in three models of experimental hypertension. *J. Hypertens.,* **14,** 365 – 372.

ZANCHI, A., AUBERT, J.F., BRUNNER, H.R. & WAEBER, B. (1995). Vascular acetylcholine response during chronic NO synthase inhibition: *in vivo* versus *in vitro*. *Cardiovasc Res.,* **30,** 122 – 129.

(Received December 2, 1997
Revised February 12, 1998
Accepted February 18, 1998)