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Involvement of nitric oxide, but not prostaglandins, in the vascular sympathoinhibitory effects of losartan in the pithed spontaneously hypertensive rat

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1 The aim of this study was to investigate whether nitric oxide (NO) and/or vasodilator prostaglandins (PGs) are involved in the sympathoinhibitory effects exerted by losartan versus the vascular responses elicited by spinal cord electrical stimulation (SCS) in pithed spontaneously hypertensive rats (SHRs).

2 SHRs were given orally and for 8 days either losartan (10 mg kg^{-1} daily) or distilled water (controls). After pithing, blood pressure, heart rate, cardiac output, renal and muscular blood flows (pulsed Doppler technique) and the corresponding vascular resistance values were measured or calculated at baseline. Then, animals from both groups were given i.v. either saline, or N^G-nitro-L-arginine methyl ester (L-NAME, 1 mg kg^{-1}), or diclofenac (4 mg kg^{-1}). Thereafter, haemodynamic parameters were determined in the six subgroups of animals in response (a) to SCS at increasing frequencies, and (b) to a noradrenaline bolus injection.

3 Losartan significantly decreased mean arterial pressure as well as renal and total peripheral resistances. In addition, losartan exhibited strong vascular sympathoinhibitory effects, significantly decreasing the systemic pressor and regional vasoconstrictor responses to SCS, but did not affect those to exogenous noradrenaline. In contrast, SCS-induced tachycardia was not modified by losartan.

4 L-NAME significantly increased total peripheral and regional vascular resistances but did not affect blood pressure and heart rate basal values. L-NAME potentiated the haemodynamic responses to SCS in control and, to a larger extent, in losartan-treated SHRs so that, with the exception of the renal vascular bed, the sympathoinhibitory effects of losartan were attenuated in all vascular beds studied. L-Arginine (300 mg kg^{-1}) caused reversal of L-NAME effects in both control and losartan-treated SHRs.

5 Diclofenac did not affect the basal values of haemodynamic parameters in control and losartan-treated SHRs. Diclofenac potentiated the pressor and vasoconstrictor responses to SCS and to a similar extent, in both control and losartan-treated SHRs, so that the sympathoinhibitory effects of losartan were fully maintained.

6 These results demonstrate that in pithed SHRs: (a) NO but not PGs contribute to the basal vasomotor tone, (b) both NO and PGs attenuate the pressor and vasoconstrictor responses to SCS, (c) NO plays a major role in the vascular sympathoinhibitory effects of losartan, except at the renal level, and (d) endogenous PGs are not involved in these sympathoinhibitory effects.

Keywords: Losartan; sympathetic nervous system; pithed SHR; prostaglandins; nitric oxide; N^G-nitro-L-arginine methyl ester; renal blood flow; muscular blood flow

Introduction

Interactions between the renin-angiotensin and the sympathetic nervous systems have been extensively investigated over the years and the concept that angiotensin II (AII) facilitates sympathetic neuronal function has been firmly established. Interruption of the renin-angiotensin system through angiotensin I converting enzyme (ACE) inhibition has been shown to result in vascular sympathoinhibitory effects, characterized in the pithed rat by a reduction of the pressor and regional vasoconstrictor responses elicited by electrical stimulation of the spinal cord (Antonaccio & Kerwin, 1981; Hatton & Clough, 1982; Richer *et al.*, 1984; 1986). More recently, the same finding has been extended to AII AT_1-receptor antagonists (Wong *et al.*, 1991; Moreau *et al.*, 1993). As AII is known to potentiate the sympathetic nervous system both centrally and in the periphery (Starke, 1971; Grant & McGrath, 1988) and to increase plasma noradrenaline levels through stimulation of prejunctional AT_1-receptors (Hilgers *et al.*, 1993; Brower & Janicki, 1994), inhibition of endogenous AII synthesis and blockade of AII AT_1-receptors have been proposed as the mechanisms underlying the sympathoinhibitory effects of ACE inhibitors and AT_1-receptor antagonists, respectively.

However, in the pithed rat, ACE inhibitors and AT_1-receptor antagonists do not affect the tachycardia developed in response to electrical stimulation of the spinal cord (Antonaccio & Kerwin, 1981; Richer *et al.*, 1984; 1986; Moreau *et al.*, 1993). This observation suggests that AII would surprisingly act as an amplifier of the sympathetic system only at the vascular but not at the cardiac level. Alternatively, mechanisms other than suppression of AII effects could be responsible for or contribute to the sympathoinhibitory effects of ACE inhibitors and AT_1-receptor antagonists at the vascular level.

Recently, nitric oxide (NO) has been reported to be involved in the vasodilator and antihypertensive effects of losartan in different experimental models (Cachofeiro *et al.*, 1992; Kumagai *et al.*, 1993; Pollock *et al.*, 1993; Sudhir *et al.*, 1993) and to modulate sympathetic neurotransmission (Zanzinger *et al.*, 1994). Moreover, in porcine cultured endothelial vascular smooth muscle cells (Jaiswal *et al.*, 1991) and in rabbit vas deferens tissue (Catalioto *et al.*, 1994), losartan has been reported to increase vasodilator prostaglandin (PGs) synthesis and/or release.

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We therefore decided to investigate whether NO and PGs might be involved in the vascular sympathoinhibitory effects displayed by losartan (Moreau *et al.*, 1993) in the pithed rat. For this purpose, the pressor and vasoconstrictor, as well as the cardiac responses to electrical stimulation of the spinal cord (SCS), were compared in control and in losartan-treated animals (a) under basal conditions, (b) after prior inhibition of NO synthesis and (c) after prior administration of a cyclo-oxygenase inhibitor.

Methods

Animals and treatments

All studies were carried out in male spontaneously hypertensive rats of the Okamoto strain (SHR) (Iffa Credo, L'Arbresle, France), aged 18–22 weeks. Half of the animals were randomly selected and given losartan orally by gavage (10 ml kg^{-1}) at the dose of 10 mg kg^{-1} per day for eight successive days, whereas the other half received distilled water (controls, 10 ml kg^{-1}) by the same route for the same duration.

All experiments were performed in accordance with the regulations edicted by the French Ministry of Agriculture for animal health care.

Systemic and regional haemodynamic measurements

On the eighth day of treatment, 2 h after the last distilled water or losartan administration, the animals were anaesthetized (sodium pentobarbitone, 50 mg kg^{-1}, i.p.), pithed, bivagotomised, intubated and ventilated with room air (Harvard respirator, model 680, Southnatick, MA, U.S.A.). Body temperature was maintained with a constant temperature heating pad. Catheters were placed in a femoral vein and a carotid artery for infusion of drugs and for measurement of arterial blood pressure via a pressure transducer (Statham P10EZ, Gould Instruments, Ballainvilliers, France), respectively.

The animals were then provided with miniaturized pulsed Doppler probes (Haywood *et al.*, 1981) which were sutured around the upper and lower abdominal aorta and the left renal artery, respectively, and connected to a pulsed Doppler flowmeter (Directional pulsed Doppler, model 545C, University of Iowa, Iowa City, IA, U.S.A.). The variations in velocity values measured by the pulsed Doppler technique have been demonstrated to be directly and linearly proportional to the changes in the corresponding flows, i.e. cardiac output (CO), hindlimb (HBF) and renal (RBF) blood flows, respectively (Hartley & Cole, 1974; Richer *et al.*, 1987). As the diameters of the vessels are not known and as the characteristics of the Doppler probes (especially the crystal angle, the probe fit, etc.) vary from one probe to another, no absolute values of the flows measured, and hence of the calculated resistances, can be given. However, the flows and resistances will hereafter and by convention be referred to as 'flows' and 'resistances' and expressed in arbitrary units (AU).

Haemodynamic signals were collected on a PC computer (Dynamit Compaq, Tokyo, Japan) with an on-line data acquisition system (PRX Software, Notocord Systems, Croissy-sur-Seine, France) and continuously displayed. Instantaneous pressure and flow signals were sampled every 2 ms and averaged over 1 s-epochs. Total peripheral resistance (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances were estimated as the mean arterial pressure (MAP) to corresponding mean flow ratios. The velocity signals, blood pressure (preamplifier 13-4615-50, Gould Instruments, Ballainvilliers, France) and heart rate (HR) (Biotach amplifier 13-4615-66, Gould Instruments, Ballainvilliers, France) were continuously recorded on a data management system (Graphtec Mark 12, Bioseb, Antony, France).

After instrumentation, the animals were given atropine sulphate (1 mg kg^{-1}, i.v.) and gallamine triiodide (20 mg kg^{-1}, i.v.) and, after a stabilization period of 20 min, baseline values of all investigated parameters were measured 165 min after the last distilled water or losartan administration.

Experimental protocols

Protocol 1: L-NAME Experiments were performed in 27 control and 28 losartan-treated SHRs. The controls were randomly divided into three subgroups: (A), (B) and (C) (n = 8 to 11) and the losartan-treated were randomly divided into three other subgroups: (D), (E) and (F) (n = 7 to 12). One hundred and sixty five minutes after the last distilled water or losartan administration, animals from groups (A) and (D) received an i.v. bolus of saline (1ml kg^{-1}) whereas animals from groups (B), (C), (E) and (F) received an i.v. bolus of L-NAME (1 mg kg^{-1}, 1ml kg^{-1}); 10 min later, i.e. when haemodynamic parameters had stabilized, animals from groups (A), (B), (D) and (E) were given an i.v. bolus of saline (1ml kg^{-1}) whereas the animals from groups (C) and (F) received an i.v. bolus of L-arginine (300 mg kg^{-1}, 1ml kg^{-1}). At that time, the different subgroups had received the following treatments: (A): distilled water, saline, saline; (B): distilled water, L-NAME, saline; (C): distilled water, L-NAME, L-arginine; (D): losartan, saline, saline; (E): losartan, L-NAME, saline; (F): losartan, L-NAME, L-arginine.

Five minutes later, i.e. 3 h after the last distilled water or losartan administration, all investigated parameters were again measured. Then systemic and regional vascular responses to noradrenaline (1 μg kg^{-1}, i.v.) and to SCS (0.125–4 Hz, 1 ms pulses, 60 V for 20 s) (stimulator ST 198, Janssen Scientific Instruments, Paris, France) were recorded.

At the end of the experiments, animals from subgroups (A) and (D) were given a bolus injection of AII (300 ng kg^{-1}).

Protocol 2: Diclofenac Experiments were performed in 26 control and 25 losartan-treated SHRs. The controls were randomly divided into two subgroups: (G) and (H) (n = 12 to 14) and the losartan-treated were randomly divided into two other subgroups: (I) and (J) (n = 11 to 14). One hundred and sixty five minutes after the last distilled water or losartan administration, animals from groups (G) and (I) received an i.v. bolus of saline (1ml kg^{-1}) whereas animals from groups (H) and (J) received diclofenac (4 mg kg^{-1}, i.v. over 1 min). At that time, the different subgroups had received the following treatments: (G): distilled water, saline; (H): distilled water, diclofenac; (I): losartan, saline; (J): losartan, diclofenac.

Fifteen minutes later, i.e. 3 h after the last distilled water or losartan administration, all investigated parameters were again measured. Then systemic and regional vascular responses to SCS (0.125–4 Hz, 1 ms pulses, 60 V for 20 s) were recorded.

Drugs

Drugs used were angiotensin II (Hypertensin, Ciba-Geigy, Basle, Switzerland), atropine sulphate (Sigma Chemical Co., Saint-Quentin-Fallavier, France), diclofenac (Ciba-Geigy, Basle, Switzerland), gallamine triiodide (Rhône-Poulenc-Rorer, Vitry-sur-Seine, France), N^G-nitro-L-arginine methylester (L-NAME, Sigma Chemical Co., Saint-Quentin-Fallavier, France), L-arginine hydrochloride (Sigma Chemical Co., Saint-Quentin-Fallavier, France), losartan (potassium salt, Merck, Sharp & Dohme, West Point, PA, U.S.A.), pentobarbitone sodium (Abbott Laboratories, Saint-Rémy sur Avre, France) and L-noradrenaline bitartrate (Sigma Chemical Co., Saint-Quentin-Fallavier, France).

All drugs administered i.v. were dissolved in saline and injected in a volume of 1ml kg^{-1}. Intravenous administration of saline alone had no consistent sustained cardiovascular effects. Losartan was dissolved in distilled water.

Statistical analysis

Results are expressed as mean ± s.e.mean. For each investigated parameter, comparison of its mean values in the

different experimental groups at baseline (i.e. 165 min after the last distilled water or losartan administration) as well as after the different pretreatments (i.e. 3 h after the last distilled water or losartan administration) was carried out by analysis of variance followed by a Student's t test with Bonferroni's correction for multiple comparisons.

Then, for each investigated parameter, the stimulation frequency-response (variations in absolute values) curves obtained in the different subgroups were compared by analysis of variance for repeated measurements using the Greenhouse-Geisser adjustment according to Ludbrook (1994) with stimulation frequency as the within-subjects factor and treatment as the between-subjects factor.

Finally, comparisons of the mean variations (in absolute values) of each parameter induced by noradrenaline and AII were compared by analysis of variance followed by a Student's t test with Bonferroni's correction for multiple comparisons.

A P value <0.05 was considered as statistically significant.

All statistical analyses were performed with a BMDP Statistical Software (BMDP, Los Angeles, CA, U.S.A.).

Results

Systemic and regional haemodynamic effects of losartan in pithed SHRs

Table 1 compares the mean values of MAP, HR, CO, RBF, HBF, TPVR, RVR and HVR determined in pithed SHRs 165 min after the last drug or distilled water administration on the 8th day of treatment. Losartan induced important reductions in MAP (−14%, $P<0.001$) and in regional vascular resistances (TPVR −21%, $P<0.01$; RVR −12%, NS; HVR −23%, $P<0.001$). HR was also decreased (−5%, $P<0.05$) but CO, RBF and HBF were not significantly affected.

L-NAME and systemic and regional haemodynamics in control and losartan-treated pithed SHRs

In control SHRs, L-NAME had no effect on MAP (Figure 1) but decreased blood flows (Table 2) and increased all investigated vascular resistances (TPVR: +74%, $P<0.05$; RVR: +61%, NS; HVR: +57%, NS) (Figure 1). L-Arginine tended to reverse these effects, except in the kidney. In losartan-treated SHRs, L-NAME slightly increased MAP but exerted almost no effect on regional blood flows and vascular resistances (Table 2, Figure 1).

L-NAME and cardiac and haemodynamic responses to spinal cord stimulation in control and losartan-treated pithed SHRs

In control SHRs, SCS led to frequency-dependent increases in MAP, TPVR, RVR and HVR (Figure 2). HR, CO and RBF but not HBF were also increased (Figure 3). In losartan-treated animals, MAP and regional vasoconstrictor responses to SCS were strongly and significantly reduced (or even abolished) (Figure 2), thus confirming the previously described vascular sympathoinhibitory effect of losartan. In contrast, SCS-induced increases in CO and RBF were strongly potentiated by losartan and HBF increased (Figure 3). Finally, the tachycardic response to SCS remained unaffected by losartan (Figure 3).

In control SHRs, L-NAME slightly increased the pressor and systemic vasoconstrictor responses to SCS but strongly and significantly potentiated the renal ($P<0.01$) and hindlimb ($P<0.05$) vasoconstrictor ones. All these effects were abolished by L-arginine (Figure 2). L-NAME had no effect on SCS-induced increases in HR and CO but suppressed the rise in RBF ($P<0.05$). The latter suppression was almost abolished by L-arginine.

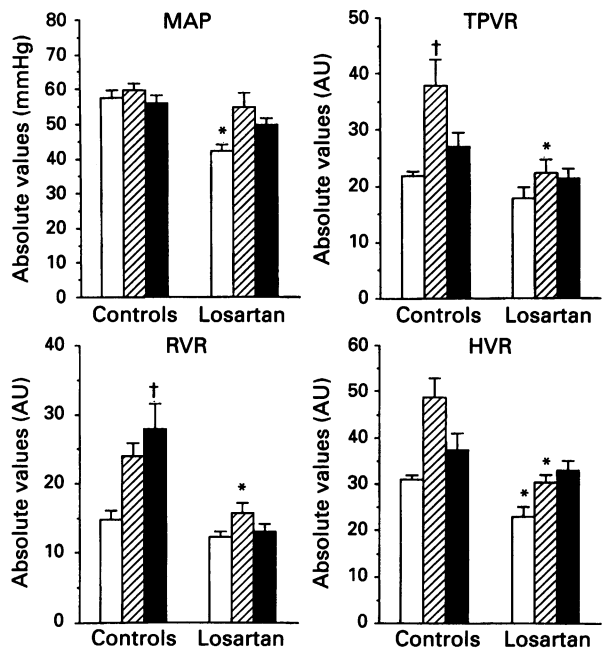

Figure 1 Mean ± s.e.mean values of mean arterial pressure (MAP), total peripheral (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances determined in control and in losartan-treated pithed SHRs after saline (open columns), or L-NAME (hatched columns), or L-NAME followed by L-arginine (solid columns). AU: arbitrary units. †Significant difference from corresponding value in animals receiving saline alone: P at least <0.05. *Significant difference from corresponding value in control animals: P at least <0.05.

Table 1 Mean ± s.e.mean values of all investigated parameters measured in control (distilled water) or losartan (10 mg kg^{-1} daily for 7 days)-treated pithed SHRs 165 min after the last distilled water or losartan administration

	Controls	*Losartan*
Mean arterial pressure (mmHg)	56.0 ± 1.2	48.3 ± 1.0*
Heart rate (beats min^{-1})	322.5 ± 5.6	291.0 ± 11.7*
Cardiac output (AU)	2.8 ± 0.1	3.0 ± 0.2
Renal blood flow (AU)	4.2 ± 0.2	3.9 ± 0.2
Hindlimb blood flow (AU)	1.9 ± 0.1	2.2 ± 0.1
Total peripheral resistance (AU)	21.5 ± 1.1	17.0 ± 0.8*
Renal vascular resistance (AU)	14.3 ± 0.7	12.7 ± 0.6
Hindlimb vascular resistance (AU)	30.4 ± 1.6	23.3 ± 11.1*

AU: arbitrary units.
*Value significantly different from corresponding control value: P at least <0.05.

Table 2 Mean ± s.e.mean absolute values of cardiac output (CO), renal (RBF) and hindlimb (HBF) blood flows determined in control and in losartan-treated pithed SHRs after saline (No pretreatment) or L-NAME (L-NAME pretreatment) or L-NAME followed by L-arginine (L-NAME + L-Arg pretreatment)

Groups	*CO* (AU)	*RBF* (AU)	*HBF* (AU)
Controls			
No pretreatment (A)	2.7 ± 0.2	3.6 ± 0.4	1.9 ± 0.1
L-NAME pretreatment (B)	1.7 ± 0.2	2.4 ± 0.3*	1.3 ± 0.1*
L-NAME + L-Arg pretreatment (C)	2.3 ± 0.2	2.3 ± 0.4*	1.6 ± 0.1
Losartan-treated			
No pretreatment (D)	2.6 ± 0.3	3.6 ± 0.3	1.8 ± 0.2
L-NAME pretreatment (E)	2.6 ± 0.3	3.7 ± 0.5†	1.8 ± 0.1†
L-NAME + L-Arg pretreatment (F)	2.5 ± 0.2	3.9 ± 0.4†	1.6 ± 0.2

AU: arbitrary units.
*Value significantly different from corresponding value in animals receiving saline (no pretreatment): P at least <0.05.
†Value significantly different from corresponding value in control animals: P at least <0.05.

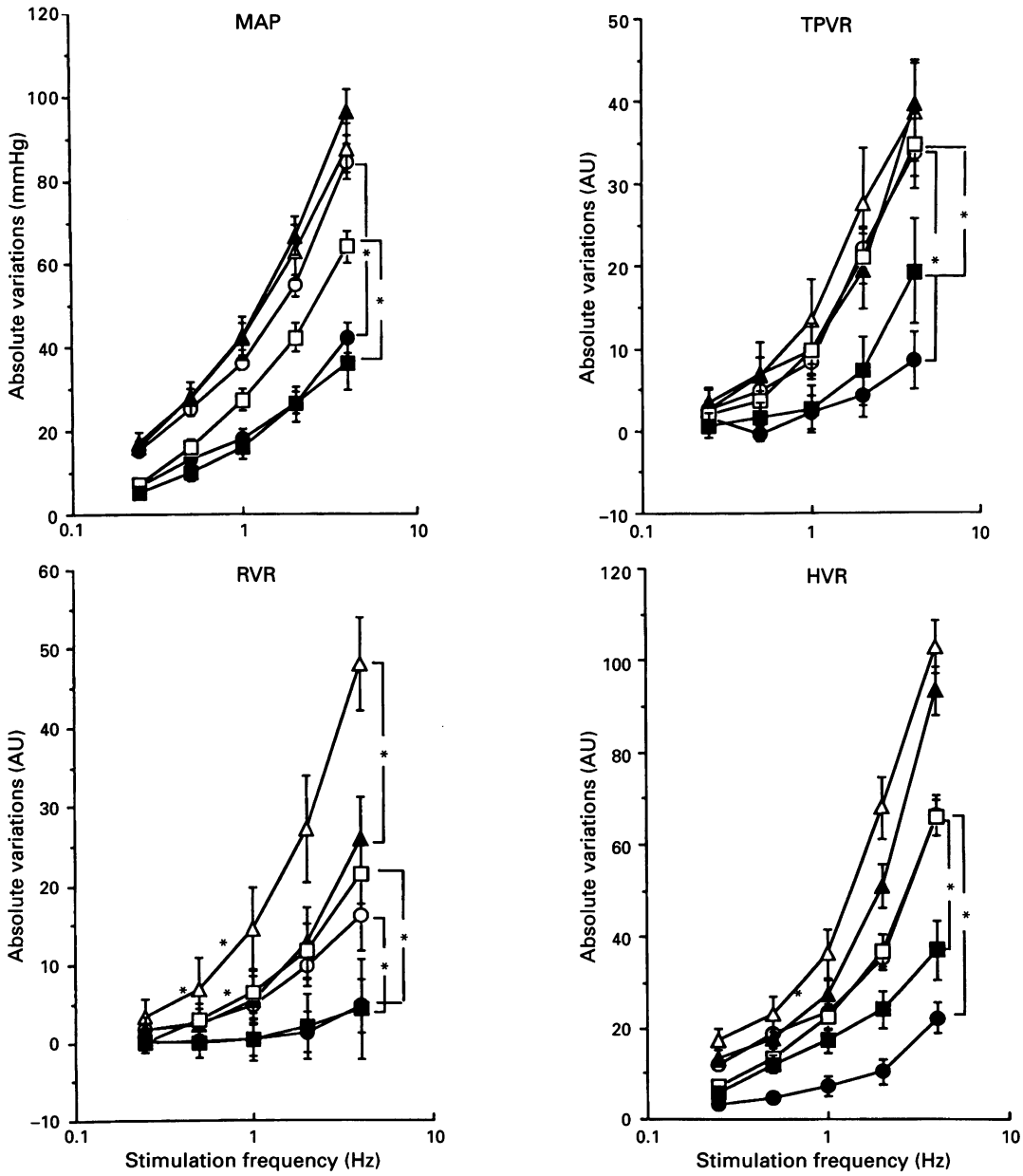

Figure 2 Mean ± s.e.mean absolute variations in mean arterial pressure (MAP), total peripheral (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances induced by electrical stimulation of the spinal cord at increasing frequencies in control animals after saline (○), after L-NAME (△) or after L-NAME followed by L-arginine (□), and in losartan-treated animals after saline (●), after L-NAME (▲) or after L-NAME followed by L-arginine (■). AU: arbitrary units. *Indicates a significant difference between the entire control and corresponding losartan-treated stimulation frequency-response curves as determined by two way analysis of variance: P at least <0.05.

In losartan-treated SHRs, L-NAME also potentiated the pressor ($P < 0.001$) and systemic ($P < 0.001$), renal ($P < 0.001$) and hindlimb ($P < 0.001$) vasoconstrictor responses to SCS but to a much greater extent than in control SHRs. As a result, after L-NAME, the SCS frequency-dependent response curves were similar for all parameters (except RVR) in control and losartan-treated animals, i.e. losartan's vascular sympathoinhibitory effects were suppressed, except at the renal level where they were fully maintained. L-Arginine suppressed all L-NAME effects, thus restoring the vascular sympathoinhibitory effects of losartan (Figure 2). In losartan-treated SHRs, L-NAME had no effect on SCS-induced tachycardia, reduced the increases in CO and RBF only at the highest SCS frequency and abolished that of HBF ($P < 0.05$). The L-NAME-induced reductions in CO, RBF and HBF were halved by L-arginine (Figure 3).

L-NAME and haemodynamic responses to exogenous noradrenaline in control and losartan-treated pithed SHRs

In control SHRs, noradrenaline (1 μg kg^{-1}) increased MAP, TPVR, RVR and HVR. These responses were strongly potentiated by L-NAME and this potentiation was abolished by L-arginine except in the kidney where it was reduced only by approximately 50% (Table 3).

In losartan-treated SHRs, the MAP, TPVR, RVR and HVR responses to noradrenaline were similar to those seen in control SHRs. This was also true in L-NAME- and in L-NAME + L-arginine-pretreated SHRs (Table 3).

L-NAME and haemodynamic responses to exogenous AII in control and losartan-treated pithed SHRs

In control SHRs, AII (300 ng kg^{-1}) increased MAP, TPVR, RVR and HVR. In contrast, in losartan-treated SHRs, AII was devoid of any haemodynamic effect, thus indicating full effectiveness of AII AT_1-receptor blockade (Table 3).

Diclofenac and systemic and regional haemodynamic effects in control and losartan-pretreated SHRs

In control SHRs, diclofenac had no effect on MAP, TPVR, RVR and HVR. This was also true in losartan-treated SHRs (Figure 4).

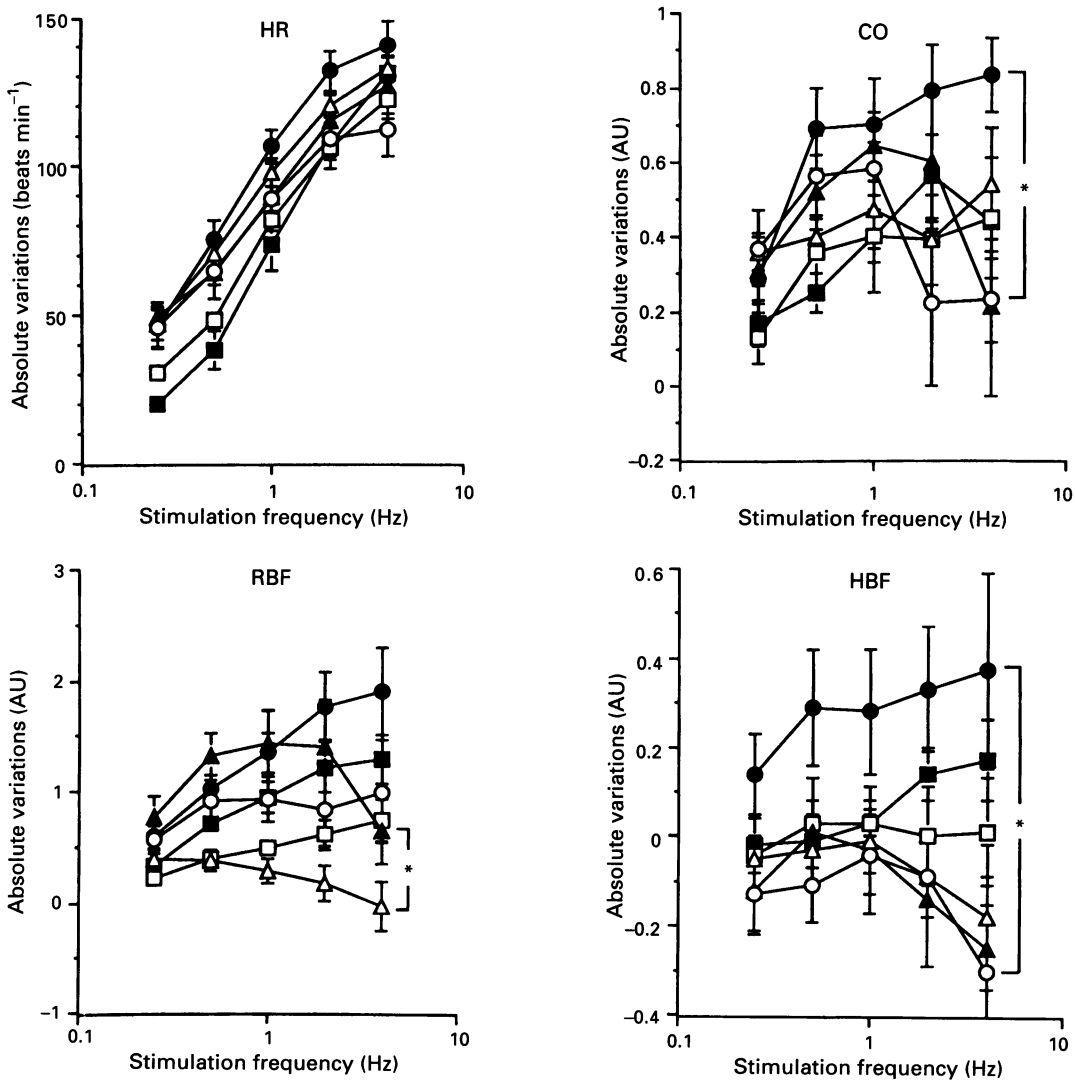

Figure 3 Mean ± s.e.mean absolute variations in heart rate (HR), cardiac output (CO), renal (RBF) and hindlimb (HBF) blood flows induced by electrical stimulation of the spinal cord at increasing frequencies in control animals after saline (○), after L-NAME (△) or after L-NAME followed by L-arginine (□), and in losartan-treated animals after saline (●), or after L-NAME (▲) or after L-NAME followed by L-arginine (■). AU: arbitrary units. *Indicates a significant difference between the entire control and corresponding losartan-treated stimulation frequency-response curves as determined by two way analysis of variance: P at least < 0.05.

Table 3 Mean ± s.e.mean absolute variations in mean arterial pressure (MAP), total peripheral resistance (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances induced by noradrenaline (1 $\mu g\ kg^{-1}$, i.v.) and angiotensin II (300 $ng\ kg^{-1}$, i.v.) in the different subgroups (A, B, C, D, E and F)

Groups	*MAP* (mmHg)	*TPVR* (AU)	*RVR* (AU)	*HVR* (AU)
Noradrenaline				
No pretreatment				
Saline (A)	49.9 ± 5.4	18.9 ± 5.8	30.5 ± 7.9	17.8 ± 2.3
Losartan (D)	41.4 ± 3.7	14.4 ± 2.5	19.7 ± 3.0	21.1 ± 3.0
L-NAME pretreatment				
Saline (B)	85.1 ± 4.9*	50.4 ± 12.9*	169.7 ± 34.4*	32.5 ± 2.4*
Losartan (E)	75.7 ± 11.9	34.0 ± 6.9	120.1 ± 37.3	25.0 ± 4.8
L-NAME + L-Arg pretreatment				
Saline (C)	37.8 ± 2.8*	19.6 ± 5.2	91.0 ± 18.2*	11.3 ± 3.5
Losartan (F)	31.2 ± 2.4	14.0 ± 2.1	56.4 ± 17.2	9.5 ± 2.6
Angiotensin II				
No pretreatment				
Saline (A)	41.3 ± 3.5	28.6 ± 4.9	158.2 ± 45.7	7.3 ± 1.1
Losartan (D)	2.9 ± 0.6*	0.1 ± 0.7*	2.1 ± 1.4*	−2.0 ± 0.2*

AU: arbitrary units.
*Value significantly different from corresponding control value in non-pretreated control animals: P at least <0.05.

Diclofenac and cardiac and haemodynamic responses to spinal cord stimulation in control and losartan-treated pithed SHRs

In control SHRs, SCS led to frequency-dependent increases in MAP, TPVR, RVR and HVR (Figure 5) and HR (data not shown). In losartan-treated SHRs, MAP and regional vasoconstrictor responses to SCS were strongly and significantly reduced, again confirming the well-documented vascular sympathoinhibitory effects of losartan. In contrast, SCS-induced tachycardia remained unaffected by losartan.

In control SHRs, diclofenac potentiated the pressor and systemic and renal vasoconstrictor responses to SCS ($P<0.01$, $P<0.001$ and $P<0.05$, respectively). In losartan-treated SHRs, diclofenac also potentiated all pressor and systemic and regional vasoconstrictor responses to SCS, and to the same extent, as in control SHRs, i.e. the vascular sympathoinhibitory effects of losartan were fully maintained. Finally, in control as well as in losartan-treated SHRs, diclofenac had no effect on the tachycardic response to SCS.

Discussion

Many *in vitro* and *in vivo* studies support the concept tht AII facilitates the sympathetic neuronal function (Starke, 1971; Hilgers *et al.*, 1993). However, a positive interaction between AII and the sympathetic neurones has been easier to demonstrate for those controlling vascular smooth muscle tone than for those controlling heart rate (Antonaccio & Kerwin, 1981; Hatton & Clough, 1982; Lanier & Malik, 1983; Vollmer *et al.*, 1984). Interestingly, the suppression of AII effects through either ACE inhibition or AT_1-receptor blockade also results in an attenuation of the vascular but not of the cardiac responses to sympathetic nervous system activation (Antonaccio & Kerwin, 1981; Hatton & Clough, 1982; Richer *et al.*, 1984; 1986; Moreau *et al.*, 1993). These *in vivo* observations of an AII-sympathetic nervous system interaction developing only at the vascular but not at the cardiac level is difficult to reconcile with the well-documented facilitatory effect of AII on noradrenergic neuronal function (Kaufman & Vollmer, 1985). Hence, the hypothesis that mechanisms other than the sole inhibition of AII effects could contribute to the vascular selective sympathoinhibitory effects observed after blockade of the renin-angiotensin system has been investigated in the present study which was conducted in the pithed SHR and used losartan as the test drug.

Losartan, a selective AII AT_1-receptor antagonist which is devoid of any agonist activity (Wong *et al.*, 1990a), was administered orally over a 1-week period at a dose of 10 $mg\ kg^{-1}$. This dose has been shown previously to demonstrate both antihypertensive properties in hypertensive rats (Wong *et al.*, 1990b) and vascular sympathoinhibitory effects in pithed SHRs (Moreau *et al.*, 1993; Chauvin *et al.*, 1995).

It appears from our study that losartan decreases blood pressure and regional vascular resistances in the pithed SHR which indicates either that AII contributes to the vascular tone in this preparation, or that losartan administration results in the release of vasodilator agents, among which NO (Cachofeiro *et al.*, 1992; Kumagai *et al.*, 1993; Sudhir *et al.*, 1993) and PGs (Jaiswal *et al.*, 1991; Catalioto *et al.*, 1994) have previously been proposed as candidates. Our results also show that losartan significantly reduces or even abolishes the systemic pressor and renal and muscular vasoconstrictor responses elicited by electrical stimulation of the spinal cord but does not affect the simultaneously induced tachycardia, thus confirming the vascular selective sympathoinhibitory effects of the drug (Moreau *et al.*, 1993). Finally, our data indicate that these sympathoinhibitory effects of losartan are not due to an effect of the drug at the level of postsynaptic α-adrenoceptors, as the hypertensive and vasoconstrictor properties of exogenous noradrenaline remained unaffected.

Losartan has been reported to stimulate *in vitro* the release of PGE_2 and to a larger extent of prostacyclin from vascular smooth muscle cells (Jaiswal *et al.*, 1991; Catalioto *et al.*, 1994), an effect not mediated through AII AT_1-receptors. Our results demonstrate that the cyclo-oxygenase inhibitor, diclofenac (a) had no effect on the basal values of blood pressure and regional vascular resistances, and (b) did not affect the blood pressure lowering effects and the vasodilator properties of losartan. It is thus most likely that PGs are not involved in the control of basal vasomotor tone in pithed SHRs and do not contribute to the vasorelaxant effects of losartan. Moreover, inhibition of PG synthesis potentiated the vasoconstrictor (but not the tachycardic) responses elicited by SCS in control as well as in losartan-treated SHRs, possibly through suppression of a vasodilator counter-regulatory mechanism through which PGs might buffer the vascular responses to sympathetic activation. Finally, diclofenac did not affect the sympathoinhibitory effects of losartan, which demonstrates that PGs are not involved in the losartan-sympathetic nervous system interaction.

Previous studies, both *in vitro* and *in vivo*, have revealed an attenuation of the vasoconstrictor effects of electrical sympa-

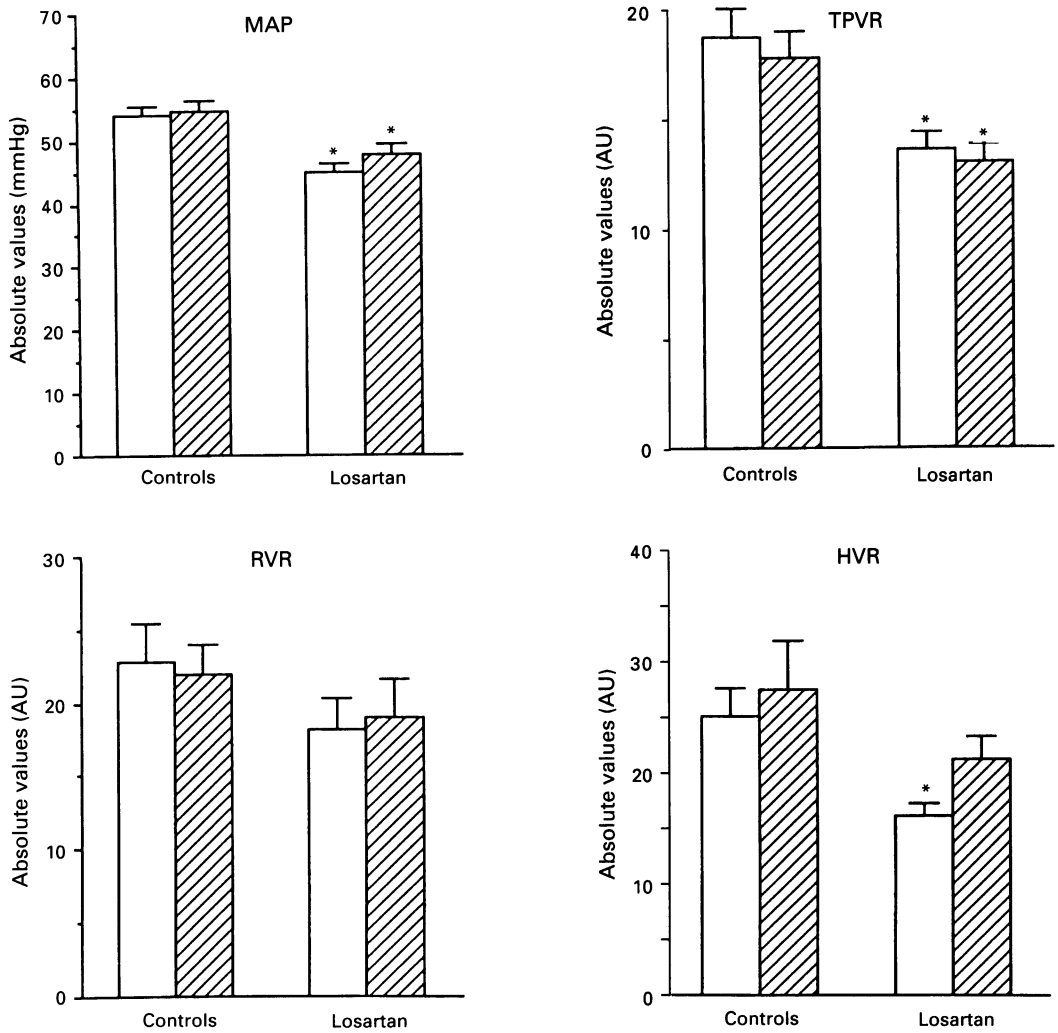

Figure 4 Mean ± s.e.mean values of mean arterial pressure (MAP), total peripheral (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances determined in control and in losartan-treated pithed SHRs after saline (open columns) or diclofenac (hatched columns). AU: arbitrary units. *Significant difference from corresponding value in control animals: P at least <0.05.

thetic nerve stimulation by endothelial NO in different experimental settings and in different animal species (Tesfeimariam *et al.*, 1987; 1989; Sakuma *et al.*, 1992; Vo *et al.*, 1992; Toda *et al.*, 1993; MacLean *et al.*, 1994), and conversely NO synthase inhibition has been shown to increase sympathetic nerve activity (Sakuma *et al.*, 1992; Harada *et al.*, 1993; Kumagai *et al.*, 1993; Toda *et al.*, 1993; Zanzinger *et al.*, 1994). The mechanisms by which NO appears to interact with the sympathetic nervous system comprise a reduction of the central sympathetic tone (decrease in peripheral sympathetic nerve activity) and an inhibition of its peripheral vasoconstrictor effects. In the periphery, NO has been shown to inhibit directly sympathetic vasoconstriction postsynaptically, reducing the pressor responses to noradrenaline which in contrast are enhanced by L-NAME (Zanzinger *et al.*, 1994). Our study confirms this potentiation both at the systemic but also at the muscular (hindlimb) and especially at the renal level.

To date, a number of studies tend to indicate that NO is somehow involved in the mechanism of the vasodilator and antihypertensive effects of losartan. Cachofeiro *et al.* (1992) have shown in the SHR that the acute reduction in blood pressure induced by the drug is reduced by approximately 60% when the animals are pretreated with L-NMMA. Similar findings have been reported by others (Kumagai *et al.*, 1993; Pollock *et al.*, 1993; Sudhir *et al.*, 1993) and in our study, L-NAME raised blood pressure and vascular resistances in losartan-treated animals. The origin of losartan-mobilized NO is still debated. Recent studies have suggested that endothelial AII AT_1-receptor blockade *per se* enhances NO production (Sudhir *et al.*, 1993) and upregulates endothelial NO synthase in elastic arteries (Holtz *et al.*, 1994). Moreover, losartan as a vasodilator increases regional blood flows which through augmented shear stress may enhance NO release (Cachofeiro *et al.*, 1992). Finally, endothelial dysfunction has been shown to be associated with hypertension (Lüscher & Vanhoutte, 1986) and the possibility cannot be excluded that an 8-day treatment with losartan may have partly restored in our SHRs their impaired ability to produce NO in response to endothelium-dependent vasodilators.

Our data show that the role of NO in the effects of losartan is even reinforced during SCS. In control animals, cardiac output and renal blood flow increased during SCS, thus resulting through augmented shear stress in an enhanced endothelial NO release, a mechanism which probably permanently limits the vasopressor and vasoconstrictor effects of SCS. But most interestingly, in losartan-treated animals, the SCS-induced increases in cardiac output and in renal blood flow are strongly potentiated, and in the hindlimb, a resistance vascular bed, muscular blood flow is greatly enhanced. Hence, it is likely that NO release is, even more during SCS than in the basal conditions, strongly involved in the effects of the drug.

Among the latter, the vascular sympathoinhibitory effects

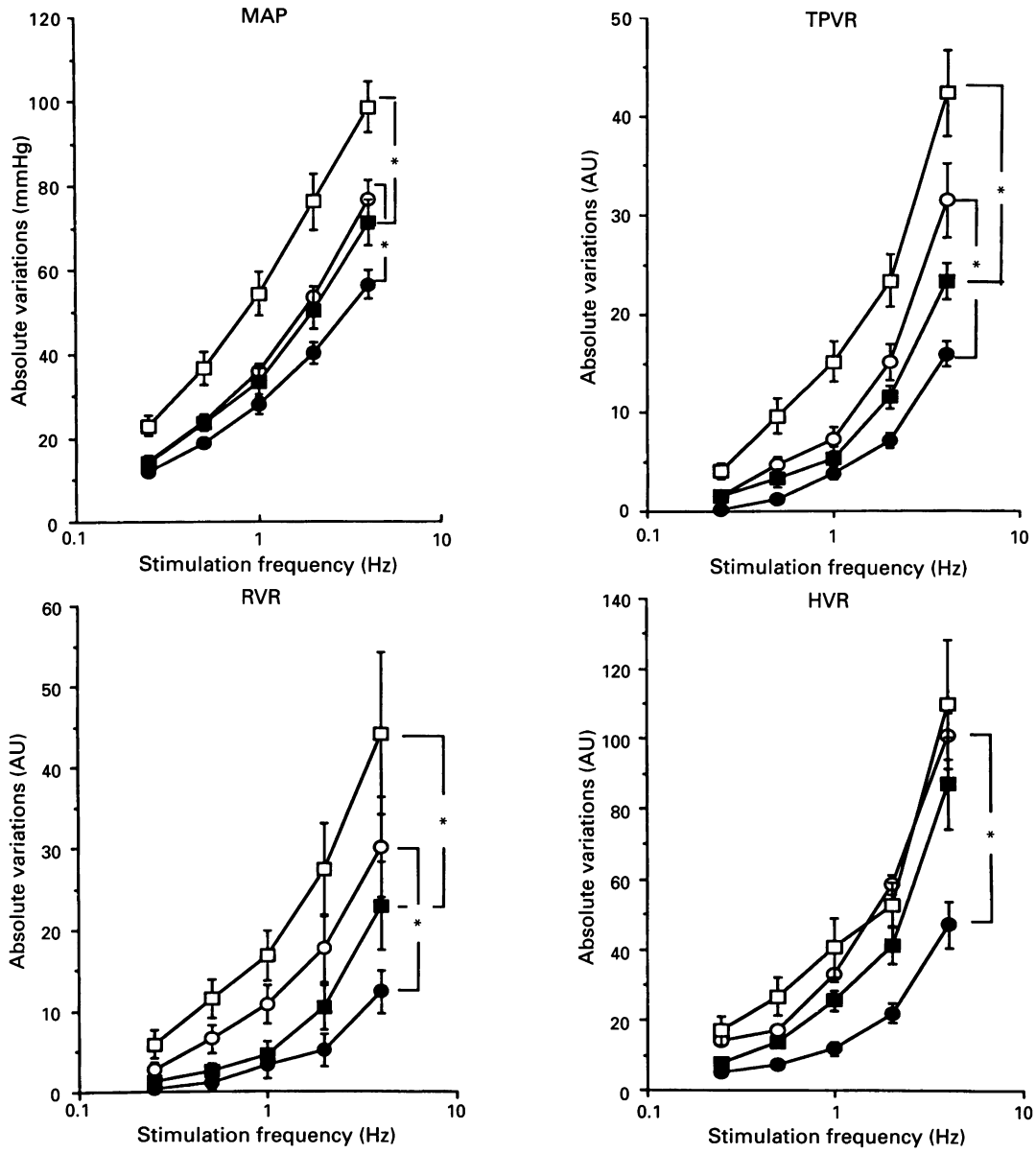

Figure 5 Mean ± s.e.mean absolute variations in mean arterial pressure (MAP), total peripheral (TPVR), renal (RVR) and hindlimb (HVR) vascular resistances induced by electrical stimulation of the spinal cord at increasing frequencies in control animals after saline (○) or after diclofenac (□), and in losartan-treated animals after saline (●) or after diclofenac (■). AU: arbitrary units. *Indicates a significant difference between the entire control and corresponding losartan-treated stimulation frequency-response curves as determined by two way analysis of variance: P at least <0.05.

of losartan are of major interest and their mechanism was not fully understood. Our study clearly demonstrates that, with the exception of the renal level, NO is most likely the major determinant of these sympathoinhibitory effects as these are abolished by NO synthase inhibition and fully restored by L-arginine. In contrast to control animals in which SCS-elicited pressor and vasoconstrictor responses were only slightly enhanced by L-NAME (maximal responses: +5, +15, +50 and +50% for MAP, TPVR, RVR and HVR, respectively, at 4 Hz), these responses were strongly enhanced by L-NAME (+140, +400, +300 and +340%, respectively) in losartan-treated animals, thus clearly underlining the much greater contribution of NO suppression to these enhancements in losartan-treated as compared to control animals. As a result of these differential enhancements, the vascular sympathoinhibitory effects of losartan were suppressed by L-NAME (except in the kidney) thus pointing out the major responsibility of NO in their development. This responsibility is furthermore emphasized by our experiments showing that when L-arginine is administered in a dose that counteracts most but not all, a finding previously reported by others (Gardiner *et al.*, 1990; Greenblatt *et al.*, 1993), L-NAME haemodynamic effects, it fully restores the sympathoinhibitory effects of losartan. Finally, in the kidney and in contrast to all other investigated vascular beds, NO appears not to be predominantly involved in the sympathoinhibitory effects of losartan. This was not because NO synthesis blockade had not been achieved at this level as renal constrictor responses to SCS were potentiated by L-NAME to the same extent as were those in the hindlimb. There thus appears a heterogeneity among the different vascular beds as to the modulating role of NO on sympathetic neurotransmission. In the kidney, a major target for AII vasoconstrictor effects, it is likely that suppression by losartan of presynaptic AII AT_1-receptor-mediated catecholamines release is the main factor responsible for the sympathoinhibitory effects of the drug but this needs further confirmation.

In conclusion, it appears from our data that in pithed SHRs: (a) NO but not PGs contribute to the basal vasomotor

tone; (b) both NO and PGs attenuate the pressor and vasoconstrictor responses to SCS; (c) NO plays a major role in the vascular sympathoinhibitory effects of losartan, except at the renal level, and (d) endogenous PGs are not involved in these sympathoinhibitory effects

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