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Lack of involvement of bradykinin in the vascular sympathoinhibitory effects of angiotensin converting enzyme inhibitors in spontaneously hypertensive rats

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1 The aim of this study was to investigate the contribution of endogenous bradykinin to the vascular sympathoinhibitory effects exerted by angiotensin I converting enzyme inhibitors (ACEIs) in the spontaneously hypertensive rat (SHR).

2 Adult SHRs were treated daily for 8 days with either perindopril (3 mg kg^{-1}), or a selective angiotensin II AT_1 receptor antagonist, losartan (10 mg kg^{-1}) both given orally – these two doses being equipotent in inhibiting angiotensin I (AI)-induced vascular responses – or distilled water (controls). After pithing, the animals were instrumented for determination of blood pressure, heart rate, cardiac output, regional (renal, mesenteric, hindlimb) blood flows (pulsed Doppler technique) and corresponding vascular resistances. Afterwards, half of the animals of each group were given the selective bradykinin B_2 receptor antagonist, icatibant, used in a dose (10 μg kg^{-1}, i.v.) that achieved B_2 receptor blockade, the other half received saline (10 μl kg^{-1}, i.v.). Haemodynamic responses to increasing frequencies of spinal cord stimulation were then measured.

3 Pressor and vasoconstrictor responses to AI were significantly and similarly reduced in both perindopril- and losartan-treated groups. Perindopril and losartan both decreased to a similar extent the pressor and vasoconstrictor responses to electrical stimulation of the spinal cord.

4 In the dose used, icatibant did not affect any of the investigated haemodynamic parameters in any of the experimental groups. Furthermore, icatibant did not affect the stimulation frequency-response curves in the control animals and did not modify the vascular sympathoinhibitory effects exerted by perindopril and by losartan.

5 Taken together, these results demonstrate that endogenous bradykinin does not, through B_2 receptor activation, contribute to the vascular sympathoinhibitory effects of ACEIs in SHRs.

Keywords: Bradykinin; angiotensin converting enzyme inhibitors; losartan; sympathetic nervous system; pithed SHR

Introduction

It has previously been demonstrated in spontaneously hypertensive rats (SHRs) that angiotensin I converting enzyme inhibitors (ACEIs) reduce the vascular responses to sympathetic stimulation and, to a lesser extent, to selective postjunctional α-adrenoceptor agonists (Antonaccio & Kerwin, 1981; De Jonge *et al.*, 1983; Richer *et al.*, 1984; 1986). Although suggesting that blockade of endogenous angiotensin II (AII) formation is the major factor responsible for the sympathoinhibitory effects of ACEIs, these results do not exclude the possibility that other mechanisms, e.g. potentiation of bradykinin, might also contribute.

ACEIs lead to bradykinin accumulation as shown by increases in its circulating and tissue levels (Campbell *et al.*, 1994). Bradykinin has been claimed to be involved in a large number of the properties of ACEIs e.g., their antihypertensive action (Thurston & Swales, 1978; Swartz *et al.*, 1979; Bao *et al.*, 1992; Cachofeiro *et al.*, 1992; Bouaziz *et al.*, 1994), their cardiac and vascular protective effects (Schölkens *et al.*, 1988; Linz & Schölkens, 1992; Gohlke *et al.*, 1994) and their inhibitory action vs neointima formation (Farhy *et al.*, 1993). Regarding sympathetic neurotransmission, the role of bradykinin remains controversial (Schwieler & Hjemdahl, 1992), this peptide having been shown, depending upon the experimental conditions, either to reduce (Starke *et al.*, 1977; Malik & Nasjletti, 1979), or to enhance (Dominiak, 1993; Schwieler *et al.*, 1993; Minshall *et al.*, 1994), or even to exert no effect on catecholamine release (Hatton & Clough, 1982).

In this context, the aim of the present study was to investigate in the pithed SHR whether ACEI-induced increased levels of endogenous bradykinin could be involved in their vascular sympathoinhibitory effects.

For this purpose, we followed two approaches. First, considering that AII AT_1 receptor antagonists are also endowed with sympathoinhibitory effects (Wong *et al.*, 1991; Moreau *et al.*, 1993), we investigated whether there were quantitative differences between an ACEI, perindopril, and an AII AT_1 receptor antagonist, losartan, in their respective effects on peripheral sympathetic neurotransmission, both drugs being used in doses equipotent in blocking AI pressor responses. Second, we investigated whether administration of a selective bradykinin B_2 receptor antagonist would limit the sympathoinhibitory effects of perindopril but not those of losartan.

Methods

All experiments were carried out in 16 week-old male SHRs of the Okamoto-Aoki strain (Iffa Credo, L'Arbresle, France). The animals were placed on a normal chow diet with water *ad libitum*. All experiments were performed in accordance with the regulations of the French Ministry of Agriculture.

Experimental protocols

Effects of icatibant on sympathetic responses to electrical stimulation of the spinal cord in control, perindopril- or losartan-treated pithed SHRs SHRs were divided randomly into three groups ($n=27-29$, each), treated daily for 8 days by gavage

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either with losartan (10 mg kg^{-1}, 0.1 ml kg^{-1}, $n=28$), or with perindopril (3 mg kg^{-1}, 0.1 ml kg^{-1}, $n=29$), or with distilled water (controls, 0.1 ml kg^{-1}, $n=27$).

On the 8th day of treatment, 2 h (perindopril) or 3 h (losartan) (i.e., at the time of the drugs' peak effects) or 3 h (controls) after the last drug or distilled water administration,

Table 1 Baseline values of mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), renal (RBF), mesenteric (MBF), hindlimb (HBF) blood flows, total peripheral (TPVR), renal (RVR), mesenteric (MVR) and hindlimb (HVR) vascular resistances in control, perindopril- and losartan-treated pithed SHRs before icatibant or saline administration

	MAP (mmHg)	*HR* (beats min^{-1})	*CO* (AU)	*Regional blood flows (AU)* *RBF*	*MBF*	*HBF*	*TPVR* (AU)	*Regional vascular resistances (AU)* *RVR*	*MVR*	*HVR*
Control	54.6	317.7	3.6	2.7	3.2	2.14	15.5	22.9	21.5	28.0
($n=27$)	±1.1	±7.7	±0.1	±0.2	±0.3	±0.17	±0.5	±2.0	±2.8	±1.5
Perindopril	40.2	299.6	3.3	2.6	2.4	2.52	14.0	16.6	18.8	17.4
($n=29$)	±0.9*	±4.9	±0.2	±0.2	±0.2	±0.16	±1.5	±0.8*	±1.5	±4.6*
Losartan	45.1	316.0	3.5	2.7	2.4	2.24	13.3	19.0	22.5	20.6
($n=28$)	±1.0*†	±6.9	±0.2	±0.2	±0.2	±0.07	±0.6*	±1.6	±1.9	±3.7*

Values are mean±s.e.mean. AU: arbitrary units; *n*: number of animals.
*Value significantly different from corresponding control value: $P<0.05$.
†Value significantly different from corresponding perindopril value: $P<0.05$.

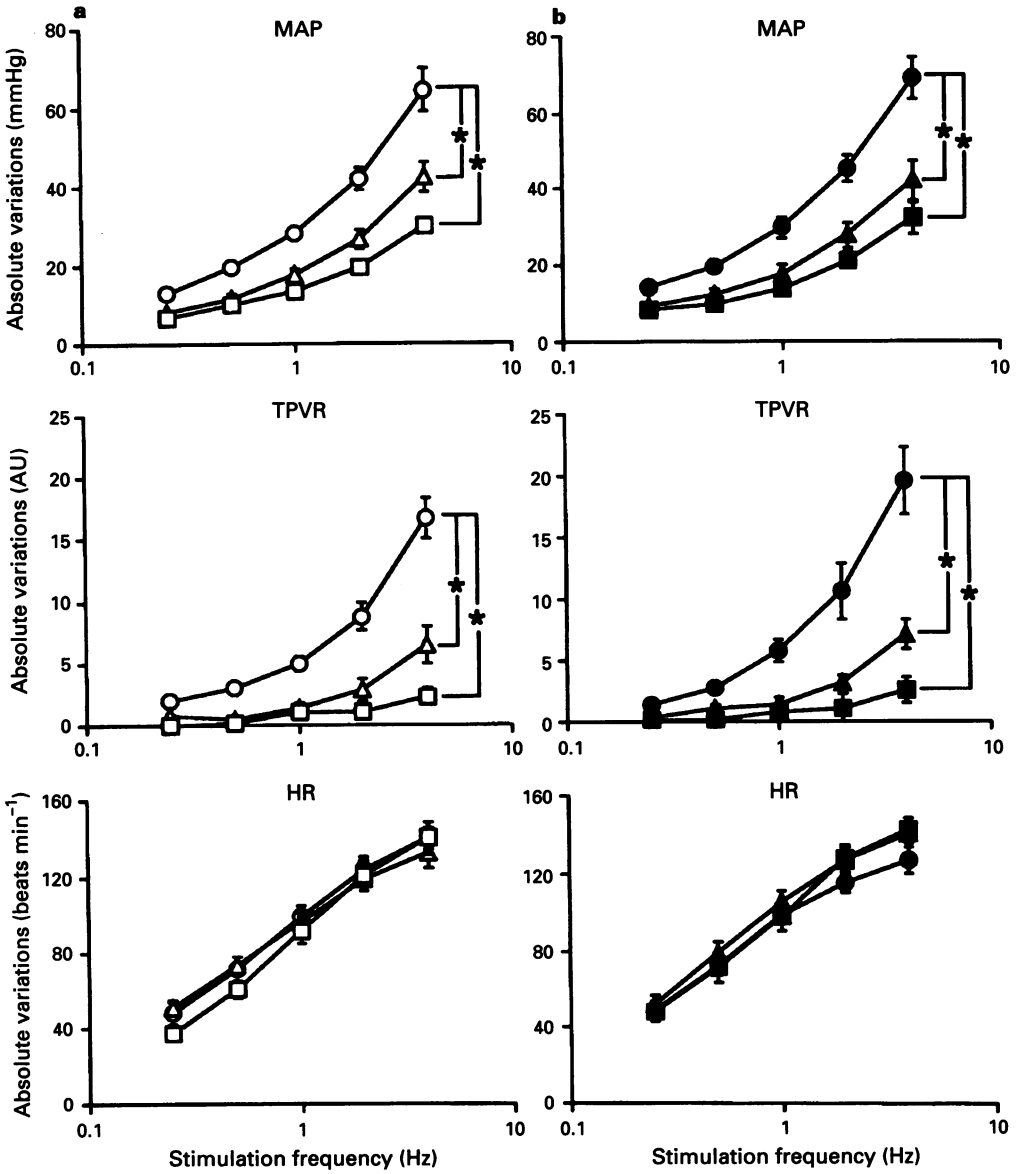

Figure 1 Mean±s.e.mean absolute variations of mean arterial pressure (MAP), total peripheral vascular resistance (TPVR) and heart rate (HR) induced by electrical stimulation of the spinal cord in control (○, ●), in perindopril-treated (□, ■) and in losartan-treated (△, ▲) pithed SHRs in the absence (a: ○, □, △) or in the presence (b: ●, ■, ▲) of icatibant. AU : arbitrary units. *Significant difference from corresponding control frequency-response curve: *P* at least <0.05.

the animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). They were pithed, bivagotomized and ventilated with room air (Harvard respirator, model 680, South Natick, MA, U.S.A.) and were instrumented for measurement of vascular flows by the pulsed Doppler technique. A carotid artery was cannulated (PE50) for blood pressure (Statham P10EZ, preamplifier model 13-4615-10, Gould Instruments, Ballainvilliers, France) and heart rate (HR) (Biotach amplifier, model 13-4615-66, Gould Instruments, Ballainvilliers, France) measurements. Miniaturized blood velocity probes were placed around the upper abdominal aorta (cardiac output), the left renal artery, the superior mesenteric artery and the lower abdominal aorta, 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 : cardiac output (CO) and renal, mesenteric and hindlimb blood flows (RBF, MBF, HBF) (Hartley & Cole, 1974; Richer *et al.*, 1987), respectively. As 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 value of the flows measured and hence of the calculated resistances can be given. However, these flows and resistances will hereafter and by convention be referred to 'flows' and 'resistances' and expressed in arbitrary units (AU). Haemodynamic signals (blood pressure and blood flows) were collected on a PC compatible computer (Dynamit Compaq, Tokyo, Japan) with an on-line data acquisition system (ANAPDR 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 periods.

Total peripheral resistance (TPVR), renal, mesenteric and hindlimb vascular resistances (RVR, MVR, HVR) were estimated as the mean arterial pressure (MAP) to corresponding mean flow ratios. Simultaneously, the four velocity signals as well as blood pressure and HR were continuously recorded (Gould Polygraph, model 6610-06, Gould Instruments, Ballainvilliers, France). The animals were then given atropine sulphate (1 mg kg^{-1}, i.v.) and gallamine (20 mg

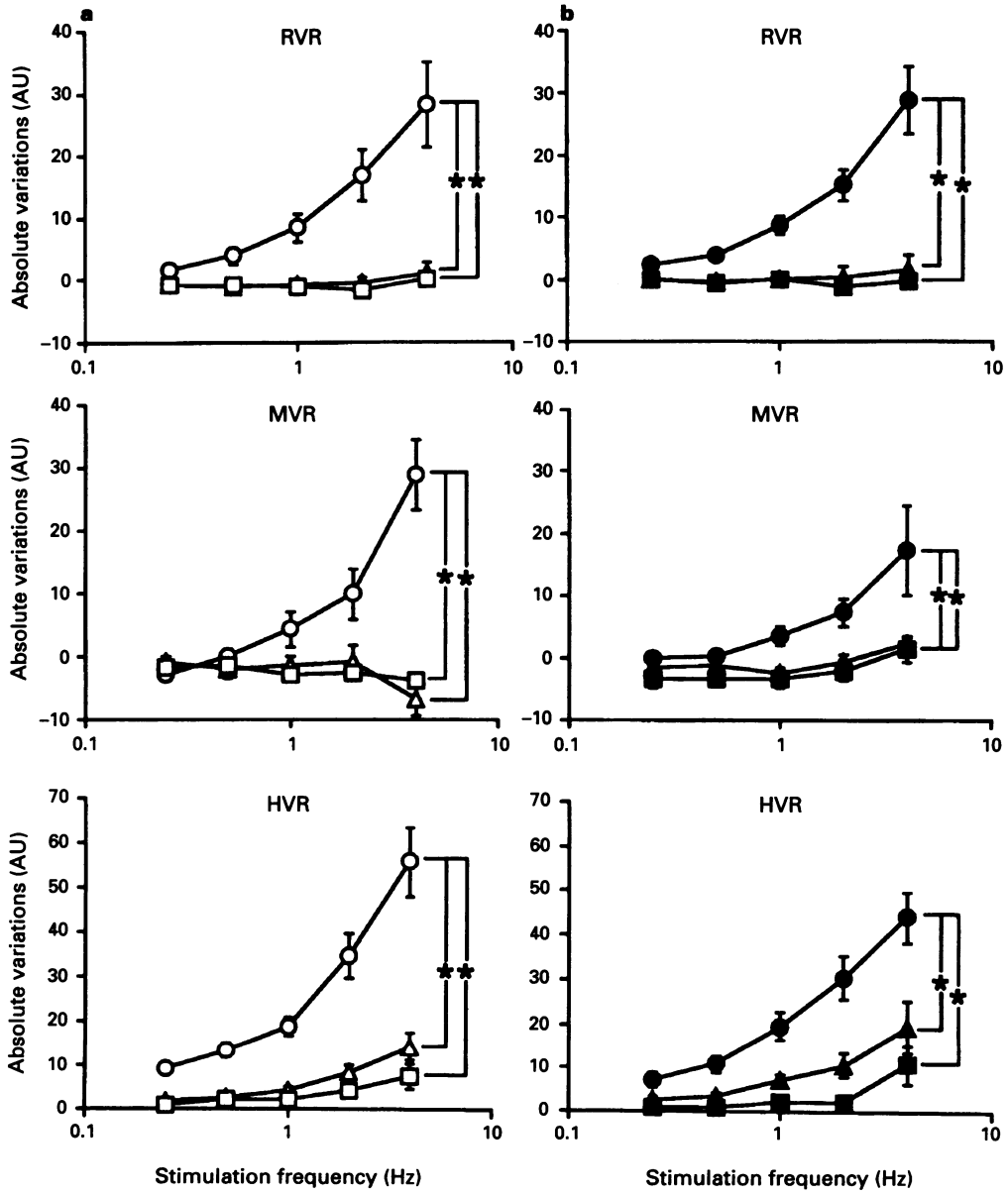

Figure 2 Mean ± s.e.mean absolute variations of renal (RVR), mesenteric (MVR) and hindlimb (HVR) vascular resistances induced by electrical stimulation of the spinal cord in control (○, ●), in perindopril-treated (□, ■) and in losartan-treated (△, ▲) pithed SHRs in the absence (a: ○, □, △) or in the presence (b: ●, ■, ▲) of icatibant. AU : arbitrary units. *Significant difference from corresponding control frequency-response curve: P at least <0.05.

kg^{-1}, i.v.). After a 15 min stabilization period, basal control values of MAP, HR, CO, RBF, MBF, HBF and corresponding resistances (TPVR, RVR, MVR, HVR) were determined. Half of the animals of each group were then given the selective bradykinin B_2 receptor antagonist, icatibant (10 $\mu g\ kg^{-1}$, i.v., $n=15-16$), the other half received saline ($n=12-14$).

Systemic pressor, tachycardic and regional vasoconstrictor responses to electrical stimulation of the spinal cord (0.25-4 Hz, 1 ms pulses, 60 V for 20 s) (Stimulator ST 198, Janssen Scientific Instruments, Paris, France) were recorded 10 min after icatibant or saline administration. At the end of the experiments, pressor, systemic and regional vascular responses to AI (300 ng kg^{-1}, i.v.) and AII (300 ng kg^{-1}, i.v.) were recorded in all animals.

Effects of icatibant on haemodynamic responses to bradykinin in intact anaesthetized SHRs Eighteen SHRs were randomized into two groups ($n=9$, each). They were anaesthetized (sodium pentobarbitone, 50 mg kg^{-1}, i.p.) and were instrumented for measurement of blood pressure, CO and HBF by the pulsed Doppler technique. They received either icatibant (10 $\mu g\ kg^{-1}$, 10 $\mu l\ kg^{-1}$, i.v.) or saline (10 $\mu l\ kg^{-1}$, i.v.). Ten minutes later, the cardiac and haemodynamic effects of increasing doses of bradykinin (1, 3, 10 and 30 $\mu g\ kg^{-1}$, i.v.) were recorded in both groups.

Calculations and statistical analysis of data

Results are expressed as means ± s.e.mean. Comparisons of mean basal values were carried out by analysis of variance followed by a Student's t test with the Bonferroni correction for multiple comparisons between groups. Mean absolute variations of the different parameters investigated induced by increasing frequencies of electrical stimulation of the spinal cord or increasing doses of bradykinin were analyzed by analysis of variance for repeated measurements using the Greenhouse-Geisser adjustment according to Ludbrook (1994) with electrical stimulation or bradykinin as a within-subject factor and treatment as a between-subject factor. Statistical comparisons for mean absolute variations induced by AI or AII were performed by analysis of variance followed by the Bonferroni test for multiple comparisons.

A P value less than 0.05 was considered to be statistically significant.

Statistical analyses were performed with a BMDP statistical software.

Drugs

Drugs used were angiotensin I (Beckman Instruments Bioproducts/Microbics, Geneva, Switzerland), angiotensin II (Hypertensin, Ciba-Geigy, Basle, Switzerland), atropine sulphate (Sigma Chemical Co., Saint-Quentin-Fallavier, France), bradykinin acetate (Sigma Chemical Co., Saint-Quentin-Fallavier, France), gallamine triiodide (Rhône-Poulenc-Rorer, Vitry-sur-Seine, France), icatibant (Hoe 140, or D-Arg-Arg-Pro-Hyp-Gly-Thr-Ser-D-Tic-Oic-Arg acetate, Hoechst AG, Frankfurt, Germany), losartan (potassium salt, Merck, Sharp & Dohme, West Point, U.S.A.), pentobarbitone sodium (Abbott, Saint-Rémy sur Avre, France) and perindopril (terbutylamine salt, Servier, Courbevoie, France). Doses are expressed in terms of the salt.

Results

Effects of icatibant on sympathetic responses to electrical stimulation of the spinal cord in control, perindopril-treated or losartan-treated pithed SHRs

Table 1 shows basal values of MAP, HR, CO, RBF, MBF, HBF, TPVR, RVR, MVR and HVR measured or calculated in

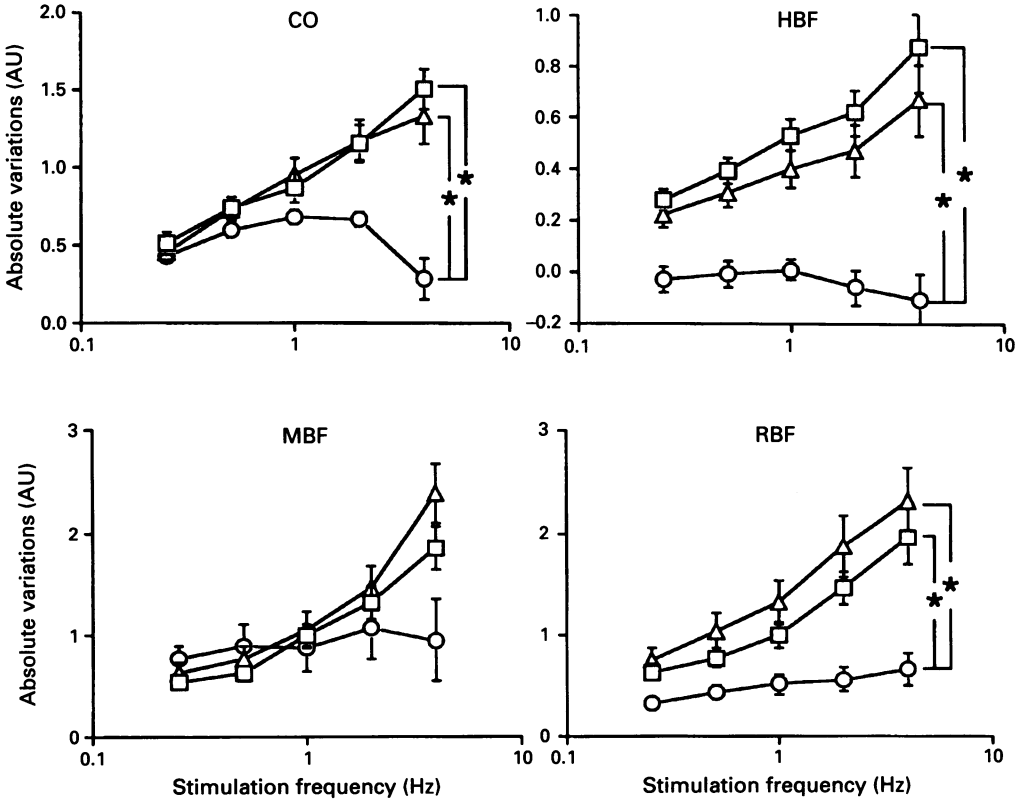

Figure 3 Mean ± s.e.mean absolute variations of cardiac output (CO), renal (RBF), mesenteric (MBF) and hindlimb (HBF) blood flows induced by electrical stimulation of the spinal cord in control (○), in perindopril-treated (□) and in losartan-treated (△) pithed SHRs in the absence of icatibant. AU: arbitrary units. *Significant difference from corresponding control frequency-response curve: P at least <0.05.

the three groups of animals after pithing and before icatibant or saline administration. Perindopril, and to a lesser extent, losartan induced a significant reduction in MAP (perindopril: −26%, $P<0.05$; losartan: −17%, $P<0.05$). TPVR (perindopril: −10%, NS; losartan: −14%, $P<0.05$), RVR (perindopril: −27%, $P<0.05$; losartan: −19%, NS) and HVR (perindopril: −37%, $P<0.05$; losartan: −27%, $P<0.05$) were also reduced whereas MVR was not affected by losartan and only slightly reduced by perindopril (−12%, NS). HR, CO and regional blood flows were never significantly affected by the drugs. In the doses used for the two drugs, there was no statistical difference between their respective haemodynamic effects, except for MAP which was decreased to a larger extent by perindopril than by losartan.

Figures 1, 2 and 3 illustrate the effects of electrical stimulation of the spinal cord in the absence (Figures 1a, 2a and 3) and in the presence (Figures 1b and 2b) of icatibant in the three experimental groups of animals. In the absence of icatibant (Figures 1a and 2a), the pressor and systemic and regional vasoconstrictor responses to electrical stimulation of the spinal cord were significantly reduced, and to a similar extent, by both perindopril and losartan, as compared to control animals. Simultaneously, spinal cord stimulation induced in control animals small and non frequency-dependent increases in CO, RBF and MBF, HBF remaining unaffected. Both losartan and perindopril potentiated the increases in CO, RBF and MBF, especially at the high stimulation frequencies, and increased significantly HBF (Figure 3). Finally, the tachycardic responses were never affected by drugs. (Figure 1).

Icatibant, 10 min after injection, did not cause any significant variation in blood pressure, blood flows and vascular resistances in any of the three experimental groups. In the presence of icatibant, perindopril and losartan still exerted significant sympathoinhibitory effects of similar magnitude (Figures 1b and 2b). Furthermore, in none of the experimental groups did these effects differ from those observed in the absence of icatibant (Figures 1b and 2b vs Figures 1a and 2a). Simultaneously, the effects of losartan and perindopril on spinal cord stimulation-induced variations in CO and regional flows were similar in the presence and in the absence of icatibant.

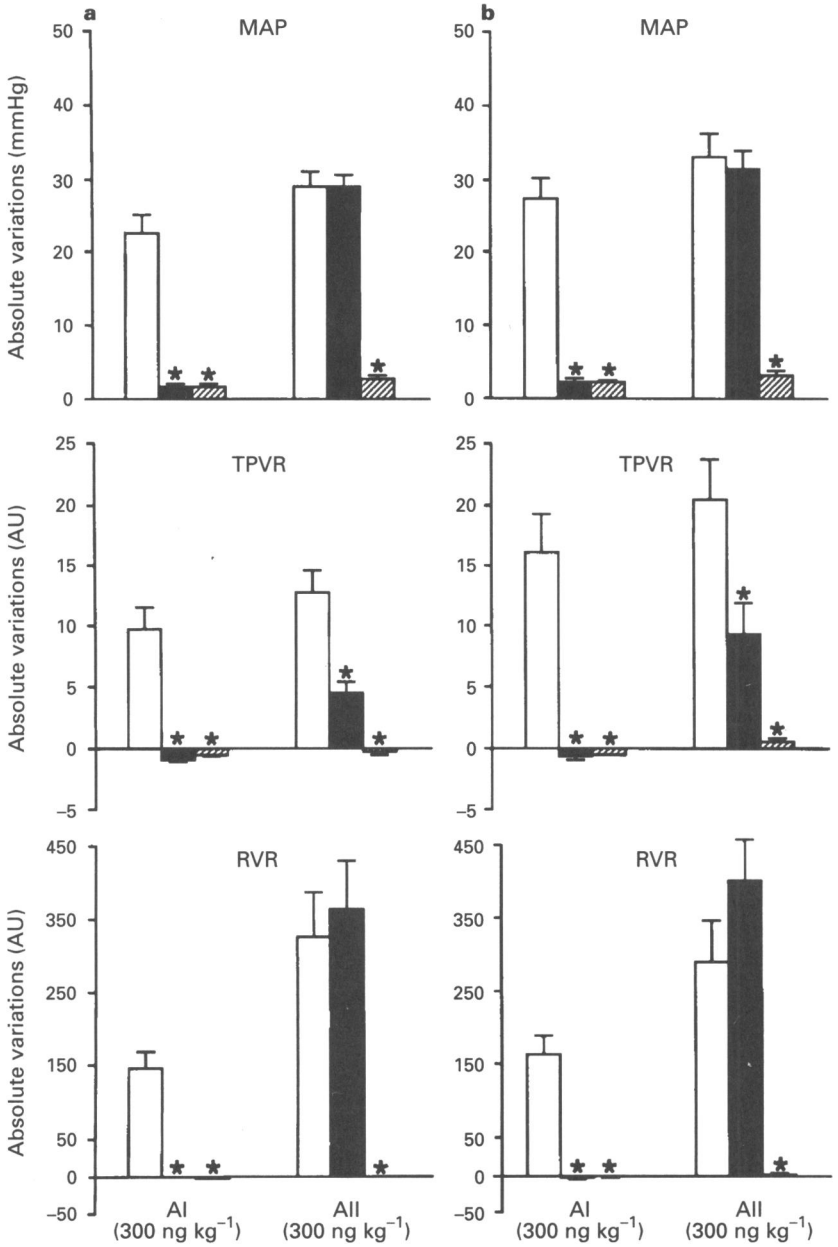

Figure 4 Mean ± s.e.mean absolute variations of mean arterial pressure (MAP), total peripheral (TPVR) and renal (RVR) vascular resistances induced by AI (300 ng kg^{-1}, i.v.) and AII (300 ng kg^{-1}, i.v.) in control (open columns), in perindopril-treated (solid columns) and in losartan-treated (hatched columns) pithed SHRs in the absence (a) or in the presence (b) of icatibant. AU: arbitrary units. *Significant difference from corresponding control value: P at least <0.05.

In the absence of icatibant (Figure 4a), perindopril significantly reduced or even abolished pressor, systemic and vasoconstrictor responses to AI, whereas pressor and renal vasoconstrictor responses to AII were maintained. However, the systemic vasoconstrictor effect of AII was surprisingly reduced. Losartan significantly abolished the haemodynamic responses to AI and to AII. Moreover, the responses to AI in the losartan group were not statistically different from those observed in the perindopril group.

Icatibant (Figure 4b) never interfered with the vascular effects of either AI or AII in any experimental group.

Blockade of haemodynamic responses to bradykinin by icatibant in intact anaesthetized SHRs

Figure 5 shows that bradykinin dose-dependently reduced MAP, TPVR and HVR. Following administration of icatibant (10 $\mu g\ kg^{-1}$, i.v.), the hypotensive and vascular responses to bradykinin were abolished (up to 10 $\mu g\ kg^{-1}$) or significantly reduced at higher doses (30 $\mu g\ kg^{-1}$).

Discussion

The aim of the present study was to evaluate the possible involvement of bradykinin in the vascular sympathoinhibitory effects of ACEIs in the pithed SHR. It appears from our data that when perindopril, which as an ACEI reduces bradykinin breakdown, and losartan, which is devoid of such an effect, are administered to SHRs in doses that inhibit to the same extent AI-induced vascular responses, these two drugs exert quantitatively similar vascular sympathoinhibitory effects. In addition, we also show that these effects are not affected by prior bradykinin B_2-receptor blockade. Taken altogether, these results demonstrate that endogenous bradykinin is not involved, through B_2-receptor activation, in the sympathoinhibitory effects of ACEIs.

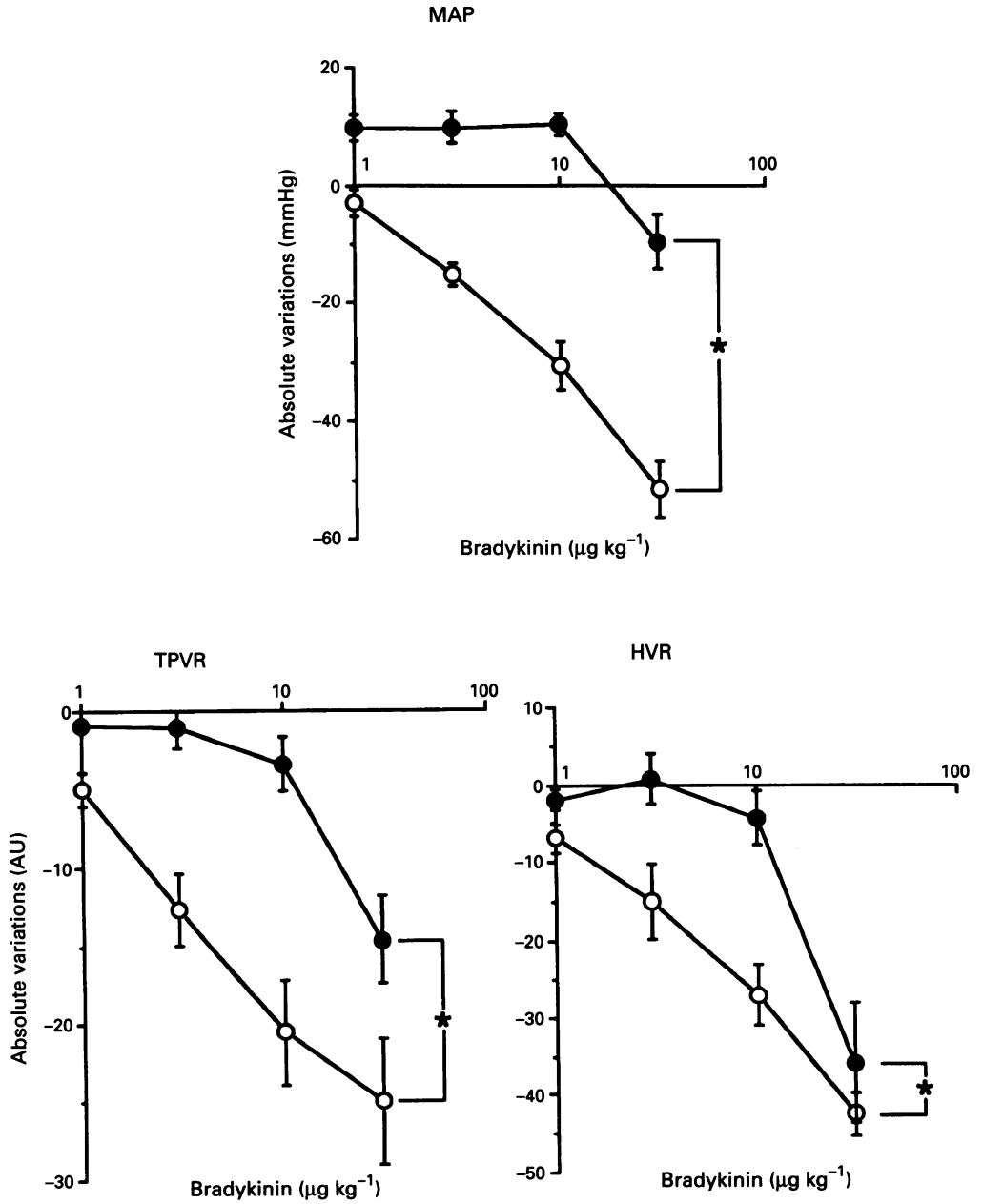

Figure 5 Mean±s.e.mean absolute variations of mean arterial pressure (MAP), total peripheral (TPVR) and hindlimb (HVR) vascular resistances induced by increasing doses of bradykinin (1 to 30 $\mu g\ kg^{-1}$) in the absence (○) or in the presence (●) of icatibant in anaesthetized intact SHRs. AU: arbitrary units. *Significant difference from corresponding control dose-response curve: P at least <0.05.

Previous findings strongly suggest that the mechanism underlying the sympathoinhibitory effects induced by ACEIs and AII AT_1 receptor antagonists consists mainly in the prejunctional suppression of AII-induced facilitation of noradrenaline release and, to a lesser extent, in an interference at the level of postsynaptic α-adrenoceptors (Antonaccio & Kerwin, 1981; Richer *et al.*, 1984; 1986; De Jonge *et al.*, 1986; Moreau *et al.*, 1993). In contrast, drug-induced reduction in basal arteriolar smooth muscle tone has clearly been shown not to be involved in these sympathoinhibitory effects (Richer *et al.*, 1984). There is to date growing evidence that some of the antihypertensive (Thurston & Swales, 1978; Swartz *et al.*, 1979; Bao *et al.*, 1992; Cachofeiro *et al* 1992; Bouaziz *et al.*, 1994), cardioprotective (Schölkens *et al.*, 1988; Gohlke *et al.*, 1994) and antiproliferative (Linz & Schölkens, 1992; Farhy *et al.*, 1993) effects of ACEIs are partly due to reduced bradykinin degradation with, as a result, an increase in endogenous bradykinin levels. Thus, the potential contributory role of bradykinin to the sympathoinhibitory effects of ACEIs needed to be investigated.

For this purpose, we compared in our study the vascular sympathoinhibitory effects of an ACEI, perindopril, with those of an AII AT_1 receptor antagonist, losartan. The first prerequisite for this study was to use doses of the two drugs equipotent in inhibiting the systemic and regional vascular responses to exogenous AI, and our data show that this goal was achieved. Moreover, perindopril and losartan were administered over a 1 week period in order to allow them to reach the tissue renin-angiotensin system, especially within the vascular wall, and to prevent the consequences of the acute stimulation of renin secretion induced by the pithing procedure (Clough *et al.*, 1982). A second prerequisite in our study was to use a dose of perindopril that really produced bradykinin accumulation. This prerequisite was most likely also fulfilled as Campbell *et al.* (1994) have recently demonstrated that a 7 day-oral administration of perindopril increases circulating and tissue levels of bradykinin in a dose-related manner starting from a threshold of 0.052 mg kg^{-1} per day, i.e., a 58 fold-lower dose than that we used in the present study.

The interaction of bradykinin itself with the sympathetic nervous system remains controversial in its mechanism (Schwieler & Hjemdahl, 1992). Exogenous bradykinin has been shown to reduce dose-dependently the vasoconstrictor responses to sympathetic nerve stimulation and to injections of noradrenaline (Malik & Nasjletti, 1979). Bradykinin might thus contribute to the sympathoinhibitory effects of ACEIs (a) by inhibiting the nerve impulse-evoked release of noradrenaline through direct activation of prejunctional B_2-receptors, and/or (b) by attenuating noradrenaline-induced vasoconstriction postjunctionally through endothelial B_2-receptor-mediated release of vasodilating factors such as prostaglandins (PGE_2) (Schwieler *et al.*, 1993; Ehring *et al.*, 1994), and/or nitric oxide (Linz *et al.*, 1992). However, it must be kept in mind that in other studies (see Introduction), bradykinin has been reported not to affect or even to enhance catecholamine secretion.

In this context, we used the selective B_2-receptor antagonist, icatibant, to investigate further the mechanism of the interaction between bradykinin and the peripheral sympathetic nervous system in the *in vivo* model of the pithed SHR, both under basal conditions and during ACE inhibition. Previous data (Bao *et al.*, 1991; Wirth *et al.*, 1991) have shown that in rats, icatibant is a highly potent and long-acting B_2-receptor antagonist, devoid of any intrinsic effect on catecholamine release. In the dose we used, icatibant completely opposed the haemodynamic effects of exogenous bradykinin up to a 10 μg kg^{-1} dose which produces plasma levels far higher than those that could be expected from endogenous sources (Campbell *et al.*, 1994). Three points appear clearly from our data. The first is that in pithed SHRs, icatibant did not affect any of the haemodynamic parameters investigated in any of the three experimental groups, especially in the perindopril-treated one in which high levels of circulating bradykinin may be expected. Hence, bradykinin does not appear to exert any tonic influence on vascular resistance in our experimental model. The second point is that in the pithed SHR, selective B_2-receptor blockade by icatibant does not affect the responses to electrical stimulation of the spinal cord in control animals. This demonstrates that endogenous bradykinin does not, through B_2-receptor activation, affect the release of noradrenaline evoked by sympathetic nerve stimulation. The final point is that icatibant does not modify in any way the sympathoinhibitory effects exerted by perindopril in pithed SHRs, which tends to exclude any participation of bradykinin in these effects.

In conclusion, endogenous bradykinin, through activation of B_2-receptors, does not seem to be involved in the sympathoinhibitory effects exerted by ACEIs in the pithed SHR.

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