A specific B₂-bradykinin receptor antagonist HOE 140 abolishes the antihypertrophic effect of ramipril

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To evaluate the role of bradykinin in the antihypertrophic effect of the angiotensin-converting enzyme (ACE) inhibitor, ramipril, we investigated the influence of HOE 140, a specific B₂-receptor antagonist, on the effects of ramipril on left ventricular hypertrophy (LVH) in rats with aortic banding. Ramipril at a dose of 1 mg kg⁻¹ day⁻¹ for 6 weeks prevented the increase in blood pressure and development of LVH after aortic banding; plasma ACE activity was significantly inhibited. A lower dose of ramipril (10 µg kg⁻¹ day⁻¹ for 6 weeks) had no effect on the increase in blood pressure or on plasma ACE activity, but prevented LVH after aortic banding. The antihypertrophic effects of the higher and the lower dose ramipril, as well as the antihypertensive action of the higher dose of ramipril were abolished by the coadministration of HOE 140 (500 µg kg⁻¹ day⁻¹). The present data show for the first time that the beneficial effects of an ACE-inhibitor on LVH in rats with hypertension caused by aortic banding can be prevented by a specific B₂-receptor antagonist.

Keywords: Left ventricular hypertrophy; angiotensin converting enzyme inhibition; ramipril; bradykinin; HOE 140

Introduction The renin angiotensin system (RAS) has been implicated in the development and maintenance of hypertension and cardiac hypertrophy (Schelling *et al.*, 1991). In the coarctation model of hypertension angiotensin-converting enzyme (ACE) inhibitors have been shown to inhibit the development, and to induce regression, of cardiac hypertrophy in doses without effects on blood pressure (Linz *et al.*, 1989). Some findings indicate that ACE inhibitors suppress the cardiac hypertrophic response by reducing the formation of angiotensin II (AII), which stimulates hypertrophy and matrix protein synthesis (Schelling *et al.*, 1991). However, since inhibition of ACE, besides reducing AII, also increases bradykinin levels, it is conceivable that bradykinin, through the subsequent generation of nitric oxide (NO) (Wiemer *et al.*, 1991), also contributes to the prevention of the hypertrophic response by ACE inhibitors.

In the present study we investigated the influence of HOE 140 (D-Arg-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸] bradykinin), a specific B₂-receptor antagonist (Wirth *et al.*, 1991) on the antihypertrophic effect of ramipril in LVH in rats made hypertensive by aortic banding.

Methods Male Sprague Dawley rats weighing 280–300 g (Møllegaard, Skensved, Denmark) were fasted for 12 h before surgery. Anaesthesia was induced by i.p. injection of 200 mg kg⁻¹ hexobarbitone (Evipan). The abdomen was opened by a cut parallel to the linea alba. The abdominal aorta was exposed above the left renal artery and a silk thread was passed under it. A cannula no. 1 (0.9 × 40 mm) was placed longitudinally on the aorta and both aorta and cannula were tied. The cannula was then removed, leaving an aortic lumen determined by the diameter of the cannula. Before the abdomen was closed with catgut, the animals received 5.5 mg rolitetracycline (Reverin, Hoechst AG, Frankfurt, Germany). The skin was closed by clipping and covered with tar spray. Control animals were subjected to the same procedure, but without aortic banding.

During the first 5 days following the operation the animals received tetracycline (1 g 350 ml⁻¹) in the drinking water. The animals were allotted to 6 groups of 20 rats each as follows: Group I, sham-operated; Group II, aortic banding without

treatment; Group III, aortic banding, and ramipril treatment with 1 mg kg⁻¹ day⁻¹; Group IV, aortic banding, ramipril treatment with 1 mg kg⁻¹ day⁻¹ and HOE 140 500 µg kg⁻¹ day⁻¹; Group V, aortic banding, and ramipril treatment with 10 µg kg⁻¹ day⁻¹; Group VI, aortic banding, ramipril treatment with 10 µg kg⁻¹ day⁻¹, and HOE 140 500 µg kg⁻¹ day⁻¹. Ramipril treatment started the day after the operation and continued for 6 weeks via the drinking water. At the end of the operation miniosmotic pumps were implanted subcutaneously in the neck of the animals to deliver HOE 140. At the end of the experiment (after 6 weeks), the animals were anaesthetized with 200 mg kg⁻¹ i.p. hexobarbitone and blood pressure was measured via catheters in the left carotid artery. The hearts were excised, cleaned of blood with saline, gently blotted to dryness, and left ventricular weight (LVW) including the septum as well as the remaining cardiac tissue representing the right ventricle (RVW) were determined (to the nearest 0.1 mg). Weights are given per 100 g body weight. Plasma ACE activity was determined radioenzymatically by use of [³H]-Hip-Gly-Gly as substrate. Ramipril and HOE 140 were dissolved in saline.

Statistical analysis was performed with ANOVA followed by the Bonferroni test when appropriate. Differences were considered significant if $P < 0.05$. Results are given as mean ± s.d.

Results The higher dose of ramipril (1 mg kg⁻¹ day⁻¹) prevented the increase in blood pressure after aortic banding as well as the development of LVH (Figure 1a,b). Plasma ACE activity was significantly inhibited (Table 1). The lower dose of the ACE inhibitor (10 µg kg⁻¹ day⁻¹) after six weeks neither prevented the increase in blood pressure nor lowered plasma ACE activity, but it prevented the development of LVH (Figure 1a,b; Table 1).

The antihypertrophic effects of the higher and lower dose of ramipril and the antihypertensive action of the higher dose of ramipril were both abolished by the coadministration of HOE 140 (Figure 1a,b). Plasma ACE activity was not changed by HOE 140 treatment (Table 1). Right ventricular weight (46–51 mg per 100 g body weight) and body weight (between 468 ± 8 and 475 ± 9) did not differ between groups.

Discussion The present data show for the first time that the beneficial effects of an ACE inhibitor on LVH in rats with

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Figure 1 Effects of orally administered ramipril ($1 \text{ mg kg}^{-1} \text{ day}^{-1}$ and $10 \mu\text{g kg}^{-1} \text{ day}^{-1}$), and coadministration of HOE 140 ($500 \mu\text{g kg}^{-1} \text{ day}^{-1} \text{ s.c.}$) on (a) mean blood pressure (MBP) and (b) left ventricular weight (LVW) in rats with aortic banding: (■) Sham; (▨) control vehicle; (▤) ramipril 1 mg kg^{-1} ; (▥) ramipril $1 \text{ mg} + \text{HOE 140}$; (▧) ramipril $10 \mu\text{g kg}^{-1}$; (▩) ramipril $10 \mu\text{g} + \text{HOE 140}$. * $P < 0.05$ vs sham, # $P < 0.05$ vs control.

hypertension caused by aortic banding can be prevented by a specific B_2 -receptor antagonist. At the end of the study the blood pressure lowering effect in the group treated with the

higher dose of ramipril was abolished by HOE 140, as observed for the acute antihypertensive actions of ramipril and their reversal by a B_2 -receptor antagonist (Danckwardt *et al.*, 1990). However, under both treatment regimens, in the present study with and without blood pressure reduction, the antihypertrophic effect of ramipril was abolished by HOE 140. This observation provides evidence for a possible involvement of bradykinin in the antihypertrophic effect of ramipril in LVH.

Both ramiprilat and bradykinin have been shown to increase nitric oxide and prostacyclin release in cultured endothelial cells (Wiemer *et al.*, 1991). Nitric oxide and nitric oxide-generating vasodilators have been demonstrated to be antimitogenic (Garg & Hassid, 1989). Similar effects are known for prostacyclin and adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Shirotani *et al.*, 1991). Thus, both nitric oxide and prostacyclin when increased by BK following ACE inhibition may participate in the beneficial effects of ACE inhibitors.

On the other hand AII has been claimed to be a myocardial growth factor (Schelling *et al.*, 1991). However, the specific blockade of AII AT_1 -receptors, seems to be less effective in this respect than ACE inhibition. In a recent study in the same experimental model, the AII AT_1 -receptor antagonist, DuP 753 (Losartan), given at a high dose that lowered blood pressure, was less effective on cardiac hypertrophy than ramipril at a low dose which did not lower blood pressure (Linz *et al.*, 1991).

In summary our data in rats with aortic banding and left ventricular hypertrophy provide evidence that the ACE inhibitor-induced potentiation of bradykinin contributes to the beneficial effects of ACE inhibitors.

Table 1 Effects of orally administered ramipril and subcutaneous coadministration of HOE 140 on plasma angiotensin converting enzyme (ACE) activity

	Sham	Control	Ramipril (1 mg)	Rami (1 mg) + HOE 140	Ramipril (10 µg)	Rami (10 µg) + HOE 140
Plasma-ACE activity	224 ± 0	219 ± 9	$61 \pm 6^{* \#}$	$78 \pm 6^{* \#}$	217 ± 7	222 ± 8

$P < 0.05$; *, vs Sham; #, vs control; plasma ACE activity: ($\text{nmol min}^{-1} \text{ ml}^{-1}$). Sham: sham-operated animals; Control: untreated controls with aortic banding; Ramipril 1 mg: ramipril $1 \text{ mg kg}^{-1} \text{ day}^{-1}$; Ramipril 10 µg: ramipril $10 \mu\text{g kg}^{-1} \text{ day}^{-1}$; HOE 140: B_2 -receptor antagonist ($500 \mu\text{g kg}^{-1} \text{ day}^{-1}$).

References

- DANCKWARDT, L., SHIMIZU, I., BÖNNER, G., RETTIG, R. & UNGER, Th. (1990). Converting enzyme inhibition in kinin-deficient brown norway rats. *Hypertension*, **16**, 429–435.
- GARG, U.C. & HASSID, A. (1989). Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J. Clin. Invest.*, **83**, 1774–1777.
- LINZ, W., SCHÖLKENS, B.A. & GANTEN, D. (1989). Converting enzyme inhibition specifically prevents the development and induces regression of cardiac hypertrophy in rats. *Clin. Exp. Hypertens.*, **A11**, 1325–1350.
- LINZ, W., HENNING, R. & SCHÖLKENS, B.A. (1991). Role of the angiotensin II receptor antagonism and converting enzyme inhibition in the progression and regression of cardiac hypertrophy in rats. *J. Hypertension*, **9** (Suppl. 6), S400–S401.
- SCHELLING, P., FISCHER, H. & GANTEN, D. (1991). Angiotensin and cell growth a link to cardiovascular hypertrophy? *J. Hypertension*, **9**, 3–15.
- SHIROTANI, P., YUI, Y., HATTORI, R. & KAWAI, C. (1991). U-61,431F, a stable prostacyclin analogue, inhibits the proliferation of bovine vascular smooth muscle cells with little antiproliferative effect on endothelial cells. *Prostaglandins*, **41**, 97–110.
- WIEMER, G., SCHÖLKENS, B.A., BECKER, R.H.A. & BUSSE, R. (1991). Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. *Hypertension*, **18**, 558–563.
- WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPOULOS, H., HENKE, St., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). HOE 140 a new potent and long acting bradykinin-antagonist: *in vivo* studies. *Br. J. Pharmacol.*, **102**, 774–777.

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