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Original article

Angiotensin II receptor blockade unmasks a depressor response to endothelin antagonists in rats

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Abstract – Endothelin (ET) antagonists do not decrease blood pressure in normal rats. Since angiotensin II (AII) and ET both induce smooth muscle cell contraction through the same transduction pathways we designed experiments to assess whether blockade of the renin angiotensin system would unmask a vasodilatory response to ET receptor antagonists in rats. For this purpose, we tested the effect on arterial blood pressure of the mixed ETA-ETB receptor antagonist bosentan or of the ETA antagonist BQ-123 in the absence or the presence of the AII receptor antagonist losartan. In control conditions bosentan did not affect arterial blood pressure. In contrast, in losartan-pretreated rats, bosentan induced a marked, dose-dependent decrease in arterial pressure (% change after bosentan 10 mg/kg: control -3 ± 3; losartan -32 ± 6; cilazapril -28 ± 3). Similarly, BQ-123 decreased blood pressure in losartan-pretreated but not in control rats. Bosentan also increased the hypotensive effect of losartan in conscious, normotensive rats. The hypotensive effect of the combination of bosentan and losartan was not associated with any changes in cardiac output or heart rate, and thus was entirely due to a decrease in total peripheral resistance. We conclude that blockade of angiotensin II, AT_1 receptors unmasks a vasodilator response to ET antagonists. This suggests that endogenous ET plays an active role in the maintenance of arterial blood pressure in rats which can be unmasked by a concomitant inhibition of the renin angiotensin system. © 2000 Éditions scientifiques et médicales Elsevier SAS

angiotensin II / endothelin / arterial pressure

1. Introduction

Although endothelin-1 (ET-1) has been shown to play a role in the maintenance of blood pressure in various pathophysiological situations, the exact role of this vasoconstrictor peptide in the control of vascular tone in normal conditions is less clear. Indeed, ET antagonists do not decrease blood pressure in normal rats [1, 2]. This could be interpreted as an indication that the endothelin system does not significantly contribute to physiological regulation of blood pressure. However, regulation of peripheral arterial tone is complex and depends on many interactions between vasomotor influences. For example in normal animals, endogenous NO permanently inhibits both the release and the vasoconstrictor effects of endothelin, and we have shown that blockade of NO synthesis unmasks an endothelin-induced vasopressor tone in rats [2]. Similarly, numerous interactions also exist between angiotensin II and endothelin. Angiotensin II stimulates the production of ET-1 [3, 4], and potentiates the contractions induced by ET-1 [5]. Moreover, ET-1 and AII induce smooth muscle cell contraction by the same transduction pathways (i.e. mainly though G protein-mediated activation of phospholipase C). Synergistic effects between angiotensin converting enzyme (ACE) inhibitors and endothelin antagonists have indeed been described in experimental models of heart failure [6] and hypertension [7]. Thus, it is conceivable that blockade of the renin angiotensin system may unmask an active contribution of ET to vasopressor tone, even in normal animals. Identification of the interactions between ET antagonists and angiotensin II antagonists or ACE inhibitors is also important given the fact that the two classes of drug may be commonly associated in the clinical setting.

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Thus, the present study was designed to assess whether combined blockade of the renin angiotensin and endothelin systems exerts vasodilatory effects that are superior to those induced by blockade of each system alone.

2. Methods

2.1. Anaesthetized animal preparation

Experiments were performed in male Wistar rats (Charles River France) weighing between 300–400 g that were anaesthetized with 50–100 mg.kg^{-1} thiopental sodium intraperitoneally. A tracheotomy was performed after midline neck incision and the rats were mechanically ventilated with room air supplemented with low-flow oxygen using a small rodent ventilator (Apelex, Massy, France) at a rate of 70 cycles per min and a tidal volume of 10 mL.kg^{-1} body weight. The respiratory rate and tidal volume were adjusted to maintain arterial blood gases within a normal range. Body temperature was maintained at 37 °C with a thermostatically controlled heating blanket connected to a rectal thermometer. The right jugular vein was cannulated for injection of drugs, which were given as i.v. bolus (0.2 mL). The left carotid artery was cannulated and a small Millar Mikrotip catheter (model SPR 407, Millar) was inserted into the artery to measure arterial blood pressure.

An ECG was also obtained with standard limb electrodes. ECG and arterial pressure were continuously recorded on a Gould Windowgraph recorder.

In experiments involving the measurement of cardiac output, rats were instrumented as described above, after which they were subjected to a left thoracotomy, and a Doppler probe was placed around the thoracic aorta. The probe was connected to a pulsed Doppler flowmeter (Triton system 6). Cardiac output and total peripheral resistance were then calculated from the measured aortic velocity.

2.2. Protocols

2.2.1. Interactions between ET antagonists and AT_1 antagonists or ACE inhibitors

Rats received saline, losartan 0.3, 1 or 3 mg.kg^{-1} or cilazapril 1 mg.kg^{-1} ($n = 6$ in each group). Fifteen minutes later, increasing doses of bosentan (0.3–10 mg.kg^{-1}) were administered. A 15 min period elapsed between each dose of bosentan, and arterial pressure and heart rate were measured 15 min after administration of each dose. In the study of the interaction between BQ-123 and losartan, only one dose of losartan was used (3 mg.kg^{-1}). In another series of experiments rats received either saline or bosentan 3 mg.kg^{-1} ($n = 6$ in each group). Fifteen minutes later, increasing doses of losartan (0.3–10 mg.kg^{-1}) were administered. In the experiments in which cardiac output was measured, rats ($n = 6$ in each group) received either saline, losartan (3 mg.kg^{-1}), bosentan (3 mg.kg^{-1}), or the combination of losartan plus bosentan.

2.2.2. Effects of bosentan on the pressor response to angiotensin II

Rats ($n = 6$ per group) received bosentan (3 mg.kg^{-1}) or saline. Fifteen minutes later, increasing doses of angiotensin II (0.01–10 μg.kg^{-1}) were administered. Arterial pressure and heart rate were measured at the peak effect of each dose.

2.2.3. Effects of losartan on the response to big ET-1

Rats ($n = 6$ per group) received losartan (3 mg.kg^{-1}) or saline. Fifteen minutes later, cumulative increasing doses of big ET-1 (0.03–1 nmol.kg^{-1}) were administered. A 15 min period elapsed between each dose of big ET-1, and arterial pressure and heart rate were measured 15 min after administration of each dose.

2.2.4. Effects of bosentan, losartan or their combination on systolic arterial pressure in conscious rats

Twelve normotensive Wistar rats received, on separate days, either vehicle, bosentan (30 mg.kg^{-1}), losartan (10 mg.kg^{-1}) or their combination (30 mg.kg^{-1} bosentan + 10 mg.kg^{-1} losartan). All drugs were administered by gavage. Three hours later systolic blood pressure and heart rate were measured by tail cuff plethysmography.

2.3. Drugs

Bosentan (sodium salt) and cilazapril were gifts from F. Hoffman-la Roche (Basel). Losartan was a gift from MSD. BQ-123, big-Endothelin (1–38) and Angiotensin II were purchased from Neosystem.

2.4. Data analysis

All results were expressed as mean ± SEM. Results were compared by unpaired *t*-tests or by repeated measures ANOVA, followed, when ANOVA

was significant, by a Tukey test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of losartan (0.3, 1, 3 mg.kg^{-1}) or cilazapril (1 mg.kg^{-1}) on the response to bosentan

The effects of losartan on the response induced by bosentan are shown in *figure 1*. Baseline values of mean arterial blood pressure (MAP) were not different in the groups (140 ± 2, 140 ± 6, 138 ± 3, and 146 ± 7 mm Hg for saline, or losartan 0.3, 1, 3 mg.kg^{-1}). Losartan alone decreased MAP at the doses of 1 (−4 ± 2 %, P = NS) and 3 mg.kg^{-1} (−7 ± 1 %, $P < 0.05$). In control rats, bosentan had no effect on MAP (1 ± 1 %). In contrast, in losartan-pretreated rats, bosentan induced a dose-dependent decrease in MAP which reached a maximum of 9 ± 4, 26 ± 5, and 32 ± 6 % after losartan 0.3 mg.kg^{-1}, 1 mg.kg^{-1} or 3 mg.kg^{-1}, respectively. Heart rate did not change at any dose. The effects of cilazapril on the response induced by increasing doses of bosentan are shown in *figure 2*. Cilazapril alone induced a significant decrease in MAP (−19 ± 6 % from a baseline value of 143 ± 6). Bosentan induced an additional decrease in MAP which reached 28 ± 3 % at the highest dose ($P < 0.05$).

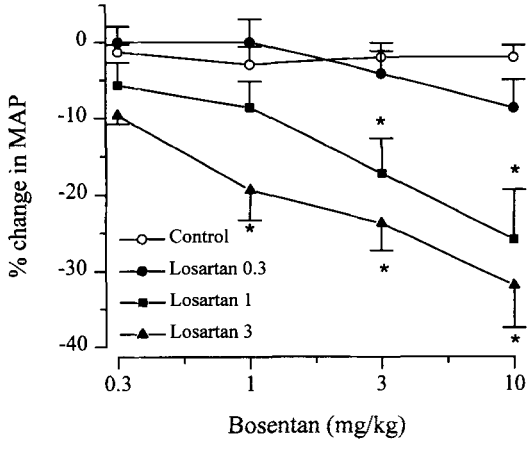

Figure 1. Effects of the AT_1 antagonist losartan (0.3, 1, 3 mg.kg^{-1}) on the response to the ET_A–ET_B antagonist bosentan in anaesthetized rats. Values are mean ± SEM of 6 rats per group. *$P < 0.05$ vs. baseline.

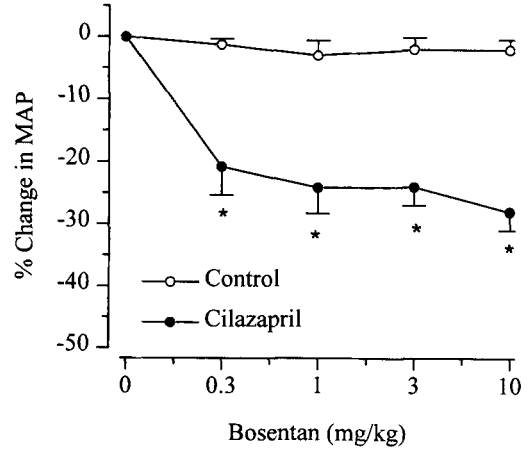

Figure 2. Effects of the angiotensin I converting enzyme inhibitor cilazapril (1 mg.kg^{-1}) on the response to the ET_A–ET_B antagonist bosentan in anaesthetized rats. Values are mean ± SEM of 6 rats per group. *$P < 0.05$ vs. baseline.

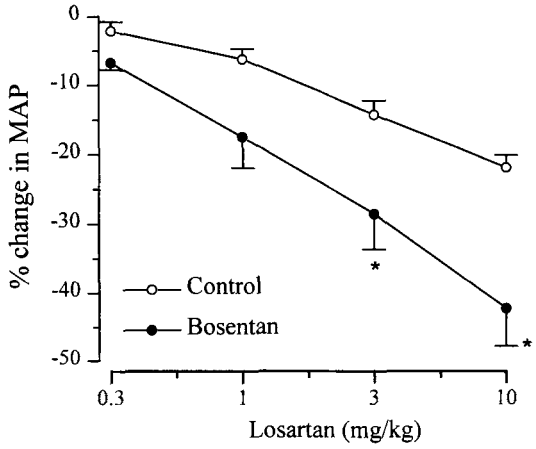

Figure 3. Effects of the ET_A–ET_B antagonist bosentan on the response to the AT_1 antagonist losartan in anaesthetized rats. Values are mean ± SEM of 6 rats per group. *$P < 0.05$ vs. baseline.

3.2. Effects of bosentan or BQ-123 on the response to losartan in anaesthetized rats

The effect of a pretreatment with bosentan on the response induced by increasing doses of losartan is shown in *figure 3*. In control conditions, losartan induced a dose-dependent decrease in MAP which reached 22 ± 2 % at the highest dose (from a baseline value of 146 ± 3 mm Hg). The effect of losartan on MAP was markedly increased in animals pretreated by bosentan. Indeed, in bosentan-treated rats, the decrease in MAP was 42 ± 6 % at the highest dose of losartan. Heart rate was slightly but not significantly decreased by the combined treatment (−9 ± 5 % after losartan 10 mg.kg^{-1}; data not shown).

BQ-123 (1 mg.kg^{-1}) also increased the response to losartan (3 mg.kg^{-1}). Indeed, losartan alone reduced MAP by 9 ± 3 %, while after BQ-123, the decrease in MAP reached 20 ± 4 % ($P < 0.05$), without a change in heart rate.

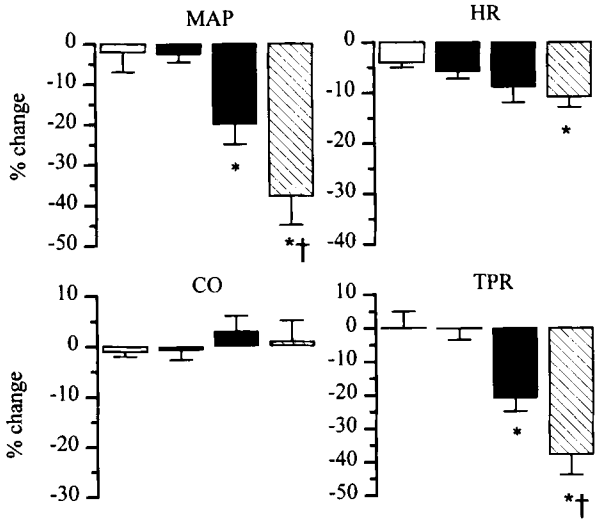

Figure 4. Effects of administration of saline (open bars), the ET_A–ET_B antagonist bosentan (3 mg.kg^{-1}, grey bars), the AT_1 antagonist losartan (3 mg.kg^{-1}, black bars), or their combination (striped bars) on mean arterial pressure (MAP), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) in open-chest anaesthetized rats. Values are mean ± SEM of 6 rats per group. *$P < 0.05$ vs. base; †$P < 0.05$ vs. losartan alone.

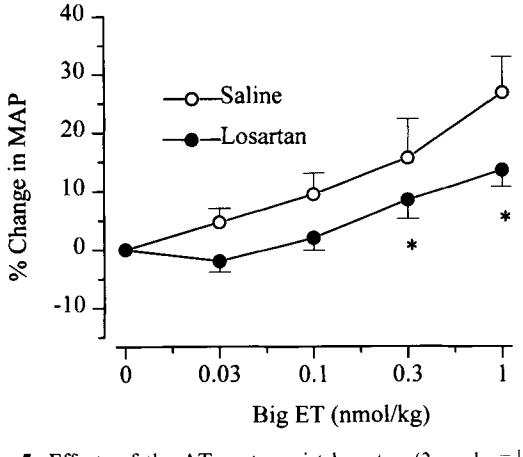

Figure 5. Effects of the AT_1 antagonist losartan (3 mg.kg^{-1}) on the pressor response induced by big endothelin-1 (0.03–1 nmol/kg) in anaesthetized rats. Values are mean ± SEM of 6 rats per group *$P < 0.05$ vs. control.

3.3. Effects of losartan, bosentan, or their combination on cardiac output (figure 4)

Neither saline nor bosentan alone affected arterial pressure, heart rate, cardiac output or total peripheral resistance. Losartan significantly reduced mean arterial pressure (20 ± 5 %) and total peripheral resistance (21 ± 4 %), without affecting cardiac output. Losartan also slightly decreased heart rate (9 ± 2 %). Combined treatment with bosentan and losartan decreased arterial pressure and total peripheral resistance, and these effects were significantly more marked than those induced by losartan alone. Indeed, the reductions in mean arterial pressure and total peripheral resistance were 38 ± 7 % and 38 ± 6 %, respectively. Heart rate was slightly decreased by the combined treatment (11 ± 2 %).

3.4. Effect of losartan on the response to big-ET (figure 5)

In control rats, big ET-1 induced a dose-dependent increase in MAP which reached 27 ± 6 % at the highest dose. This hypertensive effect of big ET-1 was markedly reduced by losartan. Indeed, after losartan, the maximal increase in MAP after big ET-1 was reduced to 13 ± 3 % ($P < 0.05$).

3.5. Effect of bosentan on the response to angiotensin II (figure 6)

Angiotensin II induced a dose-dependent increase in MAP which was not significantly affected by bosentan. Indeed, the maximal increase in MAP was 41 ± 7 % in control and 34 ± 9 % in bosentan-treated rats.

3.6. Effects of losartan, bosentan or their combination on systolic blood pressure (SBP) measured in conscious rats

Oral administration of vehicle or of bosentan (30 mg.kg^{-1}) had no effect on systolic blood pressure. Losartan (10 mg.kg^{-1}) alone slightly reduced blood pressure (6 ± 2 %). However, combined treatment with bosentan and losartan induced a decrease in systolic blood pressure which was more marked than that induced by losartan alone (15 ± 3 %; $P < 0.05$ vs. losartan).

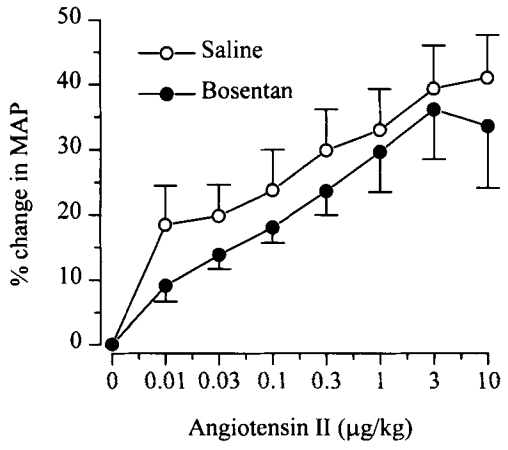

Figure 6. Effects of the ET_A–ET_B antagonist bosentan (3 $mg.kg^{-1}$) on the pressor response induced by angiotensin II (0.01–10 μg/kg) in anaesthetized rats. Values are mean ± SEM of 6 rats per group.

4. Discussion

The present study, performed in normotensive Wistar rats, with combined inhibition of angiotensin II and endothelin receptors, shows that blockade of the renin angiotensin system unmasks a potent vasodilatory response to endothelin antagonists. This suggests that endogenous ET-1 indeed contributes to the normal vasopressor tone, and that this contribution may be evidenced after blockade of the renin–angiotensin system.

The ET antagonist bosentan had no effect on blood pressure in normotensive rats, in agreement with previous results [1, 2]. However, in the presence of AT_1 blockade, bosentan induced a marked dose-dependent decrease in blood pressure. This decrease in blood pressure was entirely due to a peripheral vasodilation since in these conditions cardiac output did not change. Moreover, these results cannot be explained by pharmacological interactions between bosentan and losartan since similar results were obtained with the combination of BQ-123 and losartan, or of bosentan and cilazapril. In fact, the potentiation induced by cilazapril appeared more marked than that induced by losartan. Indeed, in the presence of cilazapril, bosentan already induced a maximal response at 0.3 $mg.kg^{-1}$, while at the same dose no effect of bosentan was observed in losartan-treated rats. Finally, the fact that we observed similar effects with the ET_A antagonist BQ-123 [8] and the mixed ET_A–ET_B antagonist bosentan [9] suggests that the vasodilatation we observed was due to blockade of the ETA receptors rather than interactions with the ET_B receptors present on the endothelium and/or the smooth muscle cells.

Synergistic effects of combined blockade of the renin angiotensin and endothelin systems have already been described in situations where both systems are activated, such as experimental chronic heart failure [6]. Similarly, local administration of BQ-123 induces forearm vasodilatation in chronic heart failure patients treated with ACE inhibitors [10]. Additional hypotensive effects of bosentan have also been recently reported in hypertensive dogs treated by an ACE inhibitor [7]. However, all these studies were performed in pathological situations characterized by activation of the endothelin system, evidenced by a vasodilatory effect of the ET antagonists administered alone [6, 11–13]. To the best of our knowledge our study is the first to report synergistic vasodilatory effects in the absence of pathological activation of the endothelin and renin angiotensin systems (although we cannot fully exclude an activation due to anaesthesia in our experiments). It must be noted, however, that in the chronic model of L-NAME-induced hypertension, blockade of the renin-angiotensin system does not unmask an endothelin-dependent vasopressor tone [14].

In order to further characterize the interactions between angiotensin II and ET-1 in vivo we performed additional experiments in which we tested the effect of losartan on the response to exogenous big ET-1, as well as those of bosentan on the response to exogenous angiotensin II. We used big ET-1 instead of ET-1 in order to mimic the endogenous release of ET-1 and avoid the overestimation of the endothelium-dependent vasodilatation which occurs with administration of ET-1. The fact that losartan markedly reduced the pressor response induced by big ET-1 suggests that endogenous stimulation of angiotensin II receptors indeed potentiates the pressor response to exogenous big ET-1. In this situation, the fact that bosentan does not affect the pressor responses to angiotensin II might be due to the fact that the endogenous stimulation of the endothelin system is too low to significantly affect the responses to exogenous angiotensin II.

The mechanisms responsible for the unmasking of a vasodilatory response to ET antagonists after losartan or cilazapril are not clear. Compensatory activation of one of the systems after blockade of the other is unlikely since in most models ET antagonists do not increase plasma renin activity, and ACE inhibitors do not increase plasma levels of endothelin, even after chronic treatment [15]. We believe that

the most probable explanation for the unmasking is related to the interactions exerted by angiotensin II and ET at the level of their common target enzymes (e.g. phospholipase C) in smooth muscle cells. Indeed, ET is probably produced in modest quantities in the present experiments, and removal of the ET-dependent component alone does not lead to a significant and detectable reduction of vascular tone. However, after blockade of the renin–angiotensin system, i.e. in a setting of moderately reduced vascular tone (as evidenced by the moderate decrease in blood pressure after losartan and cilazapril), removal of the ET-dependent component indeed leads to a large, significant decrease in the overall vasoconstrictor tone, and thus to a marked decrease in blood pressure. Thus, while there is a potentiation of the vasoconstrictor effects of angiotensin II and ET at the level of the vascular smooth muscle cells, a similar potentiation also exists between the blockers of the renin angiotensin and endothelin systems.

Our findings may have important therapeutic consequences. Indeed, ET receptor antagonists are currently evaluated in several clinical situations such as hypertension [16] or heart failure [12, 17], two situations in which ACE inhibitors or AT_1 antagonists are already prescribed. However, whether potentiation between ET and angiotensin II antagonists also occurs in these pathophysiological situations after chronic treatment remains to be determined.

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