Pharmacology of LR-B/081, a new highly potent, selective and orally active, nonpeptide angiotensin II AT₁ receptor antagonist

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1 The pharmacological profile of LR-B/081, (methyl 2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6H)-pyrimidinyl]methyl]-3-thiophenecarboxylate), a novel antagonist at the angiotensin II (AII) AT₁-receptor, was studied *in vitro* and *in vivo*.

2 In rabbit aortic strips incubated with LR-B/081 (1–1,000 nM), the concentration-response curve to AII was displaced to the right in a nonparallel fashion and the maximal contraction was progressively reduced, indicating that the compound is an insurmountable antagonist in this preparation (apparent $pK_B = 9.50 \pm 0.23$). However, the interaction of LR-B/081 with AII receptors was found to be reversible, since the maximal response to AII was restored by incubation with losartan, a surmountable AII AT₁-antagonist. Contractions elicited by KCl or phenylephrine were not affected by 10 μ M LR-B/081.

3 In rat isolated perfused kidney, LR-B/081 and losartan antagonized the AII-induced vasoconstriction [IC_{50} (95% confidence limits) = 17(13–24) and 39(32–54) nM, respectively]. The LR-B/081 antagonism was incompletely reversed by excess AII, while losartan was fully displaced. The IC_{50} values of LR-B/081 and losartan obtained against vasoconstriction induced by endothelin-1 and noradrenaline were two orders of magnitude higher.

4 In pithed rats, the intravenous administration of LR-B/081 (0.2–2 μ mol kg⁻¹) dose-dependently shifted to the right in a nonparallel fashion the dose-pressor response curve to AII. The maximal pressor response to AII was reduced by LR-B/081 in a dose-dependent fashion. The coadministration of losartan induced a progressive recovery of the maximal pressor response to AII, indicating that *in vivo* the interaction of LR-B/081 with AII receptors is reversible. LR-B/081 at 6 μ mol kg⁻¹, i.v. also did not affect the vasopressor response induced by noradrenaline in the pithed rat.

5 In conscious normotensive rats, single oral administration of LR-B/081 at 6 μ mol kg⁻¹ markedly inhibited the AII-induced pressor response; the inhibition lasted more than 24 h.

6 In conscious renal hypertensive rats, intravenous LR-B/081 appeared as potent as losartan ($ED_{40\text{mmHg}}$ (95% confidence limits) = 0.50(0.36–0.70) and 0.86(0.57–1.3) μ mol kg⁻¹, respectively). A single intravenous (2 μ mol kg⁻¹) or oral (6 μ mol kg⁻¹) administration of LR-B/081 induced a marked fall in blood pressure which lasted for at least 12 h.

7 In conscious spontaneously hypertensive rats, LR-B/081 at 20 μ mol kg⁻¹, p.o., induced a marked and sustained fall in blood pressure. The duration of the antihypertensive effect was longer than 12 h. Heart rate was not modified by LR-B/081 treatment. Repeated oral administration of 17 μ mol kg⁻¹ LR-B/081 for 16 days did not result in the development of tolerance.

8 These results demonstrate that LR-B/081 is a potent, selective and orally active antagonist of AII at the AT₁-receptor subtype, which markedly lowers the blood-pressure in conscious renal and spontaneously hypertensive rats.

Keywords: AT₁ receptor antagonists; LR-B/081; hypertension; losartan; insurmountable antagonism; angiotensin II

Introduction

The renin-angiotensin system (RAS) is important for the regulation of cardiovascular functions and body fluid composition. Angiotensin II (AII), the biologically active peptide hormone of the RAS, elicits multiple pharmacological effects, most of which seem to be mediated by the AII AT₁ receptor subtype, while the functions of the AT₂ subtype receptors are poorly understood (Philipps, 1987; Herblin *et al.*, 1991; Timmermans *et al.*, 1991). The clinical use of inhibitors of angiotensin converting enzyme (ACE) has demonstrated the relevance of the RAS in controlling blood pressure and fluid balance under physiological and pathophysiological condi-

tions (Dzau *et al.*, 1980; Williams, 1988). It has been proposed that the limited specificity of these agents for the RAS system may account for some of their clinical side-effects such as dry cough and angioedema (Williams, 1988; Chin & Buchan, 1990). Moreover, it has been recently shown that AII can also be formed in man by a chymotrypsin-like proteinase which is not affected by ACE inhibitors (Kinoshita *et al.*, 1991).

One of the possible approaches to overcome these limitations of ACE inhibitors is the specific blockade of AII receptors. The recent discovery of selective nonpeptide AII receptor antagonists (Timmermans *et al.*, 1993) provides a more selective means to control and manage hypertension. Losartan represents the prototype of this new class of agents, which is selective for the AII AT₁ receptor subtype (Chiu *et al.*, 1990; Wong *et al.*, 1990a).

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In this paper, we describe the pharmacological profile of LR-B/081 (methyl 2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1(6H)-pyrimidinyl]methyl]-3-thiophenecarboxylate; Figure 1), which belongs to a series of novel 4-pyrimidinones which are potent and selective AT₁ antagonists (Renzetti *et al.*, 1994a,b).

Methods

AII-induced contraction in rabbit isolated aorta

New Zealand White male rabbits (Pampaloni, Fauglia, Italy) weighing 2 to 3 kg were killed by cervical dislocation and exsanguinated. The thoracic aorta was removed and cleaned of adherent fat and connective tissue. The vascular endothelium was removed by gently rubbing the intimal surface of the vessel. Aortic strips (2–3 mm wide and 30 mm long) were then prepared according to Furchgott & Bhadrakom (1953) and mounted in 5 ml organ baths containing Krebs solution (composition in mM: NaCl 118, KCl 4.7, KH₂PO₄ 1.18, CaCl₂ 2.52, NaHCO₃ 25 and glucose 11.1; pH = 7.4). Strips were attached to isometric transducers connected to an 8-channel polygraph (mod. 614/2; Biomedica Mangoni, Pisa, Italy) and a resting tension of 1 g was applied to each strip. Changes in contraction were analyzed with a digital computer (IDAS BM 10000, Biomedica Mangoni). Aortic strips were allowed to equilibrate for 1 h and washed every 15 min. Two consecutive contractile-response curves to cumulative addition of AII (0.1–300 nM) were constructed. After each curve the strips were washed 4 times and allowed to relax to the baseline tension. Afterwards, each strip was incubated for 30 min with the vehicle or with a single concentration of LR-B/081 (1–10–100–1000 nM) or losartan (10–100–1000 nM) before a third concentration-response curve to AII was obtained. The results are expressed as a percentage of the maximal AII response obtained with the second curve, which served as control.

A separate series of experiments was performed in order to study the interaction between LR-B/081 and losartan. After the two AII-response curves, the tissue was incubated with 0.1 µM LR-B/081 or its vehicle. After 30 min, 1 µM losartan or vehicle was added to each experimental group and incubated for an additional 30 min. The concentration-response curve for AII was then repeated.

Pharmacological selectivity towards AII receptors was assessed by using phenylephrine (1 nM–100 µM) or KCl (10–70 mM) in the absence or in the presence of 10 µM LR-B/081 with a similar protocol.

AII-induced vasoconstriction in rat isolated perfused kidney

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 350–400 g were anaesthetized with 10% urethane i.p. Laparotomy was performed via a midline incision, one of the kidneys exposed for less than 10 min and the renal artery cannulated with a PE G-022 tubing (0.8 mm in diameter; Clay Adams, Parsippany, NJ, U.S.A.). The kidney was then removed and placed in a humid organ chamber where it was perfused with Tyrode solution (KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.04, NaHCO₃ 11.9, NaH₂PO₄ 0.42, NaCl 136.9 and glucose 11.1 mM) at 37°C and at a constant rate of 4.5 ml min⁻¹ by means of a peristaltic pump (model T 6.5, Sigmamotor, Middleport, NJ, U.S.A.). Perfusion pressure was measured with a Bentley-Trantec pressure transducer (model 60/800, American Edwards Labs., Santa Ana, CA, U.S.A.) coupled to a recorder (Unirecord 7050, U.Basile, Comerio, Italy). After a 30 min stabilization period, the Tyrode solution was replaced with a solution containing 30 nM AII, as the minimum concentration able to produce a constant and permanent increase (at least 50 mmHg) in perfusion pressure. When a stable pressure plateau was reached, increasing con-

centrations of LR-B/081 or losartan (10–300 nM) were progressively added.

To assess reversibility of the antagonism, an excess of AII (1 µM) was added to the perfusate at the end of the antagonist concentration-response curve. In separate experiments, vasoconstriction was induced by submaximal concentrations of endothelin-1 (3 nM) or noradrenaline (3 µM) (Castellucci *et al.*, 1993) to assess the selectivity of the antagonism of LR-B/081 in this preparation.

AII-induced pressor response in pithed rats

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 250–350 g were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and artificially ventilated with room air (70 strokes min⁻¹; 1 ml 100 g⁻¹, body wt.). Animals were maintained at 37°C by means of a heating pad. Subsequently, the animals were pithed through the orbit with a steel rod and the jugular and the femoral vein were cannulated for AII infusion and for vehicle or drug administration, respectively. Blood pressure was measured by means of a polyethylene catheter, inserted in the right carotid artery, connected to a pressure transducer (Transpac II, Abbot, Campoverde, Italy) coupled to a 2-channel 7070 Gemini recorder (U.Basile, Comerio, Italy).

Cumulative dose-response curves to AII (0.003–30 µg kg⁻¹; 0.5 ml kg⁻¹) were obtained after the intravenous administration of vehicle, LR-B/081 (0.2, 0.6 and 2 µmol kg⁻¹) or losartan (2, 6, 13 and 20 µmol kg⁻¹). The time interval between the administration of test compounds and the beginning of the AII dose-response curve was 5 min for LR-B/081 and 15 min for losartan. Only one full dose-response curve was obtained in each rat.

In separate experiments, the interaction between LR-B/081 and losartan was investigated. Animals were treated intravenously with LR-B/081 at 0.6 µmol kg⁻¹ and, 10 min later, with losartan at 6 or 13 µmol kg⁻¹. After an additional 5 min a dose-pressor response curve to AII was obtained.

To assess selectivity, the effects of 6 µmol kg⁻¹ LR-B/081 were tested against cumulative increasing noradrenaline (0.01–300 µg kg⁻¹, i.v.) vasopressor responses.

AII-induced pressor response in conscious normotensive rats

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 280–340 g, were premedicated with i.p. fentanyl (0.024 mg kg⁻¹) plus fluanisone (1.2 mg kg⁻¹) (Hypnorm) and anaesthetized with sodium pentobarbitone (30–35 mg kg⁻¹, i.v.). Polyethylene catheters were inserted into the left carotid artery and right jugular vein and exteriorized behind the head through a single channel swivel (U. Danuso,

Figure 1 Chemical structure of LR-B/081.

Milano, Italy). The carotid catheter was connected to a Transpac II transducer (Abbott, Campoverde, Italy) connected to a 8805 D preamplifier (Hewlett-Packard, Milan, Italy), and blood pressure and heart rate were recorded by a 7758 D polygraph (Hewlett-Packard). Data samples of variables were taken using a IDAS BM 9000 (Biomedica Mangoni) coupled to a Compaq 386/20e computer. Eighteen hours after surgery, following an overnight fast with water *ad libitum*, a submaximal pressor dose of AII ($0.105 \text{ nmol kg}^{-1}$ i.v.) was injected three times at 20 min intervals to establish the baseline response. Then vehicle, LR-B/081 or losartan ($6 \mu\text{mol kg}^{-1}$) was administered orally by stomach tube and AII bolus injections were performed every hour for 24 h by means of a computer-controlled infusion pump (mod. 22 Harvard, South Natick, MA, U.S.A.) connected to a Compaq Prolinea 3/25s personal computer.

Conscious renal hypertensive rats (RHRs)

The method of unilateral ligation of the left renal artery for development of renal hypertension in the rat has been previously described in detail (Cangiano *et al.*, 1979). Briefly, male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 270–300 g were anaesthetized with sodium pentobarbitone (45 mg kg^{-1} , i.p.) and a midline abdominal incision (about 2 cm long) was made to expose the left kidney. The renal artery was separated with care from the vein and then closed with sterile 4-0 silk. The incision was closed by careful suturing of the muscle layer and skin. Five days later, the rats were anaesthetized and instrumented as described previously. Eighteen hours after surgery, only animals with a mean arterial pressure value greater than 150 mmHg were included in the study. To evaluate the antihypertensive effect, vehicle or increasing intravenous doses (0.06 , 0.2 , 0.6 , 2 and $6 \mu\text{mol kg}^{-1}$) of LR-B/081 or losartan were cumulatively injected at appropriate time intervals (15–60 min).

To assess the efficacy and the duration of action of a single intravenous ($2 \mu\text{mol kg}^{-1}$) and oral ($6 \mu\text{mol kg}^{-1}$) dose of LR-B/081 or losartan, the respective effects on blood pressure and heart rate were monitored for 24 h following treatment.

Conscious spontaneously hypertensive rats (SHRs)

For the single dose study, male SHRs (Charles River, Calco, Italy; 22–24 weeks old) weighing 280–320 g were instrumented as described previously. Eighteen hours later, rats were treated orally with vehicle or LR-B/081 or losartan ($20 \mu\text{mol kg}^{-1}$). The effects on blood pressure and heart rate were monitored for 24 h following drug administration.

For the repeated dose study, male SHRs (Charles River, Calco, Italy; 12 weeks old) weighing 200–240 g were used. Systolic blood pressure was measured by means of the indirect tail cuff method (Digital Pressure Meter LE 5000, Letica, Barcelona, Spain). Animals were treated orally once a day for 16 days with vehicle, $17 \mu\text{mol kg}^{-1}$ LR-B/081 or $22 \mu\text{mol kg}^{-1}$ losartan. Blood pressure was measured 3 h after administration of test compounds on days 1, 4, 8, 12 and 16.

Conscious normotensive rats

Male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 270–370 g were instrumented as described in paragraph 2.4. Eighteen hours later, the animals were treated intravenously every 15 min with increasing doses of LR-B/081 (0.6 to $20 \mu\text{mol kg}^{-1}$).

Data analysis

Data in the text or figures are reported as means $\pm$ s.e.mean. The pA_2 value for losartan was determined by the method of

Arunlakshana & Schild (1959). An apparent pK_B value for LR-B/081 was derived by a double-reciprocal regression plot from at least 3–4 couples of curves (control and in the presence of the antagonist) for those concentrations of antagonist (10 – 100 – 1000 nM LR-B/081) that resulted in at least 40–50% suppression of maximal response (Kenakin, 1987). IC_{50} values and 95% confidence limits were calculated from regression lines by means of the least squares method. ED_{50} s of AII-induced pressor response in the pithed rat were determined, considering as 100% the maximum response achieved in each group. $\text{ED}_{40\text{mmHg}}$ and 95% confidence limits in renal hypertensive rats were calculated using the peak of the blood pressure lowering effect. Significance was assessed by the one-way analysis of variance followed by Bonferroni's test. A P value less than 0.05 indicated a significant difference between means.

Chemicals

LR-B/081 was synthesized by Lusofarmaco, Dept. of Medicinal Chemistry, Milan, Italy. Losartan was a gift from Dr R.D. Smith (DuPont, Wilmington, DE, U.S.A.). Angiotensin II, noradrenaline and phenylephrine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and endothelin-1 from Peninsula (Belmont, CA, U.S.A.). The antagonists were dissolved in 1% dimethylsulphoxide (DMSO) (water solution) and a stoichiometric amount of 0.01 N NaOH was added. The pH of the solution was then adjusted to 7.4–7.6 with 0.1 N HCl.

Results

Effects on AII-induced contraction in rabbit isolated aorta

The cumulative addition of AII (0.1 – 300 nM) caused a concentration-dependent contraction of rabbit aortic strips. The preincubation with LR-B/081 (1 – 1000 nM) induced concentration-related nonparallel shifts to the right of the response curve to AII and decreased the maximal contraction achievable up to 50%, yielding an apparent pK_B value of 9.50 ± 0.23 ($n = 11$) (Figure 2). No agonist activity was found with LR-B/081 at any concentration employed (data not shown). In contrast, losartan shifted to the right in a parallel fashion the concentration-response curve to AII, while leaving the maximal contraction unchanged. The Schild plot produced a pA_2 value of 8.03 ± 0.21 with a slope of 1.3 ± 0.1 (data not shown).

To investigate the nature of the insurmountable antagonism, LR-B/081 was coincubated with the surmountable

Figure 2 Effect of 1 (●, $n = 6$), 10 (□, $n = 5$), 100 (■, $n = 14$) and 1000 nM (△, $n = 8$) LR-B/081 on the contractions induced by angiotensin II (AII) in rabbit aortic strips. Results are expressed as percentage of the maximum response obtained with AII in control preparations (○, $n = 33$). Values represent the means $\pm$ s.e.mean.

antagonist losartan. LR-B/081 0.1 μM reduced the maximum contractile response to $61 \pm 3.6\%$ ($n = 12$). Addition of 1 μM losartan partly restored it to 81 ± 0.1 ($n = 7$; $P < 0.01$; Figure 3).

LR-B/081 (10 μM) did not affect the concentration-contraction curves obtained with either KCl or phenylephrine (data not shown).

Effects on AII-induced vasoconstriction in rat isolated perfused kidney

Mean resting perfusion pressure of rat isolated perfused kidney was 91 ± 6 mmHg ($n = 15$). The addition of 30 nM AII caused a sustained vasoconstriction that increased perfusion pressure by 48 ± 6 mmHg ($n = 15$) over baseline and was stable for at least 60–90 min in the control group (final value: 43 ± 5 mmHg; $n = 6$). The addition of increasing concentrations of LR-B/081 ($n = 5$) or losartan ($n = 5$) to the perfusion medium caused a progressive inhibition of AII-induced vasoconstriction. IC_{50} (95% confidence limits) values were 17(13–24) and 39(32–54) nM for LR-B/081 and losartan, respectively. The inhibition of AII-induced vasoconstriction exerted by losartan was fully reversible, since an excess of AII (1 μM) in the medium following the highest dose of antagonist caused a full recovery of the perfusion pressure. Conversely, the inhibition induced by LR-B/081 was only partially reversed by excess AII (Figure 4).

Furthermore, LR-B/081 was a weak inhibitor of vasoconstriction induced by endothelin-1 or adrenaline in this preparation: the respective IC_{50} values were 3.5 (2.6–5.2) and 2.9 (2.2–4.0) μM (data not shown).

Effects on AII-induced pressor response in pithed rats

In control rats the basal mean diastolic pressure was 40 ± 1.5 mmHg ($n = 10$) before vehicle administration and 44 ± 1.5 mmHg after 15 min. LR-B/081 at 0.2, 0.6 and 2 $\mu\text{mol kg}^{-1}$, i.v., dose-dependently decreased diastolic blood pressure (DBP) from 41 ± 0.8 ($n = 17$) to 34 ± 2.0 ($n = 6$), 29 ± 1.5 ($n = 6$) and 27 ± 1.6 mmHg ($n = 5$), 5 min after administration. No agonist activity was found with any dose employed. Losartan at 2, 6, 13 and 20 $\mu\text{mol kg}^{-1}$, i.v., lowered DBP from 41 ± 1.2 ($n = 22$) to 32 ± 4.3 ($n = 6$), 28 ± 2.8 ($n = 5$), 28 ± 1.1 ($n = 5$) and 27 ± 1.3 ($n = 6$) mmHg, respectively, 15 min after administration (data not shown).

The cumulative infusion of AII (0.003–30 $\mu\text{g kg}^{-1}$) caused a dose-dependent increase in DBP up to 108 ± 2.0 mmHg with an ED_{50} of 0.11 (0.05–0.21) $\mu\text{g kg}^{-1}$. The administration of LR-B/081 at 0.2, 0.6 and 2 $\mu\text{mol kg}^{-1}$, i.v., caused non-parallel shifts to the right of the dose-response curve to AII

and increased the ED_{50} (95% confidence limits) value to 1.5 (0.74–2.7), 5.0 (3.6–7.0) and 95 (82–111) $\mu\text{g kg}^{-1}$, respectively (Figure 5). The maximal value of DBP was progressively decreased with increasing doses of LR-B/081 (Figure 5). In contrast, losartan at 2, 6, 13 and 20 $\mu\text{mol kg}^{-1}$, i.v. induced parallel shifts to the right of the dose-response curve to AII, with ED_{50} values of 3.2 (2.4–4.3), 7.6 (4.7–9.6), 29 (14–48) and 179 (135–236) $\mu\text{g kg}^{-1}$, respectively. The maximum increase in DBP after each dose of losartan was not significantly different from that obtained with the vehicle (data not shown).

As shown in Figure 6, when 6 $\mu\text{mol kg}^{-1}$ losartan was coadministered with 0.6 $\mu\text{mol kg}^{-1}$ LR-B/081, a progressive recovery of the maximal AII-induced pressor response was observed. In the presence of 13 $\mu\text{mol kg}^{-1}$ losartan the AII-induced pressor response curve was further shifted to the right.

Noradrenaline-induced pressor responses were not affected by LR-B/081 at 6 $\mu\text{mol kg}^{-1}$, i.v. (data not shown).

Effects on AII-induced pressor response in conscious normotensive rats

In the vehicle-treated group ($n = 9$), mean basal arterial blood pressure was 113 ± 2.3 mmHg. Intravenous AII (0.105 nmol kg^{-1}) induced a pressor response (43 ± 2.3 mmHg over basal after the first injection) that

Figure 4 Representative experiments of the effects of increasing concentrations of LR-B/081 (a) and losartan (b) on angiotensin II (AII)-induced vasoconstriction in the rat isolated perfused kidney. At the end of the experiment, an excess of AII (1 μM) was added to the perfusate to test for reversibility.

Figure 3 Effect of 1 μM losartan (●, $n = 6$), 0.1 μM LR-B/081 (□, $n = 12$) and 0.1 μM LR-B/081 coincubated with 1 μM losartan (▲, $n = 7$) on the contractions induced by angiotensin II (AII) in rabbit aortic strips. Results are expressed as percentage of the maximum response obtained with AII in control preparations (○, $n = 25$). Values represent the means $\pm$ s.e.mean.

Figure 5 Effect of intravenous vehicle (○, $n = 10$), 0.2 $\mu\text{mol kg}^{-1}$ (●, $n = 6$), 0.6 $\mu\text{mol kg}^{-1}$ (▲, $n = 5$) and 2 $\mu\text{mol kg}^{-1}$ LR-B/081 on angiotensin II (AII)-induced (0.003–3,000 $\mu\text{g kg}^{-1}$, i.v.) pressor response in the pithed rat. Data represent mean values $\pm$ s.e.mean.

showed a very slow tendency to decrease during the course of the experiment (Figure 7). In LR-B/081 ($n = 8$) and losartan ($n = 8$) groups, mean basal arterial blood pressure was 113 ± 1.9 and 116 ± 2.2 mmHg and pretreatment responses to AII were 44 ± 2.4 and 40 ± 2.7 mmHg, respectively. LR-B/081 and losartan at $6 \mu\text{mol kg}^{-1}$, p.o., exerted a marked inhibitory effect on AII-induced pressor responses. Maximum inhibition was reached after 2 h with LR-B/081 ($82 \pm 3\%$) and after 4 h with losartan ($83 \pm 1.5\%$). Both compounds showed a long lasting effect, which was still significant ($P < 0.05$) 24 h after the oral dosing (Figure 7).

Effects in conscious renal hypertensive rats

Cumulative intravenous injections of LR-B/081 ($n = 6$) or losartan ($n = 12$) caused a dose-dependent decrease in mean arterial pressure in conscious RHRs. The $\text{ED}_{40\text{mmHg}}$ values (95% confidence limits) were 0.50 (0.36 – 0.70) and 0.86 (0.57 – 1.30) $\mu\text{mol kg}^{-1}$ for LR-B/081 and losartan, respectively (data not shown). A single intravenous administration of $2 \mu\text{mol kg}^{-1}$ LR-B/081 ($n = 10$) or losartan ($n = 8$) exerted a prolonged fall in blood pressure that lasted more than 12 h (Figure 8).

When given orally at $6 \mu\text{mol kg}^{-1}$ LR-B/081 ($n = 8$) produced a marked drop in MAP that lasted more than 12 h. A similar result was found with $6 \mu\text{mol kg}^{-1}$ losartan ($n = 6$; Figure 9a). Heart rate was not modified by either LR-B/081 or losartan treatment (Figure 9b).

Figure 6 Effect of intravenous vehicle ($\circ$, $n = 10$), $0.6 \mu\text{mol kg}^{-1}$ LR-B/081 alone ($\square$, $n = 6$) and coadministered with $6 \mu\text{mol kg}^{-1}$ ($\bullet$, $n = 8$) and $13 \mu\text{mol kg}^{-1}$ ($\blacksquare$, $n = 6$) losartan on angiotensin II (AII)-induced (0.003 – $3,000 \mu\text{g kg}^{-1}$, i.v.) pressor response in the pithed rat. Data represent mean values $\pm$ s.e.mean.

Figure 7 Effect of orally administered vehicle (x , $n = 9$), $6 \mu\text{mol kg}^{-1}$ LR-B/081 ($\bullet$, $n = 8$) or losartan ($\circ$, $n = 8$) on angiotensin II-induced ($0.105 \text{ nmol kg}^{-1}$, i.v.) pressor response in conscious normotensive rats. Values represent the means $\pm$ s.e. mean.

Effects in conscious spontaneously hypertensive rats (SHRs)

LR-B/081 at $20 \mu\text{mol kg}^{-1}$, p.o. ($n = 7$) caused a fall in blood pressure similar to that produced by $20 \mu\text{mol kg}^{-1}$ losartan ($n = 7$). The duration of action for both compounds exceeded

Figure 8 Effect of intravenous administration of vehicle (x , $n = 6$), $2 \mu\text{mol kg}^{-1}$ LR-B/081 ($\bullet$, $n = 10$) and losartan ($\circ$, $n = 8$) on mean arterial pressure in conscious renal hypertensive rats. Data represent the means $\pm$ s.e. mean.

Figure 9 Effect of a single oral administration of vehicle (x , $n = 11$), $6 \mu\text{mol kg}^{-1}$ LR-B/081 ($\bullet$, $n = 8$) and losartan ($\circ$, $n = 6$) on mean arterial pressure (a) and heart rate (b) in conscious renal hypertensive rats. Values represent the means $\pm$ s.e.mean.

Figure 10 Effect of single oral administration of vehicle (x, $n = 6$), $20 \mu\text{mol kg}^{-1}$ LR-B/081 (●, $n = 7$) and losartan (○, $n = 7$) on mean arterial pressure (a) and heart rate (b) in conscious spontaneously hypertensive rats. Values represent the means $\pm$ s.e.mean.

12 h (Figure 10a). Heart rate was not significantly modified by either drug (Figure 10b).

Daily oral administration of $17 \mu\text{mol kg}^{-1}$ LR-B/081 ($n = 10$) or $22 \mu\text{mol kg}^{-1}$ losartan ($n = 10$) for 16 days induced a similar sustained fall in systolic blood pressure. The decrease in systolic blood pressure measured with both compounds on the first day was not significantly different from that obtained at day 16 (Figure 11).

Effects in conscious normotensive rats

No significant difference between the effects of vehicle ($n = 6$) or cumulative dose injections of LR-B/081 (0.6 – $20 \mu\text{mol kg}^{-1}$; $n = 6$) on mean arterial pressure and heart rate were found (data not shown).

Discussion

LR-B/081 has been recently characterized in *in vitro* binding studies as a potent and selective AT_1 receptor antagonist: its affinity for receptors of rat adrenal cortex membranes ($K_i = 0.9 \pm 0.2 \text{ nM}$) was about tenfold higher than that of losartan, whereas its binding to the AT_2 receptor of bovine cerebellum membranes was negligible (Renzetti *et al.*, 1994b). In saturation experiments, LR-B/081 decreased the number of binding sites without affecting the K_D for AII. However, the compound did not influence the rate of dissociation of tritiated AII from these receptors, indicating that LR-B/081 differs from both classical competitive and allosteric antagonists (Renzetti *et al.*, 1994b).

Figure 11 Effect of repeated oral dosing of vehicle (open columns, $n = 10$), $17 \mu\text{mol kg}^{-1}$ LR-B/081 (solid columns; $n = 10$) and $22 \mu\text{mol kg}^{-1}$ losartan (hatched columns; $n = 10$) on systolic blood pressure in conscious spontaneously hypertensive rats. Figure shows the antihypertensive effect measured 3 h after treatment on days 1, 4, 8, 12 and 16. Values represent the means $\pm$ s.e.mean.

The present data agree with the above observations. Indeed, LR-B/081 appears to be an insurmountable antagonist of AII in rabbit aorta as shown by nonparallel shifts of the dose-response curves to AII and by the depression of the maximal contractile responses. Wash-out procedures for up to 2 h of rabbit aorta incubated with 10 nM LR-B/081 did not result in any recovery of the maximal contraction (unpublished data). A similar behaviour was observed in rat isolated perfused kidney, in which LR-B/081 potentially inhibited the AII-induced vasoconstriction, but an excess of AII was not able to restore the perfusion pressure to the pretreatment value. Similarly, in the pithed rat, increasing intravenous doses of LR-B/081 resulted in non-parallel shifts of the dose-pressor curves to AII with a decrease of maximal response, a behaviour consistent with insurmountable antagonism. However, the interaction of LR-B/081 with AT_1 -receptors of the vasculature appears to be reversible, since the coincubation or coadministration of losartan, a surmountable AII AT_1 antagonist, was capable of restoring the maximal response to AII both in rabbit aorta and in pithed rats, suggesting that LR-B/081 and losartan interact with the same binding site both *in vitro* or *in vivo* (Wong & Timmermans, 1991; Robertson *et al.*, 1992; Wienen *et al.*, 1992).

The molecular mechanisms that underlie this insurmountable antagonism are not clear, although a slow displacement rate of LR-B/081 from its binding sites, in tissues believed to have little or no receptor reserve for AII, is likely to be involved (Liu *et al.*, 1992; Wienen *et al.*, 1992). An insurmountable antagonism similar to that exerted by LR-B/081 has been observed for other nonpeptide AT_1 antagonists such as EXP 3174 (Wong *et al.*, 1990b), GR 117289 (Robertson *et al.*, 1992), BIBR 277 (Wienen *et al.*, 1993), CV 11974 (Shibouta *et al.*, 1993), valsartan (Criscione *et al.*, 1993) and DuP 532 (Chiu *et al.*, 1991).

In terms of potency, a precise comparison between LR-B/081 and losartan is precluded by the different nature of the antagonism, surmountable for the latter, insurmountable for the former. However, LR-B/081 appears considerably more potent in rabbit aorta and in pithed rats, in agreement with binding studies (Renzetti *et al.*, 1994b), while it was only twice as potent on rat kidney vasculature.

A selective interaction of LR-B/081 with AII receptors was substantiated by the observation that the compound was unable to affect vasoconstriction induced by either KCl or phenylephrine on rabbit aorta and inhibited the vasoconstriction induced by noradrenaline and endothelin-1 in the rat isolated perfused kidney only at high concentrations. Also *in vitro*, the antagonism of LR-B/081 appeared to be specific since vasopressor responses to noradrenaline in the pithed rat were not affected by LR-B/081.

LR-B/081, administered intravenously, lowered blood pres-

sure in the pithed rat, but did not affect blood pressure in conscious normotensive rats. The hypotensive effect is presumably due to the suppression by AT₁ antagonists of the vasoconstrictor influence of endogenous AII in the pithed rat, a model in which blood pressure is highly dependent upon activation of the renin-angiotensin system (De Jonge *et al.*, 1982). Furthermore, LR-B/081 was devoid of any agonist activity either *in vitro* or *in vivo*.

When tested orally for potency and duration of action, LR-B/081 was found to be as active as losartan either in inhibiting AII-induced pressor response or in lowering blood pressure in conscious renal and spontaneously hypertensive rats. The onset of the effect was generally shorter than that of losartan. The pressure lowering effect was not accompanied by tachycardia. Repeated administration of LR-B/081 did not result in any phenomenon of tolerance and its efficacy was retained after 16 days of treatment.

It should be noted that the maximal antihypertensive effect of LR-B/081 in RHRs did not occur concomitantly with the maximal inhibitory effect on the AII pressor response. As suggested by others (Roccon *et al.*, 1994), the AT₁ receptor antagonists, might require some time to penetrate completely into the vascular compartment to exert a maximal blockade of endogenous AII-induced vasoconstriction. Differences in inhibiting the vasoconstriction induced by exogenous or endogenous AII may involve AT₁ receptors in different layers of the vascular wall.

Although LR-B/081 was found to be more potent than losartan both in binding studies and in rabbit aortic strips or the pithed rat, its antihypertensive effects in rat models was similar to that exhibited by losartan. The reason for this discrepancy may be related to the *in vivo* effects of losartan which are attributed to the hepatically-generated metabolite, EXP 3174 (Wong *et al.*, 1990b), while LR-B/081 is active *per se in vivo*.

In summary, the data presented demonstrate that LR-B/081 is a highly selective, potent and orally active nonpeptide AII AT₁-receptor antagonist. Given orally, LR-B/081 is an effective antihypertensive agent, particularly in animals in which blood pressure is elevated as a result of activation of the renin-angiotensin system or by sustained infusion of AII. The comparison of the profile over time of the inhibitory effect of LR-B/081 on AII pressor response in normotensive rats with its antihypertensive effects in RHRs, suggests that the blockade of the vasoconstrictor effect of AII by LR-B/081 is the likely primary mechanism of its antihypertensive effect. The therapeutic potential of the LR-B/081 will be evaluated in clinical studies.

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