Pharmacological Characterization of BR-A-657, a Highly Potent Nonpeptide Angiotensin II Receptor Antagonist

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The pharmacological profile of BR-A-657, 2-n-butyl-5-dimethylamino-thiocarbonyl-methyl-6-methyl-3-[(2-(1H-tetrazole-5-yl)bibenyl-4-yl)methyl]-pyrimidin-4(3H)-one, a new nonpeptide AT 1-selective angiotensin receptor antagonist, has been investigated in a variety of in vitro and in vivo experimental models. In the present study, BR-A-657 displaced [125I]Sar1-Ile8 angiotensin II (Ang II) from its specific binding sites to AT1 subtype receptors in membrane fractions of HEK-293 cells with an IC50 of 0.16 nM. In a functional assay using isolated rabbit thoracic aorta, BR-A-657 inhibited the contractile response to Ang II (pD2; 9.15) with a significant reduction in the maximum. In conscious rats, BR-A-657 (0.01, 0.1, 1 mg/kg; intravenously (i.v.)) dose-dependently antagonized Ang II-induced pressor responses. In addition, BR-A-657 dose-dependently decreased mean arterial pressure in furosemide-treated rats and renal hypertensive rats. Moreover, BR-A-657 given orally at 1 and 3 mg/kg reduced blood pressure in conscious renal hypertensive rats. Taken together, these findings indicate that BR-A-657 is a potent and specific antagonist of Ang II at the AT1 receptor subtype, and reveal the molecular basis responsible for the marked lowering of blood pressure in conscious rats.

Key words BR-A-657; losartan; AT1 receptor; angiotensin II pressor; renal hypertensive rat

Hypertension is important not only because of its prevalence but also because it is a major modifiable risk factor of associated cardiovascular and renal complications. Current guidelines emphasize the importance of managing hypertension to reduce substantial morbidity and mortality associated with cardiovascular events.2–4 The renin angiotensin system (RAS) plays a pivotal role in cardiovascular regulation by blood pressure and fluid-electrolyte balance control. Angiotensin II (Ang II) circulates in the blood stream and is the principal mediator of the RAS system, which controls cardiovascular and renal effects in furosemide-treated rats and renal hypertensive rats. Moreover, BR-A-657 given orally at 1 and 3 mg/kg reduced blood pressure in conscious renal hypertensive rats. Taken together, these findings indicate that BR-A-657 is a potent and specific antagonist of Ang II at the AT1 receptor subtype, and reveal the molecular basis responsible for the marked lowering of blood pressure in conscious rats.

MATERIALS AND METHODS

Materials The BR-A-657 used for this study was synthesized at Boryung Pharm. Co., Ltd. (Seoul, Republic of Korea) and its chemical structure (Fig. 1A) was determined by spectral data: 1H-NMR (500 MHz, DMSO-d6) δ: 7.54–7.27 ppm, 2.52 ppm, 3.78 ppm, 3.40 ppm, 2.16 ppm, 1.58 ppm, 1.55 ppm. In a functional assay using isolated rabbit thoracic aorta, BR-A-657 inhibited the contractile response to Ang II (pD2; 9.15) with a significant reduction in the maximum. In conscious rats, BR-A-657 (0.01, 0.1, 1 mg/kg; intravenously (i.v.)) dose-dependently antagonized Ang II-induced pressor responses. In addition, BR-A-657 dose-dependently decreased mean arterial pressure in furosemide-treated rats and renal hypertensive rats. Moreover, BR-A-657 given orally at 1 and 3 mg/kg reduced blood pressure in conscious renal hypertensive rats. Taken together, these findings indicate that BR-A-657 is a potent and specific antagonist of Ang II at the AT1 receptor subtype, and reveal the molecular basis responsible for the marked lowering of blood pressure in conscious rats.

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The authors declare no conflict of interest.

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Radioligand Binding Assay  Ang II binding assays were carried out according to an adaptation and validation of the method described by Le et al. Briefly, human recombinant HEK-293 cells stably expressing the human angiotensin AT₁ receptor and AT₂ receptors were used for the radioligand binding assay. HEK-293 cells were collected by centrifugation and washed with PBS. The washed cell pellets were resuspended in buffer A (50 mM Tris–HCl pH 7.4, 5 mM ethylenediamine tetraacetic acid (EDTA), 20 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1.5 mM CaCl₂, 10 µg/mL trypsin inhibitor, 1 µg/mL leupeptin, and 75 µg/mL phenylmethylsulfonyl fluoride (PMSF)) at 4°C. After cell lysis by sonication with amplitude 60 pulsations/6s using ultrasound (Vibra-Cell 72405, Sonics & Materials, CT, U.S.A.) for 3 min at 4°C, the homogenate was further diluted to 40 mL with the same buffer and then centrifuged at 50000 × g for 15 min at 4°C. The pellet was resuspended using needles (23G then 26G) in buffer B (10% glycerol in buffer A) and protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacturer’s instruction. For a saturation binding assay, [¹²⁵I]Sar¹-Ile⁸]Ang II at 12 different concentrations ranging from 0.006 to 0.645 nM was incubated with the cell membranes (32 µg) for 120 min at 37°C in buffer (50 mM Tris–HCl pH 7.4, 5 mM MgCl₂, 1 mM EDTA, and 0.1% bovine serum albumin (BSA)). Nonspecific binding was estimated by adding 10 µM of unlabeled Ang II to the incubation mixture, and total binding of [¹²⁵I]Sar¹-Ile⁸]Ang II in the absence or presence of BR-A-657 (0.1, 0.3 nM) was tested. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with incubation buffer resulting in a final concentration of 0.02%. Following incubation, the samples were filtered rapidly under vacuum through Packard Unifilter GF/B 96-well filters (Perkin-Elmer, CA, U.S.A.) presoaked with 0.3% polyethyleneimine and rinsed several times with ice-cold 50 mM Tris–HCl (pH 7.4) using a 96-sample Packard Filtermate. The filters were dried and then counted for radioactivity in a scintillation counter (TopCount®NXT™, Packard, the Netherlands) using a scintillation cocktail (Microcint 0, Packard). The entire assay was performed in triplicate. Bound radioactivity was quantitated and corrected for nonspecific binding. Characterization of binding saturation curves (Scatchard analysis) and assessment of the number of AT₁ receptors (B₅₀) and the dissociation constant of the radioligand (K_d) were obtained using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, U.S.A.). To measure IC₅₀ values of test compounds (BR-A-657, valsartan, and losartan) on AT₁ receptors, cell membrane homogenates (8 µg protein) were incubated for 120 min at 37°C with 0.05 nM [¹²⁵I]Sar¹-Ile⁸]Ang II in the absence or presence of the test compounds in a buffer (50 mM Tris–HCl pH 7.4, 5 mM MgCl₂, 1 mM EDTA and 0.1% BSA). Test compounds dissolved in DMSO and diluted with incubation buffer resulting in a final concentration of 1 × 10⁻¹², 3 × 10⁻¹⁰, 3 × 10⁻¹¹–1 × 10⁻⁹ and 3 × 10⁻¹¹–1 × 10⁻⁸ M for BR-A-657, valsartan, and losartan, respectively. Nonspecific binding was estimated by adding 10 µM of unlabeled Ang II to the incubation mixture. IC₅₀ on AT₁ receptor was also assessed for BR-A-657 with the incubation of 0.01 nM [¹²⁵I]CGP42112A (Perkin-Elmer, NEX 324) for 240 min at 37°C with 1 × 10⁻¹⁰–1 × 10⁻⁹ M of BR-A-657. Nonspecific binding was estimated by adding 1 µM of Ang II to the incubation mixture. IC₅₀ values were determined by nonlinear least squares fitting of the inhibition curves with a sigmoid-Boltzmann equation regression analysis using GraphPad Prism 6.0.

Animals  All procedures were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHP-2006-08-15). New Zealand white male rabbits weighing 1.5–2.0 kg were supplied by Samtako (Osan, Gyeonggi, Republic of Korea). Male Sprague-Dawley rats (280–320 g) were purchased from Orient Bio (Sungnam, Gyeonggi, Republic of Korea). All animals were housed at cage and fed standard laboratory chow in an environmentally controlled animal room (20±2°C; relative humidity 40–60%) under 12 h dark/light cycle for at least two weeks. Twenty four hours before the experiment the animals were allowed water only.
account for diurnal enzyme activity variations, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and descending thoracic aortas were quickly removed and cleaned of fat and connective tissue. The thoracic aorta was dissected free of surrounding tissue in modified Kreb’s bicarbonate solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25 mM NaHCO3 and 11 mM glucose, pH 7.4) and cut into rings of 3 to 5 mm in width. Tissues were then placed between two stainless steel hooks and mounted in 10 mL organ baths. They were stretched to a resting tension of 2 g and allowed to equilibrate for 60 min during which time they were washed repeatedly with Kreb’s bicarbonate solution and the tension readjusted. Baths were maintained at 37°C and bubbled with a 95:5% O2:CO2 mixture (pH 7.4). Isometric contraction was measured on a physiograph (Grass model-7 polygraph, Grass Instrument Company, Quincy, MA, U.S.A.) with a force transducer (Grass FT03).

The antagonistic effect of the compounds was evaluated by measuring their capacity to inhibit the contractile response of the preparation to Ang II (3 x 10−7−3 x 10−10 M). The tissues were exposed to Ang II or other vasoconstrictors to verify responsiveness. After washing several times with Kreb’s bicarbonate solution and recovery of the basal tension, the first cumulative concentration–contractile response curve for Ang II was determined to obtain the maximum contractile response. The tissues were again left to recover the baseline tension by washing out several times. The second cumulative concentration contractile response curve for Ang II was established for each tissue after 30-min incubation with BR-A-657 (0.1, 1, 10 μM) or losartan (0.1, 0.3, 1 μM), respectively. Responses from rabbit aorta were expressed as a percentage of the maximum Ang II response obtained from the cumulative concentration–response curve. pD2 value for BR-A-657 was calculated as the negative logarithm of the concentration of antagonist that reduces a maximal response of agonist by 50% and pA2 value for losartan, the negative logarithm of the concentration of antagonist that requires a 2-fold increase in agonist concentration to get the same effect, was determined according to the Schild equation. The effects of BR-A-657 on contractions by KCl, norepinephrine, serotonin and histamine were also examined.

Ang II-Pressor Response in Conscious Normotensive Rats Arterial and venous catheters were inserted via the left femoral artery and vein to measure arterial blood pressures and inject drugs, respectively. Rats were placed in individual cages after surgery. In each animal on the day following surgery, an arterial catheter was connected to a Gould pressure transducer; mean arterial pressure and heart rate were continuously monitored using a Grass model-7 polygraph (Grass Instrument Company, Quincy, MA, U.S.A.). Only animals with mean arterial pressure values greater than 155 mmHg were included in this study. BR-A-657 (0.03, 0.1, 0.3 mg/kg) or losartan (1, 3 mg/kg) were administered intravenously after stabilization. Arterial pressure and heart rate were continuously monitored until 180 min following treatments.17–19 Moreover, BR-A-657 (1, 3 mg/kg) or losartan (1 mg/kg) were orally administered after achieving a stable arterial pressure. Vehicle-treated animals were administered with normal physiological saline via same routes as BR-A-657 or losartan. Blood pressure and heart rate were monitored at 30 min before and 20, 40, and 60 min after oral administration. Since then, they were measured at an interval of 30 min until 8 h and then at 23, 23.5, and 24 h.

**Statistical Analysis** All results are expressed as means ± S.E.M. Changes in response to BR-A-657 or losartan during in vivo studies were compared those shown by vehicle-treated controls over corresponding periods. One-way analysis of variance was used for intergroup comparisons and Dunnett’s test was used for multiple comparisons. SPSS ver. 8.0 (SPSS Inc., IL, U.S.A.) was used throughout, and statistical significance was accepted for p values <0.05.

**RESULTS**

**Effects of BR-A-657 on [125I][Sar2-Ile8]Ang II Binding to AT1 Receptors in Human Recombinant HEK-293 Cells** BR-A-657, valsartan and losartan at 10−12 to 10−6 M competed dose-dependently with [125I][Sar2-Ile8]Ang II for binding to AT1 subtype receptors in the membrane of HEK-293 cells with seemingly monophasic inhibition curves (Fig. 1B). The concentrations of BR-A-657, valsartan and losartan that displaced 50% of bound [125I][Sar2-Ile8]Ang II were 0.16, 2.3, and 6.6 nM, and the inhibition constants (Ki) of these compounds were 0.081, 0.11, and 0.33 nM, respectively. In addition, for
AT$_2$ receptors, IC$_{50}$ and $K_i$ values of BR-A-657 was 69 and 35 $\mu$m, respectively. As shown in Fig. 1C, the linearity of the Scatchard plot suggests that the radiolabeled Ang II bound to a single type of binding site in HEK-293 cells. BR-A-657 (0.1, 0.3 nm) displayed noncompetitive kinetics as an inhibitor of $[^{125}\text{I}]$-[Sar$^1$-Ile$^8$] Ang II binding; namely, it increased $K_d$ values (from 0.11 to 0.14, 0.22 nm, respectively) and decreases the $B_{max}$ (from 144.2 to 133.4, 116.5 fmol/mg protein, respectively) in the Scatchard plot.

Effects of BR-A-657 on Ang II-Induced Contractile Response in Isolated Rabbit Thoracic Aorta Functional in vitro study was performed to characterize the mode of interactions of BR-A-657 with the AT$_1$ receptor in rabbit thoracic aorta. BR-A-657 and losartan inhibited the Ang II-induced contractile responses in a concentration-dependent manner (Fig. 2). But the types of antagonism were different. BR-A-657 (0.1, 1, 10 nm) caused nonparallel rightward shifts in the concentration–contractile response curve to Ang II with a significant reduction in the maximal contractile response to 96.6, 47 and 18%, respectively (pD$_2$=9.15; Fig. 2A). In contrast, losartan (0.1, 0.3, 1 $\mu$m) produced parallel rightward shifts in the concentration–response curve without any changes in the maximal contractile response to Ang II (Fig. 2B), suggesting the surmountable antagonism of Ang II-induced contraction (pA$_2$=8.40). Moreover, concentrations of BR-A-657 as high as 10 $\mu$m had no inhibitory effects on the contraction induced by KCl, norepinephrine, serotonin, or histamine (data not shown).

**In Vivo Potency and Specificity of BR-A-657 in Conscious Normotensive Rats** In Sprague-Dawley rats treated with vehicle under the experimental conditions used, baseline values for diastolic arterial pressure were 120.67±0.44 (n=6) and similar in all groups of rats. Intravenous administration of Ang II (0.1 $\mu$g/kg) induced a pressor response of 41.03±0.90 mmHg (mean of 3 consecutive doses) over baseline. Intravenous administration of BR-A-657 (0.01, 0.1, 1 mg/kg) or losartan (1, 3 mg/kg) were found to have significant dose-dependent inhibitory effects on Ang II (0.1 $\mu$g/kg)-induced pressor responses. Maximum inhibitory effects of BR-A-657 (0.01, 0.1, 1 mg/kg, i.v.) on Ang II-induced pressor responses were 24, 86, and 93%, respectively, at 5 min and 17, 35, and 65% at 180 min post-treatment (Fig. 3A). The maximum inhibitory effects of losartan (1, 3 mg/kg) were 54 and 82% at 5 min post-treatment, respectively, and this inhibition was still evident (52, 70%, respectively) at 180 min post-treatment (Fig. 3B). The inhibitory effect of BR-A-657 at 1 mg/kg was similar to that of losartan at 3 mg/kg.

**Hypotensive Effects of BR-A-657 in Furosemide-Treated Rats** The effects of intravenously administrated BR-A-657...
(1, 3, 10 mg/kg) and losartan (3, 10 mg/kg) on mean arterial pressure in furosemide-treated rats are shown in Fig. 4. Mean predose values of arterial pressure and heart rate were 122.50 ± 0.82 mmHg and 332.65 ± 3.36 bpm, respectively, in furosemide-treated rats. BR-A-657 caused immediate and dose-dependent decreases in mean arterial pressure that lasted more than 180 min post-treatment (Fig. 4A). Losartan also markedly reduced mean arterial pressure with maximum effect at 120–150 min post-treatment (Fig. 4B). Maximum pressure reduction induced by BR-A-657 at 1 mg/kg and its persistence were comparable to those of losartan at 3 mg/kg. The hypotensive effects of BR-A-657 and losartan at 10 mg/kg were similar at 180 min, but BR-A-657 acted more rapidly than losartan. Neither BR-A-657 nor losartan induced a significant change in heart rate at any dose used as compared with vehicle-treated controls (data not shown).

**Antihypertensive Effects of BR-A-657 in Conscious Renal Hypertensive Rats** The effects of intravenously administered BR-A-657 (0.03, 0.1, 0.3 mg/kg) or losartan (1, 3 mg/kg) on mean arterial blood pressure in conscious renal hypertensive rats are shown in Fig. 5. The mean predose values of arterial pressure and heart rate were 167.64 ± 1.21 mmHg and 399.16 ± 4.74 bpm, respectively. BR-A-657 elicited a dose-dependent and significant decrease in mean arterial blood pressure with rapid onset (Fig. 5A). For all doses the antihypertensive effects of BR-A-657 persisted at a significant level for 180 min post-treatment. Losartan (1 mg/kg) reduced mean arterial pressure by 31 and 32 mmHg, respectively at 180 min. Furthermore, the initial immediate decrease in mean arterial pressure by BR-A-657 and losartan was accompanied by a transient increase in heart rate in the first 20–30 min, but it was not significant compared to vehicle-treated controls (data not shown).

**Antihypertensive Effects of Orally-Administered BR-A-657 in Conscious Renal Hypertensive Rats** The effects of orally administered BR-A-657 (1, 3 mg/kg) or losartan (1 mg/kg) on mean arterial blood pressure in conscious renal hypertensive rats are shown in Fig. 6. The mean value of predose arterial pressure was 168.56 ± 2.57 mmHg. BR-A-657 (1 mg/kg) caused significant and dose-dependent decreases in mean arterial blood pressure, whereas the vehicle did not alter mean arterial pressure. The onset of the antihypertensive effects was rapid (20 min) and the maximal decreases were reached at 6 to 8 h. The reduced arterial pressures were sustained significant for 24 h at both doses (p<0.05). However, losartan (1 mg/kg) showed a gradual onset of the antihypertensive effect with significant decrease after 23 h (p<0.05) and the effect was sustained until 24 h. The degrees of reduction at 24 h post dose of BR-A-657 (1, 3 mg/kg) and losartan (1 mg/kg) were 57.2 ± 7.72, 81.0 ± 13.67, and 54.3 ± 19.46 mmHg, re-
failure are mediated by AT1 receptors.\(^7,8\) In the present study, blood binding studies using the membranes of HEK-293 cells.

**Results**

The major cardiovascular effects of Ang II observed in vivo suggest that BR-A-657 specifically antagonizes Ang II responding to Ang II stems from the antagonism specific to AT\(_1\) receptors, not from a non-specific vasodilating effect, because in the absence of angiotensin II, BR-A-657 do not alter the contractile responses to KCl, norepinephrine, serotonin and histamine. By using binding assays and functional studies, we found that BR-A-657 is a potent and specific AT\(_1\) antagonist that is devoid of agonistic properties.\(^{20,24}\)

To explore in vivo antagonistic properties, we investigated the effects of intravenously administered BR-A-657 or losartan on blood pressure in three different experimental models, that is, Ang II-treated rats, furosemide-treated rats, and renal hypertensive rats. In conscious Ang II-treated rats, intravenous administration of BR-A-657 immediately and dose-dependently inhibited Ang II-induced pressor response and its effect was sustained at 3 h post-treatment. The inhibitory effect of BR-A-657 in Ang II pressor rats indicates that blockade of Ang II-induced vasoconstriction probably underlies its antihypertensive effect. Based on the above-mentioned findings, we decided to study hypotensive effects in furosemide-treated rats and renal hypertensive rats. Both of these experimental models are RAS-activated, which is known to play an important role in the development and maintenance of blood pressure. Furthermore, furosemide-treated rats represent a normal blood pressure model, whereas renal artery-ligated rats represent an elevated blood pressure model.\(^{17,18}\) In both studies, BR-A-657 exhibited a dose-dependent reduction of arterial blood pressure, which was more potent than that induced by losartan in both models. In addition, BR-A-657 had a more rapid onset than losartan, that is, maximum antihypertensive effect were observed at 20 min and 120 min, respectively, post-treatment, presumably because of the in vivo generation of the active metabolite (EXP3174) from losartan after oral administration in rats.\(^{25}\) Furthermore, the biphasic hypotensive effects of losartan in these in vivo studies are assumed to be caused by the kinetics of its active metaboliteExp3174 formation.\(^{26,27}\) Therefore, the immediate effect of BR-A-657 in vivo can probably be attributed to direct antagonism by the parent compound. A comparison with the results obtained from furosemide-treated and renal hypertensive rats showed that antihypertensive effects and haemodynamic profiles after the intravenous administration of BR-A-657 were similar, that is, rapid arrival of \(E_{\text{max}}\) and long duration. In the present study, a slight, transient increase in heart rate was observed after intravenous administration of BR-A-657 or losartan to furosemide-treated rats and renal hypertensive rats, but no significant difference in heart rates was observed versus vehicle-treated controls over 180 min post-treatment. A similar increase in heart rate has been reported after intravenous injection of valsartan in renal hypertensive rats.\(^{20}\) In the present study, it was found that BR-A-657 (10 mg/kg, i.v.) maximally reduced mean arterial pressure to the same extent as losartan at the same dose in furosemide-treated rats. These results can probably be explained by the fact that animals were given several injections of furosemide under physiological conditions, and that basal arterial pressure might have been influenced.\(^{16}\)

Further evidence for the antihypertensive effects of orally administrated BR-A-657 were demonstrated in conscious renal hypertensive rats. Given orally administrated, BR-A-657 is significantly more potent than losartan. Similar to other blockers of the RAS\(^{28}\) and nonpeptide Ang II antagonists,\(^{18}\) BR-A-657 did not increase heart rate (data not shown). In the

**DISCUSSION**

In the present study, to characterize the pharmacological specificity of the newly synthesized BR-A-657 for Ang II receptors, the affinities of BR-A-657, valsartan and losartan for these receptors were assessed and compared in radioligand binding studies using the membranes of HEK-293 cells. BR-A-657 concentration-dependently displaced specifically bound \(^{125}\)I[Sar\(^1\)-Ile\(^8\)]Ang II from HEK-293 cells membranes with an IC\(_{50}\) value of 0.16 nM. It was 14.4- and 22.5-times more potent than valsartan and losartan, respectively. Moreover, BR-A-657 showed more potent interaction with AT\(_1\) receptor (IC\(_{50}\)=0.16 nM) than AT\(_2\) receptor (IC\(_{50}\)=69 \(\mu\)M) from the membranes of HEK-293 cells (IC\(_{50}\) ratio of AT\(_2\)/AT\(_1\) is 431, 250). The major cardiovascular effects of Ang II observed in hypertension, diabetic nephropathy and congestive heart failure are mediated by AT\(_1\) receptors.\(^{7,8}\) In the present study, pressor responses to exogenous Ang II were found to be inhibited by BR-A-657. Despite the presence of both Ang II binding sites (AT\(_1\) and AT\(_2\)) in recombinant human HEK-293 cell membranes, renin secretion is enhanced by AT\(_1\) but not by AT\(_2\) antagonists.\(^{22}\) Several other studies have investigated the functional significance of these subclasses of Ang II binding sites by examining a number of Ang II responses.\(^{16,23}\) The present study also demonstrates the presence of one class of binding site for the radiolabeled Ang II in HEK-293 cells membranes. In membranes, BR-A-657 slightly decreased the \(B_{\text{max}}\) and increased the \(K_d\) of the radioligand for its receptors, suggesting that BR-A-657 is a noncompetitive inhibitor. These results suggest that BR-A-657 specifically antagonizes Ang II at AT\(_1\) subtype receptors.

In the present study, several functional in vitro and in vivo studies were performed to characterize the mode of interaction between BR-A-657 with the AT\(_1\) receptor. BR-A-657 reduced the maximal response to Ang II and caused a nonparallel shift of the concentration–response curves to the right. In contrast, losartan caused a parallel rightward shift of the concentration–response curve for Ang II without affecting the maximal response. The inhibitory effect of BR-A-657 on the

![Fig. 6. Effects of Oral Administration of BR-A-657 and Losartan on Mean Arterial Pressure in Conscious Renal Hypertensive Rats](image-url)
present study, BR-A-657 decreased blood pressure in high renin furosemide-treated and renal artery-ligated rats, which suggests an association between the RAS and the hypertensive effect of BR-A-657. Moreover, parallels over time were observed between the antihypertensive effect of BR-A-657 at each intravenous (0.03, 0.1, 0.3 mg/kg) or oral (1, 3 mg/kg) dose in renal artery-ligated rats and reduced Ang II-induced pressor response in normotensive rats, which indicates that blockade of the vasoconstrictor effect of Ang II by BR-A-657 is likely to be the primary mechanism underlying its antihypertensive effect.

In summary, the results of this study demonstrate that BR-A-657 is a potent AT₁ subtype-selective nonpeptide Ang II receptor antagonist, and suggest that it has a stronger hypertensive effect than losartan. Accordingly, we conclude that BR-A-657 is a potent candidate drug for the treatment of hypertension.

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