Nonpeptide Angiotensin II Receptor Antagonists

Studies With EXP9270 and DuP 753


A series of nonpeptide angiotensin II (Ang II) receptor antagonists was evaluated in rat adrenal cortical microsomes for their inhibitory effects on the specific binding of [3H]Ang II, in the isolated rabbit aorta bioassay for their functional antagonism of contractile response to Ang II, and in high renin, renal-hypertensive rats for their intravenous antihypertensive effects, expressed as IC50, pA2, and intravenous ED50, respectively. Highly significant linear correlations were found between IC50 and pA2 (r=—0.88), between IC50 and intravenous ED50 (r=0.79), and between pA2 and intravenous ED50 (r=—0.93). In both in vitro and in vivo functional assays, none of these antagonists exhibited agonistic effects. The orally active nonpeptide Ang II receptor antagonists EXP9270 and DuP 753 (oral ED50=3.6 and 0.59 mg/kg, respectively) were selected for further characterization. These antagonists exhibited selective and competitive Ang II antagonism in rabbit aorta and guinea pig ileum. In conscious normotensive rats, DuP 753 abolished the pressor response to saralasin, suggesting that the pressor effect of saralasin is attributed to its Ang II-like activity. In addition, DuP 753 also blocked the Ang II-induced drinking response and aldosterone release in rats. These results suggest that Ang II receptor blockade is the primary mechanism of the antihypertensive effect of these nonpeptide Ang II receptor antagonists. Further, the specificity and lack of partial agonistic effects of these molecules make them potentially useful physiological probes and therapeutic agents. (Hypertension 1990;15:823–834)

The successful development of orally active angiotensin converting enzyme (ACE) inhibitors for the treatment of hypertension and congestive heart failure has generated great interest in designing new pharmacological blockers of the renin-angiotensin system (RAS). As angiotensin II (Ang II) is the primary effector molecule of the RAS, a receptor antagonist of Ang II would provide a direct approach to block the system. Despite decades of research, however, currently available Ang II antagonists are still peptide mimetics of Ang II with limitations of short duration, poor oral bioavailability, and agonistic activities.2

Recently, we characterized the first nonpeptide Ang II receptor antagonists, S-8307 and S-8308, which were disclosed originally by Furukawa et al3,4 and found that they had poor affinities for rat adrenal cortical Ang II binding sites but exerted selective Ang II functional antagonism.5,6 However, these molecules may possess other actions in addition to Ang II receptor blockade, as captopril, saralasin, or bilateral nephrectomy reduced but did not completely abolish the hypotensive effect of S-8307 at high doses.5 Subsequently, we developed more potent and selective nonpeptide Ang II receptor antagonists, such as EXP6155 and EXP6803.7,8 Unlike saralasin, EXP6155 and EXP6803 did not have agonistic activities.7,8 Although these blockers were effective Ang II receptor antagonists in rats when given intravenously, they were inactive when given orally at 100 mg/kg.7,8 The lack of oral activity of EXP6803 in the rat appears to be due to poor absorption from the gastrointestinal tract.9 Nevertheless, EXP6803 is still a useful research tool, as it identified the blockade of Ang II formation as the primary mechanism of the short-term antihypertensive effect of captopril in renal-hypertensive rats.10 Very recently, based on these prototype molecules, orally active nonpeptide Ang II receptor antagonists with increased potency were developed.11 In this report, a series of nonpeptide Ang II receptor antagonists was evaluated in rat adrenal cortical
microsomes for their inhibitory effects on the specific binding of [3H]Ang II in the isolated rabbit aorta bioassay for their functional antagonism of Ang II and in renal-hypertensive rats for their antihypertensive effects. Further, the orally active nonpeptide Ang II receptor antagonists, 1-[(2'-carboxybiphenyl-4-yl)methyl]-2-(1-trans-butenyl)-4-chloro-5-hydroxymethylimidazole (EXP9270) and 2-n-buty1-4-chloro-5-hydroxymethyl-1-[(2'-1H-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazole, potassium salt (DuP 753), were selected for further characterization.

Methods

The animal care procedures were reviewed and approved by the Du Pont Experimental Station Animal Welfare Committee.

In Vitro Adrenal Angiotensin II Binding Assay

The Ang II binding assay has been described previously. In brief, various concentrations of each antagonist were incubated with freshly prepared rat adrenal cortical microsomes in Tris buffer containing 2 nM [3H]Ang II (27.8 Ci/mmol, Du Pont, New England Nuclear, Boston, Massachusetts). After 60 minutes at 25°C, ice-cold Tris buffer was added, and the bound and free radioactivities were separated by filtration through a glass fiber filter. The trapped radioactivity was determined in a Packard PRIAS scintillation counter (Downers Grove, Illinois). Data were calculated as specific binding, that is, the binding displaced by 10 μM unlabeled Ang II. The inhibitory concentration of each antagonist that gave 50% inhibition of the specific binding of Ang II (IC50) was determined from the displacement curves.

In Vitro Potency and Selectivity Determination in Rabbit Aorta

Rabbits were killed by stunning and cervical dislocation. The rabbit aorta helical strips were prepared, and cumulative concentration-response curves were generated as described previously. The strips were mounted in tissue baths containing Krebs' bicarbonate solution of the same composition as described previously. The Krebs' solution was kept at 37°C and bubbled continuously with 5% CO2 in O2. Initial resting tension was set and maintained at 0.5 g. After equilibration, control contractile response to Angiotensin I (Ang I, 2.6×10−9 M), Ang II (5.4×10−10 M), androgensol III (Ang III, 1×10−8 M), angiotensin III (Ang III, 1×10−8 M), bradykinin (1.1×10−8 M), acetylcholine (3×10−8 M), serotonin (1.4×10−7 M), or histamine (1.3×10−7 M) was recorded. (These concentration values represent the median effective concentrations [EC50] for Ang I, Ang II, Ang III, acetylcholine, serotonin, and histamine, respectively, and the EC50 for bradykinin determined in our pilot studies.) Each strip was tested with one agonist only. The tissue was washed and then incubated with DuP 753 for 15 minutes before the addition of Ang I, Ang II, Ang III, bradykinin, acetylcholine, serotonin, or histamine. This procedure was repeated at 15-minute intervals with increasing concentrations of DuP 753. Contraction was recorded with a Grass force-displacement transducer coupled to a Grass polygraph (Grass Instr. Co.). Response values were expressed as a percent change of control response induced by each agonist in the presence of various concentrations of DuP 753. The concentration of DuP 753 that inhibited the contractile response to Ang I, Ang II, or Ang III by 50% was determined by linear regression. Effects of captopril at 10−8−10−7 M on the contractile responses to Ang I and bradykinin were also examined. In addition, the effect of saralasin at 10−8−10−5 M concentration on the contractile response to bradykinin was studied.

Antihypertensive Effect in Conscious Renal-Hypertensive Rats

The renal-hypertensive rats (RHRs) were prepared by complete ligation of the left renal artery as described previously. Six days after ligation, the rats were anesthetized with hexobarbital (90 mg/kg i.p.), and both the right jugular vein and the carotid artery were cannulated. The catheters were passed subcutaneously to the dorsal side of the neck and exteriorized. After the rats had completely recovered from anesthesia (at least 2–2.5 hours after surgery), the carotid artery catheter was connected to a Gould pressure transducer (Gould Inc., Oxnard, California) to monitor mean arterial pressure (MAP). Heart rate was recorded by a Grass tachograph (Grass Instr. Co.). We have shown previously that hypertension in this animal model depends on activation of
the RAS because plasma renin activity is elevated in these animals and blockade of the RAS by captopril reduces blood pressure to normotensive levels.8

**Group 1.** Each of a series of nonpeptide Ang II antagonists listed in Figure 1 was given intravenously in a cumulative manner at intervals of 15 minutes or was given by gavage as a single dose in RHRs. The effective doses (ED50s) of each antagonist that decreased MAP by 30 mm Hg for both the intravenous (IV ED50) and oral (PO ED50) routes of administration were then calculated by linear regression.

**Group 2 (Figure 5).** EXP9270 was given intravenously in a cumulative manner at 0.1-10 mg/kg at intervals of 15 minutes. The ED50 of EXP9270 that decreased MAP by 30 mm Hg was then calculated by linear regression.

**Group 3 (Figure 5).** RHRs were dosed by gavage with EXP9270 at 3 or 10 mg/kg, and the experiment was monitored for 120 minutes.

**Group 4 (Figure 5).** To ascertain the specificity of EXP9270 as an Ang II receptor antagonist in RHRs, Ang II at 0.1 μg/kg i.v., norepinephrine at 0.1 μg/kg i.v., and vasopressin at 0.03 IU/kg i.v. were given at intervals of 5-10 minutes before administration of EXP9270 at 10 mg/kg i.v. and subsequently at 5, 30, and 60 minutes after EXP9270.
Effects in Conscious Normotensive Rats

Conscious male cesarean-derived Sprague-Dawley rats (300-350 g) (Charles River Labs., Inc., Kingston, New York) were used in this series of experiments. Group 1 (Figure 7). Rats were surgically prepared with arterial and venous catheters as described above 3 hours before the experiment and were pretreated with vehicle or DuP 753 at 10 mg/kg i.v. 15 minutes before the injection of saralasin. Saralasin was then given intravenously at 0.1-10 µg/kg in a cumulative manner at intervals of 15 minutes.

Group 2 (Figure 8). Rats were surgically prepared with arterial and venous catheters as described above 3 hours before the experiment. After establishing two control pressor responses to Ang II at 0.1 µg/kg, DuP 753 at 1, 3, or 10 mg/kg was given subcutaneously, and the pressor response to Ang II was again determined at 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, and 180 minutes after dosing.

Group 3 (Figure 8). To examine the effect of DuP 753 on Ang II-induced thirst, the protocol as described by Katovich et al. was used with some modifications. Rats were placed into individual metabolic cages for 2 days of habituation. One hour before the beginning of the study, DuP 753 at 3 or 10 mg/kg or its vehicle was given subcutaneously. At the beginning of the experiment, half of the vehicle-treated and all of the DuP 753-treated rats were administered Ang II at 200 µg/kg s.c., a dose derived from the study of Katovich et al. The remaining half of the vehicle-treated rats were given saline subcutaneously. Water intake was measured by weight difference of a preweighed drinking bottle 2 hours after administration of Ang II or its vehicle. To study the effect of DuP 753 alone on water intake, a separate group of rats was treated subcutaneously with vehicle or DuP 753 at 10 mg/kg 1 hour before the beginning of the experiment, and a 2-hour water intake was then determined.

Group 4 (Figure 9). To study the effect of DuP 753 on Ang II-induced aldosterone release, the protocol as described by Campbell and Pettinger was used with some modifications. Rats were surgically prepared with arterial and venous catheters 1 day before the experiment. In pilot studies, different intravenously infused doses of Ang II were evaluated for their pressor effects in conscious normotensive rats. Infusion of Ang II at 0.1 µg/min was found to cause the maximal pressor response and, therefore, was used for the following study. Fifteen minutes before infusion of Ang II, rats were pretreated with vehicle or DuP 753 at 10 mg/kg i.v. Ang II was then infused intravenously at 0.1 µg/min for 30 minutes, and at the end of the infusion, MAP was recorded. The rats were then decapitated, and aortic blood samples were collected. The blood was allowed to clot and was centrifuged at 4°C. Serum samples for aldosterone measurement were stored at -40°C until use.

Analyses and Statistics

Aldosterone was determined by radioimmunoassay with a Diagnostic Products radioimmunoassay kit (Los Angeles, California). Statistical analyses used were correlation, analysis of variance, and Duncan's new multiple-range test for multiple comparison. These analyses were performed with a computer package, STATISTICAL ANALYSIS SYSTEM (SAS Institute Inc., Cary, North Carolina), on a VAX 8650 computer. The level of significance was at p less than 0.05. All data were expressed as mean±SEM.

Drugs

Nonpeptide Ang II antagonists used in this study were dissolved in a mixture of 5% NaHCO3 and 5% dextrose (50:50) at 10-30 mg/ml and diluted with 5% dextrose to the desired concentration; however, S-8307, S-8308, EXP6155, EXP6803, EXP7367, and DuP 753 were dissolved in saline solution and water for intravenous and oral administrations, respectively. These antagonists were given intravenously at 1 ml/kg, orally at 5 ml/kg, and subcutaneously at 2 ml/kg. Captopril was injected intravenously in saline at 1 ml/kg. Ang I, Ang II, Ang III, norepinephrine, saralasin, and vasopressin were given intravenously in saline solution at 0.1 ml/kg and were obtained from Sigma Chemical Co., St. Louis, Missouri. When given subcutaneously, Ang II was given in saline at 2 ml/kg. Nonpeptide Ang II antagonists listed in Figure 1 and captopril were synthesized at E.I. du Pont de Nemours and Company, Wilmington, Delaware. Both the potassium and sodium salts of DuP 753 were used in these studies. They were water soluble and were shown to be pharmacologically equivalent in preliminary studies.

Results

Relation Between IC50 and pA2 and Between pA2 and Intravenous ED30

As shown in Figure 2, highly significant linear correlations were found between IC50 and pA2 (r=−0.88) and between pA2 and IV ED30 (r=−0.93) of a series of nonpeptide Ang II antagonists listed in Figure 1. A significant linear correlation was also found between IC50 and IV ED30 (r=0.79, p<0.001). In the isolated rabbit aorta bioassay, these antagonists all exhibited competitive selective functional
antagonism of the contractile response to Ang II. In all functional assays, none of these antagonists exhibited partial agonistic effects.

In Vitro Potency and Selectivity Determination of EXP9270 in Rabbit Aorta

In rabbit aorta, EXP9270 caused parallel shifts to the right of Ang II concentration–contractile response curves and did not alter the maximal response to Ang II at $3 \times 10^{-8}$ to $3 \times 10^{-7}$ M ($n=6$ per concentration; Figure 3). The $pA_2$ and the slope of the Schild plot were 7.93 and 1.05, respectively. A similar result was also obtained with DuP 753 (P. C. Wong, unpublished observations) for which the $pA_2$ and the slope of the Schild plot were 8.48 and 0.9, respectively. In this respect, EXP9270 and DuP 753 were 35- and 10-fold, respectively, less potent than saralasin ($pA_2=9.47$). At $10^{-5}$ M, EXP9270 did not
In Vitro Potency and Selectivity Determination of DuP 753 in Guinea Pig Ileum

In guinea pig ileum, control responses to Ang I (n=9), Ang II (n=9), Ang III (n=8), bradykinin (n=9), acetylcholine (n=6), serotonin (n=6), and histamine (n=6) averaged 1.37±0.13, 1.13±0.08, 1.32±0.25, 0.65±0.11, 1.83±0.45, 1.54±0.27, and 2.18±0.42 g, respectively. DuP 753 did not alter the contractile responses to acetylcholine, serotonin, and histamine although it effectively inhibited the responses to Ang I, Ang II, and Ang III (Figure 4). The IC50 in inhibiting the Ang I, Ang II, and Ang III responses for DuP 753 were 2.5×10⁻⁸, 1.4×10⁻⁸, and 4.3×10⁻⁹ M, respectively. A similar result was obtained with EXP9270 (data not shown). Captopril significantly inhibited the contractile response to Ang I by 35±7%, 82±1%, 99±1%, and 100±0% at 10⁻⁸, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M, respectively (p<0.05; control Ang I response =1.58±0.31 g, n=6) and significantly enhanced the contractile response to bradykinin at 10⁻⁹-10⁻⁷ M, respectively (control bradykinin response=0.51±0.07 g, n=8; Figure 4). Saralasin did not significantly change the contractile response to bradykinin at 10⁻⁹ but potentiated it at 10⁻⁵ M by 183±26% (p<0.05; control bradykinin response=0.41±0.11 g, n=6; Figure 4). In contrast, DuP 753 did not alter the contractile response to bradykinin.

Antihypertensive Effects of EXP9270 and DuP 753 in Conscious Renal-Hypertensive Rats

As shown in Figure 5, cumulative intravenous injections of EXP9270 caused a dose-dependent decrease in MAP (n=6). Given orally at 3 (n=6) and 10 (n=6) mg/kg, EXP9270 decreased MAP dose dependently. At 10 mg/kg p.o., EXP9270 decreased MAP from 167±7 to 118±8 mm Hg at 120 minutes after dosing (p<0.05). At 10 mg/kg i.v., EXP9270 decreased blood pressure significantly and inhibited the pressor response to Ang II but not to norepinephrine (AI=4). However, the pressor response to vasopressin was enhanced after administration of EXP9270.

As shown in Figure 6, compared with the vehicle-treated group (n=6), DuP 753 decreased blood pressure dose dependently at 1 (n=4) and 3 (n=5) mg/kg i.v. At 3 mg/kg i.v., DuP 753 decreased blood pressure to a normal level (p<0.05), and captopril at 3 mg/kg i.v. did not lower blood pressure further. Compared with the vehicle-treated group (n=5), DuP 753 at 3 (n=5) and 10 (n=6) mg/kg p.o. caused a dose-dependent decrease in blood pressure. At 10 mg/kg p.o., DuP 753 decreased blood pressure to a normal level at 4–6 hours after dosing (p<0.05).

Effects of DuP 753 in Conscious Normotensive Rats

As shown in Figure 7, cumulative bolus injections of saralasin caused a dose-dependent transient increase in blood pressure in vehicle-treated conscious normoten-
sive rats (n=6). Pretreatment with DuP 753 at 10 mg/kg i.v. abolished the pressor effect of saralasin (p<0.05). It should be noted that bolus injection of DuP 753 at 10 mg/kg i.v. did not increase blood pressure as did saralasin. Higher doses of DuP 753, including those at 30 and 100 mg/kg i.v., also did not increase blood pressure in this model (P. C. Wong, unpublished observation).

DuP 753 at 1, 3, and 10 mg/kg s.c. caused rapid onset of inhibition of the pressor response to Ang II at 0.1 μg/kg i.v. dose dependently (Figure 8). The inhibitory effect lasted for at least 3 hours (p<0.05). The two higher subcutaneous doses of DuP 753 were
Mean arterial pressure (mm Hg) on renal hypertensive rats.

**Figure 5.** Upper panel: Line plot showing effects of EXP9270 as a function of time (min) given cumulatively (cum) and intravenously (iv) on mean arterial pressure (mm Hg) in renal-hypertensive rats (n=6). Middle panel: Line plots showing effects of EXP9270 as a function of time (min) given by gavage (3[O] and 10 [•] mg/kg p.o.) on mean arterial pressure (mm Hg) in renal-hypertensive rats. Lower panel: Bar graphs showing effects of EXP9270 (10 mg/kg iv.) on mean arterial pressure (MAP; mm Hg, expressed as absolute change from control MAP) and pressor responses to angiotensin II, norepinephrine, and vasopressin in renal-hypertensive rats (n=4). Basal MAP of this group of renal-hypertensive rats was 192± 7 mm Hg.

Discussion

To substantiate that the antihypertensive effect of nonpeptide Ang II receptor antagonists is due to the blockade of the vasoconstrictor effect of Ang II at its receptor, correlations among their binding affinities for the adrenal cortical Ang II binding sites (IC50s), their potencies in functionally antagonizing the contractile effect of Ang II (pA2), and their intravenous antihypertensive potencies (IV ED30) in RHRs were determined. Our study shows excellent linear relations between IC50 and pA2, between pA2 and IV ED30, and between IC50 and IV ED10, suggesting that the blockade of the vasoconstrictor effect of Ang II at its receptor is the primary mechanism of the antihypertensive effects of nonpeptide Ang II receptor antagonists.

To illustrate the characteristics of this series of nonpeptide Ang II receptor antagonists, the orally active compounds EXP9270 and DuP 753 were selected for further study. In rabbit aorta, the nonpeptide Ang II receptor antagonists listed in Figure 1 all exhibited selective and competitive Ang II antagonism. For instance, EXP9270 shifted the concentration-contractile response curve for Ang II parallel to the right and did not alter the maximal response to Ang II. The slope of the Schild plot for EXP9270 was 1.05, suggesting competitive antagonism. The antagonism induced by EXP9270 was specific since at a high concentration (10^-5 M), EXP9270 did not affect the contractile responses to norepinephrine and KCl. A similar result was obtained for DuP 753 (P. C. Wong, unpublished observations). Based on pA2 values, EXP9270 and DuP 753 are more potent than the initial benzylimidazole leads S-8307, S-8308, EXP6155, and EXP6803.5-8

A similar selective Ang II antagonism was demonstrated in the guinea pig ileum. Even up to 10^-5 M, DuP 753 did not change the contractile response to bradykinin, acetylcholine, serotonin, and histamine but still blocked the responses to Ang I, Ang II, and...
Ang III effectively. Historically, Ang I has been considered an inactive precursor of Ang II. The contractile response to Ang I at the low concentrations used in this study in the guinea pig ileum is presumably due to its conversion to Ang II by ACE since captopril at 10^{-6} M completely abolished the contractile effect of Ang I. Therefore, our study supports the contention that, in addition to blocking the response to exogenous Ang II, DuP 753 is effective in inhibiting the effect of locally formed Ang II in the guinea pig ileum. Compared with our early chemical leads EXP6155 and EXP6803, DuP 753 is five to 30 times more potent in blocking Ang I and Ang II contractile responses in the guinea pig ileum.\(^8\) Potentiation of the bradykinin response in the guinea pig ileum is commonly used as a means to determine the ACE inhibitory activity as shown previously\(^8\) and in this study. As the bradykinin contractile response was not changed by DuP 753, our data, therefore, suggest that DuP 753 does not have ACE inhibitory activity. This is further supported by the finding that DuP 753 up to 10^{-5} M did not alter the kinetics of \(^{14}\)C-hippuryl-histidyl-leucine hydrolysis by rabbit lung converting enzyme (A. T. Chin, unpublished observations). Interestingly, saralasin at 10^{-5} M enhanced the contractile response to bradykinin in the guinea pig ileum, which confirms the previous finding that saralasin at high concentrations inhibits the activity of ACE.\(^7\) A direct potentiation of bradykinin by saralasin was also observed in conscious rats by Textor et al.\(^8\) This result suggests that DuP 753 is a more physiologically specific Ang II receptor antagonist than saralasin.
As a result of structure-activity relation studies to improve the oral bioavailability of our early chemical leads EXP6155 and EXP6803, EXP9270 and DuP 753 were identified to exert an antihypertensive effect after oral administration in RHRs. The mechanism of the antihypertensive effects of EXP9270 and DuP 753 in the RHR is likely attributed to their blockade of the vasoconstrictor effect of Ang II for the following reasons. First, the antihypertensive potencies of EXP9270 and DuP 753 correlate with their Ang II functional antagonistic potencies in rabbit aorta and also with their adrenal cortical Ang II binding affinities as shown above. Second, EXP9270 at an antihypertensive dose inhibited the pressor response to Ang II but not to norepinephrine, suggesting the specificity as a selective Ang II antagonist in the RHR. Further, the antihypertensive effect of DuP 753 seems to depend on the prevailing level of plasma renin activity, as DuP 753 and 10 mg/kg i.v. lowered blood pressure in neither conscious normotensive rats, a normal-renin model, nor in DOCA-hypertensive rats, a low-renin model (P. C. Wong, unpublished data). It should be noted that the antihypertensive efficacy of DuP 753 is at least similar to that of captopril since captopril at a maximal dose of 3 mg/kg i.v.\textsuperscript{10} did not reduce blood pressure further. As DuP 753 is a selective Ang II antagonist, this result also implies that the short-term antihypertensive effect of captopril in the RHR is due to the blockade of formation of Ang II, confirming our previous finding with EXP6803.\textsuperscript{10} Interestingly, the
Angiotensin II pressor response (mm Hg)

Figure 8. Upper panel: Line plot showing effects of DuP 753 at 1 (n=5), 3 (n=6), and 10 (n=5) mg/kg subcutaneously as a function of time (min) on pressor response (mm Hg) to angiotensin II (0.1 μg/kg i.v.) in conscious normotensive rats. Lower panel: (n=8 per group). Bar graph showing effects of vehicle, vehicle with angiotensin II (All, Ang II in text), and DuP 753 at 3 and 10 mg/kg s.c. with All on water intake (ml/kg) in conscious normotensive rats. Values represent mean±SEM.

Water intake (ml/kg)

Figure 9. Bar graphs showing effects of vehicle (n=5) and DuP 753 (n=6) at 10 mg/kg i.v. on the pressor (left panel) and aldosterone release (right panel) responses to angiotensin II (All, Ang II in text) at 0.1 μg/min in conscious normotensive rats. Values represent mean±SEM.
pressor response to vasopressin was found to be poten-
tiated after the injection of EXP9270. A similar result 
was also obtained previously with EXP6803. The 
mechanism of this action remains to be investigated. 

It is well established that peptide Ang II receptor 
antagonists possess agonistic effects. As shown 
previously and in this study, bolus intravenous injec-
tion of saralasin caused a transient increase in blood 
pressure. In contrast, DuP 753, as shown in this 
study, did not induce a pressor response at 10 mg/kg 
i.v. Further, pretreatment with DuP 753 at 10 mg/kg 
i.v. abolished the pressor responses to saralasin, 
reaffirming that the pressor effect of saralasin in rats 
is due to its agonistic effect. To our knowledge, this is 
the first direct proof in vivo that the pressor response 
to saralasin in rats is attributed to its Ang II-like 
activity.

Administration of Ang II has been demonstrated to 
stimulate water drinking in a variety of animal 
species (for reviews, see references 19 and 20). As 
drinking can be elicited by systemic as well as by 
central administration of Ang II, the circumventricu-
lar organs that have no blood-brain barrier appear 
to be the areas in which Ang II stimulates drinking 
(for reviews, see references 19 and 20). Our study 
confirms the previous study that Ang II at 200 
$\mu$g/kg s.c. induced a significant drinking response in 
rats. More importantly, the Ang II–induced drinking 
response was blocked dose dependently by the selec-
tive nonpeptide Ang II receptor antagonist DuP 753 
at 3 and 10 mg/kg s.c., suggesting that Ang II– 
induced drinking is mediated by its receptor activa-
tion. Since a high systemic dose of Ang II is required 
to induce a drinking response, plasma Ang II may not 
play a physiological role in drinking except in emer-
gency situations such as severe dehydration with high 
level of plasma Ang II. 

Numerous studies have provided convincing evidence 
that Ang II is an important agent in stimulat-
ing aldosterone secretion. As our nonpeptide 
Ang II receptor antagonists inhibit the specific bind-
ing of Ang II in rat adrenal cortical tissue, it is of 
interest to examine the functional consequence of this 
inhbition. This study shows that Ang II at 0.1 
$\mu$g/min increased blood pressure and aldosterone 
secretion in rats. Furthermore, these increases were 
blocked effectively by DuP 753 at 10 mg/kg i.v. Taken 
together, these results suggest that the inhibitory 
effect of DuP 753 on Ang II–induced aldosterone 
release in rats is a receptor-mediated event. 

In conclusion, our studies demonstrate that Ang II 
receptor blockade is the primary mechanism of the 
anti hypertensive effects of nonpeptide Ang II recep-
tor antagonists. As illustrated by EXP9270 and DuP 753, 
these antagonists have neither agonistic nor 
bradykinin-potentiating effects. Furthermore, orally 
active nonpeptide Ang II receptor antagonists such as 
EXP9270 and DuP 753 have been identified, 
suggesting the feasibility of designing orally active 
nonpeptide Ang II antagonists without losing speci-
ficity. Moreover, DuP 753, besides being an effective 
antagonist of the vasococontractor effect of Ang II, 
blocked aldosterone release and the drinking response 
induced by Ang II. In view of the physiological and 
pathophysiological importance of the RAS, this class of 
nonpeptide Ang II receptor antagonists could become 
potentially useful physiological probes and 
therapeutic agents.

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