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# Pharmacologic Characterization of CI-996, a New Angiotensin Receptor Antagonist

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## ABSTRACT

CI-996, a novel potent angiotensin II (Ang II) type 1 ($AT_1$) receptor antagonist was characterized in a number of *in vitro* and *in vivo* assays. In addition, CI-996 was compared with several reported $AT_1$ receptor antagonists including losartan, SK&F 108566 and L-158,809. In rat liver membranes CI-996 displaced specifically bound [$^{125}$I]Ang II with an $IC_{50}$ of 0.8 ± 0.1 nM. In isolated rabbit aorta CI-996 produced a concentration-dependent inhibition of Ang II-induced contraction and decreased the maximal contractile response to Ang II. CI-996 had no effect on the contractile responses to KCl, norepinephrine or endothelin. In anesthetized, ganglionic-blocked rats CI-996 produced dose-dependent inhibition of the Ang II pressor dose-response curve with an $IC_{50}$ of 6.2 μg/kg/min i.v. Orally administered CI-996 dose dependently lowered mean arterial blood pressure in conscious renal hypertensive rats, conscious sodium-depleted dogs, conscious sodium-depleted monkeys and conscious renal hypertensive monkeys. The duration of antihypertensive activity of CI-996 in rats was >24 hr after a single oral dose. The blood pressure lowering potency of CI-996 in dogs was less than that observed in either rats or monkeys. There was no tachyphylaxis to the antihypertensive effects of CI-996 after repeated administration in renal hypertensive monkeys. These data demonstrate that CI-996 is a potent, selective Ang II antagonist. Furthermore, CI-996 has demonstrated blood pressure-lowering activity after oral administration in rats, dogs and monkeys.

The first angiotensin receptor antagonist was described in 1971 (Pals *et al.*, 1971). Although saralasin and other related peptide antagonists served as useful tools to probe the renin-angiotensin system, they were of little therapeutic utility (Anderson *et al.*, 1977). The first nonpeptide antagonists were reported in 1982 (Furukawa *et al.*, 1982), and a strong research effort subsequently capitalized on this early lead with the design of potent, selective, orally active compounds (Carini and Duncia, 1988; Chiu *et al.*, 1990; Wong *et al.*, 1990a, 1990b). These efforts culminated in the discovery of losartan, the first nonpeptide angiotensin II (Ang II) receptor antagonist to be studied in clinical trials as an antihypertensive agent (Brunner *et al.*, 1992).

The availability of nonpeptide receptor ligands also provided evidence of angiotensin receptor subtypes. Two groups of researchers independently described the presence of Ang receptor subtypes based on receptor affinity for losartan ($AT_1$ receptors) or PD 123177 and CGP42112A ($AT_2$ receptors) (Chiu *et al.*, 1989; Whitebread *et al.*, 1989), and several groups subsequently confirmed these data (Balla *et al.*, 1991; Chang and Lotti, 1990; Chang *et al.*, 1990; De Gasparo *et al.*, 1990; Dudley *et al.*, 1990). A uniform nomenclature was proposed in 1991 (Bumpus *et al.*, 1991), which has become widely accepted. Numerous investigators have demonstrated that the classic functions of Ang II (smooth muscle contraction, aldosterone secretion, potentiation of norepinephrine) are mediated *via* the $AT_1$ receptor (Wong *et al.*, 1990c, 1991; Schwieler *et al.*, 1993). The studies reported here detail the pharmacology of a new $AT_1$ receptor antagonist, CI-996 (fig. 1). Although a number of angiotensin antagonists have been described, there are few reports of their antihypertensive efficacy and duration of action in nonrodent species. We report the blood pressure-lowering efficacy of CI-996 in a number of animal models and compare CI-996 with reference agents.

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## Materials and Methods

All experimental procedures involving animals or animal tissues were performed according to The Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, 1985 NIH Publication No. 86-23).

**Ang II receptor binding.** For assessing $AT_1$ receptor binding, rat liver membranes were freshly prepared by homogenizing tissues in 20 volumes of ice-cold 20 mM sodium phosphate buffer (pH 7.4)

**ABBREVIATIONS:** Ang II, angiotensin II; CI-996, 4-[2-(1-oxo-2,2,2-trifluoroethyl)-1H-pyrrol-1-yl]-2-propyl-1-[(2'-(1H-tetrazol-5-yl)biphen-4-yl)-methyl]-1H-imidazole-5-carboxylic acid; MABP, mean arterial blood pressure; RHR, renal hypertensive rat; RHM, renal hypertensive monkey; ANOVA, analysis of variance.

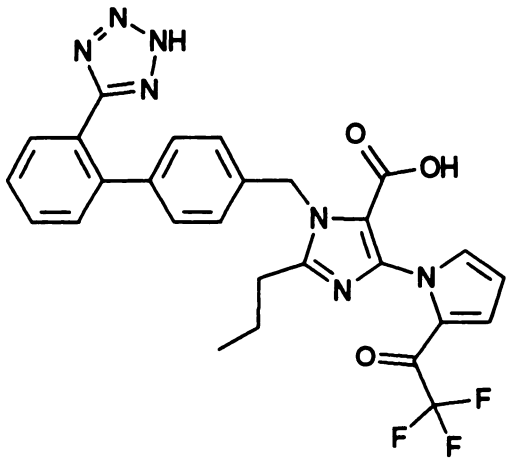

**Fig. 1.** CI-996 (4-[2-(1-oxo-2,2,2-trifluoroethyl)-1H-pyrrol-1-yl]-2-propyl-1-[(2'-(1H-tetrazol-5-yl)biphen-4-yl)methyl]-1H-imidazole-5-carboxylic acid).

using a Brinkmann Polytron PT-10 at setting 8. The homogenates were centrifuged at 48,000 × *g* for 20 min at 4°C, and the supernatant was discarded. The resulting pellet was washed once in ice-cold 20 mM sodium phosphate buffer, centrifuged as above, resuspended in 20 mM sodium phosphate buffer and used immediately. For examining $AT_2$ receptor binding, rabbit uterine membranes were prepared as above.

The binding of [$^{125}$I]Ang II to membranes was conducted in a final volume of 0.25 ml of 50 mM sodium phosphate buffer (pH 7.4) containing 100 mM NaCl, 10 mM $MgCl_2$, 100 μM EGTA, 100 μM bacitracin, 1 mg of membrane homogenate, 50 pM [$^{125}$I]Ang II and test compounds. Samples were incubated at 25°C for 1 hr and binding was terminated by filtration through Whatman GF/B glass fiber filter sheets using a Brandel 48R cell harvester. Filters were washed three times with 4 ml of Tris buffer and counted for γ radioactivity. Nonspecific binding was defined as radioactivity retained on the filters in the presence of 10 μM saralasin; specific binding was defined as total binding minus nonspecific binding. The concentration of a compound that gave 50% displacement of specifically bound [$^{125}$I]Ang II ($IC_{50}$) was calculated by weighted nonlinear regression curve-fitting to the mass action equation (Bruns *et al.*, 1986).

**Inhibition of Ang II-induced smooth muscle contraction *in vitro*.** Vascular rings were prepared from the thoracic aorta of male New Zealand white rabbits and mounted in 20-ml organ baths containing Krebs-bicarbonate solution maintained at 37°C and gassed continuously with 5% $CO_2$ in oxygen. Resting tension was adjusted to 4 g and tissues were allowed to equilibrate for 90 min before experiments were initiated. For determining $IC_{50}$ values rings were stimulated with 10 nM Ang II in the absence or presence of increasing concentrations of antagonists; inhibitory concentration response curves were generated and $IC_{50}$ values were determined graphically. In some experiments full concentration response curves were constructed for Ang II in the absence or presence of increasing concentrations of antagonist. For these experiments rings were incubated for 10 min with antagonist and increasing concentrations of Ang II added to the bath until a maximal response was achieved.

**Inhibition of Ang II-induced pressor responses.** Male CD rats (325–400 g, Charles River Laboratories, Kingston, Ont.) were anesthetized with sodium pentobarbital and instrumented with a carotid artery cannula to measure blood pressure. The jugular veins were cannulated bilaterally for i.v. drug administration and Ang II challenges. Animals were ganglionic blocked with mecamylamine (1.25 mg/kg i.v.). The maximal pressor response to an Ang II challenge (0.1 μg/kg in 0.1 ml/kg i.v.) was determined and defined as a 100% response. Animals were treated with increasing doses of antagonist or vehicle infused over 10-min intervals with an Ang II challenge at the end of each dose. Dose-response curves were constructed with data from individual animals by increasing the antagonist doses in half-log increments. The $IC_{50}$ was defined as the dose of antagonist that inhibited the Ang II pressor response by 50%.

**Antihypertensive activity in renal hypertensive rats.** Renal hypertension was induced in male CD rats (5 week old, Charles River Laboratories, Kingston, Ont.) with a silver clip placed on the right renal artery. Approximately 10 to 13 weeks later animals were prepared with indwelling aortic catheters for continuous monitoring of arterial blood pressure and heart rate. Animals were returned to their home cage on a harness swivel apparatus and allowed food and water *ad libitum*. Animals with mean arterial blood pressure (MABP) <145 mm Hg were considered borderline hypertensive and were rejected from the study. On study days separate groups of rats were dosed by oral gavage with Ang II receptor antagonists (CI-996: 1–30 mg/kg; losartan: 3–30 mg/kg; L-158,809: 1–10 mg/kg; SK&F 108856: 3–30 mg/kg) in a dose volume of 2 ml/kg. Vehicle time control rats received 2 ml/kg 0.1% methocel in water. Blood pressure and heart rate were monitored for up to 48 hr postdose by a computer-based data acquisition system.

**Blood pressure-lowering activity in sodium-restricted dogs.** Adult mongrel dogs were surgically instrumented with a telemetry device (Model TA11-PA-D70, Data Sciences, Inc., St. Paul, MN) for continuous recording of blood pressure and heart rate. The telemetry device transmitted signals to a computerized data acquisition system for analysis, display and storage while the animals moved about freely in their cages. Vehicle time control studies were conducted in each dog before and after drug studies. Animals were placed on a sodium-restricted diet (<10 mEq sodium/day) and received furosemide (5 mg/kg p.o.) every other day for 1 week before administration of test compounds. Ang II antagonists (CI-996: 10 and 30 mg/kg; losartan: 30 mg/kg) were administered as a powder in a gelatin capsule. (In vehicle studies animals received an empty gelatin capsule.) Blood pressure and heart rate were monitored for up to 36 hr postdose.

**Blood pressure-lowering activity in sodium-restricted monkeys.** Blood pressure-lowering studies were performed by methods described previously (Ryan *et al.*, 1994). Male cynomolgus monkeys (*Macaca fascicularis*, Charles River Laboratories, Wilmington, MA) weighing 5.0 to 8.8 kg were maintained on normal monkey chow (Ralston Purina, St. Louis, MO) and fruit. Monkeys were first trained to rest quietly in a basic macaque restrainer (Primate Products, Woodside, CA). Animals were then equipped with vascular access ports (Norfolk Medical Products, Skokie, IL) to measure arterial blood pressure and infuse drugs intravenously. The vascular access ports were implanted using sterile procedures and under gas anesthesia. Seven to ten days before drug or vehicle administration animals were placed on a low-sodium diet (Bio-Serv Inc, Frenchtown, NJ). Each monkey was treated with furosemide (Lasix, Inj USP 5%, Hoechst-Roussel) 2 mg/kg/day i.m. for 4 consecutive days before testing. Blood pressure and heart rate were measured directly using the arterial vascular access port connected to a Gould-Statham pressure transducer (Spectramed, Inc., Oxnard, CA). For analysis, computer-assisted data capture (Model HD-4, PO-NE-MAH Inc., Simsbury, CT) summarized data (continuous 1-min averages) at 15-min intervals. Animals were allowed to stabilize for 60 min before dosing. Compounds were dissolved in 0.5% methocel in water and administered by oral gavage using a 16-French rectal-colon tube (Davol, Cranson, RI) in a volume of 2 ml/kg. Before oral dosing animals were fasted for approximately 18 hr.

**Antihypertensive activity in renal hypertensive monkeys.** At least 30 days after implantation of vascular access ports, renin-dependent hypertension was produced by partial occlusion (approximately 60% reduction in renal blood flow) of the left renal artery using procedures described previously (Panek *et al.*, 1991). Two or more weeks later monkeys were challenged with an i.v. infusion of saralasin (20 μg/kg/min for 30 min, Sar$^1$-Val$^5$-Ala$^8$-ANG II, trifluoroacetate salt, Bachem Inc., Torrence, CA). Monkeys were included

in studies if renal occlusion raised MABP by at least 15 mm Hg above preocclusion blood pressure and MABP fell by at least 10 mm Hg during the saralasin infusion. After documenting that renal hypertension was present, the arterial vascular access port was replaced with a blood pressure telemetry unit (Model TA11-PA-D70, Data Sciences, Inc., St. Paul, MN). The implant provided a digitized signal of the arterial blood pressure that was transmitted to a receiver (Model RLA 2000, Data Sciences, Inc., St. Paul, MN) mounted inside the cage. The digitized signal from the receiver was converted to an analog signal by a digital/analog converter (Model R11CPA, Data Sciences, Inc., St. Paul, MN) and the analog signal was interfaced with a data acquisition system. Animals were chaired for oral dosing as described above.

**Statistics.** All data are expressed as mean ± S.E.M. Linear regression analysis were used to generate $IC_{50}$ values. An analysis of variance (ANOVA) with a Duncan's multiple range test or a Dunnett's test was used to compare treatment groups or to make within group comparisons to a base line, respectively. Statistical significance was defined as $P < .05$.

**Chemicals.** CI-996 (4-[2-(1-oxo-2,2,2-trifluoroethyl)-1H-pyrrol-1-yl]-2-propyl-1-[(2'-(1H-tetrazol-5-yl)biphen-4-yl)methyl]-1H-imidazole-5-carboxylic acid) (fig. 1), PD 123319 ((*S*)-1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-*c*]pyridine-6-carboxylic acid), losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole) (losartan is the assigned name (USAN) for DuP 753), SK&F 108566 (E-α-2-[2-butyl-1-(carboxyphenyl)methyl]-1H-imidazol-5-yl]methylene]-2-thiophenepropanoic acid) and L-158,809 (5,7-dimethyl-(2-ethyl-3-[[2'(1H-tetrazol-5-yl)[1,1']-biphenyl-4-yl]methyl]-$^3$H-imidazol[4,5-*b*]pyridine) were prepared by the Parke-Davis Chemistry Department according to methods described previously (Sircar *et al.*, 1993; Blankley *et al.*, 1989; Blankley *et al.*, 1991; Aldrich *et al.*, 1989; Carini and Duncia, 1988; Mantlo *et al.*, 1991; Weinstock *et al.*, 1991). Ang II and saralasin ([Sar$^1$,-Val$^5$,-Ala$^{8}$]-Ang II; Lot ZF777) were obtained from Bachem Inc. (Torrance, CA). [$^{125}$I]Ang II (2200 Ci/mmol) was obtained from New England Nuclear (Boston, MA). All dose calculations were made by use of parent molecular weight of the respective compounds.

## Results

**Ang II receptor binding activity.** The potency and selectivity of CI-996 for $AT_1$ receptors is detailed in table 1. The $IC_{50}$ for CI-996 at the $AT_1$ receptor in rat liver membranes was 0.8 ± 0.1 nM. The previously described $AT_1$ receptor antagonist, losartan, was 10-fold less potent whereas L-158,809 and SK&F 108566 were equipotent. All the nonpeptide antagonists were highly selective (>10,000-fold) for the $AT_1$ receptor *vs.* the $AT_2$ receptor compared with the peptide antagonist, saralasin, which was nonselective.

TABLE 1
**Inhibitory effect# of Ang II antagonists on specific Ang II binding *in vitro***

| Compound | $IC_{50}$ | |
|---|---|---|
| | $AT_1$[a] | $AT_2$[b] |
| | *nM* | |
| CI-996 | 0.8 ± 0.1 | >10,000 |
| Losartan | 12.1 ± 0.9 | >10,000 |
| SK&F 108566 | 3.3 ± 0.9 | >10,000 |
| L-158,809 | 0.7 ± 0.3 | >10,000 |
| Saralasin | 1.7 ± 0.7 | 1.6 ± 0.5 |

[a] Binding in rat liver homogenates.
[b] Binding in rabbit uterine homogenates.

**Inhibition of Ang II-Induced contraction *in vitro*.** The antagonist activity of CI-996 on Ang II-induced contraction *in vitro* is depicted in figure 2. CI-996 potently blocked Ang II-induced contraction ($IC_{50}$ 3.8 nM) in isolated rabbit aortic rings. The interaction of CI-996 in this *in vitro* system was insurmountable; addition of increasing concentrations of Ang II failed to restore the maximal constrictor response. CI-996 had no significant effect on contractile responses to norepinephrine or endothelin (ET-1) (data not shown). The $IC_{50}$ for losartan against Ang II-induced contraction was 89 nM.

**Inhibition of Ang-II-induced pressor responses *in vivo*.** The maximal pressor response to an i.v. challenge of Ang II (0.1 mg/kg) averaged 46 ± 4 mm Hg in anesthetized ganglionic-blocked rats before antagonist treatment. Rising concentrations of CI-996 administered at 15-min intervals dose-dependently inhibited Ang II-induced responses with maximal inhibition observed at 30 mg/kg/min i.v.; the $IC_{50}$ for CI-996 averaged 6.2 ± 0.6 μg/kg/min i.v. In contrast, losartan was approximately 10-fold less potent ($IC_{50}$ = 76 μg/kg/min i.v.).

**Antihypertensive activity in RHR.** MABP in conscious two-kidney, one-clip RHR averaged 180 mm Hg; heart rate averaged 351 beats/min. Orally administered CI-996 had dose-dependent, long-lasting blood pressure-lowering effects in conscious RHR (fig. 3). The effect of 1 mg/kg CI-996 on blood pressure was not significantly different from vehicle alone. Doses of 3, 10 and 30 mg/kg significantly lowered blood pressure ($P < .05$) producing maximal reductions in MABP of 22%, 28% and 42%, respectively. At the 3 mg/kg dose significant antihypertensive activity ($P < .05$) was first noted at 3 hr postdose; blood pressure remained significantly decreased for up to 24 hr. Significant antihypertensive effects of 30 and 100 mg/kg CI-996 were first apparent within 1 to 2 hr postdose. Maximal antihypertensive activity occurred at approximately 6 to 8 hr postdose; blood pressure decreased more rapidly with increasing doses of CI-996. Heart rate increased

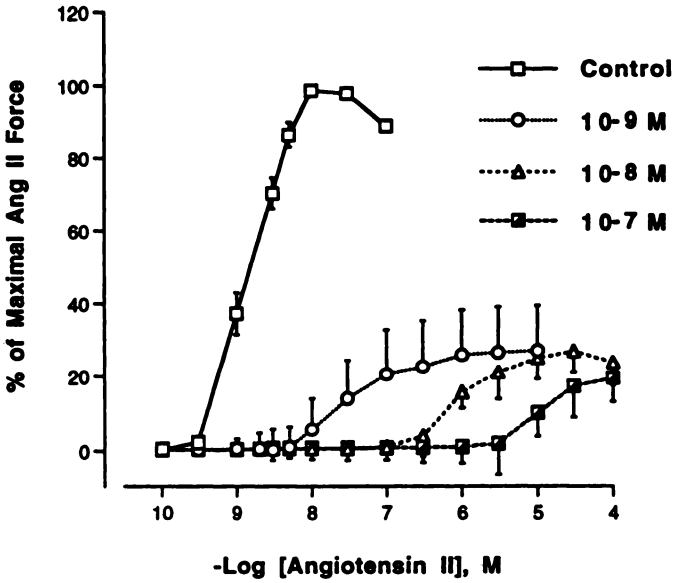


Fig. 2. Effect of increasing concentrations of CI-996 on the contractile responses to Ang II in isolated rabbit aortic rings. All values are the mean ± S.E.M. of three separate experiments performed in triplicate and expressed as the concentration required to displace 50% of maximum [$^{125}$I]Ang II binding.

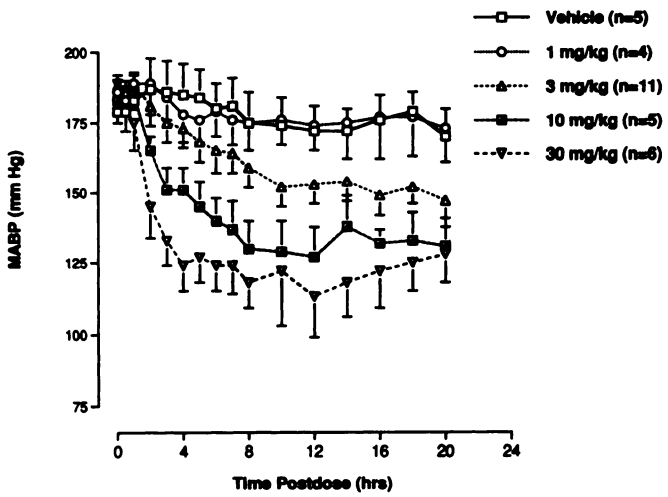


Fig. 3. Dose-related antihypertensive effects of CI-996 or vehicle in conscious renal hypertensive rats. CI-996 or vehicle was administered by oral gavage at time = 0; data are presented as group mean ± S.E.M.

modestly (+ 20 beats/min) in both CI-996- and vehicle-treated rats. The antihypertensive effects of CI-996 lasted up to 48 hr postdose in animals treated with doses ≥3 mg/kg.

Figure 4 depicts the relative effects of CI-996, losartan, L-158,809 and SK&F 108566 in conscious RHR. All of these compounds produced significant reductions in blood pressure ($P < .05$) compared with base-line values within each group or compared with a vehicle control group. In the RHR model L-158,809 seemed to be the most potent antihypertensive after oral administration; whereas SK&F 108566 was least potent when compared at a single dose of 10 mg/kg. These antagonists tended to be long acting; each had a duration of action of antihypertensive activity that was >24 hr with the exception of SK&F 108566. Blood pressure in SK&F 108566-treated rats was not significantly different from vehicle controls at time points ≥16 hr postdose.

**Blood pressure-lowering activity in sodium-depleted dogs.** The blood pressure-lowering effects of orally administered CI-996 and losartan were examined in conscious, sodium-depleted, normotensive dogs using telemetry. MABP was similar during pre- and postdrug vehicle studies and ranged from 83 ± 5 to 97 ± 2 mm Hg. In these conscious dogs control heart rates ranged from 60 to 90 beats/min. Changes in MABP during vehicle control studies varied from a decrease of −10% to an increase of +7% relative to time 0 on any given day. CI-996 dose dependently lowered blood pressure (fig. 5). MABP maximally decreased 22% ($P < .05$) with a single oral dose of 30 mg/kg and 40% ($P < .05$) after the 100 mg/kg dose. The blood pressure-lowering effects of CI-996 were maximal at 9 hr with the 30 mg/kg dose; although significant blood pressure lowering was noted at 2, 3, and 4 hr postdose. With the 100 mg/kg dose of CI-996 blood pressure was maximally lowered at 4 hr postdose and remained significantly decreased for up to 20 hr postdose. In conjunction with the hypotension heart rate increased 15 to 30% (from 66 ± 2 to 87 ± 4 beats/min, $P < .05$) after 100 mg/kg of CI-996; 30 mg/kg CI-996 had no significant effect on heart rate.

A single oral dose of losartan (30 mg/kg) significantly lowered MABP 36% ($P < .05$) within 2 hr postdose in conscious dogs (fig. 5); significant hypotension persisted up to 12 hr postdose. Tachycardia was observed with the hypotension in losartan-treated dogs; heart rate increased 62% ($P < .05$) from a base line of 81 ± 3 to 132 ± 11 beats/min at 90 min postdose.

**Blood pressure-lowering activity in sodium-depleted monkeys.** Base-line MABP in conscious, sodium-depleted, chair-restrained cynomolgus monkeys averaged 90 to 93 mm Hg and baseline heart rate averaged 188 to 196 beats/min. Saralasin, used to assess the sensitivity of these animals to renin-angiotensin system blockers, lowered MABP by 20 to 25 mm Hg. Oral CI-996 maximally reduced MABP 13 ± 2, 18 ± 1 and 27 ± 4 mm Hg at doses of 3, 10 and 30 mg/kg,

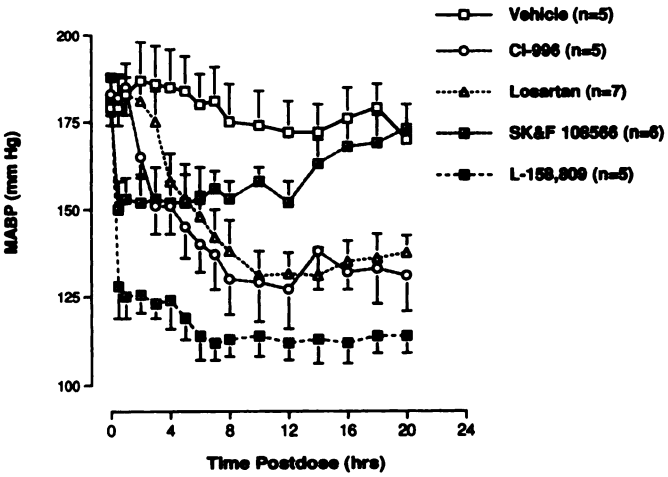


Fig. 4. Relative antihypertensive effects of CI-996, losartan, SK&F 108566 or L-158,809 in conscious renal hypertensive rats. Drugs were administered by oral gavage (10 mg/kg p.o.) at time = 0; data are presented as group mean ± S.E.M.

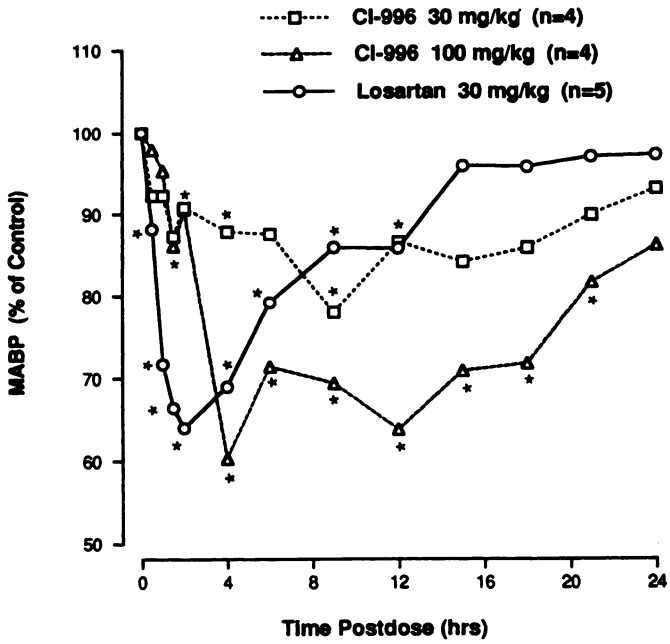


Fig. 5. Blood pressure-lowering effects of CI-996 or losartan in conscious sodium-depleted dogs. Drugs were orally administered at time = 0; mean arterial blood pressure (MABP) was monitored for up to 24 hr using radiotelemetry. Data are presented as percent of predose control values within animals; * indicates a significant change ($P < .05$) from base-line blood pressure within a group of dogs.

respectively (fig. 6). Blood pressure was significantly reduced ($P < .05$ compared with pretreatment base line) within 90 min of both the 3 and 10 mg/kg doses; the fall in blood pressure was more rapid at the 30 mg/kg which significantly lowered MABP ($P < .05$) within 30 min postdose. There was no recovery from the blood pressure-lowering effects of CI-996 during the 5-hr postdose observation period. Heart rate did not change significantly from base line in CI-996 or vehicle-treated animals.

**Antihypertensive activity in renal hypertensive monkeys.** The blood pressure-lowering effects of orally administered CI-996 and losartan were examined in conscious, renal-hypertensive cynomolgus monkeys using telemetry; the data are illustrated in figures 7 and 8. Blood pressure had a notable circadian pattern in vehicle animals ranging from 110 to 120 mm Hg between 8:00 A.M. and 6 P.M. and falling markedly within the first hour of the dark period. This pattern was observed consistently in all animals during several days of base-line data collection. CI-996 (10 mg/kg) reduced average daytime MABP about 5 mm Hg. This antihypertensive activity of the 10 mg/kg dose was statistically significant within the first 2 to 6 hr postdose and again from 10 to 14 hr postdose; during the remainder of the 24-hr interval blood pressure was indistinguishable from vehicle treatment. A dose of 30 mg/kg CI-996 reduced average daytime MABP about 15 mm Hg; these reductions in blood pressure were statistically significant ($P < .05$) within the first hour postdose, and MABP remained significantly decreased throughout the 24-hr data collection period. Thus the duration of antihypertensive action of CI-996 was approximately 20 to 24 hr in these free roaming primates. In a repeated dosing paradigm CI-996 administered for 5 consecutive days had similar blood pressure-lowering effects on the first and fifth day postdose (data not shown). Similar to blood pressure there were obvious circadian variations in heart rate; however, there were no significant effects of CI-996 on heart rate after any dose examined.

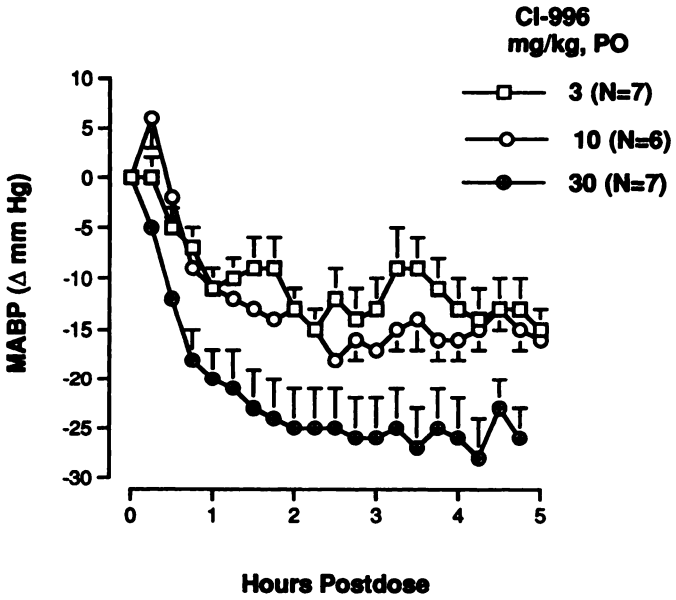


Fig. 6. Blood pressure-lowering effects of CI-996 in conscious sodium-depleted cynomolgus monkeys. CI-996 was administered by oral gavage at time = 0; mean arterial blood pressure (MABP) was monitored for up to 5 hr postdose in chair-restrained animals. Data are presented as group mean ± S.E.M.

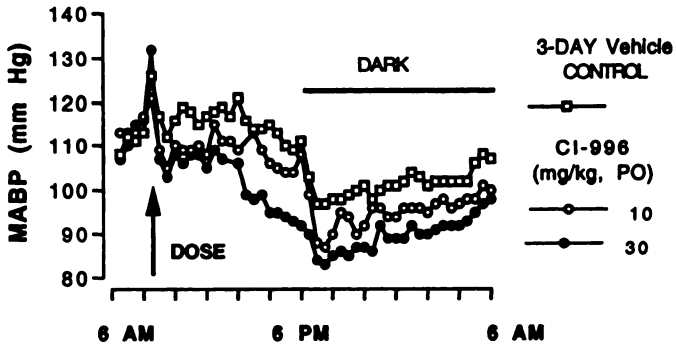


Fig. 7. Antihypertensive effects of CI-996 in conscious renal-hypertensive cynomolgus monkeys. CI-996 was administered by oral gavage; mean arterial blood pressure (MABP) was monitored continuously using radiotelemetry while animals roamed freely in their home cages. Data are presented as group mean, $n = 5$.

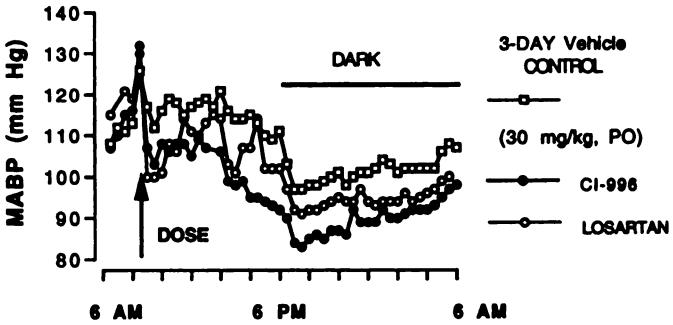


Fig. 8. Relative antihypertensive effects of CI-996 or losartan in conscious renal-hypertensive cynomolgus monkeys. Drugs were administered by oral gavage; mean arterial blood pressure (MABP) was monitored continuously using radiotelemetry while animals roamed freely in their home cages. Data are presented as group mean, $n = 5$.

The relative antihypertensive effects of CI-996 and losartan (both administered at 30 mg/kg p.o.) are depicted in figure 8 and compared to the 3-day vehicle run within these same monkeys. Both compounds significantly lowered blood pressure in the hypertensive monkeys; from a base line of 132 ± 3 mm Hg MABP decreased to a nadir of 83 ± 3 mm Hg ($P < .05$) in CI-996-treated monkeys. Losartan reduced MABP from 130 ± 4 mm Hg to a nadir of 91 ± 5 mm Hg. The decreases in blood pressure were significantly greater in CI-996 animals relative to losartan treatment during the period from 9 to 15 hr postdose. Blood pressure was significantly decreased in both groups of animals relative to vehicle controls at 20-hr postdose.

## Discussion

CI-996 is a potent and selective orally active Ang II receptor antagonist as evidenced by *in vitro* and *in vivo* data. Radioligand binding experiments using rat liver ($AT_1$) (DeGasparo *et al.*, 1990; Dudley *et al.*, 1990) or rabbit uterus ($AT_2$) (Dudley *et al.*, 1990) membranes have demonstrated that CI-996 is >10,000-fold selective for the $AT_1$ receptor.

Functional Ang II antagonism is documented by the dose-dependent inhibition of Ang II-induced contractions in rabbit aorta. CI-996 action in vascular tissue *in vitro* was characterized by a right-shift in the Ang II contractile-response

curve and a suppression of the maximal Ang II response. This "insurmountable" antagonism *in vitro* has been reported for several of the nonpeptide Ang II antagonists including EXP 3174, SR47436 and L-158,809 (Wong and Timmermans, 1991; Cazaubon *et al.*, 1993; Chang *et al.*, 1992), whereas others such as losartan seem competitive (Chiu *et al.*, 1990). Several hypotheses have been advanced to explain this insurmountable antagonism (Wong and Timmermans, 1991; Liu *et al.*, 1992); the most widely accepted hypothesis is that a slow dissociation of antagonist from the receptor in vascular tissue best explains the apparent noncompetitive action. Dissociation binding experiments with CI-996 have been performed in rat liver membranes (Overhiser *et al.*, 1992). Data from these experiments showed that a 10-min preincubation of membrane homogenates with CI-996 did not affect maximal Ang II binding density ($B_{max}$) although the affinity ($K_d$) was reduced. However, when membranes were incubated with CI-996 and then washed three times before binding experiments there was no change in either $K_d$ or $B_{max}$ indicating a rapid and reversible binding of CI-996 to $AT_1$ receptors. Therefore the prolonged *in vivo* activity of CI-996 is probably not due to noncompetitive binding of CI-996 to receptors. CI-996 alone had no effect on the basal tone in isolated rabbit aorta documenting its lack of agonist activity. CI-996 selectivity is demonstrated by the lack of effect on contractile responses induced by norepinephrine or endothelin.

CI-996 also inhibited Ang II-induced pressor responses *in vivo*. In anesthetized ganglionic-blocked rats i.v. administration of CI-996 dose dependently inhibited the increase in MABP induced by bolus administration of Ang II with no attenuation of the response to phenylephrine in these same animals. CI-996 was approximately 10-fold more potent than losartan in this model, consistent with its increased potency *in vitro* in radioligand binding experiments.

Based on the potent, selective profile of CI-996 *in vitro* we examined its blood pressure-lowering activity after oral administration to both normotensive (sodium-depleted) and hypertensive (renin dependent) animals. CI-996 dose dependently lowered MABP in two-kidney, one-clip RHR, a model in which the elevations in blood pressure are considered to be dependent on renin-angiotensin system activation. A single oral dose of 1 mg/kg CI-996 was ineffective at lowering blood pressure; however, 3 mg/kg consistently lowered blood pressure 20%, and 30 mg/kg was maximally effective in RHR. The duration of action of CI-996 in this rodent model was extremely long with blood pressure remaining suppressed for up to 48 hr after a single oral dose. CI-996 seemed roughly equipotent with losartan in RHR and slightly more potent than SK&F 108566. L-158,809 was clearly more potent than CI-996 after oral administration in rodents.

In contrast to the potent, long-acting antihypertensive effects of CI-996 in rodents (>48 hr), the blood pressure-lowering activity in sodium-depleted, normotensive, conscious dogs required higher doses and had a duration of action of about 20 to 24 hr. Whereas 3 mg/kg CI-996 consistently lowered MABP in RHR, a minimally effective dose of CI-996 in dogs was 30 mg/kg p.o. Similar results were noted with losartan. Although no pharmacokinetic data are available for CI-996, studies with losartan have documented both a low level of glucuronidation in rat liver slices and generation of an active metabolite in the rat which both contribute to a prolonged duration of action (Stearns *et al.*, 1992). In the conscious dogs in our studies, with basal heart rates averaging <80 beats/min, tachycardia was noted after blood pressure-lowering doses of both CI-996 and losartan. The change in heart rate after losartan treatment was somewhat greater perhaps due to the more rapid onset of action relative to CI-996.

Given the dissimilar profiles of CI-996 in rats and dogs we sought to characterize CI-996 activity further in conscious cynomolgus monkeys. In sodium-depleted monkeys 3 mg/kg CI-996 consistently lowered blood pressure and the effect of higher doses (10, 30 mg/kg p.o.) was dose related. The blood pressure-lowering response to oral administration of 30 mg/kg CI-996 was similar or slightly greater than that observed when monkeys were challenged with i.v. saralasin infusions suggesting that the response to CI-996 in this model is a function of Ang II antagonism. The oral potency of CI-996 in sodium-depleted monkeys was similar to that of losartan.

In conscious, two-kidney, one-clip RHM dose, related antihypertensive effects of CI-996 were noted after single oral doses of 10 and 30 mg/kg. Blood pressure was monitored in the animals using radiotelemetry while the animals roamed freely in their home cages under fixed 12-hr light/dark cycles. In this setting we observed a pronounced light/dark periodicity in blood pressure. MABP in these monkeys averaged 110 to 120 mm Hg during the "daytime" and fell approximately 15 to 20 mm Hg during "night." Animals were orally dosed with CI-996 approximately 2 to 3 hr into their "daylight" period; the antihypertensive activity was rapid in onset with peak activity noted within the first 1- to 2-hr postdose. Losartan was also antihypertensive in this model and equipotent with CI-996. There were no measurable effects of CI-996 on heart rate in this conscious unrestrained primate model. Repeated administration of 10 mg/kg CI-996 for 5 days in these monkeys produced similar antihypertensive responses on the first and last days. Although inhibition of Ang II pressor responses (Cazaubon *et al.*, 1993) and blood pressure-lowering activity in normotensive volume deplete (Siegl *et al.*, 1992) or steroid-treated primates (DeGraaf *et al.*, 1993) has been reported with other Ang II antagonists this is the first report of the antihypertensive effects of an $AT_1$ antagonist in a nonhuman primate.

In summary, CI-996 is a potent, selective, orally active $AT_1$ antagonist with antihypertensive activity in rodents and primates. CI-996 also lowers blood pressure in sodium-depleted normotensive dogs.

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