Central and Peripheral Actions of a Nonpeptidic Angiotensin II Receptor Antagonist


Nonpeptidic imidazole derivatives were recently reported to be angiotensin II receptor antagonists with acute blood pressure-lowering activity. In the present study, we characterized the angiotensin II receptor antagonist properties of one such derivative, 4'-(2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazol-1-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid (IMI). In receptor binding studies, IMI displaced bound [125I]angiotensin II from rat uterine membranes with an IC50 of 0.17 μM. In isolated rabbit aortic rings, IMI shifted the angiotensin II concentration-response curve to the right in a parallel and concentration-dependent manner. A Schild plot of these data indicated a pA2 of 7.13±0.16 and a slope of 0.94±0.06. In rat kidney slices, IMI shifted the concentration-response curve for angiotensin II-induced inhibition of renin release to the right. Antagonism of the angiotensin II pressor response by IMI was dose dependent and reversible in ganglion-blocked, anesthetized rats. The water intake and pressor responses to intracerebroventricular angiotensin II (100 pmol) were inhibited by intracerebroventricular IMI (25 or 50 nmol) in conscious Sprague-Dawley rats. Similarly, the drinking and pressor responses to intravenous angiotensin II were blocked by intravenous IMI in conscious rats. IMI alone had no effects on mean arterial pressure or drinking when administered either intravenously or intracerebroventricularly. IMI decreased mean arterial pressure throughout 5 days of infusion in spontaneously hypertensive rats. In summary, IMI was a full competitive antagonist without partial agonist activity in peripheral tissues and the central nervous system. Moreover, the chronic administration of this angiotensin II receptor antagonist was antihypertensive in spontaneously hypertensive rats. (Hypertension 1990;15:841-847)
Receptor binding studies on IMI were conducted by using a rat uterine membrane preparation. Uteri were removed from Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, Massachusetts), weighing 200–250 g; the rats were anesthetized with 80 mg/kg i.p. sodium pentobarbital. The estrous cycles of rats were not controlled. All procedures were at 4°C. Uteri were scraped free of mucosa and endometrium and homogenized in 20 vol ice-cold phosphate-buffered saline containing 5 mM EDTA. The homogenate was centrifuged at 1500g for 20 minutes, and the supernatant was recentrifuged at 100,000g for 60 minutes. The pellet was resuspended in buffer consisting of 2 mM EGTA and 50 mM Tris-HCl, pH 7.5, to a final protein concentration of 4 mg/ml. Protein concentration was measured by using the Bio-Rad protein assay kit (Cambridge, Massachusetts). The assay for receptor binding consisted of 0.25 ml of a solution containing 5 mM MgCl₂, 2 mM EGTA, 0.5% bovine serum albumin, 50 mM Tris-HCl, pH 7.5, and [¹²⁵I]Ang II (approximately 10⁶ cpm) in the absence or presence of unlabeled peptide. The reaction was initiated by the addition of 50 μg membrane protein, and the mixture was incubated at 25°C for 30 minutes. The incubation was terminated with ice-cold 50 mM Tris-HCl, pH 7.5, and the mixture was filtered to separate membrane-bound labeled peptide from free ligand. The incubation tubes and filters were washed with ice-cold buffer. Filters were assayed for radioactivity in a Micromedic gamma counter (Micromedic Systems, Horsham, Pennsylvania). Nonspecific binding was defined as binding in the presence of 10 μM unlabeled Ang II and was 15% or less of total binding. Specific binding was calculated as total binding minus nonspecific binding. Binding data were analyzed by a nonlinear least-squares curve fitting program.

The in vitro antagonist activity of IMI was tested in aortas from male New Zealand White rabbits (Doe Valley Farms, Bentonville, Arkansas), weighing 2.5–3.0 kg. Thoracic aortas were removed under 60 mg/kg i.v. sodium pentobarbital anesthesia (Nembutal, Abbott Laboratories, North Chicago, Illinois) and placed in Krebs' solution. Fat and connective tissues were removed, and intercostal arterial branches were trimmed from the aortas. The tissues were placed in oxygenated (95% O₂, 5% CO₂) Krebs' solution of the following composition (mM): Na⁺ 146, K⁺ 5, Mg²⁺ 1.2, Ca²⁺ 2.5, Cl⁻ 138, HCO₃⁻ 15, H₂PO₄⁻ 1.2, and D-glucose 11.4. The aortas were cut into 3-mm ring segments, and the endothelium was removed by gently rubbing the vessel lumen with a piece of filter paper. Rings were then placed in a 20-ml muscle bath between a moveable and fixed stainless steel wire, with the moveable end attached to an FT03 force transducer (Grass Instruments, Quincy, Massachusetts), which led to a chart recorder (model 8, Grass Instruments). Passive tension of 750 mg was applied to the rabbit aortic rings. After a 30-minute equilibration period, Ang II concentration-response curves (3 x 10⁻¹⁰ to 10⁻⁵ M) were recorded. Each concentration of Ang II was allowed to elicit its maximal contraction, and then Ang II was washed out repeatedly for 30 minutes before rechallenging with a higher concentration of the peptide. Concentration-response curves started with the lowest concentration of Ang II and increased in half-log increments. Aortas were exposed to IMI at concentrations of 10⁻⁶ to 10⁻⁴ M for 5 minutes before challenging with Ang II. Adjacent segments of the same rabbit aorta were used to construct Ang II concentration-response curves in the presence or absence of IMI.

For renin release studies, male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, Indiana), weighing 150–200 g, were decapitated, and the kidneys were immediately removed and placed in cold, oxygenated Krebs' bicarbonate buffer. The outer kidney membrane was removed, the two ends of the kidney were cut off, and the remaining tissue was mounted on a chuck for 250-μm sectioning on a Vibratome 1000 (Technical Products International, St. Louis, Missouri). The sections were further dissected, and the isolated inner and outer cortical tissues were then incubated at 37°C in a humidified incubator with an environment of 95% O₂, 5% CO₂ for 30 minutes. The buffer was then replaced, and the incubation was continued for a second 30-minute period (the control period). Aliquots of 100 μl were withdrawn from each sample and frozen on dry ice for later analysis. Fresh buffer was then added that contained Ang II (5 x 10⁻¹⁰ to 5 x 10⁻⁴ M) plus vehicle or Ang II plus IMI (50 μM). The aliquots were assayed for renin activity using a commercial plasma renin activity kit (New England Nuclear, Boston, Massachusetts) that measures the production of angiotensin I determined by radioimmunoassay. The 100-μl sample was incubated with 250 μl plasma from bilaterally nephrectomized rats as a source of angiotensinogen. Other reagents and procedures were used according to the kit.
The in vivo Ang II receptor antagonist activity of IMI was examined in ganglion-blocked male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc.), weighing 300–400 g, anesthetized with 100 mg/kg i.p. Inactin. Catheters (PE-50) were implanted in a femoral artery and vein to measure mean arterial pressure and administer compounds, respectively. A tracheal catheter maintained airway patency. For intravenous IMI experiments, autonomic neurotransmission was blocked by treatment with mecamylamine (3 mg/kg i.v.) and atropine (400 μg/kg i.v.). Ang II (30 ng/kg i.v., 20–25-μl volume) was administered four times at 10-minute intervals to establish a reproducible control pressor response. IMI was then administered at 1, 3, and 10 mg/kg in separate groups of rats as an intravenous bolus (0.2-ml volume) before rechallenging with Ang II (30 ng/kg, 20–25-μl volume) for the following 2 hours. For intraduodenal IMI experiments, rats were anesthetized as above, but ganglion blockade was not performed. Ang II was administered at 100 ng/kg i.v. (20–25-μl volume), and IMI was administered at 10, 30, and 100 mg/kg in separate groups of rats as an intraduodenal bolus (0.2-ml volume). Time control experiments in which Ang II was injected similarly over 2 hours showed that the Ang II pressor response was maintained and consistent throughout the time course of this experiment.

In Ang II water intake and pressor experiments, a guide catheter (model 900, Kopf, Tujunga, California) was stereotaxically implanted into the lateral cerebral ventricle of Sprague-Dawley rats (Harlan Sprague-Dawley, Inc.), weighing 300–350 g, under 60 mg/kg i.p. sodium pentobarbital or 400 mg/kg i.p. chloralhydrate anesthesia (Sigma Chemical Co., St. Louis, Missouri). Three to 4 days were allowed for recovery from this surgery. Separate rats were used for the arterial pressure and water intake experiments. Rats were conscious and unrestrained during the experiments. On the day before the experimental day, any rat consuming less than 3 ml water during the 20-minute period after injection of Ang II (100 pmol i.c.v.) was omitted from the study. On the experimental day, the rats were pretreated with sarmsin (500 pmol i.c.v.), saralasin (500 pmol i.c.v.), IMI (25 or 50 nmol i.c.v.), or vehicle (2 μl of 0.9% NaCl i.c.v.) immediately before reevaluating the drinking response to Ang II (100 pmol i.c.v.). For arterial pressure experiments, mean arterial pressure was recorded by means of a carotid artery catheter (PE-50) implanted under 60 mg/kg i.p. methohexital anesthesia (Brevital, Eli Lilly Co., Indianapolis, Indiana) 24–48 hours before experimentation. Arterial pressure was measured with a Statham pressure transducer (model P23Db, Oxnard, California) and a Gould chart recorder (model 3800, Cleveland, Ohio). Rats showing less than a 10 mm Hg rise in mean arterial pressure to Ang II (100 pmol i.c.v.) during both the first and last Ang II (100 pmol i.c.v.) challenges of an experiment on a single day were omitted from the study.

The ability of IMI to alter the water intake and arterial pressure responses to intravenous Ang II was examined in conscious Sprague-Dawley rats (Harlan Sprague-Dawley, Inc.) weighing 300–350 g. Arterial pressure and water intake were measured simultaneously in the same rats. In this protocol, vehicle (30 μl/min of 0.9% NaCl i.v.) or IMI (300 μg/kg/min i.v.) was infused for 40 minutes; this infusion was followed by Ang II (200 ng/kg/min i.v.) for another 60 minutes. Water intake and mean arterial pressure were measured as above during the 60-minute Ang II infusion period.

Male SHR (Harlan Sprague-Dawley, Inc.), 12–14 weeks of age, were habituated for 3–4 days in individual experimental cages, which became their home cages for the duration of the study. Rats were anesthetized with methohexital sodium (30 mg/kg i.p., supplemented as needed with 10 mg/kg i.v.) or chloral hydrate (400 mg/kg), and catheters were implanted in a femoral artery and vein. Three to 4 days after catheter implantation, a 1 ml/hr i.v. infusion (Harvard Pump 22, Harvard Apparatus, South Natick, Massachusetts) of isotonic saline was started for the duration of the 6-day experiment. Mean arterial pressure was measured continuously between 10:00 AM and 2:00 PM daily. Three groups were studied: one group served as a vehicle and time control (1 ml/hr of 0.9% NaCl i.v.), a second group received IMI (5 mg/hr i.v.), and a third group received IMI (10 mg/hr i.v.). Compounds were prepared fresh daily. Ang II receptor antagonism by IMI was tested by examining the pressor response to Ang II (100 ng/kg i.v.). In vivo data were analyzed statistically using repeated-measures analysis of variance for main effects and interactions and Tukey’s honestly significant difference test for pairwise comparisons among means.

Sarmsin and IMI were synthesized in our laboratories (Searle Research and Development, St. Louis, Missouri). Ang II, saralasin, and norepinephrine were purchased from Sigma Chemical Co.

Results

Competitive displacement of [125I]Ang II bound to rat uterine membranes by unlabeled Ang II and IMI is shown in Figure 2. The IC_{50} value for Ang II was 2.2 nM. IMI produced a biphasic displacement curve indicating the presence of high-affinity (80%) and low-affinity (20%) binding IMI sites with IC_{50} values of 0.17 μM and 33 μM, respectively (n = 3).

In the isolated rabbit aortic rings, IMI shifted (p < 0.05) the Ang II concentration-response curve to the right in a concentration-dependent and parallel manner (Figure 3). Even in the presence of 10^{-4} M IMI, the maximal response to Ang II was attainable; thus, the antagonist effects of IMI were reversible and competitive. Schild analysis indicated a slope of 0.94 ± 0.06 and a pA_{2} of 7.13 ± 0.16 (n = 3) (pA_{2} represents the concentration of IMI that produces a twofold shift to the right in the Ang II concentration-response curve). The slope of the Schild regression
was not different from unity; thus, IMI was a competitive and reversible Ang II antagonist.

In rat kidney slices, Ang II inhibited (p<0.05) renin release in a concentration-dependent manner (Figure 4). IMI (50 μM i.v.) shifted (p<0.05; n=3) the Ang II concentration-response curve to the right in a parallel manner. This effect was completely overcome at high (>5×10^{-5} M) Ang II concentrations; this result was consistent with competitive antagonism. Quantitative analysis, performed by fitting the curves to a four-parameter logistic equation, confirmed that the curves were parallel and allowed for the calculation of a pA2 of 7.1.

Unlike peptidic Ang II receptor antagonists, intravenous IMI exhibited no agonist activity in vivo but, to the contrary, immediately decreased baseline mean arterial pressure by 14±2 mmHg from 80±4 mmHg (n=6; p<0.05) when administered to ganglion-blocked, anesthetized rats. Complete and reversible antagonism of the Ang II pressor response was observed with IMI in a dose-dependent manner (Figure 5). Oral bioavailability was indicated when intraduodenally administered IMI (30 and 100 mg/kg) suppressed the pressor response to Ang II (Figure 5). Intraduodenal IMI at 10 mg/kg had no effect on the pressor response to Ang II. IMI had no effect on baseline mean arterial pressure. No transient pressor responses were observed after intravenous or intraduodenal administration of IMI.

Ang II (100 pmol i.c.v.) increased water intake similarly on day 1 and day 2 of testing (Figure 6). The
Ang II antagonists, sarmesin or saralasin (500 pmol i.c.v.), reduced (p<0.05) the drinking response to intracerebroventricular Ang II by 65% (Figure 6). Similarly, IMI at either 25 or 50 nmol i.c.v. dose-dependently attenuated (p<0.05) the intracerebroventricular Ang II water intake response (Figure 6). Water intake was not affected (less than 0.5 ml/20 min) by intracerebroventricular vehicle injection alone, sarmesin or saralasin (500 pmol i.c.v.) alone, or IMI alone (25 or 50 nmol i.c.v.). The intracerebroventricular Ang II pressor response was attenuated (p<0.05) by sarmesin (500 pmol i.c.v.) and by IMI (25 nmol i.c.v.) (Figure 7). Injection of sarmesin (500 pmol i.c.v.) reduced the pressor response to intracerebroventricular Ang II to a greater degree than did IMI (25 nmol i.c.v.). Two hours after intracerebroventricular IMI, the pressor response to intracerebroventricular Ang II was restored (Figure 7).

With vehicle infusion (n=7), intravenous Ang II increased (p<0.05) mean arterial pressure by 48±2 mm Hg and water intake by 4.4±0.1 ml/60 min. With intravenous IMI infusion (n=7), the mean arterial pressure response to intravenous Ang II was attenuated (p<0.05), increasing (p<0.05) by only 10±2 mm Hg, and the water intake response to intravenous Ang II was abolished (1.1±0.1 ml/60 min).

Chronic infusion of IMI (10 mg/kg i.v.) decreased (p<0.05) mean arterial pressure in conscious SHR throughout the 5-day period (Figure 8). Chronic infusion of IMI at 5 mg/kg lowered mean arterial pressure on days 3–5, but less than (p<0.05) IMI at 10 mg/kg. In vehicle control spontaneously hypertensive rats (0.9% NaCl), mean arterial pressure did not change over the 5-day infusion.

### Discussion

The importance of the renin-angiotensin system in the pathophysiology of hypertension is well estab-
Although angiotensin converting enzyme inhibitors have been extremely successful, there are several drawbacks to this antihypertensive therapy. First, angiotensin converting enzyme inhibitors degrade many other peptide substrates (e.g., bradykinin, enkephalins, and substance P) unrelated to the renin-angiotensin system. Second, these agents inhibit angiotensin systemically, which is unlikely to be salutary in some tissues, such as the pituitary and reproductive organs. In contrast, a number of studies have indicated that subpopulations of Ang II receptors exist within and among tissues. These findings suggest that selective Ang II receptor antagonists may be developed to target a drug to only those Ang II receptor subtypes specifically involved in the pathophysiology of hypertension.

Pepticid Ang II receptor antagonists, such as saralasin and sarmesin, are limited by partial agonist activity, short biological half-life, and lack of oral bioavailability. In the present study, we characterized the properties of a nonpepticid Ang II receptor antagonist, referred to as IMI, in peripheral tissues and the central nervous system. The competitive binding studies showed that IMI bound to a high-affinity Ang II receptor in a smooth muscle preparation. In isolated rabbit aortic rings, IMI shifted the Ang II concentration-contraction curve to the right in a concentration-dependent and parallel manner. In the kidney, Ang II inhibited renin release directly by binding to Ang II receptors on the renin-secreting juxtaglomerular granular cells. In rat kidney slices, Ang II inhibited renin release in a concentration-dependent manner; IMI shifted this curve to the right. This effect was completely overcome at high Ang II concentrations; this occurrence was consistent with competitive antagonism. Quantitative analysis confirmed that the curves were parallel and allowed for the calculation of a pA2 value of 7.1. This value is similar to that found for rabbit aorta (7.1). In comparison, sarmesin and saralasin yield pA2 values of approximately 7.6 and 9.1, respectively, in rabbit aorta (authors' unpublished observation). Thus, IMI is a moderately potent, competitive antagonist of Ang II receptors in rat uterine membranes, rabbit aorta, and renal juxtaglomerular granular cells.

IMI was evaluated as an Ang II receptor antagonist in several in vivo assays. In ganglion-blocked anesthetized rats, the intravenous Ang II-induced increase in mean arterial pressure was blocked by 10 mg/kg i.v. IMI and gradually returned to control values during the next 90–120 minutes. Lower doses of IMI (1 or 3 mg/kg i.v.) attenuated the pressor response to Ang II in a dose-dependent manner. Oral bioavailability was suggested when intraduodenal IMI (30 or 100 mg/kg) suppressed the pressor response to Ang II. Interestingly, the transient increase in mean arterial pressure seen with pepticid Ang II antagonists was not observed with IMI. Thus, IMI blocked the pressor actions of Ang II, was devoid of agonist activity, and had considerably longer half-life than pepticid Ang II receptor antagonists, and was active after intraduodenal administration.

All the components of the renin-angiotensin system have been identified in the brain. Injection of Ang II into the brain ventricles increases mean arterial pressure and water intake. Drinking is also produced by intravenous administration of Ang II, which can be abolished by lesioning the subfornical organ; this occurrence indicates a central nervous system site of action. We tested the ability of IMI to alter the drinking and pressor responses to intracerebroventricular and intraventricular Ang II administration. Both the pressor and drinking responses to intravenous Ang II were blocked by intravenous IMI. Similar to previous findings, the pepticid Ang II receptor antagonists, saralasin or saralasin (500 pmol), attenuated the water intake response to intracerebroventricular Ang II. IMI (25 and 50 nmol i.c.v.) reduced the drinking response to intracerebroventricular Ang II in a dose-dependent manner; however, to achieve an equivalent inhibition of drinking, a dose of IMI 50-fold greater than sarmesin or saralasin was required. Similarly, the intracerebroventricular Ang II pressor response was reduced 90% by 500 pmol i.c.v. sarmesin whereas the intracerebroventricular Ang II pressor response was reduced only 60% by 25 nmol i.c.v. IMI. In isolated rabbit aorta and in vivo rat blood pressure assays, IMI and saralasin had similar potencies with respect to Ang II–induced contractile and pressor responses (authors' unpublished observation). It is not clear whether the lower potency in the central nervous system is due to differences in pharmacokinetic characteristics affecting the abilities of the compounds to reach the receptors or to differences in Ang II receptors in the brain and periphery, which are revealed by the pepticid and nonpepticid antagonists. In support of the latter possibility, we have found that the Ang II analogues [Sar1]Ang II(1–7) NH2 (pA2=7.6) was similar in potency to sarmesin (pA2=7.6) in blocking Ang II–induced pressor responses in ganglion-blocked anesthetized rats but had no effect on the water intake response to intracerebroventricular Ang II in conscious rats at a dose five times greater (2.5 nmol) than the dose of saralasin (500 pmol) required to reduce intracerebroventricular Ang II drinking response by 65% (present study). These functional data are complemented by receptor binding data showing that, relative to [Asn1]Ang II or [Ile1]Ang II, the binding affinity of [Sar1]Ang II(1–7) NH2 ([Sar1Pro, desPhe2]Ang II) in the brain was 5,000-fold lower but in the uterus was only 10–30-fold lower. In contrast, Ang-(1–7) was without biological activity in peripheral tissues but stimulated the release of vasopressin from hypothalamoneurohypophysial brain tissue. Together, these data support the possibility that differences exist between central nervous system and peripheral tissue Ang II receptor populations.

Both the central nervous system and renal effects of Ang II have been implicated in the pathophysiology of hypertension. The levels of Ang II and the
density of Ang II receptors in kidneys and brain are higher in SHR than normotensive Wistar-Kyoto rats. Conversely, low level intrarenal infusion of Ang II in conscious dogs leads to chronic sodium retention and hypertension. Thus, we examined whether chronic blockade of Ang II receptors in SHR would be an effective antihypertensive treatment. Three to 5 days of IMI infusion normalized mean arterial pressure in SHR. In addition, on termination of IMI infusion, mean arterial pressure returned to control levels. These data indicate that Ang II receptor antagonists may be an effective therapeutic strategy for the treatment of hypertension.

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References


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