A Specific Competitive Antagonist of the Vascular Action of Angiotensin II

By Donald T. Pals, Frederick D. Masucci, Frank Sipos, and George S. Denning, Jr.

ABSTRACT

The effect of l-Asn-8-Ala-angiotensin II (Asn-Arg-Val-Tyr-Val-His-Pro-Ala) on the vascular action of angiotensin II was studied with rabbit aortic strips, pithed rats, and conscious rats. Dose-response (contraction) curves for angiotensin II on rabbit aortic strips shifted progressively to the right with increasing bath concentrations of l-Asn-8-Ala-angiotensin II. The pA2 value (an indication of the relative affinity of a competitive antagonist for an agonist's receptor site) was 6.84 ± 0.03. Dose-response curves for norepinephrine, serotonin, and histamine were relatively unaffected. No pA3 values could be obtained with these agonists in the concentration range tested. Partial dose-response (pressor) curves for angiotensin II in pithed rats shifted to the right during infusions of l-Asn-8-Ala-angiotensin II at 20 and 60 μg/kg min⁻¹. Partial dose-response curves for vasopressin, phenylephrine, and tyramine were unaffected. Infusions of l-Asn-8-Ala-angiotensin II counteracted the pressor effect of angiotensin II infusions in pithed and conscious rats. Infusions of l-Asn-8-Ala-angiotensin II (200 μg/kg min⁻¹) effective in reducing the blood pressure of conscious angiotensin II-infused rats caused only small pressor effects in conscious normotensive rats but reduced the blood pressure in 75% of the conscious rats with acute unilateral renal hypertension. The data presented are consistent with the conclusion that l-Asn-8-Ala-angiotensin II is a specific competitive antagonist of the vascular action of angiotensin II both in vitro and in vivo.

KEY WORDS receptor site competitive inhibition renal hypertension peptide synthesis pithed rats rabbit aortic strips conscious rats

Much effort has been expended during the last two decades to elucidate the pharmacology of angiotensin II. A notable deficiency in the available knowledge concerning the actions of angiotensin II has been caused by the lack of a specific antagonist to its stimulating properties on smooth muscle. A compound with this type of activity would provide a useful experimental tool to answer the question of whether specific receptor sites exist on smooth muscle for each pharmacologically active polypeptide. A selective angiotensin II antagonist would also aid in delineating the question of angiotensin II involvement in a number of human and animal model hypertension syndromes and be a potentially useful therapeutic agent.

Many chemical substances have been tested for their ability to reduce the angiotensin II-induced tension in smooth muscle structures. Only recently has a specific competitive inhibitor of the action of angiotensin II on isolated rat uterus and on the blood pressure of anesthetized rats been reported (1). To our knowledge, no other specific competitive antagonist of the action of angiotensin II on vascular smooth muscle has been reported. The present communication describes the preparation of and our subsequent experience...
ANGIOTEINS II ANTAGONIST

Methods

SYNTHESIS OF L-ASN-8-ALA-ANGIOTEINS II

T-butylxoycarbonyl (Boc)-alanine resin-ester (5 g, 0.5 mmol/g) was placed in a rocking Merrifield type of reaction vessel (2). The resin was swelled in chloroform (analytical grade) for 20 minutes and thereafter washed with three 50-ml portions of glacial acetic acid. All wash operations were repeated three times for 3 minutes with each solvent. The Boc-protecting group was removed by 1N HCl in anhydrous acetic acid after 40 minutes. The resin was then washed with 50-ml portions of glacial acetic acid, absolute ethanol, and N,N-dimethylformamide (DMF, "spectroquality"). The HCl was neutralized with a 10% solution of triethylamine in DMF with rocking for 10 minutes. Thereafter the resin was washed with DMF, absolute ethanol, chloroform, and dichloromethane, and a threelfold excess (8.5 mmol) of Boc-proline in dichloromethane was added. After 20 minutes of rocking, a solution of pure dicyclohexylcarbodiimide (8.5 mmol) in dichloromethane was added. The reaction mixture was rocked for 12 hours. Thereafter the whole operation starting with the acetic acid wash was repeated for each amino acid, except that a DMF-dichloromethane solvent mixture was used for the coupling of Boc-Nle-benzylhistidine and Boc-nitroarginine. The Boc-asparagine was introduced using Boc-asparagine p-nitrophenylester in 1.7-fold excess (4.3 mmol) in DMF with a 48-hour reaction time. After the last coupling step, the resin-peptide was washed with DMF, ethanol, acetic acid, and ethanol and dried in vacuo over P2O5. The weight of the resin-peptide (Boc-L-asparaginyl-N*-nitro-L-arginyl-L-valyl-O-Bzl-L-tyrosyl-L-valyl-Nle-Bzl-L-histidyl-L-prolyl-L-alanine resin-ester) was 7.3 g. The reaction mixture was then suspended in 25 ml of dry trifluoroacetic acid and a stream of dry hydrogen fluoride was passed through at a slow rate for 20 minutes. The resin was filtered off, treated again with hydrogen fluoride in trifluoroacetic acid for 40 minutes, and filtered. The filtrates were evaporated to dryness in vacuo at 20°C, and the residues were precipitated with absolute ether. The overall yield was 1.98 g of L-asparaginyl-N*-nitro-L-arginyl-L-valyl-L-tyrosyl-L-valyl-Nle-Bzl-L-histidyl-L-prolyl-L-alanine hydrobromide. The protected octapeptide hydrobromide (1.0 g) was dissolved in 20 ml of an acetic acid/dioxane/water (4:4:1, v/v) mixture and hydrogenated over Pd/BaSO4 (10%, 0.5 g) for 48 hours at atmospheric pressure. After that time, a new portion (0.2 g) of catalyst was added, and the hydrogenation was continued for 24 hours. The filtered and diluted solution was lyophilized. The yield was 0.750 g.

The crude product was purified by countercurrent distribution in a 0.58 ammonium acetate-n-butanol system and by chromatography on Sephadex G-10 using 0.2M acetic acid as the eluant. The purity of the compound (L-asparaginyl-L-arginyl-L-valyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-alanine acetate) was estimated by thin layer chromatography on Merck Cellulose F plates, Merck Silicagel 254 F plates, and Avicel CM plates with a n-butanol/acetate/water solvent system (6:2:3, v/v). Ninhydrin spray indicated one spot on each plate. The Rf values for the three solvent systems were 0.35, 0.13, and 0.2, respectively. Amino acid analyses performed on a Japan Electron Optics Laboratory analyzer (model JLC-5AH) after digestion with 6N HCl for 18 hours at 100°C yielded the following ratios: Ala 1.00, Pro 1.04, His 0.91, Val 1.94, Tyr 0.73, Arg 1.00, and Asn 1.09. The optical rotation values obtained on a Perkin Elmer model 141 polarimeter were [a]20^0 = -59.4° (C = 1, in acetic acid) and [a]20^0 = -81.1° (C = 1, in acetic acid).

RABBIT AORTIC STRIPS

Sections of thoracic aorta were obtained from rabbits (New Zealand white) following anesthesia by sodium thiopental (35 mg/kg, iv) and exsanguination via the carotid arteries. Aortic strips prepared according to the method of Furchgott and Bhadrakom (3) were mounted in 10-ml organ baths maintained at 36°C by a Haake constant temperature circulator. The tissues were bathed in aerated (95% O2, 5% CO2) Krebs-bicarbonate solution of the following composition (g/liter): NaCl 6.9, KCl 0.35, CaCl2 0.28, NaHCO3 2.1, KH2PO4 0.16, MgSO4 0.14, and glucose 1.0. Responses were recorded on a Texas Instruments recorder using a Phipps and Bird linear motion transducer (model ST2) counterbalanced with a 4 g weight. After an equilibration period of 2 hours, an agonist (angiotensin II, norepinephrine, serotonin, or histamine, 0.1 ml) was added to the bath at intervals of 30 minutes or more. The antagonist (1-Asn-8-Ala-angiotensin II) was in contact with the tissue 3 minutes before an agonist was added to the bath. Paired strips from the same rabbit were used; one was exposed to the antagonist, and the other served as a control. All pairs of strips were exposed to an initial concentration of agonist in the absence of the antagonist to determine whether both strips possessed the same...
degree of sensitivity. The subsequent dose-response curves were evaluated in terms of percent of maximum contraction of the control strip. All concentrations of drugs are expressed as moles in the bath medium (norepinephrine as the bitartrate salt, serotonin and histamine as the bases).

PITHED RATS

Sprague-Dawley rats weighing approximately 300 g were anesthetized with ether. The trachea was cannulated, and a rod 2 mm in diameter was pushed through the right orbit into the spinal cord via the foramen magnum. The animal was immediately connected to a Harvard respirator and bilaterally vagotomized. The left carotid artery was ligated, and the right carotid artery was cannulated for the recording of blood pressure with a Statham P23D transducer and a Beckman SII Dynograph recorder. The jugular veins were cannulated; one cannula was connected to a Hamilton µlter syringe containing angiotensin II, vasopressin, phenylephrine, or tyramine, and the other was connected to a Harvard infusion pump (600-910) to allow the infusion of saline or 1-Asn-8-Ala-angiotensin II at 0.004 ml/min. Infusions of 1-Asn-8-Ala-angiotensin II were utilized, because preliminary experiments indicated that the biologically effective half-life of this peptide was approximately 4 minutes and that the antagonism of angiotensin II was constant during several hours of infusion. The rectal temperature of each animal was monitored and was maintained at 35°C by the appropriate positioning of an incandescent bulb above the animal or by a heat lamp connected to a Cole-Parmer Versa-therm electronic temperature controller and rectal thermistor probe.

Each animal was left undisturbed for 90 minutes before the experiment was begun. A three-point dose-response curve to an agonist (angiotensin II, vasopressin, phenylephrine, or tyramine) was then obtained. An infusion of 1-Asn-8-Ala-angiotensin II was initiated in the appropriate experiments 10--20 minutes after the highest dose of agonist had been administered. A second three- or four-point dose-response curve to the agonist was obtained 25 minutes later. The mean and the range of the values obtained for the first dose-response curve to a particular agonist in a control group of nine or ten animals was utilized solely to determine whether subsequent preparations exhibited a similar degree of sensitivity to the agonist. Only the second dose-response curve to an agonist was used to determine the effect of treatment or nontreatment with 1-Asn-8-Ala-angiotensin II. Where applicable, drug doses are expressed as salts.

A second series of pithed rats was prepared as described. Following an initial equilibration period of 90 minutes, each animal received a continuous intravenous infusion of angiotensin II (0.30 µg/kg min⁻¹). After 60 minutes of angiotensin II infusion and while it was being continued, an infusion of 1-Asn-8-Ala-angiotensin II was initiated.

CONSCIOUS RATS

Sprague-Dawley rats weighing approximately 300 g were anesthetized with ether. A median ventral neck skin incision was made, and the left carotid artery and jugular vein were isolated. The two blood vessels were cannulated with polyethylene tubing (PE-50), and the tubes were routed subcutaneously to the back of the neck where they were exteriorized through a small skin puncture. The ventral neck skin incision was closed with wound clips. Each animal was placed in a large widemouthed glass jar and allowed to recover from the anesthesia for at least 1 hour before initiation of an experiment. The cannulas were loosely draped over the neck of the glass jar and were long enough so that the animal's movements were unrestrained. The carotid artery cannula was connected to a Statham P23D transducer fixed outside the glass jar at the animal's heart level. The jugular vein cannula was connected to a Harvard infusion pump for the infusion of angiotensin II, saline, or 1-Asn-8-Ala-angiotensin II at 0.014 ml/min.

Following the recovery period, control blood pressure recordings were made for 2 hours before initiation of an experiment. The cannulas were loosely draped over the neck of the glass jar and were long enough so that the animal's movements were unrestrained. The carotid artery cannula was connected to a Statham P23D transducer fixed outside the glass jar at the animal's heart level. The jugular vein cannula was connected to a Harvard infusion pump for the infusion of angiotensin II, saline, or 1-Asn-8-Ala-angiotensin II at 0.014 ml/min.

Following the recovery period, control blood pressure recordings were made for 1 hour before the initiation of a 3-hour infusion of angiotensin II (2.5 µg/kg min⁻¹). In some experiments, a 1-hour infusion of 1-Asn-8-Ala-angiotensin II (dissolved in the infusion solution of the angiotensin II) was interjected during the 3-hour angiotensin II infusion.

A second series of conscious rats was prepared as described above. After the recovery period and 2 hours of control blood pressure recording, a 1-hour infusion of saline or 1-Asn-8-Ala-angiotensin II was initiated. This was followed by another hour of blood pressure recording.

Unilateral renal hypertension was induced in a third series of rats by constricsting the aorta between the origins of the renal arteries (4, 5). Six to 7 days after the aortic stenosis operation, the animals were prepared as previously described for the recording of blood pressure and the infusion of saline or 1-Asn-8-Ala-angiotensin II. The experimental protocol was identical to that utilized for the second series of conscious rats.

DRUGS

The drugs employed in these studies were l-norepinephrine bitartrate, serotonin creatinine sul-
Results

RABBIT AORTIC STRIPS

Figure 1 shows dose-response (contraction) curves for angiotensin II on rabbit aortic strips. As the concentration of 1-Asn-8-Ala-angiotensin II was increased in the bath medium, the dose-response curves shifted progressively to the right. The maximal contracting effect of angiotensin II was not significantly \( (P > 0.05) \) attenuated. Concentrations of 1-Asn-8-Ala-angiotensin II greater than \( 3 \times 10^{-4} \text{M} \) were not tested, because the addition of angiotensin II at concentrations greater than \( 10^{-4} \text{M} \) to the bath containing concentrations of antagonist greater than \( 3 \times 10^{-6} \text{M} \) resulted in frothing which interfered with the determination of complete dose-response curves. The dose-response curves for norepinephrine, histamine, and serotonin were not significantly \( (P > 0.05) \) altered as the bath concentration of 1-Asn-8-Ala-angiotensin II was increased to \( 3 \times 10^{-6} \text{M} \).

The affinity, expressed as \( pA_2 \) —the negative logarithm of the molar concentration of a competitive antagonist which reduces the effect of a double concentration of agonist to that of a single one \((6)\), of 1-Asn-8-Ala-angiotensin II for the angiotensin II receptor site of rabbit aortic strips was \( 6.84 \pm 0.03 \) \((n = 12)\). Attempts to obtain \( pA_2 \) values for 1-Asn-8-Ala-angiotensin II with norepinephrine, serotonin, and histamine were uniformly unsuccessful even when the bath concentration of 1-Asn-8-Ala-angiotensin II had been raised as high as \( 10^{-4} \text{M} \). Of incidental interest was the observation that high bath concentrations \( (10^{-5} \text{M}) \) of 1-Asn-8-Ala-angiotensin II caused a potentiation of the norepinephrine-induced contractile responses of rabbit aortic strips. Subsequent preliminary experiments indicated that the norepinephrine dose-response curve exhibited a parallel shift to the left at bath concentrations of 1-Asn-8-Ala-angiotensin II on the order of \( 10^{-4} \text{M} \). Due to the limited number of experiments, attempts to interpret the latter observations were felt to be inadvisable.

Contractile activity of 1-Asn-8-Ala-angiotensin II on rabbit aortic strips was not observed at bath concentrations of less than \( 10^{-5} \text{M} \) and, except for one pair of strips from a single rabbit, was not observed at \( 10^{-6} \text{M} \).

PITVED RATS

The intravenous infusion of 1-Asn-8-Ala-angiotensin II \((6-60 \mu g/kg \text{ min}^{-1})\) caused a dose-dependent surmountable inhibition of angiotensin II-induced blood pressure effects in pitved rats. Figure 2 shows the record from a typical experiment in which 1-Asn-8-Ala-angiotensin II \((60 \mu g/kg \text{ min}^{-1})\) was infused intravenously. This figure illustrates the emergence of apparent pressor properties of 1-Asn-8-Ala-angiotensin II after the beginning and end of the infusion. These blood pressure effects were dose-dependent and were often difficult to detect at the infusion rate of 6
The record of a typical experiment on a pithed rat illustrating the surmountable inhibition of angiotensin II-induced pressor responses during the infusion of 1-Asn-8-Ala-angiotensin II (60 μg/kg min⁻¹). Note the transient pressor effects at the beginning and end of the infusion. A₁, A₂, A₃, and A₄ = angiotensin II at 0.03, 0.1, 0.3, and 1.0 μg/kg, respectively.

μg/kg min⁻¹. Bilateral adrenalectomy immediately following pithing or treatment with phenoxybenzamine did not appear to affect the occurrence of these events. Figure 2 also illustrates the consistent observation that the base-line blood pressure of pithed rats was not depressed significantly by 1-Asn-8-Ala-angiotensin II. In the few experiments where it was recorded, the heart rate of pithed rats did not appear to be affected by the infusions.

Figure 3 shows partial dose-response (pressor) curves for angiotensin II, vasopressin, phenylephrine, and tyramine in pithed rats. Infusions of 1-Asn-8-Ala-angiotensin II (20 and 60 μg/kg min⁻¹) significantly (P < 0.05) shifted the dose-response curves for angiotensin II to the right without affecting the dose-response curves for vasopressin, phenylephrine, and tyramine.

An additional series of experiments with pithed rats was designed to determine whether an elevated blood pressure due to the infusion of angiotensin II could be lowered to the control level for a prolonged period of time (1 hour) by an infusion of 1-Asn-8-Ala-angiotensin II. The results of these experiments are summarized in Table 1. Infusions of angiotensin II (0.30 μg/kg min⁻¹) caused a reasonably stable elevation of blood pressure in pithed rats for at least 2 hours. Infusions of 1-Asn-8-Ala-angiotensin II (10 μg/kg min⁻¹) lowered the angiotensin II-maintained blood pressure approximately 66%, while infusions at the rate of 20 μg/kg min⁻¹ returned the blood pressure to the control level and maintained it there throughout the duration of the infusion.

The result of infusion of 1-Asn-8-Ala-angiotensin II (20 μg/kg min⁻¹) for half an hour into a pithed rat during an infusion of angiotensin II (0.30 μg/kg min⁻¹) is shown in Figure 4. This figure illustrates the absence of a pressor effect after the beginning of the infusion and shows that blood pressure during the 1-Asn-8-Ala-angiotensin II infusion was not decreased below the preangiotensin II blood pressure level. The overshoot in blood pressure following the 1-Asn-8-Ala-angiotensin II infusion did not occur consistently.

CONSCIOUS RATS

Figure 5 (a and b) illustrates the time trend of blood pressure for conscious rats receiving an intravenous infusion of angiotensin II (2.5 μg/kg min⁻¹) and the effect of an infusion of 1-Asn-8-Ala-angiotensin II (200 μg/kg min⁻¹) during the angiotensin II infusion. It is of
TABLE 1

Effects on Blood Pressure (mm Hg) of 1-Asn-8-Ala-angiotensin II Infusion in Angiotensin II-Infused Pithed Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>90 minutes</th>
<th>120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>62 ± 1.6</td>
<td>124 ± 4.2</td>
<td>119 ± 5.8</td>
<td>118 ± 5.4</td>
<td>121 ± 5.3</td>
</tr>
<tr>
<td>1-Asn-8-Ala-angiotensin II (10 µg/kg min⁻¹)</td>
<td>60 ± 1.3</td>
<td>123 ± 6.4</td>
<td>118 ± 6.3</td>
<td>78 ± 5.1</td>
<td>82 ± 3.8</td>
</tr>
<tr>
<td>1-Asn-8-Ala-angiotensin II (20 µg/kg min⁻¹)</td>
<td>63 ± 0.9</td>
<td>125 ± 4.7</td>
<td>112 ± 4.2</td>
<td>64 ± 3.1</td>
<td>65 ± 3.1</td>
</tr>
</tbody>
</table>

Angiotensin II was infused at a rate of 0.30 µg/kg min⁻¹ from 0 to 120 minutes. Infusions of 1-Asn-8-Ala-angiotensin II were interjected at 61–120 minutes. All values are means ± SE for seven to eight animals.

Interest that the blood pressure values during the simultaneous infusion of angiotensin II and 1-Asn-8-Ala-angiotensin II did not vary more than did those during the preceding interval of angiotensin II infusion alone. This is indicative of the uniformity of the observed responses to the infusion of 1-Asn-8-Ala-angiotensin II.

The infusion of saline into conscious normotensive rats did not have an apparent effect on their blood pressure (Fig. 5c), while the infusion of 1-Asn-8-Ala-angiotensin II (200 µg/kg min⁻¹) caused a small variable pressor response which, in contrast to the transient effects noted in pithed rats, was sustained throughout and beyond the infusion period (Fig. 5d).

The time trend of blood pressure in conscious rats with acute unilateral renal hypertension is depicted in Figure 5e, and the effect of an infusion of 1-Asn-8-Ala-angiotensin II (200 µg/kg min⁻¹) on the blood pressure is shown in Figure 5f.

Blood pressure as a function of time in conscious rats. Experiments on normotensive rats are shown in a–d; e and f represent experiments on rats with acute unilateral renal hypertension. All points are means ± se from eight animals. Infusion rates were: saline 0.014 ml/min, angiotensin II 2.5 µg/kg min⁻¹, and 1-Asn-8-Ala-angiotensin II 200 µg/kg min⁻¹.
pressure of these animals is shown in Figure 5f. The variation in values during the infusion of 1-Asn-8-Ala-angiotensin II is indicative of the nonuniformity of the observed responses. In fact, 2 rats exhibited blood pressure trends which were not altered detectably by the infusion. Autopsy of the latter animals did not reveal any gross anatomical or pathological finding which was different from that observed at autopsy in the remaining 14 rats with unilateral renal hypertension.

Discussion

The structural requirements for the biological activity of angiotensin II have been extensively examined (7); small changes in the angiotensin II molecule often cause large changes in its pressor and contractile activity. We found it of interest to investigate the possibility that, although a change in the chemical structure of angiotensin II often causes a loss of intrinsic activity, the change might not cause a total loss of the analog's affinity for the angiotensin II receptor site of vascular smooth muscle (see ref. 8 for a discussion of intrinsic activity and affinity). Our efforts to test this hypothesis culminated in the synthesis and evaluation of 1-Asn-8-Ala-angiotensin II.

The evaluation of 1-Asn-8-Ala-angiotensin II utilizing rabbit aortic strips indicated that this peptide possesses negligible intrinsic activity in relation to its high affinity for the angiotensin II receptor site. Even though 1-Asn-8-Ala-angiotensin II is related structurally to angiotensin II and presents an antagonism of the action of angiotensin II which is surmountable, reasonably specific, and similar to that expected of a classical competitive inhibitor, it could not be concluded unequivocally that it is a competitive antagonist (9) of angiotensin II. On the basis of the available evidence, however, it appeared justifiable to eliminate the possibility of a functional type of antagonism (agonist and antagonist interact with different receptors and caused opposing effects). The alternative of a chemical antagonism or antagonism by neutralization could not be resolved conclusively. Experimental attempts to eliminate this possibility resulted in equivocal evidence. For example, incubation of 1-Asn-8-Ala-angiotensin II with angiotensin II for 30 minutes before their addition to the bath medium resulted in contractile responses which did not differ more than 5% from those following the simultaneous addition of angiotensin II and 1-Asn-8-Ala-angiotensin II without preincubation. Theoretical arguments also could not be used to eliminate conclusively the possibility of chemical interaction. Thus the presumptive evidence that angiotensin II exists as a monomer in dilute solution and that it is the monomer that interacts with the cellular receptor (10) suggested that a chemical interaction between angiotensin II and a compound which is almost structurally identical (except for the omission of a phenyl group) was unlikely.

The pithed rat experiments illustrated that 1-Asn-8-Ala-angiotensin II effectively antagonizes the vascular action of angiotensin II in vivo as well as in vitro. These experiments reinforce the conclusions drawn from the in vitro data by demonstrating that the antagonism of angiotensin II-induced blood pressure responses by 1-Asn-8-Ala-angiotensin II is surmountable, reasonably specific for angiotensin II, and accompanied by only minimal indications of intrinsic activity (without evidence to the contrary, it was tentatively concluded that the transient pressor responses observed at the beginning and end of 1-Asn-8-Ala-angiotensin II infusions were manifestations of the intrinsic activity of this compound). If one accepts the premise that 1-Asn-8-Ala-angiotensin II acts as a competitive antagonist of angiotensin II, it becomes difficult to reconcile these results in pithed rats with the hypothesis of Louis and Doyle (11) that angiotensin II and vasopressin share a common receptor site on blood vessels. The experiments on pithed rats also demonstrate that infusions of 1-Asn-8-Ala-angiotensin II could effectively antagonize the blood pressure elevation in pithed rats due to infusions of angiotensin II. These observations indicate that a sustained equilibrium of angiotensin II with its receptor sites could be disrupted by
the introduction of L-Asn-8-Ala-angiotensin II in appropriate concentrations.  

The experiments with conscious rats show that L-Asn-8-Ala-angiotensin II is effective in lowering the blood pressure of animals during an angiotensin II infusion but is not effective in lowering the blood pressure of normotensive animals at the dose utilized. Experiments with conscious rats in the acute stage of unilateral renal hypertension, whose ischemic atrophic left kidneys had been shown to contain large amounts of pressor substances (5), and whose blood levels of a pressor substance (presumably angiotensin II) had been shown to be significantly higher than in normal rats (4), demonstrated that L-Asn-8-Ala-angiotensin II could act as an antihypertensive agent. It must be pointed out that the infusion dose of 200 μg/kg min⁻¹ was the highest tested in these experiments due to the limited quantity of L-Asn-8-Ala-angiotensin II available. Since the antagonism of angiotensin II-induced blood pressure effects by L-Asn-8-Ala-angiotensin II is a surmountable phenomenon characteristic of a competitive inhibitor, all rats in this group might have responded positively to the infusion if the dose had been increased. The fact that all animals did not exhibit a positive response suggested that the plasma levels of angiotensin II in these animals were extremely variable, even though the blood pressure values during the control period were very similar. Alternatively, it could be postulated that animals which did not respond to L-Asn-8-Ala-angiotensin II were subjected to a rapid drug-induced change in the hemodynamic status of the left kidney, with a resultant release of renin (12) and the formation of angiotensin II in quantities sufficient to surmount the angiotensin II antagonism present and thus maintain the hypertension at predrug levels. The possibility that angiotensin II was not responsible for the maintenance of blood pressure in some of these animals cannot be discounted entirely.

A recent report (13) demonstrated that blockade of the in vivo conversion of angiotensin I to angiotensin II by a synthetic bradykinin-potentiating pentapeptide caused a reduction of the elevated blood pressure of acute hypertensive rats with unilateral renal artery constriction and an intact contralateral kidney. These authors noted that the pentapeptide did not affect the blood pressure of normotensive rats or of acute hypertensive rats with unilateral renal artery constriction and contralateral nephrectomy. Other investigators (14, 15) have shown that a phospholipid renin preinhibitor isolated from kidneys reduced the blood pressure of acute and chronic hypertensive rats with unilateral renal artery constriction and an intact contralateral kidney. The blood pressure of normotensive rats was not affected by the phospholipid renin preinhibitor. These two examples of methods that have been used to inhibit components of the renin-angiotensin system illustrate that the blood pressure of renal hypertensive rats is maintained by endogenous angiotensin II under certain conditions. The effect of L-Asn-8-Ala-angiotensin II on the blood pressure of normotensive rats and on that of rats with acute unilateral renal hypertension and an intact contralateral kidney is similar to that described in the two examples above and indicates that its actions are consistent with an antagonism of endogenous angiotensin II at vascular receptor sites.

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References

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