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# Specific inhibition of renin by an angiotensinogen analog: Studies in sodium depletion and renin-dependent hypertension

(enzyme inhibitor/acid protease/blood pressure)

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ABSTRACT **The angiotensin substrate analog Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys has no significant effect on blood pressure in sodium-replete monkeys (*Macaca fascicularis*) but blocks the pressor response to infused human renin. Pressor responses to angiotensin I and angiotensin II are not attenuated. In five studies in sodium-depleted monkeys, an infusion of 2 mg of the peptide per kg of body weight resulted in a reduction of mean arterial pressure (MAP) from 105 ± 4 to 79 ± 3 mm Hg, which is not significantly different from the response to 1 mg of the angiotensin I-converting enzyme inhibitor teprotide per kg. In uninephrectomized monkeys, inflation of a suprarenal aortic cuff caused an increase in MAP from 107 ± 3 to 131 ± 3 mm Hg. Infusion of 0.6 mg of the renin-inhibitory peptide per kg was followed by a return of blood pressure to 107 ± 4 mm Hg—a depressor response similar to that observed with teprotide. This specific *in vivo* inhibitor of renin can now be applied to a wide variety of physiologic studies.**

The renin–angiotensin system plays an important role in normal cardiovascular homeostasis and in some forms of hypertension. Clear definition of this role remains elusive, partly because of the inability to inhibit specifically the initial steps in this pathway by pharmacologic methods. Competitive inhibitors of angiotensin II binding have varying agonist/antagonist activity (1, 2), which undermines their effectiveness. Angiotensin I-converting enzyme inhibitors have been studied (3, 4), but their potential interaction with the kallikrein system (5, 6) and their inhibition of other enzymes unrelated to the renin–angiotensin system clouds interpretation of physiologic responses (7). Competitive inhibitors of renin, based on the sequence of the natural substrate (angiotensinogen), have been synthesized (8), but they have been of insufficient potency and solubility for *in vivo* studies (9). We have synthesized a decapeptide with a modification of the amino acid sequence between positions 6 and 13 of equine natural substrate. This peptide, unlike previous analogs, is not only a potent competitive inhibitor but also exhibits adequate solubility and has a prolonged *in vivo* half-life. Additionally, specific *in vivo* blockade of the pressor response to exogenous renin but not to angiotensin I or II has been demonstrated.

We report the administration of this renin inhibitory peptide to sodium-depleted normotensive and renin-dependent hypertensive monkeys. The results demonstrate the potential utility of this peptide for evaluating the role of renin in cardiovascular homeostasis and renin-dependent hypertension.

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## METHODS

**Synthesis and Characterization of the Renin-Inhibitory Peptide.** A soluble peptide with the sequence Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys was prepared as described (8). After synthesis and lyophilization under sterile conditions, the peptide was dissolved in physiologic saline (2 mg/ml) for animal studies. Plasma concentrations of infused $^3$H-labeled peptide were measured and the half-life was determined to be 3.8 min *in vivo*. Administration of the peptide was not associated with a pyrogenic response, change in body temperature, or altered behavior.

**Converting Enzyme Inhibitor.** The nonapeptide converting enzyme inhibitor, teprotide (10) (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), was synthesized for use in this study. It was dissolved in physiologic saline to a concentration of 2 mg/ml.

**Primate Studies.** Male *Macaca fascicularis* monkeys weighing 4–6 kg were used throughout the study. Chronic catheters were placed in the inferior vena cava, thoracic aorta, and lower abdominal aorta by a right iliac approach. A chronically implanted inflatable cuff was placed on the aorta above the left renal artery. Subsequently, a right nephrectomy was performed. Cuff inflation produced a pressure gradient of 60–70 mm Hg, giving a left renal artery perfusion pressure of 40–50 mm Hg. Hypertension, associated with a 2- to 4-fold increase in plasma renin activity, occurred within 1 hr (11). A regular chow diet (Purina), providing $Na^+$ and $K^+$ at 1.05 and 0.9 meq/kg per day, respectively, and supplemented with fruit and free access to water, was used. Sodium depletion was achieved by a fruit-for-chow exchange together with intravenous administration of furosemide (1 mg/kg of body weight per day) for 1 wk prior to study. This has been shown to result in a steady-state sodium depletion (11). Food was withheld the night prior to study. The monkeys were removed from their cages and placed in plexiglass restraining chairs. All studies were performed with monkeys unanesthetized and in the sitting position. A reequilibration period of 1 to several days was permitted between successive studies in the same animal. Arterial catheters were connected to Statham transducers interfaced with a Grass multichannel recorder. Mean arterial pressure (MAP) was determined as the electronically integrated mean. Heart rate [beats per min (bpm)] was recorded on a cardiotachometer. The inferior vena cava catheter was used for drug infusion and blood sampling. This method of study resulted in stable, reproducible blood pressure recordings that were within ±5 mm Hg of the mean value.

Abbreviations: MAP, mean arterial pressure; bpm, beats per min.

Five experiments were performed in three sodium-depleted normotensive monkeys and six experiments in three sodium-depleted hypertensive animals. Three normotensive sodium-replete animals were also studied.

**Biochemical Determinations.** Plasma renin activity was estimated based on the generation of angiotensin I by the method of Haber *et al.* (12). Twenty-four-hour urine collections were obtained, and aliquots were analyzed for sodium concentration (SmithKline). Values were corrected for 24-hr urine volumes and expressed as meq/day.

The paired *t* test was employed for blood pressure and heart rate analyses, with each monkey serving as his own control. All values expressed are means ± SEM.

## RESULTS

**$Na^+$ Replete, Normotensive Animals.** Three normotensive monkeys were studied in the sodium-replete state. Their baseline plasma renin activities averaged 2.4 ng/ml per hr. Renin-inhibitory peptide given as an intravenous bolus (2 mg/kg of body weight) caused an average MAP reduction of 3 mm Hg. This change was within the range of physiologic variation and comparable to the response evoked by subsequent administration of 1 mg of converting enzyme inhibitor per kg. There was no change in behavior nor fluctuation in baseline heart rate in response to the renin inhibitory peptide.

Pressor response to renin, angiotensin I, and angiotensin II was examined in five sodium-replete, normotensive animals. As can be seen in Table 1, pressor response to renin was markedly attenuated whereas that to angiotensins I and II was unaffected. At 3 times the dose of renin-inhibitory peptide (0.6 mg/kg per min) there was no additional change in the pressor response to angiotensin I. These observations indicate that at a concentration sufficient to inhibit renin, the renin-inhibitory peptide is not a converting enzyme inhibitor.

**$Na^+$ Depleted, Normotensive Animals.** A total of five studies were performed in sodium-depleted normotensive monkeys. Urinary sodium was 0.62 ± 0.46 (SD) meq/day and the plasma renin activity was 13.6 ± 5.1 (SD) mg/ml per hr. Renin-inhibitory peptide, given as an intravenous bolus (2 mg/kg) resulted in a prompt reduction in MAP from 105 ± 4 to 79 ± 3 mm Hg ($P < 0.004$) (Fig. 1). MAP gradually increased over the ensuing 15 min to a new baseline of 100 ± 4 mm Hg. The subsequent injection of converting enzyme inhibitor (1 mg/kg) resulted in reduction of MAP to 82 ± 5 mm Hg ($P < 0.006$). There was no significant difference between the hypotensive response to renin-inhibitory peptide and that of converting enzyme inhibitor, although the depressor response to teprotide is prolonged because of its longer half-life. In this group of monkeys, an important finding was the significant increase of heart rate from 180 ± 10 to 220 ± 7 bpm ($P < 0.003$) occurring during the hypotensive response to renin-inhibitory peptide. After the return of MAP to baseline, the heart rate remained at an elevated steady-state value of 196 ± 12 bpm ($P < 0.03$ compared with original heart rate of 180 ± 10 bpm). There was no further significant change in heart rate during the hypotensive response to converting enzyme inhibitor.

Table 1. Pressor response to sequential administration of purified human renin, angiotensin I, and angiotensin II*

| | Renin | AI | AII |
|---|---|---|---|
| Control | +22 ± 3 | +30 ± 1 | +30 ± 3 |
| Renin-inhibitory peptide (0.2 mg/kg per min) | + 3 ± 2† | +33 ± 3‡ | +28 ± 2‡ |

* All values are the mean ± SEM for five studies and are expressed as mm Hg increase in pressure; A, angiotensin.
† Significantly different from control ($P < 0.004$).
‡ Not significantly different from control.

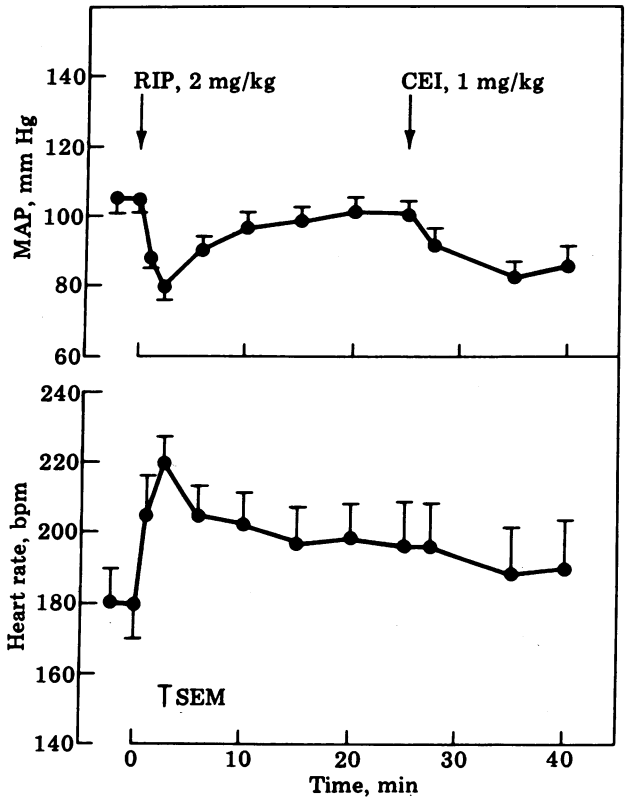


FIG. 1. MAP reduction occurred in five studies of the normotensive sodium-depleted state following a bolus injection (2 mg/kg) of renin-inhibitory peptide (RIP) ($P < 0.004$). This was associated with significant cardiac acceleration ($P < 0.003$). The MAP reduction in response to the 1 mg of converting enzyme inhibitor (CEI) per kg ($P < 0.006$) was not significantly different compared to the renin inhibitor peptide response.

**$Na^+$ Depleted, Hypertensive Animals.** Renin-inhibitory peptide was administered to sodium-depleted, renin-dependent, hypertensive monkeys. Bolus intravenous injection of peptide produced dose-dependent MAP reductions ranging from 15 mm Hg (0.5–1.0 mg/kg) to 70 mm Hg (3.0 mg/kg). The latter response was associated with a 40-bpm increase in heart rate. To gradually lower MAP, renin-inhibitory peptide was given as a graded infusion in increments of 0.2 mg/kg per min. Six studies were performed (Fig. 2). The baseline MAP was 107 ± 3 mm Hg, which increased to 131 ± 3 mm Hg ($P < 0.002$) after 1 hr of aortic cuff inflation. The plasma renin activity increased from 12.5 ± 1.7 to 33.2 ± 3.5 mg/ml per hr ($P < 0.02$). Graded infusion of renin-inhibitory peptide was initiated when MAP was stable at this hypertensive level, and prompt reduction of blood pressure occurred. At a dose of 0.4 mg/kg per min, the reduction of MAP to 121 ± 2 mm Hg was significant ($P < 0.005$). Continued infusion at the rate of 0.6 mg/kg per min resulted in reduction of MAP to 107 ± 4 mm Hg ($P < 0.004$ compared to 131 ± 3 mm Hg; $P < 0.008$ compared to 121 ± 2 mm Hg). When this prehypertensive level of MAP was achieved, the infusion was discontinued. MAP increased to 125 ± 3 mm Hg within 5 min of discontinuation, which was a hypertensive level not significantly different from 131 ± 3 mm Hg. After equilibration at 127 ± 3 mm Hg, intravenous administration of 1 mg of converting enzyme inhibitor per kg resulted in reduction of MAP to 111 ± 4 mm Hg ($P < 0.002$). The nadir hypotensive response to renin-inhibitory peptide and angiotensin I-converting enzyme inhibitor were similar. Throughout this experiment there was no appreciable change in heart rate.

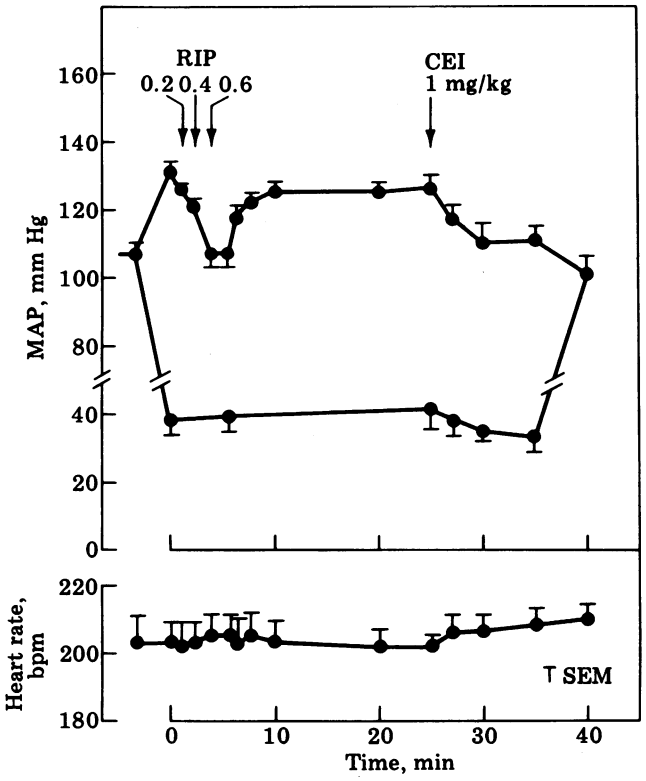


FIG. 2. After 1 hr of aortic cuff inflation, the MAP above the aortic cuff (upper curve) rose from 107 to 131 mm Hg in six studies of renin-dependent hypertension. Aortic pressure below the cuff was maintained at approximately 40 mm Hg (lower curve). Renin-inhibitory peptide (RIP) was given as a graded infusion of 0.2 mg/kg per min increments. At 0.6 mg/kg per min, MAP was restored to prehypertensive levels ($P < 0.004$). After a brief period, infusion was discontinued and MAP increased to 127 mm Hg. Converting enzyme inhibitor (CEI), 1 mg/kg, reduced MAP to 111 mm Hg ($P < 0.002$). The MAP responses to renin-inhibitory peptide and to converting enzyme inhibitor were similar (difference not statistically significant). Heart rate was consistent throughout.

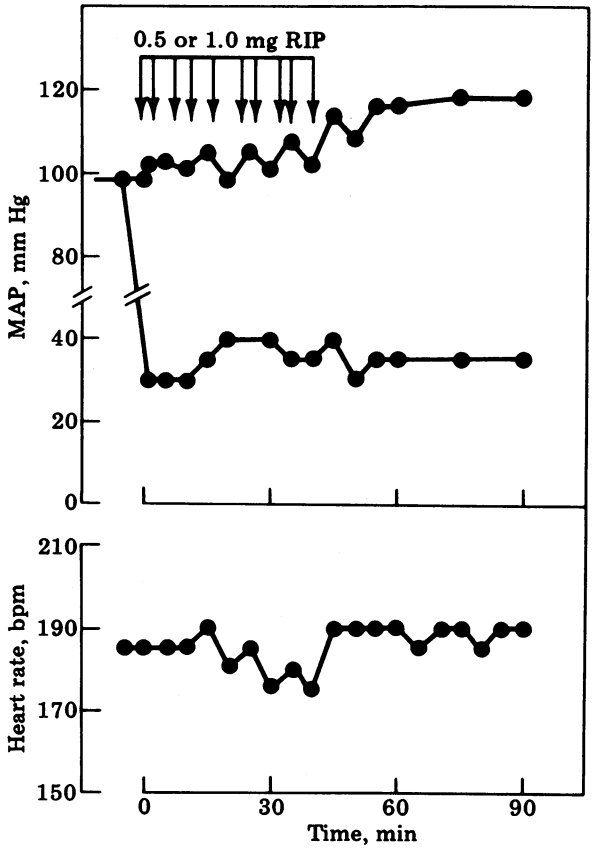


FIG. 3. Renin-inhibitory peptide (RIP) was administered as an intravenous bolus at the time of aortic cuff inflation and at subsequent 4- to 5-min intervals. The development of hypertension above the aortic cuff, which usually begins at 10 min after inflation, was delayed for 50 min by renin-inhibitory peptide and then occurred in a typical fashion when injections were discontinued. The lower curve represents pressure below the aortic cuff, which was maintained at approximately 30 mm Hg.

In two studies, bolus injections of renin-inhibitory peptide (0.5 to 1.0 mg) were given at the time of cuff inflation and at subsequent 4- to 5-min intervals. Pretreatment with peptide prevented the occurrence of hypertension. With discontinuation of peptide injections, hypertension developed in a typical fashion. A representative study is shown in Fig. 3.

## DISCUSSION

The interest in these experiments lies in the application of a highly specific inhibitor of renin. Prior studies have utilized a variety of approaches to blocking the renin system, most of which have suffered from the lack of specificity. Sympatholytic agents block renin release by the juxtaglomerular apparatus but have several other actions (13). Soluble analogs of the acid protease inhibitor pepstatin A (14) are capable of blocking renin but also may perturb other regulatory mechanisms by inhibiting various acid proteases, including cathepsin D. Angiotensin I-converting enzyme inhibitors also inhibit kininase II, possibly resulting in increased levels of the vasodilator bradykinin. Angiotensin II analogs have demonstrated agonistic as well as antagonistic activity *in vivo*.

While analogs of angiotensinogen have been demonstrated to inhibit renin (8), none have been applicable as *in vivo* inhibitors. Modifications of the octapeptide sequence found between positions 6 and 13 that increase solubility, improve binding to renin, and extend half-life *in vivo* are discussed in detail elsewhere (9). In general, addition of prolyl residues to the $NH_2$ terminus doubles solubility at physiologic pH. Replacement of the leucyl residues with phenylalanyl residues increases the inhibitory constant ($K_i$) 40-fold to yield a peptide that binds renin almost as tightly as natural substrate (8). Addition of a lysyl residue to the COOH terminus increases solubility and extends half-life in the circulation from a few seconds to almost 4 min.

Although no appreciable changes in blood pressure occurred in normotensive sodium-replete monkeys, hypotension was produced in the sodium-depleted state by renin-inhibitory peptide and by angiotensin I-converting enzyme inhibitor. Previous studies (15–17) have demonstrated the dependence of cardiovascular homeostasis on the renin–angiotensin system during salt depletion. Most recently, this was clearly shown in dogs, by utilizing a highly specific antibody to renin (18). The present study not only confirms this finding in the nonhuman primate, but also offers a potential approach for evaluating the role of renin in normal man, in whom exposure to antibodies from a heterologous species is unwarranted.

In renin-dependent hypertension, the renin-inhibitory peptide resulted in a return of blood pressure to baseline values with either bolus injection or graded infusion. When large doses were used (3–4 mg/kg), MAP decreased below baseline indicating an additional hypotensive response in the presence of sodium depletion. Reduction of MAP to the normal range was maintained with graded infusion of renin inhibitory peptide.

Furthermore, pretreatment with renin inhibitory peptide delayed the hypertensive response to aortic cuff inflation, which indicated that the peptide could prevent the genesis of renin-dependent hypertension in addition to opposing preexisting hypertension. Since the duration of the experiments was relatively brief, studies of longer duration will be required to examine chronic forms of renin-dependent hypertension.

Rapid bolus injection of renin inhibitory peptide in normotensive sodium-depleted monkeys resulted in cardiac acceleration during blood pressure reduction. Similarly, bolus injection of peptide in renin-dependent hypertension, was associated with cardiac acceleration as blood pressure was restored to prehypertensive levels. However, significant heart rate change did not occur when graded infusion of peptide gradually returned blood pressure to control levels. There are at least three explanations for the cardiac acceleration observed. The first and most likely is that this represents the efferent baroreceptor response to acute hypotension. This further suggests that the baroreceptor reflex is not qualitatively changed by renin-inhibitory peptide. Other explanations may include inhibition of vagal impulses or direct stimulation of catecholamine release. The latter explanations are unlikely, because cardiac acceleration was not observed in normotensive sodium-replete monkeys or when renin-inhibitory peptide was given as a graded infusion, thereby reducing the blood pressure gradually. Cardiac acceleration in response to blood pressure reduction has not been observed with angiotensin analogs and converting enzyme inhibitors. In fact, there has been at least one report indicating that converting enzyme inhibition may blunt the baroreceptor response to hypotension (19).

Peptides inhibiting renin will offer a very specific approach to blockade of the renin–angiotensin system. They will be valuable not only in uncovering basic mechanisms of cardiovascular homeostasis but also may provide the necessary tool for determining the role of renin in human essential hypertension.

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