H-77: A Potent New Renin Inhibitor

In Vitro and In Vivo Studies

MICHAEL SZELKE, PH.D., BRENDA J. LECKIE, PH.D., MALCOLM TREE, PH.D.,
ALLISON BROWN, PH.D., JENNETTE GRANT, ALLAN HALLETT, PH.D.,
MARIA HUGHES, DAVID M. JONES, PH.D., AND ANTHONY F. LEVER, M.D., F.R.C.P.

SUMMARY Chemical modification of the backbone at the cleavage site in the (6-13)-octapeptide of equine angiotensinogen resulted in greatly increased binding affinity and resistance to cleavage by renin. The D-His^{8}-Tyr^{9} octapeptide analog containing the reduced bond -CH_{2}-NH- instead of a peptide bond -CO-NH- at the Leu^{8}-Leu^{9} linkage (H-77) was a powerful in vitro inhibitor of canine renin (IC_{50} = 24 nM). It gave an IC_{50} of 1 μM against human renin and 0.6 μM against rat renin. In sodium-depleted conscious dogs, infusion of H-77 caused dose-related falls of plasma angiotensin I and plasma angiotensin II concentration and mean arterial pressure; the minimum effective dose was 0.1 mg · kg^{-1} · hr^{-1}. Similar infusions of H-77 in chronically catheterized rats have no effect on blood pressure or plasma angiotensin II concentration. Thus, the in vitro effect of H-77 as an inhibitor of renin in dog, human, and rat plasma was paralleled by its action in the whole animal. (Hypertension 4 (suppl II): II-59-II-69, 1982)

KEY WORDS • renin inhibitor • renin • angiotensin II • blood pressure • H-77 • angiotensinogen • octapeptide

In 1957 Skeggs and colleagues\(^1\) elucidated the N-terminal tetradecapeptide sequence of equine renin substrate (fig. 1, column 1 across). Later they found\(^4\) that the octapeptide (fig. 1, compound 2) representing the angiotensinogen (6-13)-octapeptide accounted for a large part of the binding affinity of angiotensinogen to renin. It was also the smallest fragment of the substrate still to be cleaved by renin at a significant rate. At the same time Kokubu and coworkers\(^8\) reported that the tetrapeptide ester (fig. 1, compound 2), related to the (10-13)-tetrapeptide, acted as a weak competitive in vitro inhibitor of rabbit renin. Slight inhibitory activity against rabbit renin in vivo was later demonstrated.\(^6\)

In 1971, an in vivo inhibitor of renin (fig. 1, compound 4) was prepared by reduction of the Leu-Leu bond in the tetrapeptide ester 2 (i.e., chemical trans-
formation of -CO-NH- into -CH_{2}-NH-, indicated in the formula by the symbol R over the peptide bond). This simple modification of the peptide backbone at the scissile bond endowed the molecule with greater resistance to enzymatic hydrolysis and with an increased binding affinity to renin. However, this tetrapeptide analog 4 contained only a small portion of the renin binding sites present in natural substrate and consequently possessed only modest inhibitory potency (IC_{50} approx. 800 μM)\(^*\).

More potent in vitro inhibitors of renin (K_{d} = 3-25 μM), based on the angiotensinogen (6-13)-octapeptide (compound 2), were developed by Haber and coworkers.\(^7\)\(^-\)\(^8\) However, some of these, such as the D-Leu\(^11\) substituted peptide of the angiotensinogen (6-13)-octapeptide sequence, were inactive in plasma because of insufficient solubility at physiological pH, and all were devoid of activity in vivo. Very recently, Haber and colleagues\(^9\) described the decapeptide (fig. 1, column 5) Pro\(^6\) Phe\(^7\) Phe\(^8\) Lys\(^14\) angiotensinogen (5-14)-decapeptide as an effective inhibitor of renin in vitro (K_{d} = 2 μM) and in vivo in primates.

We report here the synthesis, in vitro and in vivo activities of a potent new inhibitor of canine renin.
Methods

Synthesis of Inhibitors

All compounds listed in table 1 were synthesized by the solid-phase method, with the exception of the reduced tetrapeptide ester 4, which was prepared in solution as described previously.6 The protected reduced dipeptide isostere Boc-Leu2Leu-OH required for the synthesis of H-76, H-77, and H-79 was synthesized by methods developed in this laboratory.11 Full details of the synthesis and purification of H-76 and H-77 will be published later in 1982.12 Compounds were purified to homogeneity in one or in a combination of the following systems:

1. Gel chromatography on Sephadex G-25 in 50% aqueous acetic acid.
2. Ion exchange chromatography on Sephadex SPC 25 using 30% acetic acid and a sodium chloride gradient 0.01–1.0 M, followed by desalting according to Method 1.
3. High performance liquid chromatography (HPLC) on Partisil ODS (column measuring 500 × 10 mm) using either 0.01 M ammonium acetate-isopropanol, pH 4.0, or water-isopropanol-0.08% trifluoroacetic acid in isocratic systems of various proportions.

Thin layer chromatography (table 1) and thin layer electrophoresis (table 2) indicated that all compounds were homogeneous. For amino-acid analyses, samples were hydrolyzed in 6N HCl at 110° for 40 hours prior to running on an LKB Model 4,400 amino acid analyzer.

TABLE 1. Thin Layer Chromatography (TLC) (Merck Silica Plates) of Peptides in Three Solvent Systems

<table>
<thead>
<tr>
<th>TLC systems</th>
<th>Rf value for compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>0.14</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

TLC systems are: System 1 = n-Butanol/pyridine/acetic acid/water 30:20:6:24; System 2 = ethyl acetate/pyridine/acetic acid/water 40:20:6:11; and System 3 = ethyl acetate/pyridine/acetic acid/water 70:20:6:11; See figure 1 and table 4 for sequence of peptides.

TABLE 2. Thin Layer Electrophoresis Results (Merck Cel lulose Plates, 1000 V)

<table>
<thead>
<tr>
<th>pH</th>
<th>H-76</th>
<th>H-77</th>
<th>H-79</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>83</td>
<td>77</td>
<td>75</td>
</tr>
<tr>
<td>6.5</td>
<td>75</td>
<td>79</td>
<td>83</td>
</tr>
</tbody>
</table>

Tests for Renin Inhibition In Vitro

Plasma

Human plasma from male volunteers was collected as previously described18 and pooled. Another plasma pool was made up of samples from hypertensive patients, excluding any patients on treatment with converting enzyme inhibitors. Animal plasma was collected from dogs on either a normal or a low sodium diet, and from rats.

Renin Assay

Buffers and angiotensin I antibody for the renin assay were prepared as described previously.18,14 The assay was based on the antibody-trapping method15 in which plasma renin reacts with plasma angiotensinogen at pH 7.0 to produce angiotensin I. The incubation mixture contained 75 μl of plasma, 10 μl of a solution of the peptide inhibitor or in control incubations of solvent alone, and 15 μl of angiotensin I antibody in 3M Tris buffer, pH 6.9. For the assay of rat renin, we used 23 μl of rat plasma, 5 μl of inhibitor solution, and 15 μl of the antibody-buffer solution. The mixture was incubated at 37°C for times between 15 and 120 minutes, and the rate of angiotensin I production was found by radioimmunoassay.15 The angiotensin I produced in the presence of inhibitor was calculated as a percentage of that produced by plasma and solvent alone. Results were corrected for any angiotensin I present in the plasma before the incubation began and for any cross-reaction of the inhibitor with the angiotensin I antibody. Each dilution of inhibitor was assayed in triplicate. An “experiment” refers to a series of assays carried out with dilutions of inhibitor made from a single weighing of the dry peptide.

Inhibitors

Inhibitors were freshly weighed each day. They were dissolved either in 0.1 M HCl and diluted to give 0.01 M HCl or directly in 0.01 M HCl, which did not change the pH of the incubation mixture. The peptide H-79 was dissolved in 50% ethanol.

Tests of Renin Inhibition In Vivo in the Dog

Conscious male beagle dogs (12.7–18 kg) with previously prepared carotid artery loops were studied. Techniques used for intravenous infusion, blood pressure recording, and blood sampling have been described.18 For 3 days before each study, dogs consumed a diet low in sodium but normal in potassium (1.7 and 22.7 mEq/day respectively) and on the first day, 5 mg/kg of furosemide was injected intravenously. The study began on the 4th day. Dogs were placed on a table; their carotid artery was cannulated with a 19-gauge butterfly needle, and blood pressure was recorded continuously by mercury manometer and kymograph; pulse rate was measured by miniature electrocardiogram (Exersentry, Respironics Inc., Monroeville,
Intravenous infusions were given into a foreleg vein via a 3 F9 polythene catheter from a plastic syringe using a Harvard infusion pump. Dogs were familiar with these proceedings and stood quietly throughout.

Four-Hour Infusion of Renin Inhibitor

Two dogs were first given an intravenous infusion of 5% dextrose at 36 ml/hr for 1 hour as a control, and then H-77 dissolved in 5% dextrose at 0.1 mg • kg⁻¹ • hr⁻¹ for 4 hours. The H-77 had been filtered to sterilize it. Using millipore filters (Millex SLGS 025 BS), we took blood samples at various times for measurement of angiotensin I and II (see Results).

Dose-Response Experiment

Six dogs were studied. Each dog had five infusions on different occasions. Each infusion study began with 5% dextrose given intravenously at 36 ml/hr as a control. Then H-77 in 5% dextrose was infused at one of the chosen rates. Infusions were given for 4 hours. The H-77 had been filtered to sterilize it. Using millipore filters (Millex SLGS 025 BS), we took blood samples at various times for measurement of angiotensin I and II (see Results).
Inhibition of Renin in Plasma by Isosteric Analogs H-76 and H-77 Compared with Parent Peptides

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Compound residue no. in tetradecapeptide</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>6</td>
<td>H-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>H-Leu-Leu-Val-Phe-OMe</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>H-Pro-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Lys-OH</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>H-Pro-Phe-His-Leu-Leu-Val-Tyr-OMe</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>H-Leu-Leu-Val-Phe-OMe</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>H-Pro-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Lys-OH</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>H-Pro-Phe-His-Leu-Leu-Val-Tyr-OMe</td>
<td></td>
</tr>
</tbody>
</table>

Relationships between the (6-13)-octapeptide sequence of equine angiotensinogen and its derivatives; peptides 6 and 7, are described by Poulsen et al. and Burton et al. Peptide 4 was described by Parry et al. R indicates a reduced isosteric bond, i.e., -CH₂-NH- in place of the peptide bond -CO-NH-. OMe = methyl ester.

*Incomplete inhibition.

Octapeptide 2 showed an IC50 = 200 µM while the derivative H-76 had an IC50 = 1.0 µM. Complete inhibition of the renin enzymatic activity was obtained with H-77. However, H-109 did not give complete inhibition even at a concentration of 900 µM (fig. 2). The type of inhibition curve showed by H-109 might be obtained if the peptide were cleaved to some extent by human renin, producing a peptide that partially cross-reacted with antibody to angiotensin I. If the N-terminal histidine was omitted from the H-77 as in H-79, the IC50 increased to 17 ± 1.4 µM (mean ± SE of four experiments).

Figure 3 shows the inhibition curves we obtained with the peptides 5', 7', with the isosteric tetrapeptide 4', and with H-79. All these were comparatively poor inhibitors of the human renin-angiotensinogen reaction at pH 7.0. The results are summarized in table 4.

Figure 4 shows that H-77 inhibits the renin in plasma from normal male subjects as well as renin in plasma from hypertensive patients.
Inhibition of Renin in Animal Plasma

The effect of H-77 on renin in plasma from humans, rats, and dogs is shown in figure 5. Five experiments with H-77 in dog plasma were carried out, giving an IC₅₀ = 0.024 ± 0.004 μM (mean ± se; range, 0.01-0.03 μM). Plasma from normal dogs (renin concentration, 10 μU/ml) and dogs subjected to dietary sodium depletion (renin concentration, 84 μU/ml) gave similar values of IC₅₀. Three experiments with rat plasma gave a mean IC₅₀ = 0.63 ± 0.12 μM. The parent peptide of H-77, H-109, gave an IC₅₀ = 60 μM when tested in plasma from sodium-deplete dogs and between 20 and 50 μM in normal dog plasma to which dog renin (100 μU/ml) had been added. However, complete inhibition was not obtained with H-109 even...
at concentrations of 976 μM. Inhibition of renin was scarcely detected in plasma from normal dogs, and in fact a peptide giving partial cross reaction with antibody to angiotensin I was apparently produced. This suggests that H-109 is both a substrate and a competitive inhibitor for dog renin.

Effect of Peptidase Inhibitors on the Action of Renin Inhibitor Peptides

The renin assay system contained no peptidase inhibitors, since the angiotensin I produced during the reaction was protected by antibody-trapping rather than by chemical inhibition of peptidases. We therefore tested the effect of peptidase inhibitors on the ability of the peptides to inhibit renin. We added ethylenediaminetetra-acetic acid (disodium salt) (EDTA), 5mM; o-phenanthroline (2mM); benzamidine hydrochloride (5mM); and trasylol (50 μM) to plasma from humans and normal dogs. In the absence of peptidase inhibitors, angiotensin I (4 ng/ml) was completely destroyed after 2 hours’ incubation at 37°C. In the presence of the inhibitor mixture, 98% recovery of angiotensin I was obtained in human plasma and 80% in dog plasma over 2 hours. The rate of production of angiotensin I in the antibody-trapping renin assay was similar in the presence or absence of peptidase inhibitors.

Table 5 shows the IC₅₀ values for a single experiment in which D-His* angiotensinogen (6-13)-octapeptide (H-109) and the isosteric derivative H-77 were tested in human and in dog plasma, with and without peptidase inhibitors. Inhibition of peptidases did not affect the values of IC₅₀ obtained for H-77. The IC₅₀ for H-109 was slightly lower in the presence of peptidase inhibitors. However, the IC₅₀ for H-109 in both human and dog plasma was still higher than that for H-77.

Effect of H-77 on Renal "Acid Protease"

In a preliminary experiment we tested the effect of H-77 on "acid protease" activity in an extract of human kidney in which the release of tritium-labelled peptides from hemoglobin substrate at pH 3.3 is measured. The kidney extract contained active renin as well as acid protease, being the breakthrough peak obtained from Affigel Blue affinity chromatography in 0.02 M phosphate buffer, pH 7.0. The extract was diluted for renin assay in the 0.25 M Tris/HCl, pH 7.4 radiolmmunoassay buffer described by Millar et al. with the addition of inhibitors of metallo-peptidases and serine peptidases, as described in the previous section, and renin was measured with ox substrate. The IC₅₀ for the inhibition of renin by H-77 was 1.2 μM, but the IC₅₀ for the inhibition and acid protease was 3000 μM, indicating that H-77 is a poor inhibitor of renal acid protease.

Table 5. Values of IC₅₀ for H-109 and H-77 in Human and Dog Plasma, with and without Inhibition of Plasma Peptidases

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Peptidase inhibitor</th>
<th>IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>H-109</td>
<td>0.01</td>
</tr>
<tr>
<td>No peptide inhibitors</td>
<td>&gt;989*†</td>
<td>0.01</td>
</tr>
<tr>
<td>With peptide inhibitors</td>
<td>90*</td>
<td>0.01</td>
</tr>
<tr>
<td>Human</td>
<td>H-109</td>
<td>0.90</td>
</tr>
<tr>
<td>No peptide inhibitors</td>
<td>35*</td>
<td>0.90</td>
</tr>
<tr>
<td>With peptide inhibitors</td>
<td>22*</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Incomplete inhibition of renin.
†30% inhibition of renin at 90 μM H-109.

Figure 6. Mean arterial pressure (MAP), plasma angiotensin I concentration, and plasma angiotensin II concentration in two sodium-depleted dogs during an infusion of dextrose followed by an infusion of H-77 at 0.1 mg·kg⁻¹·hr⁻¹ for 4 hours.
Effect of Renin Inhibitors in Vivo

Our object in this part of the study was to test the renin inhibitor in conscious animals. The dog was chosen as a species likely to show a response, the rat as one in which the response was likely to be smaller.

Studies in Conscious Dogs

Two experiments were done, the first to determine the speed of onset and constancy of response during continuous intravenous infusion of inhibitor, the other to determine the dose needed to produce a response. As in earlier experiments, depletion of sodium during the 3 days before infusion produced circulating concentrations of angiotensin I and angiotensin II which were higher than those found in sodium-replete conscious beagle dogs.

Four-Hour Infusion of H-77. Infusion of H-77 reduced circulating concentrations of angiotensin I and II within 15 minutes. With the exception of angiotensin II in one dog, the effects persisted without major change throughout the 4-hour infusion (fig. 6). Blood pressure fell in both dogs but returned toward control in one during infusion of the inhibitor.

Dose-Response Study. Infusion of H-77 at 1 and 10 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1}\) markedly reduced the circulating concentrations of angiotensin I and II (fig. 7). Infusion at 0.1 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1}\) had a clear but less marked effect; infusion at 0.01 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1}\) had little effect. Control infusions of dextrose had no effect. Changes in the

![Figure 7. Plasma angiotensin I concentration and plasma angiotensin II concentration in six sodium-depleted dogs during infusion of dextrose, or H-77, at rates of 0.01, 0.1, 1.0, and 10.0 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1}\). *p < 0.02; **p < 0.01.](http://hyper.ahajournals.org/)
plasma concentration of angiotensin II were significantly correlated with changes in blood concentration of angiotensin I \( (r = 0.72 \text{ for 15-minute samples and 0.77 for 30-minute samples, } p < 0.001) \).

Arterial pressure was also reduced by H-77, and here again greater and more persistent effects were produced by higher doses (fig. 8). Changes of mean arterial pressure were also related to changes of plasma angiotensin II concentration \( (r = 0.69 \text{ in 15-minute samples and 0.72 in 30-minute samples, } p < 0.001) \). Heart rate increased significantly during periods of marked hypotension resulting from higher doses of inhibitor, but infusion at the intermediate dose of 0.1 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1} \) lowered blood pressure without increasing heart rate (fig. 8).

**Infusion of Renin Inhibitor in the Rat**

Infusion of H-77 at 1 and 10 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1} \) had no significant effect on arterial pressure or on the plasma concentration of angiotensin II. The greatest decrease of angiotensin II was from 42 to 27 pg/ml in a rat receiving H-77 at 10 mg \( \cdot \) kg\(^{-1} \cdot \) hr\(^{-1} \) (fig. 9).

**Discussion**

Our purpose was to convert the angiotensinogen \( (6-13) \)-octapeptide (compound 2) into an active inhibitor of renin by chemical modification of the peptide backbone. As our results with H-76 and H-77 indicate, reduction of the scissile bond in peptide 2 had the following effects:
The affinity of the molecule to renin apparently increased in all three species studied. The increase was most pronounced in the case of dog renin, H-77 having about 10,000 times greater affinity to the canine enzyme than the angiotensinogen (6-13)-octapeptide.

2. The modification stabilized the Leu₁⁰-Leu¹¹ link to hydrolysis by peptidases, including renin.

3. The modification also increased the solubility of the lipophilic parent molecule in aqueous solvents. Lack of solubility has hampered previous attempts to convert peptide 2 into an effective in vivo inhibitor, and maneuvers such as adding prolyl residues to the N-terminus were used by Haber. In contrast, the acetate or hydrochloride salts of H-76 and H-77 are readily soluble in aqueous buffers and in the plasma of various species.

The assay system is an important consideration when testing renin inhibitors. Parikh and Cпут the assay system is an important consideration when testing renin inhibitors. Parikh and Cuptrecasas used partially purified human renin acting on tetradecapeptide substrate 1, at pH 5.5. Kokubu et
al. used rabbit renin and rabbit angiotensinogen at pH 6.4. Haber and coworkers used human renal renin and both tetradecapeptide substrate and human substrate at pH 5.5 or 7.4. Partially purified renin preparations may contain cathepsin D which produces angiotensin I from tetradecapeptide substrate at pH levels below 6. Cathepsin D does not readily cleave natural angiotensinogen, particularly if the pH of the incubation mixture is above 6.0. Assay systems that employ partially purified renin and peptide substrates at acid pH may measure cathepsin D as well as renin, and some earlier renin inhibitors could, in fact, inhibit cathepsin D activity rather than renin. Our assay system in which plasma renin reacts with angiotensinogen at pH 7.0 provides conditions more similar to those in circulating plasma, and interference by cathepsin D is unlikely. Furthermore, measurement of acid protease activity in human kidney extracts indicated that H-77 was a poor inhibitor of acid protease, the IC₅₀ being 3000-fold greater for acid protease than for renin in the same extract. However, our assay does not exclude binding of the peptide inhibitors to plasma proteins or their destruction by peptidases.

It was interesting to find that the IC₅₀ for H-77 was identical in plasma from normal people and hypertensive patients, although the incubation time for the renin assay was 2 hours for normal plasma as against 30 minutes for the patients’ plasma. Also, plasma from sodium deplete dogs and normal dogs gave similar results whatever the time of incubation. This suggests that destruction of H-77 by peptidases did not affect the value for IC₅₀ which we obtained. More direct evidence to show that the IC₅₀ values reflected the affinity of H-77 to renin rather than the survival time of the peptide in plasma was obtained by measuring the IC₅₀ in the presence of peptidase inhibitors. The IC₅₀ of H-77 in both human and dog plasma was similar in the presence and absence of peptidase inhibitors. The parent peptide D-His⁸ angiotensinogen (6-13)-octapeptide, H-109, showed a slightly lower IC₅₀ in dog plasma when peptidase inhibitors were present. However, the inhibition was still much less than that obtained with H-77, indicating that the isosteric modification improved the affinity to renin rather than having a nonspecific effect on the susceptibility of H-77 to peptidases.

The action of enzyme inhibitors in the whole animal will depend on factors such as clearance, in addition to the affinity of the inhibitor to the enzyme. It is interesting that studies of H-77 in conscious animals accord well with the enzyme data. H-77 had an inhibitor effect 30 times greater on dog renin than on rat renin. In conscious dogs, infusion of the inhibitor at 0.1 mg • kg⁻¹ • hr⁻¹ and at higher rates lowered plasma angiotensin II concentration and arterial pressure; in the rat, infusion at 10 mg • kg⁻¹ • hr⁻¹ had no effect on angiotensin II or arterial pressure.

Inhibition of renin in the dog was rapid in onset, and the changes of angiotensin II following infusion suggest that the inhibitor has a half-life of less than 60 minutes. The slower return to control values following higher doses (fig. 2) may reflect saturation of the mechanism(s) inactivating the inhibitor.

Blood pressure was reduced by H-77 in the dog, and the relation between the decrease of plasma angiotensin II concentration and the decrease of blood pressure suggests cause and effect. On other evidence, renin and angiotensin II contribute to the maintenance of arterial pressure in the conscious sodium-deplete beagle dog. Failure of H-77 to alter angiotensin II and arterial pressure in the rat suggest that the positive response in the dog is related to its greater potency as an inhibitor of renin, rather than to some less specific action. Captopril, a converting enzyme inhibitor, also reduces blood pressure in the sodium-deplete conscious beagle, and here again the decrease of arterial pressure is related to the decrease of plasma angiotensin II concentration.

The use of renin inhibitors in physiologic studies has been reviewed by Haber and Burton. Pepstatin, an inhibitor of aspartyl proteases (which is produced by actinomycetes), has a higher affinity of cathepsin D and pepsin than for renin. Other, nonpeptide inhibitors include renin antibody, phospholipid inhibitors, and possibly various binding proteins. Peptide inhibitors based on the amino-acid sequence of angiotensinogen are likely to yield information about the renin-angiotensinogen reaction, and the physiological role of renin.

To summarize, we have shown that the isosterically modified peptide, H-77, is an effective inhibitor of canine renin both in vitro and in the conscious animal. There are prospects for it as a means of testing the role of the renin-angiotensin system in physiological and pathological states. In longer acting and more stable form, H-77 might have a future as a therapeutic agent.

Acknowledgments

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