Mammalian homeostasis is incredibly complex. It has been the historical task of the physiologist to identify specifically the contribution of each of many interacting mechanisms that together comprise a regulatory system. A traditional approach to identification of function has been ablation of the organ or structure in question. This is not possible when organs are multifunctional. As well as being an excretory organ, the kidney is a complex endocrine gland. The function of renin cannot be defined by nephrectomy alone. Not only is the source of several other vasoregulatory substances removed but also the major end organ for renin’s action. A reasonable alternative to ablation is the application of highly specific inhibitors. In this lecture I shall discuss the two very selective inhibitors that have recently become available to us. I shall outline their utility in defining the physiological and pathophysiological roles of renin in vascular homeostasis, as well as the application of one of these inhibitors in the elucidation of important problems in the biosynthesis of renin and the identification of renin from extrarenal sites.

Chemistry of the renin–substrate reaction

Renin has no known physiological effect but acts only to cleave its substrate, angiotensinogen, which is an \( \alpha_2 \)-globulin synthesized in the liver. The amino acid sequence of the N-terminal 14 residues of renin substrate is known (Fig. 1). Renin cleaves between two leucine residues at positions 10 and 11 to release the decapeptide angiotensin I. Angiotensin I is in turn split between residues 8 and 9 by a converting enzyme to yield the active pressor hormone, angiotensin II. Aminopeptidases further degrade angiotensin II by removing the N-terminal aspartic acid. The resultant heptapeptide, sometimes called angiotensin III, is believed by some investigators to be the primary mediator of adrenal-cortical aldosterone secretion (Blair-West, Coghlan, Denton, Funder, Scoggins & Wright, 1971). Angiotensin II and III have very short half-lives in the circulation and are further degraded to smaller inactive peptides (Hodge, Ng & Vane, 1967).

The past several years have seen the development of interest concerning possible renin precursors and zymogens. There exist presently a number of observations which on the surface appear contradictory. In evaluating various reports two critical questions which must be kept in mind continually are: (a) what is the definition of renin, and (b) is the enzyme that is examined in vitro the same material that is produced in vivo by the kidney?

To define renin in functional terms can be quite misleading. Many investigators depend on a pressor response in vivo. Clearly the number of potential substances giving such a response is great; even if it can be demonstrated that this response is mediated by angiotensin II, renin need not be involved. If the production in vitro of angiotensin I from renin’s protein substrate, angiotensinogen, is accepted as the sole criterion, one may be misled; other enzymes may degrade this substrate in the same way. For example, pepsin cleaves renin substrate to produce angiotensin I (Franze de Fernandez, Paladini & DeLuis, 1965).

I should like to propose a definition of renin based on two different criteria, drawing on both functional and structural considerations. Renin can be considered both as a protein molecule of unique structure and also as an enzyme of exceedingly fastidious substrate specificity. Once renin is purified, its unique structural features may be recognized by specific antibodies. How-
Renin substrate

\[
\begin{array}{cccccccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 \\
\end{array}
\]

\[H-\text{Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-}^{\text{---}}\]

\[\text{Renin}\]

\[\text{[Ile}^6\text{]Angiotensin I}\]

\[
\begin{array}{cccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \\
\end{array}
\]

\[H-\text{Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH}\]

\[\text{Converting enzyme}\]

\[\text{[Ile}^6\text{]Angiotensin II}\]

\[
\begin{array}{cccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \\
\end{array}
\]

\[H-\text{Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH}\]

\[\text{Angiotensinase}\]

\[\text{[Des-Asp-Ile}^6\text{]Angiotensin II}\]

\[\text{Angiotensinase}\]

\[\text{Angiotensin III}\]

\[\text{Angiotensinase}\]

\[\text{Inactive products}\]

\[\text{FIG. 1. Biochemistry of the renin-angiotensin system. [Ile}^1\text{]Angiotensin contains isoleucine in the 5 position and is the form of peptide that occurs in man. (By permission of the New England Journal of Medicine.)}\]

ever, in order to avoid confusion with other enzymes that may have an evolutionary relationship and thereby share common antigenic sites, it is also necessary to take advantage of renin's substrate specificity. It is perhaps the most demanding of proteolytic enzymes studied with respect to substrate sequence. Skeggs, Kahn, Lentz and Shumway (1957) showed that a defined octapeptide sequence was required for recognition and cleavage by renin. Thus a second criterion for identifying renin can be based on the construction of inhibitors that mimic the enzyme's catalytic site. An assessment of the presence and role of renin can then be made with considerably greater assurance when both structural and functional criteria may be met.

**Renin antibody as a physiological probe**

The complete purification of hog (Corvol, Devaux, Ito, Sicard, Duclox & Menard, 1977; Inagami & Murakami, 1977) and human (Yokosawa, Inagami & Haas, 1978; Slater, Cohn, Dzau & Haber, 1978; Galen, Devaux, Guyenne, Menard & Corvol, 1979) kidney renin has now been accomplished. Because the trained conscious dog has been such a useful model in the examination of the role of the renin system in a variety of deranged physiological states, we endeavoured to purify renin from canine kidney (Dzau, Slater & Haber, 1979) in order to elicit specific antibodies for use as physiological probes. If the antigen were completely homogeneous, the objection raised previously to antibodies as renin inhibitors, namely their potential for reaction with other renal components, would be obviated. Further, if antibody Fab fragments were obtained, potential problems with immune complexes or complement activation would be avoided. The kinetics of distribution and excretion of antibody Fab fragments make them ideal tools for both short-
and long-term physiological experiments. Immunogenicity of these antibody derivatives, when administered intravenously, is far less than that of intact antibody preparations (Smith, Lloyd, Spicer & Haber, 1979).

**Purification of canine renin**

Canine kidneys were subjected to an eight-step purification procedure (Dzau et al., 1979). Renal cortex was minced, freeze-dried, pulverized and extracted with acetone and water. The extract was batch concentrated with DEAE-cellulose, acidified to pH 3-0 and subjected to sodium chloride and ammonium sulphate precipitation. Renin was then separated from other proteases by a linear concentration gradient with carboxymethyl-cellulose chromatography. Pepstatin affinity chromatography then resulted in a 300-fold purification. Finally, gel filtration on Sephadex G-100 yielded a single symmetrical peak of renin activity of 4200 Goldblatt units/mg of protein and a recovery of 17% with an overall purification of 600 000-fold. Polyacrylamide-gel electrophoresis demonstrated a single homogeneous band. When slices from a simultaneously run unstained gel were assayed, a discrete peak of renin activity was recovered in a position corresponding to the stained band (Fig. 2). Sodium dodecyl sulphate-gel electrophoresis also showed a single protein band.

**Production of renin-specific antibodies**

Anti-(dog renin) antibodies were raised in goats by initial intramuscular and intradermal injections of the purified enzyme (Dzau, Kopelman, Barger & Haber, 1980). High-titre antiserum was obtained. At a dilution of 1:20 000, 100 μl of antiserum resulted in 50% inhibition of the enzymatic activity of 0-002 Goldblatt unit of standardized dog renin.

**Utilization of renin-specific antibodies in physiological studies.** Dogs were trained to lie quietly on a padded table. One group of animals were anaesthetized and subjected to unilateral nephrectomy under sterile conditions. Polyvinyl catheters were implanted chronically into the aorta, renal artery and inferior vena cava and the end of each was exteriorized. An externally inflatable silastic constricting cuff was placed around the renal artery proximal to the renal artery catheter. In some animals, an electromagnetic flow probe was secured around the origin of the renal artery. In a second group of animals, nephrectomy and the placement of the constricting cuff were omitted. Experiments on these trained conscious dogs were started 2 weeks after surgery. Systemic and renal arterial pressures were monitored with P23 Statham pressure transducers and recorded on a Grass polygraph; renal blood flow was simultaneously

---

**Fig. 2.** Profile of renin activity and protein migration on polyacrylamide disc gel electrophoresis of dog purified renal renin. Gels were 5.5 cm in length and contained 10% polyacrylamide with 2% crosslinking. Electrophoresis was performed at pH 7-8, 4°C, with a constant current of 1 mA/gel for 2 h. Protein staining was performed with Coomassie blue. Protein profiles were obtained by scanning the absorbance of the stained gel at 600 nm with the Gilford spectrophotometer. Slices (1 mm) of a simultaneously run unstained gel were assayed for renin activity determined by the method of Haber et al. (1969). Renin activity corresponded precisely to the protein band. (From Dzau et al., 1979; by permission of the American Chemical Society.)
recorded with an electromagnetic flow meter. Blood samples were collected for determination of plasma renin activity (PRA) by radioimmunoassay (Haber, Koerner, Page, Kliman & Purnode, 1969) and expressed as angiotensin I generated (ng h\(^{-1}\) ml\(^{-1}\)). Purified renin and antisera were administered as single bolus injections through the inferior vena caval catheter. Renal vascular resistance was calculated by dividing mean renal arterial pressure by renal blood flow.

**Effect of renin-specific antiserum on exogenously administered canine renin.** One Goldblatt unit of purified dog renin given intravenously resulted in a 30 mmHg rise in mean aortic pressure (MAP) and an approximately 50% increase in renal vascular resistance. These effects were completely blocked by renin-specific antiserum. The antibodies had no effect on the pressor response to systemic administration of 2 

\[
\mu g
\]

of angiotensin I or angiotensin II (Fig. 3).

The basal PRA of three dogs maintained on a diet with 80 mmol of Na and 60 mmol of K/day was 0-4 ng h\(^{-1}\) ml\(^{-1}\). Antiserum had no significant effect on MAP in the salt-replete state. Similarly, the nonapeptide angiotensin I converting enzyme inhibitor (\(<\text{Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-}\)) resulted in only a transient fall in blood pressure of 5 mmHg in these animals.

**Renin-specific antiserum in sodium depletion.** Four dogs were placed on a diet with 10 mmol of Na and 60 mmol of K/day and given 80 mg of frusemide orally for 5 days. The urinary sodium excretion at steady state was 1 mmol/day and PRA increased to 3.8 \(\pm\) 0.4 ng h\(^{-1}\) ml\(^{-1}\). An intravenous bolus of converting enzyme inhibitor (5 mg) decreased MAP by an average of 12 mmHg in these dogs. Administration of pre-immune goat serum had no effect on PRA or MAP. Antiserum reduced PRA from 3.8 \(\pm\) 0.4 to 0.4 \(\pm\) 0.1 ng h\(^{-1}\) ml\(^{-1}\), paralleled by a prompt fall in MAP from 105 \(\pm\) 2 to 94 \(\pm\) 4 mmHg within 30 min (Fig. 4). The effectiveness of renin blockade was evidenced by the lack of a pressor response to exogenous renin.

**Renin-specific antiserum in acute renovascular hypertension.** Renovascular hypertension was induced in five dogs by inflation of the silastic cuff, which reduced renal artery pressure to 50 mmHg (Fig. 5). PRA rose from 3.4 \(\pm\) 1.8 to 18.3 \(\pm\) 6.3 ng h\(^{-1}\) ml\(^{-1}\) within 40 min. Simultaneously, MAP rose from a control level of 102 \(\pm\) 6 to a plateau of 134 \(\pm\) 7 mmHg and renal vascular resistance decreased by 11%. Administration of pre-immune serum had no significant effect on these parameters. Renin-specific antiserum given 1 h after constriction caused PRA to fall to control levels in 10 min and to 1.3 \(\pm\) 0.7 ng h\(^{-1}\) ml\(^{-1}\) in 40 min. The decrease in PRA was accompanied by a similar reduction in MAP to a nadir of 102 \(\pm\) 6 mmHg (Fig. 5) and a 40% further decrease in renal vascular resistance. The duration of action of the antibody was at least 21 h, as evidenced by continued suppression of PRA and MAP at or below control levels.

![Graph](image-url)
Renin inhibitors

FIG. 4. In salt-depleted dogs, pre-immune serum had no effect whereas renin-specific antiserum lowered plasma renin activity, causing blood pressure to fall. C, Control. (From Dzau et al., 1980; by permission of the American Association for the Advancement of Science.)

Despite maintenance of renal perfusion pressure at 50 mmHg. Beyond this time PRA and MAP slowly approached their postconstriction, pre-antiserum levels (C). (From Dzau et al., 1980; by permission of the American Association for the Advancement of Science.)

FIG. 5. After renal artery constriction, pre-immune serum had no significant effects whereas specific antiserum resulted in a prompt return to aortic pressure and plasma renin activity to control levels (C). (From Dzau et al., 1980; by permission of the American Association for the Advancement of Science.)

Studied with renin-specific antibody Fab fragment

Even though the renin-specific antibody used in these studies was shown to have a very high degree of selectivity, several criticisms may be
offered concerning the use of intact antibodies in physiological studies. First, intact antibodies aggregate with antigens to form multimolecular polymers. These immune complexes together with the complement which they bind are known to influence renal function adversely. Secondly, antigen–antibody aggregates in the course of fixing complement release a variety of mediators that may in themselves be vasoactive agents. Thirdly, the use of serum introduces a wide variety of substances that may modify vaso-reactivity. Fourthly, as has been demonstrated above, the effects of antibody are very long-lived and thus do not lend themselves readily to short-term experiments.

In order to overcome these difficulties, the immune globulin fraction was first purified by DEAE-cellulose chromatography in order to isolate immunoglobulin G (IgG). This fraction was then subjected to papain digestion to yield Fab fragments (Smith, Haber, Yeatman & Butler, 1976). Fab fragments overcome all of the disadvantages of antibody. First, they do not form immune complexes of significant size. Secondly, they do not fix complement when binding antigen. Thirdly, their persistence in the circulation is brief (Smith et al., 1979).

A recapitulation of experiments in the conscious dog in both sodium depletion and acute renovascular hypertension utilizing renin-specific Fab indicates qualitatively similar results to those obtained with antiserum. Fig. 7 shows a significant hypotensive response in a sodium-depleted dog. In comparing these results with those obtained with antiserum in Fig. 4 one should note that the response is more rapid in onset and shorter in duration.

Fig. 8 shows the results of renin-specific antibody Fab fragment infused into an animal subjected to acute renovascular hypertension, the same model as was described previously being used. Again, the onset of the hypotensive response is more rapid and its duration briefer. Thus it appears that renin-specific Fab is a potent blocker of renin activity. It should now be available for further exploration of the various roles that this regulatory enzyme may play in cardiovascular homoeostasis.

The general implications of earlier experiments with saralasin and teprotide are confirmed and the results can be accepted with considerably more confidence. It is quite clear that renin does not play a major role in maintaining blood pressure in the sodium-replete animal. Sodium deprivation, however, uncovers a very different response. Significant hypotension occurs as soon as the activity of renin is inhibited. Acute renovascular hypertension in the one-kidney canine model also appears to be entirely renin-dependent. There is a great deal of work still to be done in exploring other important hypertensive models. Significant questions remain concerning the genesis of chronic renovascular hypertension. What is the difference between the one- and the two-kidney model? Is the form of chronic renovascular hypertension characterized by volume expansion associated with low or normal plasma renin and aldosterone, renin-independent, or are the levels of renin inappropriate for the degree of extracellular volume expansion that exists? What is the role of renin in malignant hypertensive models? How important is renin in the normal maintenance of sodium balance? Is angiotensin really a significant sodium-retaining agent in the normal animal, either through its direct action on the kidney or via the aldosterone pathway? I believe that we now have a tool with which to address these questions directly in the experimental animal with the full confidence that renin alone is inhibited.

**Renin inhibitors based on substrate analogues**

Although the antibody Fab fragment appears to be an excellent tool for experimental investigations in animal models, it seems unlikely that the prolonged or repetitive use of antibodies in man will be possible. Consequently we sought an agent that had the potential of high selectivity as well as compatibility with human use. As indicated previously, renin is a remarkably fastidious protease with respect to substrate requirements. Could the unique sequence of amino acids around the cleavage site be used to
Renin inhibitors

construct a very specific enzyme inhibitor? Skeggs, Lentz, Kahn & Hochstrasser (1968) defined the minimal sequence from natural protein substrate that interacts strongly with renin. The octapeptide sequence extending from histidine-6 through tyrosine-13 (Fig. 1) has kinetic parameters essentially the same as those of the full tetradecapeptide renin substrate (Skeggs et al., 1968).

Kokubu, Hwada, Ito, Ueda, Yamamura, Mizoguchi & Shigezane (1973) synthesized a number of analogues of the tetrapeptide found between residues 10 and 13 (Fig. 1) in the hope of creating an effective inhibitor. While inhibition could be shown, inhibitory constants were only in the millimolar range.

To produce more effective inhibitors, Poulsen, Burton & Haber (1973) and Burton, Poulsen & Haber (1975) synthesized analogues of a larger segment of renin substrate. Peptides were tested as renin inhibitors by using a radioimmunoassay (Poulsen & Jorgensen, 1974) to measure decreases in the generation of angiotensin I from either natural protein substrate or the tetradecapeptide. Addition of the octapeptide analogues to the standard assay mixture decreased the rate of formation of angiotensin I. Data from these tests fit the standard Michaelis-Menten equation with a high degree of precision (Poulsen et al., 1973). All of the synthetic peptides tested were competitive inhibitors. Fig. 9 shows the typical pattern of competitive inhibition found when an octapeptide analogue is used to inhibit formation of angiotensin I by renin.

The native octapeptide sequence is both a competitive inhibitor and a substrate for renin. Edman degradation of the reaction product shows that the enzyme quantitatively cleaves the leucyl-leucine bond in the octapeptide (Poulsen et al., 1973). The first modifications made in the octapeptide sequence were aimed at producing peptides that would bind but not be cleaved by renin. Replacement of either leucyl residue (10 and 11 in Fig. 1) with the D-enantiomorph yields inhibitors that are not cleaved by renin. Replacement of either leucyl residue (10 and 11 in Fig. 1) with the D-enantiomorph yields inhibitors that are not cleaved by renin. In addition, the D-Leu⁶ octapeptide binds renin one order of magnitude (3 μm) more tightly than the parent octapeptide (39 μm).

Since both a high concentration of the inhibitor and tight binding to renin are required to compete with natural substrate, the effectiveness of an inhibitor is best judged by the ratio between solubility and \( K_f \). Addition of a single prolyl residue to [Phe⁶]octapeptide doubled solubility and decreased \( K_f \) so that this ratio increased from 6 to 100 (Haber & Burton, 1979). A further improvement in the ratio was obtained by attaching a lysyl residue to the C-terminus. Solubility of the Pro-[Phe⁶,Phe⁶]octapeptide was increased eightfold with only a doubling of \( K_f \). The solubility/\( K_f \) ratio was 420. The pattern of solubility as a function of pH is also changed. The choice of the lysyl residue was dictated by the desire to increase solubility by adding a charged group without altering the conformation of the inhibitor.

\( K_f \) values of the various inhibitors can be related to the lipophilicity of amino acid residues at the cleavage site (Table 1). Replacement of the leucyl residues with phenylalanine yields an analogue which binds about 40 times as well as Pro-octapeptide (\( K_f \), 1 vs 39 μm) (Burton, Poulsen & Haber, 1978). Pro-[Phe⁶, Ph⁶]octapeptide is inactive in vivo. This peptide was cleared from circulation with a half-life considerably less than 1 min. The lysyl analogue has a half-life of 3.8 min and is lost from circulation at an exponential rate.

![Fig. 9. Competitive inhibition of human renin in plasma by (Pro)₂[Phe₆]octapeptide: Lineweaver-Burk plots of the reaction between human renin and the peptide at two concentrations as indicated. Incubation time was 2 h (37°C, pH 7.5). (From Burton et al., 1975; by permission of the American Chemical Society.)](image-url)
TABLE 1. Lipophilicity relationships of the renin inhibitors
From Burton et al., 1978; by permission of Academic Press Inc.

<table>
<thead>
<tr>
<th>Inhibitor constant, $K_r (\mu m)$</th>
<th>Observed</th>
<th>Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pro-His-Pro-Phe His-Leu Leu-Val-Tyr</td>
<td>42</td>
<td>14.7</td>
</tr>
<tr>
<td>2 Tyr</td>
<td>12</td>
<td>12.3</td>
</tr>
<tr>
<td>3 Phe</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>4 Phe-Phe</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>5 Phe(4Cl)</td>
<td>1.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Inhibition of renin in vivo by the peptide Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys was examined in the monkey (Macacus fascicularis) (Burton, Cody, Herd & Haber, 1980). When infused into normotensive sodium-replete monkeys, no significant change in blood pressure was observed. Purified human renin (Slater et al., 1978), angiotensin I and angiotensin II were then injected intravenously at concentrations sufficient to cause pressure rises of 22, 30 and 30 mmHg respectively. Infusion of the renin inhibitor at a rate of 0.2 mg min$^{-1}$ kg$^{-1}$ blocked the pressor response to human renin ($P < 0.004$), but not that of angiotensin I or II (Fig. 10). These observations indicate that the Pro-[Phe$^5$, Phe$^6$]-octapeptidyl-lysine is neither a hypotensive agent in its own right nor does it act as a converting enzyme inhibitor at the doses used. It appears to block renin specifically.

\[ \text{MAP (mmHg)} \]
\[ \text{Control} \]
\[ \text{RIP (0.2 mg min}^{-1}\text{ kg}^{-1}) \]

\[ \text{Mean arterial pressure (mmHg)} \]
\[ \text{Time (min)} \]

\[ \text{Heart rate (min}^{-1}) \]

\[ \text{SEM} \]

\[ \text{RIP (2 mg/kg)} \]
\[ \text{CEI (1 mg/kg)} \]

\[ \text{Na}^+\text{-depleted, normotensive animals} \]

A total of five studies were performed in sodium-depleted normotensive monkeys. Urinary sodium was $0.62 \pm 0.46$ mmol/day and PRA was $13.6 \pm 5.1$ ng h$^{-1}$ ml$^{-1}$. Renin inhibitory peptide, given as a 2 mg/kg intravenous bolus, resulted in a prompt reduction in MAP from 105 ± 4 to 79 ± 3 mmHg ($P < 0.004$) (Fig. 11). MAP gradually increased over the ensuing 15 min to a new baseline of 100 ± 4 mmHg. The
subsequent injection of 1 mg of converting enzyme inhibitor/kg resulted in reduction of MAP to 82 ± 5 mmHg (P < 0.006). There is no significant difference between the hypotensive response to renin inhibitory peptide and that of converting enzyme inhibitor. In this group of monkeys, an important finding was the significant increase of heart rate from 180 ± 10 to 220 ± 7 beats/min (P < 0.003) occurring during the hypotensive response to renin inhibitory peptide. After return of MAP to baseline, the heart rate remained at an elevated steady-state value of 196 ± 12 beats/min (P < 0.03, compared with original heart rate of 180 ± 10 beats/min). There was no further significant change in heart rate during the hypotensive response to converting enzyme inhibitor.

**Na+-depleted, hypertensive animals**

Renin inhibitory peptide was administered to sodium-depleted, renin-dependent, hypertensive monkeys. In this model, the right kidney was removed and an inflatable cuff placed around the aorta immediately above the left renal artery. Bolus intravenous injection of peptide produced dose-dependent MAP reductions ranging from 15 mmHg (0.5–1.0 mg/kg) to 70 mmHg (3.0 mg/kg). The latter response was associated with a 40 beats/min increase in heart rate. To lower MAP gradually, renin inhibitory peptide was given as a graded infusion in 0.2 mg min⁻¹ kg⁻¹ increments. Six studies were performed (Fig. 12). The baseline MAP was 107 ± 3 mmHg, increasing to 131 ± 3 mmHg (P < 0.002) after 1 h of aortic cuff inflation. PRA increased from 12.5 ± 1.7 to 33.2 ± 3.5 mg h⁻¹ ml⁻¹ (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 min⁻¹ kg⁻¹ the reduction of MAP to 121 ± 2 mmHg was significant (P < 0.005). Continued infusion at the rate of 0.6 mg min⁻¹ kg⁻¹ resulted in reduction of MAP to 107 ± 4 mmHg (P < 0.004 compared with 131 ± 3 mmHg, P < 0.008 compared with 212 ± 2 mmHg). When this prehypertensive level of MAP was achieved, the infusion was discontinued. MAP increased to 125 ± 3 mmHg within 5 min of discontinuation, which was a hypertensive level not significantly different from 131 ± 3 mmHg. After equilibration at 127 ± 3 mmHg, intravenous administration of 1 mg of converting enzyme inhibitor/kg resulted in reduction of MAP to 111 ± 4 mmHg (P < 0.002). The nadir hypotensive responses to renin inhibitory peptide and converting enzyme inhibitor were comparable (P = N.S.). Throughout this experiment there was no appreciable change in heart rate.

These experiments closely duplicate the observations previously detailed with renin-specific antibody. The substrate inhibitor peptide acts specifically on renin and does not inhibit the pressor action of either angiotensin I or II. It appears to be well tolerated without any haemodynamic consequences in the normotensive sodium-replete animal. The role of renin in maintaining blood pressure in the sodium-depleted state as well as its importance in acute renovascular hypertension is again clearly demonstrated. The potential of the peptide lies particularly in its selectivity for human and subhuman primate renin. The substrate analogue peptides we have studied have very little inhibitory activity with respect to animal renins.
Apart from the evident potential for exploring the role of renin in normal human cardiovascular homeostasis, and in certain forms of renovascular and renal hypertension, the opportunity exists for defining the role of this enzyme in the genesis and maintenance of essential hypertension in man. There are highly provocative reports demonstrating a hypotensive effect of the converting enzyme inhibitor, captopril, in essential hypertension (Gavras, Brunner, Turini, Kershaw, Tiff, Cuttelod, Gavras, Vukovich & McKinstry, 1978). Are these effects related to renin inhibition or the actions of the converting enzyme inhibitor on kinins, or yet another system? Utilization of a specific renin inhibitor will serve to clarify this issue.

**Biosynthesis of renin**

Many enzymes are synthesized first in the form of an inactive zymogen which is then subject to proteolytic cleavage before eventual secretion in active form. Does renin have a zymogen? Is the inactive renin that is observed in plasma a precursor of renin? Does the transformation of inactive to active renin represent a mode of regulating renin activity?

Renal and plasma renin have a molecular weight of approximately 40,000. However, many investigators have identified less active higher-molecular-weight forms of renin in the kidneys of several species as well as in plasma. Do these higher-molecular-weight forms represent the biosynthetic precursors of renin, or do they represent postsynthetic modification?

To answer this question, one must first define the major storage form of renin in the granules of the juxtaglomerular cells. If renin is stored in precursor form and also excreted as such, one could entertain the concept that transformation of prorenin into renin is a possible regulatory mechanism. If on the other hand the principal storage form of renin is the active, molecular weight 40,000, molecule, circulating inactive renins are likely to be postsynthetic derivatives and consequently of lesser physiological importance. Poulsen, Vuust, Lykkegaard, Høj Nielsen & Lund (1979) isolated messenger RNA from mouse submaxillary gland. This message was translated in a cell-free system either from reticulocytes or from wheat germ. A single protein reacting with renin-specific antibody was isolated and shown to have a molecular weight of 50,000. Subsequently similar experiments were completed with mouse kidney RNA (K. Poulsen, personal communication). Thus it appears that renin is first synthesized as a higher-molecular-weight form, probably a pre-pro form of the molecule. With this result firmly established, one simply needs to determine the relative distribution between the precursor and the active form of the enzyme existing in the kidney.

Carlson and collaborators in our laboratory (Carlson, Quay, Dzau, Kreisberg, Slater & Haber, 1980) addressed this question by examining the biosynthesis of renin in isolated fresh canine glomeruli. These glomeruli have renin-producing juxtaglomerular cells still attached, and are able to incorporate labelled amino acids into proteins for periods up to 24 h. After incubation for various periods with [³⁵S]-methionine, glomeruli were homogenized and the supernatant of the homogenate was precipitated with renin-specific antibody. The proteins thus isolated were separated by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. As seen in the densitometric tracings of the gel radioautographs in Fig. 13, the major immunoreactive species appear at molecular weights 55,000 and 38,000. After 2 h of incubation the principal immunoreactive species is of higher molecular weight.
molecular weight, but, as time proceeds, the lower-molecular-weight species becomes dominant. These observations indicate that prorenin exists within the renin-producing cell for a finite period of time. They do not, however, indicate what fraction of stored renin is of higher molecular weight nor whether or not the higher molecular-weight species is secreted. Further applications of this method of studying renin biosynthesis clearly have the potential for answering these questions and thereby determining whether or not the inactive renins found in plasma bear a relationship to the biosynthetically demonstrable prorenin.

**Vascular wall renin**

There has been considerable interest surrounding the possibility that extrarenal sources of renin exist and perhaps are important in physiological regulation (Skeggs, Lentz, Kahn, Doer & Levine, 1969; Reinharz & Roth, 1971; Day & Reid, 1976; Hackenthal, Hackenthal & Hilgenfeldt, 1978). The presence of renin has been inferred by demonstrating that tissue homogenates can

---

**FIG. 14.** Polyacrylamide-gel electrophoresis of immunoprecipitates prepared from $^{35}$S-methionine-labelled canine smooth muscle cells. Cultured canine arterial smooth muscle cells (approximately $10^6$ cells) were washed with phosphate-buffered saline and then exposed to methionine-deficient media to which 1 mCi of $^{35}$S-methionine or of $^3$H-labelled amino acids was added. The cells were then incubated for 4 h. At the end of that time an excess of complete medium was added and the incubation continued for an additional 20 h. The cells were then harvested, frozen, thawed on ice, sonicated and divided into aliquots. After incubation with normal rabbit serum or rabbit renin-specific antiserum, the antigen–antibody complexes formed were precipitated by *Staphylococcus aureus* protein A. These immunoprecipitates were then subjected to polyacrylamide-gel electrophoresis and subsequently to fluorography. The experimental conditions were: (A), (C), normal rabbit serum; (B), (D), renin-specific antiserum; (E), renin-specific antiserum adsorbed with pure renin; (F), molecular-weight standards, Pharmacia (Coomassie blue); (G), authentic renal renin (Coomassie blue). Arrows, renin; O, origin; DF, dye front.
generate angiotensin I from plasma angiotensinogen. Interpretation of these results, however, is clouded by the appreciation that many proteolytic enzymes can also cleave renin substrate to release angiotensin I. There is also the possibility that the renin activity demonstrated in these tissues has in some way been concentrated from plasma renin. It should be possible to overcome both these problems by utilizing a more specific marker for identifying renin than its enzymatic activity, and to isolate growing cells from plasma by cell culture.

R. Re, J. Fallon, V. J. Dzau, S. Quay & E. Haber (unpublished abstract, Renin synthesis by arterial smooth muscle cells, presented at the Council for High Blood Pressure Research, Cleveland 1979) cultured arterial smooth muscle cells derived from explants of canine aortic media. Homogenates of these cultured cells produced angiotensin I when incubated with renin substrate at neutral pH. Cytological reactivity of the cell cultures was examined by utilizing the specific antiserum to pure canine renin (Dzau et al., 1979). With cells that had been deprived of calf serum for 3 days, renin-specific antiserum produced granular cytoplasmic staining, whereas the pre-immune serum produced only slight nuclear staining and weak cytoplasmic fluorescence. Adsorption of renin-specific antiserum with foetal calf serum did not diminish granular cytoplasmic staining, which excludes the possibility that renin-specific antiserum was binding to bovine renin from the foetal calf serum. Adsorption of the antiserum with purified canine renin, however, markedly diminished the granular cytoplasmic immunofluorescence. As control cells, cultured canine dermal fibroblasts did not produce granular cytoplasmic immunofluorescence when studied with renin-specific antiserum. Thus the presence of intracellular renin immunoreactivity does not appear to be a general property of all cells.

In order to examine whether or not the renin-like material is biosynthesized by smooth muscle cells, the cells were incubated in the presence of $^{[15}S]$methionine or $^3$H-labelled amino acid. The cells were homogenized and immunoreactive proteins precipitated with renin-specific antibody. The radiolabelled proteins were then examined by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. The results are shown in Fig. 14. It is apparent that renin-specific antiserum can precipitate a protein with an apparent molecular weight of 41 000 and that purified renin can block the immunoprecipitation of this band.

Thus it appears that vascular smooth muscle cells in culture synthesize a renin-like enzyme whose identity may be inferred by its enzymatic activity and its recognition by renin-specific antibody. Whether or not the protein is identical in structure with renal renin or is a similar but structurally related enzyme remains to be determined.

Conclusion

I have attempted to demonstrate the singular value of specific reagents in solving several problems related to the importance of the renin-angiotensin system both in physiological regulation and relative to its mode of biosynthesis and distribution in various tissues. Renin-specific antibody, particularly antibody Fab, appears to be an extremely potent physiological tool for demonstrating the role of renin both in normal vascular homeostasis and in the genesis of experimental forms of hypertension. A decapetide analogue of renin substrate is also a specific inhibitor in vivo, particularly suited to experiments in subhuman primates and possibly in man. This blocker may permit the dissection of the role of renin in human physiology and in hypertension. The antibody has also proved to be of exceptional value in biosynthetic studies. A biosynthetic precursor form of renin has been identified, in organ cultures of renal glomerli, that appears to be degraded intracellularly to a lower-molecular-weight form. It is of particular interest that the higher-molecular-weight form persists within the cell for a number of hours and is not simply a highly transient intermediate. The antibody has also been useful in demonstrating the biosynthesis of a renin-like molecule in vascular smooth muscle.

Acknowledgments

The following investigators participated in the work summarized in this lecture: A. Clifford Barger, James Burton, William Carlson, Robert Cody, Victor Dzau, John Fallon, Allan Herd, Richard Kopelman, Knud Poulsen, Steven Quay, Richard Re and Eve Slater. The work was supported by NIH grant HL-19517 and a grant from the Reynolds Industries.

References


