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Review

Renin Inhibitors

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Pharmacological intervention in the renin–angiotensin system (RAS) by inhibition of angiotensin-converting enzyme (ACE) is an effective therapy for the majority of hypertensive patients and a major advance in the treatment of hypertension and congestive heart failure. The success achieved with ACE inhibitors has increased interest in inhibitors of renin. Renin catalyzes the first and rate-limiting step of the RAS and, unlike ACE, has a high specificity for its endogenous protein substrate. A therapeutic agent that inhibits this specific reaction could have advantages over antihypertensive drugs with less specific modes of action. Although inhibitors of renin have been studied for over two decades, only recently has substantial progress been made toward potent, low molecular weight inhibitors likely to become useful therapeutic agents. Recent advances in the development of renin inhibitors, especially progress toward clinically useful inhibitors, is reviewed.

KEY WORDS: renin inhibitors; renin–angiotensin system; antihypertensives; peptide drugs; enzyme inhibitors.

INTRODUCTION

The renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure and in the maintenance of sodium and volume homeostasis. The formation of the potent vasoconstrictor angiotensin II, the end product of the RAS, is outlined in Fig. 1. Possible modes of intervention in this process include inhibition of renin release, renin inhibition, angiotensin-converting enzyme (ACE) inhibition, and angiotensin II receptor antagonism. Peptide analogues of angiotensin II inhibit the effects of this hormone by competitive blockade at its receptors, but their experimental and clinical applications have been limited by partial agonist activity and limited oral absorption (1,2). Inhibition of ACE is an effective therapy for the majority of hypertensive patients and is a major advance in the treatment of hypertension and congestive heart failure (3–7). Nevertheless, ACE is not specific for angiotensin I and cleaves kinins and other endogenous peptides. Although the inhibition of the formation of angiotensin II appears to be the most important action of ACE inhibitors, the potentiation of kinins in plasma or in tissues may also contribute to their antihypertensive effects.

The therapeutic effectiveness of ACE inhibitors in both normal and high-renin hypertensive patients has increased interest in inhibitors of renin. The cleavage of angiotensinogen by renin to form angiotensin I is the first and rate-limiting step in angiotensin II generation (Fig. 1). Since angiotensinogen is the only known naturally occurring substrate for renin, inhibition of this specific reaction may have advantages over ACE inhibition (8,9). Renin inhibitors could produce fewer side effects than other antihypertensive drugs with a less specific mode of action. Other possible advantages of renin inhibitors have been summarized by Boger (9). The development of renin inhibitors has been reviewed (9–13). The present review focuses on recent advances in the area, especially progress toward clinically useful renin inhibitors.

Renin–Angiotensin Pathway and Physiology

Angiotensinogen, which is the initial substrate for the RAS, is an α-2-globulin synthesized in the liver and found in the general circulation. Renin, synthesized and released by the kidneys in response to decreased renal perfusion, cleaves the amino terminal decapeptide from angiotensinogen to yield angiotensin I (Fig. 1). ACE, both in the plasma and on the outer membrane of endothelial cells, catalyzes the removal of the carboxy-terminal dipeptide to form angiotensin II. This short-lived octapeptide, which is cleaved to inactive fragments, acts via its receptors to constrict the vasculature, to stimulate the release of aldosterone from the adrenals, and, in a direct negative feedback loop, to inhibit the further release of renin from the kidney (14–17). Concentrations of renin in the blood are variable, depending on the physiological state of the organism. As expected for an enzyme important in controlling a homeostatic process, renin is rapidly turned over, with the $t_{1/2}$ for clearance of exogenously administered renin estimated at 13 min in rats (18). Although the renin–angiotensin system may exist in other tissues (19–21), its precise role in the arterial wall or in the central nervous system, for example, remains controversial.

Properties of Renin (EC 3.4.99.19)

Renin is an aspartic proteinase but differs from the other members of this class (e.g., pepsin, cathepsin D, gastricin, and the fungal proteinases penicillopepsin, endothio-

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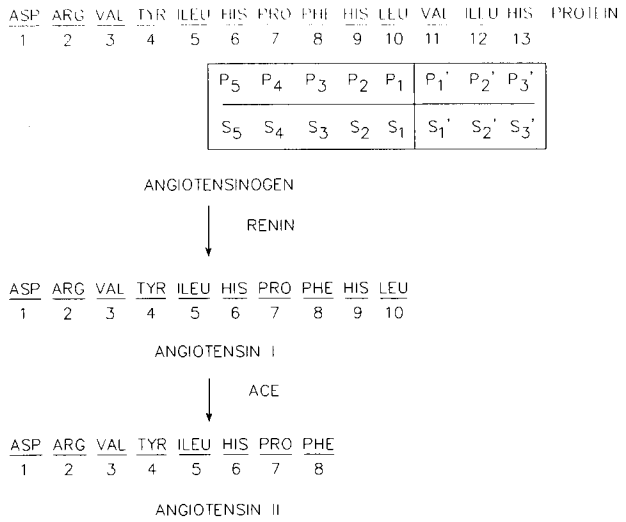


Fig. 1. Renin–angiotensin system: Formation of angiotensin II by the action of renin and angiotensin-converting enzyme. Substrate side-chain positions (P_5 through P_3') and renin binding sites (S_5 through S_3') on amino- and carboxy-terminal sides of scissile bond are illustrated in the notation of Schechter and Berger (60).

pepsin, and *Rhizopus* pepsin) in two important respects. While the other aspartic proteinases show maximum activity at a low pH, renin has its pH optimum closer to physiological pH (17). Although other members of the class have little specificity, renin has a remarkably narrow substrate specificity, limited to a single bond in angiotensinogen. The human substrate scissile bond is Leu–Val, while it is Leu–Leu for other mammals (22,23). Even this minimal substrate difference is significant, in that while the human enzyme will cleave human and other mammalian substrates, only human renin will cleave human substrate (14).

Structure and Mechanism of Renin

The aspartic proteinases share the functional presence of carboxyl residues in the active site, many common structural features, and a striking homology in sequence (24–27). Although the three-dimensional structure of renin has not been determined, those of related aspartic proteinases show the active-site aspartic acids centered in a long cleft, capable of binding at least eight amino acids. The aspartic acids are hydrogen bonded to each other and (for penicillopepsin and *Rhizopus* pepsin) to an electron density believed to be a water molecule or an ammonium ion (28,29). The ionization state of the catalytically active aspartic acids of renin at physiological pH is not known, but one may remain protonated. Renin, like the other aspartic proteinases, has a flap region which can open and close upon substrates or inhibitors. Although the sequence homology of renin to other aspartic proteinases suggest similarities in mechanism, the unique specificity and pH optimum of renin remain to be explained.

The general features of the mechanism for cleavage of amide bonds by renin and other aspartic proteinases are not yet established. A stereochemical proposal for the catalytic mechanism of penicillopepsin, based on an X-ray structure of an enzyme–inhibitor complex, has been reported (28). Other mechanistic studies of aspartic proteinases have been reviewed by Rich (30). The results to date are most consistent with a general acid–base mechanism, in which water attacks the carbonyl of the scissile peptide bond, with the active-site carboxylates mediating the required proton transfers.

Purification and *in Vitro* Assays

Although multiple forms of enzyme and the glycoprotein nature of renin have complicated its purification, homogeneous enzyme has been obtained from a variety of sources including human kidney (31–35), dog kidney (36), hog kidney (37), rat kidney (38), and mouse submaxillary gland (39–41). Affinity columns prepared using pepstatin and other renin inhibitors have been used. Recombinant human renin has recently been expressed in mammalian cells and pure enzyme isolated in quantity (42).

A variety of *in vitro* assays is used to determine activities of renin inhibitors. Among these are human kidney renin (HKR) assays which use partially or extensively purified enzyme and human angiotensinogen; other mammalian angiotensinogens and synthetic peptides have been used as substrates. In human plasma renin assays, angiotensin II, cleaved from endogenous substrate by endogenous enzyme, is measured by radioimmunoassay. A variety of assay conditions (pH, concentration of substrate, presence of other proteinase inhibitors, detection method) has been reported for *in vitro* renin assays. As a result, comparisons between literature IC_{50} values are difficult; the literature references cited in this review should be consulted when small differences in IC_{50} values are interpreted.

IN VITRO INHIBITION OF RENIN

Knowledge of substrate sequence, mechanistic hypotheses based on structural information drawn from several aspartic proteinases, and the discovery of pepstatin, a naturally occurring inhibitor of this class of proteinases, have all contributed to the development of renin inhibitors. In the last several years, work on renin inhibitor design has yielded several classes of potent inhibitors, the best of which are active at nanomolar concentrations. Steady progress has been made toward lower molecular weight (MW) inhibitors which are more likely to become useful therapeutic agents. Inhibition of renin has been achieved with renin antibodies, substrate analogues, and several classes of transition-state analogues.

Renin Antibodies

Inhibitory renin antibodies provided the first evidence that interference in the action of the renin–angiotensin system had therapeutic potential (43). Although these first renin antibodies were impure, recently polyclonal, monoclonal, and F_{AB} antibodies prepared using purified renins have shown potent (K_i = 0.01–1.0 n*M*) renin inhibition (44–48) and hypotensive effects when administered iv to salt-depleted monkeys (see Intravenous Administration of Renin Inhibitors, below).

Substrate Analogues and Other Peptide Inhibitors

The success achieved in development of substrate analogues as renin inhibitors has provided a basis for recent

Table I. Inhibitors of Human Renin[a]

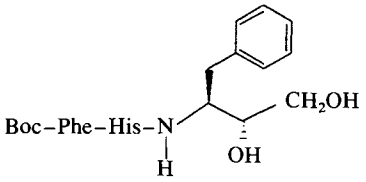
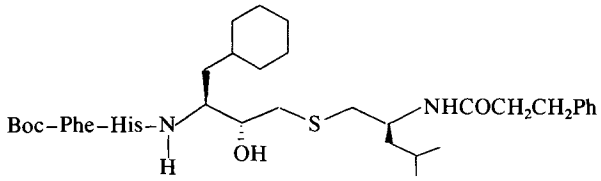
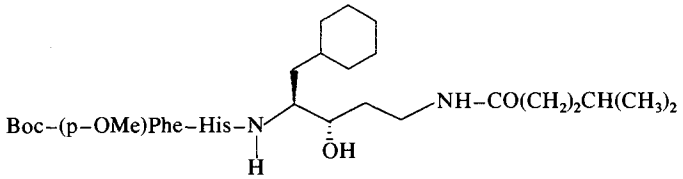
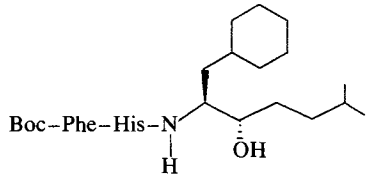
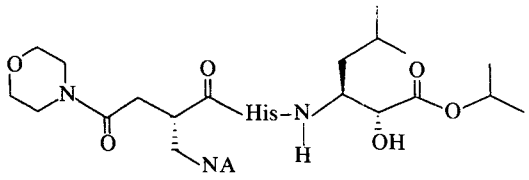
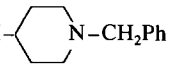
Inhibitor	Structure (6 P_5 7 P_4 8 P_3 9 P_2 10 P_1 11 P_1' 12 P_2' 13 P_3')	IC_{50} (n*M*)
Minimum substrate	His-Pro-Phe-His-Leu—Leu-Val-Tyr	
Human sequence (1)	His-Pro-Phe-His-Leu-Val-Ile-His	(313,000)
RIP (2)	Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys	(2,000)
3	(N-Et)Phe-D-Met-Gly-Phe-NH-(2-Ad)	90,000
H-142 (4)	Pro-His-Pro-Phe-His-Leu(R)-Val-Ile-His-Lys	10
5	Boc-Phe-His-Cal(R)-Val-NH-2(S)-methylbutyl	(8.6)
H-261 (6)	Boc-His-Pro-Phe-His-Leu(OH)-Val-Ile-His	0.70
U-71,038 (7)	Boc-Pro-Phe-(N-Me)His-Leu(OH)-Val-Ile-NH-CH_2(2-pyr)	0.39
8	*t*-$BuCOCH_2CH(CH_2Ph)CO$-His-Cal(OH)-Val-NH-CH_3	(2)
Pepstatin A (9)	Iva-Val-Val-Sta——Ala-Sta	22,000
SR 41,128 (10)	Iva-Phe-Nle-Sta——Ala-Sta	28
SCRIP (11)	Iva-His-Pro-Phe-His-Sta——Leu-Phe-NH_2	16
CGP-29,287 (12)	Z-Arg-Arg-Pro-Phe-His-Sta——Ile-His-Lys(Boc)-OCH_3	1.0
13	His-Pro-Phe-His-Asta-Val-Ile-Phe	(60)
ES-305 (14)	BNMA-His-Sta-NH-2(S)-methylbutyl	9.2
ACRIP (15)	Iva-His-Pro-Phe-His-ACHPA-Leu-Phe-NH_2	0.17
16	Boc-Phe-His-ACHPA-NH-2(S)-methylbutyl	(4)
RRM-188 (17)	Z-Nal-His-Leu-al	(80)
18		2,600
19	Boc-His-Pro-Phe-His-Leu(K)-Val-Ile-His	6
20	Boc-Phe-His-Sto——Ile-$NHCH_2$(2-pyr)	34
21	Boc-Phe-His-(di-F)Sto——Ile-$NHCH_2$(2-pyr)	1.4
22		(7)
23		(4)
24		(10)
KRI-1230 (25)		7.8

Table I. Continued

Inhibitor	Structure: 6 P_5	7 P_4	8 P_3	9 P_2	10 P_1	11 P_1'	12 P_2'	13 P_3'	IC_{50} (n*M*)
26	Boc–Phe–His–Leu–Gly–Ile–His–OCH_3								(31)
27	Iva–His–D–Pro–Phe–His–Sta–AA–Leu–$NHCH_2Ph$								150
28	Boc–Phe–His–Sta–AA–Leu–$NH(CH_2)_2NHC(=NH)NH_2$								64
29	Boc–Phe–His–Sta–AA–Leu–NH– 								25

[a] References may be found in the text. IC_{50} values in parentheses are for pure human kidney renin assays; others are for plasma renin assays. An R over a bond indicates a "reduced" peptide isostere; an OH, an "hydroxy" isostere; a K, a "ketone" isostere; an AA, an "amino alcohol" isostere (see Fig. 2). Ad, adamantyl; Asta, aminostatine; BNMA, bis-naphthylmethylacetyl; Boc, t-butoxycarbonyl; Cal, 3-cyclohexylalanine; Et, ethyl; Iva, isovaleroyl; Leu-al, 2(S)-amino-4-methylpentanaldehyde; Me, methyl; NA, l-naphthyl; Nal, 3-(1-naphthyl)alanine; Ph, phenyl; pyr, pyridine; Sta, statine; Sto, statone; Z, carbobenzoxy.

work on potent transition-state inhibitors. Early substrate analogues, based on residues 10–13 of angiotensinogen, were renin-stable inhibitors with K_i values in the millimolar range (49). Burton and colleagues began with the minimum substrate sequence (Table I) as determined by Skeggs *et al.* (50) and synthesized a series of peptide inhibitors including a decapeptide (2), which they named RIP (renin inhibitory peptide). The N-terminal proline and C-terminal lysine of 2 contribute to water solubility, and the substitution of Phe–Phe for Leu–Leu prevents cleavage by renin (51,52). More recently, this approach has led to a shorter sequence, Phe–Phe(4-I)–Val–Tyr–Lys (RI-103), reported to be as potent as RIP (53). Several peptides such as 3, which have structures related to those of stabilized enkephalin analogues, are competitive inhibitors of human renin with a modest potency (54). Peptide 3 has been suggested to bind in such a way that the C-terminal amide bond replaces the scissile bond of substrate. Peptides derived from the prosegment of human prorenin are competitive inhibitors of human renin, the best being Boc–Leu–Lys–Arg–Met–Pro–OCH_3 with an IC_{50} of 16.6 μ*M* (55).

Transition-State Inhibitors of Renin

The design of enzyme inhibitors which resemble the transition state for the enzyme-catalyzed reaction has been described by Wolfenden (56). The transition state is the molecular arrangement of the highest free energy along the reaction pathway. It is believed that the acceleration of reactions brought about by enzymes is a result of tight binding and stabilization of the transition-state species by the enzyme, and thus it is expected that inhibitors whose designs approximate the transition state will bind more tightly than substrates or products.

"Reduced Peptide" Isostere

Using the transition-state inhibitor approach, Szelke and co-workers designed potent inhibitors of renin in which the scissile bond of substrate analogues is replaced by a "reduced peptide" isostere ($-CH_2NH_2-$) as illustrated in Fig. 2 (57). This functionality is intended to mimic the tetrahedral intermediate for the peptide bond hydrolysis, a structure which is presumed to approximate that of the transition state. Although the "reduced peptide" isostere shares the tetrahedral geometry of the tetrahedral intermediate (Fig. 2), it lacks both hydroxyl groups. The high potency for inhibitors containing this isostere may be due to an electrostatic interaction between the basic amine and the active-site aspartic acids. The "reduced peptide" analogue (4) of the human angiotensinogen sequence is 30,000 times more potent than the human octapeptide substrate analogue (1) from which it was derived (58). The high potency of inhibitor 5 (59) demonstrates the effectiveness of the reduced peptide design in low MW inhibitors.

"Hydroxy" Isostere

A second transition-state design introduced by the Szelke group is exemplified by the potent inhibitor 6, which incorporates a "hydroxy" isostere ($-CHOHCH_2-$) of the Leu–Val peptide bond as illustrated in Fig. 2 (57). The hydroxy group may serve as a mimic for the hydrated carbonyl of the tetrahedral intermediate for hydrolysis, while the Leu and Val side chains are correctly positioned to make binding interactions in the S_1 and S_1' subsites of renin (notation of Schechter and Berger, Ref. 60). The hydroxy isostere gives a high potency in smaller inhibitors such as pentapeptide 7 (61) and "dipeptide" 8 (62).

Inhibitors Containing Statine and Its Analogues

The naturally occurring aspartic proteinase inhibitor pepstatin A (9) (63) is an exceedingly potent (K_i = 0.05 n*M*) inhibitor of pepsin (64) but a much weaker inhibitor (Table I) of human renin (65). The central statine [4(S)-amino-3(S)-hydroxy-6-methylheptanoic acid] element of pepstatin A (Fig. 2) has been proposed to serve as a mimic of the transition state for hydrolysis (66) or, alternatively, as a "collected substrate" analogue, where the substrates are peptide and water (67). The importance of the 3S hydroxyl group of statine in statine-derived inhibitors is demonstrated by the 1000-fold drop in inhibitory activity upon changing to the 3R configuration (65). Rich has proposed that an enzyme-bound water molecule is displaced by the hydroxy group of statine upon binding of inhibitor (67). The resulting gain in entropy may contribute to the overall binding energy.

Derivatives of pepstatin with increased solubility have been prepared as inhibitors of human renin, the best being pepstatin–Glu, with a K_i of 5.8 μ*M* (68). More recently, the

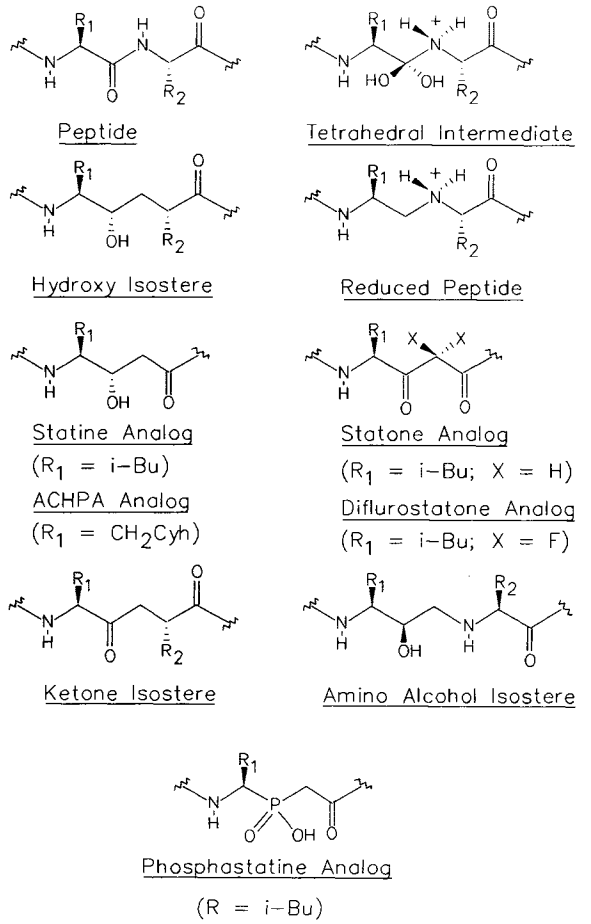


Fig. 2. Stereochemical relationship of substrate and tetrahedral intermediate for hydrolysis (proposed to resemble the transition state for amide bond cleavage) to peptide bond isosteres incorporated into renin inhibitors as transition-state mimics (R^1 and R^2 are alkyl groups corresponding to the sidechains of Leu and Val in the substrate).

Sta–Ala–Sta sequence has been incorporated into highly potent pentapeptides such as **10** (69).

Since renin is a highly specific proteinase, Boger and co-workers incorporated statine into the angiotensinogen sequence hoping to find more potent renin inhibitors (65). Powers *et al.*, noting similarities between the structure of pepstatin and that of substrates of aspartic proteinases, suggested that statine replaces both amino acids around the cleavage site (70), a conclusion also reached by Boger *et al.* (65) using molecular modeling methods (see Molecular Modeling in Renin Inhibitor Design, below). Peptides such as **11** (statine-containing, renin-inhibitory peptide; SCRIP), synthesized on the basis of this hypothesis, are potent inhibitors of human renin and are selective for renin versus other aspartic proteinases (65). Incorporation of statine modeled as a single amino acid replacement gave less potent inhibitors. Inhibitors such as **12** have demonstrated that IC_{50}'s of 1 n*M* can be obtained with statine-containing renin inhibitors (71).

Inhibitors such as **13**, containing an analogue of statine ("aminostatine") in which the statine hydroxyl is replaced by an amino group, have been reported (72, 73). One might expect the amino group of these inhibitors to make a favorable electrostatic interaction with the active-site aspartic acids. A cation binding site near the aspartic acids of penicillopepsin has been located in an X-ray crystallographic study of an enzyme:inhibitor complex (28). However, inhibitors incorporating aminostatine show no advantage in potency. A favorable ionic interaction with the active-site aspartic acids may be balanced by the energy required for desolvation of the ammonium group as inhibitor binds to renin (73). Moreover, the fact that the 3R and 3S diastereomers of aminostatine give nearly equipotent inhibitors (with those containing the 3R diastereomer being slightly more potent) suggests different binding modes for 3R aminostatine and 3R statine.

Continual progress has been made toward potent lower MW inhibitors containing statine or its analogues. These include dipeptide inhibitor **14**, which incorporates the bis-naphthylmethylacetyl (BNMA) group (74). Boger and coworkers used molecular modeling (see Molecular Modeling in Renin Inhibitor Design, below) to predict that inhibitors containing the statine analogue ACHPA [4(S)-amino-5-cyclohexyl-3(S)-hydroxypentanoic acid] would have increased potency. The ACHPA-containing analogue ACRIP (**15**) corresponding to SCRIP was in fact more potent (75), and many recently reported renin inhibitor designs have featured the cyclohexyl modification. ACHPA-containing inhibitors as small as tripeptide **16** are highly potent (76). Inhibitor **8**, which contains a single naturally occurring amino acid, has been mentioned. It is likely that the cyclohexyl of the "hydroxy" isostere present in **8** contributes to its high potency.

In another approach to increased potency, analogues of statine and ACHPA bearing 2R or 2S alkyl substituents have been prepared and incorporated into peptide inhibitors. The additional alkyl group is intended to replace the S_1' side chain, which is absent when statine or ACHPA is used as a dipeptide mimic. Although replacement of statine in a large peptide inhibitor (iBu–His–Pro–Phe–Phe–Sta–Leu–Phe–NH_2) by 2R and 2S-isobutyl substituted statines gave no advantage in potency (77), incorporation of a 2-allyl substituent into smaller inhibitors such as the unsubstituted ACHPA-containing tripeptide **16** did lead to more potent inhibitors (78).

Peptide Aldehydes and Glycols

Peptide aldehydes such as **17**, which utilize binding sites only to the amino-terminal side of the cleavage site, have been reported to be potent inhibitors of renin (79,80). They may bind in the hydrated form and, as such, mimic the transition state for substrate cleavage. Peptide aldehydes were the first small inhibitors with a high potency against human renin to be reported, although potent tripeptide inhibitors incorporating statine and other transition-state designs were described soon after. Since the aldehyde function is rapidly metabolized, peptide aldehydes are not expected to have potential as *in vivo* inhibitors of renin. Peptide glycols such as **18** also utilize binding sites only toward the amino side of the cleavage site but position a metabolically more stable diol group so as to interact with the active-site aspartic acids (81). Diols with an additional alkyl substituent capable of binding in the renin S_1' subsite have appeared in the patent literature (82,83).

Peptide Ketones

Renin inhibitors that contain a ketone function have been reported. Many of these are more potent against human renin than peptide aldehyde **17**, reflecting the fact that they can make binding interactions on both sides of the cleavage site. Although inhibitor **19**, which incorporates a "ketone" isostere of the Leu–Val peptide bond (Fig. 2), is highly potent, the corresponding analogue without an amino-terminal Boc group unexpectedly has a markedly reduced potency (IC_{50} = 500 n*M*) (58). It is unclear whether the "ketone" isostere can be effective in lower MW inhibitors. Inhibition of renin by a peptide (**20**) containing a ketone analogue of statine ("statone") has been reported (84). Studies of the binding of a similar analogue to pepsin by Rich *et al.* (67,85,86) have established its conversion to a tetrahedral ketal by enzyme-catalyzed addition of water. The statone-containing peptide **20** shows a 20-fold reduction in potency compared to the corresponding statine analogue, a difference which may reflect the energy required for hydration of the ketone (84). Inhibitor **21**, which incorporates a more easily hydrated difluorinated ketone analogue of statine ("difluorostatone"), is 65 times more potent than **20**. Interestingly, a difluoroketone analogue of ACHPA gave less potent inhibitors than those containing difluorostatone, which suggests that the mode of binding of the difluoroketone inhibitors differs from that of inhibitors containing statine or ACHPA (84).

Other Transition-State Inhibitor Designs

Several classes of inhibitors which incorporate nonpeptide C-terminal elements have been reported. Inhibitors such as sulfide **22** (87) and retro-amide analogue **23** (88) may derive their potency by positioning hydrophobic groups (isobutyl, phenethyl) for productive interactions in the S_2' and/or S_3' subsites of renin. Other designs of this type incorporate carboxy-terminal ether, sulfoxide, or sulfone elements. Remarkably, even an inhibitor (**24**) with a C-terminal hydrocarbon chain has a similar potency (89). Binding of the isoamyl moiety of this inhibitor in the S_1' subsite seems likely to contribute to its potency. An additional inhibitor design featuring a nonpeptide C terminus is exemplified by **25**, which incorporates a shortened statine (norstatine) ester (90).

Potent renin inhibitors such as **26** have been obtained using an "amino alcohol" isostere, another mimic of the tetrahedral intermediate for peptide bond hydrolysis (Fig. 2) incorporating elements of both "hydroxy" and "reduced peptide" isosteres (91,92). The more potent diastereomer is often that with a hydroxyl configuration (R) opposite to that preferred for statine and the hydroxy isostere, suggesting that, for one or both diastereomers, the secondary amine is involved in binding (91). Bartlett and Kezer (93) have reported the synthesis of an analogue of statine ("phosphastatine") in which a phosphinic acid moiety replaces the statine hydroxyl group (Fig. 2). Although this design gives potent inhibitors of pepsin, it fails for human renin. At the higher (physiological) pH at which renin inhibition is determined, the phosphorus acid (pK_a = 2) is completely ionized and binding of the anionic form may be energetically unfavorable (94).

MOLECULAR MODELING IN RENIN INHIBITOR DESIGN

Although a crystal structure of renin is not yet available to serve as a basis for molecular modeling studies, exciting progress has been made toward this goal. Preliminary X-ray crystallographic data on mouse submaxillary gland renin and inhibitor complexes have been described by Navia *et al.* (95). Human renin obtained by recombinant DNA techniques has provided crystals of native enzyme and enzyme–inhibitor complexes (42), but as yet no X-ray crystal structure data have been reported. X-ray crystal structures of the related aspartic proteinases pepsin (96), penicillopepsin (28), and endothiapepsin (97) have been determined. An X-ray crystal structure of a complex of pepstatin with the aspartic proteinase from *Rhizopus chinensis* (*Rhizopus* pepsin) is also available (29). X-ray structures have been described for a complex of inhibitor **4** with endothiapepsin (98) and of a pepstatin analogue (Iva–Val–Val–Sta–OEt) with penicillopepsin (100). Models of mouse renin (99) and human renin (59,100–104) have been derived from their known primary sequences and one or more of these structures.

Renin models have played an important part in the design of renin inhibitors. Boger has described the use of modeling based on the *Rhizopus* pepsin: pepstatin structure of Bott *et al.* in the design of ACHPA, in the prediction that statine would serve best as a dipeptide mimic, and in the design of two classes of conformationally-restricted cyclic statine-containing inhibitors (105,106). A detailed description of uses of a renin model in visualizing enzyme–inhibitor complexes has been reported by Raddatz *et al.* (104). A proposed network of hydrogen-bonding interactions between enzyme and the peptide backbone of a renin inhibitor has been derived from a human renin model at Abbott (59). Additional contributions of modeling to inhibitor design are anticipated, especially when an X-ray structure of human renin with an inhibitor bound becomes available.

IN VIVO INHIBITION OF RENIN

To be successful as a drug, a renin inhibitor will have to be specific for renin, survive numerous proteinases in the gastrointestinal tract to pass across the gut wall intact, and possess pharmacokinetic properties that will ensure a reasonable duration of action at an acceptable dose. Progress has been made in achieving each of these goals.

Specificity of Renin Inhibitors

Clinically useful inhibitors of renin should not inhibit other proteinases, including the related aspartic proteinases pepsin and cathepsin D. A high specificity for human renin has been reported for inhibitors containing statine and other peptide bond isosteres, including inhibitors **14** (74), **17** (79), **23** (88), and **25** (90). The presence of an aromatic amino acid (or an equivalent, as in **14**) at the Phe(8) position and the presence of His(9) appear to be important determinants for this specificity.

Stability of Renin Inhibitors

Stability toward peptidases in *in vitro* experiments has been reported for several inhibitors, suggesting that they

may prove to be stable *in vivo*. Amide bonds involving the statine moiety have been shown to be stable toward cleavage *in vivo* (107); thus the stability to proteases reported for statine-containing inhibitor **14**, for example, is not surprising. The Phe(8)–His(9) bond of **22** was found to be cleaved by a pancreatic homogenate (87). Stabilization of this bond has been achieved by incorporating *p*-methoxyphenylalanine into inhibitor **23** (88) and D-Pro into inhibitor **27** (107). Inhibitor **25**, in which the labile Phe(8)–His(9) bond has been eliminated, is stable to several proteinases (90). The potent pentapeptide **7**, which incorporates a metabolically stable N-methylated Phe(8)–His(9) bond, was inert to the action of a liver homogenate (61). An inhibitor similar in structure to **7**, but which incorporates an (α-methyl)Pro–Phe–His sequence replacing Pro–Phe–(*N*-methyl)His, is stable to chymotrypsin, elastase, porcine pepsin, and rat liver homogenate (108). Stability toward peptidases would be assured in a wholly nonpeptide renin inhibitor, and progress toward totally nonpeptide inhibitors has been achieved by incorporation of nonpeptide elements at amino (e.g., compounds **8**, **14**, and **25**) and carboxy (e.g., compounds **22**, **23**, and **25**) termini.

Physical Properties of Renin Inhibitors

Lipophilic side chains are preferred in the S_3, S_2, S_1, and S_1' binding sites of renin, and inhibitors based on the current transition-state designs must make favorable binding interactions in at least the first three of these sites in order to achieve a high potency. The resulting low aqueous solubility for many inhibitors is likely to limit their oral bioavailability (109). Although a nonaqueous formulation enhances oral absorption of the highly lipophilic cyclic peptide cyclosporin (110), it is unclear whether a similar strategy would be effective and practical for an insoluble renin inhibitor.

In general, the incorporation of less lipophilic (polar and/or ionic) side chains into renin inhibitors to increase the aqueous solubility results in an unacceptable loss in potency. It appears, however, that the introduction of solubilizing groups in appropriate locations can yield potent inhibitors with a high water solubility. Analogues of pepstatin A (**9**) with increased potency and solubility have been derived by the addition of charged amino acids (Glu and Arg) to the carboxy terminus (68). Inhibitors incorporating aminostatine have increased solubility compared to the corresponding statine analogues (73). The contributions of N-terminal Pro and C-terminal Lys to the solubility of RIP (**2**) have been mentioned (see Inhibitors Containing Statine and Its Analogues, above). The carboxy-terminal pyridylmethyl amide of inhibitor **7** likely contributes to its high solubility (30 mg/ml in 0.1 *M* citric acid) (61). Solubilities for a series of potent statine and ACHPA-containing inhibitors incorporating solubilizing groups at the carboxy terminus have been reported (111); an example is inhibitor **28** (18 mg/ml in H_2O). Nonapeptide **12**, which contains two arginine residues, is also quite soluble in water (100 mg/ml) (71).

Plasma Binding

In several cases, potency differences have been noted when human renin inhibition was compared in plasma and human kidney renin assays (55,90). Degradation of inhibitor in plasma or differences in assay conditions (see Purification and *in Vivo* Assays, above) may be responsible for these differences. Inhibitor **29** and related analogues were reported to have high potency in a pure enzyme assay but remarkably reduced activity in a plasma renin assay. This led to the hypothesis that plasma factors bind inhibitor and prevent its interaction with renin (112). Although direct evidence for binding of **29** by plasma components was obtained, subsequent work has shown its high potency in the pure enzyme assay to be artifactual (113). It is possible that plasma binding of renin inhibitors, a subject not yet explored, could be an important determinant for their pharmacokinetics.

In Vivo Models

There is no ideal animal model for *in vivo* evaluation of renin inhibitors. Although various rat models have been used extensively for the *in vivo* testing of ACE inhibitors, rat renin is insensitive to many of the potent classes of human renin inhibitors. This necessitates the administration (and inhibition) of exogenous renin (hog or human) to rats in order to determine the response of blood pressure (BP) to inhibitor (114,115). Renin must be administered by infusion due to its rapid clearance. Salt-depleted dogs (116–118), monkeys (87,114,119,120), baboons (120,121), and marmosets (71,122) have been used for *in vivo* evaluation of inhibitors. In these salt-depleted models, the accompanying volume depletion results in activation of the renin–angiotensin system and increased plasma renin activity (PRA). Under these conditions, a component of systemic blood pressure is renin dependent, and inhibition of renin can be measured by the reduction in BP. Marmosets have the advantage of small size (300 g) and the high degree of renin dependency which can be achieved in their BP (122). Nevertheless, salt-deficient animals are not true hypertensive models (in fact their BP is often lower than in the salt-replete state) and their relevance to hypertension in humans is uncertain. Evidence that inhibiting renin–angiotensin systems in vascular or other tissues or organs may be important in the control of hypertension (19–21) raises additional questions about basing the evaluation of inhibitors solely on their effect on plasma renin activities. Extensive evaluation in hypertensive humans may be necessary to assess accurately the potential of renin inhibitors and their value relative to ACE inhibitors.

Intravenous Administration of Renin Inhibitors

The intravenous (iv) administration of renin antibodies to inhibit renin has been discussed by Haber (123). Their lack of oral activity and restriction to the plasma compartment limit their use as therapeutic agents. *In vivo* studies of peptide-based inhibitors of renin have been reviewed by Boger (9) and by Hofbauer and Wood (12). Effective inhibition of PRA and lowering of BP have been obtained in renin-dependent models, including primates, with the administration of inhibitor by infusion or iv bolus. The duration of action is usually quite short but can be increased with larger doses of inhibitor.

Several inhibitors with a longer duration of action have been described. CGP-29,287 (**12**), despite its large size, lowered BP for 1–3 hr after iv administration (0.1–10 mg/kg);

the protecting groups at amino and carboxy termini are believed to contribute to its prolonged duration of action (71). The potent inhibitor U-71,038 (7) lowered PRA and BP for over 3 hr after a single iv dose (5 mg/kg; infusion over 17 min) (61). SR 42,128A (**10**; arginine salt) completely inhibited PRA for 3 hr after iv administration (3 mg/kg) to cynomolgous monkeys (119); stability to proteinases due to its two statine residues (see Stability of Renin Inhibitors, above) may play a role.

Two renin inhibitors, **2** and **4**, have been studied in humans. Compound **2** administered by infusion lowered BP effectively in sodium-depleted monkeys, but in salt-depleted humans, produced hypotension accompanied by bradycardia at the highest dose (0.5 mg/kg/min) (123). Infusions of **4** into sodium-deficient humans produced only a slight lowering of systolic BP but a clear decrease in diastolic BP. A modest increase in heart rate was noted (124).

Oral Administration of Renin Inhibitors

Several reports of orally active renin inhibitors have appeared. In spite of its large size, nonoapeptide **12** reduced PRA and BP for over 1 hr after oral administration of a high (100 mg/kg) dose to salt-deficient marmosets (71). Inhibitor **7**, when administered orally to salt-depleted monkeys (50 mg/kg), produced a drop in BP and PRA which persisted for over 5 hr (114). Oral administration of **25** to monkeys (30 mg/kg) reduced PRA and BP for 6 hr, after a 1-hr induction period, presumably due to a slow rate of absorption (90). Recently, oral activity in marmosets was reported for **5**, at a dose of 10 mg/kg; the duration of action was about 2 hr (62).

Renin Release

Inhibition of the RAS brings about release of renin from the kidney by suppressing the negative feedback mechanism mediated by angiotensin II (125). An enhanced release of renin which can overcome inhibition of renin has been of concern with regard to the potential use of renin inhibitors as therapeutic agents. However, in marmosets the maximum level of renin (presumed steady state) was the same whether renin or ACE was blocked (126). Moreover, no "breakthrough" of the inhibitory effect of **11** by newly released renin was found during a 48-hr infusion into sodium-deficient dogs (117).

Renin Inhibitors Versus ACE Inhibitors

Comparisons in salt-depleted animals of the hypotensive effects of renin inhibitors versus those of ACE inhibitors have shown either little difference (121,127,128) or a slightly greater effect for an ACE inhibitor (116,118). The latter result is consistent with the hypothesis that ACE inhibitors may also potentiate bradykinin and other hypotensive peptides. Possible advantages of renin inhibitors relative to ACE inhibitors, due to the high specificity of renin, have been mentioned (See Introduction); it is likely that extensive clinical trials will be necessary to evaluate relative effectiveness and side-effect profiles of these two classes of therapeutic agents.

BIOAVAILABILITY OF RENIN INHIBITORS

Low oral absorption and rapid elimination or inactivation of inhibitor appear to be major limitations to bioavailability for known renin inhibitors. The high oral doses required to obtain hypotensive activity and the short duration of action after iv administration of all but high doses are consistent with poor bioavailability. Improving the bioavailability of renin inhibitors based on current inhibitor designs is a major challenge, due to their high molecular weight and peptide character.

Oral Absorption

Although intestinal absorption of amino acids and dipeptides is well documented, that of larger peptides is not (129). Direct (130,131) and indirect (132) evidence for transport of large peptides exists, but low or negligible absorption has been found for many bioactive peptides, even when they are stabilized toward proteolytic degradation (133,134). Factors determining whether or not a given peptide (if stable) will be well absorbed are unclear (135). Although gastrointestinal absorption of peptides can be enhanced by the addition of absorption promoters (136,137), potentially irreversible effects of promotors on membranes must be avoided. A combination of chemical and formulation modifications may be required to maximize oral absorption of a peptide-based renin inhibitor.

Biliary Excretion

With a few exceptions (see *In Vivo* Models, above), renin inhibitors reported to date have a short duration of action after iv administration. While proteolytic cleavage may contribute, it is likely that biliary excretion of intact inhibitor is the major factor in their rapid disappearance. After iv administration of the stabilized statine-containing inhibitor (Iva–His–D-Pro–Phe–His–Sta–Leu benzylamide), 63% of the inhibitor appeared undegraded in bile within 2 hr (107). Pepstatin A-[^{14}C]glycine was rapidly excreted intact in the bile (and urine) after intravenous administration to rats (138). Ample precedent exists for rapid biliary excretion of intact peptides (139,140). Nevertheless, factors determining whether or not a drug is eliminated in bile are poorly defined. In humans, biliary excretion of drugs becomes increasingly likely as the molecular weight (MW) rises above a "threshold" of 500 (141). All potent renin inhibitors reported to date have a MW exceeding this "threshold" (e.g., inhibitor **25**, MW = 649). Examples of orally effective, high MW drugs that are more slowly excreted (e.g., erythromycin, cyclosporin, reserpine) demonstrate that factors other than MW are important. If current inhibitor designs, as exemplified in Table I, fail to yield potent inhibitors of lower MW, other approaches, such as adjustment of physical properties (solubility, lipophilicity, ionic state), may be necessary to address the problem of biliary excretion. At present, little information is available to suggest how this should be done.

SUMMARY

Renin inhibitors are interesting pharmacological tools for study of the renin–angiotensin system. Persistent, insightful inhibitor design and evaluation have yielded potent inhibitors with promising activity in several *in vivo* models. The development of orally effective and long-acting inhibitors will enable their long-term antihypertensive efficacy

and possible advantages over ACE inhibitors to be investigated. Moreover, the success achieved with renin inhibitors will increase our knowledge of the pharmacokinetic properties of small peptides, information which may be applicable to the development of other peptide-based drugs.

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