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Perspective

Inhibition of the Renin-Angiotensin System. A New Approach to the Therapy of Hypertension

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The renin-angiotensin system is one of the humoral mechanisms involved in the regulation of blood pressure. Even though the preliminary investigations can be traced as far back as 1898, it was only with the pioneering efforts of the research groups of Page in the United States and Braun Menendez in Argentina that we began to understand the molecular basis of this blood pressure regulating mechanism.[1]

Renin is a proteolytic enzyme, produced mainly in the juxtaglomerular apparatus of the kidney, which acts on the circulating α-globulin angiotensinogen, produced by the liver (Figure 1). The result of this enzymatic action is the formation of the decapeptide angiotensin I, which has very little, if any, biological activity. Removal of the C-terminal dipeptide histidylleucine from this decapeptide by the angiotensin-converting enzyme present in lungs and other organs yields the octapeptide angiotensin II. This peptide is a very potent vasoconstrictor agent and also the main physiological stimulus for the release of aldosterone from the adrenal gland. This mineralocorticoid, in turn, induces sodium and water retention, leading, therefore, to an increase in blood pressure by a "volume" mechanism.[2]

The relevance of the renin-angiotensin system in blood pressure regulation under normal or pathological circumstances has been very controversial. Studies with animal models and clinical observations with patients of varied etiology indicate a clear involvement of the renin-angiotensin system[3,4] in hypertension of renovascular origin. However, the role of this system in human hypertension of unknown origin (essential hypertension) and in those animal models that are assumed to be a correlate of essential hypertension, i.e., the spontaneous or genetic hypertensive rat, is still the subject of considerable debate.

Clarification of the role of the renin-angiotensin system in animal models of hypertension by measuring circulating levels of the different components has been always one of the main goals of research in this area. However, the relevance of circulating levels in comparison with intratissue or intraorgan levels has been questioned. There is clear evidence that renin and angiotensin-converting enzyme are present in blood-vessel walls.[3] Therefore, the intravascular formation of angiotensin II could play a more important role than that of circulating angiotensin II produced by plasma renin.[5] If the level of circulating renin is to be considered as the most reliable yardstick for renin-angiotensin system involvement, then only animal models of acute renovascular hypertension, such as the two kidney-one clip hypertensive rat, may be considered renin dependent. Chronic renovascular models, like the one kidney-one clip renal hypertensive rat or the several weeks old two kidney-one clip renal hypertensive rat, show normal levels of renin and, therefore, could not be considered renin dependent. It has, however, been shown[6,7] that sodium depletion restores the renin dependence of these chronic hypertensive animals, and this observation explains why, as we shall see later, renin-angiotensin system blockers are more effective when utilized in conjunction with diuretics or low sodium diets.

In human hypertension a large proportion of patients, mostly those classified as essential hypertensive, show "normal" renin levels and a small number have even low renin levels.[3] Their hypertension is, therefore, not apparently supported by the renin-angiotensin system. Laragh, however, has raised the point that in hypertensive patients the levels of renin should be lower than normal if the renin-angiotensin system were not involved, since blood pressure exerts a negative feedback effect on renin levels.[8]

An alternate approach to the study of the role of the renin-angiotensin system than that of measuring blood levels of intermediates is the use of specific blockers, and a detailed evaluation of this approach and its implications for the treatment of human hypertension is the subject of this article.

Blockers of the Renin-Angiotensin System

Careful consideration of the different steps involved in the activation of the renin-angiotensin system indicates

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(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
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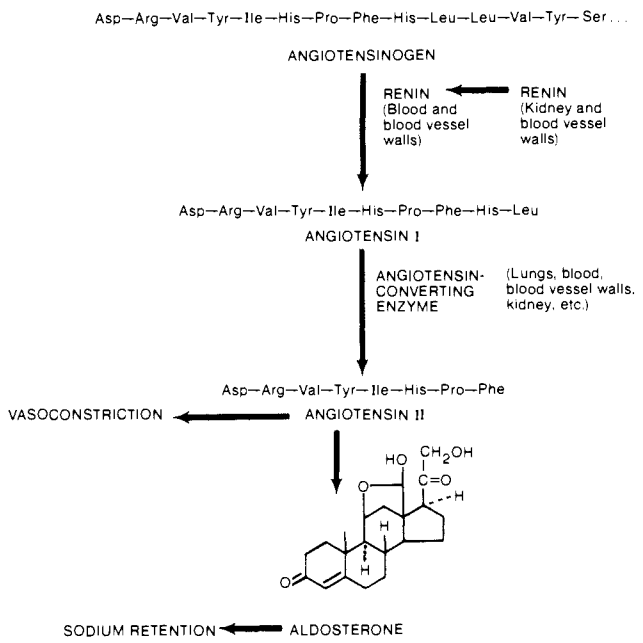

Figure 1.

that the possibility exists of blocking this activation at various points; namely: (a) renin release, (b) renin activation, (c) angiotensin I formation, (d) angiotensin II formation, and (e) angiotensin II action on receptors.

Steps "b", "c", and "d" are known to be enzymatically mediated, while "a" and "e" involve interactions at the receptor level in different tissues or target organs. The reason for making this distinction is that the knowledge of the molecular events involved in enzyme catalysis is far more advanced than the knowledge of the molecular events governing agonist–receptor interaction. In the latter case, the only molecular information available to the medicinal chemist for antagonist or drug design is the structure of the agonist. In the case of an enzyme, not only the structure of the substrate is available but also that of the products and, in addition, knowledge of the enzyme functional groups implicated in catalysis. Therefore, the possibilities for inhibitor or drug design are greater in the case of enzymatic reactions than in agonist–receptor interactions.

A more detail evaluation of the different steps of the activation process outlined above will allow us to focus on the potentials and disadvantages involved in the inhibition of the renin–angiotensin system at each particular level.

Renin Release

Renin release from the kidney is under the control of baroreceptors in the juxtaglomerular apparatus and chemoreceptors in the macula densa.[9] Stimulation of renal β-adrenergic receptors or blockage of α-adrenergic receptors leads to renin release.[10] Angiotensin II also exerts an inhibitory effect on renin release by a feedback mechanism, possibly mediated by another specific receptor. No specific inhibitors of renin release have yet been developed, but the antihypertensive properties of β-adrenergic receptor blockers are assumed to be due, at least partially, to inhibition of renin release.[10] However, since they also influence blood pressure by acting on extrarenal β-adrenergic receptors, the contribution of the renin inhibition to their total antihypertensive effect is still controversial.[11] It is possible that one could design an angiotensin analogue that would inhibit renin release without having agonistic or antagonistic activity on other angiotensin II receptors, but this approach has not yet been explored.

Renin Activation

As mentioned above, the main site of plasma renin production is the kidney, but enzymes with similar specificity have been found in other tissues.[12] Even though there is still a great deal of confusion in the literature concerning the different molecular forms of renin,[4,12] it is clear that there are inactive forms of this enzyme or "prorenins" present in different tissues and in plasma. Most investigators have reported the isolation of forms of renin with molecular weights in the neighborhood of 60 000 or 40 000. The 60 000-dalton form can be activated to different degrees, with or without change in molecular weight; but the low-molecular-weight forms cannot usually be further activated. It has been recently postulated that the "big renins" of normal human plasma could be mixtures of active and inactive forms of the same molecular weight.[13]

The "prorenins" of human plasma can be activated by incubation at low temperatures (cryoactivation), at acid pH's, or with exogeneous proteases.[14] Cryoactivation and low pH activation are apparently mediated by endogenous neutral serine proteases.[12] Urinary (renal) and plasma kallikreins have been postulated as the most likely physiological activators of prorenin.[14-18] Plasma kallikrein is a neutral serine protease that cleaves the plasma protein kininogen to liberate the vasodepressor nonapeptide bradykinin (Figure 2). Renal kalikrein generates the decapeptide lysyl-bradykinin, which is converted to bradykinin by tissue aminopeptidases. These enzymes can be inhibited by polyvalent bovine protease inhibitors and by synthetic active-site-directed inhibitors.[19] The enzyme kallikrein might, therefore, constitute a physiological link between the renin–angiotensin system and the kallikrein–kinin system,[14] and, as we shall see later, angiotensin-converting enzyme establishes a second link between these two important vasoactive systems.

If activation by an enzyme mechanism plays a regulating role in the generation of renin, an inhibitor of this activation could represent a viable alternative for the blockade of the renin–angiotensin system. However, the relevance of renin activation to hypertension is still not clear.

Angiotensin I Formation

Renin, the enzyme that hydrolyzes angiotensinogen to generate angiotensin I, belongs to the group of acidic peptidases like pepsin, even though the optimal pH varies between 5 and 8 depending on the origin, the substrates,

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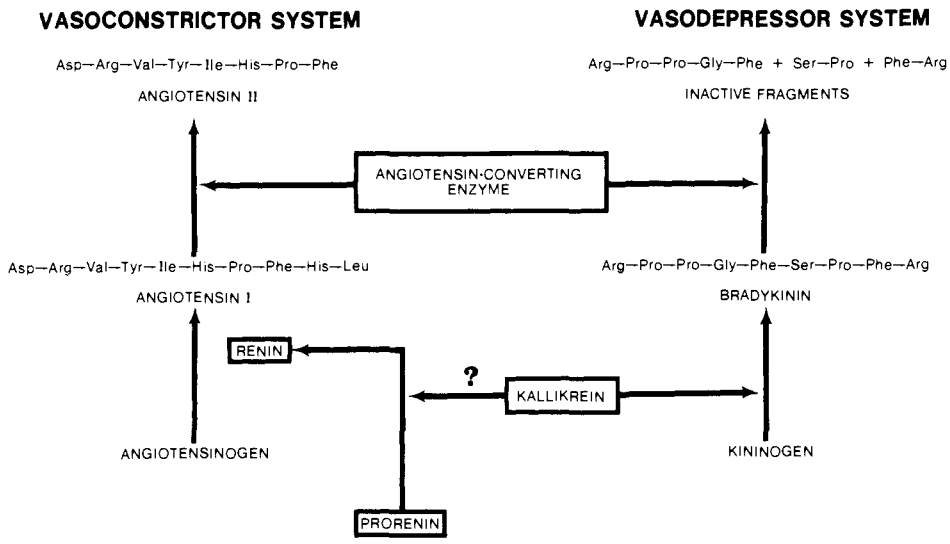
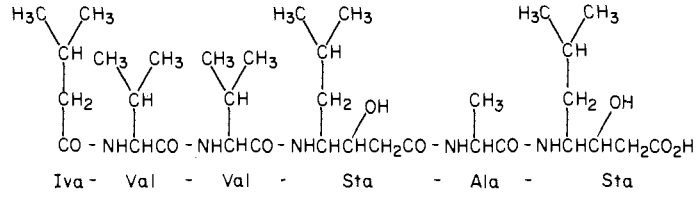
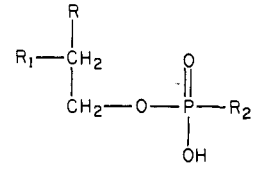


Figure 2.

Table I. Renin Inhibitors

	K_i, μM
Substrate Analogues[a]	
Pro-His-Pro-Phe-His-Leu-Leu-Val-Tyr	42
Pro——————Phe———	4
Pro——————Phe-Phe———	1
Pro——————Phe-Phe———Lys	2
Pepstatin and Derivatives[b]	
	0.1
Iva - Val - Val - Sta - Ala - Sta	
pepstatin	
Iva-Val-Val-Sta-Ala-Sta·Arg	0.1
Iva-Val-Val-Sta-Ala-Sta-Arg-OCH_3	0.3
Phospholipids	
	400-2000
general structure	

[a] References 21 and 22. [b] References 23-25.

and the assay method used.[20] Human renin is active against angiotensinogen from most mammalian species, but human angiotensinogen is only hydrolyzed by primate or human renin.

Three different types of renin inhibitors have been developed so far: synthetic peptides related to the substrate; modified peptides like pepstatin, isolated from fermentation broths; and phospholipids. Structures of some of the inhibitors of each type are shown in Table I. The development of substrate analogues as renin inhibitors has been hindered by the fact that the increase in hydrophobicity required to decrease the K_i (Table I) has an adverse effect on the solubility, which has resulted in a lack of in vivo activity.[21] However, the most recent results reported by Cody et al.[22] give hope that a solution to this dilema can be found. The addition of a C-terminal lysyl residue to one of the most active analogues in vitro yielded a decapeptide (Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys) which retains very good in vitro activity, good solubility, and it is capable of inhibiting renin-induced hypertension when administered iv at 0.2 (mg/kg)/min.

Pepstatin, a peptide-like acid protease inhibitor isolated from a *Streptomyces* species,[23] also suffers from the

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(21) Burton, J.; Poulsen, K.; Haber, E. In "Polymeric Drugs"; Donaruma, L. G.; Vogl, O., Eds.; Academic Press: New York, 1978; p 219.

(22) Cody, R. J.; Burton, J.; Herd, J. A.; Slater, E.; Haber, E. *Clin. Res.* **1980**, *20*, 499A.

(23) Umezawa, H. "Enzyme Inhibitors of Microbial Origin"; University Park Press: Baltimore, London, and Tokyo, 1972.

drawback of low solubility. Pepstatin[24] and its more soluble derivatives[25] are only active in vivo when administered by the parenteral route, and they have relatively short duration of action. Structural modifications of pepstatin are being pursued with the aim of clarifying the mechanism of its inhibitory activity on pepsin and renin.[26] It is hoped that these studies will lead to major improvements in the design of potent and specific inhibitors.

Phospholipid renin inhibitors can show substantial antihypertensive activity in several animal models of hypertension,[27,28] but they are not very potent inhibitors of renin in vitro. This discrepancy raises the question of whether their antihypertensive activity is completely related to their renin-inhibitory activity.[26]

Specific antibodies to canine renin have demonstrated clear antihypertensive activity in renin-dependent hypertensive dogs.[29] This approach is of great value in studying the mechanism of hypertension, but is not likely to have therapeutic application.

The very significant advances in renin isolation and purification brought about by the use of affinity chromatography techniques has made possible the purification of the enzyme from several sources[30,31] and the initiation of detailed studies on the active site,[20] which in many respects resembles that of pepsin. This knowledge will eventually lead to the development of active-site-directed inhibitors.

Since renin is an enzyme of high substrate specificity, it is very unlikely that any of its specific inhibitors should affect the kinin system, and it is to be expected that they would be free of the ambiguity, as far as mechanism of antihypertension activity is concerned, that characterizes inhibitors of the angiotensin-converting enzyme.

Angiotensin II Formation

The formation of angiotensin II constitutes the final and critical step in the activation of the renin–angiotensin system, since its immediate precursor, angiotensin I, is for all practical purposes biologically inactive. The enzyme responsible for this transformation, angiotensin-converting enzyme, plays a key role in the activation of this pressor system. However, angiotensin is not the only biologically important substrate of this enzyme. The studies of Erdös and other investigators have clearly demonstrated that angiotensin-converting enzyme is identical to kininase II, one of the most important of all the enzymes involved in the inactivation of bradykinin, the final mediator of the kallikrein–kinin system[32] (Figure 2).

Angiotensin-converting enzyme is a peptidyl dipeptide carboxyhydrolase that cleaves off dipeptides from the C-terminal end of peptide chains without any major specificity for the peptide sequence involved.[33] The hydrolysis of the C-terminal dipeptide from the angiotensin I decapeptide yields the potent vasopressor octapeptide

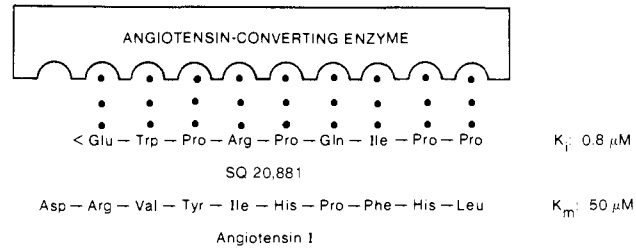

Figure 3.

angiotensin II, while the hydrolysis of the C-terminal dipeptide from the hypotensive nonapeptide bradykinin destroys its blood pressure lowering effect. Therefore, the total effect of angiotensin-converting enzyme is a pressor effect, and the inhibitors of this enzyme will have an antihypertensive effect that may be mediated by two different mechanisms, inhibition of angiotensin II formation and potentiation of bradykinin.[34]

This dual effect of angiotensin-converting enzyme inhibitors was clearly demonstrated with the peptide inhibitors isolated from the venom of *Bothrops jararaca* and their synthetic analogues. The most extensively studied of these peptide inhibitors was the nonapeptide teprotide (SQ 20 881), also designated as BPP_{9a} by Ferreira, Greene, and collaborators.[28] This nonapeptide is a competitive inhibitor that binds to the active site of angiotensin-converting enzyme by multiple interactions with different subsites, which results in tighter binding than that observed with the substrate angiotensin I (Figure 3).[35] The duration of this inhibition in vivo, as judged by the blockade of the angiotensin I hypertensive activity, is considerable, particularly for a peptide.[36]

Teprotide and its analogues show various degrees of antihypertensive activity in different animal models of hypertension.[5,34] The argument that this antihypertensive effect is a proof of the involvement of the renin–angiotensin system has been highly controversial, mainly because of the bradykinin potentiating activity of these inhibitors. However, since angiotensin antagonists are also effective in many or all of the same animal models,[1] it is very likely that most of the hypertensive activity of teprotide and analogues is mediated by blockade of the renin–angiotensin system.

A similar situation developed in the interpretation of the very significant clinical results obtained with teprotide. Besides its striking antihypertensive activity in high renin patients, teprotide also lowers blood pressure in patients with "normal" renin levels.[37] These results have been interpreted by Laragh and collaborators as an indication that in this group of patients, which constitute the largest group in essential hypertension, the renin–angiotensin system is involved in maintaining the blood pressure elevation. Other investigators have countered that the antihypertensive effect in "normal" renin patients could be a consequence of the potentiation of endogenous bradykinin, which is, in turn, a consequence of the inhibition of the kininase II inactivating mechanism.[38] However, the evidence presented for the involvement of a kinin is not

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(29) Dzau, V. J.; Kopelman, R. I.; Barger, A. C.; Haber, E. *Science* **1980**, *207*, 1091.
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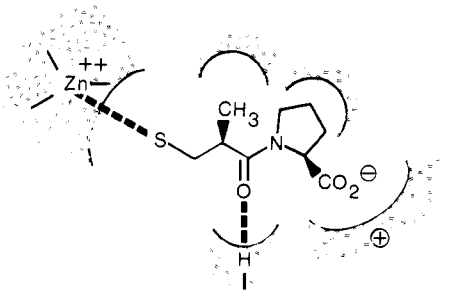


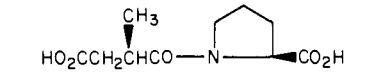
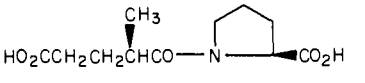
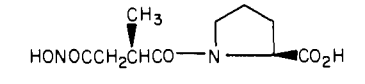
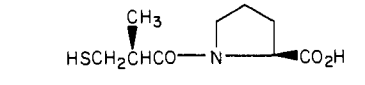
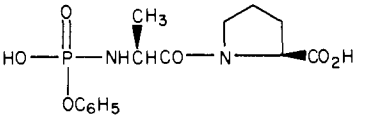
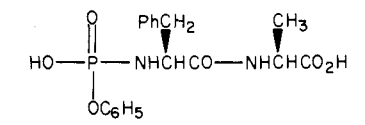
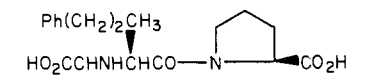
Figure 4.

very strong, and it is not very likely that conclusive evidence in favor of this hypothesis will be forthcoming in the near future.

Whether the antihypertensive effect of angiotensin-converting enzyme inhibitors is mediated exclusively by their effect on the renin–angiotensin system or by the combination of their effect on this and the kallikrein–kinin system, the studies with teprotide served to demonstrate that compounds of this type could offer a new approach to the treatment of hypertension.

This promise was fulfilled with the development of captopril. This novel therapeutic agent is an example of drug design based on the knowledge of the possible molecular interactions between the drug and its receptor, in this case the angiotensin-converting enzyme active site.[39,40] Two factors played a critical role in this approach to ab initio drug design: first, the knowledge accumulated in the structure studies with peptidic inhibitors of angiotensin-converting enzyme; second, the possibility of developing potent inhibitors of this enzyme due to the similarity of its mechanism of action with that of carboxypeptidase A. The detailed story of the design of the new class of angiotensin-converting enzyme inhibitors represented by captopril has already been told. Here it suffices to emphasize that captopril is a potent and specific inhibitor of the angiotensin-converting enzyme because it combines in one molecule functional groups that can establish effective regio- and stereospecific interactions with other functional groups on the enzyme surface (Figure 4). The carboxyl group is involved in an electrostatic interaction with a cationic group, probably derived from an arginine residue on the enzyme surface. The amide bond carbonyl is most likely hydrogen bonded, with the oxygen atom functioning as hydrogen bond acceptor for a hydrogen bond donor on the enzyme, and the sulfhydryl group becomes one of the ligands of the zinc ion complexed at the active site. The pyrrolidine ring of the proline residue and the α-methyl substituent of the mercaptopropanoyl moiety contribute to strengthen the interaction with the enzyme by restricting the mobility or degrees of freedom of the inhibitor molecule and, probably, also through hydrophobic and dispersion interactions with the enzyme surface. The importance of these multiple interactions has been demonstrated by the synthesis of suitably modified analogues[41-45]

Table II. Angiotensin-converting Enzyme Inhibitors

	K_i, μM
	2.5[a]
	0.8[a]
	0.6[a]
	0.0017[a]
	0.001[b]
	2.2[c]
	0.0012[d]

[a] Reference 45. [b] Reference 50. [c] Reference 49. [d] I_{50}; ref 57.

and by successfully extending the same rationale to the design of potent inhibitors of other zinc-containing peptidases, such as carboxypeptidases A and B.[46]

Other investigators have applied a similar rationale, namely, a zinc-ligand moiety joined to specific substrate-like moieties, to develop inhibitors for zinc-containing peptidases like thermolysin[47] and *Pseudomonas aeruginosa* elastase.[48] Utilizing sulfhydryl-containing inhibitors and the cobalt form of carboxypeptidase A and thermolysin, Holmquist and Vallee[49] have shown that the sulfur atom of these inhibitors is bound in the first sphere to coordination of the metal as postulated by Ondetti et al.[39,45] These investigators[47,49] and more recently Galardy and Fernandez[50] have also shown that *N*-phosphoryl dipeptides are potent inhibitors of angiotensin-converting enzyme and thermolysin (Table II). In the case of thermolysin the phosphoryl moiety of these inhibitors had been shown to be bound to the active-site zinc atom.[51]

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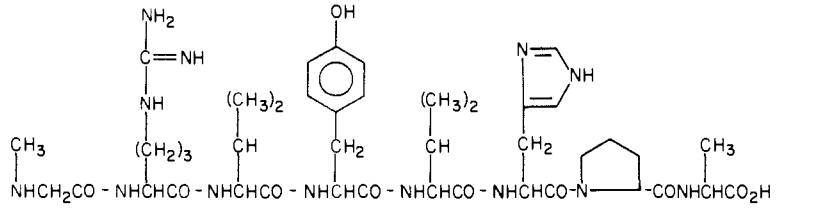
Chart I



saralasin: Sar - Arg - Val - Tyr - Val - His - Pro - Ala

Sar^1,Ile^5,Ile^8-AII: Sar - Arg - Val - Tyr - Ile - His - Pro - Ile

Sar^1,Ile^5,Thr^8-AII: Sar - Arg - Val - Tyr - Ile - His - Pro - Thr

The multiplicity of regio- and stereospecific interactions with the enzyme surface explains the specificity of captopril, which does not inhibit to any significant extent other proteolytic enzymes and does not interfere with the action of a large variety of agonists on smooth muscle, with the exception of angiotensin and bradykinin.[5] All the antihypertensive effects of captopril can and should, therefore, be explained by its inhibition of angiotensin-converting enzyme, which leads to a modulation of the levels of angiotensin II and bradykinin and, eventually, of aldosterone (Figures 1 and 2). Some authors have attempted to find antihypertensive properties in captopril that are independent from the inhibition of angiotensin-converting enzyme, but convincing evidence is still lacking.

Captopril has shown significant antihypertensive activity in acute and chronic animal models of renovascular hypertension and also in spontaneously hypertensive rats, indicating that clinical efficacy should be expected not only in renovascular but also in essential hypertension.[5] The clinical experience accumulated so far has amply confirmed these expectations.[52,53] Whether the widespread antihypertensive activity of this angiotensin-converting enzyme inhibitor is only the result of the blockade of the renin–angiotensin system remains still controversial, since a large number of patients that respond to captopril therapy have "normal" levels of plasma renin. However, as indicated above, circulating levels of renin might not be the best monitor of renin–angiotensin system activation.

Captopril has now been studied in well over 1000 patients and in some cases for periods longer than 1 year. Normalization of blood pressure has been observed in approximately 50% of the patients. Combined therapy with diuretics extends considerably the antihypertensive effects of captopril, particularly in mild to moderate hypertension where the rate of responders to this combination therapy can reach 90% or higher.

One of the most exciting developments in the therapeutic application of angiotensin-converting enzyme inhibitors has been the demonstration of their considerable usefulness in the treatment of congestive heart failure, either with the nonapeptide teprotide[54] or with captopril.[55,56]

Even though several types of side effects have been observed during therapy with captopril,[52] this new antihypertensive therapy lacks the side effects associated with other common antihypertensive agents that interact with the central and peripheral nervous systems.

The outstanding antihypertensive activity of captopril and the fact that its development was the result of a rational ab initio approach to drug design have attracted the attention of all medicinal chemists involved in cardiovascular research, and new types of angiotensin-converting enzyme inhibitors will undoubtedly reach clinical experimentation in the near future. Patchett and collaborators have recently disclosed the development of angiotensin-converting enzyme inhibitors based on the principle of establishing multiple and specific interactions with the active site to achieve a high degree of binding. The preliminary pharmacological evaluation indicates that these inhibitors are very effective antihypertensive agents in animal models of renovascular and essential hypertension.[57]

Angiotensin II Antagonists

Angiotensin II plays a pivotal role in the renin–angiotensin system, since it is through the interaction of this octapeptide with different tissue receptors that all the actions of the system are mediated.

One of the first approaches to the blockage of the renin–angiotensin system was the development of angiotensin II antagonists, and this development was the fulfillment of quite a few years of research on the structure–activity relationships among angiotensin II analogues. The historical sequence of these studies has been reviewed recently.[1] The angiotensin II antagonists developed so far are all analogues of angiotensin II in which the phenylalanine residue in the 8 or C-terminal position has been replaced by another amino acid, almost always an aliphatic one branched at the β or α position. Since the original observation of Pals et al.[58] that substitution of the aspartic acid in position 1 increased the in vivo antagonist activity of 8-Ala-angiotensin II, probably by a combination of increased binding affinity and decreased susceptibility to enzymatic degradation, most of the potent antagonists developed so far contain this substitution.

The antagonist that has been most widely studied in laboratory animals and in man is [Sar^1,Val^5,Ala^8]-angiotensin II (saralasin) (Chart I),[59] but other analogues, e.g., Sar^1,Ile^5,Ile^8-angiotensin II and Sar^1,Ile^5,Thr^8-angiotensin II, have also received a considerable amount of attention.[1] All of the antagonists developed so far have retained variable amounts of agonistic activity.

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Angiotensin antagonists can exert a substantial antihypertensive effect on animal models of renovascular hypertension, particularly during the early phase, which has, therefore, been assumed to be renin dependent. During the chronic phase of these hypertensive models, angiotensin II antagonists are devoided of antihypertensive effect, unless the animal is sodium depleted.

In the spontaneously hypertensive rats, the results have been somewhat contradictory, since utilizing different analogues some investigators have claimed significant blood pressure lowering effects, while others have not been able to observe any significant effect.[1]

The availability of antagonists of angiotensin II has played an important role in the clarification of the mechanism of activation of the renin–angiotensin system and this role will certainly be increased if tissue- or organ-specific antagonists can be developed. Several investigators have advocated their use as a diagnostic tool to classify hypertensive patients and determine which ones have angiotensin II dependent hypertension, even though other investigators would prefer to use angiotensin-converting enzyme inhibitors for the same purpose.[60] The therapeutic application of angiotensin antagonists in hypertension has been thwarted by the need of utilizing a parenteral route for their application and by the brief duration of their action.

Where does the future lie in angiotensin antagonists? Some investigators would like to develop angiotensin analogues that have antagonistic activity and tissue specificity. This is a very worthwhile research goal, but it is unlikely that peptidic analogues of angiotensin II could lead to therapeutically useful antihypertensive agents, specifically, agents that can be used orally and have a reasonable duration of action. Attempts to uncover angiotensin antagonists by a standard screening procedure have met with failure.[61] It is tempting to speculate that with the increased understanding on the conformation of angiotensin II in solution[1] it might become possible to design analogues that will combine the binding determinants of the octapeptide in a nonpeptidic structure that could lead to specific and orally active angiotensin antagonists.

Conclusion

The decade of the 1960's witnessed an increased interest in the physiological and pathological role of the renin–angiotensin system based on the pioneering discoveries made in the 1940's and 1950's. In the 1970's, the development of blockers of the renin–angiotensin system, namely, angiotensin antagonists and angiotensin-converting enzyme inhibitors, showed that this system plays, indeed, an important role in the pathogenesis of hypertension. The 1980's will see a more clear definition of this role and establish angiotensin-converting enzyme inhibitors and, perhaps, new renin inhibitors and angiotensin antagonists as a distinctive group of antihypertensive agents, equally or more important than diuretics, β-blockers, vasodilators, and centrally acting antihypertensive agents.

In the search for new inhibitors of the renin-angiotensin system, a thorough understanding of the physicochemistry of drug–target interactions will play the most critical role, and the medicinal chemists will certainly rise to meet this new challenge in drug design.

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