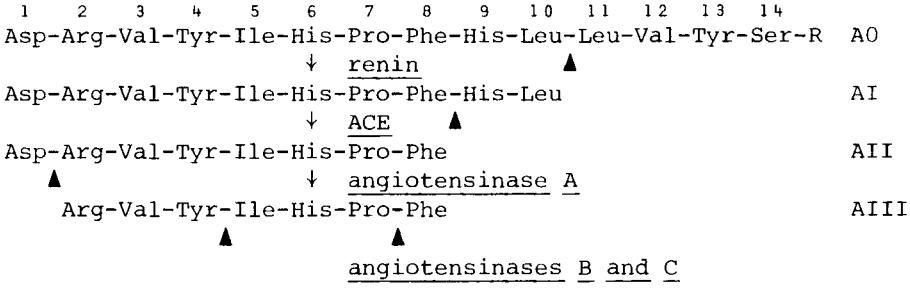
Chapter 9. Inhibitors of the Renin-Angiotensin System

Miguel A. Ondetti and David W. Cushman
The Squibb Institute for Medical Research
P.O. Box 4000, Princeton, N.J. 08540

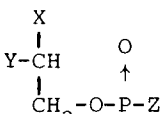
Introduction: The renin-angiotensin system[1-9] consists of blood-borne and tissue-bound peptidases and their protein or peptide substrates that participate in the maintenance of blood pressure in low volume or low sodium states, and in the elevation of blood pressure in certain forms of hypertensive disease. Renin[3-5], the rate-limiting enzyme, is released into the blood from the kidney in response to hyponatremia or low blood pressure; it cleaves a blood glycoprotein angiotensinogen (AO) between leucine residues 10 and 11 to liberate the biologically inactive decapeptide angiotensin I (AI). Circulating AI is converted in lung, kidney and other tissues to the pressor octapeptide angiotensin II (AII) by a peptidyldipeptide hydrolase commonly referred to as angiotensin-converting enzyme (ACE)[6,7]. Circulating AII directly constricts arterioles and produces an immediate elevation of blood pressure; either AII or its des-Asp[1]-heptapeptide derivative angiotensin III (AIII)[8] stimulates release from the adrenal gland of the sodium-retaining steroid aldosterone which causes a more gradual increase in extracellular fluid volume and a sustained increase in blood pressure. Angiotensins I-III are quickly destroyed by blood and tissue enzymes referred to as angiotensinases[9]. Pharmacological interruption of the renin-angiotensin system has been a powerful tool for elucidating its role in hypertensive disease. In this brief review we will discuss various approaches to the inhibition of different components of this system, but we will concentrate on recent developments concerning those agents that have the greatest potential for therapeutic use.

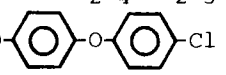


Renin Inhibitors: Renin is inhibited *in vitro* by high concentrations (0.4 to 2 mM) of naturally-occurring renal lysophosphatidylethanolamines[10,12], synthetic phosphatidylethanolamines[11] and lysophosphatidylethanolamine analogs[12,13], and also by neutral lipids and phosphatidylcho-

Copyright © 1978 by Academic Press, Inc.
All rights of reproduction in any form reserved.
ISBN 0-12-040513-X

line derivatives[14]. In spite of their weak inhibitory activity *in vitro*, several of these compounds significantly lower blood pressure in the Goldblatt two-kidney renal hypertensive rat (RHR), a renin-mediated hypertensive model in which one renal artery is constricted and the other kidney is left intact. The renal phospholipid and synthetic analogs 1 and 2 given by daily intramuscular injections (7-50 mg/kg) produced a progressive lowering of blood pressure that reached a maximum of 40-50 mm Hg within 2-4 days[10,12]. Analog 2 also produced an 85% inhibition of plasma renin activity in the RHR[12]. The phosphorylethanolamine analog PE-104 (3) was shown to be a specific competitive inhibitor of renin *in vitro* (K_i=2 mM). Upon intravenous infusion at a total dose of 200 mg/kg, it produced a 26 mm Hg lowering of blood pressure and a partial

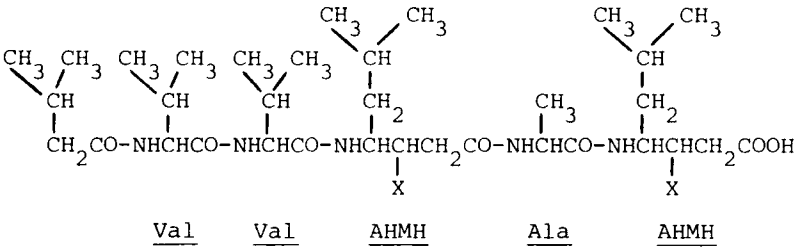


	X	Y	Z
1	$-CH_2O_2C(CH_2)_{16}CH_3$	$CH_3(CH_2CH{=}CH)_3(CH_2)_7CO_2-$	$-OCH_2CH_2NH_2$
2	$-CH_2CH_2(CH{=}CHCH_2)_4(CH_2)_3CH_3$	H-	$-CH_2CH_2NH_2$
3	$-NHCOCH_2O$- 	H-	$-OCH_2CH_2NH_2$

inhibition of renin activity in RHR[13]; its renin inhibitory activity was reported to be of short duration. It remains to be conclusively demonstrated whether or not the antihypertensive actions of analogs 1-3 are related to their weak renin inhibitory activity.

4: X=OH

5: X=H



Val Val AHMH Ala AHMH

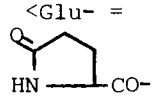
Pepstatin A (4) is an N-acetylated pentapeptide of microbial origin that contains two residues of the unusual amino acid 4-amino-3-hydroxy-6-methylheptanoic acid (AHMH) and is a potent inhibitor of acid proteases such as pepsin, cathepsin D, and renin[15-17]. Structure-activity studies[17,18] indicate that the hydrophobic side-chains and the first AHMH residue of pepstatin are important for its inhibitory activity. Such results have led to the suggestion that pepstatin is a transition-state analog, with the hydroxyl group of the first AHMH residue mimicking the tetrahedral transition state of the scissile peptide carbonyl. In apparent support of this concept, the dideoxy analog 5 is a competitive inhibitor of pepsin, with a 1000-fold lower inhibitory action than pepstatin[18]. In rats, intravenous doses of 0.2 to 0.8 mg/kg (limited by the poor aqueous solubility of pepstatin) inhibit the pressor response to infused renin, and lower blood pressure in RHRs[16,19], but with a duration of only 10 to 15 minutes.

Several structural analogs of the renin substrate are reasonably potent competitive inhibitors of renin *in vitro*; but none has yet been shown to inhibit significantly the action of renin *in vivo*. Tetrapeptide ester 6 and similar analogs[20] were shown to be weak competitive inhibitors of renin at pH 6.4 (I_{50}=0.5 to 1.0 mM), and inhibited only partially

6	Leu-Leu-Val-Phe-OEt	
7	His-Pro-Phe-His-Leu-*Leu*-Val-Tyr	*Leu* = D-Leu
8	Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-*Leu*-Val-Tyr-Ser	
9	Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr	

the blood pressure response to renin at a total intravenous dose of 50 mg in rabbits. Octapeptide analog 7 and tridecapeptide analog 8, with D-leucine incorporated into the bond normally cleaved by renin, are potent inhibitors of renin at pH 5.5 (K_i=3 μM and 7 μM, respectively)[21,22]; analog 7, however, was shown to be inactive at neutral pH[23]. Recently the phenylalanine substituted analog (9) was shown to be a potent inhibitor of human renin at physiological pH (K_i=1.0 μM), but a poor inhibitor of non-primate renins[23,24].

Inhibitors of Angiotensin-Converting Enzyme: The peptidyldipeptide hydrolase usually referred to as angiotensin-converting enzyme (ACE) also inactivates the vasodepressor nonapeptide bradykinin (BK)[25]; for this reason, all inhibitors of ACE have two different but related biological activities: inhibition of AI actions and augmentation or prolongation of BK actions. Both of these activities, however, can lead to lowering of blood pressure. Two distinct classes of specific competitive inhibitors of ACE have been useful in clinical studies: peptides originally isolated from snake venoms, and acylamino acids designed for specific interaction with ACE.

10 (BPP_{5a})	<Glu-Lys-Trp-Ala-Pro	<Glu- = 
11 (teprotide)	<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	

A mixture of bradykinin-potentiating peptides (BPP) from the venom of the Brazilian pit viper *Bothrops jararaca*[26] was shown in 1968 to inhibit ACE[27]. A number of peptides were isolated from this venom as bradykinin potentiators[28] or as ACE inhibitors[29]; and similar bradykinin potentiating peptides were isolated from the Asian viper *Agkistrodon halys Blomhoffii*[30]. One of the bradykinin-potentiating peptides from *B. jararaca* (BPP_{5a}, 10) was sequenced, synthesized, and shown also to be a potent inhibitor of ACE[28,31]. Six of the ACE inhibitors from *B. jararaca* were also sequenced, synthesized, and shown to be bradykinin-potentiating peptides as well as competitive inhibitors of ACE[29,32,33]; the most potent and thoroughly characterized of these was the nonapeptide SQ 20,881 (11). Peptide 11, employed as a bradykinin-potentiating peptide, has also been referred to as BPP_{9a} (see Table II of reference 33), or sometimes as CEI (converting enzyme inhibitor)[34]. Structure-activity studies with analogs of 10 and 11 have revealed the importance of the free carboxyl group and the C-terminal tripeptide sequence for competitive binding at the active site of ACE; certain of the other (N-terminal) amino acid residues also apparently bind

to the enzyme and contribute to the overall inhibitory potency[35]. Replacement of the glutamine in 11 by lysine appears to increase tissue binding *in vitro*[36].

The *Agkistrodon* peptides[30,37] have not been extensively tested *in vivo*. The pentapeptide 10 was found to inhibit vasopressor responses to AI and augment vasodepressor responses to BK in rats, and also to lower blood pressure in two-kidney RHR; but its potency and duration of action were much less than those of the nonapeptide 11, which has been the only peptidic inhibitor of ACE used in clinical studies. At intravenous doses of 3 to 25 mg/kg, 11 produced a dramatic and prolonged lowering of blood pressure in two-kidney RHR[32]. Similar doses lowered blood pressure of dogs made hypertensive by constriction of one renal artery[34], and produced marked increases in plasma renin activity, presumably by interrupting a direct negative feedback action of AII on renin release. The nonapeptide 11 was not hypotensive in normal animals, but significantly lowered blood pressure in sodium depleted rats[34,38,39] and prevented the increase in plasma aldosterone that normally results from sodium depletion[38,39].

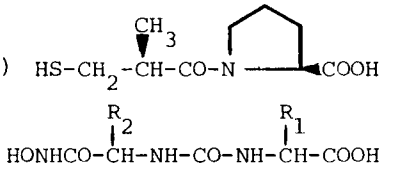
Compound 11 was also not hypotensive in normal human volunteers; but at an intravenous dose of 0.25 mg/kg a marked hypotensive response was obtained in sodium depleted subjects after a rapid 70° tilt. Plasma renin activity rose dramatically, but the normal increase in aldosterone was blocked[34]. In 65 hypertensive patients of various etiologies including renovascular, malignant, and essential, all high-renin patients and more than 90% of the "normal-renin" patients showed a lowering of blood pressure after intravenous administration of 11 at 1 mg/kg[40]. In earlier studies[41], its duration of action, at doses of 1-4 mg/kg, was shown to be as great as 16 hours, and the antihypertensive action was shown to be potentiated by mild sodium depletion.

Since the renin-angiotensin and kallikrein-kinin systems may increase in parallel in response to stimuli such as sodium depletion, it is not easy to assess the relative contributions of AII inhibition and BK augmentation to the antihypertensive action of 11. No increase in plasma BK was found during the hypotension produced by 11 in sodium depleted subjects[42], but a 2.3-fold increase in plasma BK was observed in sodium depleted essential hypertensive patients[43]. Unlike most antihypertensive drugs, this nonapeptide increased renal blood flow in spite of its lowering of blood pressure[43,44]; this intrarenal effect was not associated with an increase in BK[44].

Other potential uses of ACE inhibitors have been explored. Several investigators (see reference 45) have suggested the possible use of the nonapeptide 11 for treatment of shock. At 1 mg/kg, 11 protected dogs from death following hemorrhagic shock; AII levels during shock were decreased 10-fold, and increases in vasopressin were unaffected. Studies with 11 have also indicated that the renin-angiotensin system is essential for the compensatory response to circulatory insufficiency in an animal model of congestive heart failure[46]. In patients with established heart failure, this nonapeptide improved cardiac function by decreasing

peripheral resistance and afterload on the heart[47].

Recently, a second class of potent and specific inhibitors of ACE has been developed that has the additional advantage of oral activity[48]. These compounds, carboxyalkanoyl and mercaptoalkanoyl amino acid derivatives, were designed for optimal binding to the active site of ACE as visualized in a hypothetical model based on the known active site of a similar exopeptidase, carboxypeptidase A[48,49], which, like ACE, has a Zn ion at the active site. The most potent inhibitor of this class is SQ 14,225 [D-3-mercapto-2-methylpropanoyl-L-proline (S,S), 12], a competitive inhibitor with a K_i value of 1.7×10^{-9} M. The sulfhydryl function, amide carbonyl, and carboxylic acid group of 12 are thought to bind to the zinc ion, a hydrogen bonding group, and a positively charged residue, respectively, at the active site of ACE[48,49]. Structure-activity studies[49] have confirmed the great importance of these three structural features of this inhibitor and have also indicated that the acyl chain length, the 2-methyl substituent, and the pyrrolidine ring are optimal for binding to the enzyme. Hydroxamic acid derivatives related to 12[50] and ureidoacetohydroxamates (13)[51] have also been described as ACE inhibitors. In the interaction of these compounds with the enzyme, the hydroxamic acid functionality probably binds to the Zn ion.



12 (captopril)

13

In rats, 12 administered orally was at least ten times more inhibitory, on a weight basis, than the nonapeptide 11 administered parenterally[52]; it specifically inhibited AI vasopressor activity and augmented BK vasodepressor activity in rats, cats, dogs, and rabbits[52-55]. Administered centrally, 12 did not affect blood pressure, but did inhibit central effects of AI; the compound, however, did not readily cross the blood-brain barrier[55]. At 3-30 mg/kg oral doses, it rapidly lowered blood pressure by 50 to 60 mm Hg in two-kidney RHR. At the higher dose, blood pressure did not return to normal in 24 hours[56]. The drug lowered more slowly the blood pressure of spontaneously hypertensive rats (SHR)[56,57], with about a 30 mm Hg drop, but did not appreciably affect pressures of normotensive rats. Only a mild tachycardia was observed with 12; this was not related to the degree or suddenness of the blood pressure drop. After 11 days of treatment in spontaneously hypertensive rats, no evidence was obtained for decreased responsiveness[56]. Compound 12 also inhibited the vasopressor activity of AI in human volunteers[58]; total blockade for 2 hours was obtained at an oral dose of 0.3 mg/kg. No untoward side effects or changes in biochemical parameters were observed in this first clinical study.

Angiotensin-Receptor Antagonists: Extensive structure-activity studies on analogs of AII carried out since its original synthesis in 1957 led in 1970 to the development of specific AII receptor antagonists: [Ile^5, Ala^8]-AII was shown to inhibit the contractile response of guinea pig ileum to AII[59], and [Phe^4, Val^5, Tyr^8]-AII was shown to be a competitive inhibitor of the AII actions *in vitro* and *in vivo*[60]. The Ala^8 analogs of

Ile^5-AII and Asn^1-Val^5-AII were later reported to inhibit the vasopressor action of AII in rats[61,62] but not in cats[63].

A major development in the search for useful AII antagonists was the observation that replacement of the asparagine residue in position 1 of the Asn^1-Val^5-Ala^8-AII analog by sarcosine[64] led to a substantial increase in the antagonistic activity. Subsequently, similar observations were made with the Ile^8, Leu^8, and Phg^8 analogs[65-67]. Since that time, a considerable effort has been devoted to the development of new and more specific antagonists and to the reduction of the intrinsic agonistic activity of these compounds. Structure-activity studies have been recently reviewed[37,68], and several papers have appeared since these reviews that have further refined our understanding of the interactions between AII receptors and agonists or antagonists[69-71]. Based on these studies the following conclusions can be drawn. 1) Replacement of the Phe^8 residue of AII by an aliphatic amino acid is the single most important modification required to obtain an antagonist or to decrease substantially the agonistic activity. 2) Replacement of the N-terminal aspartic acid residue with sarcosine increases the agonistic or antagonistic potency *in vitro* and *in vivo*, mostly due to a stronger binding to, and a slower dissociation from, the receptor, but also and to a smaller extent by increasing resistance to enzymatic degradation[72]. 3) None of the antagonists developed so far is completely devoid of agonistic activity[73], and some authors[74] have emphasized that these compounds should be more correctly referred to as weak competitive agonists. 4) Some of the most commonly studied AII antagonists are Ile^5 analogs, and some are Val^5 analogs. Where this substitution was specifically examined[75,76], no major differences were observed, even though minor ones do appear to exist[76].

14	(saralasin)	Sar-Arg-Val-Tyr-Val-His-Pro-Ala
15	(Sar^1-Ala^8-AII)	Sar-Arg-Val-Tyr-Ile-His-Pro-Ala
16	(Sar^1-Ile^8-AII)	Sar-Arg-Val-Tyr-Ile-His-Pro-Ile
17	(Sar^1-Thr^8-AII)	Sar-Arg-Val-Tyr-Ile-His-Pro-Thr
18	(Ile^8-AIII)	Arg-Val-Tyr-Ile-His-Pro-Ile

The angiotensin antagonists that have been most thoroughly studied *in vivo* are saralasin (14), Sar^1-Ala^8-AII (15), Sar^1-Ile^8-AII (16), and Sar^1-Thr^8-AII (17). The Sar^1-Ile^8 and Sar^1-Thr^8 analogs are the most potent antagonists of the blood pressure increase induced by exogeneous AII[65,77,78]. Sar^1-Ile^8-AII has equal or more agonistic activity than Sar^1-Ala^8-AII, but Sar^1-Thr^8-AII has significantly less agonistic activity than either[77,78]. A more recently synthesized analog [Sar^1-Thr(Me)^8]-AII, has an even higher antagonist activity than the Thr^8 analog, but this modification unfortunately also increases significantly the agonistic activity[79]. The same comparative degree of agonistic activity of these analogs can be also observed in their stimulation of aldosterone release from the adrenal cortex[39,78] and catecholamine release from adrenal medulla[79]. All AII antagonists developed so far have a comparatively short duration of action *in vivo* and must be administered by continuous infusion.

Angiotensin antagonists (saralasin, Sar^1-Ile^8-AII, and Sar^1-Thr^8-AII) have been shown to lower blood pressure in the acute hypertensive phase of two-kidney RHR[64,80,81] and one-kidney RHR (unclipped kidney removed)[81] when infused at a maximal dose of 10 to 60 μg/kg/min. The acute (renin dependent) hypertensive phase in the one-kidney model is so short that in many cases angiotensin antagonists and other renin-angiotensin system blockers have been found inactive in this model. In the chronic phase of both models the renin dependency disappears, and angiotensin antagonists are inactive[64,80,81]. Similar results were obtained in a new model of two-kidney hypertension in conscious dogs[82]. Renin dependency and, therefore, response to angiotensin antagonists in these models can be reinstated by sodium depletion[80]. In the SHR, saralasin[64] and several analogs of Phg^8-AII[67] have been found inactive. However, Sar^1-Ile^8-AII (5.5 μg/kg/min)[83] and Sar^1-Thr^8-AII (1 μg/kg/min)[84] lower blood pressure in this model, and, in the case of Sar^1-Thr^8-AII, this effect appeared to be dependent on the age of the rats.

The availability of saralasin has prompted a considerable number of clinical studies with this angiotensin antagonist in normal subjects and hypertensive patients. Saralasin is usually administered by infusion, but bolus intravenous injection has been studied[85,86] for diagnostic purposes in hypertensive patients. In normal man, saralasin (10-20 μg/kg/min) has generally been found to lower blood pressure significantly only under the combined stimulus of sodium restriction and upright posture[87-89], and this response depended on the degree of sodium balance[90]. When Sar^1-Ile^8-AII and Sar^1-Ala^8-AII were compared in normal subjects[91] in the supine position, it was found that both analogs had vasopressor activity after salt repletion or regular diet, and that Sar^1-Ile^8-AII had vasopressor activity even in salt depleted subjects, thus confirming the higher agonistic activity of this analog found in animals.

In hypertensive patients, saralasin has been shown to produce consistent lowering of blood pressure only in those patients with high plasma renin activity[74,92-95], and similar results were obtained with Sar^1-Ile^8-AII[96,97]. Low-renin hypertensive patients showed vasopressor responses after saralasin infusion[74,88-90], and normal-renin patients have shown either no response, or a mildly vasopressor response[74]. Due to their residual agonistic activity, the angiotensin antagonists so far available for clinical studies can only be used reliably in the diagnosis of the renin component of hypertension in those cases where a clear vasodepressor response has been obtained. The usefulness of prior sodium depletion for this diagnosis has been proposed[92], and questioned[74].

The analogs obtained by replacing the Phe^8 residue of the heptapeptide AIII by an aliphatic amino acid residue are more specific antagonists of the AII-stimulated aldosterone secretion than the corresponding octapeptide analogs[68]. The Ile^8-AIII analog (18) has now been shown to have both agonistic and antagonistic activities on blood pressure and aldosterone secretion in normal human subjects[98], even though its effect is more pronounced on the adrenal function than on the peripheral vasculature. The data gathered so far utilizing this new heptapeptide inhibitor have

not provided conclusive evidence for the hypothesis that the conversion of AII to AIII is a necessary step in the stimulation of aldosterone biosynthesis[73]. The recent finding[99] that rat adrenal tissue has binding sites with high affinity, specific only for AIII and sites with lower affinity that can bind both AII and AIII, suggests, however, that under physiological conditions AIII may be the normal stimulus for aldosterone secretion.

Conclusions: Renin inhibitors have a unique potential for studying the pathophysiological function of the renin-angiotensin system, since they lack the ambiguity of angiotensin antagonists (partial agonism) or ACE inhibitors (bradykinin potentiation). However, none of the inhibitors of renin that have been developed to date have been thoroughly evaluated *in vivo*. Parenterally administered AII antagonists and ACE inhibitors have contributed significantly to our understanding of the renin angiotensin system and its role in human hypertension. The orally active ACE inhibitors show great promise as a novel type of antihypertensive drug.

References

1. S. Oparil, "Renin 1976", Eden Press, Montreal, 1976.
2. M. J. Peach, Physiol. Rev., 57, 313 (1977).
3. L. T. Skeggs, F. E. Dorer, J. R. Kahn, K. E. Lentz, and M. Levine, Am. J. Med., 60, 737 (1976).
4. L. T. Skeggs, M. Levine, K. E. Lentz, J. R. Kahn, and F. E. Dorer, Fed. Proc., 36, 1755 (1977).
5. E. Haber, Circulation, 54, 849 (1976).
6. R. L. Soffer, Ann. Rev. Biochem., 45, 73 (1976).
7. E. G. Erdös, Fed. Proc., 36, 1760 (1977).
8. J. O. Davis and R. H. Freeman, Biochem. Pharmacol., 26, 93 (1977).
9. P. A. Khairallah and M. M. Hall in "Hypertension: Physiopathology and Treatment", J. Genest, E. Koiw, O. Kuchel, Eds., McGraw-Hill, New York, N.Y., 1977, p. 179.
10. S. Sen, R. R. Smeby, and F. M. Bumpus, Biochemistry, 6, 1572 (1967).
11. M. Miyazaki and K. Yamamoto, Proc. Soc. Exp. Biol. Med., 155, 468 (1977).
12. J. C. Turcotte, C. S. Yu, H. L. Lee, S. K. Pavanaram, S. Sen, and R. R. Smeby, J. Med. Chem., 18, 1184 (1975).
13. K. Hosoki, M. Miyazaki, and K. Yamamoto, J. Pharmacol. Exp. Therap., 203, 485 (1977).
14. M. Overturf, E. R. Druilhet, and W. M. Kirkendall, Biochem. Pharmacol., 25, 2443 (1976).
15. F. Gross, J. Lazar, and H. Orth, Science, 175, 656 (1972).
16. R. P. Miller, C. J. Poper, C. W. Wilson, and E. DeVito, Biochem. Pharmacol., 21, 2941 (1972).
17. T. Aoyagi, H. Morishima, R. Nishizawa, S. Kunimoto, T. Takeuchi, and H. Umezawa, J. Antibiot., 25, 689 (1972).
18. D. H. Rich, E. Sun, and J. Singh, Biochem. Biophys. Res. Commun., 74, 762 (1977).
19. B. A. Schölkens and W. Jung, Arch. Int. Pharmacodyn., 208, 24 (1974).
20. T. Kokubu, K. Hiwada, T. Ito, E. Ueda, Y. Yamamura, T. Mizoguchi, and K. Shigezane, Biochem. Pharmacol., 22, 3217 (1973).
21. K. Poulsen, J. Burton, and E. Haber, Biochemistry, 12, 3877 (1973).
22. I. Parikh and P. Cuatrecasas, Biochem. Biophys. Res. Commun., 54, 1356 (1973).
23. K. Poulsen, J. Burton, and E. Haber, Prog. Biochem. Pharmacol., 12, 135 (1976).
24. K. Poulsen, E. Haber, and J. Burton, Biochim. Biophys. Acta, 452, 533 (1976).
25. E. G. Erdös, Biochem. Pharmacol., 25, 1563 (1976).
26. S. H. Ferreira, Brit. J. Pharmacol., 24, 163 (1965).
27. Y. S. Bakhle, Nature, 220, 919 (1968).
28. S. H. Ferreira, D. C. Bartelt, and L. J. Greene, Biochemistry, 9, 2583 (1970).
29. M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluščec, E. R. Weaver, and O. Kocy, Biochemistry, 10, 4033 (1971).
30. H. Kato and T. Suzuki, Biochemistry, 10, 972 (1971).
31. L. J. Greene, A. C. M. Camargo, E. Krieger, J. M. Stewart, and S. H. Ferreira, Circ. Res., 30, II-62 (1972).

32. S. L. Engel, T. R. Schaeffer, M. H. Waugh, and B. Rubin, Proc. Soc. Exp. Biol. Med., 143, 483 (1973).
33. H. S. Cheung and D. W. Cushman, Biochim. Biophys. Acta, 293, 451 (1973).
34. E. Haber and C. Barger, Prog. Biochem. Pharmacol., 12, 16 (1976).
35. D. W. Cushman, J. Pluščec, N. J. Williams, E. R. Weaver, E. F. Sabo, O. Kocy, H. S. Cheung, and M. A. Ondetti, Experientia, 29, 1032 (1973).
36. E. B. Sabia, M. Tomenaga, A. C. M. Paiva, and T. B. Paiva, J. Med. Chem., 20, 1679 (1977).
37. G. R. Marshall, Fed. Proc., 35, 2494 (1976).
38. G. A. Stephens, J. O. Davis, R. H. Freeman, B. E. Watkins, and M. C. Khosla, Endocrinology, 101, 378 (1977).
39. R. E. McCaa, Circ. Res., 40, I-157 (1977).
40. D. B. Case, J. M. Wallace, H. J. Keim, M. A. Weber, J. E. Sealey, and J. H. Laragh, New Eng. J. Med., 296, 641 (1977).
41. H. Gavras, H. R. Brunner, J. H. Laragh, J. E. Sealey, I. Gavras, and R. Vukovich, New Eng. J. Med., 291, 817 (1974).
42. J. Sancho, R. Re, J. Burton, A. C. Barger, and E. Haber, Circulation, 53, 400 (1976).
43. G. H. Williams and N. K. Hollenberg, New Eng. J. Med., 297, 184 (1977).
44. N. K. Hollenberg, G. H. Williams, K. J. Taub, I. Ishikawa, C. Brown, and D. F. Adams, Kidney Int., 12, 285 (1977).
45. J. J. Morton, P. F. Semple, I. M. Ledingham, B. Stuart, M. A. Tehrani, A. R. Garcia, and G. McGarrity, Circ. Res., 41, 301 (1977).
46. A. C. Barger, Agents and Actions, 6, 538 (1976).
47. P. Faxon, J. Berkoben, H. Gavras, P. Flessas, and T. J. Ryan, Circulation, 56, 117, (1977).
48. M. A. Ondetti, B. Rubin, and D. W. Cushman, Science, 196, 441 (1977).
49. D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, Biochemistry, 16, 5484 (1977).
50. M. A. Ondetti, E. F. Sabo, K. A. Losee, H. S. Cheung, D. W. Cushman, and B. Rubin, "Peptides, Proceedings of the Fifth American Peptide Symposium", M. Goodman and J. Meienhofer, Eds., John Wiley & Sons, New York, N.Y., 1977, p. 576.
51. D. C. Fessler, G. A. Heavner, and T. H. Massay, U.S. Patent #4,028,401, June 7, 1977.
52. B. Rubin, R. J. Laffan, D. G. Kotler, E. H. O'Keefe, D. DeMaio, and M. E. Goldberg, J. Pharmacol. Exp. Therap. (In Press).
53. V. S. Murthy, T. L. Waldron, M. E. Goldberg, and R. R. Vollmer, Eur. J. Pharmacol., 46, 207 (1977).
54. V. S. Murthy, T. L. Waldron, and M. E. Goldberg, Proc. Soc. Exp. Biol. Med., 157, 121 (1978).
55. R. R. Vollmer and J. A. Boccagno, Eur. J. Pharmacol., 45, 117 (1977).
56. R. J. Laffan, M. E. Goldberg, J. P. High, T. R. Schaeffer, M. H. Waugh, and B. Rubin, J. Pharmacol. Exp. Therap. (In Press).
57. E. E. Muirhead, R. L. Prewitt, B. Brooks, and W. L. Brosius, Clin. Res., 25, A603 (1977).
58. R. K. Ferguson, G. A. Turini, H. R. Brunner, H. Gavras, and D. McKinstry, Lancet, 775, (1977).
59. P. A. Khairallah, A. Toth, and F. M. Bumpus, J. Med. Chem., 13, 181 (1970).
60. G. R. Marshall, W. Vine, and P. Needleman, Proc. Nat. Acad. Sci., 67, 1624 (1970).
61. W. K. Park and D. Regoli, Brit, J. Pharmacol., 43, 418P (1971).
62. D. T. Pals, F. D. Masucci, F. Sipös, and G. S. Denning, Jr., Circ. Res., 29, 664 (1971).
63. R. K. Türker, M. Yamamoto, P. A. Khairallah, and F. M. Bumpus, Eur. J. Pharmacol., 15, 285 (1971).
64. D. T. Pals, F. D. Masucci, G. S. Denning, Jr., F. Sipos, and D. C. Fessler, Circ. Res., 29, 673 (1971).
65. R. K. Türker, M. M. Hall, M. Yamamoto, C. S. Sweet, and F. M. Bumpus, Science, 177, 1203 (1972).
66. D. Regoli, F. Rioux, and N. K. Park. Rev. Can. Biol., 31, 73 (1972).
67. B. A. Schoelkens, Clin. Sci. Mol. Med., 48, 19s (1975).
68. F. M. Bumpus and M. C. Khosla in "Hypertension: Physiopathology and Treatment", J. Genest, E. Koiw, O. Kuchel, Eds., McGraw-Hill, New York, N.Y., 1977, p. 183.
69. M. C. Khosla, M. M. Hall, H. Muñoz-Ramírez, P. A. Khairallah, F. M. Bumpus, J. H. Seely, and A. M. Thomas, J. Med. Chem., 20, 253 (1977).
70. M. C. Khosla, H. Muñoz-Ramírez, M. M. Hall, P. A. Khairallah, and F. M. Bumpus, J. Med. Chem., 20 , 1051 (1977).
71. A. C. M. Paiva, V. L. A. Nouailhetas, and T. B. Paiva, J. Med. Chem., 20, 898 (1977).
72. D. Regoli, F. Rioux, W. K. Park, and C. Choi, Can. J. Physiol. Pharmacol., 52, 39 (1974).

73. F. M. Bumpus, Fed. Proc., 36, 2128 (1977).
74. D. B. Case, J. M. Wallace, H. J. Keim, J. E. Sealey, J. H. Laragh, Am. J. Med., 60, 825 (1976).
75. D. Regoli, W. K. Park. F. Rioux, Pharmacol. Rev., 26, 69 (1974).
76. B. A. Schoelkens, H. Wissmann, E. Lindner, R. Geiger, Z. Physiol. Chem., 357, 825 (1976).
77. H. Muñoz-Ramírez, M. C. Khosla, M. M. Hall, F. M. Bumpus, and P. A. Khairallah, Res. Commun. Chem. Path. Pharmacol., 13, 649 (1976).
78. E. L. Bravo, M. C. Khosla, and F. M. Bumpus, Prog. Biochem. Pharmacol., 12, 33 (1976).
79. M. C. Khosla, H. Muñoz-Ramírez, M. M. Hall, R. R. Smeby, P. A. Khairallah, F. M. Bumpus, and M. J. Peach, J. Med. Chem., 19, 244 (1976).
80. H. Gavras, H. R. Brunner, H. Thurston, and J. H. Laragh, Science, 188, 1316 (1975).
81. C. M. Ferrario, F. M. Bumpus, Z. Masaki, M. C. Khosla, and J. W. McCubbin, Prog. Biochem. Pharmacol., 12, 86 (1976).
82. Z. Masaki, C. M. Ferrario, F. M. Bumpus, E. L. Bravo, and M. C. Khosla, Clin. Sci. Mol. Med., 52, 163 (1977).
83. T. G. Dimitrov, D. I. Kiprov, A. I. Maleeva, and S. A. Terziyska, Compt. Rend. Acad. Bulg. Sci., 30, 771 (1977).
84. H. Muñoz-Ramírez, M. C. Khosla, F. M. Bumpus, and P. A. Khairallah, Pharmacologist, 18, 229 (1976).
85. L. S. Marks, M. H. Maxwell, and J. J. Kaufman, Lancet, 2, 784 (1975).
86. D. H. P. Streeton, G. H. Anderson, Jr., and T. G. Dalakos, Am. J. Med., 60, 817 (1976).
87. R. H. Noth, S. Y. Tan, P. J. Mulrow, J. Clin. Endocrinol. Metab., 45, 10 (1977).
88. G. A. MacGregor and P. M. Dawes, Clin. Sci. Mol. Med., 51, 193s (1976).
89. J. J. Brown, W. C. B. Brown, R. Fraser, A. F. Lever, J. J. Morton, J. I. S. Robertson, E. A. Rosei, and P. M. Trust, Aust. N.Z. J. Med., 6, Suppl. 3, 48 (1976).
90. H. R. Brunner, H. Gavras, A. B. Ribeiro, L. Posternak, Prog. Biochem. Pharmacol., 12, 145 (1976).
91. T. Ogihara, T. Hata, H. Mikami, T. Mandai, and Y. Kumahara, Life Sci., 20, 1855 (1977).
92. D. H. P. Streeten, T. G. Dalakos, and G. H. Anderson, Jr., Prog. Biochem. Pharmacol., 12, 214 (1976).
93. Th. Philipp, H. Zschiedrich, and A. Distler, Klin, Wochensch., 55, 917 (1977).
94. R. Fagard, A. Amery, P. Lijnen, and T. Reybrouck, Eur. J. Clin. Pharmacol., 12, 1 (1977).
95. W. A. Pettinger, H. C. Mitchell, Fed. Proc., 35, 2521 (1976).
96. T. Yamamoto, T. Ogihara, T. Hata, K. Ichihara, Y. Kumahara, Jap. J. Med., 16, 117 (1977).
97. M. Yasujima, K. Abe, N. Irokawa, K. Ritsu, M. Seino, K. Saito, Y. Sakurai, S. Chiba, T. Ito, and K. Yoshinaga, Tohuka J. Exp. Med., 122, 191 (1977).
98. T. Kono, F. Oseko, F. Ikeda, M. Nanno, and J. Endo, Acta Endocrinol., 86, 156 (1977).
99. M. Devynck, M. Pernollet, P. G. Matthews, M. C. Khosla, F. M. Bumpus, and P. Meyer, Proc. Nat. Acad, Sci., 74, 4029 (1977).