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COMMENTARY

INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME FOR TREATMENT OF HYPERTENSION

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One elusive goal of biochemical pharmacologists has been the design of drugs for precise fit with their macromolecular receptors. At the present time, however, the only potential drug receptors for which small molecule interactions are understood in any detail are the active-sites of enzymes, and most of the best characterized enzymes do not appear to play essential roles in disease processes. However, catalytic mechanisms of enzymes tend to fall into general classes, and it is often possible by studying various properties of a biologically important enzyme to deduce the general nature of its active-site. In attempting to design specific inhibitors of such an enzyme, one usually need be concerned only with binding of the potential drug to its receptor (activesite), and not with secondary conformational changes that are important for 'activation' of membrane-bound receptors such as those mediating hormone action.

The history of development of specific inhibitors of an unusual peptidase known historically as 'angiotensin-converting enzyme' (EC 3.4.15.1) provides an instructive model for the study, in general, of enzyme inhibitors as drugs. In the study of inhibitors of this enzyme, it has been possible to develop a number of assay procedures *in vitro* and *in vivo* that have been of great importance for logical stepwise expansion from inhibitory action of a compound in cell-free extract to the ultimate use of inhibitors for treatment of human disease. It has also been possible, by considering the probable nature of the active-site of this enzyme, to optimize the interactions of inhibitors with the enzyme, and to develop drugs with outstanding potency and specificity.

Angiotensin-converting enzyme: a key enzyme in blood pressure regulation

Enzyme inhibitors can have therapeutic value only if the enzyme target plays a key role in some disease process. However, one cannot usually be certain of the pathophysiological importance of a particular enzyme until specific inhibitors have been developed and tested in animal models of the disease in question. Angiotensin-converting enzyme is potentially of great importance for regulation of blood pressure by virtue of either of two different reactions that it catalyzes (Fig. 1). Angiotensin I, the inactive decapeptide product of the action of renin (EC 3.4.99.19), is 'converted' to the potent hypertensive octapeptide angiotensin II, with the dipeptide His-Leu released as a byproduct [1, 2]. The same enzyme inactivates bradykinin, the hypotensive nonapeptide product of kallikrein (EC 3.4.21.8), again by hydrolytic release of one or more carboxyl-terminal dipeptide residues [2, 3]. Both reactions can contribute to increased blood pressure in the intact organism, and, thus, specific inhibitors of the enzyme have potential antihypertensive action.

Discovery and design of specific inhibitors

Two rather different inhibitors of angiotensin-converting enzyme have been studied as antihypertensive drugs (Fig. 2). Teprotide (SQ 20,881) is a nonapeptide that owes its potency and selectivity as a converting enzyme inhibitor to natural selection, since it is a normal component of the venom of a Brazilian viper, *Bothrops jararaca* [4, 5]. The evolutionary advantage of such an inhibitor to the snake

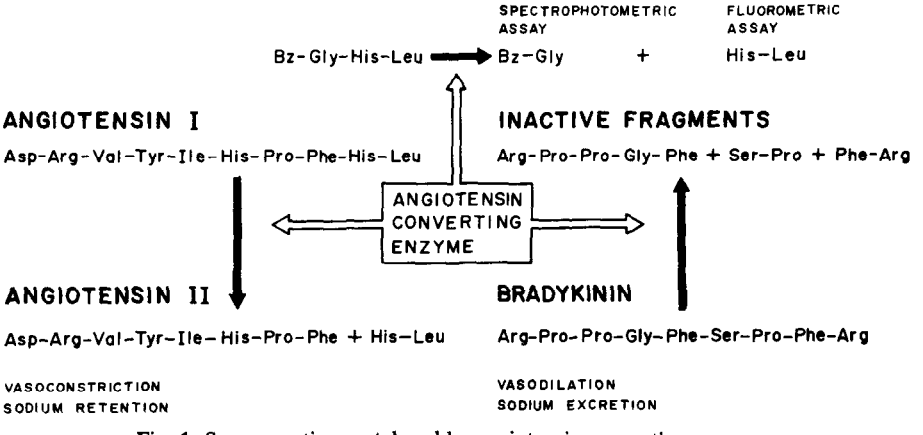

Fig. 1. Some reactions catalyzed by angiotensin-converting enzyme.

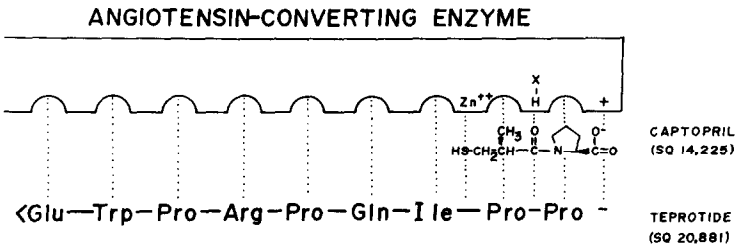

Fig. 2. Specific inhibitors of angiotensin-converting enzyme and their probable enzyme-binding interactions. The circular clefts represent subsites on or near the active-site of angiotensin-converting enzyme that may interact to varying degrees with the side-chains of amino acid residues of peptide substrates or inhibitors.

may be its ability to enhance the capillary permeability effect of another venom constituent, bradykinin, and thus to facilitate distribution of the toxic venom factors within the body of the victim. Captopril (SQ 14,225) owes its potency and selectivity to chemical design, guided by a hypothetical active-site model that was based on the observed properties of angiotensin-converting enzyme and on analogy to the known active-site of a related zinc-containing peptidase [6, 7].

The binding of teprotide to the active-site of angiotensin-converting enzyme has been studied mostly by testing the inhibitory activities of analogs of this nonapeptide with one or more amino acid substitutions and of similar analogs of the more potent but short-acting venom pentapeptide BPP_{5a} (<Glu-Lys-Trp-Ala-Pro). Such studies [8] have indicated that both of these competitive peptide inhibitors bind to angiotensin-converting enzyme as shown for teprotide in Fig. 2, with the negatively charged, terminal carboxyl group binding ionically with a postively charged residue on the enzyme, and with the carboxyl-terminal amino acid sequence lined up on the active-site of the enzyme in the same manner as the analogous sequence of a peptide substrate. A tripeptide with a free terminal carboxyl group is the minimal structural requirement for binding and cleavage of substrates by angiotensin-converting enzyme, and the analogous tripeptide sequence is of great importance for competitive binding of venom peptide inhibitors such as teprotide. The optimal tripeptide sequence for such competitive binding appears to be that found in BPP_{5a}, Trp-Ala-Pro, but the less favorable sequence, Ile-Pro-Pro, found in teprotide provides much greater biological stability [8]. Teprotide binds more tightly to angiotensin-converting enzyme than typical substrates because many of its amino acid residues beyond the carboxyl-terminal tripeptide residue interact with 'subsites', represented as circular clefts in Fig. 2, that do not interact as strongly with amino acid residues of substrates such as angiotensin I or bradykinin.

The binding of captopril to angiotensin-converting enzyme (Fig. 2) has been studied in much greater detail than that of teprotide. In fact, the relatively simple structure of captopril is the end result of an extensive series of structure–activity correlations designed to study and optimize each of five postulated interactions with the active-site of angiotensin-converting enzyme [6–8]. The resulting structure of captopril is partially analogous to the favorable carboxyl-terminal dipeptide residue, Ala-Pro, of BPP_{5a}, and four of its five strong interactions with the enzyme are similar to those of the two terminal amino acid residues of this venom pentapeptide. Captopril, however, cannot be cleaved by angiotensin-converting enzyme or by most other peptidases, and it has a fifth and very strong interaction of its sulfhydryl group with the tightly bound zinc ion of angiotensin-converting enzyme that is entirely different from any binding interactions of the snake venom peptides.

Development of inhibitors as antihypertensive drugs

It is usually not difficult to uncover reasonably potent inhibitors of any enzyme, or to quantitatively compare inhibitory activities of large numbers of compounds or structural analogs, if a reliable assay method is available for measuring enzyme activity in cell-free extract. However, other much less common properties that are essential for use of inhibitors as drugs, such as specificity, biological stability and adequate distribution within the whole organism, are not revealed by such a simple test *in vitro*, and tests in whole animals are usually much too variable and time consuming for use in searching for such favorable properties among large numbers of compounds. For development of inhibitors of angiotensin-converting enzyme, it has been of great importance to have available a series of test systems, each representing a level of biological complexity greater than the preceding one. These test systems have allowed not only accurate screening of large numbers of inhibitors, but also rapid elimination from consideration of those inhibitors with properties that would compromise their eventual use as antihypertensive drugs. This sequence of test systems provides a good model for systematic development of inhibitors of any biologically important enzyme, even though a complete logical series of such testing systems may be difficult to develop for certain enzymes.

Inhibition of the isolated enzyme. A simple quantitative assay procedure, essential for enzyme purification and characterization and for the initial testing of inhibitors, may not always be available for a particular biologically important enzyme that has not been 'popular' with enzymologists. Before 1970, the activity of angiotensin-converting enzyme was usually assayed by a bioassay method that took advantage of the difference in biological activity between angiotensins I and II. The enzyme was often studied by investigators with a physiological bias, to whom

angiotensin I appeared to be the 'natural' substrate for this enzyme, a bias that is not supported by the phylogeny or the presently understood substrate specificity of this particular enzyme. The development of simple spectrophotometric [9] and fluorometric [10] methods for quantitative assay of angiotensin-converting enzyme, using small peptide substrates such as Bz-Gly-His-Leu (hippuryl-L-histidyl-L-leucine) and Z-Phe-His-Leu (benzyloxycarbonyl-L-phenylalanyl-L-histidyl-L-leucine), has been essential for quantitative studies of properties of the enzyme. Bz-Gly-His-Leu (Fig. 1) is particularly useful for assay of angiotensin-converting enzyme activity under a great variety of circumstances. Unlike the angiotensins, it is not cleaved by most other types of peptidases present in tissue extracts. The most stable product, hippuric acid (Bz-Gly), can be measured spectrophotometrically with great accuracy, after extraction from assay incubation mixtures employing the crudest extracts as source of the enzyme. The other product, His-Leu, can be measured fluorometrically with great sensitivity, using slightly more purified preparations of the enzyme [11]. Such simple chemical assay methods were essential for fractionation of angiotensin-converting enzyme inhibitory peptides such as teprotide from the venom of *B. jararaca* [5, 11], and for the exhaustive structure–activity correlations used to optimize the structure of captopril for specific inhibition of angiotensin-converting enzyme [6–8].

Inhibition of the enzyme in excised smooth muscle. The quantitative inhibitory activities (IC_{50}* or K_i values) obtained for compounds by study of their inhibition of angiotensin-converting enzyme in cell-free extract give us a measure of their potency, but tell us little about their specificity as inhibitors. The evaluation of specificity by studying the inhibitory actions of compounds on several closely related peptidases (Table 1) is a time-consuming operation that cannot be performed routinely. In an excised smooth muscle such as guinea pig ileum, angiotensin-converting enzyme is present in its most natural (hydrophobic) environment, attached to cell membranes, and may, in this environment, show a somewhat different susceptibility to inhibitors than does the isolated, soluble enzyme. Guinea pig ileum also contains a complement of metabolizing enzymes that should be capable of inactivating many biologically unstable inhibitors, especially since activity of angiotensin-converting enzyme in this system cannot be artificially favored by manipulation of assay incubation conditions, as is the case with the isolated enzyme. Such a smooth muscle assay system represents a step toward greater biological complexity without any major problems of drug absorption or distribution. It also allows one to test readily for specificity of action of inhibitors of angiotensin-converting enzyme.

Contraction of a guinea pig ileum strip induced by angiotensin I is due to conversion of this decapeptide to the active contractile agent, angiotensin II, by converting enzyme within the tissue. Thus, specific inhibitors of angiotensin-converting enzyme, that can maintain their action within the muscle strip, inhibit contractions induced by angiotensin I, but not those induced by angiotensin II or most other biologically active agents that contract guinea pig ileum. However, since the same enzyme inactivates bradykinin, the contractile action normally observed with this peptide is greatly enhanced and prolonged by specific inhibitors of angiotensin-converting enzyme. Table 1 shows the specific effects obtained with teprotide and captopril [8, 12] on various agonists contracting guinea pig ileum. Both inhibitors at low concentrations block the contractile action of angiotensin I and augment that of bradykinin, but neither, at 500–20,000 times higher concentrations, has any effect on the contractile actions of a variety of other biologically active agents. Augmentation of bradykinin action is a particularly good index of

* Concentration of inhibitor causing 50 per cent inhibition.

Table 1. Actions *in vitro* of specific inhibitors of angiotensin-converting enzyme*

Test system	EC_{50} (μM)	
	Teprotide	Captopril
Peptidase inhibition		
Angiotensin-converting enzyme	0.56	0.02
Carboxypeptidase A	1800	1500
Carboxypeptidase B	520	800
Leucine aminopeptidase	31,000	69
Trypsin	1000	> 1000
Chymotrypsin	380	> 1000
Effect on myotropic action in guinea pig ileum		
Angiotensin I inhibition	0.060	0.023
Bradykinin potentiation	0.002	0.003
Angiotensin II	> 30	> 500
Acetylcholine	> 30	> 500
Histamine	> 30	> 500
Prostaglandin E_2	> 30	> 500

* EC_{50} is defined as the concentration producing a 50 per cent inhibition of peptidase activity, a 50 per cent augmentation of bradykinin-induced contraction of guinea pig ileum, or a 50 per cent inhibition of contraction induced by angiotensin I or other agonists.

specificity of action of inhibitors of angiotensin-converting enzyme, since non-specific compounds selected at random usually inhibit rather than potentiate the contractile action of this nonapeptide. A 50 per cent inhibition of the converting enzyme of guinea pig ileum usually leads to well over a 100 per cent augmentation of bradykinin action; thus, the EC_{50} values for bradykinin potentiation listed in Table 1 are much lower values (and more variable) than the IC_{50} values for inhibition of angiotensin I action.

Inhibition of the enzyme in vivo. The relatively few inhibitors of angiotensin-converting enzyme that retain their activity in guinea pig ileum and show the requisite specificity of action are tested for their primary enzyme inhibitory action in whole animals by studying their ability to inhibit blood pressure elevation produced by exogenous angiotensin I in the rat. A similar study of the effects of these compounds on the actions of bradykinin and other vasoactive agents allows confirmation *in vivo* of the specific action of a particular inhibitor. This initial test of inhibitors *in vivo* also provides a preliminary indication of their absorption, bioavailability, biological stability, duration of action, and toxicity or side effects.

Teprotide administered intravenously to rats at a dose of 0.5 mg/kg produced an 80 per cent inhibition of the vasopressor response to angiotensin I and a 150 per cent augmentation of the vasodepressor response of the animal to bradykinin, without affecting the blood pressure increase induced by angiotensin II [13]. This nonapeptide was also active by subcutaneous or intramuscular routes of administration, but was not orally active. Captopril, which is orally active, inhibited the pressor action of angiotensin I by 50 per cent and augmented the depressor action of bradykinin by at least 100 per cent at an oral dose of 0.17 mg/kg in the rat [12, 14]; it did not alter the blood pressure effects of angiotensin II or acetylcholine when administered at 1 mg/kg. In other species, captopril has been shown to have a similar specificity *in vivo*, and also to be without effect on blood pressure changes induced by catecholamines or prostaglandins [14]. The duration of the enzyme inhibitory action of teprotide or captopril in rats was dose dependent. After oral administration at 1 mg/kg, captopril maintained its maximal (85–100 per cent) inhibitory action for about 1 hr in rats [12, 14]. A similar maximal inhibitory effect of 1-hr duration would appear to require the intravenous administration of teprotide at about 5–10 mg/kg [13].

Antihypertensive action. Inhibitors of angiotensin-converting enzyme that meet the standards of potency, specificity, absorption, distribution and duration of action in the *in vitro* and *in vivo* tests describe above can then be tested for their desired therapeutic action in animal models of the disease against which they were initially targeted. Several animal models of hypertensive disease have been developed in rats and other species. The two kidney, one clip renal hypertensive rat appears to be a good model of renovascular hypertension in humans, with the elevated blood pressure associated with increased activity of the renin–angiotensin system during at least the first few weeks following the initial restriction of blood flow to one kidney [15]. This has been the primary model employed for detailed study of the antihypertensive activity of inhibitors of angiotensin-converting enzyme. Another useful model has been the spontaneously hypertensive rat, which develops high blood pressure of genetic origin without any obvious activation of the renin–angiotensin system [16]; this has often been considered to be a good model of human essential hypertension, that is, hypertension of unknown etiology.

Teprotide administered subcutaneously to two kidney, one clip renal hypertensive rats at doses of 30 or 100 mg/kg produced a dramatic lowering of mean blood pressure from about 190 mm Hg to 135–140 mm Hg [14, 17]. A similar acute antihypertensive effect was observed with captopril given orally at 10–30 mg/kg; at the highest dose, the maximal antihypertensive effect persisted for 6 hr, and the blood pressure remained significantly depressed even after 24 hr [14, 17]. Captopril did not appreciably lower the blood pressure (*ca.* 120 mmHg) of normotensive rats in sodium balance [14, 17]. Sodium-depleted normotensive animals, however, have a greater than normal dependence on the renin–angiotensin system for maintenance of blood pressure, and show an appreciable hypotensive response to converting enzyme inhibitors such as teprotide and captopril [18].

Captopril has been shown to be remarkably effective for long-term treatment of high blood pressure in animals models of hypertension. In two kidney, one clip renal hypertensive rats, captopril given orally at 30 mg/kg maintained its effective antihypertensive action for at least 1 year, produced a dramatic increase in survival, and reversed cardiac hypertrophy [14, 19, 20], effects that were even more pronounced if the action of the drug was combined with the sodium-depleting action of a diuretic. Administration of such high doses of captopril over a major portion of the life span of the rat was not associated with any obvious toxicity or side effects. Intermittent removal of the drug did not cause 'rebound hypertension'; in fact, several days were required for the blood pressure to increase back to the pre-drug hypertensive level. Captopril has also been shown to normalize blood pressure in the spontaneously hypertensive rat after several months of treatment [14, 21], despite the prevailing opinion that the renin–angiotensin system plays no role in this hypertensive animal model.

Clinical studies with inhibitors as antihypertensive drugs

Teprotide and captopril have both been shown to be effective antihypertensive drugs in a high percentage of the human hypertensive population, and their actions parallel those obtained in preclinical studies with animal models. Both converting enzyme inhibitors have few, if any, acute side effects. Teprotide, the first such inhibitor to be studied in the clinic, produced marked lowering of blood pressure, with a total duration of up to 16 hr, when given intravenously at doses of 0.25 to 4 mg/kg [22–24]. Its antihypertensive action was enhanced by coadministration of a diuretic, and, although tested in a relatively few patients, it appeared to lower blood pressure in most patients with either high or normal

levels of plasma renin. Studies with teprotide in man have confirmed the utility of inhibitors of angiotensin-converting enzyme for use as novel antihypertensive drugs, and have greatly stimulated the basic research studies that led to development of the orally active drug captopril, an inhibitor of angiotensin-converting enzyme that can be conveniently employed for long-term control of high blood pressure.

Captopril has now been studied in hundreds of patients, in some cases for more than 1 year. Given orally two to four times a day at total doses of 3–14 mg/kg, captopril has been found to normalize blood pressure in about 50 per cent of all patients tested, and to produce a significant reduction of blood pressure in about 80 per cent [25, 26]. As with teprotide, the antihypertensive action of captopril is augmented, in patients whose blood pressures were not already normalized, by coadministration of a diuretic. The antihypertensive effect does not diminish, and may increase, after several months or more than a year of treatment [25–28]. It is probable that lower doses that those presently employed will be effective for chronic lowering of blood pressure in most hypertensive patients. Chronic drug-related side effects of captopril include a reversible rash syndrome and occasional loss of taste discrimination, but the drug is free from most of the more burdensome side effects often encountered with antihypertensive drugs that act on the central or sympathetic nervous systems. Hypotension, even at high doses, is a problem only in severely volume depleted patients. The clinical profile of the action of captopril is essentially that which would be predicted from preclinical animal studies. Its relative freedom from side effects is related at least in part to its logical development, and to the careful optimization of its chemical structure for maximum potency and specificity.

Mechanism of antihypertensive action of inhibitors

Captopril was designed and developed as a potent and specific enzyme inhibitor, not primarily as an antihypertensive drug, although blood pressure lowering was tested for certain intermediate compounds in the succession that led to captopril. Captopril is the end result of a process of selection and optimization that resulted in a 25,000-fold increase in enzyme inhibitory potency over that of the initial prototype [6–8]. Thus, as an enzyme inhibitor, captopril has an extremely selective action, but, as a drug postulated to act by any other mechanism, it would have to be considered a purely random choice. The extremely low probability of existence of an antihypertensive mechanism unrelated to enzyme inhibition, however, has not deterred some investigators from suggesting such an action, based on certain results obtained in whole animals [29–32].

Captopril does lower blood pressure in anesthetized normotensive dogs even after nephrectomy or administration of the angiotensin receptor blocker saralasin, results that have been interpreted to involve an antihypertensive mechanism unrelated to inhibition of the renin–angiotensin system [29, 30]. Saralasin, however, is not as effective as captopril in blocking the activity of the renin–angiotensin system, both due to its intrinsic weak agonistic action and to the fact that it binds to its receptor with no greater affinity that its competitive agonist, angiotensin II, whereas captopril binds to its receptor, the active-site of angiotensin-converting enzyme, 1000 times more effectively than angiotensin I, with which it competes. Of course, saralasin also cannot potentiate bradykinin actions. The unexpected effectiveness of captopril in nephrectomized animals may be due to the presence of a functional renin–angiotensin system in vascular walls [32, 33]. Another argument put forward for an unknown antihypertensive mechanism for captopril [30] is the fact that captopril does not lower blood pressure over the same range of concentrations for which it produces effective inhibition of angiotensin-converting enzyme *in vivo*. But it is not at all surprising that near maximal inhibition of the enzyme is necessary to achieve the decrease in the steady-state blood level of angiotensin II required for even a weak blood pressure lowering effect. One group of investigators [31] observed a good antihypertensive action of captopril in patients, apparently in the absence of any inhibition of angiotensin-converting enzyme. However, their measure of inhibition *in vivo* was inhibition of the normal activity of plasma angiotensin-converting enzyme in blood samples withdrawn from the patient during the antihypertensive response, and captopril is quite susceptible to inactivation by oxidation to its disulfide dimer under certain conditions, including those obtaining in plasma samples [33]. Certainly, no antihypertensive mechanism for captopril or teprotide unrelated to their enzyme inhibitory activity has ever been directly demonstrated. Neither drug is a direct vasodilator, has any direct effect on the sympathetic nervous system, or is even significantly distributed into the central nervous system [34, 35].

Even when one accepts the high probability that captopril lowers blood pressure by inhibiting the angiotensin-converting enzyme, the precise mechanisms of its acute and chronic antihypertensive actions are far from being clear. Blood pressure decrease could be due to elimination (via decreased synthesis) of the direct constrictor action of angiotensin II on blood vessels, or to elimination of the intrarenal or aldosterone-mediated actions of this peptide that produce increased effective blood volume via sodium and fluid retention. Coverting enzyme inhibitors may also prolong the direct vasodilatory effect of bradykinin or the intrarenal action of this peptide as a stimulator of the release of fatty acid precursors of vasodilatory and natriuretic prostaglandins or prostacyclin.

In hypertensive patients there is at least a rough correlation between pretreatment plasma renin activity and the acute blood pressure lowering obtained with inhibitors of angiotensin-converting enzyme [22–24, 26–28, 36]. The blood level of angiotensin II also decreases [36–38], but it is difficult to measure very low levels of this octapeptide, especially in the presence of up to 30-fold increased levels of the decapeptide angiotensin I [36–38], which cross-reacts with angiotensin II in the radioimmunoassay [36, 39]. Administration of converting enzyme inhibitors has usually been shown to reduce the

slightly elevated aldosterone levels of hypertensive patients back to the level associated with normal blood pressure [22, 27, 28, 36–38]. Most investigators have not observed increased blood levels of bradykinin accompanying the antihypertensive action of converting enzyme inhibitors [36–38], and the small increases that have been observed [40] seem insufficient to maintain a lowered blood pressure through direct peripheral vasodilation. Within the kidney, however, a much greater accumulation of bradykinin may occur in response to converting enzyme inhibition, and lead to a greatly increased production of the precursors of prostaglandins or prostacyclin [41, 42]. Increased urinary kinin secretion and increased plasma prostaglandin E_2 levels have been observed in hypertensive patients treated with teprotide [38].

It will probably not be possible in the near future to adequately study all of the potential consequences of specific inhibition of angiotensin-converting enzyme in hypertensive patients or animal models. Use of such specifically acting drugs is probably the best method available for unraveling complex pathological mechanisms, but in this case, as in many others, there are several important secondary consequences of the specific drug action. Although improved methods for measuring blood or tissue levels of bradykinin, angiotensins I and II, and prostaglandins continue to be developed, it is likely that the development of additional specific drugs affecting the renin–angiotensin or kallikrein–kinin systems will be the most effective method for clarifying the roles of these systems in hypertension and in the antihypertensive action of drugs such as captopril. Potent and specific inhibitors of renin, for instance, would prevent angiotensin formation without affecting bradykinin and its important secondary effects on sodium excretion and prostaglandin synthesis. A specific bradykinin receptor antagonist might similarly help to resolve many of the ambiguities encountered with the converting enzyme inhibitors. From a therapeutic point of view, however, the inhibitors of angiotensin-converting enzyme, whatever their complex mechanism of action, or perhaps because of it, are remarkable antihypertensive drugs both in their effectiveness and in their relative freedom from side effects or toxicity.

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