Chronicles of Drug Discovery
Volume 2

Edited by
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CHRONICLES OF DRUG DISCOVERY

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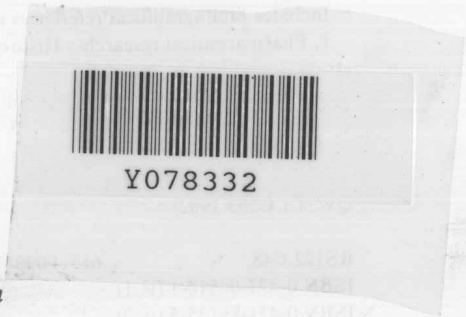

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Preface

When we set in motion the project which resulted in "Chronicles of Drug Discovery", the scope of the undertaking was not at all clear. We had no idea, for example, whether discoverers of drugs would even be willing to take the trouble to write about their experience. We were thus surprised and gratified to find that such a high proportion of individuals who were approached agreed to contribute to the volume. The acceptance rate was, in fact, so high that the contributions could not be contained in a single book. At about the same time too, some additional new drugs came to our attention which demanded inclusion. It thus became clear that we might well be faced with at least an additional book and perhaps a series rather than a single volume.

The book in hand is thus the second of what we hope will be a continuing series of volumes describing new drugs. Since the process of drug development is somewhat sporadic in nature, timing of subsequent additions to this series is expected to also be uneven. Books will be organized and published each time a sufficient volume of new material becomes available.

The first volume discussed drugs belonging to a rather broad selection of therapeutic areas; many different approaches to the discovery process were represented. The present volume mirrors both the breadth in content and in philosophy. The reader will thus meet an antihypertensive agent, a CNS drug, five antibiotics, and one drug each for the treatment of helminthiasis and fungal infections. We have, for the first time, included cancer chemotherapy agents; two are included in this book.

Approaches to the discovery process cover a similarly broad range. To name a few, several chapters describe the development of a new drug based on very directed synthesis. At the other end of the spectrum several sections describe drugs which were the results of serendipitous discovery. It is of note that these very different approaches and philosophies are apparently equally fruitful in producing new drugs.

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Captopril

Miguel A. Ondetti, David W. Cushman and Bernard Rubin

1. INTRODUCTION

The observations of Tigerstedt and Bergman in 1898 on the extraction of a hypertensive principle from the kidney[1] and the development of an experimental model of renal hypertension by Goldblatt and collaborators in 1934[2] are the most significant milestones in the research trail that led to an understanding of the role of the kidney in hypertension. The independent and convergent work of Braun-Menendez and coworkers[3] and of Page and Helmer[4] during the late thirties and early forties established on solid foundations the nature of the humoral mechanism that supports this important role of the kidneys in hypertension, and that is now known as the renin-angiotensin system. The chemical characterization of the final steps of this sequence (Figure 1) had to await the isolation studies of Bumpus et al.,[5] Peart,[6] and Skeggs et al.,[7] the identification of the two forms of angiotensin and the isolation of the angiotensin-converting enzyme by Skeggs et al.,[8] and finally the synthetic studies that confirmed the structure proposed for angiotensin II.[9,10]

Angiotensin II was thus established as one of the most potent vasoconstrictor agents known, but its significance as a mediator of elevated blood pressure in clinical or experimental renovascular hypertension was still unclear. In 1960 and 1961 several independent groups of investigators[11-15] provided conclusive proof that angiotensin was involved in the release of the sodium-retaining hormone aldosterone by the adrenal gland, thus establishing that the renin-angiotensin system could affect blood pressure by a dual mechanism of vasoconstriction and sodium retention.

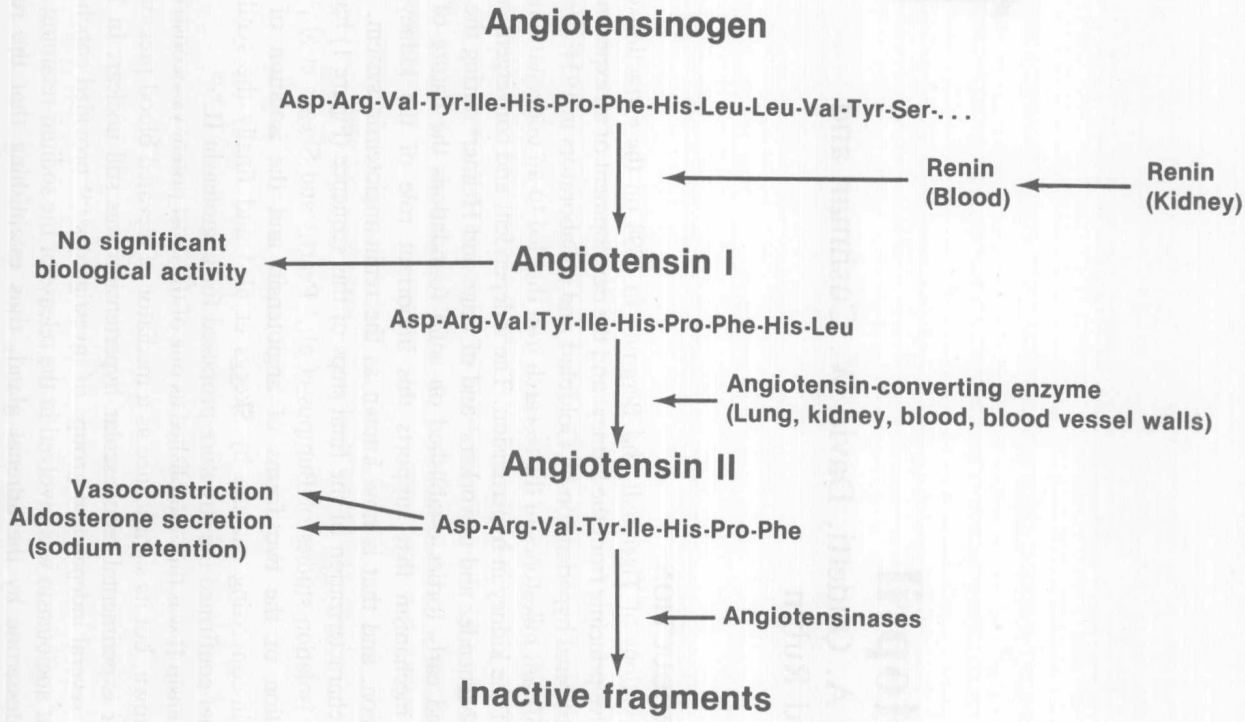


Figure 1. The renin-angiotensin system.

In spite of these brilliant contributions, the role of the renin-angiotensin system in relation to the elevated blood pressure in experimental and human hypertension remained doubtful, particularly when the blood levels of renin and/or angiotensin were elevated only slightly or not at all. To quote W. S. Peart[16]: "it seems unlikely that the system usually operates as a direct pressor system, but the various ways in which the pressor effects can be modified by electrolyte changes, by aldosterone, and by interaction with the nervous system would allow widely varying quantitative relations in different physiological and pathological states."

2. THE SEARCH FOR ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

Against this background of potential biological significance and inconclusive therapeutic relevance, we initiated our studies directed at blocking the renin-angiotensin system through inhibition of angiotensin-converting enzyme. In retrospect, we can say that these studies exemplify three different approaches to drug discovery: (a) identification of a naturally occurring biologically active lead, followed by total synthesis of the original structure and analogues; (b) random screening of a wide variety of chemical structures to obtain a new biologically active lead suitable to further chemical modification; and (c) *ab initio* drug design based on a molecular model of the receptor site. No explanations are necessary for the first two approaches, but it is important to point out that the expression "drug design" has been normally applied to the optimization of a biologically active lead obtained from approach *a* or *b* by a careful study of the physicochemical parameters implicated in the drug action.[17] In the development of the orally active angiotensin-converting enzyme inhibitors, the process of design was critically applied in *generating the leads,* before any optimization process could take place. Thus the expression "*ab initio* drug design" has been coined to distinguish this approach from that usually implied with the use of the "drug design" terminology.

2.1 The Natural Product Approach

One of us (D.W.C.) had already begun in 1968 to study the properties of angiotensin-converting enzyme, at that time a rather novel and poorly characterized peptidase. However, the great impetus for beginning a search for inhibitors was provided by the observation of Ng and Vane[18] and Bakhle[19] that crude extracts of the venom of *Bothrops jararaca* inhibited the conversion of angiotensin I to II. Ferreira had shown in 1965 that these extracts potentiated the biological activities of the nonapeptide bradykinin,[20] particularly its hypotensive effect. In collaboration with L. J. Greene, he undertook the fractionation of the crude venom extract, with the aim of isolating "bradykinin potentiating peptides."[21-23]

Table 1
Angiotensin-Converting Enzyme Inhibitors (Bradykinin Potentiators) Isolated from *Bothrops jararaca*

Designation		Structure[a]	ACE I_{50}[b] Cheung *et al.*[27]	ACE I_{50}[b] Bakhle *et al.*[26,28]	BkP[c]	References
SQ 20,475	BBP_{5a}	<Glu-Lys-Trp-Ala-Pro	0.06	0.9	100	26, 27, 29
SQ 20,661		<Glu-Trp-Pro-Arg-Pro-Thr-Pro-Gln-Ile-Pro-Pro	5.0		34	27, 29
SQ 20,881	BBP_{9a}	<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	0.56	0.02	410	26, 27, 29
SQ 20,858		<Glu-Asn-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro	5.8	0.02	47	26, 27, 29
SQ 20,859	BPP_{10a}	<Glu-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro	34.0	3.2	80	26, 27, 29, 47
SQ 20,861		<Glu-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	2.5		100	27, 29
SQ 20,718	BPP_{13a}	<Glu-Gly-Gly-Trp-Pro-Arg-Pro-Gly-Pro-Glu-Ile-Pro-Pro	14	0.2	200	27, 29, 47
	V-3-B	<Glu-Ser-Trp-Pro-Gly-Pro[d]		>5.0	9	28, 29
	V-1-A	<Glu-Glx-Trp-Ala-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro[d]			380	28, 29, 47
	BPP_{13b}	<Glu-Gly-Gly-Leu-Pro-Arg-Pro-Gly-Pro-Glu-Ile-Pro-Pro[d]		0.4	90	

[a]The standard three-letter abbreviations for amino acids have been used. All amino acids are of the L-configuration.

[b]Micromolar concentration required to inhibit 50% of the activity of angiotensin-converting enzyme.

[c]Relative specific activity, on a molar basis, for the potentiation of the action of bradykinin on guinea-pig ileum. The activity of BPP_{5a} is arbitrarily taken as 100.

[d]The sequence of these peptides was not determined, but was deduced from physical properties and comparison with related peptides.[29]

Since it was not quite clear to us that bradykinin potentiation would be a desirable property for an antihypertensive agent, in view of the putative pro-inflammatory actions of bradykinin, we began our studies on the fractionation of the *Bothrops jararaca* venom, following not the bradykinin potentiating activity but the angiotensin-converting enzyme inhibitory activity.[24,25] As it turned out, both activities were found to reside in the same peptides, and the amino acid compositions of peptides that we isolated were identical to those of the peptides isolated by Greene and Ferreira (Table 1).[26-29] A synthetic sample of one of the most active peptides we isolated (the nonapeptide SQ 20,881) was found to be identical with the nonapeptide BPP_{9a} (bradykinin potentiating peptide 9a), the most active bradykinin potentiator isolated by Greene, Ferreira, and collaborators.[28] In addition to the peptide inhibitors that were isolated from the venom, all their synthetic analogues showed both activities in a guinea pig ileum test; that is, all inhibited the contractile response of angiotensin I and potentiated the contractile activity of bradykinin without affecting the action of any other smooth muscle agonist, (*e.g.*, angiotensin II and acetylcholine). Finally, the studies of Erdös, Sofer, and other investigators utilizing homogeneous angiotensin-converting enzyme showed that both peptides, angiotensin I and bradykinin, are substrates for this enzyme and that the bradykinin-hydrolyzing enzyme previously designated as kininase II is indeed angiotensin-converting enzyme.[30,104]

The structure-activity relationships elaborated on the basis of inhibitory activity versus isolated angiotensin-converting enzyme correlate extremely well with those obtained using the bradykinin potentiating activity in guinea pig ileum,[98] supporting the contention that the effects of these inhibitors on the agonistic activities of angiotensin I and bradykinin in this smooth muscle preparation are due to their interaction with the converting enzyme present in this tissue. Thus the isolated smooth muscle test became a very useful tool in discriminating between specific angiotensin-converting enzyme inhibitors and compounds that nonspecifically antagonize a variety of other agonists in addition to angiotensin I.

Extensive *in vivo* studies were carried out with SQ 20,881 (teprotide) in our own laboratories, and those of other investigators.[31-33] Both inhibition of hypertension induced by exogenous angiotensin I and potentiation of hypotension induced by bradykinin were observed in several animal species. The duration of action after parenteral administration was considerable, keeping in mind that SQ 20,881 is a peptide and that peptides are often known to have rather fleeting activity.

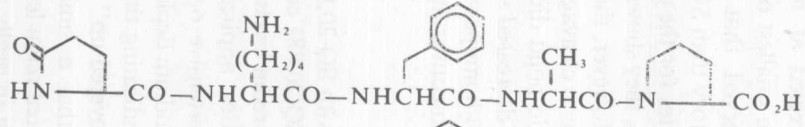
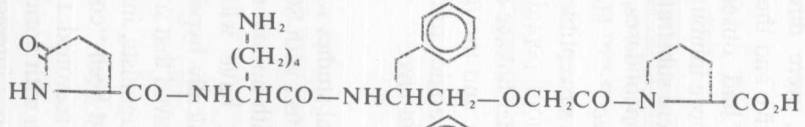
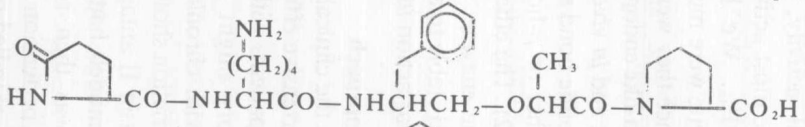
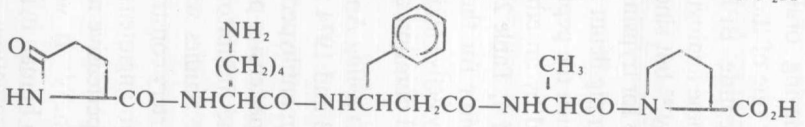
When tested in animal models of hypertension, SQ 20,881 produced the most significant lowering of blood pressure in those that had been suspected to be dependent on the renin-angiotensin system for the maintenance of elevated

blood pressure, such as the two-kidney one-clip classical Goldblatt model.[34,35] In the one-kidney one-clip dog model, the antihypertensive effect was only manifested for a brief period after renal artery constriction.[36] The first studies in the spontaneously hypertensive rat (SHR), using the same doses that were effective in the renal hypertensive rat, showed no hypotensive effect.[32] However, it was later shown that larger doses do indeed produce a modest but significant lowering of blood pressure.[37] These results correlated fairly well with the then prevailing understanding of the functioning of the renin-angiotensin system. Based on circulating levels of renin, the only animal model of hypertension in which the renin-angiotensin system was thought to be important for the maintenance of blood pressure was the two-kidney one-clip renal hypertensive rat, the classical Goldblatt model. In the one-kidney one-clip model or in the SHR, the levels of renin were normal or returned to normal very soon after renal artery constriction and nephrectomy.[36,38]

More or less concurrently with our studies on angiotensin-converting enzyme inhibitors, researchers in other laboratories had finally developed angiotensin II receptor antagonists, thereby opening a new avenue for the blockade of the renin-angiotensin system.[39-41] From the point of view of potency and duration of action, the most effective of these antagonists was saralasin, the 1-sarcosine-5-valine-8-alanine angiotensin II.[41] This agent was also shown to lower blood pressure in the two-kidney one-clip rats, but not in the one-kidney one-clip or the SHR.[41-43] These results, being in good agreement with those obtained with angiotensin-converting enzyme inhibitors, confirmed the unique but restricted importance of the renin-angiotensin system in hypertension. Since the duration of the antihypertensive activity of these angiotensin II antagonists was shorter than that of SQ 20,881, and since they still retained a small amount of agonistic activity, we felt that converting enzyme inhibition was still the preferable approach to renin-angiotensin system blockade, in spite of the ambiguity introduced by the potentiation of the kinin system.

Studies in human volunteers confirmed that potent and fairly long lasting inhibition of the pressor activity of exogenous angiotensin I could be obtained with reasonable intravenous doses of SQ 20,881,[44] and the first clinical report on the use of this converting enzyme inhibitor showed that lowering of blood pressure could be obtained in those patients with elevated blood renin levels.[45,46] These observations demonstrated a point of the utmost importance, namely, that any compound capable of inhibiting isolated angiotensin-converting enzyme and specifically antagonizing angiotensin I contractile activity in smooth muscle preparations had potential for use as an antihypertensive agent. However, two very basic problems were still unresolved: (1) SQ 20,881 and any of its peptide analogues could only be used parenterally, and any serious attempt at the development of an antihypertensive agent for

Table 2
Peptidic Angiotensin-Converting Enzyme Inhibitors with Modified Peptide Bonds[48]

No.	Structure[a]	Rabbit lung ACE I_{50}[b]
1		0.1
2		44
3		34
4		0.4

[a]All amino acids are of the L-configuration.

[b]Concentration (μM) required to inhibit 50% of rabbit lung angiotensin-converting enzyme (ACE) activity.

chronic use required oral administration; and (2) if blockade of the renin-angiotensin system was only effective in reducing blood pressure in those patients with elevated plasma renin levels, this approach was going to be of limited utility, since hypertensive patients with high plasma renin constitute only 10-20% of the total hypertensive population. Further clinical studies by Laragh and collaborators were to provide some very important clues to resolve the second problem, but it was the first that constituted the major challenge for the medicinal chemist.

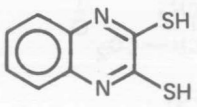
Our first attempts at increasing the stability of peptidic inhibitors, with the aim of achieving oral activity, were directed at modifying the peptidic backbone of one of the most active and the smallest of the venom inhibitors, the pentapeptide BPP_{5a}. We had observed that this peptide and its 3-phenylalanine analogue were more inhibitory than SQ 20,881 in the isolated enzyme assay,[27] but since they were substrates for the converting enzyme, and possibly also for trypsin-like endopeptidases, they showed a rather poor activity in the guinea pig ileum and *in vivo*.[23,32,47] However, the synthetic analogues of BPP_{5a} in which the peptide bond susceptible to cleavage by converting enzyme was replaced by an ether or β-homopeptide bond did not lead to useful inhibitors.[48] (**1-4**, Table 2). The ether linkage decreased substantially the affinity of the inhibitor for the enzyme, and the β-homopeptide derivative (**4**), even though fairly active and stable to angiotensin-converting enzyme, did not show an improved duration of action *in vivo*.

2.2 The Screening Approach

During 1973 and 1974 the clinical studies with SQ 20,881 (teprotide) began to show that an antihypertensive effect with SQ 20,881 could also be observed in essential hypertensive patients with normal renin levels, indicating that converting enzyme inhibitors might have wider applicability than originally thought.[26,49] Studies with chronically hypertensive one- and two-kidney rats with renal artery constriction showed that sodium depletion made these models responsive to angiotensin II antagonists, indicating that the renin dependence of these hypertensive models had been "covered up" by a volume-dependent hypertension.[50,51] It was then reasoned that a similar situation might be operative in human hypertension with normal renin levels.

These observations rekindled our interest in orally active and longer lasting angiotensin-converting enzyme inhibitors and prompted us to expand the previously initiated screening of a large variety of nonpeptidic chemical structures, utilizing an *in vitro* assay with isolated rabbit lung enzyme. The results of this screening were rather disappointing, since out of approximately 2000 compounds only a few showed any significant inhibitory activity, and practically all

of them were not specific when tested in the guinea pig ileum smooth muscle assay, that is, besides inhibiting the contractile activity of angiotensin I, they also inhibited that of bradykinin and usually that of other agonists such as acetylcholine and angiotensin II. Only one compound, 2,3-quinoxalinedithiol (**5**),[52] showed the typical specificity of an angiotensin-converting enzyme inhibitor in the smooth muscle test. The metal-chelating ability of this compound



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explains its inhibitory activity on angiotensin-converting enzyme, a metalloprotease. However, the significant toxicity of the compound precluded any further development of this lead.

It is interesting to speculate why the approach of random screening did not produce any significant lead. Our later studies on the design of active-site-directed specific inhibitors of this enzyme have shown that considerable attention must be paid to the detailed molecular architecture and functionality to obtain potent inhibitors. The presence of at least one acidic group is essential to obtain significant interaction with the enzyme and specificity in the smooth muscle assay. Compounds of this type of functionality were very poorly represented among the different types of chemicals available for random screening. Besides, the two main groups of compounds that we later found to provide efficient and specific inhibitors, namely, carboxyalkanoyl and mercaptoalkanoyl amino acids, had been the subject of very few literature reports before we started our studies, and therefore, it would have been very unlikely for us to come across representative samples of these classes during the random screening process.

2.3 The Design of Active-Site-Directed Inhibitors

2.3.1 Preliminary Studies In March of 1974, shortly after Byers and Wolfenden published their studies on benzylsuccinic acid as a biproduct analogue inhibitor of carboxypeptidase A,[53] we initiated a program aimed at the design of active-site-directed inhibitors of angiotensin-converting enzyme, utilizing the rationale expounded by those investigators.

According to Byers and Wolfenden, benzylsuccinic acid is a potent inhibitor of carboxypeptidase A because it combines in one molecule the modes of binding of the two products of the enzymatic reaction: the original C-terminal