



Surfing the Heart

*World Health Organization – The European Heart Network
Clinical Trials Through the National Institutes of Health – Yahoo! Webring*

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Web sites of important health organizations often provide fresh perspectives on cardiovascular diseases with respect to those offered by professional societies. The web site of the **World Health Organization (WHO)** is a prime example. WHO's *Programme on Cardiovascular Diseases* includes various strategies aimed at the prevention, management, and monitoring of the disease at a global level, and these are extensively described at www.who.int. The section on cardiovascular diseases is found under *Health Topics* among the list of *Noncommunicable Diseases*, and can easily be accessed at www.who.int/ncd/cvd/index.htm. This represents an enormous source of epidemiological data and facts on the impact of heart disease on health. The home page defines cardiovascular disease, and describes its risk factors and social and economic consequences. There are several subsections, including a *Publications* area, where it is possible to download an impressive amount of material on the subject found in WHO publications, and, under *Country Profiles*, the powerful *Global Cardiovascular Infobase*. The section entitled *News* provides comprehensive information about important selected topics. At the end of your visit, you will understand why, I quote, "heart disease has no geographic, gender or socio-economic boundaries." A great web site!



"Health and Politics cannot be separated" is stated in the front page of the European Heart Network (EHN) web site. This site constitutes a useful resource for health or media professionals. The information provided includes annual reports, newsletters, and useful links.



Clinical trials through the
National Institutes of Health

www.clinicaltrials.gov

The US National Institutes of Health, through the National Library of Medicine, has developed www.clinicaltrials.gov to provide patients, family members, and members of the public with current information about clinical research trials. This web site currently contains information on approximately 5200 clinical studies sponsored primarily by the National Institutes of Health and other US federal agencies. The powerful search engine can be utilized to find the needle in the haystack of available knowledge: the database can be interrogated using keywords or browsed by conditions. A similar web site would be useful in Europe!



Science Humor Webring

[http://nav.webring.yahoo.com/hub?
ring=sciencehumor&page=1&list](http://nav.webring.yahoo.com/hub?ring=sciencehumor&page=1&list)

The Internet is rapidly changing and new ways of communicating are constantly being developed. A webring is a series of linked sites about the same subject: here's one dedicated to humor about science or scientists. In this ring, you can find out which ancient language gave rise to the chemical nomenclature, what happens if you eat cesium, why you should NOT allow your children to study chemistry, what is the newly discovered evolutionary transition between the car and the airplane, how NOT to write a scientific paper, and many other things. For more information or to submit material, go to <http://www.xs4all.nl/~jcdverha/sciHum/webring.html>. This site is well worth a visit for scientists or physicians needing a break from a frantic day!

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Trails of Discovery

A close call: the discovery of the ACE inhibitors

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The path leading to the discovery and development of the first angiotensin-converting enzyme (ACE) inhibitor, captopril, as a valuable agent in the management of cardiovascular disease provides a typical example of the complexities and uncertainties that surround novel drug research. The trail starts in 1898 with Tigerstedt's¹ discovery of the pressor effects of a renal extract, renin, which was able to constrict resistance vessels in the arterial tree without altering cardiac output. He subsequently showed that renal extracts caused a marked pressor effect in nephrectomized animals. However, other investigators in the field were unable to repeat his observations, and so he abandoned studies on the action of renin. The reason for the failure to replicate Tigerstedt's experiments is probably that the renin in the extracts of other investigators was easily destroyed by keeping it at room temperature with or without the additional effects of bacterial contamination.

Little further interest was shown in the biological effects of renal extracts until 1934 when Goldblatt showed, in dogs, that clipping the renal artery raised blood pressure with a hemodynamic profile similar to that in human hyper-



Figure 1. *Bothrops jararaca*, an extremely poisonous pit viper that occurs in South America, is the snake that started it all...
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tension.² This was associated with an increase in plasma vasopressor activity. When plasma renin levels were subsequently measured in patients with essential hypertension, it was found that, in contrast to Goldblatt's experiments, the plasma renin levels were normal or low. They were elevated in less than 10% of patients, and so Goldblatt's renin hypertension hypothesis was rejected (*Table I*).

The field of renin and essential hypertension was confounded for over 30 years from 1950 onwards by methodological problems in measuring renin in human plasma. One immunoassay (Haber) did not control pH adequately, leading to a lack of sensitivity and accuracy. An alternative assay, in which plasma was routinely acidified, led to conversion of prorenin to active renin, so that small differences in the in vivo levels of renin were submerged by the in vitro formation of excess renin. Subsequently, an appropriate assay was developed.³

The purpose in providing this background to the evolution of the renin hypertension hypothesis is to emphasize that, when research started in the Squibb laboratories in 1968 with the purpose of finding specific inhibitors of ACE, there was little, if any, support for the importance of renin in essential hypertension by the cardiovascular clinical science constituency except for Page,⁴ Skeggs,⁵ Braun-Menendez,⁶ and, subsequently, Laragh.⁷

EARLY STAGES OF ACE RESEARCH

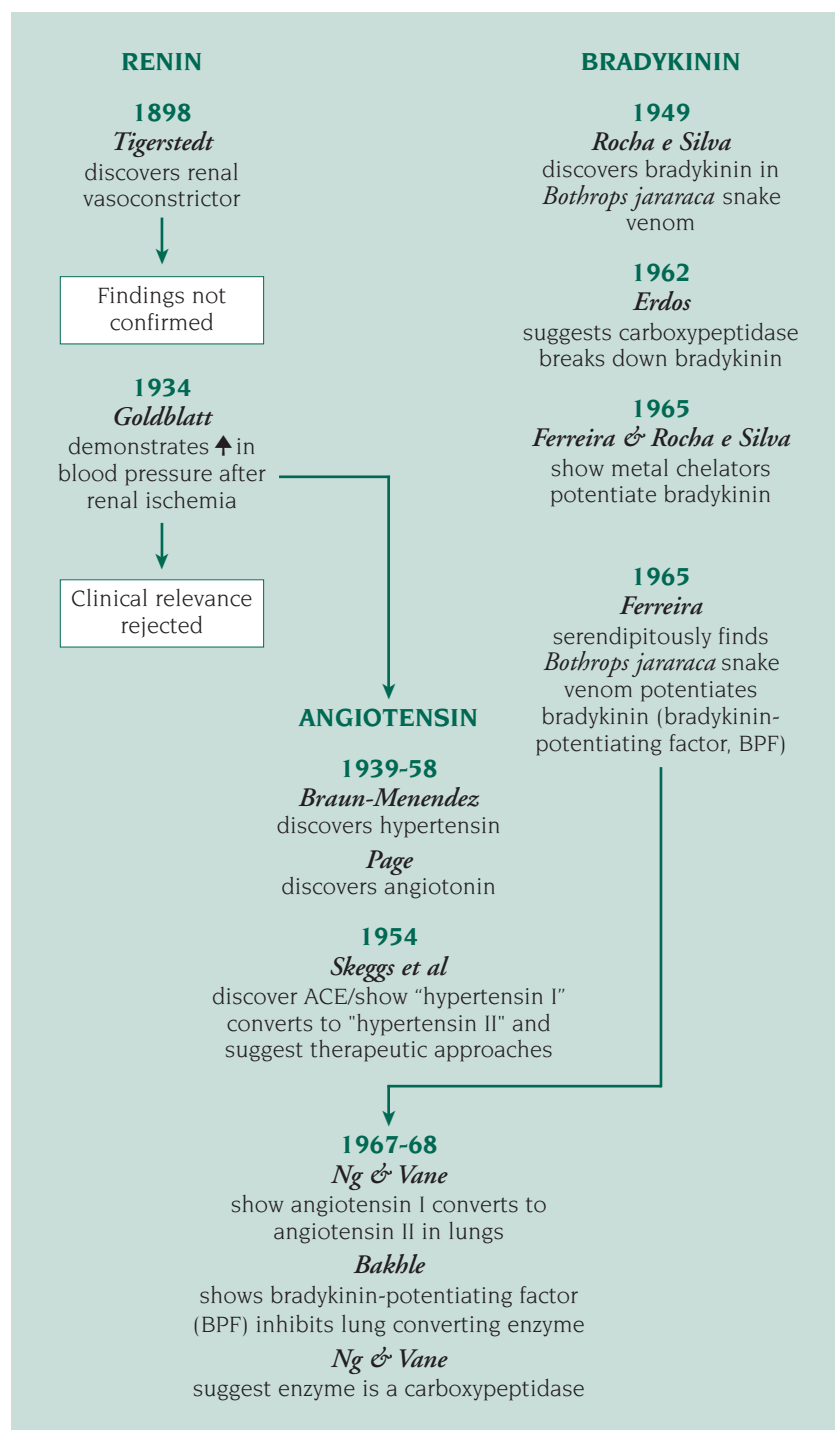
There are three interrelated research themes that led to the discovery of specific ACE inhibitors (*Table I*). These are: (i) the discovery of bradykinin in snake venom; (ii) the discovery of angiotensin I and II; and (iii) the discovery of carboxypeptidase-mediated conversion of angiotensin I to angiotensin II, as well as the degradation of bradykinin.

BRADYKININ

Snake poisons were known to cause hypotension, shock, and hypovolemic death. In 1949, Rocha e Silva identified bradykinin in snake venom and he proposed that this was responsible for the vascular collapse.⁸ Bradykinin appeared to be rapidly broken down in venom, making it difficult to study. Thirteen years later, Ferreira,⁹ working in the same laboratory, used metal-

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binding agents to inhibit the hypothesized enzyme, carboxypeptidase, as suggested by Erdos.¹⁰ Not only did these agents reduce bradykinin breakdown, but, subsequently, Ferreira found that the venom itself could potentiate the action of bradykinin, which sug-

gested that the venom contained an inhibitor of the degrading enzyme.¹¹ Two years later, Ferreira was then working in Vane's laboratory in the Royal College of Surgeons, London, examining the disappearance of bradykinin from vascular beds, including the pul-

Table I. Research trials leading to the first angiotensin-converting enzyme (ACE) inhibitor: identification of biotransformation of angiotensin/bradykinin.

monary circulation. Bradykinin was rapidly destroyed in a single passage through the lungs. He subsequently isolated 22 protein fractions from the snake *Bothrops jararaca* (Figure 1), and eventually isolated and synthesized a small peptide (BPP5A), which not only prolonged the action of bradykinin in the circulation, but also increased the amount of angiotensin I that had to be administered to raise blood pressure in anesthetized rats.¹² At the same time, Bakhle,¹³ also working in Vane's laboratory, showed that the original bradykinin-potentiating factor (BPF) of Ferreira was a competitive inhibitor of the enzyme that converted angiotensin I to angiotensin II in dog lung and also protected bradykinin against inactivation.

ANGIOTENSIN

The generation of a pressor substance by the action of renin on plasma was described in 1939 and 1940, both by Page and Helmer, working in Indianapolis City Hospital, and Braun-Menendez from the Institute of Physiology in Buenos Aires. Page called the pressor substance *angiotonin* and Braun-Menendez called it *hypertensin*. This dual nomenclature caused continuing confusion among workers in the field and was only resolved by renaming the subsequently characterized peptide as *angiotensin* in 1958.¹⁴

Pivotal studies by Skeggs et al,^{15,16} working in Cleveland from 1950 onwards, showed that the dialysate of plasma from patients with *malignant* hypertension contained *hypertensin*, but only some patients with essential *benign* hypertension had *hypertensin* in the plasma. During their studies, these authors made a vital, serendipitous, discovery. In preparing *hypertensin* from blood, the purification

procedure involved dialysis against distilled water. For a reason that has never been clear, in one experiment, dialysis was against 1.05 mol/L sodium chloride. The material resulting from this incubation had a very different distribution pattern in the homemade countercurrent distribution apparatus from the usual distribution curve. Skeggs et al concluded that *hypertensin* was present in plasma in two forms, and that one was converted to the other by ACE, which is a chloride-activated enzyme.¹⁷ They showed that when *hypertensin I* is injected into the perfused rat kidney, there was no change in pressure, whereas *hypertensin II* caused a marked rise in pressure. In the same time frame, Page's group¹⁸ in Cleveland described the structure of *angiotensin II* obtained from hog plasma, and Elliott and Peart,¹⁹ in London, described the structure of *hypertensin* isolated from bovine plasma. The discussion in the paper of Skeggs et al,¹⁷ 1956, contains some prophetic remarks. Despite the general view that renin was not important as a cause of essential hypertension, they wrote:

It is of paramount interest to discover a therapeutic method of lowering the blood pressure of human beings afflicted with hypertensive cardiovascular disease. Owing to the knowledge now available, concerning the structure of hypertensin I, hypertensin II, and the converting enzyme, it becomes possible for the first time to approach this problem upon rational grounds. It may now be possible to discover and protect by structural analogues the bond which the enzyme renin dissociates when hypertensin I is formed from renin substrate... It may also be possible to prevent the formation of hypertensin II from hypertensin I by the converting enzyme, for example, by providing a structural analogue of the phenylalanyl-histidyl-leucine bond... Finally, it may be possible to prevent the vasoconstrictive action of hypertensin II upon smooth muscle.

Over the past 25 years, all three approaches have been explored by different research groups within the

pharmaceutical industry. Subsequently, Skeggs's group spent much time exploring approaches to finding an inhibitor of renin.

TEPROTIDE AND CAPTOPRIL

In 1968, Vane, a consultant for Squibb, had just published two key papers showing that angiotensin I was converted to angiotensin II when passing through the canine lung, and speculated that the enzyme responsible for the conversion was a carboxypeptidase. He met with the Director of Pharmacology, Horowitz, Head of Peptide Chemistry, Ondetti, and Cushman, a gifted biochemist. Based on that discussion, it was decided to mount a speculative research program in order to find an inhibitor of ACE with potential application in essential hypertension (*Table II*). This was a courageous decision for several reasons. Despite Goldblatt's demonstration in 1934 that renal artery clip hypertension in dogs had the same hemodynamic profiles as patients with essential hypertension, studies in humans suggested that blood renin values were mostly either normal or low and that only a minority had truly high values. With few exceptions, most investigators assumed that renin had nothing to do with the etiology of most forms of essential hypertension. The basis for such opinions is exemplified by Gross, of Heidelberg,²⁰ an authority on the renin-angiotensin system, who concluded that the renin-angiotensin system had little, if any, significance in the pathogenesis of essential hypertension and that interference with the renin-angiotensin system would not lead to new antihypertensive drugs. He based his opinion on the fact that angiotensin II antagonists such as saralasin caused vasoconstriction and only lowered blood pressure when patients were in negative sodium balance, and that the ACE inhibitor teprotide had to be given by injection.

The research program in Squibb made rapid progress for two reasons. Firstly, Cushman developed a spectrophotometric assay that permitted careful structure–function studies on any peptides made by Ondetti. Secondly, the chemistry program could be based on the published findings of Ferreira et al¹² who had shown that snake venom contained inhibitors of the breakdown, not only of bradykinin, but also angiotensin I. The Squibb group was the first not only to characterize, but synthesize, specific peptide inhibitors isolated from snake venom. One of them, SQ20881, was shown to be the same as the bradykinin-potentiating factor described by Ferreira. This nonapeptide was called teprotide and taken forward for human studies.²¹ In 1972, Laragh's group used teprotide to undertake a series of studies of its effects in animals and humans. This group was the first to show that inhibition of ACE with teprotide could reduce blood pressure in patients with hypertension.⁷ Structure–function studies showed that the minimum requirement for inhibition was a tripeptide, Phe-Ala-Pro and its acylated derivatives. Attempts to discover an orally active inhibitor led to repeated failures, and even random screening of 2000 compounds did not provide any lead. So, in 1973, the ACE-inhibitory project was stopped by the research directors. One of the factors influencing the decision to stop the ACE-inhibitor program was that the provisional sales estimates for an orally active ACE inhibitor were \$20 million annually. The current sales exceed several billion dollars.

In March 1974, Cushman read a paper by Byers and Wolfenden²² on carboxypeptidase inhibitors, showing that benzylsuccinic acid was a potent inhibitor. Horowitz called a meeting with Ondetti and Cushman, and it was decided to make some custom-designed molecules *without official sanction*. The then Director of Phar-



macology, Goldberg, recalls that, as he was entering a meeting room to give a strategic research presentation to the senior management, the Vice-President of R&D said: “Do not discuss anything about the ACE work, these guys have been told the program is dead and buried.” Cushman and Ondetti firmly believed that they could achieve an orally active ACE inhibitor

based on drug design considerations. They speculated that the compound, α -D-methylsuccinyl-L-proline would be active, and this proved to be the case. Based on theoretical considerations, Ondetti and Cushman also wanted to make a sulfhydryl-substituted analogue. From the chemical synthesis point of view, this was very difficult to do. Progress was only made

possible by the chance reading of a biochemical product brochure showing that there was a new reagent that would make it much easier to introduce sulfhydryl groups on proteins. Using this new reagent, a compound, 3-mercaptopropanoyl-L-proline, was made, which was 3000 times more potent than the initial lead compounds and was active orally as well as intravenously. The α -methyl analogue was named captopril.²³ By 1976, studies in human volunteers showed that 1 to 10 mg orally could block the pressor effects of angiotensin I for several hours. Captopril was taken into full clinical development, and after following several blind alleys in the clinical studies, it was eventually shown that low doses of captopril were safe and efficacious in controlling blood pressure in 50% to 60% of hypertensive patients. In April 1981, captopril was approved for the treatment of heart failure. Finally, in July 1985, it was approved for treatment of all types of hypertension.

The publication of the hypotensive effects of teprotide, in man, triggered the interest of many other pharmaceutical companies, especially Merck in the US and Servier and Hoechst in Europe. The Merck research program started in 1974, but it was 5 years before enalapril and lisinopril were identified. The target was to identify a compound with a much longer duration of action than captopril and without sulfhydryl groups, since it was believed at that time that some of the side effects (rashes, taste alteration) were due to this substituent. In 1981, it was shown that enalapril maleate (Vasotec), 20 to 40 mg once daily, controlled blood pressure. I had twice the systemic bioavailability of the analogue lisinopril. Enalapril maleate was marketed in 1985 and lisinopril in 1987. By 1990, perindopril (Servier) and ramipril (Hoechst) had been marketed and there were 30 other ACE inhibitors in clinical development.

1967	<ul style="list-style-type: none"> • Squibb peptide research redirected by <i>Welch</i> from gastrointestinal hormones
1968-70	<ul style="list-style-type: none"> • <i>Vane</i> suggests ACE as a target • <i>Horowitz</i> = Biology Director; <i>Ondetti</i> = Chemist; <i>Cushman</i> = Biochemist at Squibb • <i>Cushman</i> devises spectrophotometric assay for ACE • <i>Ferreira et al</i> isolate inhibitory peptide from <i>Bothrops jararaca</i> snake venom (<Glu-Lys-Trp-Ala-Pro) • <i>Ondetti et al</i> confirm and synthesize 6 specific peptide inhibitors, of which SQ20881 = <i>Ferreira's</i> bradykinin-potentiating factor (BPF_A) = TEPROTIDE • <i>Erdo</i> shows bradykinin and angiotensin I are identical substrates for ACE
1971-72	<ul style="list-style-type: none"> • TEPROTIDE lowers blood pressure in Goldblatt rat model of hypertension as does 1st angiotensin II antagonist saralasin
1973	<ul style="list-style-type: none"> • <i>Collier, Robinson, and Van</i> show in humans that TEPROTIDE inhibits angiotensin I rise in blood pressure
Squibb stops ACE program in 1973	
1974	<ul style="list-style-type: none"> • <i>Laragh's</i> group show TEPROTIDE lowers blood pressure in hypertensive patients • Search for oral TEPROTIDE fails after 2000 compounds are tested • <i>Cushman</i> sees <i>Byers</i> and <i>Wolfenden</i> paper on carboxypeptidase inhibitor • <i>Ondetti</i> agrees to make analogues of succinyl-L-proline in antibiotic program!
1975	<ul style="list-style-type: none"> • Chance information that a new reagent (propiothiolactone) enhances synthesis of sulfhydryl groups on proteins • 3-mercaptopropanoyl-L-proline found to be 3000 times more potent as ACE inhibitor: α-methyl analogue was CAPTOPRIL
1976	<ul style="list-style-type: none"> • CAPTOPRIL ↓ blood pressure in volunteers
1981	<ul style="list-style-type: none"> • CAPTOPRIL marketed
1981	<ul style="list-style-type: none"> • CAPTOPRIL gets chronic heart failure indication
1991	<ul style="list-style-type: none"> • CAPTOPRIL shown to decrease chronic heart failure morbidity

Table II. Angiotensin-converting enzyme (ACE) inhibitor program.

CONCLUSION

The history of the discovery of the ACE inhibitors provides interesting examples of certain general principles in speculative drug research. One of the more important principles is that most breakthroughs in therapeutics are preceded by lengthy incubation periods. The 1956 paper by Skeggs et al¹⁷ laid out the target options for modulating the renin-angiotensin system, but it was 25 years before captopril was marketed. Another feature is the need for persistence and commitment from drug researchers so that, even when the projects are stopped because of lack of progress, attention remains focused on solving the problem some time, even when working on a different therapeutic target. The relevance of the Byers and Wolfenden²² paper was grasped by Cushman, which led to the drug design model that he and Ondetti devised. A further lesson is the critical importance of clinical scientists as exemplified by Laragh's group in not only studying the pathophysiology of disease, but also having a willingness to undertake proof-of-concept studies to validate novel drug target concepts. The award of the Lasker prize to Ondetti and Cushman († in 1999) provides dilatory, but welcome, recognition of their great contribution to cardiovascular therapeutics.

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