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Review article

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THE HISTORY OF INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME

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This review paper by Sir John Vane, The Nobel Prize Laureate for the first time reveals the insides of discovery of inhibitors of angiotensin converting enzyme (ACE-1), presently known as important drugs for the treatment of hypertension, congestive heart failure and coronary artery disease.

Key words: ACE-1, angiotensin I, angiotensin II, bradykinin, metabolic functions of pulmonary circulation.

Just over 100 years ago Felix Hoffman synthesised acetylsalicylic acid, which was marketed as Aspirin by the Bayer Company. It is now selling more than 45,000 tonnes per year. The development of the aspirin-like drugs (1) is a fascinating one, in which our discovery that they inhibit the formation of prostaglandins (2) was a key stepping stone for progress. Now we have selective COX-2 inhibitors, which have the promise of being excellent anti-inflammatory drugs without the side effects on the stomach (3).

Here, I will relate our involvement in a different field, that of the renin angiotensin system. At about the same time that Felix Hoffmann was synthesizing aspirin, Tigerstedt and Bergman in 1898 (4) found that crude saline extracts of the kidney contained a long-acting pressor substance, which they named renin. This fundamental observation led, over the next 100 years to the elucidation of the complex renin angiotensin system, involving such famous names as Goldblatt, Braun-Menendez, Bumpus and Page, Skeggs, Gross, Peart and many others. We became involved in the late 1960's and our research work played an essential role in the discovery of angiotensin converting enzyme inhibitors.

First, what is the renin angiotensin system? Renin is an enzyme stored by the granular juxtaglomerular cells that lie in the walls of the afferent arterioles

as they enter the glomerulae. Renin is a protease, the principle natural substrate being the circulating α_2-globulin called angiotensinogen. The active form of renin is a glycoprotein that contains 340 aminoacids. This is synthesised as a pre-pro enzyme of 460 aminoacids but is processed to pro-renin a mature, but inactive form of the enzyme.

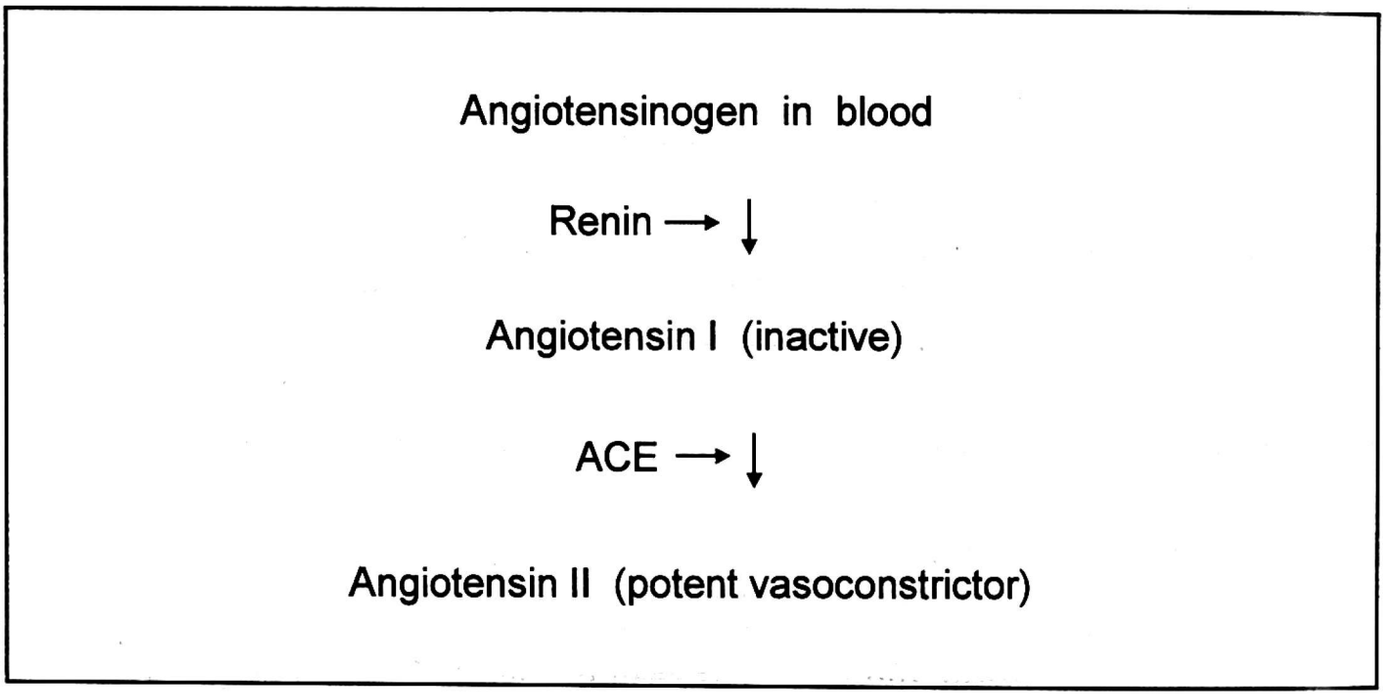

Fig. 1

When renin is secreted from the kidney into the bloodstream, it chops the decapeptide angiotensin-1 from the aminoterminal end of angiotensinogen, a 452-aminoacid plasma protein.

Angiotensin converting enzyme (ACE) is the second enzyme (*Fig. 1*) in the cascade. It was discovered in plasma serendipitously by Skeggs in 1956 (5) as the factor responsible for conversion of the inactive decapeptide, angiotensin-I to the potent pressor octapeptide, angiotensin-II. Human ACE contains 1278 aminoacid residues and is rather non-specific in that it cleaves dipeptide units from substrates with diverse aminoacid sequences, including angiotensin I and bradykinin.

When we started our work in the field it was assumed that angiotensin I was converted to the potent pressor substance angiotensin II by ACE in the plasma. This was because when angiotensin I was injected intravenously there was a rapid rise in blood pressure due to the formation of angiotensin II, suggesting that the plasma enzyme was a potent one. We tested this hypothesis using the blood-bathed organ technique (6). In this method, different isolated organs are superfused continuously with heparinised blood taken at a rate of 10ml/min from an anaesthetised cat or dog and then returned into the animal intravenously. Over the years we chose a series of different isolated organs

which would react differently to endogenous hormones so that we could measure adrenaline by a rat stomach strip and a chick rectum, angiotensin-II by a rat colon, bradykinin by a cat jejunum and so on (7).

We wanted to measure the rate of conversion of angiotensin-I to angiotensin-II in blood so we used an incubating circuit into which we could infuse either substance with a defined delay of anything up to three minutes before it reached the assay tissues (8). We were surprised to find that there was little or no conversion of angiotensin I to angiotensin II in the blood. For instance, there was only 27% conversion of angiotensin I to angiotensin II after 15s. incubation with blood, 40% after 60s. and 93% after 180s. We, therefore, began to examine infusions of the angiotensins into different parts of the circulation. We found that when infused intravenously, using a rat colon bathed in femoral arterial blood to measure the effects after passage through the lungs, there was an increase in activity as the angiotensin I, went through the lungs (*Fig. 2*). We found that there was a strong conversion of angiotensin I to angiotensin II in the few seconds it takes to traverse the pulmonary circulation. We repeated this experiment in several ways, as well as measuring the lack of conversion of angiotensin I across other vascular beds (8). To make sure the plasma had no effect on this process, we perfused isolated lungs from guinea pigs with Krebs' solution and found, as seen in *Fig. 3*, the same conversion (9).

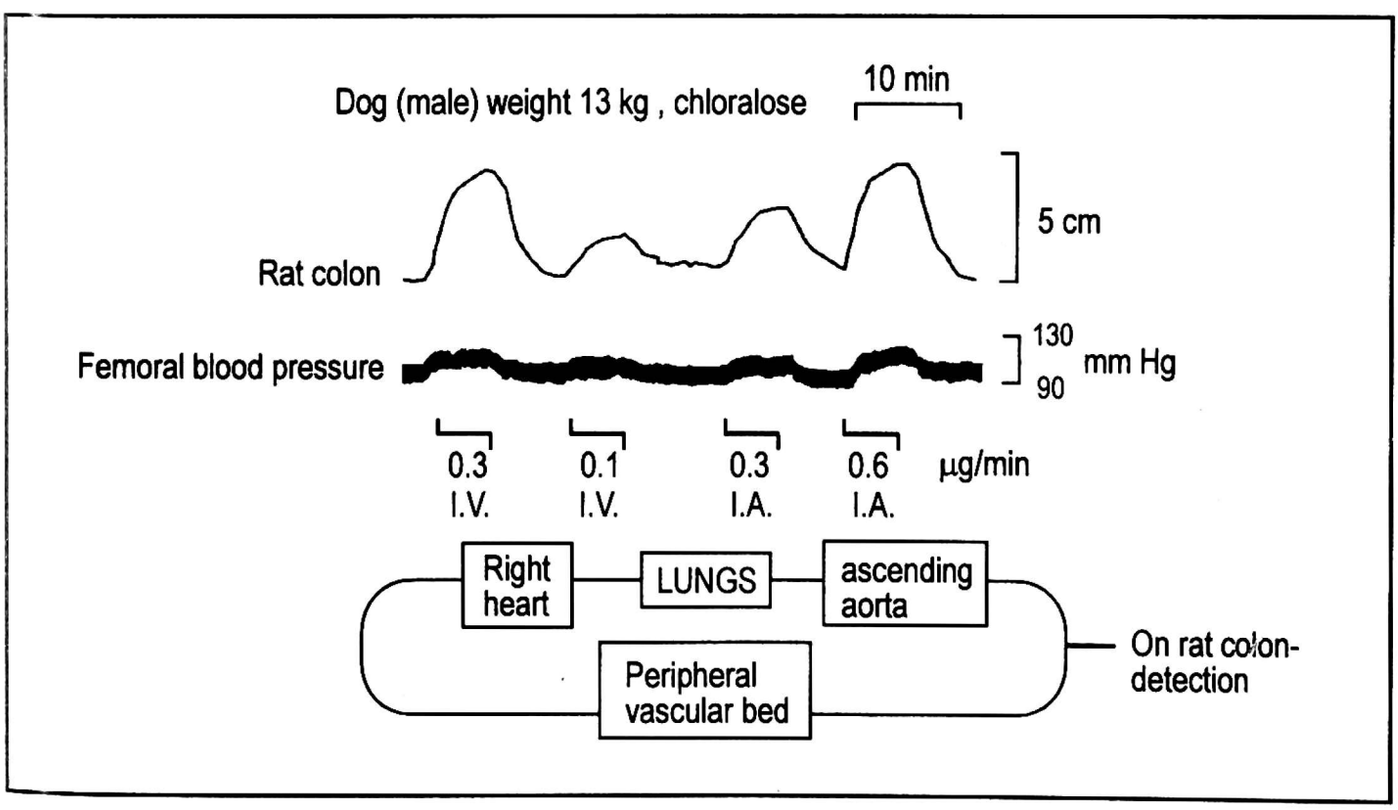

Fig. 2. Increased activity of angiotensin I when passed through pulmonary vascular bed *in vivo*. Tracings show responses of isolated rat colon superfused with blood from dog femoral artery and changes in blood pressure of the dog. Angiotensin I was given to right heart (I.V.) or ascending aorta (I.A.). Angiotensin I at a dose of 0.3 µg/min, when given I.V., exerted greater response than the same dose given I.A. The dose of 0.3 µg/min of angiotensin I, when passed through pulmonary vascular bed, is eguivalent to two-fold greater dose (0.6 µg/min) given I.A.

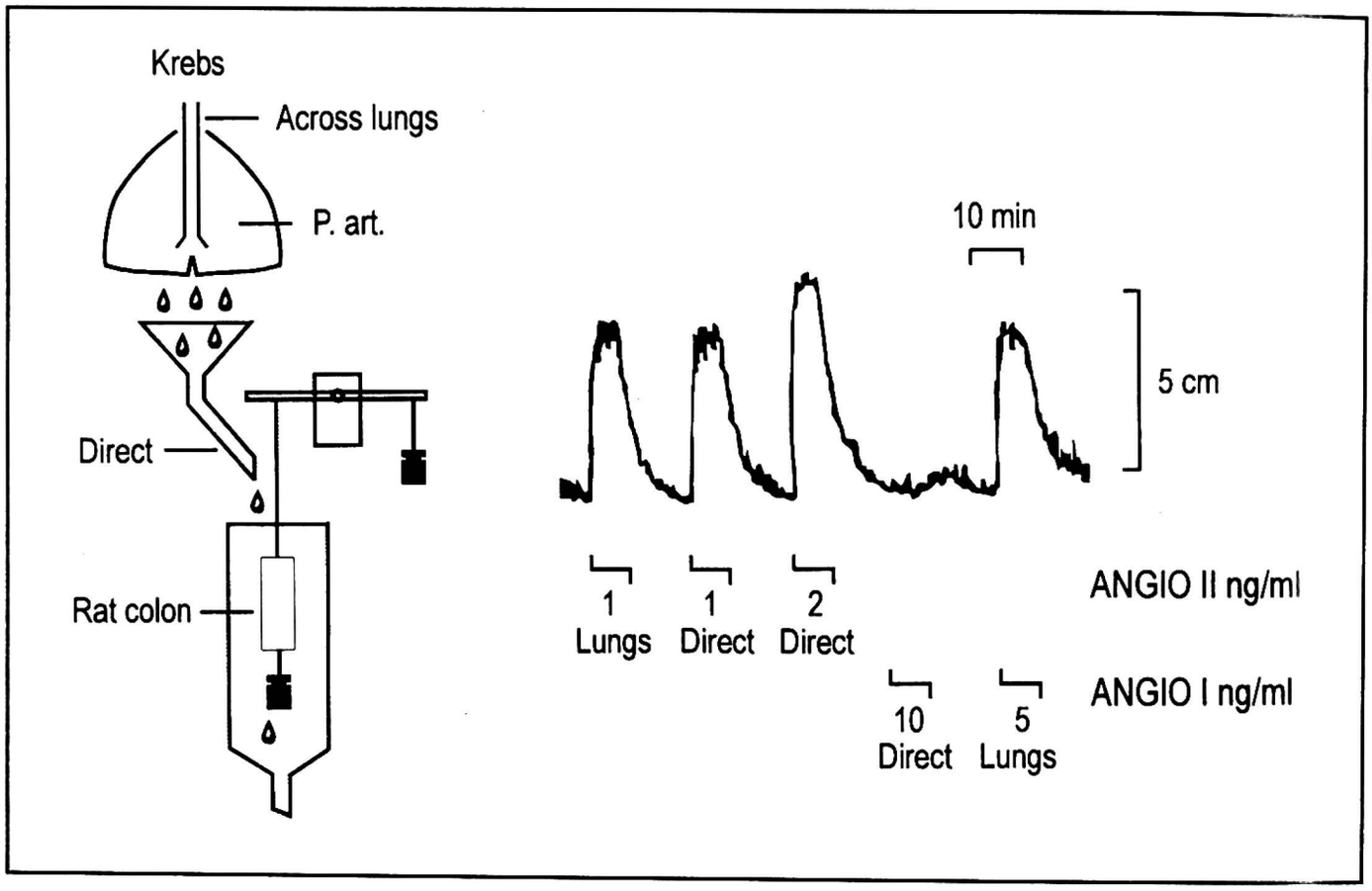

Fig. 3. Increased activity of angiotensin I when passed through guinea-pig isolated lungs. The diagram on the left shows the experimental procedure. Infusions of angiotensin II (Angio II) through the lungs or direct to the rat colon gave the same response of the rat colon, showing that there was no destruction in the lungs. An infusion of Angiotensin I (Angio I) at 10 ng/ml direct to the rat colon gave the minimal response, but then half of this concentration (5ng/ml) was infused through the lungs there was a much greater contraction of the rat colon. (From Bakhle *et al.* 1969).

Our discovery that ACE largely resided in the pulmonary tissues was an exciting one. We did not know which cell contained ACE but we did speculate that, because bradykinin was also largely inactivated in the pulmonary circulation, ACE and bradykininase may be the same enzyme (10). Erdos and his colleagues (11) then showed that ACE was identical to kininase II. It was left to Una Ryan et al (12) to localise the enzyme on the luminal surface of the vascular endothelial cells. There followed an explosion of research on the metabolic function of the lung (7), which are largely associated with the endothelial cells (13, 14). This monolayer lining of the blood vasculature is responsible, not only for angiotensin conversion, but also for generating the vasodilator and anti-platelet prostacyclin, as well as nitric oxide and endothelin. Furthermore, the endothelium inactivates PGE_1, PGE_2, 5-HT, bradykinin and so on (13, 14).

We also measured the release of angiotensin II by various manipulations of the circulation. For instance, when we inflated a balloon in the aorta of the dog in order to reduce renal arterial pressure, there was an outpouring of renin, which caused generation of angiotensin II (15). Similarly, when we removed

blood from the dog in controlled haemorrhages, there was also a release of angiotensin II followed later by a secretion of adrenaline as shown by relaxation of the rat stomach strip (16). We also showed an increase of circulating angiotensin II during carotid occlusion in the dog (17).

In the mid 1960's, a Brazilian, Sergio Ferreira, came to work with me as a post-doc. He was carrying in his pocket an extract of the venom of the poisonous Brazilian viper *Bothrops Jararaca.* He came from the laboratory of Mauricio Roche-e-Silva, who discovered bradykinin in the venom of the snake. Working on the old principle that venom sometimes contained not only noxious substances, but also others that potentiate their effects. Ferreira for his PhD thesis isolated in 1965 from the venom of *Bothrops Jararaca* a factor that he called bradykinin potentiating factor or BPF (18).

Enthusiastically, I suggested to Sergio that he should examine his venom extract on the renin angiotensin system, because we were already immersed in projects involving angiotensin-I and angiotensin-II. He wanted to go on with his work on bradykinin, using the blood-bathed organ technique and being a strong personality, he persuaded me to join him in this work. We published several interesting papers together on the fate of bradykinin in the circulation, including the fact that bradykinin was largely inactivated in a single passage through the pulmonary circulation (19—21).

Eventually, two years later, I persuaded another colleague, Mick Bakhle to test the snake venom extract on angiotensin-converting enzyme and he found it to be a potent inhibitor (22). We followed this up on various bioassay preparations and also in the whole animal. As described above, we had already shown, not only *in vivo* in the dog, but also in isolated lung preparations, that angiotensin I was largely converted to angiotensin II in the pulmonary circulation. It was in such preparations that we also showed that BPF inhibited angiotensin converting enzyme (23).

Interestingly, as shown in *Fig. 4*, the converting enzyme activity in isolated arterial vessel strips varied from vessel to vessel, but not much from species to species. Pulmonary arteries showed the highest activity, then rat aorta and then the carotid arteries. Coeliac, renal, femoral and axillary arteries had little or no activity. In the light of the localisation of ACE on the surface of the endothelial cell, these observations suggest that the levels of ACE vary according to the locality in the circulation.

In 1953, for my own post-doc experience, I went to work at Yale University with Arnold Welch, who had just become the Professor of Pharmacology and I spent two happy years there. By the mid 1960's, he had moved on to become the R&D Director for the pharmaceutical company Squibb in New Jersey and he asked me to be a consultant to them. On one of my early visits I suggested that they should study this snake venom extract which we then knew to be a mixture of peptides. At that time, no one knew whether angiotensin II

formation contributed to high blood pressure. With the help of one of the purified peptides from it, I proposed that they could test whether or not angiotensin II was important in high blood pressure and if it were, then they would have a starting point for a new therapy. Indeed, in parallel work when he returned to Brazil (now convinced that ACE inhibition was important), Ferreira showed that BPF ablated the rise in blood pressure in cats which was caused by the massive release of renin when the blood supply to a kidney was restored after being clamped for six hours (24). BPF also worked in other experimental models of hypertension.

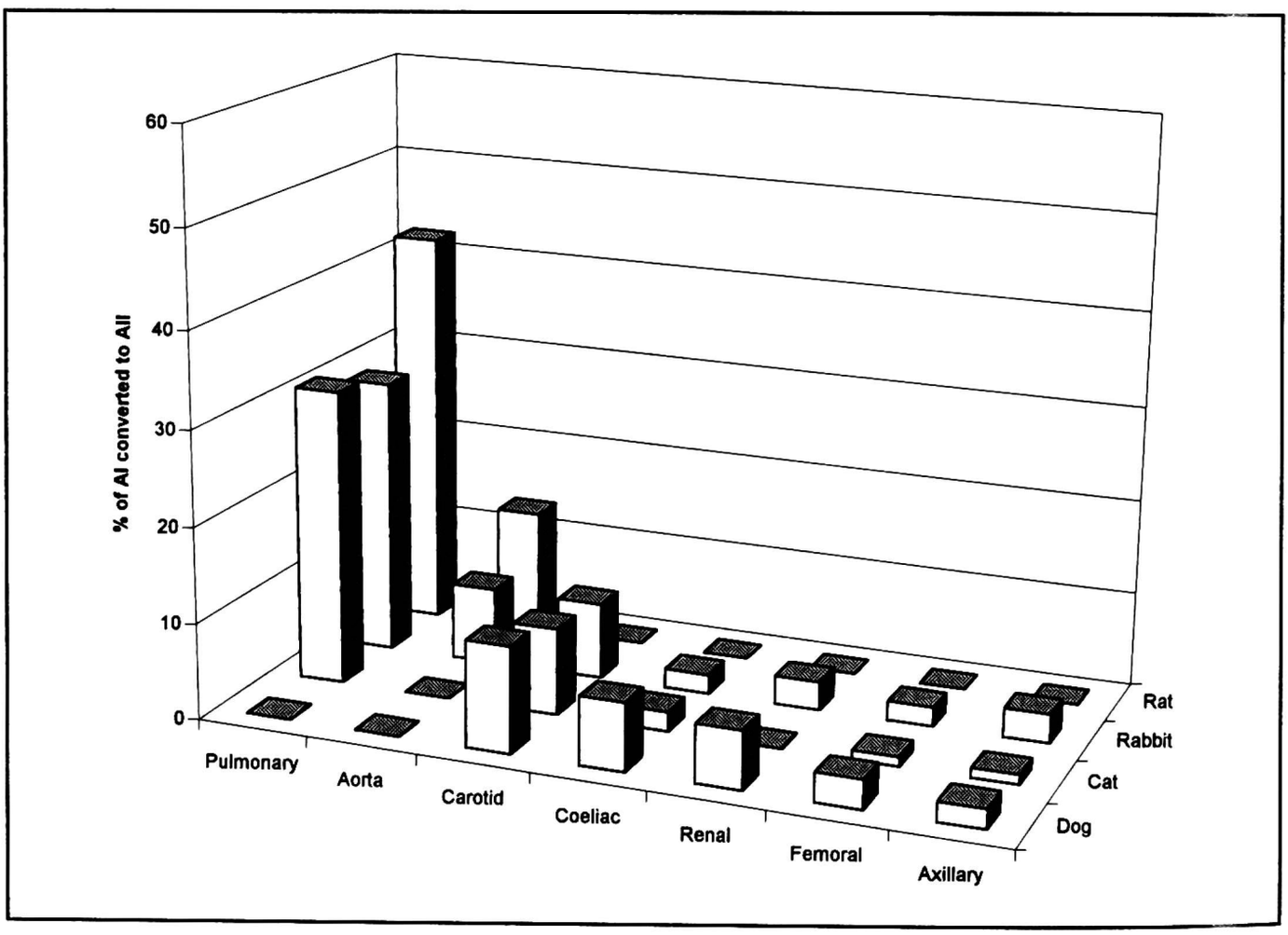

Fig. 4. The diagram illustrates the differences in converting enzyme activity shown by arterial stripes from rat, rabbit, cat and dog. Pulmonary arteries showed the highest activity, then aorta and then the carotid arteries. Coeliac, renal, femoral and axillary arteries had little or no activity.

I visited Squibb three times a year and each time found that their initial enthusiasm for the project was waning, mainly because their marketing people did not comprehend that proving a concept with an extract of snake venom could possibly lead to a new drug. Peptides, they argued, have to be injected rather than given orally and they emphatically reiterated that there was no market for an anti-hypertensive drug that had to be injected. They were in the business of selling drugs and not of proving concepts. Nevertheless, my two

main scientific contacts at Squibb, Arnold Welch, the Vice-President in charge of research, and his deputy Chuck Smith, remained enthusiastic, as did Dave Cushman and Miguel Ondetti who were the bench scientists.

Working independently, Ferreira *et al.* (25) and Ondetti *et al.* (26) characterised the various peptides in BPF. Ferreira concentrated on a pentapeptide, BPP_{5a}, whereas the research of Ondetti *et al.* led them to a nonapeptide, which had a longer duration of action. Both groups synthesised the peptides and showed that they were potent inhibitors of ACE. Squibb eventually synthesized (at a cost of some $50,000) 1Kg of the nonapeptide, which they called teprotide.

In London, in 1973, we showed (27) that when volunteers were given Teprotide intravenously, the conversion of angiotensin I to II was inhibited, so confirming its mode of action Squibb also sent teprotide to John Laragh in New York, who was one of the leading specialists in the USA in hypertension. He injected it into some of his hypertensive patients and was delighted to find that the blood pressure was reduced (28, 29). Thus, the concept was proved: angiotensin was important in hypertension.

In 1973, I left the Royal College of Surgeons to become the R&D Director at the drug company known as Burroughs Wellcome in the States or as the Wellcome Foundation in the UK. I could not continue my relationship with Squibb but Dave Cushman and Miguel Ondetti carried on with the ACE work.

The breakthrough came for Squibb on Wednesday 13th March 1974, when Ondetti and Cushman were discussing a paper describing inhibitors of the enzyme carboxypeptidase (30). A number of properties of ACE suggested to them that it was an exopeptidase with an active site similar to that of carboxypeptidase-A, presumably including the presence of zinc iron. They reasoned that the major difference between the exopeptidases was that the active site of ACE had evolved to accommodate a dipeptide residue rather than a single aminoacid residue as the leaving group for the peptidolytic reaction that is catalysed (*Fig. 5*). The compound they first made as a potential inhibitor was D-2-methylsuccinyl-L-proline. This did indeed inhibit ACE, but it had a disappointingly low activity (31).

They then found that the D-2-methyl derivative of succinyl-L-proline was about 15 times more potent and were encouraged when it showed oral activity for ACE inhibition in animal experiments. A further breakthrough was achieved when the carboxylate was replaced by a simple sulphhydryl function giving a 2000 fold increase in potency as an inhibitor of ACE (31).

Captopril was the final product of these studies in which only 60 compounds were synthesised and tested logically (32). Interestingly, they had also set up a screen for inhibitors of ACE and tested over 2000 random compounds. There were no positive leads.

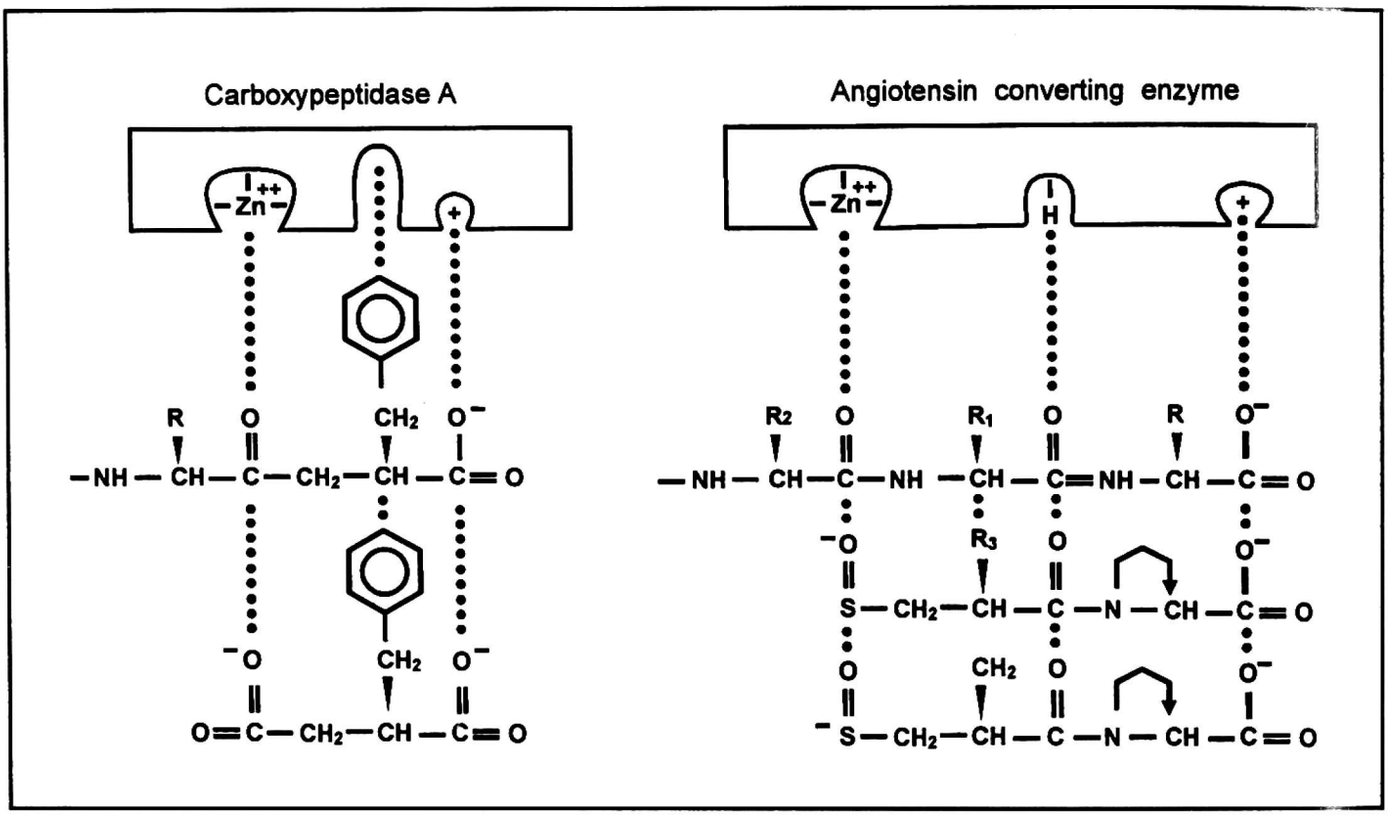

Fig. 5. Schematic representation of substrates and inhibitors binding to active site of pancreatic carboxypeptidase A and to hypothetical active site of ACE.

Merck was not far behind and they developed Enalapril and Lisinopril (32). Here, then, was the birth of a new class of anti-hypertensive drugs, the ACE inhibitors. Between them, the first two alone (Captopril and Enalapril) sell billions of dollars each year. There are now at least 10 other ACE inhibitors available in even more preparations and presentations (*Table 1*).

Table 1. Marketed ACE inhibitors.

● Captopril	● Moexapril
● Cilazapril	● Perindopril
● Fosinopril	● Quinapril
● Fosinopril	● Ramipril
● Imidapril	● Trandolapril
● Lisinopril	

There are many interesting aspects of this scientific trail from snake venom to ACE inhibitors as valuable therapeutic agents. Serendipity, chance and coincidence all played a part. In the first place, Sergio Ferreira wanted to go to Oxford for his post-doc. studies to work with Bill Paton. However, his wife wanted to take a PhD at the London School of Economics, which was just

round the corner from my laboratories in the Royal College of Surgeons and so he came to work with me. Had he gone to Bill Paton, Captopril may not exist.

And then the bioassay and the blood-bathed organ technique were very powerful tools in the mid-1960's and 1970's for making important new discoveries. Without them, we would not have discovered how aspirin works (2), the metabolic functions of the pulmonary circulation (7), the conversion of angiotensin-I to II in the lungs (8—10), prostacyclin (33), and Salvador Moncada would not have discovered the identity of EDRF as nitric oxide (34).

This story, along with many others involving new block-busting drugs also demonstrates the clash between science and marketing. In this instance, as well as with H_2 antagonists, beta-blockers and many other new drugs, the marketing arm of the company gave them no support because they could not define what the market would be. Marketing research works on data from the past and to predict whether a new drug with a new mode of action will be a success was then and is still very difficult.

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