Personal and Historical Perspectives

Discovery of the Two Angiotensin Peptides and the Angiotensin Converting Enzyme

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I have long believed that most important scientific discoveries are in large part accidental. Very few of us are like Christopher Columbus, who reasoned that the world was round and set out with his three little ships to prove that he was right.

In 1953, our group at the Cleveland Veterans Administration Hospital (associated with Western Reserve University, now Case Western Reserve University) consisted of Joseph R. Kahn, a pathologist who had worked with Harry Goldblatt during his important discoveries in the field of hypertension, Walter Marsh, and myself. Walt Marsh and I had recently received our PhD degrees in biochemistry from Western Reserve.

These were exciting times for us. We were in what we considered to be a race to isolate and determine the structure of angiotensin with Merlin Bumpus and his group at the Cleveland Clinic and Stanley Peart in London.

I must explain, parenthetically, that angiotensin was known to us, being followers of Harry Goldblatt and Eduardo Braun-Menendez, as hypertensin. Merlin Bumpus knew angiotensin as angiotonin. This was natural because Merlin worked with Irvine Page, who had coined the name angiotonin. It was a number of years later that Page and Braun-Menendez agreed on the name angiotensin, and all the rest of us used the new name.

We prepared very crude renin from hog kidneys and an equally crude substrate from horse blood. Both preparations were thoroughly dialyzed against distilled water, and the insoluble proteins formed during dialysis were removed by centrifugation. The renin was then reacted with its substrate to produce angiotensin. The reaction was terminated by the addition of 3 volumes of alcohol. After filtration, the alcohol was removed by evaporation, and the cloudy, aqueous solution was extracted with ethyl ether.

The resulting solution was then subjected to countercurrent distribution between *n*-butanol and phosphate or bicarbonate buffer. Countercurrent distribution was then newly developed by Lyman Craig at Rockefeller University, and it proved very useful to us because none of the chromatographic methods that were available at the time worked well with angiotensin. The results of the distribution could also be mathematically analyzed to obtain an estimate of a component's purity. We later used the method to good advantage in our purification of angiotensin I, angiotensin II, and the tetradecapeptide renin substrate.

At the time, the hospital (known locally as the Crile VA Hospital) was located in a sprawling, one-floor brick and wood structure that had been hurriedly put together during World War II as an Army general hospital for wounded veterans from the war in Europe and the Pacific. We had two small rooms in the clinical laboratory in which to do our work. We were soon overflowing into and up and down the narrow hallway outside our doors with our tanks, centrifuges, and other equipment. Evidently, we were such a nuisance that the hospital manager moved us to an empty, unused former mess hall with 3,600 square feet of uncluttered space.

Moving completely interrupted our work, and we got nothing done for 2 or 3 weeks. Finally, we were able to start work again in our new location. We prepared more crude renin and substrate. In preparing the new substrate, I made a small change in the procedure. Instead of dialyzing the preparation against distilled water, I used saline. I don't remember why I did this. Perhaps I thought I could avoid the necessity of removing insoluble proteins by centrifugation.

We prepared angiotensin with the new renin and substrate. After the necessary preparatory steps, we submitted the batch to countercurrent distribution. In this instance, the distribution was carried out manually in twenty 1-L separatory funnels, and the process took all day for two of us. We then prepared samples from each separatory funnel for bioassay using an anesthetized rat. To our utter consternation and surprise, the active band was not located in the middle of the countercurrent distribution as it always had been in previous experiments. Instead, it was found in the very first separatory funnels. In spite of having carried out twenty extractions, the active pressor material was still in the first one or two funnels!

We then spent a number of days trying to discover what we had done to obtain such a remarkably different result. It finally came to me that we had dialyzed our substrate against 0.9% NaCl instead of water. Subsequent experiments showed that when renin was reacted with our crude substrate in the absence of chloride, the product was found in the middle of the distribution and had a distribution coefficient close to 1.0. In the presence of chloride, the product was found in the first few funnels with a coefficient close to 0.1. The first product we called angiotensin I, the second angiotensin II. Both were active in our rat bioassay.

We then found that renin had nothing to do with determining whether we obtained angiotensin I or an-

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giotensin II; angiotensin I could be converted to angiotensin II by incubation with our crude substrate in the presence (but not absence) of chloride ions. After considering these results, I developed the theory that there was an enzyme in our crude substrate that was activated by chloride ions and that converted angiotensin I to angiotensin II. Although it appears obvious now that my theory was correct and that this was the first demonstration of the angiotensin converting enzyme, it did not seem clear to me at the time. I remember spending several hours with Harland Wood, who was then chairman of the Department of Biochemistry at the medical school at Western Reserve University. I remember distinctly that after 2 or 3 hours of going over our data and expressing my concern about my theory being correct, he told me (probably out of exasperation) not to worry so much about being wrong, that we all make mistakes, and that if I was wrong, it wouldn't be fatal.

My good friend, Oscar Helmer, then wrote me from the Eli Lilly Laboratories. He had also been working on the purification of angiotensin. He informed me that he had found that incubating his angiotensin preparations with plasma greatly increased their activity in his aortic strip bioassay and wondered if this could be due to our angiotensin converting enzyme producing an active angiotensin II from an inactive angiotensin I.

We put this possibility to the test without delay, using an isolated perfused rat kidney. In this way, we discovered that angiotensin I does not vasoconstrict the isolated kidney perfused with Ringer's solution, whereas angiotensin II does. Here again we were fortunate in picking as a test tissue the rat kidney that did not contain the angiotensin converting enzyme, thus allowing us to discover that angiotensin I is the inactive precursor of angiotensin II.

The finding that there were two forms of angiotensin, a biologically inactive decapeptide and a highly active octapeptide, and the enzyme, the angiotensin converting enzyme that transforms one peptide to the other, was an immediate stimulus to further progress. The structure of both peptides was quickly determined, and angiotensin II was successfully synthesized within the year. Suddenly, large amounts of synthetic angiotensin II were easily available. Just as suddenly, there were hundreds of researchers working in hypertension, rather than the handful of investigators in Europe, South America, and the United States that preceded them.

Later, inhibitors of the angiotensin converting enzyme were developed and used to demonstrate conclusively that the renin-angiotensin system causes the rise in blood pressure in most people who have high blood pressure. These inhibitors are in widespread use for the treatment of hypertension today.

In most cases, those who have made an important discovery are not entitled to harbor any conceit whatsoever as to their intelligence. They are, however, privileged to experience the very real and lasting joy that comes with an unexpected discovery.

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