Personal and Historical Perspectives

The Experimental Observation That Led to Discovery of Angiotensin
1939 Buenos Aires, Argentina

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This article is a short story of the work done by our group in Dr. Bernardo Houssay’s laboratories in Buenos Aires 50 years ago that led to the discovery of angiotensin.

In 1934 I was 23 years old and a student at the Faculty of Medicine of Buenos Aires University. I was paying for my studies by working as an undergraduate assistant teaching physiology to medical students at the “Instituto de Fisiología” directed by Dr. Houssay. At that time, the Instituto de Fisiología was very active in various fields of research, such as carbohydrate metabolism and diabetes, cardiology, endocrinology, and others.

To acquire a medical degree, I had to present a thesis that had to be approved by a special jury. I asked Dr. Houssay to supervise my doctoral thesis. He suggested experimental renal hypertension as the subject of my thesis.

Attempts to induce sustained renal hypertension in laboratory animals had failed despite the different techniques devised such as reduction of renal parenchyma, occlusion of branches of the renal arteries, irradiation of the kidneys with x-rays, passive congestion of the kidney by venous obstruction, and many others. Conflicting results were published by various investigators. An initial rise of the blood pressure followed some of these procedures, but the rise was short-lived and prevented the study of the mechanisms involved. Sustained neurogenic hypertension, however, had already been induced by Koch and Mies in 1929 by the removal of the carotid and sinus nerves.

While I was striving to induce arterial hypertension in rats by burning the cortex of the kidneys and facing problems with the measurement of the blood pressure, I came across the paper published by Goldblatt and coworkers in 1934. They showed that the reduction of the blood flow through the kidneys induced permanent hypertension in dogs. The introduction of a reliable method to induce permanent renal hypertension opened a new era in the studies of arterial hypertension.

I gave up my experiments in rats and, with the approval of Dr. Houssay, I began to produce hypertensive dogs with the method described by Goldblatt. After initial difficulties, I was able to induce benign and malignant hypertension in dogs as previously shown by Goldblatt. I studied the role played by the nonischemic kidney and the adrenal glands on the development of renal hypertension, as well as the anatomic alterations of the blood vessels.

The role played by the nervous system had been studied by Goldblatt and other investigators. It was shown that hypertension due to renal ischemia was not due to a nervous mechanism, such as a reflex of renal origin or hyperactivity of the vasoconstrictor nerves. Thus, the increase in the peripheral resistance seemed due to a humoral mechanism. Goldblatt explained his view with these words: “The view that in the pathogenesis of hypertension due to renal ischemia, a humoral mechanism involving a hypothetical effective substance of renal origin plays a part of primary importance is based almost entirely upon indirect evidence.”

The idea of a pressor substance being the cause of hypertension was not new. Volhard in 1923 postulated a vasospastic factor as the cause of “pale” hypertension (malignant hypertension). According to him, the presence of a vasospastic substance was shown by the blanching of the skin and by ocular and cerebral symptoms. One of his collaborators, Bohn, reported that a vasoconstrictor substance was present in the blood of hypertensive patients, but his results were disproved by the work of other investigators.

Searches for the pressor substance in the blood of hypertensive animals were fraught with difficulties and negative or inconclusive results presented by various investigators.

The search for pressor substances in the ischemic kidney was also pursued. Crude saline extracts of normal and ischemic kidneys injected intravenously in dogs and rats induced an initial fall of the blood pressure that was followed by a rise. Usually the increases in pressure induced by the extracts of ischemic kidneys were greater than those produced.
by extracts of normal kidneys, but it was difficult to make a quantitative estimate of the results.

I reasoned that, if a pressor substance was continuously released by the kidney, the amount found in the kidney extracts might not be increased if the turnover was rapid. I was lucky that in these days Drs. Houssay and Foglia were working on the secretion of insulin by grafting the pancreas to pancreatectomized dogs. To graft a kidney was simpler than to graft a pancreas, and it seemed to me that such a technique could yield more direct evidence about the release of a pressor substance from the ischemic kidney.

Dr. Houssay showed me how to do the renal grafting. The renal artery of an ischemic kidney from a Goldblatt hypertensive dog was connected to the carotid artery of an anesthetized dog, and the renal vein was connected to the jugular vein using a special type of cannula. The endothelium of the carotid artery and jugular vein contacted those of the renal artery and vein, so the use of anticoagulants was unnecessary. Recipient dogs were nephrectomized because we suspected that the normal kidneys might inactivate the pressor substance.

We were rewarded with a beautiful experiment. Once the clip on the carotid artery was opened and the blood began perfusing the grafted kidney, the arterial pressure of the recipient dog began to rise slowly. The increase, more than 40 mm Hg, was maintained during 30 minutes or more after the perfusion was discontinued. The same kidney was grafted to another dog and also induced a spectacular rise of the arterial pressure (Figure 1). This experiment indicated that the kidneys were secreting a pressor substance, as the increase of the arterial pressure caused by the graft of the same kidney to a second dog could not be attributed to washout of pressor material retained in the kidney. Similar experiments confirmed these results while the grafting of kidneys from nonhypertensive dogs was usually unable to increase the arterial pressure of recipient dogs.7,8

Taquin19 showed that the blood of the renal vein of the ischemic kidneys of hypertensive dogs had vasoconstrictor activity. He perfused the diluted plasma through the hind legs of toads, the so-called Lawn-Trendelenburg preparation. He found that, although the blood of normal dogs was ineffective, that of hypertensive animals induced strong vasoconstriction.

These results convinced us that the increase of the arterial pressure that followed renal ischemia was caused by the release of a pressor and vasoconstrictor substance secreted by the kidney. Thus, the kidneys appeared to have an endocrine function. The function seemed to us related to the regulation of the blood pressure inside the glomeruli. The clamp placed on the renal artery reduced its intrarenal pressure and impaired glomerular filtration. The release of the pressor substance would raise pressure within the glomerulus by increasing the arterial pressure. Hypertension due to renal ischemia thus seemed to be a compensatory mechanism.

Naturally, we needed to learn more about the pressor substance to study its physiological activity, to purify it, and eventually to isolate it in pure form. I realized that I could not do that alone. Fortunately, Dr. Eduardo Braun-Menéndez, who had just returned to Argentina after spending a year in England, was interested and offered his help. Thus began a cooperative effort that lasted for several years and a friendship that lasted until his tragic death in an airplane accident in 1959.

To accomplish our project, we needed an abundant source of renal venous blood from ischemic kidneys. The preparation of hypertensive dogs, however, was laborious and difficult. If it had been necessary to use the hypertensive dog as a source of pressor material, progress would have been slow and difficult. Fortunately, we found that dog kidneys, either grafted in the neck of recipient dogs or perfused with defibrinated blood with a Schuster-Dale pump, released a pressor substance within minutes after 80–90% reduction of the blood flow.10 The pressor activity of the venous blood was tested by intravenous injection of 20–40 ml of the venous blood of these kidneys into nephrectomized dogs. The blood pressure of the recipient dogs rose slowly, and the elevation persisted for more than 30 minutes, resembling the effect found by grafting of ischemic kidneys. The pressor activity persisted after bilateral vagotomy, atropine, cocaine, or injection of the sympatholytic drug Fourneau 933. Nephrectomized dogs were more

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**Figure 1.** Smoked paper record of the arterial pressure of two nephrectomized dogs. Gift of kidney of a hypertensive dog was first made to dog A at 1; kidney was withdrawn at 2 to be grafted to dog B at 1 and removed at 2 to be grafted again to dog A at 3. Pressure in millimeters of mercury, time in minutes. Reprinted with permission from Fasciolo JC: Hipertensión Arterial Nefrógena. Estudio Experimental. Buenos Aires, "El Ateneo," 1939
sensitive to the pressor substance. At this point, we had developed a fairly abundant source of the pressor substance and a semiquantitative method to estimate the pressor activity of the blood.

We were now ready to proceed to the next step: The identification of the pressor substance. Two friends of Braun-Menéndez and myself, Drs. L.F. Leloir and J.M. Muñoz, were at that time working at the Institute of Physiology on biochemical aspects of alcohol metabolism. They had followed the ups and downs of our work and became interested in joining us in the project of purifying the pressor substance. We welcomed their help, and a four-person team was formed; Leloir and Muñoz worked mainly on the chemical aspects, and Braun-Menéndez and I worked on the pharmacological aspects.

After trying the toad preparation that had been previously used by Taquini to test for the activity of the various extracts, we decided to use dogs, a more expensive but a more reliable method of assay. Almost every day we discussed the successes or failures of our experiments with optimism and good humor. Dr. Houssay was aware of the progress of our research and helped us with his advice and his constant support.

Extracts of the venous blood from the ischemic kidneys made by the addition of 3 volumes of acetone were found to increase arterial pressure of the dogs, whereas extracts made from venous blood of normal kidneys failed to raise pressure. The blood pressure rose abruptly, but the increase lasted for only a few minutes. We were puzzled, as we expected a long-lasting rise similar to that induced by grafting an ischemic kidney or by the injection of the venous blood from an ischemic kidney.

The pressor substance was thermostable, insoluble in ether, and had a vasoconstrictor action on the vascular bed of the toad. Its chemical and pharmacological properties were different from those of other pressor substances known at that time. We called it hypertensin because we believed it to be the humoral mediator of renal hypertension.

Hypertensin was quite different from renin, the pressor substance discovered in 1898 by Tigerstedt and Bergman[11] in the renal cortex. Renin was thermostable, did not dialyze, and its pressor action was prolonged, whereas hypertensin was thermostable, dialyzable, and had a short pressor action.

Grafting of an ischemic kidney as well as the injection of the blood coming from acutely ischemic kidneys produced a prolonged pressor effect that was more easily attributable to renin than to hypertensin. If renin was the substance causing the rise of blood pressure in both cases, it was difficult to explain the presence of hypertensin in the renal venous blood.

We made attempts to produce hypertensin in vitro by incubating renal cortex slices with blood serum under conditions of anoxia, but there was no evidence for the formation of any pressor substance. We now know that these negative results were probably due to the presence in the kidney slices of enzymes that would destroy any hypertensin formed.

An apparently foolish experiment was tried later. A crude extract containing renin was incubated at 37°C with blood serum, and to our surprise, a pressor substance was formed that seemed identical to hypertensin (Figure 2). Several incubation periods of the renin preparation with blood serum were tried. When the incubation time was prolonged, the pressor activity was found to be less and to completely disappear after longer incubations. Clearly, the pressor substance was being destroyed by enzymes present during the incubation. These results were published in 1939 in the "Revista de la Sociedad Argentina de Biología."[12] The conclusion stated:

A hypertensive substance, soluble in 95% acetone and in glacial acetic acid, insoluble in ether and amyl alcohol, dialyzable and very resistant to acid hydrolysis (until 2 hours in 1N HCl) was extracted.
from the venous blood of ischemic kidneys. By incubating renin with blood serum or with its pseudoglobulins, the same substance was formed for which was proposed the name of hypertensin. The intravenous injection of hypertensin, either formed “in vitro” or extracted from venous blood induced an increase in the arterial pressure not abolished by Fourneau 933. Chemical and pharmacological properties of hypertensin differ from those of renin, pitressin, urohaptensin, adrenaline, and tyramine. Renin appears to be an enzyme of the papain type, its substrate a protein in the pseudoglobulin fraction and the product hypertensin.

The nomenclature adopted was the enzyme, renin; the substrate, hypertensinogen; the product, hypertensin; and the enzymes that destroyed it, hypertensinases.

To our dismay, we learned, a couple of months after the publication of our paper, that the same substance was reported in 1940 by Page and Helmer,13 who followed an entirely different approach. Kohlsteadt, Helmer, and Page14 found that purified renin preparations produced a rise of blood pressure when injected intravenously into intact animals but did not produce vasoconstriction if it was dissolved in Ringer's solution and perfused through the vessels of the dog's tail or the rabbit's ear. They showed that the vasoconstrictor activity could be reestablished by the addition of plasma proteins. To this plasma protein they gave the name of renin activator, indicating that renin was inactive as a pressor substance without its activator. The combination of purified renin and its activator gave rise to a substance with a vasoconstrictor action. Page and Helmer13 called this vasoconstrictor substance angiotensin. Hypertensin and angiotensin were the same substance, and hypertensinogen and renin activator were synonyms.

There were differences between both laboratories concerning interpretations and nomenclature. Hypertensin and angiotensin were indeed the same substance, but to have different names complicated the nomenclature. Braun-Menéndez and Page met in Ann Arbor in 1958 and agreed on a new name with 50% of the letters of the two previous names. The new word “angiotensin” was accepted by all the investigators working in this field. In a letter that Dr. Page sent to me in June 1985, he made this reflection: “Too bad we can't leave a historical puzzle so some younger can write a book about a controversy which did not occur. I hope the hypertensin story can be a model for future scientists to show how difficult situations can be solved like gentlemen and friends.”

In the years that followed the discovery of angiotensin, we studied its enzymatic release from angiotensinogen, identified angiotensin as a peptide, studied the secretion of renin by the kidneys, the formation of angiotensinogen by the liver, the pharmacology of angiotensin, and other subjects.15-17 Dr. Taquini rejoined us after a year in Boston.

One important contribution was the finding that pig renin did not produce angiotensin when incubated with human plasma, whereas human renin did.18 On the other hand, when injected in humans, pig renin was inactive, whereas human renin increased the arterial pressure. These experiments showed that renin has physiological activity only through the release of angiotensin.

Dr. Lewis Dexter from Harvard Medical School was interested in our work and came to Buenos Aires in 1940 to spend a year in our laboratory. He was an intelligent and open-minded young person whom we liked very much. He translated into English the book Hipertensin Arterial Nefrógéna, which we published in 1943.19 This book was written by Braun-Menéndez, Leloir, Muñoz, Taquini, and myself when we had to stop our experimental work after Dr. Houssay was expelled from the University for political reasons. According to Dr. M. Bumpus, our book served as a reference source during the decade after its publication for scientists interested in hypertension research.

In the years that followed the discovery of the renin-angiotensin system, the main goal was to establish its role in renal hypertension, human or experimental. Studies were handicapped by the lack of methods sensitive enough to estimate either the renin or the angiotensin content of the blood.

Important discoveries made later showed that the renin-angiotensin system was more complex than previously thought and its physiological role wider.

Although the role played by the renin-angiotensin system in hypertension is controversial, the inhibition of angiotensin II release through the blockade of the angiotensin converting enzyme is at present one of the most effective treatments for hypertension.

I'm glad that the pioneering work of our group in Argentina contributed to a better understanding of the mechanisms of arterial hypertension and to its treatment.

Acknowledgments

I wish to render homage to the people to whom I feel indebted: Dr. A.B. Houssay, who introduced me to research; Drs. E. Braun-Menéndez, L.F. Leloir, and J.M. Muñoz, with whom I shared the discovery of angiotensin; and Dr. A.C. Taquini, who did crucial experiments and was a part of our group. I also thank Dr. Allyn Mark for his invitation to write an article on angiotensin discovery and Dr. J.C. Romero for revising my manuscript.

References


8. Fasciolo JC, Houssay BA, Taquini AC: The blood pressure raising secretion of the ischemic kidney. J Physiol (Lond) 1938;94:281-293


15. Leolir LF, Munoz JM, Braun-Menendez E, Fasciolo JC: La secreción de renina y la formación de hipertensina. Rev Soc Argent Biol 1940;16:75-80


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