Presence of Renin in Plasma of Patients with Arterial Hypertension

By Oscar M. Helmer, Ph.D.

I was asked to give a brief historical summary of the discovery of angiotensin before presenting my current work. In the collaborative work with Dr. Irvine Page and Dr. Kenneth Kohlstaedt, which began in 1937, my task was to prepare renin from hog kidneys. While I was so engaged, Dr. Kohlstaedt and Mr. Wilson were perfusing an isolated dog's tail with Ringer's solution in an effort to determine whether plasma from hypertensive dogs had greater constrictor activity than plasma from normotensive dogs. Late one afternoon, I climbed the stairs to their laboratory with a renin preparation of which I was quite proud and suggested that we put some of this potent extract through the dog's tail to see what would happen. Since they were through with their work for the day and were ready to discard the preparation anyway, and since they were not finding any differences between the "normotensive" and "hypertensive" plasmas, they agreed to the suggestion. We found that this potent kidney extract containing renin, which was known to cause a strong elevation in blood pressure when given intravenously to dogs, was inactive in the dog's tail perfused with saline.

The next step was obvious. The renin solution was mixed with plasma and put through the tail. An intense vasoconstriction occurred. Since we knew that renin was a protein, it was boiled and mixed with plasma. This mixture was inactive. Similarly, a mixture of renin with boiled plasma caused no constriction. However, when renin and plasma were incubated together, even at room temperature, and boiled and the coagulum centrifuged off, the resulting solution caused a strong constriction of the vessels of the isolated dog's tail. The substance in the plasma which reacted with renin we called renin-activator. Soon thereafter, Dr. Page and I reported a new substance formed by the interaction of renin and renin-activator. We called it angiotonin.

Shortly before his untimely death, Braun-Menendez, in a lecture at the Indiana University School of Medicine, described the steps which led to the discovery of hypertensin in the Institute of Physiology at the University of Buenos Aires. Houssay had become interested in hypertension in 1923. The motivation for this research was the death of one of his most brilliant pupils, who died of malignant hypertension at the age of 33 years. Houssay and Fasciolo, in 1937, were the first to demonstrate that a humoral mechanism was involved in the type of experimental hypertension produced by Goldblatt and his coworkers. They produced hypertension in a dog by Goldblatt's technic and grafted 1 of its kidneys into the neck of a nephrectomized dog. The blood pressure of the latter dog began to rise in a few minutes, and after the elevated pressure was established, it remained at a high level for 2 to 3 hours, even after the removal of the grafted kidney.

Houssay then called Braun-Menendez, Fasciolo, and Leloir, a biochemist, for a conference. He told them, "Well, here we have a very important observation. Something is poured into the blood by the kidney. This substance we must isolate." As Braun-Menendez remarked, "It was an easy thing to say but not so easy to do." The experiment which led to their discovery of a substance, which they named hypertensin, was the grafting of a normal kidney into the neck of a nephrectomized animal and constricting the renal artery. The renal vein blood of this preparation had pressor properties. Acetone extracts

From the Lilly Laboratory for Clinical Research, Marion County General Hospital, Indianapolis, Indiana.
Table 1

Preparation of Blood for Assay of Renin

1. Blood is collected with heparin as anticoagulant and centrifuged immediately.
2. If plasma not dialyzed immediately, it is frozen.
3. Plasma adjusted to pH 5.5 with HCl and dialyzed overnight in “Visking” casings.
4. After dialysis, plasma made isotonic with NaCl and centrifuged to remove insoluble protein. Supernatant adjusted to pH 5.5 with HCl and stored in frozen state.
5. Before being added to muscle chamber, plasma adjusted to approximately pH 7.2 with NaOH.

Menendez agreed on the term angiotensin for the pressor substance and angiotensinogen for the substrate upon which renin acts.

Since then, the polypeptide nature of angiotensin has been proved by the determination of its amino acid sequence; that of ox angiotensin by Elliott and Peart and of horse angiotensin I and II by Skeggs, Lentz, Kahn, Shumway, and Woods. Helmer and Skeggs, Kahn, and Shumway have presented evidence that the true pressor agent is not the decapeptide but the octapeptide. Angiotensin I is equally active in vivo because it is rapidly converted, when given intravenously, by a halide-activated enzyme in plasma, called “converting enzyme” by Skeggs.

Recently, Dr. Judson and I reported the presence of vasoconstrictor and vasopressor activity in renal venous and peripheral blood of patients with arterial hypertension. In this paper, I wish to present evidence that we are measuring renin activity.

Since all of the components for the production of angiotensin—renin, renin-substrate, and converting enzyme—are proteins and, therefore, nondialyzable, it is possible to remove by dialysis vasoactive agents not related to this system. No attempt was made to remove angiotensinase because we wanted to modify the plasma as little as possible.

Table 1 shows how the blood was treated. The plasmas were assayed for vasoconstrictor activity in a spirally cut strip of rabbit aorta or for vasopressor action by intravenous injection in a 2-day postnephrectomized cat. In figure 1 is shown a comparison of the constrictor activity of plasmas from the left and right renal vein.
right renal veins of a patient with accelerated hypertension and those from a normotensive patient. A strong contraction was produced by 0.5 ml. of renal venous plasma from the patient with hypertension, whereas 2.0 ml. of renal venous plasma from the normotensive patient was inactive.

In figure 2, 5 ml of brachial artery plasma from the same 2 patients were injected intravenously in a 2-day postnephrectomized cat. The plasma from the hypertensive patient (J.S.) caused a marked elevation in pressure, whereas the plasma from the normotensive subject (D.W.) showed only a slight change as a result of volume effect. With these plasmas, as well as with all others tested, there was a close parallel between the constrictor activity on the aortic strip and the pressor activity in the pithed cat. The contour of the pressure curve of the active plasma differed from that of the angiotensin standard in that the pressure remained above the baseline. This response to plasma may be due to renin in the plasma and its reaction product, angiotensin. Evidence that the enzyme in the plasma had the characteristics of renin and that its reaction product behaved like angiotensin will be presented in the following sections.

Nature of the Vasoactive Factors

The aortic strip is an excellent preparation for working out factors concerned in the formation of the vasoactive agent in plasma.

Human renin or renin from other species does not cause a contraction of the muscle. Furthermore, renin-substrate and angiotensin I are inactive on the strip. In figure 3, a response to dialyzed plasma from a patient with malignant hypertension is compared with that to angiotensin. Sometimes, depending on the sensitivity of the strip and the activity of the plasma, the contour of the response is identical to that with angiotensin II. However, as in this figure, the delay in shortening often is longer, and the rate of contraction is slower.

In figure 3, 0.15 of our unit caused a recorded 31-mm. contraction. In some strips, 0.1 unit causes as great or even greater shortening. Our angiotensin has been standardized against a pure sample of Skeggs’s natural angiotensin II, 1 μg. of which is equivalent to 2.38 Goldblatt units (1 Goldblatt unit is equivalent to 0.42 μg.). Since our unit is one-sixth of a Goldblatt unit, our unit is equal to 0.07 μg. of angiotensin II. As little as 0.05 of our unit, equivalent to 0.0035 μg. of angiotensin, can be readily detected.

The activity of freshly dialyzed plasma is destroyed by boiling. After incubation at 37
C. at pH 5.5 for 1 to 2 hours, the pressor and constrictor activity of a boiled and unboiled active plasma are the same. The vasoactivity is due to the liberation of a heat-stable substance by an enzyme which, from our studies to date, behaves identically to renin, as briefly summarized in the following paragraph.

The enzyme kinetics are the same as renin. The optimum activity of the enzyme in dialyzed plasma is approximately pH 5.5. When human plasma was incubated with angiotensin for 1 hour at 37 C. at pH 4.5 to 8.5 in increments of 1.0 pH unit, the maximum inactivation occurred at pH 7.5. This may partially explain the greater activity of plasma when incubated in vitro at pH 5.5 rather than at pH 7.4. It also behaves like human renin in that its activity is increased tremendously by a rise of reaction temperature from 2 to 37 C. The enzyme is of renal origin. It is always found in greater quantity in the renal venous blood than in peripheral blood. Samples of peripheral plasma from patients with unilateral renal vascular disease have a high content of renin. After removal of the involved kidney, the activity of the peripheral plasma showed very low or no demonstrable constrictor activity.

Further evidence for the identity of the enzyme in plasma of hypertensive patients with renin was obtained by incubating an “active” plasma and an “inactive” plasma for 4 hours at 37 C. at pH 5.5. Any angiotensin formed during the incubation was removed by adsorption on animal charcoal. Before this procedure, the renin-substrate in terms of angiotensin units was 8.4 AU/ml. After the incubation and charcoal treatment, the renin-substrate was reduced to 6.6 AU/ml.

The vasoactive heat-stable factor liberated by the action of the renin-like enzyme has the following properties which are similar to angiotensin: It has the properties of a polypeptide. It is destroyed, as shown in figure 4, by incubation with crystalline trypsin and chymotrypsin under the same conditions as angiotensin. The vasoactive substance is also inactivated by kidney and red cell angiotensinase. These properties, as well as the comparatively short incubation time necessary for its release and its formation when incubation is performed in the presence of ethylenediaminetetra-acetic acid, differentiate it from vasopressor lipids found in plasma. The contour of the pressure curve cannot be differentiated from angiotensin (fig. 4). Other characteristics of this polypeptide which are also identical to angiotensin are the rate of dialysis through “Visking” casings, adsorption on animal charcoal, and elution by glacial acetic acid. Pharmacologically, too, the constrictor activity of dialyzed plasma on the aortic strip is similar to angiotensin II. Neither is blocked by phentolamine. Aortic strips which are insensitive to angiotensin II are also insensitive to “active” plasma, even when the strip responds strongly to catecholamines.

The presence of the converting enzyme and the 2 forms of angiotensin can also be demonstrated in the dialyzed plasma of patients with arterial hypertension, as shown in figure 5. The plasma was dialyzed in a cold room for 4 days with running double-distilled water at 4 C. Aliquots were incubated for 1 hour at 37 C. at pH 5.5, with and without the addition of NaCl, and boiled to stop the reaction. The coagulum was removed by centrifugation and the supernatant assayed for constrictor activity on the aortic strip. The dialyzed plasma incubated without NaCl was inactive like angiotensin I. The sample incubated with NaCl behaved like angiotensin II.
I feel that the data presented in this paper demonstrate the presence of renin in the blood of patients with arterial hypertension. By means of kinetic studies based on the renin-angiotensin system, it is possible to express the renin content of dialyzed plasma in terms of Goldblatt units per L. For example, the renal venous plasma from the involved kidney of a patient with unilateral kidney disease contained 7.0 Goldblatt units of renin per L. The brachial artery blood, drawn 5 days before, had a value of 1.5 units. These values are compatible with a blood pressure of 200/130. Two hours after the removal of the involved kidney, no renin could be detected in the peripheral blood of this patient.

Dr. Judson and I have assayed approximately 400 samples of plasma from patients with arterial hypertension. The highest values for renin are found in accelerated hypertension and in patients with renal vascular occlusive disease. In the latter condition, it has proved quite useful in determining which is the involved kidney. The plasmas of many patients with essential hypertension have an elevated renin content. However, the plasmas from some patients with high pressure are inactive. Since such small amounts of plasma are needed to do the assay, it is hoped that the application of the technics described in this paper will help to clarify the role of renin in arterial hypertension.

References
Presence of Renin in Plasma of Patients with Arterial Hypertension

OSCAR M. HELMER

doi: 10.1161/01.CIR.25.1.169

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/25/1/169.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/