A Plasma Humoral Factor of Extrarenal Origin Causing Release of Reninlike Activity in Hypotensive Dogs

By E. De Vito, C. Wilson, R. E. Shipley, R. P. Miller, and B. L. Martz

ABSTRACT
Plasma reninlike activity significantly increased in anesthetized and unanesthetized normal dogs after intravenous injection of plasma from nephrectomized and normal dogs that have been made hypotensive by controlled hemorrhage. The injection of plasma from nephrectomized, nonhypotensive dogs or the in vitro incubation of normal plasma with calculated proportions of plasma from nephrectomized, hypotensive dogs did not produce any changes in plasma renin concentration or angiotensin production. These results suggest the presence of renin-releasing factor of extrarenal origin which is produced and circulated in the plasma of dogs subjected to periods of hypotension by means of controlled hemorrhage. A direct or indirect action of the renin-releasing factor on the kidney in association with or in conjunction with one or more of the other mechanisms that have been hypothesized to explain the control of renin release is not as yet known. An alternative hypothesis should also be considered. It is possible that the renin-releasing factor may be an activator of the conversion of prerenin to renin and of its release from the kidney into the bloodstream. The source and chemical nature of the renin-releasing factor are not as yet known.

KEY WORDS plasma renin-releasing factor hemorrhagic hypotension bilateral nephrectomy plasma renin activity angiotensin renin-substrate concentration renin-angiotensin system

Several hypotheses involving baroreceptors, the macula densa, and electrolyte shifts, as well as other mechanisms, have been proposed to explain the control of renal renin release (1). None of the hypotheses appears to be consistent with all of the experimental findings, and the mechanism(s) causing renin release very quickly after the onset of hypotension due to hemorrhage is still obscure.

In 1966, Fasciolo et al. (2) suggested that the presence of a humoral factor associated with the action of intrarenal baroreceptors could be involved in renin release. An extensive search for substances that would accelerate the release of renin in anesthetized normal dogs (2) and from kidney slices (3) was not successful.

In experiments designed to study the physiological properties of a "sustained pressor principle" described by Shipley et al. (4), anesthetized normal dogs were injected with plasma from nephrectomized hypotensive dogs. Plasma reninlike activity increased substantially after the injection, and the probability that this increase was due to a humoral factor was studied. The results of these experiments are reported in this paper.

Methods
Male or female mongrel dogs weighing 5.6-14.5 kg were anesthetized with secobarbital sodium (30 mg/kg iv). A bilateral nephrectomy was performed through a retroperitoneal incision under aseptic conditions. Forty-eight hours after nephrectomy, the dogs were anesthetized with phenobarbital sodium (150 mg/kg), and blood samples were drawn through a polyethylene catheter inserted into the femoral artery and collected in cold tubes.
containing heparin. Plasma was separated in a refrigerated centrifuge. The dogs were then subjected to acute hemorrhagic hypotension (mean blood pressure, 55–80 mm Hg) for 15–30 minutes. After hemorrhage, a blood sample was drawn, and plasma was separated by centrifugation. Plasma obtained under similar conditions before and after hypotension from nonnephrectomized dogs was used as a control. All arterial blood pressures were recorded on a kymograph with a mercury manometer connected to the femoral artery.

**Protocol 1.**—Experiments were performed on 14 normal dogs (8 male and 6 female) weighing 7.2–15.0 kg and maintained on standard laboratory chow. Each was anesthetized with phenobarbital sodium (150 mg/kg), and arterial blood pressure was recorded as previously described. A 15-ml blood sample for control determinations of reninlike activity and angiotensinogen was taken from the carotid artery through a polyethylene catheter before and several times after the injection of one of several plasma preparations. Injections were made through a polyethylene catheter in the femoral vein in accordance with the following experimental plans:

1. Plasma from 48-hour nephrectomized dogs before hemorrhage.
2. Plasma from 48-hour nephrectomized dogs after hemorrhage.
3. Plasma from nonnephrectomized dogs before hemorrhage.
4. Plasma from nonnephrectomized dogs after hemorrhage.
5. Plasma from 48-hour nephrectomized dogs after hemorrhage and following extensive dialysis of the plasma against normal saline.

**Protocol 2.**—In four normal, unanesthetized dogs, trained to lie quietly on a board, blood was taken by puncture of the femoral artery. Plasma renin activity was measured before and after the injection of 15 ml of plasma collected from 48-hour nephrectomized dogs before and after hemorrhage.

**Reninlike Activity.**—Reninlike activity was measured according to the method of De Vito and Fasciolo (5), and the results were expressed as ng angiotensinlike activity/ml plasma.

**Angiotensinogen Determination.**—Reninsubstrate content in the plasma was measured by incubating 0.5 ml of plasma for 1 hour at 37°C in the presence of 0.2 Goldblatt units of hog renin obtained by the method of Miller et al. during the purification of sustained pressor principle, and free of this substance (6, 7). The volume of each sample was made up to 2.5 ml with the following buffer solution: NaCl, 0.9 g/100 ml, 75 ml; 0.1M phosphate citrate buffer, pH 5.4, 25 ml; EDTA disodium salt, 150 mg. The reaction was stopped by placing the tubes in a boiling water bath, and the precipitates were removed by centrifugation. The supernatant fluid was brought to pH 7 and assayed against synthetic angiotensin amide (Hypertensin, CIBA) in pentolinium-treated anesthetized rats. The results were expressed as ng angiotensinlike activity/ml plasma.

**Results**

Figure 1 shows the results of 10 experiments carried out according to protocol 1. A significant increase in reninlike activity was found in all the experiments after the injection of plasma from nephrectomized, hypotensive dogs (Ab, Bb, C, D, Eb, Fb). Also a significant increase was found after the injection of plasma from a nonnephrectomized, hypotensive dog (G,H). No increases were found in reninlike activity when the injected plasma was taken from normotensive normal or nephrectomized dogs (Aa, Ba, Ea, Fa).

Extensive dialysis against normal saline did not remove the ability of plasma from nephrectomized, hypotensive dogs to increase circulating reninlike activity in the recipient dog. Plasma from a 48-hour nephrectomized dog subjected to hemorrhage of 37 ml/kg body weight was placed in Visking casing and dialyzed with gentle stirring against 4 liters of normal saline for 36 hours at 5°C. Normal saline was changed eight times. Twenty ml of
dialyzed plasma was injected into each of two normal anesthetized dogs (Fig. 1: I, J). The effects of plasma from normal and from nephrectomized dogs, normal dog plasma

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The possibility that the observed increases in renin activity were due to an increase of substrate concentration or to the presence of an activator of the renin-substrate reaction was ruled out by adding, in vitro, a calculated proportion of plasma from nephrectomized, hypotensive dogs to the control plasma (first collected samples). Assuming that plasma volume is 4.5% of the whole body weight, 0.15-0.40 ml of plasma from nephrectomized, hypotensive dogs was added to 5 ml of the control plasma to obtain the same approximate dilution as would exist when donor plasma was injected into the bloodstream of the recipient dogs. No difference in angiotensin production was found in the incubated samples with or without the addition of plasma from nephrectomized, hypotensive dogs in each of five experiments.

An extrarenal source of reninlike activity in the recipient dog was ruled out when 20 ml of plasma from nephrectomized, hypotensive dogs was injected into two 48-hour nephrectomized dogs, and no reninlike activity was
In four normal, board-trained dogs, plasma renin activity and renin-substrate concentration were measured before and after the injection of 15 ml of plasma from 48-hour nephrectomized, hypotensive dogs, fresh plasma in A and B and week-old, frozen plasma in C and D. The dogs from A and B were used 10 days later, but in these experiments, 15 ml of normal plasma and 15 ml of nephrectomized, normotensive dog plasma were injected in E and F, respectively.

Significant increases in reninlike activity were found in the samples taken 20 and 60 minutes after injection of 15 ml of plasma from nephrectomized, hypotensive dogs into normal, unanesthetized, board-trained dogs (Fig. 3: A, B, C, D). Plasma from normal or nephrectomized, nonhypotensive dogs caused no change in the renin activity levels of the normal recipient dogs (Fig. 3: E, F). No significant changes in plasma-substrate levels were observed.

Discussion
A significant increase in the plasma reninlike activity was found in anesthetized and unanesthetized normal dogs after intravenous injection of plasma from nephrectomized and normal dogs that had been made

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hypotensive by controlled hemorrhage. An increase in renin-substrate concentration in the recipient dogs as the result of the injection of plasma with high concentrations of substrate cannot explain the increase of plasma renin activity because: (1) Plasma from nephrectomized, nonhypotensive dogs with the same high substrate concentrations did not produce any change in plasma renin concentration when injected into normal dogs. (2) The in vitro incubation of normal dog plasma with plasma from nephrectomized, hypotensive dogs in the calculated proportion (considering the dog's plasma volume) produced an amount of angiotensin similar to that produced by normal plasma alone. The amount of substrate injected was not more than 10% of the substrate originally present in the recipient dog's plasma. The in vitro results also rule out the presence of an activator of the renin-substrate reaction.

The present experiments suggest that a renin-releasing factor is produced and is circulated in the plasma of dogs that have been subjected to periods of hypotension over a period of 15-30 minutes. Plasma from dogs that had previously been nephrectomized and then subjected to hypotension caused renin release, indicating that the source of the renin-releasing factor was extrarenal.

The finding that an extrarenal renin-releasing factor is elaborated during hypotension does not exclude or invalidate other mechanisms that have been postulated to explain the control of renin release. It is possible that the extrarenal renin-releasing factor may operate in association with or in conjunction with one or more of the other mechanisms that have been hypothesized. In the present experiments, the increase in plasma renin activity following the injection of plasma from hypotensive dogs (nephrectomized or not) was detectable about 30 minutes after injection and usually continued to increase over at least 2 hours. Furthermore, the action of renin-releasing factor may not be directly on the kidney, rather it may be mediated indirectly through an effect on some extrarenal structure. Also the rate of renin release may increase as a result of a small, but persistent, direct effect on some renal structure.

An alternate hypothesis should also be considered. Previous work (8) strongly supports the concept that there is an enzymatically inactive "prerenin" in kidney tissue that can be converted to an enzymatically active reninlike material. It is therefore possible that the renin-releasing factor may be an activator of prerenin and can accelerate its conversion to renin and its release from the kidney into the bloodstream. That renin-releasing factor itself may be a small molecule or active peptide can be ruled out, because it retains full activity after extended dialysis. The source of the renin-releasing factor and the factor's chemical nature are not as yet known.

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References

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