Does Renotropin Have a Role in the Pathogenesis of Hypertension?

Harry G. Preuss

The existence of an organ-specific, circulating renal growth factor, renotropin, has been established through various bioassays. However, renotropin may be more than a growth factor. The pathogenesis of essential hypertension is unknown, although a humoral agent related to renal metabolism is believed to be responsible, at least in part. In parallel with the development of a renotropin assay, we have measured vasomotor and natriuretic serum activities. Our data suggest that renotropin possesses vasomotor and natriuretic properties. Through one or both properties, renotropin may play an important role in essential hypertension.

This possibility is strengthened by observations that: a) many methods to produce experimental hypertension involve elevations of renotropin—a reduction of renal mass and/or prevention of compensatory growth; b) high renotropin activity has been demonstrated in genetically hypertensive rats (SHR); and c) sera containing high renotropic activity stimulate contraction of isolated rat aortic rings and influence organic anion and cation transport. Am J Hypertens 1989;2:65-71

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Because Goldblatt et al reported that renal artery constriction induces hypertension in dogs, the pathogenesis behind many forms of hypertension has been hypothesized to have renal origins. In support, the hypertension in various strains of genetically hypertensive rats was linked to the kidneys. This renal involvement was demonstrated by the discovery that crosstransplantation of kidneys from hypertensive rats into matched normotensive control rats raised the blood pressure, whereas transplantation of the normotensive rats’ kidneys to the hypertensive rats normalized the blood pressure of the recipient.

How is the kidney involved in hypertension? Frequently, hypertension can not be related to changes in the renin-angiotensin system or to perturbations of renal sodium-water handling. Plasma renin activity is not elevated in many patients with renal vascular hypertension. In some cases of secondary aldosteronism, normal blood pressures exist despite high levels of renin. In addition, passive immunization against renin and angiotensin have provided conflicting results.

While it is generally accepted that volume and sodium overload can cause or, at least, augment blood pressure, a consistent association of hypertension with these perturbations has not been observed. Likewise, disturbances in renal production of vasodilators (prostaglandins, bradykinins) do not generally correlate with blood pressure. Therefore, another renal mechanism must be considered.

Because renal growth occurs in many experimental situations and under many conditions that produce hypertension, Braun-Menendez postulated that increased concentrations of a humoral renal growth factor, renotropin, not only initiates and regulates renal growth but is the basis for the hypertension as well. His
hypothesis states that besides initiating renal growth, renotropin has a secondary constrictive effect on blood vessels. According to his theory, hypertension develops when the remaining renal tissue is unable to remove or inactivate the growth stimulator, which in turn causes an increase in the concentrations of the growth factor and greater vasoconstriction.

Braun-Menendez proposed his hypothesis over 30 years ago; it was not accepted for many reasons. Among the reasons was that another mechanism was generally favored. The major research thrust centered on the prehypertensive actions of the kidney, such as the renin-angiotensin system. Although the antihypertensive actions of the kidney were generally recognized (renoprival hypertension), few connected the renotropic system with this phenomenon. Importantly, there was no proof that renotropin existed. The passage of time has corrected this.

**Circulating Renotropin** It is generally accepted that renotropin exists; two historically important experiments suggested this early on. In 1896, Sacerdotti infused blood from bilaterally nephrectomized dogs into normal dogs and produced renal growth. Moreugo reported in 1980 that bilateral nephrectomy in one animal of a parabiotic pair caused renal growth in the normal partner. Because we recently summarized modern evidence for the existence of renotropin, only a brief overview will follow.

Studies using parabiotic and in vivo serum injections indicate the presence of circulating renotropin. Kurnick and Lindsay established a common peritoneal cavity between mice and demonstrated limited compensatory renal growth in the normal mouse after the partner underwent renal mass extirpation. More convincing evidence in favor of the renotropin hypothesis was obtained in subsequent parabiotic experiments utilizing vascular cross-circulation techniques, ie, linking the carotid artery of one rat to the jugular vein of another and vice versa. Lowenstein and Stern, using multiple intraperitoneal injections, demonstrated by autoradiography that sera from rats 48 hours after uninephrectomy increase H-thymidine uptake in kidney nuclei of intact rats. Although some studies utilizing fewer subcutaneous and/or intraperitoneal injections failed to provide evidence for a serum renotropic factor following uninephrectomy, enhanced renal ODC activity in 24 of 25 paired experiments (+42.5% ± 9.0 (SEM), P < .01. This methodology corroborated the previous findings, because sera from unilaterally nephrectomized rats significantly increased the number of labeled nuclei. In addition, ornithine decarboxylase (ODC) activity is a means to estimate growth without using isotopes. Using renal fragments, we found that uninephrectomized sera stimulated H-thymidine incorporation into the DNA of incubating rat renal medullary tissue. Our laboratory has performed more studies on renotropin than any other. In 1970, we used an in vivo assay to demonstrate the humoral renotropic factor. Using kidneys slices, the initial studies revealed that plasma (plasma and sera are interchangeable) from uninephrectomized rats compared to plasma from sham-operated rats stimulate isotope incorporation into DNA and RNA (DNA results shown in Figure 1). Further, this phenomenon was evident only in renal cortex, not tissue from other organs or even renal medulla (Figure 1).

Compared to plasma from sham-operated rats, plasma from uninephrectomized rats (24 hours postoperative) in concentrations between 8% to 50% v/v augmented isotope incorporation into DNA of kidney slices. A finding that intrigued us during these studies was that azotemic plasma from rats 24 hours after bilateral nephrectomy, unlike plasma from uninephrectomized rats, did not stimulate the incorporation of H-thymidine (Figure 1). Although azotemic sera from rats bilaterally nephrectomized 24 hours earlier did not stimulate H-thymidine incorporation into renal fragment DNA, stimulation was present after sera were dialyzed for 24 hours. From this we conclude that augmented concentrations of renotropin are present in sera from rats made azotemic by having both kidneys removed 24 hours earlier, and this renotropic factor can remain active even after 24-hour dialysis. Because both kidneys were absent for 24 hours before obtaining sera, it seems unlikely that kidneys produce renotropin.

Many in vivo studies concerned with the incorporation of labeled nucleotides into DNA have been criticized on the grounds that a varying dilution of isotope in the circulation or in the kidney milieu could create a situation where there appeared to be increased DNA production when there was actually none. To obviate the above objections, we used autoradiography in our assay and measured nuclear labeling to assess renotropic stimulation. This methodology corroborated the previous findings, because sera from unilaterally nephrectomized rats significantly increased the number of labeled nuclei. In addition, ornithine decarboxylase (ODC) activity is a means to estimate growth without using isotopes. Using renal fragments, we found that uninephrectomized sera compared to sham-operated sera enhanced renal ODC activity in 24 of 25 paired experiments (+42.5% ± 9.0 (SEM), P < .01. Lyons and coworkers used hamster kidney cells in culture, Kanetake and Yamamoto worked with primary rabbit kidney cell cultures and Hansen examined primary rat kidney cultures to establish the existence of renotropin. Accordingly, all evidence points to the existence of a specific renal growth regulator.
FIGURE 1. Histogram showing nucleic acid incorporation into rat kidney slice halves under a test situation as compared to a control situation. In the first two bars, slice halves were placed in the same environment with one being arbitrarily labeled test and the other control. In the next two bars, the ability of plasma from uninephrectomized or bilateral nephrectomized rats compared to plasma from sham-operated rats to alter incorporation is depicted. The isotope incorporation into the DNA of slice pairs from various organs in the presence of uni- and sham-sera is depicted in the last bars. Only results from kidney cortex and liver are statistically significant. The number of slice pairs tested is in parentheses under each bar. NS means not significant.

Role of Renotropin in Hypertension During our investigations of the specific renal growth factors, we became intrigued with its role in hypertension.14–17,47,48 We believe that understanding the relationship between renotropin and hypertension is important, because many methods to produce elevated blood pressures involve a reduction in functional renal tissue and/or interference with normal compensatory renal growth.49,50

Although prohypertensive renal influences of the renin-angiotensin system are apparent in many experimental models, antihypertensive effects are also present, ie, the “protective action” of the normal kidney against hypertension is evident.51 Kolff52 found that bilateral nephrectomy in dogs resulted in hypertension. Obviously, this was not secondary to release of a renal pressor substance. Importantly, hypertension did not develop if the ureters were anastomosed to the inferior vena cava instead of removing kidneys. Even though the same fluid load and retention of excretory products occurred, the presence of two normal-sized kidneys prevented hypertension. The veracity of the above was corroborated when it was further shown that transplantation of normal kidneys into bilaterally nephrectomized dogs returned elevated pressures to normal. Hypertension associated with the lack of normal functioning renal tissue became known as “renoprival hypertension.”

Following uninephrectomy, removal of the contralateral untouched kidney converts the hypertension from a transient to a permanent form.53,54 This suggested to Greenwood et al55 that removal of the normal kidney (renoprival effect) increased even more the production of a pressor factor by the ischemic one. They encased a normal intact dog kidney in gauze soaked with colloidion and removed its normal partner. As predicted, hypertension, which they attributed to a humoral mechanism, ensued. Pickering and Prinzmetal55 produced unilateral renal ischemia in the rabbit by clamping the renal vessel. With time they noticed that the clamped kidney had a normal consistency but became small, whereas the contralateral kidney enlarged. The transient early hypertension decreased as the uninvolved kidney enlarged.

With the above observations in mind, Braun-
Menendez\textsuperscript{14-17} offered his explanation for renopral hypertension. He postulated that an increased concentration of a humoral renal growth factor, renotropin, not only initiates and regulates renal growth but produces hypertension, because renotropin has a secondary constrictive effect on blood vessels. Hypertension develops when the remaining renal tissue is unable to remove or inactivate the growth stimulator, which, in turn, causes an increase in the concentration of the growth factor and greater vasoconstriction. In support, various conditions and substances stimulating renal growth also elevate blood pressure. Braun-Menendez\textsuperscript{14-17} listed decreases in renal mass, high protein diets, and injections of growth hormone, thyroxin, and testosterone as methods to increase both renal mass and blood pressure. We add that high sodium and low potassium intakes influence both parameters, ie, augment renal weight and elevate blood pressure.\textsuperscript{56}

In addition to a renotropic factor, circulating vasoconstrictor and natriuretic factors also exist.\textsuperscript{57-64} Various studies have demonstrated a serum factor from hypertensive animals and humans that render bioassay animals hypertensive. Further, the vasoconstrictive potential of the serum factor was shown in isolated vessels incubating in vivo.\textsuperscript{58} The active substance has a molecular weight between 1,000–20,000 daltons, is heat insensitive, and can be frozen and thawed without losing activity.\textsuperscript{56}

We showed that rat renotropin has an approximate molecular weight between 6,000–25,000 daltons, is heat insensitive, and can be frozen and thawed without losing activity.

As a first approximation, we tested rat sera removed 17 to 24 hours postuninephrectomy on the constrictive response of isolated rat aorta. Both uninephrectomized and normal sera showed constriction in a dose-dependent manner; however, uninephrectomized sera were approximately twice as active. In six experiments, diazylsera were found to retain roughly the same constrictive potential as before, indicating that the effect was not brought on by small molecules such as potassium or calcium and that the vasoconstrictive factor was nondialyzable, the same as renotropin. Heating of the serum decreased vasoconstrictive activity of the test and control sera about eightfold; however, the percent increase in tension of uninephrectomized compared to sham-operated sera remained the same. Significant stimulation was found only at 24 hours, not six hours, 30 hours, 48 hours, seven days and 14 days postunilateral nephrectomy, simulating the brief period of renotropic stimulation seen with sera from uninephrectomized rats and sham-operated rats at same time after operation. Average \pm SEM and number of experiments are shown. For comparison, the temporal relationship of renotropic activity in sera from uninephrectomized rats compared to sham-operated rats (solid line) is shown.

**Renotropin and Sodium Metabolism** Abnormal sodium metabolism plays a critical role in hypertension. Enhanced sodium ingestion raises blood pressure,\textsuperscript{65-71} and abnormalities in red and white cell electrolyte concentrations are noted in the hypertensive state.\textsuperscript{72-74} Accordingly, it has been postulated that the potentiator for vasoconstriction works through an influence on membrane transport.\textsuperscript{75} In 1969, Dahl and co-workers\textsuperscript{76} first proposed that a circulating saluterec substance elevates arterial pressures in salt-sensitive rats. Using the organic anion and cation transport systems, Preuss et al\textsuperscript{77} in 1974 postulated that elevated levels of a natriuretic hormone existed in spontaneously hypertensive rats, (SHR). Haddy and Overbeck\textsuperscript{78} in 1976 applied this observation, ie, they proposed a role for a natriuretic factor in the blood pressure elevations produced by primary aldosteronism, cortisone administration, chronic renal failure, and some forms of experimental hypertension.

Similar to SHR, sera from animals with experimental hypertension and from humans with essential hypertension possess a factor that influences membrane transport.\textsuperscript{62,78} The humoral agent may operate by inhibiting Na\textsuperscript{+}, K\textsuperscript{+} ATPase in blood vessels.\textsuperscript{75,78} Haddy showed that the Na\textsuperscript{+}, K\textsuperscript{+} pump is suppressed during hypertension in all elements of the cardiovascular system — arteries, veins, and heart — and that the suppression is not secondary to increased pressure. Hamlyn et al\textsuperscript{79} demonstrated increased circulating concentrations of an inhibitor to Na\textsuperscript{+}, K\textsuperscript{+} ATPase (natriuretic hormone) in hypertension. Repression of the Na\textsuperscript{+}, H\textsuperscript{+} transporter could accomplish the same end. Inhibition of sodium pumps leads to a net accumulation or redistribution of various cations like Ca\textsuperscript{2+}, Na\textsuperscript{+} in intracellular smooth muscle.\textsuperscript{75} Evidence for feasibility of the hypothesis lies in the fact that inhibition of Na pumps in vascular tissue by ouabain causes increased resting tone and enhanced reactivity to catecholamines.\textsuperscript{80}

Changes in organic anion transport (PAH) occur with
renal hypertrophy. Although we observed an increased PAH uptake in the medium concentration (S/M), in 1966, we reported a factor in urine that inhibited PAH transport. 

Believe that some of this effect was due to a natriuretic factor, ie, a hormone that influences PAH as well. We also observed that serum from volume-expanded dogs not only inhibited sodium transport but PAH transport as well and attributed this to a natriuretic hormone. In accordance, we corroborated their volume-expanded dog results in salt-loaded rats using PAH S/M ratios.

Sera from saline-drinking rats compared to sera from water-drinking rats depressed not only 3H-PAH but 14C-TEA (tetraethylammonium, an organic cation) uptake. Likewise, sera from rats 17 to 24 hours after uninephrectomy also depressed 3H-PAH and 14C-TEA S/M. Interestingly, this is the same time frame when renotropic and vasoconstrictive activities are highest in this model. In contrast to the serum effects, renal tissues from these rats when incubated in vitro, free of the serum influence, showed enhanced PAH and TEA uptake over one to two days. It is attractive to hypothesize that these opposing effects, which have also been reported in SH rats, develop (sera and tissue) to offset each other.

Conclusion The random associations made in the last few sections are consistent with the possibility that similar or even common factors are responsible, at least to some extent, for renal growth, renal sodium transport alterations, vasoconstrictive effects, and hypertension.

REFERENCES


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