Vasoactive Peptides and Substrates and Their Relation to Protein Handling by the Kidney

By Ramon B. Rosas, M.D.

The interrelationships between circulating vasoactive peptide substrate, excreted vasoactive peptides, renal handling of proteins, and arterial blood pressure have become a focus of investigation. Information derived in these areas may assist in clarifying the role of the kidney in the handling of proteins and, possibly, in hypertension.

VASOACTIVE PEPTIDES FROM SERUM

Many peptides with vasoactive properties have been obtained by a variety of methods from animal serum. When serum is incubated at 38 C for 3 to 48 hrs at pH 3.8 to 4.0, "anephrotensin," a polypeptide which alters the contractility of smooth muscle, is liberated.1 The anephrotensins released from serums of several animal species are not all identical in their pharmacological properties, but they are alike in causing constriction of the smooth muscle of the uterus, ileum, and duodenum.2 All anephrotensins studied have been effective in raising the blood pressure of nephrectomized rats and of intact rats in which the sympathetic system has been blocked with pentolinium.

Since the reaction which liberates anephrotensin exhibits optimal temperature and pH relationships, it has been assumed that it is an enzymatic reaction, that an enzyme in the serum liberates the anephrotensin from a protein substrate, anephrotensinogen. The enzyme which liberates anephrotensin has not been identified.

Anephrotensin has not been demonstrated in the normal circulation. However, since the maximum amount obtainable from the serum varies with the experimental conditions, the amount of circulating substrate must also vary. The effects of nephrectomy and desoxycorticosterone (DCA) treatment on the amount of anephrotensin released from serum are described below.

SUBSTRATES IN NEPHRECTOMIZED ANIMALS

Serum from nephrectomized animals yielded more anephrotensin than serum from normal animals. Serum obtained from rats 24 hrs after nephrectomy yielded three to five times as much anephrotensin as serum from normal rats. Serum from 48-hr nephrectomized dogs yielded five to six times as much anephrotensin as that of the controls. An increase in
anephrotensinogen may result from deficiency of some endocrine function of the kidney and not from a lack of its excretory action, since elimination of renal excretory function by ureter ligation does not increase anephrotensinogen.

The amount of angiotensinogen also is higher in serum of nephrectomized animals. The rate of increase with time after nephrectomy is about the same for anephrotensinogen and angiotensinogen. This observation suggests that a common factor is involved in the production of anephrotensin and angiotensin from the serum.

Parallel changes in the yield of anephrotensin and of angiotensin from serum of nephrectomized and normal dogs and rats were found. Both substrates were measured in the serum of DCA-treated rats. When a change in the amount of anephrotensinogen was seen, there was a similar change in the angiotensinogen (fig. 2). These findings raise the possibility that anephrotensinogen and angiotensinogen may be the same substance.

In the dog, other substrates which produce polypeptides, such as bradykinin and kallidin, are not increased after nephrectomy. We have found this result also with rat serum. In seven experiments in which bradykinin was exhausted by the method of Diniz et al. and the bradykinin liberated was assayed by its effect on blood pressure of normal rats, the substrate producing bradykinin was not increased after nephrectomy.

ANEPHROTENSINOGEN IN DCA-TREATED RATS

Administration of DCA to rats given 1% NaCl as drinking solution produced only a slight decrease in the amount of anephrotensinogen in the serum during the early weeks of treatment; later, however, there was an increase. The time interval before the increase in anephrotensinogen varied with the amount of DCA given.

When DCA (1 mg/day) in the form of microcrystals was administered intramuscularly to uninephrectomized rats, the increase in blood pressure and in anephrotensinogen was found to be chronologically related. In other studies on intact rats injected subcutaneously with a suspension of DCA, in doses of 2.5 or 10 mg/kg/day, and in uninephrectomized rats implanted with DCA pellets (40 mg), an increase in blood pressure, as measured by tail microphone, was seen before the increase in anephrotensinogen became evident (fig. 3). This difference may be accounted for by the difference in dosage.
**VASOACTIVE PEPTIDES**

**Blood Pressure Response to (A) Angiotensin and (B) Anephrotensin from Serum of Normal, Nephrectomized and DCA treated Rats**

![Graph showing blood pressure responses to angiotensin and anephrotensin](image)

**FIGURE 2**

Blood pressure responses of 24-hr nephrectomized, pentolinium-blocked rat to angiotensin (A) and to anephrotensin (B) obtained from serum of normal, nephrectomized (Nx), and DCA-treated, uninephrectomized rat.

**FIGURE 3**

Anephrotensinogen in 1 ml serum (Aneph), vasoaetive urine peptides in 24 hrs/kg rat (V.U.P), each in terms of μg of angiotensin, and blood pressure in mm Hg of control (C), and DCA-treated (40-mg pellets), uninephrectomized rats (B).
VASOACTIVE PEPTIDES FROM URINE OF CONTROL AND DCA-TREATED RATS

Urine peptides were measured in all the animals involved in the anephrotensinogen studies to discover any possible relationships between the peptides and the changes in yield of anephrotensin from the serum or the development of hypertension. Overnight urine collections were made at weekly intervals and extracted according to the method of Noble, Rinderknecht, and Williams. Blood pressures were measured on the same day. Biological activity of each of the urine extracts was assayed by its effect on the blood pressure of a previously nephrectomized rat anesthetized with pentobarbital Na (40 mg/kg) and blocked with pentolinium (5 mg/kg), and by its contractile effect on the isolated rat uterus. Synthetic angiotensin and oxytocin were used as standards for the tests.

An increase of vasoactive peptides was found in the urine of DCA-treated rats. After six weeks the increase was more than 100-fold in rats injected with 10 mg DCA/kg/day. With this dose of DCA the urine peptides were found to be increased chronologically with the development of hypertension. With administration of smaller doses of DCA (2.5 mg/kg/day), however, the development of hypertension preceded the increase in activity of the urine peptides (figs. 3 and 4).

When urine extracts from control and DCA-treated rats were assayed by means of the isolated rat uterus, the differences were found to be even greater than the differences in pressor activity (fig. 5).

From the several experiments with DCA-treated rats, it is possible to conclude that when serum anephrotensinogen is increased, urine peptide excretion is also elevated (fig. 3); the increase in urine peptides may pre-
cede the increase in anephrotensinogen. This chronological relationship between anephrotensinogen and urine peptides suggests that the vasoactive urine peptide may be anephrotensin. This suggestion is supported by the finding that rat urine peptides and rat anephrotensin are similar in their effects on rat blood pressure and on the isolated uterus.12

**URINE PROTEIN EXCRETION IN DCA-TREATED RATS**

DCA-treated rats develop proteinuria, and it appears before an increase in vasoactive peptide excretion is seen. This proteinuria is accompanied by a change in the urine albumin/globulin ratio from 0.23 in the normal rat to 1.95 in DCA-treated rats (fig. 6). Agar gel electrophoresis16 shows that the main change in the protein composition of the urine is an increase in the albumin, which is present in only very small amounts in normal rat urine. Small amounts of gamma globulin also appear in the urine of DCA-treated rats (fig. 7). These results are in accord with the findings of Lewis et al.17

**PROTEIN HANDLING BY THE KIDNEY**

Since the changes brought about by DCA treatment, in the amount of anephrotensin obtained from the serum and in urine excretion of peptides and proteins, appear late in the treatment period, and since an increase in anephrotensinogen is also seen in nephrectomized animals, it seems possible that these changes may be related to a loss of some kidney function, possibly that related to protein metabolism. The magnitude of its role is an unsettled question, but all authors seem to agree that the kidney does play some part in protein metabolism. This subject will be reviewed briefly in the following paragraphs.

Wearn and Richards18 were not able to demonstrate that proteins were filtered in the frog glomerulus and concluded that if protein filtration does occur, the amount must be of the order of less than 30 mg/100 ml of ultrafiltrate. Proteins appear in low concentrations in the glomerular filtrate of rat,19 dog,20 mouse,21 and guinea pig,22 but because of the large volume of ultrafiltrate, appreciable amounts are filtered. It has been estimated that ultrafiltration of albumins and globulins in normal man is about 18 g in 24 hrs23; however, only between 39 and 71 mg of this material appear in the urine27-29 probably because of reabsorption in the renal tubules.
The fact that the albumin/globulin ratio in human urine is between 0.51 and 0.65 strongly suggests that albumin is preferentially reabsorbed in the tubules. The findings of Oliver, MacDowell, and Lee concerning the formation of droplets in the cells of the proximal tubules when the ultrafiltrate has an excess of proteins or proteins of different nature support this idea.

It has been demonstrated that in rabbits and dogs protein concentration in the blood of the renal vein is 1 to 5% lower than in arterial blood. Albumin is decreased 3 to 7%, which is greater than the decrease in globulin. Apparently the only such studies which have been done on hypertensive animals are those of Gerbi. To 13 rabbits made hypertensive by wrapping silk around one or both kidneys, he was unable to find any differences in protein concentration between arterial and renal venous blood; such differences were present in blood of normal rabbits. Since renal blood flow is high, the amount of protein reaching the kidney tissue in a given time may be very high. This difference in protein concentration in the blood of the renal vein and in arterial blood cannot be explained (1) by difference in hematocrit, since hematocrit is practically unchanged; (2) by the elimination of proteins in the urine, since only about 300 mg are excreted during the same period of time; or (3) by the reabsorption of proteins through the lymphatic system, which accounts for only about 25 g in the same period.

The renal arteriovenous difference in protein concentration is, therefore, probably due to protein breakdown by the kidney. Eliasch et al. reported that the concentrations of amino-acid nitrogen and peptide nitrogen were higher in renal venous plasma of the rabbit than in arterial plasma. Harms et al. have found this difference also in dogs, although Latham and Benjamin did not.

**ANEPHTOTENSINOGEN AND ANGIOGENE-**

**SINOGEN HANDLING BY THE KIDNEY**

Croxatto suggested that the increased amounts of anephrotensinogen and angiotensinogen in nephrectomized animals result from the absence of the protein catabolism.
VASOACTIVE PEPTIDES

NORMAL ANIMAL

Nx ANIMAL

DCA-TREATED ANIMAL

FIGURE 8

Schematic representation of possible manner of renal handling of anephrotensinogen and angiotensinogen. Substrates are built up in the liver and broken down in the kidney. With nephrectomy or DCA administration, breakdown does not occur, and substrate accumulates. Numbers represent relative amounts of substrate.

Fig. 8. Schematic representation of possible manner of renal handling of anephrotensinogen and angiotensinogen. Substrates are built up in the liver and broken down in the kidney. With nephrectomy or DCA administration, breakdown does not occur, and substrate accumulates. Numbers represent relative amounts of substrate.

normally carried on by the kidney. The increase in protein substrate, or substrates, for anephrotensin and angiotensin in the serum of DCA-treated rats may also be accounted for in this way (fig. 8). The other explanation that has been advanced, that the build-up of substrate necessary for the production of angiotensin is the result of a lack of renin, is apparently inadequate, since in the frog, which has no renin in the kidney, there is an increase in anephrotensin substrate after nephrectomy. In addition, in DCA-treated rats the disappearance of renin from the kidneys occurs many weeks before the increase in the substrate producing anephrotensin and angiotensin. It has been shown that in DCA-treated rats the decrease in renin release precedes the decrease in renin content.

URINE PROTEIN AND PEPTIDE HANDLING BY THE KIDNEY

The increase in vasoactive peptides excreted in the urine also may be explained by a decrease in tubular reabsorption of the vasoactive peptide itself or of its substrate; this substrate may liberate the active peptide through the action of an enzyme in the urine itself (fig. 9), much as kallikrein liberates kallidin. Another possibility to account for the increase of vasoactive urine peptides is that there may be an increase in the blood of the vasoactive peptides or of the substrate producing these peptides. In this case, the increase of circulating substances would bring about increased renal clearance of these substances. As yet we do not have convincing evidence supporting one of these mechanisms to the exclusion of the others.

The increase in both anephrotensinogen in the blood and renal excretion of vasoactive peptides and proteins in DCA-treated rats is in accord with the possibility that DCA produces a diminution or loss of some renal function normally involved in the handling of proteins.

The relationships outlined above permit the speculation that the role which the kidney plays in protein metabolism may be related to experimental hypertension and that hypertension during the later period of DCA treatment resembles renoprival hypertension.

Summary

The properties of anephrotensin, a polypeptide liberated by acid incubation of serum, are reviewed. Experiments with antirenin rule out the possibility that renin is the enzyme that liberates anephrotensin.

Serum anephrotensinogen increases after bilateral nephrectomy or after several weeks.
of treatment with desoxycorticosterone (DCA). There are parallel changes under these conditions in the amounts of anephro-
tensinogen and angiotensinogen in the serum, suggesting that a common substrate may be involved. The increase in substrate is in ac-
cord with the possibility that DCA treatment results in some deficiency in the handling of proteins by the kidney.

There is an increase in urine vasoactive peptides and total proteins in DCA-treated rats. These increases are probably related to
deficiency in tubular reabsorption. The in-
crease in the albumin/globulin ratio of the
urine proteins under these circumstances sup-
ports this idea.

The changes described above are not neces-
sarily related to the onset of hypertension.

Whether the changes in protein handling by
the kidney are related to the hypertension
produced by DCA treatment is an unsettled
question.

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VA$O$ACTIVE PEPTIDES


Circulation Research, Volume XII, May 1963
Dr. James O. Hoppe, New York: Have you any information on the amino acids involved in anephrotensin, and do they resemble or are they different in any important way from angiotensin?

Dr. Oscar Helmer, Indianapolis: Dr. Judson and I have assayed the renin substrate or angiotensinogen in several hundred patients with various forms of hypertension, and we have found remarkable changes in this factor. In some hypertensives, there is no more than normal, but those with a higher renin content will have a higher substrate content; in unilateral vascular occlusive disease, along with elevated renin content, we have found a higher substrate. In pre eclampsia we have found 10 times more renin substrate. We have to think not only of the renin release, but also we have to consider the changes in renin substrate. Interestingly enough, we have found a lower content of renin substrate in the renal vein blood than that of the contralateral kidney. I think that renin substrate is going to be as important in the study of hypertension as the liberation of renin by itself.

Dr. Jean Oliver, Summit, New Jersey: There is no question that it has been shown that the proximal tubule of the nephron can reabsorb certain proteins from the tubule fluid and break them down. The obvious case is hemoglobin; this is a very old observation which has been confirmed by many studies, including electron microscopy, in the last few years. The exact cytological details of how this degradation occurs are perhaps not entirely agreed on by everyone, but if it is admitted that the kidney does reabsorb protein and can break it down, the question arises as to what the products of degradation might be and whether they might produce physiological effects. One thinks at once of arteriovenous differences as a method of examining these questions. If something were taken out of the blood or if something were produced by synthesis in the renal cells, then one would expect to find some evidence of that deduction.
or addition in renal veins. As Dr. Rosas mentioned, several studies have shown quantitative differences in the protein or polypeptide content in artery and vein. But the difference is so minute (and I might add that it must be minute, because if it were not, with the volume of blood that goes through the kidney, the whole protein system of the blood would be turned over in no time at all)—the arteriovenous difference is so small as to be perhaps not so convincing as one would desire.

When we were studying the problem at Long Island, it occurred to us that a qualitative comparison of arterial and venous renal blood might be helpful. Using paper chromatography, to put the hypothesis crudely, one might expect a differing pattern of spots from serum of the blood that was going into the kidney from that which was coming out. In the first few experiments (they terminated for reasons beyond our control), there were differences. We could find spots in the venous chromatogram that were not in the artery and vice versa. Now it seems to me that an elaboration of this method of examination might answer some specific questions. If it could be definitely shown that the kidney could actually remove or put something into the blood that is passing through it, we would be a little bit ahead. "With improvements in chromatographic techniques, it is even possible that some of these additional spots could be eluted, examined, and identified. Also, the blood might be recirculated, the same blood passing through an isolated kidney many times. In this way one would avoid the undeniable action on the blood of all the other tissues in the body which are known to have a protein metabolic activity, such as the reticuloendothelial system. The kidney alone would be acting, and by recirculating the blood a concentration or increasing deficiency of some unsuspected body might become apparent that had been masked by the activity of extrarenal tissues acting on the proteins circulating in the intact animal.

It is gratifying to see that the attention of physiologists is directed toward this problem of what is happening in the kidney cells. Until very recently they have attended only to what might be called the gross aspects of the handling of protein by kidney; for example, such matters as the possible lack of reabsorption of protein by the tubule as a cause of proteinuria. This question is apparently settled. Although there is no question that it is a logical necessity, as Thomas Addis pointed out, that if tubules which normally reabsorb protein fail to do so, there will be an increase in the amount put out, still, in the actual day-to-day clinical situation, there always seems to be glomerular "damage" and consequently increased filtration, and the changes in tubular reabsorption are probably relatively minor in their effect.

The fact that Dr. Rosas' paper is exploring a new field is most interesting, and I hope it will lead to stimulation of more efforts to see what the cells of the nephrons are doing to the protein bodies which they undoubtedly reabsorb.

Dr. Sydney M. Friedman, Vancouver: The evolution of the changes which Dr. Rosas described reminded me of the evolution of the changes in renal function which my wife and I described years ago in DGA-treated rats. For what the evidence in the rat is worth, there is first of all an increase in filtration rate followed by a maintained filtration rate despite a gradual decline in renal blood flow, and that in turn is followed by a fall in filtration rate and severe decline in renal blood flow. With a fall in blood flow, the reabsorptive processes would necessarily be very much impaired. The early increase in filtration might result in quite a proteinuria.

Much more recently, the competitive nature of amino acid reabsorption has been studied. Byers and his group and, more recently, Pitts and his collaborators have pointed out that the reabsorption of amino acids is a competitive process so that the amount of one peptide that may be absorbed is very much dependent on others present at the same time. If one adds this fact to the evolution of func-
tional changes I have mentioned, I am not entirely sure that one has to look for specific changes in the kidney affecting the actual metabolic breakdown of proteins. It may well be that the ordinary mechanisms are slowed up and that competitive inhibition of one type or another occurs. What, incidentally, are the characteristics that distinguish anephrotensin from vasopressin?

Dr. Jacques Genest, Montreal: Two questions, Dr. Rosas: What is your method of preparation of urine peptides, and secondly, are these peptides inactivated by trypsin?

Dr. Ramon B. Rosas, Ann Arbor, Michigan: Because anephrotensin has been characterized only pharmacologically and has not been isolated in a pure state, no amino acid composition studies have been done. In addition to the active polypeptide or polypeptides, there are probably many inactive peptides that make studies of this kind invalid. For this reason I cannot say whether anephrotensin contains some of the amino acids present in angiotensin.

Anephrotensin is not vasopressin, because trypsin totally destroys vasopressin's antidiuretic and pressor activity and destroys only about 50% of the anephrotensin activity. Anephrotensin is not angiotensin, because trypsin and pepsin, which totally destroy the activity of angiotensin, only partially destroy the activity of anephrotensin. The fact that some proteolytic enzymes partially destroy the biological activity of anephrotensin may mean that anephrotensin is not a single active polypeptide but that some active polypeptide is liberated at pH 4. Some anephrotensins, like those obtained from dog, frog, or chicken serum, raise blood pressure in normal and in nephrectomized animals; other anephrotensins, like those obtained from rat serum, lower the blood pressure in normal rats and increase the blood pressure in nephrectomized rats. These pharmacological properties are not in agreement with those of angiotensin. I would like to point out that anephrotensin is obtainable from blood serum of a variety of animals. It is possible to obtain vasoactive polypeptides by incubation, at pH 4, of serum from mammals, birds, reptiles, and fish, but it may be that polypeptides liberated from the serum of each are slightly different in their amino acid composition, because of the differences in pharmacological properties. For this reason any comparison between anephrotensin and some of the known vasoactive polypeptides is related strictly to the species from which it came.

The method used to extract peptides from urine consists of bringing the urine to pH 5 and density 1.010, precipitating all peptides with 2 N ZnSO4 and 2.25 N K4Fe(CN)6, and then extracting the precipitate three times with 1% ammonium in 80% alcohol. After evaporating the alcohol, the powder is dissolved in either distilled water or in saline. Sometimes I did a butanol extraction, in which these peptides are soluble, and I was also able to dialyze them through a cellophane membrane. Therefore, I am able to say that these are small peptides. I cannot say what the action of trypsin on urine polypeptides is, because as yet I have not studied this.

In addition to the DGA-treated rats, experiments in figure-of-eight rats also showed that the onset of hypertension is not necessarily related to the increase in the excretion of vasoactive urine peptides, but the latter probably play a role in the development of the hypertension. I agree with Dr. Friedman that to explain the role of the kidney in protein metabolism through a single mechanism, like the breakdown of proteins, is only an incomplete approach; other mechanisms also play a role. Different mechanisms for the breakdown of various proteins by the kidney have been described in the literature. Renal vein blood has more fibrinolytic action than arterial blood because of the liberation of an activator. Kidneys directly liberate angiotensin from angiotensinogen by the production of an enzyme, renin.

Reference
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