Evidence for Humoral Factors in Renoprival Hypertension

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Plasma of renoprival hypertensive rats was found to have an exaggerated constrictor effect on isolated rat aorta strips, however, no pressor effect was produced in acutely nephrectomized rats cross-circulated with renoprival hypertensive animals. It is suggested that if the constrictor element of the plasma from renoprival hypertensive rats is not just an artifact of the aorta strip assay technic but does exist and play a role in renoprival hypertension, it does so by acting on arterioles sensitized by the chronic a-renal condition.

Speculations as to the basic mechanism of hypertension generally follow one of two lines of thought: that increased peripheral resistance is caused (1) by increased levels of humoral constrictor material or (2) by increased responsiveness of the arterioles to normal constrictor influences. The study of renoprival hypertension is especially interesting in exploring the first supposition in that the most frequently postulated source of abnormal pressor principle, the kidney, is absent throughout the development of this acute, severe hypertensive disease. Such studies seem the more pertinent in consideration of the large body of evidence\(^1\)\(^-\)\(^9\) which suggests that the ultimate causative mechanism is the same for both renal and renoprival hypertension.

We have prepared renoprival hypertensive rats using the technic developed by Kolff and Page.\(^10\) Female rats of 225 to 250 Gm. are bilaterally nephrectomized and maintained by daily peritoneal lavage. Hypertension usually develops by the third day. Systolic pressures increase to 150 to 180 mm. Hg or more and are maintained until immediately before death. On the sixth to tenth day after nephrectomy animals are used for one of two types of experiments designed to disclose the presence of humoral constrictor material.

In the first group of experiments the constrictor ability of plasma from normal and renoprival hypertensive rats was compared using the aortic strip method of Furchgott.\(^11\) A strip cut spirally from the thoracic aorta of a rat is suspended from a light muscle lever and immersed in a bath of balanced salt solution. The strip contracts in response to the addition to the bath of many vasoactive materials. A record of the contractions of such a strip in response to serotonin, epinephrine, serum and plasma is illustrated in figure 1. The response to plasma samples taken at each of 3 successive intervals during rapid exsanguination are also illustrated. The increase in constrictor activity of the plasma as bleeding continues presumably reflects the increased sympathoadrenal discharge in response to severe hypotension.

In comparisons of strip response to plasma from normal and from renoprival hypertensive rats, although standardized, relatively atraumatic bleeding technics were used, the hypertensive plasma consistently showed greater constrictor activity than the normal plasma. Data from two series of experiments are summarized in table 1. In each series the hypertensive plasma showed greater constrictor activity than the normotensive plasma tested concurrently. More recently attempts have been made to quantify this difference by comparing, with each strip, plasma constrictor activity and the constrictor activity of epinephrine. The activity of the plasma can then be stated in epinephrine equivalents, i.e., as micrograms of epinephrine per liter of

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plasma if the constrictor activity were due entirely to epinephrine.

Since it is clear that the constrictor activity of plasma is influenced by factors involved in obtaining it, the possibility exists that the difference between the plasmas of renoprival and normal rats may be due simply to epinephrine being released more readily from the renoprival hypertensive animal during bleeding. Attempts have been made to determine the nature of the constrictor material. Identification by means of adrenergic-blocking agents has not proved fruitful since the agents used produced an equal reduction in the response to both normal and hypertensive plasma. Instead we have used a bizarre differential responsiveness that has become evident between rabbit and rat aortas.

A typical experiment, in which the responses of rabbit aorta are compared with the responses of rat aorta, is illustrated in figure 2. Epinephrine sensitivity of these strips is very similar. Contractions of the 2 strips in response to the various plasmas, however, are not similar. Hypertensive plasma produces greater contraction of the rat strip than of the rabbit strip whereas normal plasma produces greater contraction of the rabbit than of the rat strip. To eliminate from our considerations differences in reactivity between the 2 strips, epinephrine-equivalent concentrations have been calculated. Comparison of the strips with respect to these values indicates that the rabbit strip was half as responsive to hypertensive plasma as was the rat strip, but 4 times as responsive to the normal plasma. The fact that the plasmas influence the strips differently suggests that the constrictor principles in the 2 types of plasma are fundamentally different.

![Figure 1](http://circ.ahajournals.org/)

**Fig. 1.** Upper record, contractile responses of a spirally cut rat aortic strip to several vasoactive materials. Lower record, the height of the contraction produced by equal amounts of plasma varies directly with the length of the bleeding procedure and probably reflects increasing titers of humoral adrenergic vasoactive agents.

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<th>Table 1.—Plasma Constrictor Activity from Normal and Hypertensive Rats</th>
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Some indication of the nature of this difference may be obtained by considering the epinephrine equivalent value for each of the plasmas on the paired strips. Epinephrine is made the denominator for reducing the reactivity of the 2 strips to a common value. Comparison of the reactivity of the 2 strips to plasma should, therefore, yield a rabbit strip : rat strip ratio of 1 if epinephrine in the plasma is the stimulus for contraction. Conversely a ratio other than 1 indicates that the plasma constrictor is not epinephrine-like.

The data, summarized in table 2, indicate that higher levels of constrictor activity of hypertensive than of normal plasma are detectable with rat aorta but not with rabbit aorta. The high average rabbit : rat activity ratio (8.7) of control plasma taken early in the bleeding procedure indicates that its constrictor material affects the strips quite differently from epinephrine. The average activity ratio of plasma taken late in the bleeding procedure (which would be expected to contain additional quantities of epinephrine) is much lower (2.9) and suggests that a higher proportion of the total contractile stimulus is due to epinephrine-like materials. Hypertensive plasma, though it was drawn as 'early plasma,' showed a rabbit : rat sensitivity ratio of 1.5, even closer to that which would be expected if epinephrine were the constrictor agent.

These observations suggest that the abnormal constrictor activity of renoprival hypertensive plasma is due to an increase in circulating epinephrine-like material. We can not ignore the possibility, however, that there is in renoprival hypertensive plasma an increased amount of a material which affects the 2 strips in a manner just opposite to that of the constrictors of normal plasma, i.e., a material which has a rabbit : rat activity ratio of less than unity. The question remains as to whether this epinephrine-like material is released more readily by the renoprival hypertensive animal on bleeding than by the normal, or whether, in this form of hypertension, the concentration of such circulating pressor amines is chronically elevated.

A second group of experiments has been performed in an attempt to differentiate between these possibilities. An isovolemic controlled cross-circulation system has been arranged by means of which a renoprival hypertensive animal is cross-circulated with a normal assay animal. To maintain stable and normal blood volumes the animals were arranged on the 2 pans of a double-pan balance (fig. 3) sufficiently sensitive to detect a volume change of the order of 0.1 ml. By adjusting the valves in the cross-circulation
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Fig. 3. Diagram of mechanical arrangement in cross-circulation experiments. Flow rate was measured by measuring rate of weight gain in the recipient when one arteriovenous coupling was clamped.

tubing, the flow rate in the 2 directions could be kept equal. The arteriovenous couplings permitted a rate of flow between the animals sufficient to exchange their blood volumes 6 to 10 times during the average cross-circulation period of 70 minutes. This allowed the extracellular fluid of the two rats to approach equilibrium. Blood pressure was recorded from the carotid artery via small volume mercury manometers. Should the elevation in humoral constrictor material indicated by the strip assay be responsible for the elevated blood pressure of the renoprival hypertensive animal, the free intermixing of blood would result in a fall in blood pressure of the renoprival hypertensive or a pressor response in the assay animal, or perhaps both. Since it has been shown that normally circulated kidneys reduce or prevent renoprival hypertension,12-14 the assay rats were bilaterally nephrectomized immediately before the experiment.

The upper record, figure 4, illustrates the response to cross-circulation of a pair of control animals. Both animals were nephrectomized a short time before cross-circulation began. Evaluation of the responses to cross-circulation in 5 such control experiments indicate that blood pressure changes were minimal. Furthermore, the variations that were seen were apparently unrelated to the cross-circulation procedure since the same type of variability was seen before, during, and after cross-circulation periods.

In the lower record, figure 4, blood pressure changes during cross-circulation between a renoprival hypertensive rat and an acutely nephrectomized assay animal are illustrated.
There was a gradual, slight elevation of blood pressure in the assay animal during the period of cross-circulation. This record illustrates the only observation suggesting any pressor influence from the blood of hypertensive rats cross-circulated with acutely nephrectomized assay animals. Evaluation of 5 such experiments indicate that fluctuations in blood pressure during cross-circulation were quite similar to those seen in the control group.

SUMMARY

Evidence obtained by means of the rat aortic strip assay indicates that there is exaggerated constrictor activity in plasma from renoprival hypertensive rats. It was impossible, however, to detect any pressor influence of this plasma by cross-circulation experiments. These apparently conflicting results can be reconciled if any of the following possibilities hold: 1. If the high constrictor activity found in this plasma represents a more rapid release of constrictor material by the renoprival hypertensive rat than by the normal rat during bleeding. 2. If this material in the plasma of the renoprival hypertensive rat selectively constricts the isolated aortic smooth muscle but fails to cause constriction of the vascular smooth muscle governing total peripheral resistance (arteriolar) in vivo. 3. If this humoral constrictor factor in renoprival rats acts only on arterioles sensitized in some way by the protracted a-renal condition.

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