The potentiating effect of nephrectomy on the pressor response to renin injection has been known since the discovery of renin by Tigerstedt and Bergman in 1898, and has been used occasionally as a device for increasing the sensitivity of the assay animal in biological determinations of renin or angiotensin. The response of nephrectomized rats to injected renin differs from that of control animals in two distinct, and perhaps etiologically separate, respects: 1) in increased peak pressure, 2) in increased duration. The former has been shown to be due, in part, to increased elaboration of angiotensin in the blood of nephrectomized animals, probably resulting from an increase in substrate (angiotensinogen) concentration in the blood of these animals. It is toward the latter, less well studied, persistent element of the pressor response that this study has been directed.

The maintained, stable, moderate pressure elevation which occurs in nephrectomized animals after renin injection was first thought to be due to a reduced rate of excretion of exogenous renin, but the observation that the potentiation does not appear until several hours after nephrectomy, and a second observation, that a similar potentiated renin response occurs in the presence of functionally adequate kidneys, as in renal hypertension and DCA-treated rats, each suggests that the phenomenon is due to abnormalities more subtle than the absence of excretion of renin.

In this study the capacity of angiotensin itself to produce the persistent pressure elevation is described, the humoral and neurogenic components of the angiotensin response are evaluated, and the hemodynamic basis of the pressure elevation is established.

**Methods**

**PREPARATION OF ANIMALS**

Male and female rats, 210 to 360 g, of several strains and from several commercial suppliers, were used unselectively in different parts of the study. Bilateral nephrectomy or a sham operation was performed through flank incisions under light ether anesthesia 18 to 24 hr before an experiment was done in order to permit development of the increased sensitivity. For anesthesia at the time of the experiment, a single dose of urethane (1.4 g/kg body wt) was given intramuscularly. Animals were given 100 units (USP) heparin in 1 ml saline.

**ANGIOTENSIN INFUSION**

Angiotensin was administered iv, through a catheter in the femoral vein. An infusion schedule was used which produced, in a nephrectomized rat, a pattern of blood pressure (BP) response mimicking that induced by a standard injection of renin. It was assumed that a rate of injection of angiotensin which duplicates, in initial rise and subsequent slight regression, the response to an injection of renin, duplicates, approximately, the rate at which angiotensin is liberated by that renin injection. The two patterns of infusion used were: 1) Ten .05 μg injections at 10-sec intervals, followed by ten .05 μg injections at 15-sec intervals, and finally ten .05 μg injections at 20-sec intervals. 2) Ten 1.0 μg injections at 5-sec intervals, then thirty 1.0 μg injections at 10-sec intervals. In the first sequence the total volume infused was 0.3 ml, in the second, 0.4 ml. Injections were made from % or 1 ml tuberculin syringe or micro-injector.

**CROSS-CIRCULATION PROCEDURE**

The cross-circulation procedure has been described previously. For each experiment one

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Supported by the Michigan Heart Association and by Grant HTS-5465 from the National Institutes of Health.

Accepted for publication December 10, 1965.
femoral artery of each of a pair of rats was connected to a femoral vein of the other with polyethylene tubing through which flow could be regulated individually by means of clamps. Isovolemia was maintained by placing the animals on the pans of a balance and adjusting the tubing clamps so that the exchange rates between the two animals were equal. Exchange rates were determined by closing one clamp for a timed interval and measuring the resulting weight change. In all studies mean arterial pressure was recorded from the contralateral femoral artery by transducer.

**CARDIAC OUTPUT DETERMINATION**

Cardiac output (CO) was measured using the thermodilution method as modified and described by Rosas et al.\(^1\) A small thermistor was positioned in the ascending aorta. Temperature changes at this site resulting from the injection of .1 ml of 147 mm NaCl at room temperature into the central venous pool were recorded, together with right atrial and systemic arterial pressures. A thermal dilution curve was obtained and analyzed, and the CO calculated from the formula derived by Fegler:\(^1\)

\[
CO = \frac{Vi \times \Delta T \times 60 \times C}{A \times f}
\]

where,

CO = cardiac output in ml/min  
Vi = volume of injectate in ml minus dead space of the venous catheter  
\(\Delta T\) = temperature difference between the rat's blood and the injectate, in °C  
C = correction factor of 1.1  
A = area under the curve in mm sec  
f = calibration coefficient in °C/mm deflection

Using the values for CO so derived, total peripheral resistance (TPR) was calculated according to the formula:

\[
TPR = \frac{\text{mean arterial pressure in mm Hg}}{\text{CO in liters/min/m}^2}
\]

Body surface was determined by the equation, \(S = \text{wt}^{0.6} \times 12.54\). Since most animals used for CO determinations were near the average weight, 258 g, CO expressed per kg body wt is approximately 0.137 times the value per m\(^2\) of body surface.

Right atrial pressure was recorded continuously from a cannula placed in the right atrium via the right jugular vein.

Determinations were made as follows: 1) four determinations at 2-min intervals during angiotensin infusion, the first being made 30 sec after the start of the infusion, 2) four determinations at 5-min intervals after the end of the angiotensin infusion. All CO experiments were performed in an air-conditioned room, 74 ± 2°F, to avoid circulatory changes associated with body temperature regulation. Correct positioning of the thermistor and the right atrial catheter were verified by autopsy at the end of the experiment.

**GANGLIONIC BLOCKADE**

An initial dose of 5 mg/kg of pentolinium bitartrate was given to block sympathetic ganglia. Half the dose was given intravenously, the other half intramuscularly. The persistence of the blockade was evaluated in two ways: 1) In one group of animals blood pressure was recorded continuously for 40 min after administering the blocking dose, to establish the absence of recovery. 2) Animals were given a second dose, 2.5 mg/kg iv, at the end of the experiment to determine whether the blockade was complete at that time.

**HEMATOCRIT AND VISCOSITY DETERMINATIONS**

Blood samples for hematocrit and viscosity determinations were obtained from a catheter in the femoral artery; the first 2 to 4 drops of flow were discarded. Samples were collected in standard microcapillary tubes and centrifuged for 5 to 10 min at 13,000 \(\times\) g. Viscosity of whole blood was determined with a Hellige viscometer no. 1200 with a tube of < 400 μ i.d. The viscosity value given for each sample is the mean of three or more determinations on the same blood sample.

**VARIABILITY**

Variability is expressed throughout as standard error of the mean (SEM).

**Results**

**CAPACITY OF ANGIOTENSIN TO INDUCE A PERSISTENT PRESSOR RESPONSE**

In 23 studies of the response of nephrectomized rats to angiotensin, the total amount of drug injected was varied from 0.5 to 200 μg and the duration of the infusion was similarly widely varied from 2 or 3 sec to 7.5 min. The characteristic response was an initial rise in BP followed by a slight regression then stabilization until after the infusion was stopped. Subsequently the BP fell further, but in no case did the BP of the nephrectomized rat return to the pre-injection level during the observation period of from 15 to 50 minutes.
The average increase over pre-injection BP was 24.7 ± 3.3 mm Hg. A representative tracing obtained with one of the two angiotensin dosage schedules most frequently used, together with a tracing obtained after an injection of renin, is illustrated in figure 1. Unoperated and sham operated animals showed a similar initial rise in BP which continued until shortly after the injection was stopped, at which time the BP did not plateau but began to fall, reaching the base line in less than 10 min.

**SPECIFICITY OF ANGIOTENSIN FOR THE RESPONSE**

Ten 20-hr nephrectomized rats injected with epinephrine, 0.1 μg every 5 sec, then 0.1 μg every 10 sec for 5 min, for a total dose of 4 μg, failed to show a persisting pressor response; in each case the blood pressure returned quickly to the pre-injection level or lower (fig. 2). These same animals, when

---

**FIGURE 1**

Comparison of blood pressure responses to renin and to angiotensin. Upper record: Typical blood pressure response of 20-hr bilaterally nephrectomized rat to i.v. renin. Middle and lower records: Simultaneous recordings of blood pressure from two 20-hr nephrectomized rats prepared for cross-circulation. X-CIRC indicates period of blood exchange. No change in blood pressure is seen in either animal during control exchange period. After 8 min one animal is given angiotensin infusion to mimic blood pressure response to renin shown in upper trace. After infusion, blood pressure plateaus 50 mm Hg above base level; subsequent blood exchange induces no change in blood pressure in either animal.

---

**FIGURE 2**

Pressor responses to epinephrine and angiotensin infusion. Values are averages obtained from 10 experiments. n: number of rats. Brackets indicate SEM. Dashed line: Sham nephrectomized rats show no persistent response to angiotensin. Solid line: 20-hr nephrectomized rats show no protracted response to epinephrine, but subsequent angiotensin infusion causes persistent increase over preinfusion level.
given a subsequent angiotensin infusion, showed the typical protracted pressor response (fig. 2). The control group of 10 sham-nephrectomized animals which received the same angiotensin infusion alone, failed to develop the protracted response (fig. 2). Norepinephrine infusion likewise failed to produce the prolonged response in nephrectomized animals (in this case blocked with 5 mg/kg pentolinium) (fig. 3, upper record).

ABSENCE OF CIRCULATING PRESSOR SUBSTANCE IN ANGIOTENSIN-TREATED NEPHRECTOMIZED RATS

In seven experiments a nephrectomized, untreated assay animal was cross-circulated with an angiotensin-treated, nephrectomized rat 3 to 28 min after injection (fig. 1). At the time of cross-circulation the BP of the angiotensin-treated animals (plateau pressure) was between 12 and 50 mm Hg (avg 27.4 ± 12.9) above the pre-injection control level. In no case was constrictor material detected in the blood of angiotensin-treated nephrectomized animals. The exchange of blood, roughly equal to the total blood volume (avg 15.9 ml), did not produce a pressor response in any assay animal or reduce the BP of any injected animal (fig. 1).

Effect of ganglionic blockade on angiotensin responses of nephrectomized and non-nephrectomized rats. n: number of rats. Brackets indicate SEM. Upper curves, dashed line: Blood pressure of non-nephrectomized animals returns to pre-injection level after angiotensin infusion both before and after ganglionic blockade. Angiotensin bracket after blockade refers to this curve only. Solid line: After ganglionic blockade norepinephrine infusion is followed by a quick return of blood pressure to pre-injection levels in nephrectomized animals. Norepinephrine bracket refers only to this line. Lower curves, solid line: After ganglionic blockade, angiotensin infusion induces a protracted pressor response. Broken line: Blocked animals not infused with angiotensin. Little spontaneous return toward control pressure is seen during the period of the experiment. Terminal pentolinium injection indicates block is persistent.

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To determine whether greater amounts of angiotensin in intact rats might produce the protracted response seen in nephrectomized animals, eight animals, male and female, were given angiotensin infusions of 2 to 120 μg. The peak BP elevations attained ranged from 107 to 186 mm Hg, but in no experiment was there a significant maintained increase in baseline pressure after the infusion was discontinued (table 1).

**TABLE 1**

*Angiotensin Infusions in Non-nephrectomized Rats*

<table>
<thead>
<tr>
<th>Total dose of angiotensin (μg)</th>
<th>Number of trials (no.)</th>
<th>Δ BP (mm Hg ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>19</td>
<td>+ .21 ± .98</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>+2.6 ± .73</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>+ .66 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>+4.7 ± 1.4</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>−20 ± 1.7</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>+1.5 ± 1.2</td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>+3.0 ± .86</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>+3.0 —</td>
</tr>
</tbody>
</table>

**GANGLIONIC BLOCKADE**

The response of nephrectomized and sham-operated animals to angiotensin infusion was not altered by ganglionic blockade. In blocked sham-nephrectomized animals the BP at the termination of angiotensin infusion returned quickly to the pre-injection level (fig. 3, upper record); in nephrectomized blocked animals BP remained elevated after the infusion (fig. 3, lower record). When blocked nephrectomized rats were infused with noradrenaline, however, BP returned to pre-injection values immediately after the injection period.

**CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE**

The average pre-infusion BP of the nephrectomized group was 83.6 ± 5.11 mm Hg; that of the sham-operated controls was 110.8 ± 6.73 mm Hg (fig. 4). Average peak BP of the nephrectomized group was essentially the same as that of the control animals. Twenty minutes after cessation of angiotensin infusion, average BP of the nephrectomized...
TABLE 2
Viscosity and Hematocrit Before and After Angiotensin in 24-Hr Sham-operated and Nephrectomized Rats

<table>
<thead>
<tr>
<th></th>
<th>Before injection</th>
<th>sham-operated</th>
<th>SEM</th>
<th>Hematocrit (%)</th>
<th>BP (mm Hg)</th>
<th>After injection</th>
<th>SEM</th>
<th>Hematocrit (%)</th>
<th>BP (mm Hg)</th>
<th>Effect of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated, n = 9</td>
<td>47.3</td>
<td>4.3</td>
<td>115</td>
<td></td>
<td>45.8</td>
<td>4.1</td>
<td>115</td>
<td></td>
<td>-3.2%</td>
<td>-4.0%</td>
</tr>
<tr>
<td>Nephrectomized, n = 8</td>
<td>43.7</td>
<td>3.8</td>
<td>74.1</td>
<td></td>
<td>43.1</td>
<td>3.7</td>
<td>100</td>
<td></td>
<td>-1.4%</td>
<td>-2.6%</td>
</tr>
</tbody>
</table>

Effects of nephrectomy

-7.6% -11.6% -35.4% -5.9% -9.8% -12.8%

p < 0.025 p < 0.05 p < 0.0005 p < 0.05 p < 0.10 p < 0.005

Discussion

The observation that the classical potentiated response to renin does not develop until several hours after nephrectomy suggests that this phenomenon is not due simply to the loss of renin excretion and the associated continued elaboration of angiotensin, but must involve a change in responsiveness of the cardiovascular system itself. This is further substantiated by the observation that the protracted response can be induced by angiotensin injection. When angiotensin is infused at a rate which produces a BP response similar in height and duration to that induced by an injection of renin it will produce a prolonged, stable pressor response which persists long after the infusion is stopped.
The prolonged response is not merely the aftermath of a severe hypertensive episode since after infusions of epinephrine or noradrenaline, which produce responses duplicating typical renin responses, BP returns rapidly to pre-infusion levels or below (figs. 2 and 3).

CHANGES IN ANGIOTENSIN METABOLISM

A delayed rate of destruction of angiotensin might be postulated as the cause of the protracted response. However, when a single small (0.05 μg) dose of angiotensin is given to a nephrectomized rat, the pressor response is very short-lived and the BP returns rapidly to, or near to, base line. This denotes rapid destruction of angiotensin. Evidence from cross-circulation experiments also indicates that there is no reduction in angiotensinase activity; even large infusions of angiotensin did not overwhelm the angiotensin-inactivating system for the infused constrictor did not long remain in the circulation. When circulating constrictor material such as epinephrine or angiotensin or endogenous or exogenous renin is present in one of a pair of cross-circulated animals, there is an increase in the BP of the assay animal and, in the case of the direct-acting agents, some decrease in that of the test animal. By this cross-circulation procedure blood levels of angiotensin which elevate the arterial pressure of the donor animal only a few mm Hg can be detected. Since no pressor substance was detected in rats’ blood three or more minutes after infusion of angiotensin was stopped, it is unlikely that decreased angiotensinase activity is responsible for the potentiation. The fact that the BP of the infused animal remains elevated long after circulating constrictor material falls to undetectable levels, implies some non-humoral basis for the response.

There is considerable evidence that nephrectomy and DCA treatment alter the blood in some way that favors the production of angiotensin or other pressor peptides when renin or acid is added. Although increased production of angiotensin in nephrectomized rats may well explain much of the increase in peak response to renin injection, it alone cannot account for the prolonged elevation, for when intact animals are given renin-mimicking infusions of angiotensin, even in amounts far in excess of that elaborated by renin in nephrectomized rats, no prolonged elevation occurs (table 2).

CHANGES IN NEUROGENIC BUFFER SYSTEM

In nephrectomized animals the rate of the fall in BP after the pressor response to renin injection appears exponential and becomes asymptotic to the plateau (fig. 1). A similar fall occurs immediately after angiotensin infusion is stopped (fig. 1). This suggests that after each episode the pressure returns to a new, stable, elevated, regulated level. This might seem to indicate some neurogenic regulation but no evidence for the involvement of a neurogenic mechanism was obtained. Ganglionic blockade with pentolinium had no influence on: a) the development of the protracted response to angiotensin in nephrectomized animals, b) the failure of angiotensin to induce the response in sham-operated animals (fig. 3), or c) the failure of catecholamines to induce the response in nephrectomized animals.

HEMODYNAMIC MECHANISMS

The hemodynamic basis of the pressor response, both the acute constrictor phase and the protracted phase, is, apparently, increased TPR (fig. 4). The evidence from the CO studies does not deny a cardiotonic action of angiotensin, but indicates that if any such action occurs under these conditions it is masked by the increase in myocardial workload induced by the large increase in TPR. This increased resistance could be caused by a change in the vasculature, producing a greater hindrance to blood flow, or by increased blood viscosity. To determine which of these factors was dominant in causing the lower TPR in nephrectomized animals before angiotensin infusion and the subsequent increase in TPR in these animals after infusion, hematocrit and blood viscosity were measured. Although the viscosity of blood from 20-hr nephrectomized animals was less than that of sham-operated animals, this reduction could
account for only part of the lower TPR and mean systemic pressure seen in these animals prior to angiotensin infusion. In neither nephrectomized nor sham-operated animals was there any evidence that hematocrit or viscosity was changed by angiotensin infusion (table 2).

Evidently, then, the persistent increase in TPR after angiotensin infusion is the result of a net increase in geometric resistance, or hindrance. The locus of the change has not been identified, but some sites have been ruled out. The alteration obviously does not involve the renal circulation. Nor does it involve a significant change in flow through the thermoregulatory, cutaneous circuits for there is no change in body temperature nor any change in color of feet or ears associated with angiotensin infusion.* Neither the central venous bed nor the pulmonary circuit were sufficiently affected by the angiotensin infusion to alter right atrial pressure, for this value remains stable under all experimental conditions.

The apparent independence of this persistent decrease in vessel diameter of the usual systemic tone-regulating systems raises the question of its local cellular basis. The changes could be “structural,” in the sense that water bound to components of the wall is a structural component. Structural changes such as might be induced by fluid or electrolyte redistributions within the walls of the vessel could increase wall volume and thereby encroach on the lumen. The changes could be due to muscular contraction, but if neither humoral nor neurogenic in origin, they must be mediated by a third mechanism involving changes in contractility intrinsic to the muscle cell. The possibility cannot be ignored that, under renin or angiotensin deprivation, angiotensin becomes attached to its receptor and continues to act as a constrictor agent, immune to attack by the angiotensinase which quickly destroys the circulating peptide.

This study has shown that under experimental conditions involving interruption of renal excretory and incretory functions and the renin-angiotensin system, an increase in vascular resistance occurs whose maintenance appears to be independent of humoral and neurogenic mechanisms. It would seem reasonable to suggest that the mechanisms involved here may have a role in the increased vascular resistance of experimental renal and essential hypertension, in which, likewise, there is an increase in TPR without an elevation in circulating renin or angiotensin.

Summary

The pressor response of nephrectomized rats to an injection of renin is marked by a prolonged stable plateau. A similar, prolonged response to angiotensin has been demonstrated and the following aspects of this pressor effect were evaluated.

1. The protracted response was shown not to be due to a persistence of angiotensin in the blood of nephrectomized animals. There are several indications from unrelated studies that angiotensin is destroyed rapidly. Cross-circulation experiments indicate that during the protracted pressor phase blood levels of constrictor materials have fallen to undetectably low levels.

2. No evidence could be obtained to indicate that the neurogenic buffer system is involved in the development of the protracted response. Ganglionic blockade was ineffective in preventing its development.

3. The hemodynamic basis for the response must be a persistent increase in total peripheral resistance (TPR) since there is no increase in cardiac output (CO) in response to the angiotensin infusion. Before infusion, the CO of nephrectomized animals is higher than that of sham-operated control animals.

4. The increase in TPR must be due to an increase in geometric hindrance for no changes in hematocrit or in viscosity result from angiotensin infusion. Blood viscosity is lower in nephrectomized than in sham-operated animals, but this can account for only part of the lower pre-injection control pressure seen in this group.

*Control period, 37.7 ± 0.34°C; after infusion, 37.8 ± 0.30°C.
It appears that in animals depleted of endogenous renin, a large infusion of angiotensin increases vascular resistance by some non-neurogenic, non-humoral mechanism which may represent another type of vascular tone.

Acknowledgment
We are indebted to Sandra J. Foulke and to Thomas R. Colladay for technical assistance, and to Dr. Ruth B. McVaugh for editorial counsel.

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Edward H. Gabelman and Paul A. Rondell

Circ Res. 1966;18:705-713
doi: 10.1161/01.RES.18.6.705

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