Reduction in Renal Vascular Responses to Angiotensin and Norepinephrine during Carotid Sinus Stimulation

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ABSTRACT

In dogs anesthetized with morphine and chloralose, the renal vascular responses to angiotensin and norepinephrine were studied under conditions of altered sympathetic nervous activity. The kidneys and one or both carotid sinuses were perfused at a constant rate of flow. Sympathetic activity was altered by changing the mean carotid sinus perfusion pressure from approximately 30 to 200 mm Hg. During maximal carotid sinus pressure, the renal vasoconstrictor responses to angiotensin and norepinephrine were reduced by an average of 52% and 47%, respectively. Similar results were obtained in innervated, chronically denervated, and excised kidneys perfused with the dogs' own blood. Increasing carotid sinus pressure alters the responsiveness of the renal vasculature to angiotensin and norepinephrine by some mechanism that is not dependent on the renal sympathetic nerves or on norepinephrine. It is proposed that raising mean carotid sinus pressure alters the concentration of some bloodborne substance and so reduces the vasoconstrictor responses to these vasoactive agents in the renal vascular bed.

ADDITIONAL KEY WORDS

vasopressin innervated kidneys
denervated kidneys (excised)
renephrenal pressure
denervated kidneys (in situ)
anesthetized dogs

The relation between the vasoconstrictor activity of angiotensin and the sympathetic nervous system has been reported by several workers. Zimmerman (1) showed that the vasoconstrictor response to angiotensin in the perfused hindquarters of the dog is reduced by acute sympathectomy, and Laverty (2) confirmed this for the rat hind limb. Angiotensin has been reported to release catecholamines from the adrenal medulla (3-4), to potentiate the action of tyramine (5), and to enhance the contraction of the vas deferens during stimulation of the hypogastric nerve (6). Day, McCubbin, and Page (7) showed that the compensatory cardiovascular reflexes limit the pressure rise to infusions of angiotensin.

Constriction of the renal vascular bed by angiotensin depends partly on sympathetic nervous activity and on the level of circulating sympathetic neurohormones (8). In the experiments reported here, the renal vascular changes to angiotensin were studied during low and high carotid sinus pressures. In early experiments in intact dogs, we observed that renal blood flow decreased when angiotensin was given either intravenously or into the renal artery. However, during carotid sinus stimulation, it either decreased less or increased during administration of angiotensin. Because McGiff and Itskovitz (9) reported that the vasoconstrictor activity of angiotensin is reduced during renal ischemia and because carotid sinus stimulation reduces systemic
pressure and renal blood flow, we have repeated our early experiments with a constant rate of flow through the renal arteries that was considered to be near normal.

**Methods**

**A. Perfusion of Innervated Kidneys**

Twenty-one mongrel dogs (14 to 30 kg) were anesthetized with morphine (3 mg/kg) subcutaneously and chloralose (100 mg/kg) intravenously. Both kidneys were perfused by a Sigmamotor pump at a constant rate of flow (240 to 360 ml/min). Flow was adjusted so that renal perfusion pressure initially was about the same as systemic pressure. Figure 1 illustrates the perfusion arrangement. The aorta was dissected free for a distance of about 1 cm above the right renal artery and 5 cm below the left renal artery. The lumbar arteries in this segment were tied off. Following the intravenous injection of heparin (3 to 5 mg/kg), a cannula consisting of two concentric glass tubes was inserted into the aorta about 5 cm below the left renal artery and advanced until the outer shell was at the level of the renal arteries and the inner shell extended in the aorta 3 to 5 cm above the origin of the right renal artery. Ligatures were tied on the cannula above and below the renal arteries, which were then perfused by a Sigmamotor pump that received blood from a common carotid artery and pumped it through the outer shell of the cannula. Blood flowed through the inner shell to maintain flow to the hindquarters. With the exception of an 8-inch length of rubber tubing in the pump head, 6-inch Tygon tubing was used to make all connections; its approximate internal volume was 50 ml. The tubing was filled with 0.9% saline. This method of perfusion was selected because we believe that it preserves renal innervation, never interrupts renal blood flow, and allows hindquarter circulation to be reestablished. Since the kidneys are perfused at a constant rate of flow, renal perfusion pressure reflects renal vascular resistance.

**B. Perfusion of Denervated Kidneys**

1. **In Situ.**—Four dogs were anesthetized and their kidneys denervated 5 days before being perfused. Denervation consisted of stripping the renal arteries, renal veins, and ureters and applying 95% phenol to these structures and to the aorta, which was also stripped in this region. The animals were allowed to recover, and experiments as outlined above were conducted.

2. **Excised.**—In three dogs, one kidney was acutely excised and its artery perfused by a pump with blood from the common carotid artery. Venous blood was returned to the femoral vein.

In 16 experiments, one carotid sinus was isolated from systemic pressure fluctuations by ligating the internal and external carotid arteries, the occipital artery (near its origin), and all other arteries found in this region. The common carotid was then tied, and a cannula directed toward the heart was inserted into it; this provided a source of blood for renal perfusion. No attempt was made either to destroy or preserve the innervation of this carotid sinus.

The other carotid sinus, isolated in a similar manner, was perfused with blood from its common carotid artery at a constant rate (75 ml/min) by a pulsatile Sigmamotor pump that pumped blood back into this vessel just below the carotid sinus. The external carotid artery was cannulated to provide an outflow into the external jugular vein. A screw clamp on the outflow tubing provided a means of changing pressure in this carotid sinus. Mean carotid sinus pressure was altered from about 30 mm Hg to about 200 mm Hg. Pulse pressure within the sinus was approximately 50 and 80 mm Hg respectively at the two levels of mean perfusion pressure.

In addition to the above experiments, both carotid sinuses were perfused as described above in five vagotomized dogs in an attempt to implicate or eliminate some factors that could be involved in the results obtained. Vagotomy enhanced the systemic pressure response to carotid sinus stimulation, but was usually avoided because it often resulted in large pressure fluctuations associated with breathing that necessitated artificial ventilation to reduce them. Neither vagotomy nor artificial ventilation affected the experimental results.

Mean systemic blood pressure was measured from a branch of the femoral artery. Renal perfusion pressure and carotid sinus perfusion pressure were measured from side tubes off the appropriate cannulas. Statham strain gauges re-
RENAL RESPONSES TO VASOACTIVE DRUGS

According to an Offner oscillograph were used. Solutions (1.0 and 0.1 μg/ml) of angiotensin amide (Hypertensin-Ciba) and norepinephrine (L-arterenol bitartrate, Winthrop Stearns) were made, and doses of 0.01-1.0 μg were injected directly into the renal perfusion circuit.

Results

Innervated Perfused Kidneys

In kidneys perfused at a constant rate of flow, the vasoconstrictor response to intra-arterial angiotensin (0.5 to 1.0 μg) during maximal carotid sinus pressure (200 mg Hg) was, on the average, 52% less than the response to the same dose during minimal carotid sinus pressure (30 mm Hg). Figure 2 shows typical changes in renal perfusion pressure in response to angiotensin. In this experiment, the kidneys were perfused at a rate of 340 ml/min. Raising mean carotid sinus perfusion pressure from about 30 to 200 mm Hg caused the expected fall in systemic pressure. Renal perfusion pressure in this case fell slightly. In some cases, no change in renal perfusion pressure was observed in response to carotid sinus stimulation, while in others, a large fall was seen. In general, the reduction in the response to angiotensin appeared to be unrelated to the change in renal perfusion pressure.

A similar reduction in the renal vasoconstrictor response to intra-arterial norepinephrine was observed during increased carotid sinus pressure. The renal vasoconstrictor responses to intravenous injections of these drugs were also reduced during maximal carotid sinus pressure. Bilateral adrenalectomy did not alter these results. Table 1 summarizes the data. The discrepancies between the number of dogs shown in this table and the total number used result from excluding data from dogs in which fluctuations in pressure made measurement of the renal vasoconstrictor responses to the drugs impossible and from early termination of some experiments caused by failure of the cardiovascular system. In one experiment, a depressor response to angiotensin occurred during maximal carotid sinus pressure.

The reduction in the vasoconstrictor response to angiotensin does not appear to be due to tachyphylaxis since the reduction was independent of the sequence of drug administration and was always related to carotid sinus pressure.

Denervated Perfused Kidneys

1. In Situ.—In chronically denervated kidneys, the vasoconstrictor response to angiotensin was reduced an average of 59% during maximal carotid sinus pressure (Table 1). The response to norepinephrine was reduced 33%. Figure 3 shows the typical results of one experiment.

2. Excised.—The perfused excised kidneys demonstrated enhanced sensitivity to angiotensin and norepinephrine. For this reason, the dose of these drugs was reduced in order to obtain responses similar in magnitude to those obtained above. The response to angio-

\[ \text{FIGURE 2} \]

Vasoconstrictor responses to angiotensin in innervated kidneys perfused at a rate of 340 ml/min. Carotid sinus pressure was increased from 30 to 200 mm Hg. Angiotensin given into the renal perfusion circuit at A. Responses to 0.5 μg angiotensin shown in upper panel; responses to 1.0 μg angiotensin in lower panel.

\[ \text{FIGURE 3} \]

Vasoconstrictor responses to 0.5 μg angiotensin (A) and norepinephrine (N) in chronically denervated kidneys perfused at a rate of 346 ml/min. Drugs given into the renal perfusion circuit.
Average Renal Responses to Angiotensin and Norepinephrine before and during Carotid Sinus Stimulation in 16 Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dose (µg)</th>
<th>Carotid Sinus Pressure</th>
<th>Renal pump output (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerves intact</td>
<td>0.5-1.0</td>
<td>Minimal</td>
<td>280 ± 6</td>
</tr>
<tr>
<td>Denervated</td>
<td>0.5-1.0</td>
<td>Minimal</td>
<td>346 ± 0</td>
</tr>
<tr>
<td>Excised†</td>
<td>0.01-0.1</td>
<td>Minimal</td>
<td>135 ± 4</td>
</tr>
<tr>
<td>Nerves intact</td>
<td>0.5-1.0</td>
<td>Maximal</td>
<td>288 ± 10</td>
</tr>
<tr>
<td>Denervated</td>
<td>1.0</td>
<td>Maximal</td>
<td>346 ± 0</td>
</tr>
<tr>
<td>Excised†</td>
<td>0.1-0.5</td>
<td>Maximal</td>
<td>145 ± 5</td>
</tr>
</tbody>
</table>

*Range in parentheses. All values are ± SEM.
†Values based on flow through one kidney.

Angiotensin in these kidneys (three dogs) was reduced on the average 43% during maximal carotid sinus pressure. The response to norepinephrine was reduced 33%. Figure 4 shows the typical results.

Pressure in the renal vein was monitored in two of these experiments. This pressure did not change in response to carotid sinus stimulation, angiotensin, or norepinephrine. Raising renal venous pressure (0 to 30 mm Hg) by clamping the outflow tubing did not alter the results.

Humoral Factors

In five vagotomized dogs (not included in Table 1) we studied some humoral factors that possibly might affect the sensitivity of the renal vascular bed to angiotensin. In these dogs both carotid sinuses were perfused.

Blood Gas Composition

Arterial blood samples were taken before and 5 min after raising mean carotid sinus pressure to 200 mm Hg. No consistent changes were observed in the arterial blood Pco₂, Po₂, or pH as a result of this procedure. The blood gas tensions in these anesthetized, spontaneously breathing animals were found to be in the range reported in the Biological Handbook.

FIGURE 4

Vasoconstrictor responses to 0.1 µg angiotensin (A) and norepinephrine (N) in one excised kidney perfused at a rate of 145 ml/min. Drugs given into the renal artery. Vagi cut; artificial ventilation.
RENAL RESPONSES TO VASOACTIVE DRUGS

<table>
<thead>
<tr>
<th>Renal perfusion pressure (mm Hg)</th>
<th>Increase</th>
<th>Renal resistance (pump output X 100)</th>
<th>Initial</th>
<th>Increase</th>
<th>Percent reduction of renal vasoconstrictor response during carotid sinus stimulation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>112 ± 6</td>
<td>54 ± 4</td>
<td>41 ± 2</td>
<td>20 ± 1</td>
<td>52 ± 8</td>
<td>P &lt; 0.01 (7-120)</td>
</tr>
<tr>
<td>105 ± 6</td>
<td>25 ± 4</td>
<td>39 ± 3</td>
<td>9 ± 2</td>
<td>59 ± 7</td>
<td>P &lt; 0.01 (40-84)</td>
</tr>
<tr>
<td>101 ± 3</td>
<td>68 ± 12</td>
<td>29 ± 1</td>
<td>20 ± 4</td>
<td>43 ± 6</td>
<td>P &lt; 0.01 (25-50)</td>
</tr>
<tr>
<td>99 ± 3</td>
<td>29 ± 6</td>
<td>28 ± 1</td>
<td>9 ± 2</td>
<td>33 ± 7</td>
<td>P &lt; 0.01 (10-75)</td>
</tr>
<tr>
<td>101 ± 4</td>
<td>45 ± 5</td>
<td>76 ± 4</td>
<td>34 ± 4</td>
<td>33 ± 5</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
<tr>
<td>86 ± 9</td>
<td>26 ± 3</td>
<td>64 ± 6</td>
<td>22 ± 4</td>
<td>28 ± 6</td>
<td>P &lt; 0.01 (20-47)</td>
</tr>
<tr>
<td>123 ± 8</td>
<td>53 ± 6</td>
<td>46 ± 3</td>
<td>19 ± 3</td>
<td>47 ± 7</td>
<td>P &lt; 0.01 (10-75)</td>
</tr>
<tr>
<td>113 ± 7</td>
<td>28 ± 6</td>
<td>43 ± 3</td>
<td>10 ± 2</td>
<td>33 ± 7</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
<tr>
<td>104 ± 6</td>
<td>42 ± 1</td>
<td>30 ± 2</td>
<td>12 ± 1</td>
<td>33 ± 5</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
<tr>
<td>100 ± 3</td>
<td>28 ± 3</td>
<td>29 ± 1</td>
<td>8 ± 1</td>
<td>33 ± 5</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
<tr>
<td>87 ± 2</td>
<td>54 ± 3</td>
<td>62 ± 2</td>
<td>38 ± 6</td>
<td>28 ± 6</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
<tr>
<td>82 ± 3</td>
<td>37 ± 4</td>
<td>58 ± 2</td>
<td>26 ± 4</td>
<td>28 ± 6</td>
<td>P &lt; 0.01 (20-53)</td>
</tr>
</tbody>
</table>

book (10). Blood pH was low (7.28 to 7.3), partly because of respiratory acidosis under chloralose anesthesia. Alteration of the gaseous composition and pH of the arterial blood by artificial ventilation was without effect on the results.

Noradrenaline

The experiments on denervated kidneys and adrenalectomized dogs appear to rule out catecholamines of adrenal or renal sympathetic nerve origin as factors that change the sensitivity of the renal vascular bed to angiotensin. Dibenzyline (5 mg/kg) infused into the renal artery over a period of 30 min did not abolish the reduction of the renal response to angiotensin (Fig. 5) during maximal carotid sinus pressure, although the response to a test dose of norepinephrine was abolished. This finding would appear to eliminate circulating norepinephrine from any source as a factor in the results.

The use of bretylium (10 mg/kg iv) in several dogs was unsuccessful because of the severe fall in systemic pressure and consequent failure to maintain the renal and carotid

![Figure 5](http://circres.ahajournals.org/)

Vasoconstrictor responses to 0.5 μg angiotensin (A) given intra-arterially to the kidneys, perfused at a rate of 280 ml/min after Dibenzyline (5 mg/kg body wt) had been infused into the renal artery over a period of 30 min. Vagi cut; both carotid sinuses perfused.
sinus perfusions. In one experiment in which perfusion was maintained following bretylium the reduction in the renal angiotensin response was not observed during carotid sinus stimulation.

**Pitressin**

Share and Levy (11) have reported that carotid sinus stimulation decreases the rate of ADH (antidiuretic hormone) release. During a continuous infusion (10 milliunits/min) of Pitressin (Parke, Davis & Co.) into the renal artery of two dogs, the renal vasoconstrictor response to angiotensin was not changed. These experiments were abandoned because the renal vascular bed rapidly became refractory to Pitressin.

**Discussion**

The role of the sympathetic nervous system in the regulation of the renal vascular bed is not clear. Heymans (12) cited inconsistent effects of carotid sinus baroreceptor activity on renal blood flow. Carotid occlusion may either increase or decrease renal blood flow. Carotid sinus stimulation always decreased or caused no change in renal blood flow. Khajutin (13) reported weak dilation of the renal vascular bed during carotid sinus stimulation, but strong constriction during carotid artery occlusion.

In most of our experiments, increasing the carotid sinus pressure caused a small decrease in renal perfusion pressure; however, in some experiments there was a large decrease. The reduction of the renal vasoconstrictor response to angiotensin and norepinephrine was not related to the changes in renal perfusion pressure that resulted from carotid sinus stimulation. Increasing pump output so that renal perfusion pressure remained constant during carotid sinus stimulation was without effect on the results. We observed a small decrease in renal perfusion pressure in both denervated and excised kidneys. This is possibly due to a slight decrease in pump output resulting from the lowered systemic pressure produced by carotid sinus stimulation.

The renal vascular activity of angiotensin is related to the activity of the autonomic nervous system. McGiff and Fasy (8) reported that sympathetic denervation of the kidneys and sympathetic blocking drugs reduce the renal vascular response to angiotensin. This suggests to them that angiotensin exerts a part of its action on the renal vascular bed by acting on the stores of the sympathetic neurotransmitter. Our results do not support their finding that autonomic denervation of the kidneys reduces the pressor response to angiotensin. The response was greater in the chronically denervated kidneys and in the excised kidneys than in the intact kidneys. As indicated in Table 1, much smaller doses were used to cause responses comparable to those in the innervated kidneys. We have no explanation for these contrasting results.

In our experiments, increasing the carotid sinus pressure decreased the vasoconstrictor response to angiotensin and norepinephrine in the renal vascular bed. This reduction appears to result from a change in the level of some bloodborne substance, since it is not dependent on the integrity of the renal sympathetic nerves. This is supported by the similar results obtained in experiments conducted on dogs with chronically denervated kidneys and on excised kidneys perfused by the dogs' own blood.

Experiments conducted in an attempt to eliminate other possible factors appear to rule out norepinephrine and changes in the gaseous composition of the arterial blood. In our studies, mechanical changes or reflex activity resulting from changes in breathing did not appear to be implicated. Opening the chest and hyperventilating the dog until its breathing movements ceased did not change the results. Nor did changes in abdominal muscle contractions appear to be involved, since the angiotensin response was reduced in the excised kidney removed from the abdominal cavity. Variation of venous pressure in the excised kidney also failed to affect the results.

Raising the mean carotid sinus perfusion pressure from 30 mm Hg to 200 mm Hg increased pulse pressure by about 30 mm Hg. To eliminate the effects of pulse pressure, an air chamber was introduced in the carotid
sinus perfusion circuit in one animal. Depulsing the carotid sinus pressure did not alter the results.

Although the occipital artery was tied near its origin in all animals, no tests were conducted to ensure that the carotid bodies were vascularly isolated. For this reason it is possible that during elevated carotid sinus pressure, blood flow to the carotid bodies changed. This change could alter chemoreceptor activity, and chemoreceptor reflexes rather than baroreceptor reflexes may bring about the change in the renal vascular response to angiotensin. In either case, it would appear that the effect must be mediated by some humoral mechanism.

Speculation as to the humoral mechanism involved is not warranted by the experiments reported here. This phenomenon could result in a less severe reduction and possible improvement of renal blood flow in the presence of substances like angiotensin that are general vasoconstrictors.

Acknowledgment

The authors express sincere appreciation to David Enerson, who was associated with this project on a summer fellowship and who rendered invaluable technical assistance, and to Dr. Q. R. Murphy for advice and criticism in preparation of this manuscript.

References

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Circ Res. 1967;20:321-327
doi: 10.1161/01.RES.20.3.321

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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