Effect of Angiotensin on Juxtaglomerular Cells and Vessels of the Kidney

By Yale J. Katz, Ph.D., M.D., Paul R. Patek, Ph.D., and Sol Bernick, Ph.D.

With the technical assistance of Raymond S. Moore, B.S., and Celia delNoyos, B.S.

Angiotensin, considered to be a product of renal ischemia, appears itself to promote additional renal ischemia. It has been shown to cause a decrease in renal blood flow by investigators using the clearance method, the electromagnetic flowmeter, and the rotameter. McQueen and Morrison found that with the equal pressor doses, angiotensin caused a greater decrease in renal plasma flow than did epinephrine. Peart has shown that even in nonpressor doses, angiotensin alters water and electrolyte excretion.

The electric impedance method has been applied to the measurement of the pulse of the kidney, and with this method in some preliminary studies, one of us noted that angiotensin caused a decrease, while norepinephrine caused an increase in the amplitude of the kidney pulse wave. Because constriction of the renal artery sufficient to lead to hypertension in the dog caused a similar decline in the amplitude of the kidney pulse wave, the suggestion was made that angiotensin might cause a change in the renal circulation similar to that of renal artery constriction and might be involved in a feedback mechanism as follows:

Renal ischemia

\[ \text{Increased} \leftarrow \text{Increased} \]

angiotensin renin formation

As one test of this concept, we determined to give rats repeated subcutaneous injections of pressor doses of angiotensin and to observe the effect on the juxtaglomerular (JG) cells, since Tobian et al. had previously shown that renal artery constriction in the rat produces an increase in the juxtaglomerular index (JGI).

Methods

Seventy-seven rats, first the Wistar strain and later the Holtzman strain males averaging 265 Gm., were used for this study. They were maintained on Golden State rat food, McGill University formula, and tap water. Two \( \mu \)g. angiotensin II dissolved in 0.2 cc. of 0.9 per cent NaCl were given subcutaneously at approximately 8:30 A.M., 12:30 P.M., and 5:30 P.M. daily to a total of 43 animals. This dose of angiotensin produces a 30-mm. rise in blood pressure for 1½ hours, as measured by the Friedman-Freed tail microphonograph method. Nineteen animals were given 20 \( \mu \)g. epinephrine in 0.2 cc. of saline, and 15 animals were given saline only. Twenty \( \mu \)g. epinephrine produce a pressor response similar to 2 \( \mu \)g. angiotensin II. The animals were killed on schedule (see Results), and the kidney, heart, aorta, liver, brain, and spleen were removed. Blocks of tissues were placed in Bouin's fixative and prepared for paraffin embedding in the routine manner. Alternate sections cut at 5 \( \mu \) to 7 \( \mu \) were stained by the Alcian blue-PAS method and routine hematoxylin and eosin, PAS, and aldehyde fuchsin. The JG cells were evaluated, and the JG indices were calculated by the method of Hartroft and Hartroft. This method gives relative weights to the units of JG cells per 100 glomeruli according to the concentration of the PAS-positive granules with JG units of one plus.

From the Departments of Medicine and Anatomy, University of Southern California, Los Angeles, California.

Supported by grants from the American Heart Association, Los Angeles County Heart Association, and Southern California Chapter National Kidney Disease Foundation and U.S. Public Health Service Grant H-4867 C-1.

Dr. Katz's work was done during tenure of Established Investigatorship, American Heart Association.

Some of this material was presented to the American Physiological Society at the Fall Meeting, 1961 (Physiologist 4: 58, 1961), and a preliminary note appeared in Lancet 2: 602, 1961.

Received for publication June 19, 1962.
FIGURE 1
Section of kidney of saline control animal. Note the few JG cells which contain PAS-positive granules. X 250. Alcian blue-PAS stain.

FIGURE 2
Section of kidney from a rat killed one day after injection of angiotensin. Note the increase in both the size of the juxtaglomerular cells as well as the number of their granules. X 500. Alcian blue-PAS.

Results

EFFECT OF ANGIOTENSIN ON JG CELLS

Figure 1 is of a group of JG cells, or JG unit, at the glomerular pole in a control animal. The PAS-positive granules stain red in the histological material and appear black in these figures. Table 1 lists the results of JG cell appraisal in 16 rats given angiotensin for from 1 to 14 days, and four rats given saline only. The histological material was selected at random for duplicate or triplicate cell counting from among 44 rats given angiotensin and 15 given saline only.

The weighted JG index of the saline control animals averaged 16.7, with a range of 13 to 19. On the first day following administration of angiotensin there was an increase in the number of JG units and the granularity of the cells (table 1 and fig. 2). The average weighted index calculated as 81.5 with a range of 72 to 97. PAS-positive granules appeared in cells in the media of the afferent arteriole.

This figure compares well with that of Hartroft and Hartroft but is lower than that of Tobian et al. and Demopoulos, Kaley, and Zweifach. The difference may be due to the higher Na content of the present diet as compared with that used by the latter two groups of authors.

*This figure compares well with that of Hartroft and Hartroft but is lower than that of Tobian et al. and Demopoulos, Kaley, and Zweifach. The difference may be due to the higher Na content of the present diet as compared with that used by the latter two groups of authors.

Circulation Research, Volume XI, December 1963
not only adjacent to the glomerulus, but also in the media at some distance from the glomerulus (fig. 3). The granules in these cells did not take the aldehyde fuchsin stain, and in addition, because the cells are clearly in the media of the vessel, they are probably not tissue mast cells.

Figure 4 is from an animal given angiotensin for three days. Note the greater number of JG cells in this complex than in that of the control animal (fig. 1).

Animals given angiotensin for seven days exhibited somewhat of a decrease in the number of JG units; however, most of those present were of the four plus grouping. The JG index ranged from 70 to 85, with an average of 77.

Figure 5 demonstrates an afferent arteriole in cross-section from an animal given angiotensin for seven days. The lumen of the vessel is surrounded by a cuff of cells containing PAS-positive granules. The granules are so numerous as to obscure the cell nuclei.

Continuation of angiotensin injection for 14 days was followed by a marked lessening of the content of granules of the JG cells; the cells, however, retained their swollen appearance (fig. 6). Based on content of granules the cells were classified as one plus, giving a drop in the weighted index to 21, with a range of 17 to 28.

**EFFECTS OF ANGIOTENSIN ON RENAL VESSELS**

Animals killed 24 hours after receiving three pressor doses of angiotensin exhibited some changes in the media of their medium-sized intrarenal arteries. Figure 7 is from such an animal. There is a loss of nuclear staining in the muscle cells of the media and the appearance of amorphous PAS-positive material in the ground substance of the media. After seven days of angiotensin injections, the degenerative lesions in the media appear much advanced. In figure 8, vacuoles may be seen scattered throughout the media; no

---

**TABLE 1**

*Effect of 6 μg. Angiotensin Daily on the Juxtaglomerular Cells of the Rat*

<table>
<thead>
<tr>
<th>No. rats</th>
<th>Days of angiotensin</th>
<th>Total JG units/100 glomeruli</th>
<th>Average no. of JG units of each type</th>
<th>JG Index</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>10.2</td>
<td>3.8  3.2  1.4  0</td>
<td>16.7</td>
<td>13 to 19</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>22.8</td>
<td>2.5  8.7  10.5  1.5</td>
<td>51.6</td>
<td>72 to 97</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>21.2</td>
<td>1   12   7.2  1</td>
<td>83.5</td>
<td>63 to 74</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>13.5</td>
<td>2.5  .75  2.2  13.5</td>
<td>77.0</td>
<td>70 to 85</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>15.0</td>
<td>11  2.8  1.3  0</td>
<td>21.8</td>
<td>17 to 23</td>
</tr>
</tbody>
</table>

_Circulation Research, Volume XI, December 1968_
Section of kidney from a rat injected with angiotensin for two weeks. The juxtaglomerular cells are still swollen but degranulated. Note the apparent constricted portion of the arteriole and the dilatation distal to this extreme narrowing of the lumen. X 500. Alcian blue-PAS.

Section of interlobar artery from a rat killed one day after injection of angiotensin. Note the PAS-positive material deposited in the media of the vessel. X 500. Alcian blue-PAS.

longer can fine elastic fibers be seen, and the internal elastic membrane appears fragmented.

Figure 9 is from animals given angiotensin for two weeks. Note the extensive vacuolization, necrosis of muscle cells, and proliferation of both cellular and fibrous elements of the intima.

In addition to the changes in the intrarenal arteries, the afferent arterioles exhibited alterations. It was noted in the previous section that after two weeks of angiotensin injection, the granules tend to disappear from the JG cells, but the cells remain swollen. This swelling of the cells apparently leads to a narrowing of the afferent arteriole at the glomerular pole as in figure 6.*

Figure 8
Section of an intrarenal artery from a rat given angiotensin for seven days. Note vacuoles scattered throughout the media, loss of fine elastic fibers of the media, and fragmentation of the internal elastic membrane. X 500. Alcian blue-PAS.

Angiotensin Effects on Other Vessels
Examination of the vessels of the brain, heart, aorta, liver, and spleen of animals given angiotensin in pressor doses for two-week periods failed to show the degenerative vascular lesions seen in the intrarenal vessels.

Effects of Epinephrine
Nineteen animals were given 20 μg. epinephrine three times a day. Groups of two to four were sacrificed at two or three-day intervals over a period of 14 days. There was no obvious change in the JG cells or their degree of granulation. The JG index range was 15 to 21. The intrarenal vessels did not show the degenerative changes that were seen in the animals given angiotensin over the same period of time.

Discussion
In these studies, it is seen that subcutaneous

*It is interesting to recall that Goormaghtigh observed, after renal artery constriction in the dog and rabbit, that the lumen of the afferent arteriole is often narrowed when the JG cells become enlarged."

Circulation Research, Volume XI, December 1968
ANGIOTENSIN AND KIDNEY

injections of pressor doses of angiotensin cause at first an increase in the number and size of the JG cells and their PAS-staining granules; after a week of these injections, the granules decrease in number, but the cells retain their swollen appearance. The renin content of the rat kidney has been shown to parallel the JG index, so that one may tentatively conclude that during the first week of its administration angiotensin increases the renin content of the kidney and, as in a feedback mechanism, appears to promote its own increased formation.

Tobian et al. have shown that the JG index in the rat varies inversely with the kidney perfusion pressure. If angiotensin causes sufficient constriction in vessels proximal to the afferent arterioles, it might lower the pressure in these vessels even while raising the pressure elsewhere and perhaps in this way cause the increase in the JG index.

Repeated subcutaneous injections of angiotensin (but not epinephrine) caused progressive degenerative lesions in the vessels proximal to the afferent arterioles. If these vessels are those undergoing vasoconstriction, perhaps the lesions are the result of the repeated stimulation by angiotensin, or possibly the lesions result from the combined effects of vasoconstriction and heightened systemic pressure.

Assali and Westersten and McGiff and Aviado have shown that angiotensin has a preferential constrictor action on the renal vessels. Possibly this preferential effect of angiotensin on the renal vessels accounts for the presence of vascular lesions in the kidney and their absence in other organs.

Since angiotensin stimulates the secretion of aldosterone and increases sodium retention, the secondary decrease in PAS granules during the course of angiotensin administration may result from one or both of these effects. Hartroft and Hartroft showed that a high sodium diet caused a decrease in the JG index, and we have found that by giving aldosterone together with angiotensin, the usual increase in the JG index that follows angiotensin injections does not take place.

While epinephrine increases blood pressure and produces renal vasoconstriction, it causes neither the JG changes nor the vascular changes seen following angiotensin administration. It may be that these changes did not occur because epinephrine does not cause the same degree of vasoconstriction in the vessels proximal to the afferent arterioles as does angiotensin. The histological lesions and the marked vasoconstriction may both be the consequence of a unique sensitivity of the renal vessels for angiotensin.

The renal vascular lesions that follow re-
peated injections of angiotensin resemble those found by Wilson and Byrom to develop in the unclamped kidney of rats made hypertensive by unilateral renal artery clamping. The present study suggests that the renal vascular lesions in the hypertensive rat could result from the effects on the renal vessels of increased angiotensin produced by the clamped kidney—and the heightened pressure.

It is doubtful that the vascular lesions are due to the effects of a secondary increase in aldosterone secretion alone. Gornall, Grundy, and Koladich produced hypertension in rats by giving them aldosterone and saline over a three-month period; yet the animals failed to develop renal-vascular lesions.

Summary

Angiotensin II, given subcutaneously in pressor doses to rats three times a day, causes a prompt increase in the size and granulation of the juxtaglomerular cells. After one week of angiotensin injections, there is some loss of granules from these cells. Concurrently, degenerative changes develop in the intrarenal arteries. These changes may result from vasoconstriction in the vessels proximal to the afferent arterioles.

Epinephrine given in an amount similar in pressor effect to angiotensin II did not produce these changes. Vascular lesions were not seen in the heart, lung, brain, aorta, liver, and spleen of animals given angiotensin over the two-week injection period. This study provides additional evidence that the vessels of the kidney are uniquely sensitive to angiotensin.

References

Effect of Angiotensin on Juxtaglomerular Cells and Vessels of the Kidney
Yale J. Katz, Paul R. Patek and Sol Bernick

Circ Res. 1962;11:955-960
doi: 10.1161/01.RES.11.6.955
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/11/6/955

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/