Juxtaglomerular Cell Changes
in Dogs with Antirenin Titers

By Herman E. Schmid, Jr., M.D., and Leonard A. Graham, Ph.D.

With the technical assistance of Mrs. D. M. Schmid

Increasing evidence has accumulated to support the juxtaglomerular cell rather than the proximal tubular cell as being the site of renin formation in the kidney. The determination of the site of renin is important to the elucidation of the mechanism of stimulation of the renin-angiotensin pressor system. Goormaghtigh first demonstrated changes in the juxtaglomerular cell which indicated a secretory function and this led him to hypothesize this cell as the source of renin. More recent microdissection and fluorescent antibody studies are in accord with Goormaghtigh's findings.

Parallel changes in juxtaglomerular cell granularity and renal renin content have been found following procedures which affect renal hemodynamics or electrolyte metabolism. The majority of these studies were conducted in the rat, which advantageously has, under normal conditions, a well-defined juxtaglomerular cell with granules. These studies have been limited by relatively small changes in renal renin concentrations between the control and experimental kidneys.

This report studies changes in juxtaglomerular cells of dog kidney over a wide range of renal renin concentrations produced by the formation of antirenin. The renin content of kidney is significantly increased above control levels in the presence of antirenin which is capable of neutralizing the endogenous renin of the dog.

Methods

Specimens of dog kidney used in this histological study were selected from a group of dogs used in an earlier study of the relationship between antirenin titer and renal renin content. Specimens were selected on the basis of renal renin content to provide a wide range of values from 1 to 22 Goldblatt Dog Units for special staining of the juxtaglomerular cell. All dogs had been subjected to bilateral renal artery constriction by the Goldblatt technique in an amount known to produce a sustained experimental renal hypertension.

Dogs 1 to 3 (table 1) received injections of saline following the production of experimental renal hypertension and served as control specimens. Dogs 4 to 8 received injections of hog renin, six days per week, after the production of hypertension and were sacrificed at different levels of antirenin titer. Dogs 9 to 11 received injections of hog renin and formed antirenin before renal artery constriction and were sacrificed one month after application of the second Goldblatt clamp. Injections of hog renin and control saline solution were continued until the day of sacrifice. Blood pressures were followed weekly by direct femoral artery puncture.

Renal renin and antirenin concentrations were determined by bioassay according to a standard procedure in nephrectomized dogs and reported in Goldblatt Dog Units of renin (DU) per Gm. kidney tissue and Antirenin Units (AU) per cc. plasma. One Antirenin Unit, by definition, is that amount of antibody capable of neutralizing the pressor effect of one Dog Unit of renin. Both the renin and antirenin values reported in table 1 have been rounded off to the nearest whole number.

Thin slices of right kidney were placed, at the time of sacrifice, into 10 per cent formalin and the remaining kidney tissue processed for renin content. Tissue sections were stained by both the Bowie method as described by Pitcock and Hartroft, and the periodic acid technique (PAS) as modified by Hotchkiss. Changes in juxtaglomerular cells were evaluated by assigning a plus 1 to

*Goldblatt Dog Unit of renin (DU): That amount of renin which will give a 30 to 35 mm. Hg rise in mean arterial pressure on intravenous injection into a trained, unanesthetized dog.
TABLE 1

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Treatment</th>
<th>Blood pressure (mm. Hg) (mean ± S.D.)</th>
<th>Period postconstriction (months)</th>
<th>Antigen (AU/cc)</th>
<th>Renal renin (DU/Gm. kidney)</th>
<th>JGA rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>165 ± 13.2</td>
<td>37</td>
<td>0</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>Saline</td>
<td>161 ± 10.4</td>
<td>70</td>
<td>0</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>Saline</td>
<td>139 ± 7.9</td>
<td>48</td>
<td>0</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>Hog renin</td>
<td>116 ± 6.7</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>5</td>
<td>Hog renin</td>
<td>106 ± 10.7</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>6</td>
<td>Hog renin</td>
<td>147 ± 12.7</td>
<td>15</td>
<td>20</td>
<td>8</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>Hog renin</td>
<td>111 ± 8.5</td>
<td>26</td>
<td>21</td>
<td>11</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>Hog renin</td>
<td>147 ± 5.7</td>
<td>8</td>
<td>16</td>
<td>12</td>
<td>2.7</td>
</tr>
<tr>
<td>9</td>
<td>Hog renin</td>
<td>99 ± 11.3</td>
<td>1</td>
<td>66</td>
<td>14</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>Hog renin</td>
<td>121 ± 13.5</td>
<td>1</td>
<td>33</td>
<td>19</td>
<td>3.7</td>
</tr>
<tr>
<td>11</td>
<td>Hog renin</td>
<td>116 ± 6.2</td>
<td>1</td>
<td>58</td>
<td>22</td>
<td>3.5</td>
</tr>
</tbody>
</table>

plus 5 rating to each of 50 glomeruli sectioned through their vascular poles and observed consecutively under high dry magnification. The average of the individual values was reported as the juxtaglomerular rating in table 1. The following criteria were used to determine individual values:

Plus 1: A normal-appearing vascular pole area with smooth muscle cells predominating in the walls of the arteriole without marked hyperplasia or increased density of stain (fig. 1A).

Plus 2: An enlargement and increased stainability of the vascular pole area with definite hyperplasia of the medial cells of the arteriole and without any cytoplasmic granules (fig. 1B).

Plus 3: In addition to the hyperplasia of the medial cells, the appearance of clearly discernible cytoplasmic granules in the juxtaglomerular cells at one or two locations in the arteriole at the vascular pole (fig. 1C).

Plus 4: Numerous granules in juxtaglomerular cells at several locations at the vascular pole of the glomerulus (fig. 1D).

Plus 5: Granules heavily scattered or clumped in approximately one-half or more of the juxtaglomerular cells (fig. 1E and F).

Results

Sections of kidney from renal artery-constricted dogs with serum-antirenin titers demonstrated cellular hyperplasia of the vascular pole area of the glomeruli, associated with the appearance of granulated juxtaglomerular cells. These changes, as evaluated by the juxtaglomerular rating described under Methods, closely paralleled the increased renin content of these kidneys (table 1). The relationship between antirenin titer and renal renin content is also apparent in table 1 and has been reported in detail earlier.6

The saline-injected control dogs (table 1, nos. 1 to 3) had little hyperplasia of the vascular pole (fig. 1A) and only seldom was a well-defined juxtaglomerular cell present. In contrast, specimens from dogs with increased renal renin content demonstrated increasing numbers of juxtaglomerular cells and granules (fig. 1B to E). The cells of the macula densa appeared hypertrophied in several of the specimens with high renin content, but the cytoplasm remained free of stain. The macula densa was found in most instances to be associated with the most heavily granulated region of the juxtaglomerular cell complex, but granules were also usually present at other locations. Apparently, under the conditions of this experiment and associated with a marked increase in renal renin content, juxtaglomerular cells with granules were found associated with both the afferent and efferent arterioles of the glomerulus (fig. 1F).

Blood pressure levels were consistent with previously reported findings.6 Control dogs (table 1, nos. 1 to 3) had pressure elevations, secondary to bilateral renal artery constriction, in the hypertensive range at the time of nephrectomy. Dogs (nos. 4 to 8), injected with hog renin after the establishment of experimental renal hypertension, demonstrated various levels of blood pressure at sacrifice, which
Wakerlin and associates\textsuperscript{11} have shown to be inversely related to the antirenin titer. Dogs 9 to 11, injected with hog renin and forming antirenin before bilateral renal artery constriction, apparently were protected against an elevation in systemic blood pressure.

**Discussion**

The parallel relationship found between juxtaglomerular cell changes and renin in the renal artery-constricted dog kidney confirms the earlier findings of Marshall and Wakerlin\textsuperscript{14} and provides additional support to the hypothesis of Goormaghtigh\textsuperscript{2} that the juxtaglomerular cell is the site of renin formation.

As noted in table 1, the juxtaglomerular rating used in this study to evaluate changes at the vascular pole of the glomerulus was found to plateau, while the renal renin continued to increase in a linear manner. This most likely reflects an error inherent in this method of evaluation. It is difficult to measure changes in a three-dimensional structure by means of a two-dimensional observation. \textcite{Hartroft 16} attempted to circumvent this problem by means of the juxtaglomerular-index method of evaluation. The juxtaglomerular index, however, is most useful for animals that demonstrate, under control conditions, a well-defined juxtaglomerular complex with granules. This is not the case with the dog.

No attempt was made to study enzyme changes of the macula densa. \textcite{Others 18} have reported changes in the concentration of glucose-6-phosphate dehydrogenase after experimental procedures which affected the renin content.

The juxtaglomerular cell changes and increased renal renin could theoretically be secondary to a reduction in renal artery pressure distal to the constricting clamp. The effect of antirenin on experimental renal hypertension could further reduce this pressure. Although changes in the juxtaglomerular cell have not been studied in normotensive dogs with titers, a similar relationship between antirenin and increased renal renin has been found to exist in dog and rabbit.\textsuperscript{8, 9}

In a recent review, Tobian\textsuperscript{17} presented several interesting speculations regarding the interrelationship between electrolytes, pressure, juxtaglomerular cells, and renin content. In general, experimental procedures which altered systemic and renal hemodynamics were associated with parallel changes in juxtaglomerular cell granularity and renal renin concentration. Goormaghtigh originally suggested a possible regulatory action of this cell on renal arteriolar tone. Possibly, the juxtaglomerular cell responds to changes in glomerular filtration pressure with a release of renin which could act to alter postglomerular resistance in the kidney. This release by means of a pressure feedback system would serve to regulate glomerular filtration pressure.\textsuperscript{18} The blocking of such a mechanism by antirenin might account for the marked juxtaglomerular cell changes which represent a compensatory response.

The production of increased renal renin and the accompanying juxtaglomerular cell changes might serve as a useful laboratory model in the dog for further study and evaluation of factors related to renin formation.
Summary

Renin concentration and juxtaglomerular cell changes, as demonstrated by the Bowie and periodic acid stains, were studied in 11 dogs with partial renal artery constrictions of 1 to 70 months' duration. Three received injections of saline, had renal renin contents averaging 2 Goldblatt Dog Units per Gm. kidney tissue, and had juxtaglomerular cell ratings averaging 1.5, as evaluated on a plus 1 to plus 5 basis. Eight dogs injected with hog renin developed antirenin titers ranging from 1 to 83 per cc. plasma, had an increase in renal renin content ranging from 3 to 22 Dog Units, and had a juxtaglomerular cell rating ranging from 1.7 to 4.0. The juxtaglomerular cells in the high-renin specimens were markedly hypertrophied and densely packed with cytoplasmic granules. Also, juxtaglomerular cells with granules were found associated with both the afferent and efferent vessels at the vascular pole of the glomerulus. This study supports the concept that the juxtaglomerular cell is the site of renin formation.

Acknowledgment

The authors wish to express their appreciation to Dr. H. E. Catechopol and Dr. G. E. Wakerlin for their assistance and helpful suggestions.

References

Juxtaglomerular Cell Changes in Dogs with Antirenin Titers
Herman E. Schmid, Jr. and Leonard A. Graham

Circ Res. 1962;11:853-856
doi: 10.1161/01.RES.11.5.853

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/11/5/853

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/