Mechanisms by Which Nephrectomy Stimulates Adrenal Renin

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SUMMARY Renin has been identified in the adrenal gland by several investigators. Nephrectomy is the most potent stimulator of adrenal renin, and in the present study we investigated the mechanism by which nephrectomy stimulates adrenal renin. The pituitary plays a permissive role since hypophysectomy abolished the response of adrenal renin to nephrectomy (from 117.3 ± 14.55 to 10.37 ± 1.63 ng angiotensin I/mg protein/hr) and adrenocorticotropic hormone (ACTH) treatment restored the response to nephrectomy in hypophysectomized rats to 120.26 ± 20.62 ng angiotensin I/mg protein/hr. However, large doses of ACTH given to intact rats did not increase adrenal renin to the high level observed after nephrectomy. Potassium also plays an important role, since prevention of hyperkalemia after nephrectomy by treatment with a cation exchange resin, sodium polystyrene sulfonate (Kayexalate), significantly reduced the adrenal renin response to nephrectomy. A third factor involved is the lack of negative feedback by plasma angiotensin II. Infusion of angiotensin II intraperitoneally prevented the rise in adrenal renin after nephrectomy (from 65.25 ± 7.60 to 9.27 ± 0.99 ng angiotensin I/mg protein/hr) despite an increase in plasma potassium and corticosterone. In conclusion, three factors influence the response of adrenal renin to nephrectomy: 1) the pituitary through the release of ACTH, 2) a direct stimulation by high plasma potassium levels, 3) the lack of angiotensin II feedback inhibition. Whether the high adrenal renin contributes to the high aldosterone observed in rats after nephrectomy remains to be established. (Hypertension 8: 997-1002, 1986)

KEY WORDS • extrarenal renin • adrenal renin • angiotensin II • potassium • adrenocorticotropic hormone • hypophysectomy

RENIN isoenzymes have been found in tissues as varied as salivary glands,1,2 uterus,3,4 brain,5,6 and blood vessel walls.7-11 In 1967, Ryan12 identified the presence of a reninlike enzyme in the adrenal glands of rabbits. Since then, the presence of active renin in adrenal glands has been reported in various species by other investigators.13-15 Naruse and Inagami16 demonstrated the presence of active renin in the adrenal gland in spontaneously hypertensive rats and Wistar-Kyoto rats. The adrenal renin level was higher in spontaneously hypertensive rats and increased markedly after nephrectomy. This adrenal enzyme was inactivated by specific renin antibodies.17 Previously, our laboratory also showed that adrenal renin is primarily located in the zona glomerulosa cells and that adrenal renin increases after low sodium or high potassium diet and after nephrectomy. There is a positive correlation between adrenal renin and aldosterone, suggesting that adrenal renin may be a local hormone involved in the regulation of aldosterone production.18-20 The presence of immunoreactive angiotensin II in rat adrenal capsular portion21-23 is suggestive of the existence of a local renin-angiotensin-aldosterone system in the adrenal glomerulosa cells.

Nephrectomy is the most potent stimulator of adrenal renin, resulting in a 10- to 20-fold increase in renin activity in the glomerulosa cells after 20 hours. Our previous study found little detectable renin activity in the fasciculata medullary cells, and this concentration did not change after nephrectomy.24 25 In the present study we investigated the mechanisms by which nephrectomy stimulates adrenal renin. Since circulating corticosterone levels are elevated after bilateral nephrectomy,26 we investigated the role of adrenocorticotropic hormone (ACTH) in the adrenal renin response to nephrectomy. Next, we studied the effect of potassium, since the serum potassium rises significantly after nephrectomy and we have shown previously that potassium loading for 5 days markedly stimulated adrenal zona glomerulosa renin concentration.27,28 Since angiotensin II has a strong negative feedback on
kidney renin, we also studied whether the lack of angiotensin II feedback could play a role in the adrenal renin response to nephrectomy.

Materials and Methods

Female Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 190 to 210 g were used for the experiments. The rats were maintained on a regular Purina chow diet (St. Louis, MO, USA). Bilateral nephrectomy was performed with the rats under pentobarbital anesthesia (30 mg/kg) 20 hours before they were killed. The conscious rats were quickly killed by decapitation. In some rats, the blood was sampled by heart puncture under ether anesthesia for measurement of plasma potassium and corticosterone, and then the rats were killed by decapitation. Blood was removed from the adrenals by flushing with normal saline through the thoracic aorta. The adrenals were then removed, and the capsules were separated from the fasciculata medulla by a previously described technique. In previous experiments using this technique, we showed that the degree of contamination of capsular cells with fasciculata medullary cells was approximately 5%. The separated portions were homogenized with 0.1 M tris(hydroxymethyl)aminomethane (Tris) acetate buffer, pH 7.4, at 4°C for 30 seconds with a Teflon-glass homogenizer and then centrifuged for 30 minutes at 1800 g at 4°C. The supernatant was aspirated, and renin activity was measured by radioimmunoassay using a Becton-Dickinson kit (Orangeburg, NY, USA). In brief, the supernatant (50 μl) was incubated with 5 μl of 8-hydroxyquinoline, 5 μl of dimercaprol, 25 μl of 4% ethylenediaminetetraacetic acid, and 90 μl of Tris-lysozyme acetate buffer (pH 7.4) using 25 μl of nephrectomized rat plasma as substrate, as previously described. The amount of substrate used during the incubation was less than 3%. After incubation for 1 hour at 37°C, a 50-μl aliquot was taken for radioimmunoassay of generated angiotensin I. Protein concentration of the extract was measured by the method of Lowry et al. Plasma corticosterone was measured after extraction with dichloromethane using a highly specific antibody obtained from Endocrine Science (Tarfzana, CA, USA) by a previously described method. Plasma potassium was measured by flame photometer.

Anti–hog kidney renin monoclonal antibody was kindly donated by Dr. L. T. Skeggs of the Department of Biochemistry, Case Western Reserve University (Cleveland, OH, USA). This antibody completely inhibited renin activity in a rat kidney extract at a dilution of 1:100, and a 1:1000 dilution caused a 50% inhibition of renin activity. For the antirenin assay, 100 μl of the adrenal capsular extract of rat was incubated with 100 μl (1:100 dilution) of the monoclonal antirenin antibody or 100 μl of Tris acetate buffer for 15 minutes at 37°C at pH 7.4. Then, a 50-μl aliquot was taken, and renin activity was measured at pH 7.4 in Tris acetate buffer using nephrectomized rat plasma as substrate, as already described.

The ACTH (H.P. Acthar Gel, Armour Pharmaceutical Company, Phoenix, AZ, USA) was injected subcutaneously at a dose of 1, 5, and 10 U/day for 2 or 4 days into intact rats. Control rats were injected subcutaneously with an equal volume of normal saline. Hypophysectomized rats were treated with ACTH at a dose of 10 U/day per rat for 2 days before the experiment. The effect of hypophysectomy on the adrenal renin response to nephrectomy also was investigated. Nephrectomy was performed 4 days after hypophysectomy. A group of hypophysectomized rats was treated with ACTH subcutaneously at a dose of 10 U/day per rat for 2 days before nephrectomy.

Rats were bilaterally nephrectomized under pentobarbital anesthesia (30 mg/kg) and received the cation exchange resin sodium polystyrene sulfonate (Kayexalate; Breon Laboratory, New York, NY, USA) at a dose of 0.25, 1.0, and 2.0 g/kg by gastric gavage immediately after nephrectomy and every 9 hours thereafter for a total of three doses. Control rats with sham operation (flank incision with kidneys left intact) and nephrectomized rats without treatment received the same amount of distilled water. The blood was sampled by heart puncture with the rats under ether anesthesia for measurement of plasma potassium 20 hours after nephrectomy, and the rats then were killed by decapitation.

To study the effect of angiotensin II infusions on the adrenal renin response to nephrectomy, a group of rats was bilaterally nephrectomized under pentobarbital anesthesia (30 mg/kg) and then immediately infused intraperitoneal with synthetic angiotensin II (Sigma Chemical, St. Louis, MO, USA) at a rate of 200 ng/min through an osmotic minipump (Alza Corp., Palo Alto, CA, USA). Before implantation the minipumps were placed in a 37°C water bath for 7 hours to remove the dead space. Sham-operated rats and nephrectomized rats also were infused with normal saline through the osmotic minipumps. Twenty hours after the operation, the blood was sampled by heart puncture with the rats under ether anesthesia for measurement of plasma potassium and corticosterone, and the rats then were killed by decapitation.

The results of the experiments are expressed as the mean ± SE. Each adrenal was studied separately. Statistical analysis were made using the Student's unpaired t test, analysis of variance, and Scheffe's F test for multiple-range analysis, and significance was defined as a p value of less than 0.05.

Results

Nephrectomy had a striking effect on the renin concentration of the adrenal capsular or glomerulosa zone: the concentration increased from 4.34 ± 1.49 before nephrectomy to 71.5 ± 10.6 ng angiotensin I/mg pro-
tein/hour 20 hours after nephrectomy. The activity of these adrenal capsular renins in rats before and after nephrectomy was inhibited more than 95% by the specific anti-hog kidney renin monoclonal antibody (data not shown).

Figure 1 shows the effect of ACTH treatment for 2 and 4 days in intact rats. The adrenals were considerably enlarged by this treatment, and the adrenal capsular renin also increased significantly even at a dose of 1 U/day of ACTH for both 2 and 4 days. However, despite a large dose of ACTH for 2 or 4 days, the adrenal capsular renin levels could not be stimulated up to the level observed in nephrectomized rats.

To further study the possible role of ACTH, we investigated the effect of hypophysectomy on adrenal capsular renin. Figure 2 shows the effect of hypophysectomy or ACTH on adrenal capsular renin in intact rats. The adrenals were quite atrophied by hypophysectomy (from 27.9 to 15.4 mg per adrenal); however, there was no significant difference in adrenal capsular renin between control and hypophysectomized rats. When a large dose of ACTH was administered to hypophysectomized rats, the adrenal capsular renin levels increased significantly (control, 4.34 ± 1.53; hypophysectomy, 6.95 ± 1.06; hypophysectomy + ACTH, 40.54 ± 6.78 ng angiotensin I/mg protein/hr).

Figure 3 shows the effect of hypophysectomy or ACTH on the adrenal capsular renin response to nephrectomy. When the rats were hypophysectomized 4 days before nephrectomy, hypophysectomy prevented the response of adrenal capsular renin to nephrectomy (from 117.3 ± 14.55 after nephrectomy to 10.37 ± 1.63 ng angiotensin I/mg protein/hr after nephrectomy and hypophysectomy). However, when hypophysectomized nephrectomized rats were treated with a large dose of ACTH, the response of adrenal capsular renin reached the levels observed after nephrectomy in normal rats (i.e., increased to 120.26 ± 20.62 ng angiotensin I/mg protein/hr).

Figure 4 shows the effect of reduction in serum potassium on the response of adrenal capsular renin to nephrectomy. The sham-operated rats had a serum potassium of 4.45 ± 0.14 mEq/L, and 20 hours after nephrectomy, rats had a serum potassium of 6.90 ± 0.18 mEq/L. When the rise in serum potassium was prevented by Kayexalate, the concentration of adrenal capsular renin was reduced in a dose-dependent fashion, and normalization of serum potassium by the largest dose of Kayexalate abolished the adrenal renin response to nephrectomy.

Figure 5 shows the effect of angiotensin II on the response of adrenal capsular renin to nephrectomy. Adrenal capsular renin was significantly elevated 20
FIGURE 4. Effect of reduction in serum potassium on the response of adrenal capsular renin to nephrectomy (Nepex). Bar represents the mean ± SE. Prevention of hyperkalemia after nephrectomy by Kayexalate (Kayex) treatment significantly reduced the adrenal renin response to nephrectomy. AI = angiotensin I.

hours after nephrectomy. However, when a large dose of angiotensin II was infused immediately after nephrectomy, the response of adrenal capsular renin to nephrectomy was abolished (i.e., decreased from 65.25 ± 7.60 after nephrectomy to 9.27 ± 0.99 ng angiotensin I/mg protein/hr after nephrectomy + angiotensin II). Table 1 shows the levels of plasma corticosterone and potassium in the control rats and in nephrectomized rats with and without angiotensin II infusion. Plasma corticosterone was significantly increased after nephrectomy from 1.4 ± 0.12 to 28.58 ± 2.71 μg/dl (p < 0.001), and treatment with angiotensin II further increased plasma corticosterone levels to 39.77 ± 2.16 μg/dl (p < 0.01), suggesting release of ACTH by angiotensin II. Plasma potassium was also markedly increased after nephrectomy but did not change after the treatment with angiotensin II.

Discussion

In 1967, Ryan20 reported the presence of a reninlike enzyme in the adrenal glands of rabbits. Since then adrenal renin has been studied by several investigators.13'21'29 Recently, Naruse and Inagami and colleagues26' further characterized this enzyme and showed that it was inactivated by a specific antirenin antibody. In this study, we incubated the enzyme at pH 7.4 to avoid any contamination with cathepsinlike enzymes.29'43 Furthermore, this enzyme could be inhibited more than 95% by a specific anti-hog kidney renin monoclonal antibody both before and after nephrectomy, indicating that the measured enzymatic activity is immunologically related to renin and can be differentiated from cathepsinlike enzyme.

The location of reninlike enzyme in the adrenal gland has been studied by several investigators.21'26'46 The main site of origin of adrenal renin has been reported to be the adrenal cortex in the rat.23'24 Our laboratory also showed that renin concentration of dispersed glomerulosa cells of sodium-deficient or nephrectomized rats is significantly higher than that of the fasciculata medullary cells.30'31 These data suggest that the main site of origin of adrenal renin is the glomerulosa cell. Recently, Naruse et al.34 demonstrated the localization of renin in the inner cortical region of mouse adrenal gland by immunohistochemical techniques. In that study, however, the biochemical determination of reninlike enzyme in the separated portions of the cortex and the medulla was not performed. The different localization of adrenal renin in the mouse

### TABLE 1. Effect of Angiotensin II on Adrenal Renin, Plasma Corticosterone, and Serum Potassium in Nephrectomized Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 6)</th>
<th>Nepex (n = 6)</th>
<th>Nepex + ANG II (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal renin (ng ANG I/mg protein/hr)</td>
<td>12.59 ± 0.90</td>
<td>58.1 ± 8.78*</td>
<td>9.29 ± 1.03†</td>
</tr>
<tr>
<td>Plasma corticosterone (μg/dl)</td>
<td>1.4 ± 0.12</td>
<td>28.58 ± 2.71*</td>
<td>39.77 ± 2.16‡</td>
</tr>
<tr>
<td>Plasma potassium (mEq/L)</td>
<td>4.1 ± 0.21</td>
<td>7.52 ± 0.38*</td>
<td>7.41 ± 0.22</td>
</tr>
</tbody>
</table>

Results are means ± SE. Plasma corticosterone increased significantly after nephrectomy, and angiotensin II further increased plasma corticosterone levels. Serum potassium did not change significantly after angiotensin II infusion in nephrectomized rats. Nepex = nephrectomy; ANG II = angiotensin II.

* p < 0.001, compared with values in control rats; † p < 0.001, ‡ p < 0.01, compared with values in nephrectomized rats.
may be due to species difference or to the limited sensitivity of the histochemical technique.

The effect of nephrectomy on adrenal renin has been studied by Naruse and Inagami. They showed a markedly elevated specific renin level in the adrenal glands of genetically hypertensive rats after nephrectomy. Our laboratory showed that adrenal renin increases after low sodium or high potassium and after nephrectomy. A most interesting finding was a strong positive correlation between the adrenal renin concentration and adrenal aldosterone concentration. Nephrectomy was the most potent stimulator of adrenal renin, resulting in a 10- to 20-fold increase by 20 hours. Our laboratory has previously shown that dexamethasone treatment before nephrectomy partially blunts the rise in adrenal renin, suggesting a role for the pituitary in the response of adrenal renin to nephrectomy. In 1975, however, Ganten et al. showed that hypophysectomy increases adrenal iso-renin concentration within 1 week, and recently, Naruse et al. also suggested that adrenal renin concentration may be independent of the pituitary.

In the present study, hypophysectomy abolished the response of adrenal renin to nephrectomy and ACTH treatment restored the response to nephrectomy in hypophysectomized rats. Our results indicate that the marked elevation in adrenal renin after nephrectomy may partly be due to the increase of ACTH in response to the stress of nephrectomy. However, this effect of ACTH appears to be a permissive action, since administration of large doses of ACTH to intact rats for several days did not increase adrenal renin concentration to the high level observed after nephrectomy.

A high potassium diet has been shown to increase plasma aldosterone and decrease plasma renin activity. Our laboratory has previously shown that potassium loading for 5 days markedly stimulates adrenal renin. In our study, prevention of hyperkalemia after nephrectomy abolished the increase in adrenal renin in response to nephrectomy. These data indicate that potassium is another important stimulus responsible for the rise of adrenal renin after nephrectomy.

Recently, the presence of immunoreactive angiotensin in the rat adrenal capsule has been demonstrated by Aguilera et al. and Mendelson. Aguilera et al. showed that adrenal capsular angiotensin II was not decreased after nephrectomy, but that infusion of angiotensin II decreased adrenal angiotensin II after nephrectomy. These data are suggestive of the presence of a local renin-angiotensin-aldosterone system in the adrenal capsular portion. Angiotensin II has a strong negative feedback effect on kidney renin. In our study, angiotensin II treatment abolished the adrenal renin response to nephrectomy. Plasma corticosterone was increased by the infusion of angiotensin II after nephrectomy, suggesting the release of ACTH by angiotensin II. Plasma potassium also increased in nephrectomized rats after infusion of angiotensin II. These data indicate that prevention of the adrenal renin response to nephrectomy by infusion of angiotensin II has no relation to ACTH or potassium and may be due to a negative feedback mechanism of angiotensin II on adrenal renin similar to its effect on kidney renin. Therefore, the lack of angiotensin II feedback inhibition may play a role in the adrenal renin response to nephrectomy.

In conclusion, three factors influence the response of adrenal capsular renin to nephrectomy: 1) the pituitary through the release of ACTH, 2) a direct stimulation by high plasma potassium, and 3) the lack of angiotensin II feedback inhibition. Whether this high adrenal renin concentration contributes to the high aldosterone concentration observed in rats after nephrectomy remains to be established.

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