

Mechanism of Adrenal Angiotensin II Receptor Changes after Nephrectomy in Rats

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ABSTRACT At 48 h after bilateral nephrectomy in rats there is a two- to threefold increase in the number of adrenal angiotensin II receptors and a decrease in K_d of smooth muscle angiotensin II receptors. These changes have been attributed to the absence of circulating angiotensin II. Serum K^+ , which increases after nephrectomy may be an important and overlooked modulator. Therefore, the present experiments were designed to assess the role of K^+ as a regulator of angiotensin II receptors after nephrectomy. Serum K^+ was controlled with Na polystyrene sulfonate (Kayexalate), a resin designed to exchange Na^+ for K^+ in the gastrointestinal tract. Acutely nephrectomized rats were divided into two groups: experimental animals received Kayexalate resin every 12 h for four doses, and controls received Kayexalate exchanged with KCl in vitro before gavage. There was a significant positive correlation serum K^+ and aldosterone ($r = 0.78$, $P < 0.001$). Kayexalate maintained a normal serum K^+ of 5.9 ± 0.2 meq/liter ($n = 27$), aldosterone 25 ± 3 ng/dl ($n = 27$) and adrenal receptor concentration of 934 ± 156 fmol/mg protein ($n = 4$). Control animals had significantly higher serum K^+ of 10.5 ± 0.4 meq/liter ($n = 23$), aldosterone 435 ± 32 ($n = 23$), and adrenal receptors of 2726 ± 235 fmol/mg protein ($n = 4$). There was a linear relationship between serum K^+ and number of adrenal receptors ($r = 0.87$). No such relationship was present in uterine smooth muscle. Therefore, these studies demonstrate that K^+ modulates the number of adrenal but not smooth muscle angiotensin II receptors after nephrectomy. This is the first evidence that potassium modulates angiotensin II receptors independently of changes in angiotensin II blood levels.

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INTRODUCTION

An increment in angiotensin II receptors is present in rats at 48 h after bilateral nephrectomy (1, 2). These changes have been demonstrated in both adrenal glomerulosa and uterine smooth muscle and have been presumed to occur secondary to the elimination of renin and angiotensin II from the circulation. However, these receptor changes are atypical of what has been demonstrated to accompany other means of manipulating angiotensin II blood levels. Specifically, low sodium intake (which stimulates renin and angiotensin II) and angiotensin II infusion both increase (3–5), whereas high sodium intake decreases the number of adrenal angiotensin II receptors (3). It therefore seems possible that an alternative explanation, such as the change in ionic composition of plasma, might be responsible for the observed receptor changes after nephrectomy. Nephrectomized rats have a rise of serum potassium of 3 meq as early as 10–12 h after nephrectomy (6). In addition, we have suggested that potassium acts directly on the adrenal glomerulosa during dietary K^+ -loading to increase angiotensin II receptors (7). The effects of potassium seemed to occur independently of angiotensin II.

In view of these findings, the present studies were undertaken to evaluate the role of potassium on the control of angiotensin II receptors in rats after bilateral nephrectomy. These studies were also designed to evaluate the relationship between serum electrolytes and aldosterone in rats after nephrectomy.

METHODS

Materials. Synthetic (Asp¹ Ile⁵)-angiotensin II was obtained from Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif. The [¹²⁵I]monoiodo-angiotensin II was prepared by New England Nuclear (Boston, Mass.) with a specific activity of 800–1,000 μ Ci/ μ g. Female Sprague-Dawley rats (225–250 g) were obtained from Zivic Miller, Pittsburgh, Pa.

Experimental protocols. Food was withheld from rats for 12 h before and 48 h after nephrectomy. During this period they received only distilled-deionized water to drink ad lib. One group of nephrectomized rats received 2 g/kg of sodium polystyrene sulfonate (Kayexalate; Winthrop Laboratories, Sterling Drug Co., New York) with 0.7 g/kg of sorbitol and 1 cm³ of magnesium citrate by gavage every 12 h for four doses. The first dose was administered immediately after nephrectomy. Control animals received the same quantity of the resin exchanged in vitro with 167 mg KCl/g of Kayexalate. The resin exchanged in vitro was mixed with a similar amount of sorbitol and magnesium citrate and administered at the same intervals. At death, blood was collected by aortic puncture under ether anesthesia for determination of serum electrolytes by flame photometry and aldosterone by radioimmunoassay (8).

Tissue preparation and receptor binding studies. Experiments were conducted such that receptor binding by tissues from Kayexalate-treated animals was compared with the receptor binding in animals treated with resin exchanged in vitro on the same day. Capsules were stripped from pooled rat adrenals collected in medium 199 at 4°C, homogenized in 20 mM sodium bicarbonate with a Teflon-glass homogenizer, and filtered through nylon gauze. This method has been described elsewhere and verified for use with electrolyte-modified diets (3). Subcellular fractions were collected between 10,000 and 100,000 g for studies of adrenal receptor binding. Smooth muscle receptors were prepared by a modification of the technique of Rouzaire-Dubois et al. (9), described in detail elsewhere (10). Pooled rat uteri were stripped of serosa, minced, homogenized with a Kinematica, PT 10–20 homogenizer (Kinematica Corp., Barrington, Ill.) for 4 s, filtered through nylon gauze and sedimented at 700–20,000 g. The fraction was used for the radioreceptor assay. Incubations for receptor binding were performed in 50 mM Tris HCl (pH 7.4) with 5–15 fmol of ¹²⁵I-angiotensin II, 1% bovine serum albumin, 5 mM dithiothreitol, 120 mM NaCl, and 125–175 µg of smooth muscle or 10–30 µg of adrenal receptor protein in a final vol of 300 µl. Varying concentrations of unlabeled angiotensin II between 10 and 0.1 nM were added to generate full binding-inhibition curves. All binding is reported as specific binding corrected for binding of tracer in the presence of excess unlabeled angiotensin II (0.3 µM). Binding studies were performed at 22°C for 30 min with smooth muscle and 60 min with adrenal tissues. Separation of receptor-bound angiotensin II was achieved by Millipore filtration (Millipore Corp., Bedford, Mass.) using HAWP (0.45 µm) filters. Under these conditions, nonspecific binding represents <10% of the total binding in the adrenal glomerulosa

and <40% of the total binding in smooth muscle. Samples were counted using a Searle gamma-spectrometer with efficiency of 75% (Searle Radiographics Inc., Des Plaines, Ill.). Binding constants were determined by Scatchard analysis (11). Linear regression analysis used to determine binding constants was considered significant if *r* exceeded 0.90. Proteins were determined by the method of Lowry et al. (12) as modified by Cuatrecasas (13). Results are reported as means±SE and statistical significance determined using paired Student's *t* test for comparisons of receptor binding constants and unpaired Student's *t* test for other comparisons (14).

RESULTS

Effect of nephrectomy on angiotensin II receptors. At 48 h after nephrectomy, rats had a significant increase in serum potassium and aldosterone (Table I). After nephrectomy there were 2,341±797 fmol of angiotensin II bound per mg of adrenal protein (*n* = 10) compared to 499±135, *n* = 10 (*P* < 0.05) for control sham-operated animals. In uterine smooth muscle, there was no significant change in the number of receptors. However, there was a slight but significantly lower *K*_d of 1.7±0.3 nM (*n* = 4) as compared to the control value of 2.4±0.3 nM, *n* = 4, (*P* < 0.05).

Cation-exchange resin administration. When acutely nephrectomized rats were administered 2 g/kg of Kayexalate resin by gavage, it was found that serum potassium and aldosterone were at or below normal values. The serum electrolytes and aldosterones of these animals, who were killed 12 h after the last Kayexalate dosage, are summarized in Table II. Animals receiving Kayexalate had significantly higher serum sodium, lower serum potassium, and lower serum aldosterone than control animals that received resin exchanged in vitro. Logarithmic changes in serum aldosterone were significantly correlated with linear changes in serum potassium (Fig. 1). The resultant regression analysis was: $\log y = 0.2x + 0.39$, *r* = 0.78, *P* < 0.001.

Fig. 2 illustrates representative Scatchard plots of

TABLE I
Serum Electrolytes, Aldosterone, and Angiotensin II Receptor Constants at 48 h after Nephrectomy in Rats

	Serum potassium	Serum aldosterone	Adrenal		Smooth muscle	
			Number	<i>K</i> _d	Number	<i>K</i> _d
	meq/liter	ng/100 ml	fmol/mg protein	nM	fmol/mg protein	nM
Sham-operated	5.3±0.3 (23)	53±8.6 (12)	499±135 (10)	1.3±0.1 (10)	55±7 (4)	2.4±0.3 (4)
Nephrectomized	9.3±0.3 (13)	329±30 (16)	2,341±797 (10)	1.6±0.4 (10)	62±5 (4)	1.7±0.3 (4)
<i>P</i> value	<0.001	<0.001	<0.05	NS	NS	<0.05

TABLE II
Kayexalate Effect on Serum Electrolytes and Aldosterone in Nephrectomized Rats

	Serum Na ⁺	Serum K ⁺	Serum aldosterone
	meq/liter	meq/liter	ng/100 ml
Kayexalate (2 g/kg)	138.5±1.8 (28)	5.9±0.2 (27)	25±3 (27)
Kayexalate exchanged in vitro (2 g/kg)	130.5±1.7 (24)	10.5±0.4 (23)	435±32 (23)
P value	<0.005	<0.001	<0.001

adrenal radioreceptor assays of rats given Kayexalate or resin exchanged in vitro. This dosage prevented the increase in adrenal receptor concentration that usually occurred after nephrectomy. The receptor concentration was 680 fmol/mg protein in Kayexalate-treated rats contrasted with 2,327 fmol/mg protein in animals receiving resin exchanged in vitro. K_d were not significantly different.

Table III comprises a summary of results from four experiments. There was a significantly lower number of adrenal receptors in Kayexalate-treated rats as compared to control animals receiving resin exchanged in vitro. There was, however, no significant differ-

ence in K_d of adrenal and smooth muscle receptors or number of smooth muscle receptors. There was a linear relationship between serum K⁺ and adrenal receptor number with both groups of animals combined (Fig. 3). The resultant regression analysis was $y = 0.0023x + 4$, $r = 0.87$, $P < 0.005$. There was no such relationship between the level of serum sodium and number of angiotensin II receptors.

DISCUSSION

These data demonstrate that there is a direct correlation between the level of serum potassium and the number of adrenal angiotensin II receptors in nephrectomized rats. When serum potassium was prevented from increasing through the use of a cation-exchange resin, the number of adrenal receptors remained at a normal concentration. However, control animals whose serum potassiums were allowed to rise by administering resin exchanged in vitro had significantly higher concentrations of angiotensin II receptors than normal.

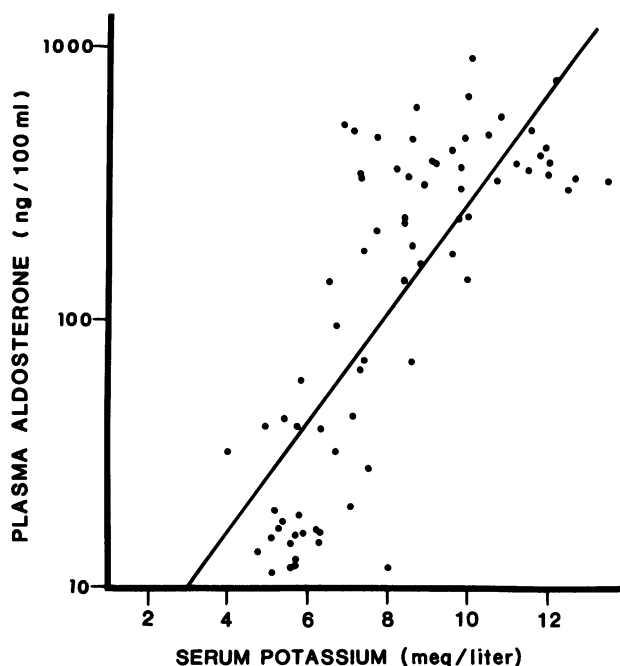


FIGURE 1 Linear regression analysis of semilogarithmic plasma aldosterone and linear serum potassium from nephrectomized rats. Data was collected from two groups of animals: group one received Kayexalate resin exchanged in vitro with KCl (control) and a second group received Kayexalate resin (experimental). $r = 0.78$; $P < 0.001$.

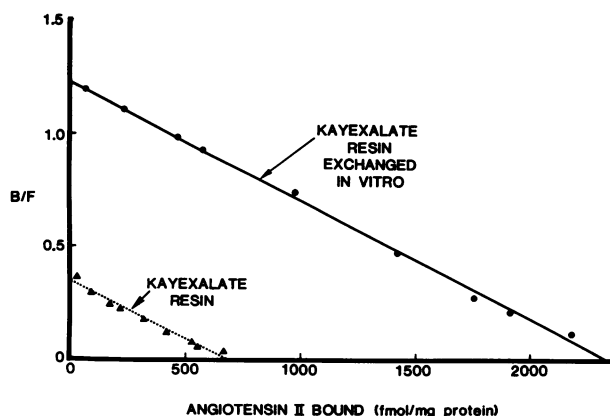


FIGURE 2 Scatchard analysis of adrenal receptor binding of ¹²⁵I-angiotensin II. Values are the mean of triplicate determinations from a single experiment. Adrenal glomerulosa from Kayexalate-treated rats bound 680 fmol/mg protein with a K_d of 0.64 nM compared with 2,327 fmol/mg protein with a K_d of 0.58 from controls treated with preloaded Kayexalate resin.

TABLE III
Kayexalate Effect on Angiotensin II Receptors of Nephrectomized Rats

	Adrenal receptors		Smooth muscle receptors	
	Number	K_d	Number	K_d
	<i>fmol/mg protein</i>	<i>nM</i>	<i>fmol/mg protein</i>	<i>nM</i>
Kayexalate (2 g/kg)	934±156 (4)	0.83±0.07 (4)	95±19 (4)	2.2±0.2 (4)
Kayexalate exchanged in vitro (2 g/kg)	2,726±235 (4)	0.85±0.1 (4)	66±17 (4)	1.7±0.5 (4)
<i>P</i> value	<0.01	NS	NS	NS

The serum potassium level and receptor concentration of this group of animals was not significantly different from that of animals nephrectomized and given nothing. The cation exchange resin employed primarily exchanged sodium for potassium and as a result the serum sodium was significantly higher in the Kayexalate-treated rats as compared with those receiving the resin exchanged in vitro. Therefore, there was a remote possibility that the serum sodium may have in some way contributed to the receptor changes observed. However, no significant relationship was present between serum sodium and number of angiotensin II receptors.

An increment in serum potassium now seems to be the major factor responsible for the increment in adrenal receptors after nephrectomy (1, 2) and with dietary potassium loading (3, 7, 10). In both of these conditions, there was absent or low renin and angio-

tensin II, and similar increments in receptors as occurs with the high angiotensin states of sodium depletion (3) and angiotensin infusion (4). In the former situations, potassium was the glomerulotrophic factor that directly stimulated an increment in angiotensin II receptors, independent of angiotensin II. On the other hand, after sodium depletion or angiotensin II infusion, angiotensin served as the major adrenal stimulus for the increment in receptor sites. The changes in adrenal angiotensin II receptors correlated better with the level of serum aldosterone than with the circulating angiotensin II blood level as is illustrated in Fig. 4. With changes in potassium balance or bilateral nephrectomy there was an inverse correlation between the number of adrenal receptors and the level of circulating angiotensin II. In contrast, with changes in sodium balance or angiotensin II infusion, there was a direct correlation between the number of adrenal receptors and the level of circulating angiotensin II. However, one unifying factor in all six states listed in Fig. 4 is that the level of aldosterone varied directly with the number of adrenal receptors. A direct role for mineralocorticoid in the receptor regulatory process is unlikely because deoxycorticosterone acetate administration to rats in a concentration sufficient to cause suppression of renin and hypertension caused a decrease in the number of adrenal angiotensin II receptors (15).

Smooth muscle receptors differed significantly from adrenal receptors in that no correlation was present between the changes in number or affinity of receptor sites and level of serum potassium. Previous studies have demonstrated that angiotensin serves as a major regulator of the number and affinity of uterine smooth muscle receptors (10). The present studies demonstrated a 30% decrease in the K_d of smooth muscle receptors for angiotensin II after nephrectomy, which correlated with the decrease in one-half the effective dose (ED_{50}) of angiotensin II of 55% in uterine myometrium after nephrectomy (16). This receptor

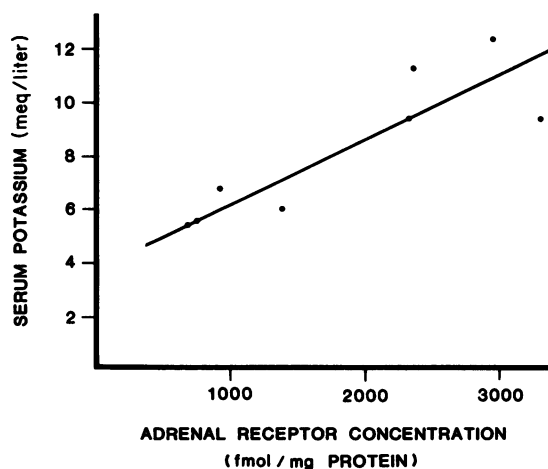


FIGURE 3 Linear regression analysis of serum potassium and adrenal receptor concentration from nephrectomized rats that received either Kayexalate resin exchanged in vitro with KCl (control) or Kayexalate resin (experimental). $r = 0.87$, $P < 0.005$.

	CIRCULATING ANGIOTENSIN II	SERUM K ⁺	SERUM ALDOSTERONE	ADRENAL RECEPTORS
POST-NEPHRECTOMY	↓	↑	↑	↑
POTASSIUM LOADING	↓	↑	↑	↑
POTASSIUM DEPLETION	↑	↓	↓	↓
ANGIOTENSIN II INFUSION	↑	↔	↑	↑
SODIUM DEPLETION	↑	↔	↑	↑
SODIUM LOADING	↓	↔	↓	↓

FIGURE 4 Correlations between changes in circulating angiotensin II, serum potassium, aldosterone, and adrenal receptor concentration in rats.

change could in part explain the long-standing observation of enhanced pressor responsiveness to angiotensin II after nephrectomy (6, 17, 18). Such a comparison between angiotensin II binding properties of uterine smooth muscle and vascular responses to angiotensin II has validity. We have demonstrated excellent correlations between angiotensin II antagonist binding properties in uterine smooth muscle and inhibitory properties in the rat pressor assay and rabbit aortic strip (19). Additionally, alterations in receptor affinity in uterine myometrium accompanying K⁺-loading in rats correlate with changes in in vivo pressor response to angiotensin II in the rat (20).

The present data are consistent with the interpretation that after nephrectomy, the acute increment of serum potassium caused a logarithmic increase in adrenal sensitivity as measured by aldosterone release. The positive correlation between changes in serum potassium and aldosterone ($r = 0.78$) was greater than the negative correlation between sodium and aldosterone ($r = -0.42$). Therefore, a role for sodium could not be substantiated. Previous studies in nephrectomized humans (21–24), and animals (25–28) have demonstrated that the adrenal glomerulosa retains the capacity to respond independently to changes in serum potassium and sodium in spite of the absence of renin and angiotensin II. It is of interest that the serum aldosterone of nephrectomized rats with normal serum potassium (25 ± 3 ng/dl, $n = 27$) was lower than rats with kidneys (53 ± 8.6 ng/dl, $n = 12$). Both groups had similar serum potassiums. This suggests that the renin-angiotensin system, acutely stimulated by anesthesia used for blood collection, in rats with kidneys was the major factor responsible for the difference in the two groups.

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