

Regulation of Angiotensin II Receptors in the Rat Adrenal Cortex by Dietary Electrolytes

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ABSTRACT The binding affinity and concentration of specific angiotensin II receptor sites of rat adrenal cortical cells and homogenates were determined after 1 and 6 wk of altered sodium and potassium intake. Sodium deprivation caused marked increases in plasma renin, blood angiotensin II, and plasma aldosterone, and was accompanied by a significant increase (+74%) in the number of specific angiotensin II receptor sites per adrenal cortical cell. High potassium intake was followed by increased serum potassium and markedly elevated plasma aldosterone, with subnormal levels of renin and angiotensin II and a 170% increase in the number of angiotensin II receptors per cell after 1 wk. Sodium loading and potassium deprivation were followed by the opposite effect upon adrenal receptors, with reduction of the angiotensin II-binding capacity. None of the dietary electrolyte changes were accompanied by an increase in receptor affinity above the control value of 2 nM⁻¹. A decrease in receptor affinity was noted after 6 wk of either low sodium or low potassium intake, when the renin and angiotensin II levels were increased by 104–129%.

The adrenals of normal rats infused acutely with synthetic angiotensin II, or anesthetized with ether or sodium pentobarbital, which markedly increased plasma renin activity, contained fewer angiotensin receptors. These reductions in binding site concentration were not accompanied by changes in affinity and were attributed to occupancy by angiotensin II.

These studies have demonstrated that chronic changes in sodium or potassium balance and acute changes in blood angiotensin II levels can exert modulating effects upon the adrenal content and/or affinity of specific receptor sites for angiotensin II.

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INTRODUCTION

The production of aldosterone by the zona glomerulosa of the adrenal cortex during changes in electrolyte balance is believed to be controlled predominantly by the circulating levels of angiotensin II and by the extracellular concentrations of potassium and sodium (1, 2). Aldosterone production is also influenced by ACTH, but the role of this peptide under physiological conditions is probably minor. Of these proximate modulators of glomerulosa cell function, angiotensin II and potassium appear to exert the most important regulatory effects upon aldosterone secretion *in vivo*.

With the development of methods for quantitative analysis of angiotensin II receptors in the adrenal cortex (3), it has become possible to examine the relationships between angiotensin II receptors and dietary electrolyte changes that markedly affect aldosterone secretion. Such studies are readily performed in the rat, a species in which the effects of altered electrolyte intake upon adrenal morphology (4) and function have been extensively studied (5). Although circulating angiotensin II and potassium concentrations have been shown to be important stimuli of aldosterone secretion in several animal species, the glomerulosa cells of the rat adrenal also appear to be highly responsive to ACTH. The sensitivity of the rat adrenal to ACTH has sometimes masked the action of angiotensin II upon aldosterone secretion in this species, unless care was taken to block stress-induced ACTH release during experimental procedures performed upon anesthetized animals (6). More recently, administration of angiotensin II to conscious rats has been shown to produce a marked rise in plasma aldosterone (7, 8). Also, cells prepared from the capsular layer of the rat adrenal have been shown to produce aldosterone in response to physiological concentrations of angiotensin II *in vitro* (9). For these

TABLE I
Effects of Dietary Sodium and Potassium Intake upon Body Weight, Adrenal Glomerulosa Width, Serum Electrolytes, and Blood Angiotensin II

Treatment (6 wk)	Diet analysis	Weight gain	Glomerulosa width	Serum		Blood angiotensin II
				Sodium	Potassium	
			μm	meq/liter	meq/liter	$\mu\text{g/ml}$
Controls	Na, 0.31–0.37%	30%	77±3.2	132±0.6	4.5±0.1	48±7
	K, 0.77–0.81%			(9)	(9)	(13)
Low sodium	Na, 0.06–0.10%	21%	156±2.6	132±1.1	4.8±0.2	98±11
	K, 0.73–0.76%			(6)	(6)	(11)
High sodium	Na, 2.8–3.1%	33%	54±1.8	131±0.5	4.7±0.1	13±5
	K, 0.83–1.0%			(11)	(11)	(4)
High potassium	Na, 0.46–0.50%	24%	157±3.2	134±1.2	7.9±0.4	7.7±3
	K, 7.7–9.2%			(3)	(3)	(6)
Low potassium	Na, 0.36%	8%	51±0.8	132±0.7	2.9±0.3	110±36
	K, 0.03%			(6)	(5)	(7)

reasons, angiotensin II is probably an important regulatory factor in the control of aldosterone secretion in the rat, as in other species.

In contrast to the actions of sodium balance, which appear to act primarily via the renin-angiotensin system upon aldosterone secretion, changes in potassium balance are believed to modulate aldosterone synthesis through a direct action upon the cells of the zona glomerulosa. This effect operates through local changes in extracellular potassium concentration, which influence aldosterone production *in vivo* and *in vitro* (10). In contrast to the effect of high circulating levels of renin and angiotensin II that accompany sodium deprivation, the increased aldosterone secretion during potassium loading is characterized by a reduction in plasma renin and angiotensin II levels (11).

The present study was undertaken to ascertain whether the changes in circulating angiotensin II and aldosterone that accompany alterations in sodium and potassium balance are associated with significant alterations in the binding affinity and concentration of adrenal cortex receptor sites for angiotensin II. In addition, the acute effects of anesthesia, angiotensin II infusion, and nephrectomy upon the number and affinity of adrenal receptors were examined. These experiments have shown that adrenal receptors for angiotensin II are significantly influenced by dietary electrolyte changes and by alterations in the circulating levels of angiotensin II.

METHODS

Synthetic [Asp¹, Ile⁶] angiotensin II was obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. and from Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif. The concentration of angiotensin II standard solutions was calibrated by reference to the ultraviolet

absorption of tyrosine, for which the optical density of a 1-mM solution at 275 nm was taken as 1.34. Monoiodinated ¹²⁵I-angiotensin II was obtained from Schwarz/Mann; the specific activity of each tracer preparation was determined by radioimmunoassay and radioligand-receptor assay and ranged from 1,200 to 1,700 $\mu\text{Ci}/\mu\text{g}$.

Male Sprague-Dawley rats (200–250 g) obtained from Holtzman Co., Madison, Wis. were maintained on diets of constant electrolyte composition for 1–6 wk. Each group of 15–20 rats received diets containing accurately defined sodium and potassium concentrations, as listed in Table I. The diets were prepared by Zeigler Brothers, Inc., Gardeners, Pa. The rats were allowed free access to tap water and were weighed at weekly intervals during each experiment.

Blood samples for measurement of plasma renin activity (PRA)¹ and plasma aldosterone were collected from the jugular veins under sodium pentobarbital anesthesia or from the neck during the first 10 s after decapitation. Disodium EDTA was used as an anticoagulant for all blood samples at a final concentration of 5 mM. PRA and plasma aldosterone concentration were determined in individual plasma samples by previously described assay methods (12, 13) and expressed as nanograms angiotensin I per milliliter per hour, and nanograms aldosterone per 100 ml, respectively. Angiotensin II was measured in blood samples collected into acetone, by extraction and radioimmunoassay, as previously described (14). Blood for serum sodium and potassium determinations was collected by aortic puncture; the serum was separated immediately and frozen until assay by flame photometry.

Angiotensin II infusions were performed by injecting the synthetic peptide at a rate of 50 ng/min for 20 min through polyethylene tubing into the saphenous vein of rats during sodium pentobarbital anesthesia. Control animals were infused with saline over the same time. Blood samples for extraction and radioimmunoassay of angiotensin II were collected into acetone at the completion of the infusions (14). Rats were sacrificed after the infusions, and adrenals

¹ *Abbreviations used in this paper:* K_a , equilibrium association constant; PRA, plasma renin activity.

from each group of animals were pooled and homogenized for preparation of binding fractions. During experiments to determine the effects of nephrectomy on angiotensin II receptors, rats on low sodium diet were maintained for 18 h after nephrectomy, before sacrifice by decapitation and preparation of adrenal particles.

For histologic examination of the adrenals and measurement of the width of the zona glomerulosa, whole adrenals were fixed in 10% formalin, and 10- μ m paraffin sections were prepared and stained with hematoxylin and eosin.

Angiotensin II binding studies. Adrenals were collected from rats under sodium pentobarbital anesthesia or after decapitation and immediately placed into ice-cold medium 199 containing dextrose (2 mg/ml), 10 mM Hepes buffer, and bovine serum albumin (5 mg/ml). Either whole adrenals or adrenal capsules (obtained by incising the capsule and expressing the inner layers) from groups of 10-15 rats were homogenized with 30 ml of 20 mM sodium bicarbonate in a Dounce homogenizer with five strokes of a loose pestle and five strokes of a tight pestle. The homogenate was stirred on ice for 10 min, filtered twice through nylon gauze, and centrifuged at 600 *g* for 20 min. The supernate was then centrifuged at 20,000 *g* for 30 min. The 600-20,000 *g* pellet was suspended in 50 mM Tris-HCl buffer (pH 7.4) to give a final protein concentration of 6-8 mg/ml. Collagenase-dispersed capsular (predominately glomerulosa) adrenal cells were prepared by methods described elsewhere for canine adrenal cells (9). Incubations were performed at 37°C for 45 min with 1-ml aliquots of approximately 200,000 cells in medium 199 containing bovine serum albumin (2 mg/ml).

Binding assays were performed with 200-300 μ g of adrenal particles in 120 mM NaCl, 5 mM dithiothreitol, 0.2% bovine serum albumin, 50 mM Tris-HCl (pH 7.4), and ¹²⁵I-angiotensin II in a final volume of 0.25 ml. Incubations were performed in 12 \times 75-mm glass tubes at 22°C for 45 min or at 4°C for 4 h. At the end of the incubation, each assay tube was filled rapidly with 4 ml of ice-cold phosphate-buffered saline, pH 7.4, and bound and free angiotensin II were separated immediately by Millipore filtration (Millipore Corp., Bedford, Mass.) through nitrocellulose HAWP (0.45 μ m) filters. Each filter was washed twice with 4 ml of cold phosphate-buffered saline to remove unbound ¹²⁵I-angiotensin II. Millipore filters were placed in polyethylene counting vials (Packard Instrument Co., Inc., Downers Grove, Ill.) and bound radioactivity was measured in an automatic gamma spectrometer with counting efficiency of 65%. Binding assays were performed by both tracer saturation and displacement methods, and the equilibrium constants and binding capacities were calculated by a curve-fitting computer program, as previously described (15).

Proteins were determined according to Lowry et al. (16), with bovine serum albumin as the standard. The number of receptor sites, calculated by either direct analysis of the binding curve or Scatchard analysis, was corrected for the amount of protein per assay tube. When only adrenal capsules were employed protein concentration ranged from 50 to 100 μ g/tube; with whole adrenals protein values were from 300 to 400 μ g/tube.

RESULTS

Dietary effects on body weight, adrenal weight, glomerulosa width, and serum electrolytes. All groups of animals gained weight during the 6 wk period of dietary treatment (Table I). The control group showed a mean

weight gain of 30%, and the weight gain of animals subjected to low potassium diet was markedly retarded (8%). Rats on low sodium and high potassium diets gained 21 and 24%, respectively.

The widths of the zona glomerulosa in animals after the various dietary regimens are summarized in Table I. Each value represents the mean of at least 50 observations. A marked increase in zona glomerulosa width was observed in animals maintained on low sodium or high potassium intake ($P < 0.001$). Conversely, a significant decrease in zona glomerulosa width was observed in animals given the high sodium or low potassium diets ($P < 0.001$). The changes in glomerulosa width after 1 wk paralleled the changes noted after 6 wk of modified diet.

The high and low potassium diets were accompanied by striking changes in serum potassium concentration, with mean values of 7.9 ± 0.4 and 2.9 ± 0.3 meq/liter, respectively. These levels were significantly different from the normal value of 4.5 ± 0.1 meq/liter ($P < 0.001$). No significant differences in serum sodium concentration were observed after any of the electrolyte-modified diets.

Dietary effects on PRA, angiotensin II, and aldosterone. The mean values for PRA and aldosterone in animals maintained on modified electrolyte intakes for 6 wk are illustrated in Fig. 1. When blood samples were obtained after decapitation, the control PRA was 2.8 ± 0.3 ng/ml per h, and plasma aldosterone was 14.1 ± 2.0 ng/100 ml. Low sodium intake caused a significant stimulation of both plasma renin and aldosterone to 4.8 ± 0.5 ng/ml per h and 297 ± 104 ng/100 ml ($P < 0.01$), while high sodium intake caused by significant suppression of PRA to 0.6 ± 0.1 ng/ml per h, and of plasma aldosterone to 7.6 ± 0.8 ng/100 ml ($P < 0.01$). After high potassium intake, PRA was suppressed to 0.9 ± 0.2 ng/ml per h, comparable to the values seen after high sodium diet, but plasma aldosterone was markedly elevated to 425 ± 91 ng/100 ml. Low potassium intake was accompanied by a marked increase in PRA to 20.5 ± 2.2 ng/ml per h, while the mean plasma aldosterone of 14.4 ± 1.5 ng/100 ml was not significantly different from the normal value.

The prominent changes in PRA and plasma aldosterone in response to the various electrolyte intakes were apparent even during the acute elevations of these values induced by pentobarbital anesthesia (Fig. 1). Neither the increase in renin and aldosterone induced by low sodium nor the suppression by high sodium diet was obscured by anesthesia, and both changes were significantly different from the corresponding control values ($P < 0.01$). The basal value for PRA was elevated from 2.8 ± 0.3 ng/ml per h in samples obtained by decapitation to 19.5 ± 2.7 ng/ml per h when sodium pen-

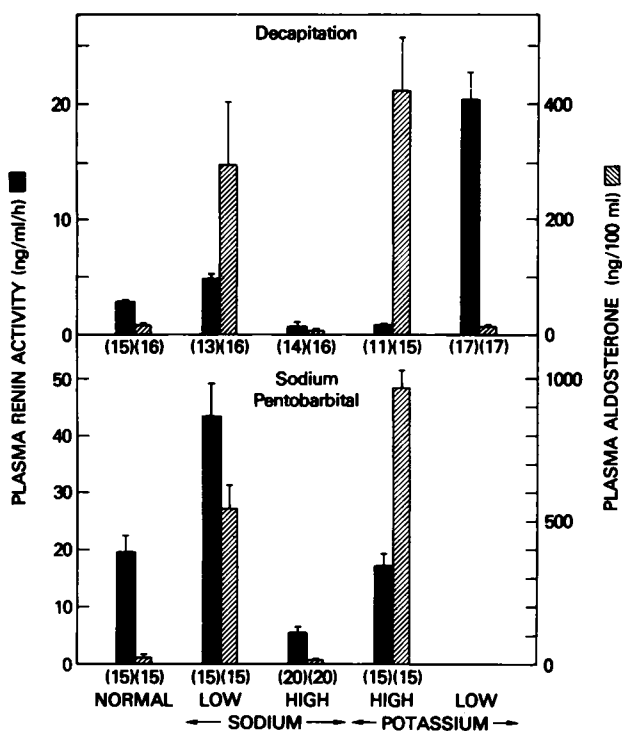


FIGURE 1 The effects of dietary electrolyte changes upon PRA and plasma aldosterone in normal rats. The estimations shown in the upper panel were performed upon blood samples collected after decapitation; those in the lower panel were performed upon samples collected under pentobarbital anesthesia. The number of animals included in each group is shown in parentheses under each column. All values represent mean \pm SE.

pression of angiotensin II concentrations by 73% (with two of four values at the lower level of detectability) and high potassium diet caused an 83% decrease compared to the controls with five of six values at the lower limit of detectability of the assay (5 pg/ml).

Binding affinity of angiotensin II receptors. The mean equilibrium association constant (K_a) of adrenal receptors for angiotensin II, determined in membrane-rich homogenates prepared from control rats on normal diet, was $2.0 \pm 0.4 \text{ nM}^{-1}$ ($n=9$). Both Scatchard plots and direct analysis of the binding data demonstrated the presence of a second order of lower-affinity binding sites, with K_a of $0.15 \pm 0.07 \text{ nM}^{-1}$. The equilibrium association constants were identical when either whole adrenal glands or capsular tissues were used to prepare the binding fractions. Although striking changes in PRA and plasma aldosterone were induced by anesthesia, no significant difference was noted in the equilibrium association constant for angiotensin II binding by the adrenal receptor sites. The values for K_a obtained with adrenal homogenates from rats killed with ether, sodium pentobarbital, or decapitation were 1.8–2.5 nM^{-1} .

The equilibrium association constants after 1 and 6 wk of dietary electrolyte alteration are listed in Table II. In animals maintained on normal diet, the mean K_a value obtained for the two series of experiments was $2.0 \pm 0.4 \text{ nM}^{-1}$. During dietary manipulations, the only significant changes in receptor affinity were observed after 1 wk of high potassium diet, and after 6 wk of low sodium or low potassium intake. After 1 wk of high potassium intake, there was a significant decrease in receptor affinity (to $0.7 \pm 0.1 \text{ nM}^{-1}$) when compared to re-

tobarbital was used. The changes in plasma aldosterone during anesthesia were less striking than the alterations in PRA, with mean values of $24 \pm 4 \text{ ng/100 ml}$ during anesthesia and $14.1 \pm 2 \text{ ng/100 ml}$ after decapitation on normal sodium and potassium diet. In contrast with the significant suppression of PRA by high potassium intake, apparent in plasma samples obtained after decapitation, the less marked fall to $17.2 \pm 2.2 \text{ ng/ml per h}$ during pentobarbital anesthesia was not statistically significant. The direct stimulation by high potassium intake of aldosterone secretion was clearly shown by the striking elevations of plasma aldosterone to $967 \pm 59 \text{ ng/100 ml}$ during anesthesia and to $425 \pm 91 \text{ ng/100 ml}$ after decapitation.

The blood angiotensin II values determined after 6 wk on the electrolyte-modified diets are listed in Table I. The relative changes in blood angiotensin II during the various dietary electrolyte intakes were consistent with the corresponding changes in PRA. Thus, blood angiotensin II concentrations were increased by a mean of 104% in animals on low sodium intake and 129% on low potassium intake. High sodium diet caused sup-

TABLE II
Equilibrium Association Constants of Adrenal Receptors for Angiotensin II during Changes in Sodium and Potassium Intake

Treatment	High affinity	Low affinity
Control	2.0 ± 0.4	150 ± 70
1 wk*		
Low sodium	1.9 ± 0.2	57 ± 4
High sodium	1.9 ± 0.4	270 ± 240
High potassium	0.7 ± 0.1	72 ± 59
Low potassium	2.5 ± 0.5	76 ± 34
6 wk†		
Low sodium	0.5 ± 0.01	—
High sodium	1.7 ± 0.6	120 ± 120
High potassium	2.5 ± 1.6	110 ± 53
Low potassium	1.0 ± 0.03	—

* Mean \pm SEM.

† Values represent the mean of two experiments.

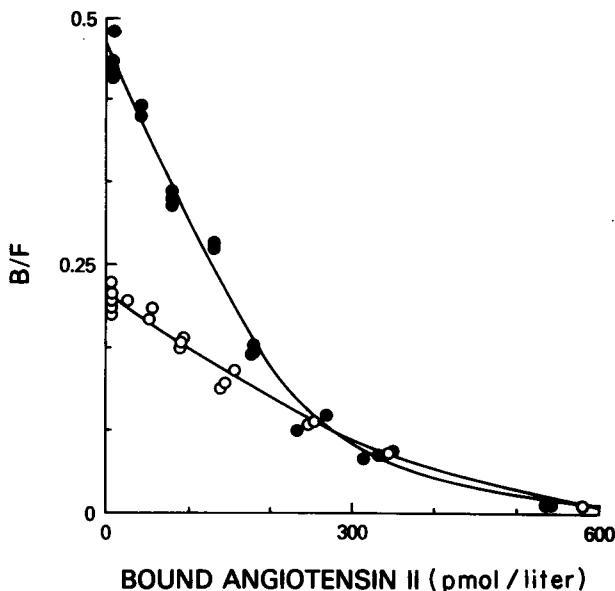


FIGURE 2 Scatchard plots of angiotensin binding data derived after 1 wk of low-sodium diet (●-●) and after 1 wk of high potassium intake (○-○).

ceptors from rats of the other groups. The differences in the Scatchard plots of the receptors from low sodium and high potassium animals are illustrated in Fig. 2, the steepest slope being associated with low sodium intake. The number of angiotensin II receptor sites was similar in each group of animals. After 6 wk of low sodium intake, only a single set of binding sites was present, with association constant of 0.5 nM^{-1} . The adrenals of animals on high potassium and high sodium intakes contained both high and low affinity binding sites. During high sodium diet, an increased proportion (57–66%) of high affinity sites was present at both 1 and 6 wk, by comparison with the proportion of 33–46% measured during normal sodium intake. The effect of low potassium diet upon receptor affinity was broadly comparable to that of low sodium intake, with a single set of sites and slightly reduced affinity after 6 wk.

Adrenal content of angiotensin II receptor sites. In addition to changes in receptor affinity, dietary electro-

TABLE III

Angiotensin II Receptor Concentrations in Adrenal Particles during Changes in Sodium and Potassium Intake

Duration	Low sodium	High sodium	High potassium	Low potassium
1 wk	3.0	0.7	2.0	0.7
6 wk	1.7	0.8	3.0	0.5

Values represent the mean from two to four experiments. Normal diet = 1.0.

lyte alterations were accompanied by quite marked changes in receptor concentration in the adrenal glands. The ratios between adrenal receptor concentrations of experimental groups and control animals after 1 and 6 wk of dietary modifications are listed in Table III. After 1 wk of low sodium intake, the number of receptor sites was increased to three times the control value (Fig. 3). After 1 wk on high potassium diet, an increase to twice the normal receptor concentration was apparent. By contrast, both high sodium and low potassium diets were accompanied by a significant decrease in the total number of receptor sites. In collagenase-dispersed intact cells from rats maintained on control diet, angiotensin II binding sites have been found to be more concentrated in the capsular zona glomerulosa cells, with 30% more receptors per cell (9). Capsular cells from rats maintained on a low sodium diet for either 1 or 3 days, before the onset of cell hyperplasia, exhibited a negligible increase (+7%) in the number of receptors per cell. However, after 1 wk, there was a $74 \pm 10\%$ ($n = 3$) increase in the number of receptors per cell, which paralleled the increase noted in the adrenal homogenate preparations. A comparison of angiotensin II binding to cells from animals maintained on normal sodium and low sodium intake for 1 wk is shown in Fig. 4. Also, high potassium diet for 1 wk was found to increase the number of receptors per cell by 170% ($n = 2$).

After 6 wk, the effects of low sodium diet were still apparent, but the increase in angiotensin II receptors

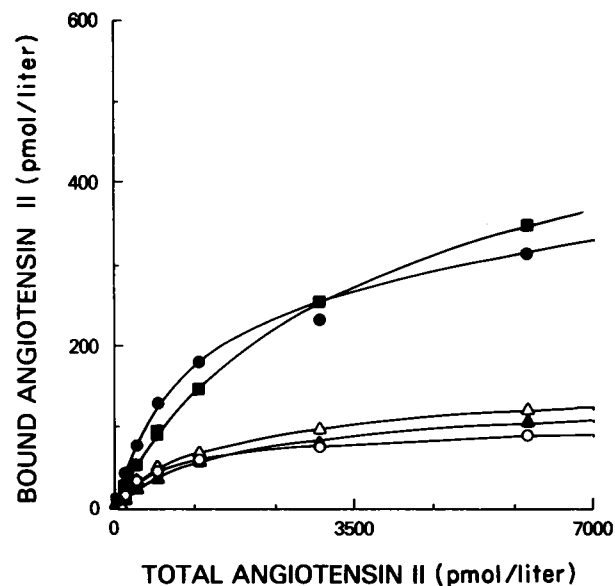


FIGURE 3 Saturation curves derived by direct analysis and computer fitting of angiotensin binding data after 1 wk of electrolyte-modified diets. Δ - Δ Control; \bullet - \bullet low-sodium diet; \blacksquare - \blacksquare high-potassium diet; \blacktriangle - \blacktriangle high-sodium diet; \circ - \circ low-potassium diet.

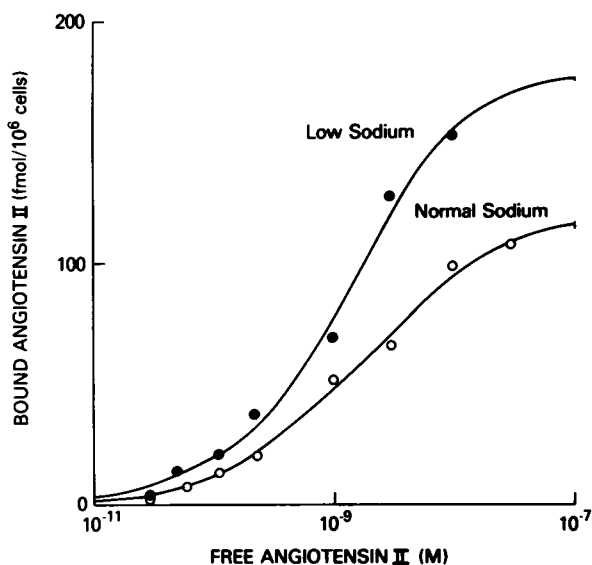


FIGURE 4 Angiotensin II binding to adrenal glomerulosa cells from rats maintained on normal sodium (○—○) and low sodium diet (●—●) for 1 wk. The concentration of binding sites in each preparation is expressed as femtomoles per 10^6 cells.

was less marked, being approximately 1.7 times the normal value. At this time, adrenal receptors of rats on high potassium diet were increased to three times the control value. Conversely, high sodium diet was consistently associated with a significant decrease in the tissue concentration of angiotensin II receptor sites. However, no significant change in receptor sites was apparent in the adrenals of animals on high sodium or low potassium intake for 6 wk. The effects of sodium pentobarbital anesthesia and decapitation on the number of receptor sites measured after 6 wk on the electrolyte-modified diets are illustrated in Fig. 5. In both circumstances, the most striking increases in angiotensin II receptor concentration were observed with high potassium and low sodium intakes.

Effect of circulating angiotensin II levels upon adrenal receptor content. Alterations in the concentration of receptor sites accompanied the changes in PRA and aldosterone induced by anesthesia. The number of receptor sites in adrenals obtained after decapitation (32 pmol/g protein) was significantly higher than the values observed after pentobarbital anesthesia (26 and 22 pmol/g, respectively; $P < 0.01$). This difference was probably attributable to occupancy of receptor sites by the high endogenous angiotensin II levels induced by anesthesia.

To determine the effect of acute elevations of circulating angiotensin II levels on the number of adrenal receptor sites, angiotensin II infusions were performed in 10 rats for 20 min, followed by subsequent adrenalectomy and binding analysis.

The mean blood angiotensin II concentration in the control rats was 33.3 pg/ml, and the mean level after angiotensin infusion was elevated to 763 pg/ml. The equilibrium association constant for angiotensin II was identical (2 nM^{-1}) in both control and angiotensin-treated groups of rats, while the concentration of receptor sites was reduced from 80 pmol/g protein in the control group to 64 pmol/g protein in rats receiving angiotensin II infusion ($P < 0.01$).

To examine the effects of an acute decrease in circulating angiotensin II levels upon adrenal receptor sites, nephrectomies were performed upon rats receiving low sodium diets for variable periods of time. Plasma samples obtained from nephrectomized rats 16–18 h after operation did not contain detectable quantities of angiotensin II when extracted and subjected to radioimmunoassay (less than 5 pg/ml). After 1 and 2 wk of low sodium diet, high and low affinity sites with K_a values of 1.5 nM^{-1} and $80 \mu\text{M}^{-1}$ were present in adrenal particulate fractions prepared before and after nephrectomy. The absence of a change in receptor affinity after weeks of low sodium diet is illustrated by the parallel Scatchard plots derived from several such experiments (Fig. 6). After 6 wk of low-sodium diet, when only a single class of angiotensin II receptors with reduced affinity was present, nephrectomy was followed by the appearance of high and low affinity sites with K_a values of 1.2 nM^{-1}

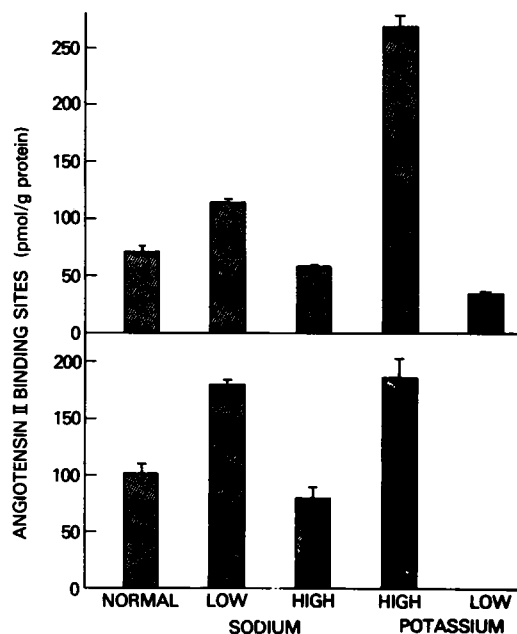


FIGURE 5 The effects of electrolyte intake on the adrenal content of angiotensin II receptors after 6 wk of altered sodium and potassium diets. The data in the upper panel were obtained from animals killed by decapitation; those in the lower panel were from animals killed with sodium pentobarbital.

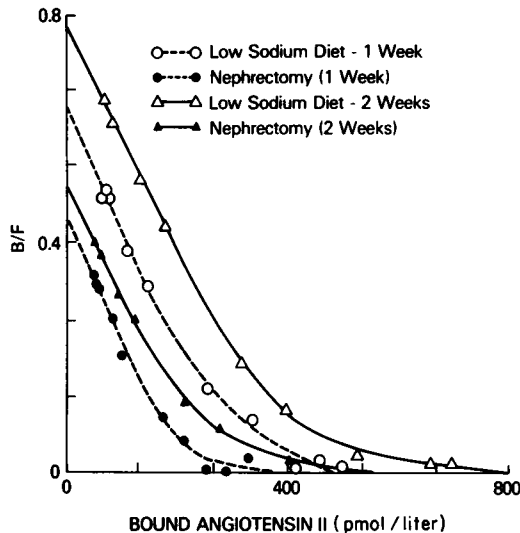


FIGURE 6 Scatchard plots of binding data derived with adrenal particles from rats maintained on low-sodium diet for 1 and 2 wk, and after nephrectomy at each time interval.

and $92 \mu\text{M}^{-1}$. Adrenal receptor sites were progressively increased after 1 and 2 wk of low sodium diet, then no significant further change occurred between 2 and 6 wk of sodium restriction. Bilateral nephrectomy during sodium deficiency caused a significant decrease in the number of adrenal receptor sites at 1 and 2 wk, with a 44% fall at 1 wk and a 40% fall at 2 wk. At 6 wk, only a slight decrease in receptor sites occurred after nephrectomy. In addition to these observations, performed 18 h after nephrectomy, a significant reduction in receptor site concentration was also observed after a shorter interval, when adrenals were analyzed 6 h after nephrectomy in animals subjected to 2 wk of sodium deprivation.

DISCUSSION

These experiments have demonstrated that the angiotensin II receptors of the rat adrenal cortex are quantitatively and qualitatively modified by changes in dietary electrolyte content, and by acute changes in the circulating level of angiotensin II. The major effects of the dietary maneuvers upon adrenal receptors were: (a) an increase in the number of receptor sites during low sodium or high potassium intake; (b) a decrease in the number of receptor sites during high sodium or low potassium intake; and (c) a decrease in the affinity of adrenal receptors for angiotensin II after 6 wk of low sodium or low potassium diet and after 1 wk of high potassium diet. Changes in the number of receptor sites per gram of adrenal cortical tissue during low sodium and high potassium diets were paralleled by increased numbers of receptors per cell after sodium restriction and potassium loading for 1 wk. Thus, the changes in receptor content were not merely a reflection of hy-

perplasia of the zona glomerulosa at 1 and 6 wk, but could be attributed to an absolute increase in receptors per glomerulosa cell.

The changes in PRA, blood angiotensin II, and plasma aldosterone that accompany alterations in dietary sodium and potassium in rats have not previously been analyzed in detail. In general, blood angiotensin II levels were found to reflect the changes in PRA. During changes in sodium intake, there was a direct relationship between renin, angiotensin II, and aldosterone. PRA and angiotensin II levels increased twofold after low sodium diet and were suppressed by high sodium intake. Low potassium diet was accompanied by a sixfold elevation in PRA and a twofold increase in angiotensin II, but plasma aldosterone concentration remained normal. Although the levels of renin activity induced by low potassium intake were even higher than after low sodium diet, and the blood angiotensin II concentrations were comparable, the plasma aldosterone concentration was only 5% of that observed during low sodium diet. This disparity indicates that adequate dietary potassium or another factor is necessary to permit the adrenal hyperplasia and elevated aldosterone secretion that accompanies low sodium intake. It is evident that increased blood angiotensin II is not an effective glomerulotropic stimulus when potassium intake is reduced.

The increase in the number of angiotensin receptors per cell during low sodium diet may be secondary to receptor induction by high circulating levels of angiotensin II. However, this mechanism could not account for the increase in receptors with high potassium intake, when angiotensin II levels were approximately 10% of those seen with sodium deprivation. The striking elevation in serum potassium to 7.9 meq/liter may have induced changes in angiotensin II receptors by a direct action on the adrenal or through interaction with the small amount of circulating angiotensin II. In contrast, with low potassium intake and subnormal serum potassium levels, the very high circulating angiotensin II levels did not induce angiotensin II receptors. Therefore, it is possible that the factor or factors responsible for hyperplasia of the zona glomerulosa during low sodium or high potassium intake also control the angiotensin II receptor concentration.

The equilibrium association constant of adrenal receptors for angiotensin II was reduced after 1 wk of high potassium intake, to about one-third of the K_d of receptors from animals maintained on normal or low sodium intake. The slopes of the Scatchard plots illustrated in Fig. 2 are significantly different, and emphasize the lower affinity of adrenal receptors during high potassium intake when compared to low sodium intake. However, after 6 wk of high potassium intake, adrenal receptor sites of higher affinity were again present. The

K_d of angiotensin receptors was then similar to the normal control value and was about five times higher than that observed during low sodium intake. During recent *in vitro* observations upon collagenase-dispersed dog adrenal glomerulosa cells, we have observed that increasing potassium concentrations from 2.5 to 7.5 mM were associated with increasing sensitivity to angiotensin II in the absence of a change in receptor affinity (17). These findings suggest an additional relationship between the actions of potassium and angiotensin II upon the target cell, operating synergistically beyond the receptor site.

Although the proportions of high and low affinity sites for angiotensin II were not significantly altered after 1 wk of low sodium intake, only a single set of sites with intermediate affinity was detected after 6 wk of sodium restriction. This finding could result from a conformational change in the receptors induced by prolonged exposure to high endogenous angiotensin II levels. The existence of negative cooperativity has been demonstrated for several hormone receptors (18), particularly those for ligands that exert rapid and marked physiologic effects. The presence of concave Scatchard plots during angiotensin II binding studies could result from negative interactions between receptors with increasing saturation, rather than from the presence of two orders of binding sites. Our findings of high receptor affinity during normal and high sodium diet, and reduced affinity during low sodium or low potassium diet, could be attributed to an *in vivo* effect of high endogenous angiotensin II levels upon receptor affinity subsequently measured *in vitro*. Further studies are in progress to investigate the extent to which site-site interactions are responsible for the apparent changes in affinity during dietary electrolyte alterations.

During sodium deficiency, receptor concentration was elevated to three times the normal value after 1 wk, and remained increased to 1.7 times the control level at 6 wk. A simultaneous alteration in receptor affinity was apparent, with a decrease from 2 nM^{-1} at 1 wk to 0.5 nM^{-1} , at 6 wk of sodium deficiency. A decrease of both receptor number and affinity was noted after 6 wk of low potassium intake, with similar elevation of blood angiotensin II levels. In both conditions, it is not possible to exclude completely the presence of a high-affinity site which is masked by occupancy due to the high circulating levels of angiotensin II.

We have demonstrated that *in vivo* occupancy of adrenal receptor sites, achieved by infusion of angiotensin II to give a blood concentration of 20 times the normal level, caused a decrease in the number of binding sites measured by binding assay with ^{125}I -angiotensin II *in vitro*. This mechanism could also account for the decrease in the number of receptor sites measured dur-

ing anesthesia with accompanying high PRA, as compared to the number measured after decapitation. The apparent loss of sites attributable to masking of receptors by acutely elevated levels of exogenous or endogenous angiotensin II was not accompanied by the changes in receptor affinity observed after more chronic induction of high endogenous angiotensin II levels by dietary modifications. A comparable reduction in angiotensin II binding sites of rat uterine smooth muscle after angiotensin II infusion has been recently demonstrated (19). In addition, it has been suggested that occupancy of vascular receptors by high circulating levels of angiotensin II may be a major cause of the decrease in vascular responsiveness to angiotensin II during sodium depletion (20).

However, with changes in sodium balance, the adrenal content of receptor sites was directly proportional to the blood angiotensin II levels, rather than inversely proportional as in the studies mentioned above. Elevations in blood angiotensin II levels caused by sodium deprivation for 1, 2, and 6 wk were accompanied by, and possibly contribute to, an increase in the number of adrenal receptor sites for angiotensin II. Conversely, a decrease in angiotensin II concentration after nephrectomy or during chronic sodium loading was followed by a decrease in adrenal receptor concentration. There appeared to be no "down regulation" in the number of receptors per cell with high endogenous ligand concentrations, as has been demonstrated for the binding of insulin (21) and growth hormone (22) to lymphocyte receptors. In the adrenal, the opposite relationship is observed between angiotensin II receptors and blood angiotensin levels during changes in electrolyte balance. In addition, the decreased affinity of the receptors may act as a buffer or protective mechanism against the high circulating levels of angiotensin II. After high sodium intake, the adrenal content of receptor sites was reduced to 70% and 80% of control values at 1 and 6 wk of salt loading. This decrease in receptor sites was comparable with the decrease observed after nephrectomy, when the change was concomitant with an abrupt decrease in circulating angiotensin II levels. Such changes suggest that the adrenal receptors are subject to relatively rapid alterations in concentration, and that the sites may show acute alterations in response to changes in circulating angiotensin II levels.

The terms "angiotensin II receptors" and "angiotensin II binding sites" have been used interchangeably, since we have previously demonstrated correlations between angiotensin II binding and aldosterone production in glomerulosa cells from the dog and rat adrenal glands (9, 23). This functional correlation of steroidogenesis with peptide binding, as well as the biological specificity and other features of the adrenal binding

sites (3), form the basis for their validation as receptor sites for angiotensin II. Binding studies in isolated adrenal cells have also demonstrated the presence of both high and low affinity receptor sites for angiotensin II. The association constant of the high affinity sites observed in rat adrenal cells prepared from animals on normal electrolyte intake is 20 nM^{-1} , commensurate with the mean concentration of circulating angiotensin II in rat blood of about 50 pM. The lower affinity of the receptor sites observed in subcellular preparations may be due to physical or enzymatic factors that modify receptor affinity during cell fractionation.

It is of interest to compare these changes in adrenal receptor sites with the changes in adrenal sensitivity to angiotensin II during alterations in sodium balance. Enhanced adrenal sensitivity to angiotensin II during sodium deficiency has been demonstrated in rat (24, 25), dog (26), and man (27, 28). Conversely, positive sodium balance has been shown to reduce the aldosterone response to angiotensin II in rat (29), dog (30), and sheep (31). The present studies suggest that altered receptor concentration and binding affinity are potentially significant modulating factors in the control of adrenal sensitivity to angiotensin II with changes in sodium intake. The exact degree to which changes at the receptor level and at more distal sites in the biosynthetic pathway contribute to adrenal sensitivity during altered electrolyte intake has yet to be determined. However, it can be concluded that alterations in dietary sodium and potassium are accompanied by significant changes in both the binding affinity and tissue concentration of angiotensin II receptors in the adrenal cortex.

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