Dietary Intake of Sodium Chloride in the Rat Influences [^{3}H]Nitrendipine Binding to Adrenal Glomerulosa Cell Membranes But Does Not Alter Binding to Vascular Smooth Muscle Membranes

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Abstract

Angiotensin II-stimulated secretion by adrenal glomerulosa cells and contraction by vascular smooth muscle (VSM) are dependent on calcium influx through membrane calcium channels. We have examined the hypothesis that the altered responsiveness of adrenal glomerulosa cells and VSM to angiotensin II during NaCl restriction may be associated with a change in membrane calcium channel number. To test this hypothesis, female rats were placed on a high or low NaCl diet. On the 14th day, membranes were prepared from the zona glomerulosa, aorta, mesenteric artery, and uterus. [^{3}H]Nitrendipine binding was used to monitor calcium channel number. The [^{3}H]nitrendipine binding capacity was observed to be higher in the zona glomerulosa during NaCl restriction than during high NaCl intake (83±18 vs. 49±9 fmol/mg protein, $P < 0.025$, $n = 6$ paired experiments). The binding capacities of [^{3}H]nitrendipine on the low and high NaCl diet were similar in the mesenteric artery (10±1 vs. 9±1 fmol/mg protein, $n = 8$), aorta (33±5 vs. 35±8 fmol/mg protein, $n = 5$), or uterus (87±15 vs. 85±16 fmol/mg protein, $n = 4$), respectively. The dissociation constants of [^{3}H]nitrendipine binding did not differ on a low or high NaCl intake in the zona glomerulosa (0.84±.12 vs. 0.79±.10 nM), mesenteric artery (0.82±.06 vs. 83±.05 nM), aorta (0.90±.11 vs. 0.92±.12 nM), or uterus (0.55±.12 vs. 0.56±.10 nM), respectively. We conclude that the blunted response of VSM to angiotensin II during NaCl restriction is best explained by the previously reported lower number of angiotensin II receptors since calcium channel number does not change. In the adrenal glomerulosa cell, NaCl restriction is associated with a higher number of membrane calcium channels and angiotensin II receptors. The increase in calcium channel number may reflect the influence of an unknown factor(s) believed to be necessary for the full expression of the adrenal glomerulosa cell response to NaCl restriction.

Introduction

Aldosterone secretion and vascular smooth muscle reactivity are influenced by dietary NaCl intake. When NaCl is deficient in the diet, aldosterone secretion is enhanced (1–7). This increase in aldosterone secretion by the adrenal glomerulosa cell is associated with an increase in membrane-bound angiotensin II, suggesting an increase in angiotensin II receptor number (8). Reciprocal events occur in vascular smooth muscle during NaCl restriction where the pressor response to angiotensin II is blunted, which is associated with a fall in angiotensin II binding to vascular smooth muscle membranes (1–3, 9). The lower angiotensin II binding during NaCl restriction in vascular smooth muscle is also mirrored in uterine smooth muscle (10).

Angiotensin II-stimulated aldosterone secretion and vascular smooth muscle contraction are inhibited by calcium channel antagonists, suggesting that calcium influx through the calcium channel is a necessary element of the angiotensin II stimulatory signal (11–15). This observation suggests that tissue responsiveness to angiotensin II may be modulated by changes in membrane calcium channel number as well as angiotensin II receptor number. The present study was designed to examine the hypothesis that the altered responsiveness of the adrenal glomerulosa cell and vascular smooth muscle to angiotensin II during NaCl restriction is associated with a change in membrane calcium channel number. Thus, the number of calcium channels may be higher in the adrenal glomerulosa cell, and lower in vascular smooth muscle during NaCl restriction. This hypothesis was tested by examining binding of the dihydropyridine calcium channel antagonist [^{3}H]nitrendipine to rat adrenal glomerulosa cells, aorta, mesenteric artery, and uterine smooth muscle.

Methods

Tissue preparation. Female Sprague-Dawley rats, weight 200–225 g, were fed a low NaCl diet (<0.1% sodium and 0.5% chloride; ICN Nutritional Biochemicals, Cleveland, OH) and were given deionized water or NaCl 0.9% as drinking water. Rats given deionized drinking water are designated as the low NaCl diet group and rats given NaCl 0.9% drinking water as the high NaCl diet group. On the 14th day of the diet, 18 animals from each group were killed by decapitation. Blood was collected from the stump for measurement of aldosterone and corticosterone. Several animals from each group were anesthetized with ether, and blood was drawn from the inferior vena cava only for measurement of serum sodium and potassium. 18 aortas, 18 mesenteric arteries, 10 uteri, or 36 adrenal glands were harvested from each group and placed in a homogenizing buffer (sucrose, 250 mM; Tris, 50 mM, pH 7.4) on ice. The aortas were cleaned of adhering tissue and the serosa was stripped from each uterus. Mesenteric arteries were prepared as previously described (16). Briefly, the main trunk of the mesenteric vein was stripped away from the artery in situ. The mesenteric artery was removed and adhering fat and remaining mesenteric vein were scraped free. Residual fat was removed using a loose-fitting, 9-ml glass-Teflon homogenizer set at the slowest turning speed. The adrenals were cleaned of adhering fat, bivalved, and the capsules containing the zona glomerulosa were stripped from the remainder

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of the gland. Aortic tissue in 9 ml of buffer and uterine tissue in 9 ml were homogenized by four 15-s bursts with a polytron set at maximal speed. Mesenteric arteries in 9 ml were homogenized with the polytron set at 60% of maximal speed for five 15-s bursts followed by five 15-s bursts at a speed setting of 80%. Adrenal capsules were homogenized with a tight-fitting, 9-ml glass-Teflon homogenizer set at maximal speed with seven passes over 20 s in 4.5 ml. An enriched membrane fraction was obtained by differential centrifugation. The differential centrifugation technique ultimately used for all the binding studies was determined from a preliminary study. In this study, the membrane marker 5′ nucleotidase activity and [^{3}H]nitrendipine binding were examined using adrenal capsular tissue. These two parameters were measured on homogenate pellets obtained by centrifugation at the following sequential speeds: 1,500 *g* for 10 min, 5,000 *g* for 10 min, 10,000 *g* for 10 min, 30,000 *g* for 30 min, and 243,000 *g* for 20 min. The 1,500 *g* pellet was discarded. The Scatchard plots of [^{3}H]nitrendipine binding to each protein fraction obtained by differential centrifugation were parallel. The binding capacity of the protein fractions increased threefold at the two highest centrifugal speeds when expressed as amount bound per milligram of protein. 5′ Nucleotidase activity also increased threefold, parallel to that of the [^{3}H]nitrendipine binding capacity again at the two highest speed spins. Thus, the protein pellet obtained between the 10,000 *g* 10-min spin and the 243,000 *g* 20-min spin was used for [^{3}H]nitrendipine binding studies on all tissues. After centrifugation, the protein pellets were suspended in deionized water, using a loose-fitting, 9-ml glass-Teflon homogenizer at 50% of maximal speed. The entire protein pellet from each tissue was used for the binding studies except for the uterus where only one-third of the protein content of the pellet was used. The protein concentration was determined by the method of Lowry using bovine serum albumin (BSA) as a standard (17). 5′ Nucleotidase activity was assayed as previously described (18).

[^{3}H]Nitrendipine binding. [^{3}H]Nitrendipine, specific activity 75.9 Ci/mmol, was obtained from New England Nuclear (Boston, MA). Its purity was 94% as determined by high performance liquid chromatography. [^{3}H]Nitrendipine binding was examined at seven concentrations of the ligand from 0.1 to 3.0 nM for the adrenal capsular and uterine membranes. Five concentrations of ligand were examined with aortic and mesenteric artery membranes from 0.25 to 2.0 nM. Each point on the binding curve was performed in triplicate. Nonspecific binding was determined in the presence of 1 μM of unlabeled nitrendipine (gift of Dr. Alexander Scriabine, Miles Laboratories, Inc., New Haven, CT). The ligand buffer consisted of 100 mM Tris (pH 7.4) and 0.2% albumin. Adding albumin prevented ligand adsorption to glass and plastic, which in its absence was ~10% for glass and 25% for plastic. The ligand in a 200-μl vol was added to 200 μl of the membrane preparation for a total incubate volume of 400 μl. The amounts of protein used for each binding assay for low and high NaCl diets were 87±8 and 97±8; 95±6 and 93±9; 69±10 and 61±13; and 80±11 and 90±15 μg for adrenal capsular, aortic, uterine, and mesenteric artery membrane fractions, respectively. The samples were incubated in 12 × 75-mm polypropylene tubes for 60 min at 22°C, in a dimly lit room, since nitrendipine is reported to undergo 50% degradation in 7 h under fluorescent lights. Degradation of [^{3}H]nitrendipine was <2% when incubated with glomerulosa cell membranes in the presence or absence of unlabeled nitrendipine for 60 min as determined by high performance liquid chromatography. Bound and free ligand were separated by filtration through a 13-mm glass fiber filter (Schleicher and Schuell, Inc., Keene, NH). Filters were washed with 12 ml of buffer containing Tris 50 mM (pH 7.4) and albumin 0.1%, and allowed to air dry. Aqueous counting scintillant (Amersham Corp., Arlington Heights, IL) was added and the radioactivity of each sample was quantitated with a beta counter (Beckman Instruments, Inc., Fullerton, CA). Nonspecific binding as a percent of specific binding was 20, 200, 170, and 7% for zona glomerulosa, mesenteric artery, aorta and uterine membranes.

^{125}I-Angiotensin II binding studies. Uterine and adrenal capsular tissue were prepared as described above. Tissues were homogenized in a buffer containing 50 mM Tris (pH 7.4), 2 mM EDTA, and 250 mM sucrose. The ^{125}I-angiotensin II binding assays were performed as previously described with minor modification of the buffers (19–21). Zona glomerulosa cell membranes were incubated in a 50-mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM dithiothreitol, 0.1% BSA, and 5 mM $MgCl_2$. The Tris buffer for the uterus binding studies contained 5 mM dithiothreitol, 0.1% BSA, and 5 mM $MgCl_2$. ^{125}I-Angiotensin II (specific activity 1,880 μCi/μg; New England Nuclear, Boston, MA) 100,000 dpm was incubated with eight different concentrations of unlabeled angiotensin II 0.2–10 nM each in triplicate in a total volume of 400 μl. Nonspecific binding was determined in the presence of 1 μM unlabeled angiotensin II. The amount of protein used for the ^{125}I-angiotensin II binding studies averaged 145±6 μg for the adrenal and 107±5 μg for uterine tissues. Incubations were for 45 min at 22°C. Bound and free ligand were separated as described above. Radioactivity was determined with a gamma counter (Packard Instrument Co., Downers Grove, IL).

Table I. Steroid and Electrolyte Measurements after 14 d of Dietary Sodium Restriction or during High Sodium Intake

Diet	Serum sodium	Serum potassium	Plasma aldosterone	Plasma corticosterone
	meq/liter	*meq/liter*	*ng/dl*	*μg/dl*
Low sodium	138±1	4.1±0.1	269±54*	48±4
High sodium	138±1	4.0±0.1	39±5	46±5

Results are expressed as the mean±SEM (n = 19 for electrolyte measurements and n = 25 for steroid measurements). All animals were fed a low sodium diet. The low sodium diet group received deionized drinking water and the high sodium diet group received 0.9% sodium chloride in their drinking water.
* $P < 0.001$.

Serum electrolyte and steroid determinations. The serum sodium and potassium concentrations were determined by flame photometry. Plasma aldosterone and corticosterone concentrations were determined by radioimmunoassay (RIA) as previously described with two minor modifications (22). Each sample was purified by paper chromatography in a Bush V system (benzene/methanol/water, 15:4:4). Recovery averaged 35% for aldosterone and 40% for corticosterone. Antibody bound and free steroid were separated by the dextran-coated charcoal technique.

Calculations and statistics. Binding data were analyzed by the computer program SCATFIT (23). RIA results were computed by the RIA-PROG-1980 program (24). Comparisons were made by the unpaired t test or the analysis of variance program of Statistical Analysis Systems (SAS Institute, Inc., Cary, NC). Results are expressed as the mean±SEM.

Results

After 14 d, serum sodium and potassium were similar in rats on a high or low NaCl diet (Table I). As expected, plasma aldosterone was significantly higher ($P < 0.001$) in rats on the low NaCl diet (269±54 ng/dl) than those on a high NaCl diet (39±5 ng/dl). Plasma corticosterone measurements were similar in each group. On the low and high NaCl diets, the rats gained 21±3 and 19±2 g/14 d (n = 23) and ate 6.1±.2 and 6.0±.2 g/100 g body weight of food per day (n = 12), respectively.

In Fig. 1 is plotted the specific binding of [^{3}H]nitrendipine to glomerulosa cell membranes as a function of time. Equilibrium was achieved by 15–20 min and remained stable for 60 min. Equilibrium binding studies were not performed on the smooth muscle membrane preparations since [^{3}H]nitrendipine binding to a variety of tissues achieves equilibrium by 30 min and remains stable for at least 60 min (25–27). In Fig. 2 is illustrated the equilibrium binding of [^{3}H]nitrendipine to glomerulosa cell membranes. Nonspecific binding increased linearly

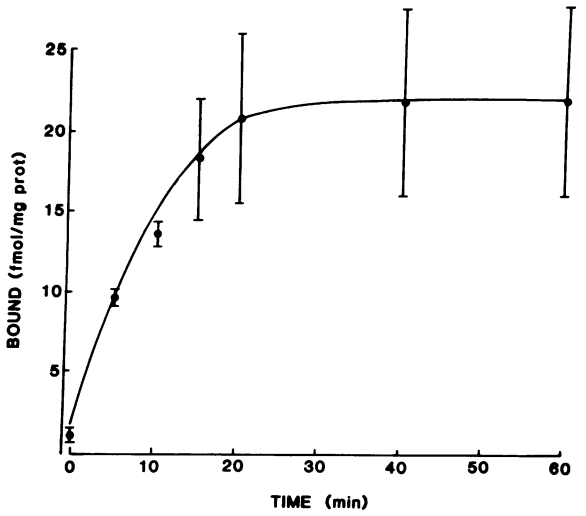

Figure 1. Specific binding of 1 nM [^{3}H]nitrendipine to adrenal zona glomerulosa membranes obtained from rats on a normal NaCl diet. Binding was examined at pH 7.4 at 22°C (mean±SEM, n = 2 experiments). Nonspecific binding determined in the presence of 1 μM unlabeled nitrendipine, reached equilibrium by 10 min.

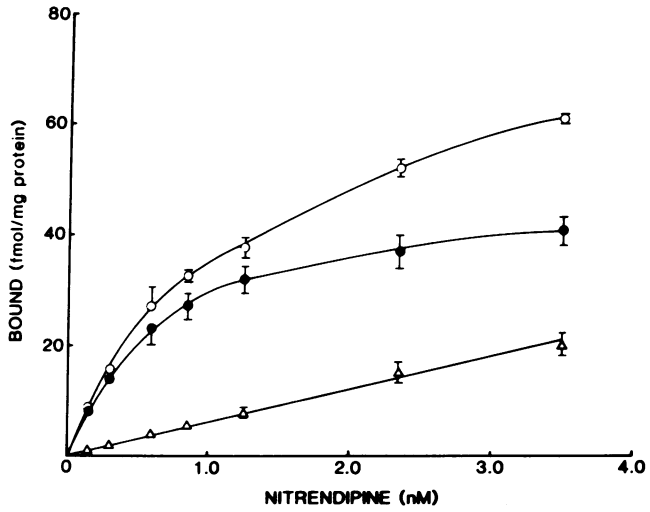

Figure 2. Equilibrium binding of [^{3}H]nitrendipine to rat adrenal zona glomerulosa membranes obtained from rats on a normal NaCl diet. Binding was examined as described in Fig. 1 for 60 min: total binding (○), specific binding (●), and nonspecific binding (△) (mean±SEM, n = 2 experiments). The Scatchard plot of these data are presented in Fig. 3.

with the concentration of [^{3}H]nitrendipine, whereas the specific binding, defined as the difference between total and nonspecific binding, was a saturable function of the [^{3}H]nitrendipine concentration. The linearity of the Scatchard plot of these data (Fig. 3) is consistent with specific binding to a single class of sites. Representative Scatchard plots of [^{3}H]nitrendipine binding to mesenteric artery, aorta, and uterine membranes are also shown in Fig. 3.

In Table II are contained the binding capacity (B_{max})[1] and equilibrium dissociation constant (K_d) of [^{3}H]nitrendipine binding to membrane preparations from adrenal zona glomerulosa, mesenteric artery, aorta, and uterine tissues. These studies were performed on the 14th day of a low or high NaCl diet. The dissociation constants of [^{3}H]nitrendipine binding to the tissues examined were not significantly influenced by dietary NaCl intake. However, NaCl intake did significantly influence the B_{max} of [^{3}H]nitrendipine binding to the adrenal zona glomerulosa membrane preparation. In this tissue, [^{3}H]nitrendipine binding was 1.7-fold higher during NaCl restriction. However, the binding capacities of [^{3}H]nitrendipine binding to vascular smooth muscle and uterine membranes were not influenced by dietary NaCl intake.

Activity measurements of the presumably stable membrane marker 5′ nucleotidase were determined in the membrane fractions used for the [^{3}H]nitrendipine binding studies. There was no significant difference in 5′ nucleotidase activity of the membrane preparations obtained while on a low or high NaCl diet (Table II). The 5′ nucleotidase activity of the membrane fraction used for the binding studies was compared with that of the crude homogenate in the zona glomerulosa. 5′ Nucleotidase enrichment was 3.9±0.2-fold (mean±SEM, n = 4) during the low NaCl diet and 4.1±0.3-fold on the high NaCl diet. Also included as a control for the [^{3}H]nitrendipine studies was an examination of ^{125}I-angiotensin II binding to adrenal capsular and uterine membranes. ^{125}I-Angiotensin II binding was examined in three separate experiments after 14 d of a high or low NaCl diet. As anticipated, the B_{max} of ^{125}I-angiotensin II binding to adrenal capsular tissue was higher during NaCl restriction relative to a high NaCl diet, 1,804±193 versus 1,392±162 fmol/mg protein

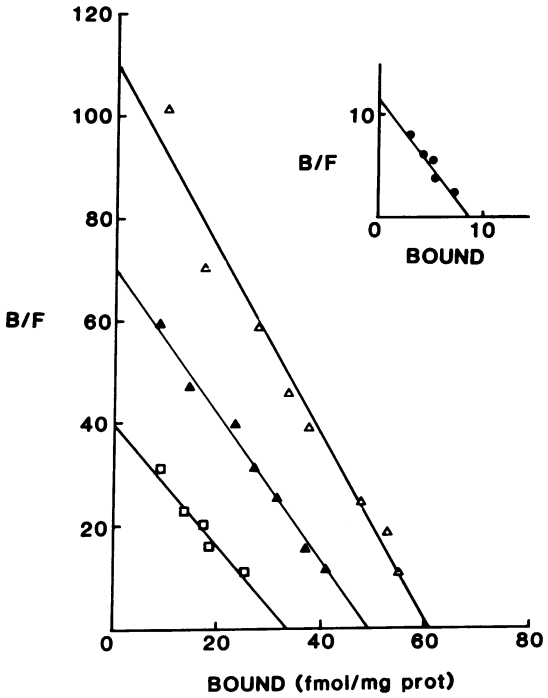

Figure 3. Representative Scatchard plots of binding data. Scatchard plot of [^{3}H]nitrendipine binding to membranes of rat mesenteric artery (●), aorta (□), adrenal zona glomerulosa (▲), and uterus (△). Binding studies were performed as in Fig. 2. Mesenteric artery, aorta, and uterine tissues were obtained during NaCl restriction and adrenal zona glomerulosa during a normal NaCl diet.

1. *Abbreviations used in this paper:* B_{max}, binding capacity.

Table II. [^{3}H]Nitrendipine Binding Characteristics and 5' Nucleotidase Activity of Enriched Membrane Preparations from Different Tissues during Dietary Sodium Restriction or High Sodium Intake

Tissue	*n*	Sodium diet	Binding capacity	K_d	5' Nucleotidase activity
			fmol/mg prot	*nM*	*nmol/min per mg prot*
Zona glomerulosa	6	Low	83±18*	0.84±.12	64±8
		High	49±9	0.79±.10	63±7
Mesenteric artery	8	Low	10±1	0.82±.06	69±7
		High	9±1	0.83±.05	76±5
Aorta	5	Low	33±5	0.90±.11	90±25
		High	35±8	0.92±.12	97±18
Uterus	4	Low	87±15	0.55±.12	163±30
		High	85±16	0.56±.10	172±34

Mean values of *n* experiments±SEM are given. Dietary groups are described in Table I. * $P < 0.025$ by analysis of variance.

($P < 0.04$), respectively. Contrariwise, ^{125}I-angiotensin II binding to uterine smooth muscle membranes was greater during the high NaCl diet than the low NaCl diet, 69±2 vs. 48±8 fmol/mg protein ($P = .05$), respectively.

Discussion

The observation that calcium channel number of a tissue may change in response to chronic stimulation or chronic suppression has not been previously reported to our knowledge. In the present study, we observed that dietary NaCl restriction is associated with a greater number of calcium channels in glomerulosa cell membranes as suggested by the higher B_{max} for [^{3}H]nitrendipine during NaCl restriction relative to that observed during a high NaCl intake. The physiological importance of this observation is suggested by the essential role the calcium channel plays in angiotensin II-stimulated aldosterone secretion. Angiotensin II stimulation of the glomerulosa cell increases calcium influx (12). Calcium influx and aldosterone secretion are both inhibited by blockade of the calcium channels (11–14). Also, the rise in cytosolic calcium that occurs with angiotensin II stimulation of the glomerulosa cell is markedly blunted by nifedipine and is associated with a limited aldosterone secretory response (14). Thus, these observations suggest that the calcium channel is an essential and integral part of the angiotensin II stimulatory pathway.

The dihydropyridine calcium channel antagonist, [^{3}H]-nitrendipine, was chosen to monitor changes in membrane calcium channels in preference to other commercially available labeled calcium channel ligands because dihydropyridines have been demonstrated to bind to an active site of the calcium channel (28). This conclusion is derived from the observation that a dihydropyridine analogue, Bay k 8644, is a calcium channel agonist or activator (29). More specifically, membrane binding of Bay k 8644 is competitively inhibited by nitrendipine (30) and [^{3}H]nitrendipine binding is competitively displaced by Bay k 8644 (31). Functionally, there is competitive antagonism between Bay k 8644 and nifedipine, whereas the structurally dissimilar calcium channel antagonists, verapamil and diltiazem, produce only a functional, noncompetitive inhibition of the effects of Bay k 8644 (32). Collectively, these observations suggest that nitrendipine binds to an active site in the calcium channel.

The mechanism whereby angiotensin II activates or opens the calcium channel is not known. However, two mechanisms of calcium channel activation are presently recognized, receptor activated and voltage activated (33). Angiotensin II may open a receptor-activated channel or may even indirectly open a voltage-activated channel since angiotensin II has been observed to lower the membrane potential of zona glomerulosa cells (34). The relatively weak correlation between the 1.3-fold increase in angiotensin II binding and the 1.7-fold increase in [^{3}H]nitrendipine binding to zona glomerulosa cell membranes in this study does not exclude a receptor-activated channel mechanism for angiotensin II, as the stoichiometry of a receptor-calcium channel relationship is not known.

During dietary NaCl restriction, adrenal glomerulosa cell sensitivity to angiotensin II is enhanced since aldosterone secretion occurs at lower concentrations of angiotensin II (2–7). This increased glomerulosa cell sensitivity to angiotensin II may reflect an increase in the rate of calcium influx at lower concentrations of angiotensin II. Total calcium influx through calcium channels is governed by three known mechanisms: number of calcium channels, number of open channels, and rate of calcium influx through each channel (35). Under normal physiological conditions, the rate of calcium influx through each channel does not change and therefore the overall rate of calcium influx is dictated by the number of calcium channels and the probability of their being open. Thus, the enhanced glomerulosa cell sensitivity to angiotensin II during dietary NaCl restriction may reflect an enhanced overall cellular rate of calcium influx, mediated by an increased number of calcium channels. In addition, there may be a higher probability of open channels from the increased number of angiotensin II receptors.

The mechanism responsible for modulating the response of vascular smooth muscle to a variable NaCl intake is believed to be the renin-angiotensin system under normal physiological conditions. High circulating levels of angiotensin II, as seen during NaCl restriction, "down-regulate" or lower angiotensin II receptor number (9). The lower number of angiotensin II receptors during NaCl restriction is associated with a blunted contractile response to angiotensin II (1–3). The opposite occurs during a high NaCl diet (1–3). Furthermore, studies in which the converting enzyme inhibitor, captopril, was administered during NaCl restriction suggest that the influence of dietary NaCl

intake on angiotensin II receptor number and responsiveness of vascular smooth muscle is solely mediated by the circulating angiotensin II levels and not by other factors during NaCl restriction (9).

In the present study, the influence of dietary NaCl intake on [^{3}H]nitrendipine binding to vascular smooth muscle was examined using three different tissues. The need to study more than just the mesenteric artery was prompted by the high nonspecific binding and low binding capacity of the mesenteric artery for [^{3}H]nitrendipine. In order to derive meaningful data, eight experiments were performed on the mesenteric artery. Dietary NaCl intake failed to influence [^{3}H]nitrendipine binding to the mesenteric artery. This observation was corroborated by similar findings in aortic and uterine tissues where the nonspecific binding was lower and B_{max} higher. Thus, the observation that calcium channel number does not change in vascular smooth muscle in response to alterations in dietary NaCl intake is consistent with the thesis that angiotensin II receptor number in vascular smooth muscle may be the major limiting determinate in the response to angiotensin II.

The events occurring at the level of the adrenal glomerulosa cell in response to changes in NaCl intake are more complex. The circulating angiotensin II level does not appear to be the sole determinant of the adrenal glomerulosa cell response to NaCl restriction, as suggested by the following observations. Chronic infusion of angiotensin II results in a subnormal aldosterone biosynthetic response when compared with the response induced by NaCl restriction (6, 36). Anterior pituitary insufficiency is associated with a blunted aldosterone response to NaCl restriction (37–39). The introduction of captopril during NaCl restriction does not decrease the glomerulosa cell response to infused angiotensin II (3). However, captopril administration from the onset of NaCl restriction does block the adrenal response to an NaCl deficient diet (40). These observations suggest that angiotensin II is necessary but not sufficient for the induction of a normal response to NaCl restriction; however, it is not necessary once the response is established. Thus, another factor(s) appears to be essential for the adrenal glomerulosa cell response to an NaCl restricted diet. The complexity of the adrenal glomerulosa response to NaCl restriction may reflect a necessity to increase both angiotensin II receptor number and calcium channel number. A failure to increase one but not the other may result in a blunted response. If future studies reveal that calcium channel number is controlled by a factor(s) other than angiotensin II, then the above phenomena regarding the complexity of the adrenal glomerulosa cell response to NaCl restriction may be better understood. Thus, a normal glomerulosa cell response to NaCl restriction may require two membrane events, each governed separately by two different hormonal systems.

In summary, [^{3}H]nitrendipine binding to glomerulosa cells is higher during dietary NaCl restriction than during high NaCl intake. Contrariwise, the NaCl content of the diet does not alter the B_{max} of [^{3}H]nitrendipine to vascular smooth muscle. The tissue selective difference in response to dietary NaCl intake, although unknown, may relate to the apparent involvement of the pituitary or factor(s) other than angiotensin II in the glomerulosa cell response to NaCl restriction which is not shared by vascular smooth muscle. This suggests that angiotensin II may not influence calcium channel number by itself and that the unknown factor(s) involved with the response to NaCl restriction may be responsible for influencing membrane calcium channel number in the glomerulosa cell. Vascular smooth muscle may not respond to this unknown factor(s) for lack of a receptor.

In conclusion, we have made the novel observation that chronic stimulation or chronic suppression may influence the number of calcium channels in the target tissue. A higher number of calcium channels may enhance or amplify a stimulus that is calcium channel-dependent. Thus, a previously unrecognized membrane event has been observed to occur and suggests a new area of investigation in monitoring tissue response to chronic stimulation or suppression.

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