Blood Pressure Development of the Spontaneously Hypertensive Rat after Concurrent Manipulations of Dietary Ca^{2+} and Na^+

Relation to Intestinal Ca^{2+} Fluxes

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Abstract

The blood pressure of the spontaneously hypertensive rat (SHR) is influenced by the Ca^{2+} content of its diet. As the SHR's greater dependence on dietary calcium may reflect a defect in intestinal calcium absorption, we measured in vitro unidirectional Ca^{2+} flux (J) in the duodenum-jejunum (four segments each) of the SHR (n = 6) and the normotensive Wistar-Kyoto rat (WKY; n = 6) by a modified Ussing apparatus. Because of the known and postulated interactions between Ca^{2+} and Na^+ in both intestinal and vascular tissue, we assessed in vivo the influence of a concurrent manipulation of Na^+ intake (three levels: 0.25%, 0.45%, and 1.0%) on the blood pressure development of SHRs (n = 35) and WKYs (n = 35), between 6 and 20 wk of age, exposed to three levels of dietary calcium (0.1, 1.0, and 2%).

Net calcium flux (J_{net}) (mean±SEM) was significantly ($P < 0.01$) lower in the SHR (−2.8±6.3 nmol/cm^2 · h) than in the WKY (34.6±8.8 nmol/cm^2 · h). The SHR's decreased J_{net} resulted from a significantly ($P < 0.03$) lower mucosa-to-serosa flux ($J_{m\text{-}s}$) in the SHR (41.0±5.6 nmol/cm^2 · h) compared with the $J_{m\text{-}s}$ of the WKY (70.1±9.1 nmol/cm^2 · h). Serosa-to-mucosa flux for calcium did not differ between the SHR (43.8±6.6 nmol/cm^2 · h) and the WKY (35.5±8.0 nmol/cm^2 · h). The SHR's decreased ($P < 0.002$) $J_{m\text{-}s}$ was confirmed by additional measurements in SHRs and WKYs. $J_{m\text{-}s}$ was 36.2±3.7 nmol/cm^2 · h in the SHRs (n = 11) and 64.4±6.7 nmol/cm^2 · h in the WKYs (n = 9).

The provision of an increased dietary Ca^{2+} (2% by weight) and increased Na^+ (1%) to the SHR prevented the emergence of hypertension ($P < 0.001$) (mean±SEM systolic blood pressure at 20 wk of age; 135±5 mmHg for the 2% Ca^{2+}, 1% Na^+ SHR vs. 164±2 mmHg for the control diet SHR). Ca^{2+} (0.1%) and Na^+ (0.25%) restriction accelerated the SHR's hypertension (192±2 mmHg) ($P < 0.001$) and was associated with higher pressures in the WKY (146±4 mmHg in the restricted WKY vs. 134±4 mmHg in the control WKY). In a parallel group of 24 SHRs and 24 WKYs fed one of three diets (2% Ca^{2+}/1% Na^+; 1% Ca^{2+}/0.45% Na^+; or 0.1% Ca^{2+}/0.25% Na^+), the heart ($P < 0.05$) and kidney ($P = 0.08$) weight of the SHRs varied depending on the diet at 20 wk of age. Low Ca^{2+} and Na^+ intake was associated with increased heart weight (1.6±0.9 g) compared with the normal diet for SHR (1.51±0.07 g). Increased Ca^{2+} and Na^+ intake was associated with a significantly ($P = 0.05$) lower heart weight in the SHR (1.37±0.03 g) and in the WKY (1.35±0.06 g) compared with their normal diet controls. These findings show one mechanism for the SHR's depressor response to supplemental dietary Ca^{2+} and, in part, explain the sodium dependence of calcium's cardiovascular protective effect.

Introduction

Abnormally elevated arterial pressure in humans and experimental animals has been attributed, in part, to specific nutrients (1). Excessive dietary sodium has been the nutritional pattern most frequently implicated (2). The spontaneously hypertensive rat (SHR)[1] is one of the most widely studied genetic models of human hypertension, having been characterized as closely paralleling the human disorder in its hemodynamic, endocrine, and end-organ manifestations (3). The development of the SHR's hypertension is not sodium dependent, though modifying Na^+ intake may produce modest blood pressure changes (4). Reports have noted, though, a sensitivity of the adult SHR's pressure to manipulations of Ca^{2+} intake (5). The addition of Ca^{2+} to the SHR's diet attenuates the rise of the young SHR's blood pressure (6, 7) and will reverse "fixed" hypertension in the adult animal (5).

Several theoretical mechanisms might account for the SHR's greater dependence on dietary calcium. A variety of abnormalities of cellular Ca^{2+} metabolism have been identified in the SHR (5, 8–16). In vascular tissue, these have included an increased membrane permeability (8, 10), decreased vesicular uptake of Ca^{2+} (12, 13), and reduced Ca^{2+} ATPase activity (12, 13), defects consistent with a primary failure of Ca^{2+} calmodulin activation (11) of membrane stabilization and Ca^{2+} sequestration in contractile tissue. Independent of these defects of vascular tissue handling of Ca^{2+}, the SHR's Ca^{2+} dependence may reflect the animal's end-organ abnormalities, which would impair its ability to maintain external Ca^{2+} balance. Reports have identified an abnormality of renal Ca^{2+} reabsorption in the SHR (5, 14) that would potentially result in negative Ca^{2+} balance were its net intestinal Ca^{2+} transport either normal or reduced compared with that of the Wistar-Kyoto rat (WKY). In this latter area of intestinal Ca^{2+} transport, the published reports are conflicting (14–16). The most rigorous in vitro evaluation demonstrated an ~50% reduction of the SHR's duodenal Ca^{2+} transport (16).

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1. *Abbreviations used in this paper:* G, conductance; H, high; I_{sc}, short-circuit current; J, flux; $J_{m\text{-}s}$, mucosa-to-serosa flux; J_{net}, net intestinal calcium flux; $J_{s\text{-}m}$, serosa-to-mucosa flux; L, low; PD, potential difference; SBP, systolic blood pressures; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.

Other studies, though, have suggested that net Ca^{2+} transport may be increased in the SHR (14, 15).

In the first part of the study, we assessed in vitro, unidirectional calcium flux (J) in both SHRs and WKYs using a modified Ussing apparatus. Based upon the previous observations noted, as well as on the accepted and hypothesized link between Ca^{2+} and Na^+ in both intestinal Ca^{2+} absorption (17, 18) and in the regulation of vascular smooth muscle function (19, 20), the second part of the study was undertaken. Concurrent modifications of Ca^{2+} and Na^+ intake within the physiological range of the rat diet (21) were used to assess the influence of moderate restriction and/or enhancement of the intake of these two nutrients on the development of genetic hypertension in the SHR and the emergence of end-organ involvement with hypertensive cardiovascular disease.

Methods

In vitro calcium J measurements. Six SHRs and six WKYs (Charles River Breeding Laboratories, Inc., Wilmington, MA) were raised on a 1% Ca^{2+}/0.45% Na^+ (percent of dry weight) (20% protein as casein; vitamin D_2, 2,200 U/kg of food) diet until 12–14 wk of age. At that age, intestinal transmural calcium J was determined using the modified Ussing apparatus (22). Duodenal-jejunal segments, 15 cm immediately distal to the pylorus, were rinsed in situ with ice-cold Krebs-Ringer bicarbonate solution. The segments were then excised and opened along the mesenteric border and mounted unstripped in modified Ussing chambers. Four chambers were used in each rat. Tissue appentures were 0.5 cm^2. Tissues from SHR and WKY were studied in random order and blinded fashion, the sequence of which was unknown to the operator.

The mounted intestinal segment was bathed in a phosphate-free Krebs-Ringer bicarbonate solution (23, 24) maintained at 37°C that had the following electrolyte concentrations (mM): 145 Na^+, 4.7 K^+, 1.25 Ca^{2+}, 1.2 Mg^{2+}, 127.2 Cl^-, 25 HCO_3^-, 1.2 SO_4^{-2}, and 11 D-glucose. Each hemichamber compartment contained 10 ml of the electrolyte solution and was aerated with 95% O_2/5% CO_2 and maintained at pH 7.4 and 37°C. The solutions bathing the mucosal and serosal sides were connected via agar bridges to calomel electrodes for the direct measurement of the potential difference (PD) and to Ag-AgCl electrodes for the passage of direct current through the tissue, using an automatic voltage clamp (dual voltage clamp 616C-3, bioengineering, the University of Iowa, Iowa City, IA). The short-circuit current (I_{sc}) was recorded in the absence of PD after appropriate corrections for the resistance of the fluid between the two PD-sensing bridges (25). Conductance (G) was calculated from I_{sc} and PD. Total resistance and conductance were monitored at regular intervals during the entire experiment. Mucosa-to-serosa ($J_{m\text{-}s}$) and serosa-to-mucosa ($J_{s\text{-}m}$) J's were paired across adjacent pieces of intestine using the 30% conductance-matching criterion used by Walling and Kimberg (26).

After the steady state had been reached (60 min), $^{45}Ca^{2+}$ was added to either the serosal or mucosal side of the paired chambers. 1-ml samples were taken from the "cold" side at 20-min intervals for 1 h. Each aliquot removed was replaced with the same solution but without the $^{45}Ca^{2+}$. Each aliquot removed was counted in 5 ml of scintillation fluid (Pico-Fluor TM 30; Packard Instrument Co., Inc., United Technologies, Downers Grove, IL) with a liquid scintillation counter (model 1212 Rack Beta Wallace; LKB Instruments, Inc., Gaithersburg, MD). Calcium J was calculated as described by Schultz and Zalusky (27) and expressed as nanomoles per centimeter squared per hour. Steady state values were reached after 20 min and are reported as the mean of the J in the two subsequent 20-min periods. Net intestinal calcium J (J_{net}) was calculated on paired tissues from the same animal as $J_{net} = J_{m\text{-}s} - J_{s\text{-}m}$. At the completion of the initial six WKYs and six SHRs, eight additional rats (three WKY, five SHR) were studied to verify the $J_{m\text{-}s}$ data.

Chronic dietary manipulations. 35 SHRs and 35 WKYs were raised on standard rat chow until 6 wk of age. At that time, the SHRs and WKYs were randomized to one of five diets (Teklad, Madison, WI): control, standard rat chow with a 1% Ca^{2+}/0.45% Na^+ (percent dry weight); high (2%) Ca^{2+}/high (1%) Na^+; low (0.1%) Ca^{2+}/high Na^+; high Ca^{2+}/low (0.25%) Na^+; low Ca^{2+}/low Na^+. The standard diet composition of protein, carbohydrate, fat, vitamins, and minerals was maintained except for alterations in the Ca^{2+} (as $CaCO_3$) and Na^+ (as NaCl). The potassium concentration of 0.6% was increased to 1.1% in the two high Na^+ (1%) diets. Chloride concentration varied as a function of Na^+. Beginning at 10 wk of age, tail-cuff systolic blood pressures (SBP) and weights were measured every 2 wk. Serum and urine chemistries were also measured at 10 and 20 wk of age. A second group of 24 SHRs and 24 WKYs was begun on one of three diets (2% Ca^{2+}/1% Na^+; 1% Ca^{2+}/0.45% Na^+; 0.1% Ca^{2+}/0.25% Na^+) at 6 wk of age. The diets were identical to those used for the first group.

For the first group, tail-cuff blood pressures were measured in the unanesthetized animal using standard techniques (Narco Bio-Systems, Healthdyne Co., Houston, TX). An average of four readings was recorded for each animal. Weights were obtained at all blood pressure determinations. Within 24 h after the blood pressure recordings, 3.0–3.5 ml of blood was removed via a subclavian venipuncture under light ether anesthesia. Normal saline replacement, 3–3.5 ml, was given subcutaneously after phlebotomy. 1 d before blood pressure measurements, the rats were placed in individual metabolic cages, and timed urines were collected.

At 20 wk of age, the second group of animals was anesthetized and then killed by exsanguination. The heart and kidneys were removed, cleaned of extra tissue, weighed, and then submitted for routine histologic examination. Standard methods were used to assess the blood and urine samples: calcium and magnesium by atomic absorption, sodium and potassium by flame photometry, and phosphorus and creatinine by autoanalyzer. Blood pressure, body weight, tail length (as an index of skeletal growth) (28), and chemistry data were analyzed statistically using analysis of variance-repeated measures design and multiple range testing (Student-Newman-Keuls). The two-tailed Mann-Whitney U test was used for statistical evaluation of the differences in intestinal calcium J. Values reported represent the means±SEM.

Results

J_{net} was significantly ($P < 0.01$) reduced in SHR as compared with WKY rats (Fig. 1). J_{net} of the SHR approached a value consistent with no active transport in the duodenum and proximal jejunum of this animal, as a result of a significant ($P < 0.03$) reduction in the SHR's $J_{m\text{-}s}$. There was no difference in $J_{s\text{-}m}$ between SHR and WKY rats. Electrical parameters (I_{sc}, G) were comparable in the intestinal segments from the two strains

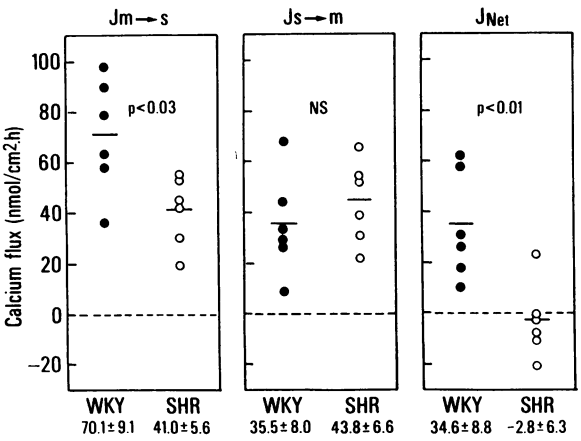

Figure 1. Calcium J across duodenal–jejunal segments expressed as mean±SEM. •, WKY (normal control rats); ○, SHR.

Table I. Electrical Parameters in Ussing Chamber Experiments

Rat strain	G		I_{sc}	
	m → s	s → m	m → s	s → m
	mS/cm²	*mS/cm²*	*μA/cm²*	*μA/cm²*
WKY	27.8±3.8	27.0±4.3	145.2±18.5	145.4±23.4
SHR	30.6±3.6	31.0±3.4	121.6±18.0	127.6±14.2

of rats (Table I). No correlation was found between calcium $J_{m\text{-}s}$ and G ($r = 0.27$, NS). The additional three WKYs and five SHRs in which $J_{m\text{-}s}$ was measured provided confirmation of the SHR's significant reduction in $J_{m\text{-}s}$. For the 11 SHRs studied, $J_{m\text{-}s}$ was 36.2±3.7 nmol/cm² · h, while the WKYs' was 64.6±6.7 nmol/cm² · h ($P < 0.002$).

The mean (±SEM) SBP at 2-wk intervals for each strain and diet group is shown in Table II. Within both strains, there was a significant ($P < 0.001$) difference in SBP based upon the dietary content of Ca^{2+} and Na^+. Between strains there was also a significant ($P < 0.0001$) difference in the blood pressure response to the dietary modifications. Fig. 2 depicts the mean SBP of the WKY and SHR on the control diet and high Ca^{2+}/Na^+ diets. SBP in the control SHR increased with age and was significantly higher ($P < 0.001$) than that of both the WKY control and the high Ca^{2+}/high Na^+ SHR. Importantly, there was no statistical difference throughout the period of observation between the SBP of the control diet WKY and the modestly increased high Ca^{2+}/high Na^+ SHR. The SBP of this genetically predisposed SHR was essentially identical to its normotensive control, the WKY. The normotensive WKY also demonstrated a beneficial effect from the diets as the WKY receiving the higher Ca^{2+}/Na^+ diet exhibited consistently lower blood pressures than the control diet WKY.

As depicted in Fig. 3, the impact of these diet modifications on the SBP at 20 wk of age of both the WKY and SHR was consistent in the relative rank order and magnitude. The restricted (low) Ca^{2+}/Na^+ SHR (192±2 mmHg) was higher ($P < 0.001$) than each of the other SHR diet groups. The low Ca^{2+}/

*Table II. Tail Cuff Pressures on Varying Ca/Na Diets**

Rat strain	Week of age	Diets				
		Control	HCa/HNa	HCa/LNa	LCa/HNa	LCa/LNa
SHR	10	158±6	129±8‡‡	143±2‡	125±7	141±3
	12	154±5	131±7	156±8	171±9	202±7‡
	14	162±6	134±7	165±8‡	159±5	191±2
	16	174±6	141±3	169±9	166±3	158±4
	18	170±5	135±5	158±3	192±8	204±5
	20	164±2	135±5	159±3	176±3	192±2
	n	7	5	5	7	6
WKY	10	127±6	120±9	122±4‡	121±5‡	116±5
	12	126±4	123±4	128±6	133±4	138±5
	14	131±3	123±3	119±3	130±5	134±2
	16	134±3	128±2	128±3	121±3	115±4
	18	135±2	125±2	123±2	147±5	137±6
	20	134±4	119±3	129±7	134±2	146±4
	n	7	7	6	6	7

* Mean±SEM.

‡ Ether-phlebotomy-associated death (‡‡, two deaths occurred).

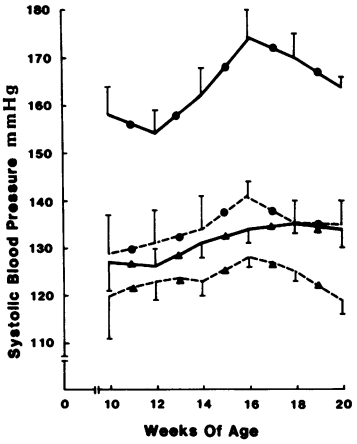

Figure 2. Tail cuff SBP (mean±SEM) (mmHg) in SHRs (●) and WKYs (▲) between 10 and 20 wk of age. Animals received either a standard rat chow (——) that contained 1% Ca^{2+} and 0.45% Na^+ by weight or a modified diet that had increased Ca^{2+} (2%) and Na^+ (1%) in it (- - -). The modified diet was introduced at 6 wk of age. At 10 wk of age and onwards, the control SHR's blood pressure was significantly higher ($P < 0.001$) than that of the HCa^{2+}/HNa^+ SHRs, whose blood pressure was virtually identical to the normotensive WKY. HCa^{2+}/HNa^+ was also associated with lower blood pressures in the WKY.

high Na^+ SHR (176±3 mmHg) was lower than the low Ca^{2+}/low Na^+, but higher than the control diet SHR (164±2 mmHg) ($P < 0.01$). The high Ca^{2+}/low Na^+ SHR's SBP (159±3 mmHg) was not significantly lower than the control animal, but was higher than the high Ca^{2+}/high Na^+ SHR (135±5 mmHg) ($P < 0.01$). The rank order of the diet groups' SBP was identical in the WKY's, though the magnitude of the differences was smaller. Nevertheless, Ca^{2+} and Na^+ restriction in the WKY produced in the animal an SBP (146±4 mmHg) higher than those of both the Ca^{2+}/Na^+-supplemented WKY (119±3 mmHg) ($P < 0.001$) and the high Ca^{2+}/low Na^+ animal (129±7 mmHg) ($P < 0.05$).

Consistent with previous reports, the WKY was heavier (355±5 g) than the SHR (323±7 g) at 20 wk. This was also reflected in the WKY's slightly longer tail length (WKY, 19.1±0.1 cm; SHR, 17.5±0.1 cm). Tail length as an indicator of skeletal maturation and growth did not vary significantly

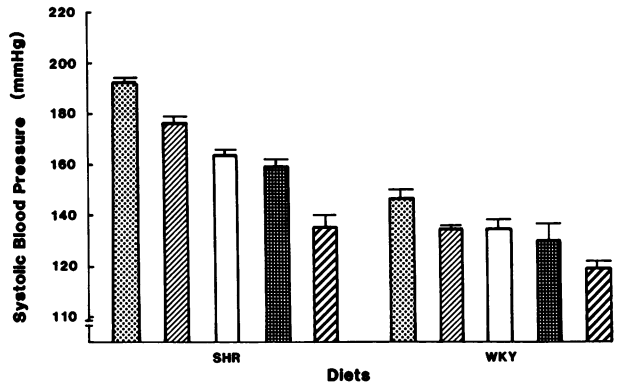

Figure 3. Mean (± SEM) tail cuff systolic blood pressures at 20 wk of age in SHRs and WKYs fed one of the five modified diets that differed in their Ca^{2+} and Na^+ content (control, 1% Ca^{2+}/0.45 Na^+, □; LCa^{2+}/HNa^+, 0.1% Ca^{2+}/0.25 Na^+, ▨; LCa^{2+}/LNa^+, 0.1% Ca^{2+}/1% Na^+, ▨; HCa^{2+}/LNa^+, 2% Ca^{2+}/0.25% Na^+, ■; or HCa^{2+}/HNa^+, 2% Ca^{2+}/1% Na^+, □). Diets were consumed for 16 wk. The rank order was identical for the five diets within both strains. The SHR, though, demonstrated a greater sensitivity to the diet manipulations ($P < 0.001$), with both a higher Ca^{2+} and higher Na^+, producing the best blood pressure effect ($P < 0.001$). A similar effect was seen in the WKY, as the blood pressure response varied significantly ($P < 0.001$) at the extremes of Ca^{2+} and Na^+ intake.

among the various diet groups within each strain. By analysis of variance, observed variations in weight or tail length (Table III) were independent of the observed differences in blood pressure for the respective diet groups. The serum and urine chemistries at 20 wk are shown in Table IV. Serum total calcium concentration varied with the diet composition ($P < 0.001$) for both strains. Across diet groups, serum calcium was lower, but did not reach statistical significance ($P < 0.06$) in the SHR. Serum magnesium values were higher in the SHR ($P < 0.001$) and for both strains varied significantly ($P < 0.001$) with dietary Ca^{2+} modification. A significant diet-strain interaction ($P < 0.002$) existed for magnesium. The diets resulted in predictable ($P < 0.001$) alterations in serum phosphorous levels: as the calcium content was decreased, serum phosphorous rose. Increased Ca^{2+} was associated with a modest decline in serum phosphorous that was not significant. Serum sodium differed by strain ($P < 0.002$) and differed from control in both the SHR and WKY on the high calcium/low sodium diet ($P < 0.005$). Serum potassium also differed by strain ($P < 0.0001$). Except for the high calcium/high sodium diet (both SHR, $P < 0.05$, and WKY, $P < 0.05$), serum potassium levels in manipulated animals did not differ from those of controls. Serum creatinines were similar for the two strains and were unaffected by the diets. Urinary calcium excretion ($U_{Ca}V$) varied significantly ($P < 0.001$) with the diets and between strains ($P < 0.02$). This was evident in the case of the higher Ca^{2+} intake where the SHR exhibited greater $U_{Ca}V$. Urinary sodium excretion ($U_{Na}V$) was lower in the SHR as compared with the WKY for all diets except the high Ca^{2+}/high Na^+. Urinary potassium excretion (U_KV), likewise, increased in the SHR as dietary Ca^{2+} increased and as a function of greater dietary potassium content of the high Na^+ diets.

Heart weight (Fig. 4) varied significantly ($P < 0.05$) based on the Ca^{2+} and Na^+ diet content. The low Ca^{2+}/low Na^+ animals had increased heart weights, while the high Ca^{2+}/high Na^+ animals' heart weights were indistinguishable from the comparable diet group of WKYs. Likewise, kidney weight (Fig. 5) in the SHR ($P = 0.08$) but not in the WKY was modified by the diet. Specifically, the low Ca^{2+}/Na^+ SHRs developed greater kidney weight. Microscopic examination did not reveal any evidence of altered cardiac or renal histology among the five diet groups.

Discussion

Concurrent modifications of the Ca^{2+} and Na^+ intake in both normotensive and spontaneously hypertensive rats produced significant alterations in both the pattern of SBP and maximal SBP attained. The consistency within and between strains of the SBP at 20 wk of age suggests that simultaneous reductions in both Ca^{2+} and Na^+ accelerate the SHR's hypertension and also produce a higher SBP in the normotensive WKY. The rank order of the diets indicates that maintenance of an increased Na^+ diet in the face of Ca^{2+} restriction is modestly protective, though it is still associated with an SBP higher than that seen with the control diet. The consumption of a higher Ca^{2+} intake was associated with some reduction in SBP when Na^+ content was reduced, but the provision of a diet increased in both Ca^{2+} and Na^+ resulted in the optimal effect on the SBP of both the hypertensive and the normotensive rat. Calcium's net effect appears to be greater than that of Na^+, though the latter nutrient is essential for the full expression of calcium's antihypertensive actions in the SHR. These observations complement the recent report of a nonpressor effect of dietary sodium in the absence of chloride in the deoxycorticosterone acetate rat model (29). Those investigators suggested that Cl^- rather than Na^+ might be the causative factor in that experimental model, and more recently, preliminary reports have indicated that Cl^- action may be mediated by an effect on calcium balance (30). The results of our investigation in this genetic model of hypertension not only imply a protective interaction of Na^+ with Ca^{2+} on blood pressure development, but also suggest that in the SHR Cl^- consumption may not have adverse effects provided Ca^{2+} status is protected, as Cl^- intake was greatest in those SHRs and WKYs with the lowest blood pressure on the high Ca^{2+} diets.

The administration of a higher Ca^{2+} and Na^+ diet to the SHR prevented the development of genetic hypertension. Previous studies had documented the adult SHR's blood pressure

Table III. Body Weight in Grams and Tail Length in Centimeters

Rat strain	Weeks of age	Control	HCa/HNa	HCa/LNa	LCa/HNa	LCa/LNa
SHR	10	256.4±6.7	228.0±2.6	224.4±6.8	223.4±8.3	242.3±7.2
	12	279.4±7.0	249.5±2.4	229.6±6.6	243.7±6.4	260.3±9.0
	14	305.3±10.1	265.5±5.8	268.0±11.6	269.1±6.4	292.3±9.1
	16	327.1±9.2	285.0±5.1	309.6±17.4	289.1±7.4	313.8±7.7
	18	336.7±9.7	290.0±4.8	296.8±11.4	304.3±8.3	329.3±8.7
	20	355.1±11.0	307.5±6.1	316.4±13.1	307.4±6.8	321.5±21.6
Tail length	20	17.5±0.3	17.2±0.3	17.4±0.1	17.5±0.1	17.9±0.1
WKY	10	259.3±7.3	228.0±4.8	235.6±6.0	226.0±8.6	220.6±4.4
	12	289.3±8.6	258.6±5.8	247.2±6.3	241.7±8.5	240.9±4.9
	14	321.3±10.6	283.4±5.2	289.2±8.4	283.7±7.4	285.7±5.6
	16	341.5±10.9	303.6±4.8	313.8±11.6	304.3±8.9	328.5±12.3
	18	362.7±13.5	314.7±5.5	334.2±10.9	324.8±8.4	337.7±6.9
	20	385.5±16.0	332.9±6.7	360.4±11.6	347.7±9.4	348.1±7.8
Tail length	20	19.0±0.2	18.8±0.2	19.5±0.3	18.8±0.2	19.3±0.1

Mean±SEM.

Table IV. Chemistries at 20 Wk of Age

Rat strain	Diets	Serum						Urine		
		Total Ca^{2+}	Magnesium	Phosphorus	Creatinine	Potassium	Sodium	Ca^{2+}	Na^+	K^+
		meq/liter	*meq/liter*	*mg/dl*	*mg/dl*	*meq/liter*	*meq/liter*	*meq/24 h*	*meq/24 h*	*meq/24 h*
SHR	LCa/LNa	4.77±0.04	2.00±0.06	4.82±0.19	0.32±0.13	4.33±0.06	148.1±0.5	0.006±0.0004	0.10±0.02	0.31±0.03
	LCa/HNa	4.60±0.04	2.04±0.04	5.56±0.16	0.42±0.10	4.36±0.23	145.8±0.6	0.003±0.0003	0.36±0.17	0.59±0.15
	Control	4.89±0.06	1.93±0.03	4.84±0.37	0.45±0.08	4.36±0.09	147.5±0.4	0.145±0.017	0.30±0.06	0.46±0.02
	HCa/LNa	5.18±0.07	1.26±0.07	3.10±0.39	0.46±0.17	4.30±0.15	141.5±0.3	0.443±0.099	0.40±0.08	0.56±0.09
	HCa/HNa	4.70±0.20	1.27±0.18	4.10±0.35	0.47±0.03	5.02±0.11	148.4±2.6	0.517±0.066	0.96±0.30	0.80±0.13
WKY	LCa/LNa	4.89±0.03	1.60±0.08	4.84±0.09	0.42±0.07	3.99±0.06	146.7±0.5	0.006±0.0014	0.48±0.13	0.35±0.08
	LCa/HNa	4.67±0.06	1.82±0.06	5.48±0.13	0.45±0.13	3.92±0.05	144.6±0.9	0.004±0.0018	0.59±0.15	0.45±0.08
	Control	5.02±0.03	1.60±0.05	4.60±0.18	0.40±0.08	3.76±0.07	147.0±0.3	0.126±0.016	0.50±0.12	0.44±0.09
	HCa/LNa	5.05±0.04	1.33±0.07	3.40±0.14	0.53±0.10	3.88±0.09	142.0±1.5	0.280±0.098	0.66±0.42	0.28±0.12
	HCa/HNa	4.90±0.08	1.33±0.09	4.59±0.19	0.47±0.02	4.10±0.05	147.2±0.4	0.247±0.060	0.67±0.17	0.42±0.09

Mean±SEM.

sensitivity to a supplemented Ca^{2+} diet (5–7). However, to our knowledge, the results of this study represent the first time hypertension has been prevented in the SHR by an intervention that represents a physiologic and nutritionally relevant maneuver. Furthermore, this intervention was associated with no detectable adverse effects on growth or biochemical parameters. In fact, cardiac weight as an index of possible end-organ involvement from chronic hypertension was favorably modified in the SHR consuming the higher Ca^{2+} and Na^+ diet.

Multiple abnormalities of cellular calcium metabolism have been identified in a wide variety of cell types of the SHR (5, 7–16). We chose to focus on intestinal calcium transport in the SHR for several reasons. The SHR's established dependence on supplemental dietary calcium suggested that the failure of the animal to maintain external calcium balance might be one pathological mechanism for which supplemental dietary calcium might compensate. Several reports have documented the presence of a hypercalciuric state in the adolescent and adult SHR (5, 7, 14). If the SHR's intestinal calcium transport were either normal or reduced compared with that of the WKY, its renal abnormality would obligate the animal to being in a state of chronic negative calcium balance, a situation consistent with our preliminary report that in both the mature and aged SHR bone density is reduced (30). Long-term provision of supplemental Ca^{2+} and Na^+ favorably modifies the SHR's bone density, while Ca^{2+} restriction lowers its bone density and Ca^{2+} content (31, 32).

We elected to study the SHR's epithelial calcium transport in the intestine using the modified Ussing chamber technique (22), which is unique in that it permits the study of active calcium transport under electrically controlled conditions, in addition to permitting the continuous monitoring of tissue viability. Intestinal calcium absorption has been investigated in the SHR, primarily in vivo. The results, though, have been conflicting (14–16). Schedl et al. (16) reported decreased absorption, while Toraason and Wright (15) and Lau and co-workers (14) observed increased calcium transport.

Our findings indicate a profound reduction in the SHR's net duodenal-jejunal flux. In fact, we observed a zero J_{net} in the SHR compared with ~30 nmol/cm^2 · h in the WKY, the latter figure being comparable to that obtained by other investigators

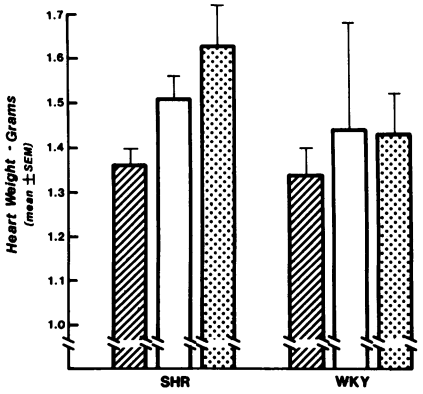

Figure 4. SHR's (n = 24) and WKY's (n = 24) heart weights in grams randomized at 6 wk of age to one of three diets: 2% Ca^{2+}/1% Na^+, ▨; 1% Ca^{2+}/0.45% Na^+, □; or 0.1% Ca^{2+}/0.25% Na^+, ■. Heart weight was determined at death at 20 wk of age. Heart weight for the SHR (P < 0.05) varied significantly based upon the diet consumed.

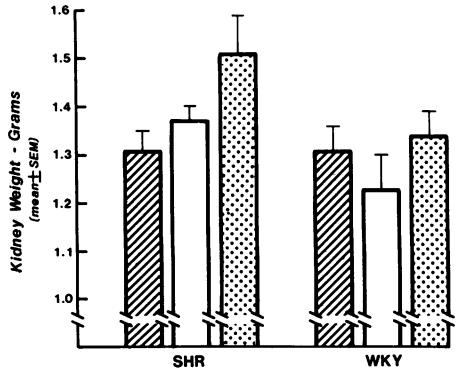

Figure 5. SHR's (n = 24) and WKY's (n = 24) kidney weights in grams randomized at 6 wk of age to one of three diets: 2% Ca^{2+}/1% Na^+, ▨; 1% Ca^{2+}/0.45% Na^+, □; or 0.1% Ca^{2+}/0.25% Na^+, ■. Kidney weight was determined at death at 20 wk of age. Kidney weight for the SHR (P < 0.05) varied significantly based upon the diet consumed.

studying normal rats (33). The difference in J_{net} between the SHR and WKY is virtually entirely accounted for by the lower $J_{m\text{-}s}$ of the SHR. That this decrease in the SHR's J_{net} represents a difference in active rather than passive calcium transport is supported by our finding of an absence of a correlation between $J_{m\text{-}s}$ and tissue conductance. Thus, our observations are consistent with the recent report of Schedl et al. (16), who used both everted duodenal sacs in vitro and in situ duodenal luminal perfusion in vivo to document an ~50% reduction in maximal duodenal transport of calcium. Our results, based upon direct measurements of unidirectional J, are in close agreement with this earlier report. We cannot account for the differences between these findings and those of either Toraason and Wright (15) or Lau et al. (14), who used different techniques to measure intestinal Ca^{2+} transport.

The intracellular mechanism(s) contributing to the SHR's abnormal J_{net}, revealed by our in vitro experiments, cannot yet be defined, though several mechanisms can be considered for future investigation. Currently, active calcium transport by the small intestine is thought to be dependent upon two principal mechanisms, a Ca^{2+}/Mg^{2+} ATPase (34, 35) that has been localized to the basolateral membrane of the enterocyte of the rat (36–38), and Na^+/Ca^{2+} exchange (34, 35), also localized to this same site. Intestinal Ca^{2+} ATPase exhibits enzymatic characteristics similar to those of erythrocyte Ca^{2+} ATPase (37). Abnormal Ca^{2+} ATPase activation has previously been reported in the SHR's erythrocytes (11) and a similar defect may exist in its enterocytes. The partial dependence of the SHR's response to calcium supplementation on sodium may indirectly argue against the defect residing in the Na^+/Ca^{2+} exchanger. Additional mechanisms to be considered would include an abnormality of the $1,25(OH)_2$ vitamin D_3-controlled brush border permeability to calcium or to a failure of normal intracellular Ca^{2+} binding to its binding protein, which could alter both intracellular transport of calcium and Ca^{2+} calmodulin activation of Ca^{2+} ATPase. Future studies will be needed to clarify these various issues.

By reducing net calcium absorption, defective active intestinal transport provides one clear mechanism for the SHR's dependence on supplemental dietary calcium intake and its tendency for negative calcium balance. The addition of dietary calcium would be expected to overcome, in part, the reduction in active transport by means of an increased absorption via passive mechanisms (24, 39). Furthermore, the defect in active intestinal calcium transport we have characterized provides a theoretical explanation for the SHR's dependence on adequate exposure to dietary sodium, which we have also identified. Earlier studies have demonstrated the importance of sodium in the regulation of calcium absorption in the rat small intestine (17, 18, 34, 35). The SHR's requiring sodium for the expression of calcium's antihypertensive action suggests that this animal is as dependent on Na^+/Ca^{2+} exchange as is the WKY to ensure an adequate absorption of the divalent cation. Our results do not exclude the possibility that Ca^{2+} absorption increased as a consequence of increased Na^+ absorption and advent drag.

Our observation that the WKY's blood pressure was also sensitive, though less so to concurrent manipulations of dietary Na^+ and Ca^{2+}, suggests that mechanisms other than ones involving solely the intestinal absorption of calcium must be involved. Calcium exerts multiple effects on vascular smooth muscle function, serving to control both contraction and relaxation (40). This dual effect was initially characterized in vitro by Bohr (40), who noted that at higher extracellular Ca^{2+} concentrations, aortic strips relaxed as opposed to the observed contraction at lower ambient concentrations of the cation. Subsequent investigations linked calcium's vasorelaxing properties to a stabilization of the vascular smooth muscle cell membrane (41). Calcium exerts these biphasic effects on vascular smooth muscle cells via its regulation of membrane ion fluxes (42), including its own (43–45), modulation of both the recruitment and the storage of Ca^{2+} within the cell (42, 45, 46), and activation of enzymatic pathways that both stimulate and inhibit the actin-myosin interaction (40, 42, 46). These regulatory functions of Ca^{2+} are dependent in part on the cation reversibly binding to calmodulin, its intracellular binding protein (46, 47). In accordance with these in vitro findings are the observations from in vivo studies that have demonstrated both vasoconstricting and vasodilating effects of Ca^{2+} infused into regional vascular beds of experimental animals (48, 49).

The apparent synergistic action between dietary Ca^{2+} and Na^+ in normalizing the SHR's blood pressure has been suggested by earlier studies. Webb and Bohr (41) noted that the smooth-muscle–relaxing, membrane-stabilizing effects of Ca^{2+} were, in part, Na^+-dependent. When the Na^+ content of the extracellular fluid was reduced, maximal Ca^{2+}-mediated relaxation did not occur. In both vascular (19) and nonvascular tissue (50), a close interaction between the membrane J of these two ions has been demonstrated repeatedly. The uptake of Ca^{2+} by smooth muscle plasma membranes has been shown to be both Ca^{2+}-dependent and Na^+-requiring (52) for maximal effect. In cardiac tissue, high extracellular Ca^{2+} stimulates Na^+ efflux and is associated with membrane stabilization and reduced contractile force (53). Sodium's enhancement of calcium's relaxation of vascular tissue has been attributed to the Ca^{2+}-Na^+ exchange across the plasma membrane (19, 51–53). With our report, the synergistic action of sodium and calcium to relax vascular tissue is encompassed by observations in cellular preparations, whole organs, and now the intact animal.

An alternative mechanism to explain calcium's effect on blood pressure would include the cation's natriuretic action (54). Increasing calcium excretion by the kidney will promote Na^+ excretion. If natriuresis were principally responsible for calcium's antihypertensive action, then the addition of Na^+ to the diet would be expected to dampen, rather than enhance as we observed, the blood pressure–lowering effect of calcium. Lau et al. (55) have demonstrated that calcium's salutory effect on the SHR's blood pressure is not due to changes in sodium balance. These same authors, however, suggested that calcium's antihypertensive effect may be in part due to phosphorus depletion. In the current studies, serum phosphorus was maintained in the higher Ca^{2+} animals who experienced the best blood pressure effect and antihypertensive action. Collectively, these observations appear to dissociate calcium's effects on blood pressure from induced changes in either Na^+ or PO_4^- balance of the animals.

A marked sensitivity of the SHR to maneuvers that may serve to modify membrane handling of Ca^{2+} and Na^+ is consistent with the numerous observations that the SHR's membrane Ca^{2+} kinetics are abnormal (7–13, 56). These abnormalities presumably result in the animal's exhibiting a variety of biochemical and organ defects related to Ca^{2+} metabolism (5, 7–9, 14). Besides the SHR's having an apparent dependence upon receiving a sufficient dietary Ca^{2+} exposure, reports have suggested a de-

pendence of the SHR on an adequate Na^+ intake (57, 58), as sodium restriction in the young SHR was noted to result in growth retardation and premature mortality (57–59).

Dietary potassium does not appear to be a significant factor in the SHRs' blood pressure responses in this study (60). Chronic manipulation within the range of this study (0.6–1.1%) has not been noted to affect blood pressure in the SHR (60). In addition, the low Ca^{2+}/high Na^+ SHRs were exposed to the same K^+ content of the diet as the high Ca^{2+}/high Na^+ animals and did not experience a hypotensive response.

Interpretations of human disorders based on extrapolations of animal data must always be done cautiously. Given that consideration, the current findings are consistent with observations in humans. Several reports have documented that Ca^{2+} intake is lower in subjects who have established hypertension (61, 62) or who are at risk of developing hypertension (62–64). Furthermore, the provision of supplemental Ca^{2+} to normal and hypertensive individuals has been associated with the lowering of blood pressure (65, 66). Finally, nutritional patterns in adult Americans that reflect greater Na^+ exposure in the diet are associated with a lower prevalence of high blood pressure (64, 67).

Emerging evidence suggests a potentially critical role for the maintenance of a relatively high dietary intake of Ca^{2+} and a "normal" intake of Na^+ for the optimal regulation of blood pressure in experimental animals and in humans. The results of this study demonstrate that in a genetic model of hypertension, the SHR, blood pressure development, and end-organ compromise from hypertensive cardiovascular disease can be favorably modified by such a dietary maneuver. Our findings of a significant impairment of intestinal Ca^{2+} transport in the SHR may provide one pathological mechanism that accounts, in part, for blood pressure dependence on supplemental dietary calcium and sodium.

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