High Calcium Diet Reduces Blood Pressure in Dahl Salt-Sensitive Rats by Neural Mechanisms

JACOB D. PEULER, DONALD A. MORGAN, AND ALLYN L. MARK

SUMMARY We tested the hypothesis that high dietary calcium attenuates hypertension in Dahl salt-sensitive rats by neural as opposed to vascular mechanisms. Four-week-old Dahl salt-sensitive rats were fed a high salt diet (3.3% sodium) with either high (4.0%; n = 21) or normal (0.4%; n = 21) calcium content until they were 10 to 11 weeks old. Total plasma calcium concentration was increased and plasma phosphorus concentration was decreased by the high calcium diet. At 10 weeks, food intake and intestinal absorption of sodium were not altered by the high calcium diet. There were three major observations. First, mean arterial pressure was lower in awake rats fed a high versus normal calcium diet (137 ± 7, n=11, vs 165 ± 6 mm Hg, n=10, respectively; p<0.05). This pressure difference was dependent on intact autonomic transmission, since ganglionic blockade eliminated the significant difference between pressures in rats fed high (78 ± 5 mm Hg) and normal (85 ± 6 mm Hg) calcium diets. Second, high calcium intake augmented baroreceptor reflex inhibition of renal sympathetic nerve activity and heart rate during ramp increase in arterial pressure produced by infusion of phenylephrine. Reflex suppression of renal sympathetic nerve activity was twofold greater in rats fed the high (vs normal) calcium diet (−2.79 ± 0.25 vs −1.34 ± 0.14 %ΔΔ mm Hg, respectively; n = 9 rats per group; p<0.05). Third, high calcium intake did not attenuate vascular responsiveness, since pressor responses to norepinephrine and angiotensin II did not differ between rats fed high and normal calcium diets after ganglionic blockade. In conclusion, we found that high calcium feeding attenuated salt-induced hypertension in Dahl salt-sensitive rats by reducing the neural contribution to hypertension rather than by reducing vascular responsiveness to vasoconstrictor stimuli.

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KEY WORDS • calcium • salt • Dahl rats • hypertension • autonomic nervous system • sympathetic nervous system • phosphorus

Increasing information implicates calcium deficiency (hypocalcemia) in human and experimental hypertension and suggests that increased calcium intake may lower blood pressure by restoring normal calcium homeostasis. Efforts to explain these effects have concentrated thus far on actions of calcium on vascular smooth muscle. Little attention has been given to the relation of calcium to neural hypertensive mechanisms. Dahl salt-sensitive rats (DS) display multiple abnormalities in neural control of vascular resistance and arterial pressure, including alterations in baroreceptor function and central neural pressor mechanisms. In contrast, DS do not demonstrate alterations in membrane electrogensis or vascular reactivity to norepinephrine in prehypertensive or early hypertensive stages. Thus, the Dahl strain is a useful model of hypertension in which to determine whether a high calcium diet can attenuate hypertension by influencing neural in addition to vascular mechanisms.

Urinary calcium excretion is higher in DS than in Dahl salt-resistant rats (DR) fed a normal sodium diet. This increase is associated with transient hypocalcemia coincident with the onset of hypertension in DS. In addition, compared to DR, DS exhibit sustained hypercalciuria when sodium is supplemented in the diet. The foregoing observations raise the possibility that DS have alterations in calcium metabolism that might be involved in altered control of neurocirculatory

From the Veterans Administration Medical Center and Cardiovascular Center, Department of Medicine, College of Medicine, University of Iowa, Iowa City, Iowa.

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Address for reprints: Dr. Jacob D. Peuler, Cardiovascular Research Labs, 10W-20, Veterans Administration Medical Center, Iowa City, IA 52240.
mechanisms and arterial pressure. Based on these findings, we tested two hypotheses: first, that dietary calcium supplementation attenuates salt (NaCl)-induced hypertension in DS, and second, that such attenuation occurs by neural rather than vascular mechanisms.

**Methods**

Four-week-old female DS from the Brookhaven Laboratory (Upton, NY, USA) were fed diets (Costum formulations, ICN Biochemicals, Cleveland, OH, USA) that contained normal amounts of potassium (0.4% K), inorganic phosphorus (1.08% P), and other elements, but high amounts of sodium chloride (3.3% Na). The diets contained either high levels of calcium (4.0% Ca; n = 21) or normal levels of calcium (0.4% Ca; n = 21) as calcium carbonate. The National Research Council's third revised edition of Nutrient Requirements of Laboratory Animals (Washington, DC, 1978:16–19) was used to select these levels. When the rats were 10 weeks old, they were individually housed and their ad libitum intake of food and water was measured. For a separate small group of DS (n = 4) fed each diet in metabolism cages, 24-hour sodium output in both urine and feces (ashed) was measured by flame photometry.

When they were between 10 and 11 weeks of age, the awake and unrestrained rats' arterial pressure was monitored by tail catheter inserted under anesthesia (methohexital sodium, 40 mg/kg i.p.) two days before recording. A femoral venous catheter was inserted at the same time for injection of vasoactive agents. Baroreceptor reflex control of heart rate was determined in these rats by linear regression analysis of bradycardic response to intravenous injections of PE, norepinephrine (NE), and angiotensin II. These procedures were conducted in accordance with institutional guidelines for studies with conscious animals.

Three to four days later, after the rats recovered from ganglionic blockade, we studied baroreceptor reflex control of renal sympathetic nerve activity (RSNA) and heart rate in anesthetized rats. Anesthesia was induced with methohexital sodium (40 mg/kg i.p.) and maintained with chloralose (25 mg/kg/hr, intra-arterial infusion). The slow rate of intra-arterial infusion (0.01 ml/min) did not interfere with arterial pressure recording. The renal nerve was prepared for recording,25,26 and RSNA was recorded as impulses per second (Hz).22 as described previously. Arterial baroreceptor reflex inhibition of RSNA and heart rate was produced by linearly increasing infusion of PE (1–20 µg/kg/in/min i.v.), which was sufficient to induce slow pressor ramps of less than 1 mm Hg/sec for 2 to 2.5 minutes. Infusion of vehicle (0.02–0.40 ml/min) for this period of time did not increase MAP or decrease RSNA and heart rate. RSNA was corrected for background noise observed after ganglionic blockade. Linear regression analysis was employed to obtain best-fit (linearly correlated) baroreceptor reflex slopes describing dependencies of RSNA (percent of change from control) and heart rate (change in beats/min) on MAP (change in mm Hg) for each rat. These slopes were summarized for each group of rats to assess effects of dietary calcium.

At the end of these protocols, blood samples were taken from the rats for subsequent analysis of total plasma calcium, phosphorus, and magnesium concentrations.27,28 Plasma creatinine (Jaffé reaction) and monovalent cation (Na⁺, K⁺) concentrations (ion-selective electrodes) were determined by Beckman ASTRA 8 (Brea, CA, USA). Rats were killed with methohexital sodium and excision of the heart. Cardiac ventricles were weighed after flushing with saline and weighed again after drying for one week at 60°C.

Data are expressed as means ± SE. Statistical evaluations were performed with linear correlation and regression (slope) analyses and unpaired t tests as described previously.29 Statistical significance for group mean differences was considered as p < 0.05.

**Results**

Arterial pressures measured in conscious, unrestrained rats are shown in Figure 1. NaCl-induced hypertension in DS was attenuated (p < 0.05) nearly 30 mm Hg by the high calcium intake. Incidence of sudden death preceded by signs of stroke (seizures) or pulmonary edema was 0 of 21 DS fed the high calcium diet as opposed to 4 of 21 DS fed the normal calcium diet. MAP was substantially reduced by ganglionic blockade in both groups to levels that were no longer significantly different between awake DS fed high and normal calcium diets (see Figure 1). Heart rate was similar in DS on high versus normal calcium diet both before (429±12 vs 422±15 beats/min, respectively) and after ganglionic blockade (398±8 vs 397±13 beats/min, respectively).

**Figure 1.** Mean arterial pressure (MAP) in awake, unrestrained DS before (control) and after ganglionic blockade. Entries are means ± SEM. Numbers of rats are listed in the bottom of each bar.
Arterial pressor responses to adrenergic and nonadrenergic pressor agents (after ganglionic blockade) were not influenced by dietary calcium (Figure 2). Similarly, tachycardic responses to multiple doses of NE were not influenced by dietary calcium.

A typical PE ramp-induced change in MAP, RSNA, and heart rate in a chloralose-anesthetized DS is shown in Figure 3. Pressor ramps were slow, linear ($r > 0.97$), and similar for rats fed both high and normal calcium diets (0.46 ± 0.05 vs 0.51 ± 0.08 mm Hg/sec, $n = 9$ rats per group). Baroreceptor reflex inhibition of RSNA and heart rate was significantly augmented in high calcium versus normal calcium–fed DS (Figures 4 and 5). This enhancement in reflex control of RSNA was particularly evident near arterial pressures exhibited by these rats while awake (4 days prior to study under anesthesia) despite similarity (parallelism) in the slopes for reflex control of RSNA at much lower arterial pressures (see Figure 4). The increase in baroreceptor reflex control of heart rate was evident in both conscious and chloralose-anesthetized rats (see Figure 5). Linear correlation coefficients ($r$) relating decreases in RSNA and heart rate to increases in MAP in each rat were all statistically significant ($p < 0.05$), ranging from −0.91 to −0.98.

Total plasma calcium concentrations were higher and phosphorus concentrations were lower in high calcium-fed DS (Table 1). Plasma magnesium, creatinine, sodium, and potassium levels were not altered.

At 10 weeks old, ad libitum intakes were similar in DS on high and normal calcium diets (food, 17 ± 1 vs 14 ± 1 g/day; water, 97 ± 6 vs 89 ± 8 ml/day, respectively; $n = 8$ rats per group). Similar results from DS ($n = 4$) fed high and normal calcium diets in metabo-
Arterial and cardiopulmonary baroreceptor reflex control of autonomic function is impaired in DS compared to DR even in prehypertensive rats fed a very low NaCl diet. Arterial baroreceptor reflex impairment has been traced to the level of baroreceptor discharge in DS. High calcium intake may have directly corrected baroreceptor dysfunction in our high NaCl-fed DS, an action that could have contributed to the antihypertensive effect of calcium. However, threshold pressure for baroreceptor discharge is normally increased when ionized extracellular calcium concentration is acutely elevated from less than to greater than normal (physiological) plasma levels. Furthermore, recent work suggests that this acute effect of calcium on baroreceptor threshold is enhanced in prehypertensive DS compared to DR. Thus we might expect that, if ionized plasma calcium concentration were increased (even chronically) during high calcium intake, then baroreceptor function would not be improved in DS. In another, ionized concentrations of plasma sodium and potassium, which could also influence baroreceptor activity, were not altered by high calcium intake (see Table 1). Thus, the augmentation of baroreceptor reflex function in DS fed the high calcium diet in our study was either secondary to attenuation of hypertension or related to some effect of the high calcium diet other than increased plasma calcium concentration per se.

Calcium deficiency may be present in DS. The high calcium diet elevated total plasma calcium from approximately 8 to 10 mg/dl (see Table 1). The latter and not the former value is similar to the normal total plasma calcium concentration reported for Sprague-Dawley rats, the parent strain of Dahl rats. Therefore, the DS fed high NaCl and normal calcium in our study may have been hypocalcemic. Compared to DR, DS are reported to be transiently hypocalcemic in early development when fed a normal sodium diet. We suspect that, compared to DR, DS fed a high sodium diet will be chronically hypocalcemic. Reduced plasma calcium (total and ionized), along with increased parathyroid hormone concentrations, are reported for human hypertensive subjects and for spontaneously hypertensive rats (SHR). The relative weight of the parathyroid gland is also increased during development of hypertension in young SHR. The role of these abnormalities in development and maintenance of hypertension is presently the subject of considerable investigation and debate.

Two factors of renal origin, hypercalciuria and hyperphosphatemia, may contribute to hypocalcemia in DS. First, the addition of high sodium to a normal calcium diet was dependent on intact autonomic transmission, since it was abolished by ganglionic blockade (see Figure 1). Second, baroreceptor reflex control of heart rate and renal nerve activity was significantly augmented in rats fed high versus normal calcium diets (see Figures 4 and 5). Third, after ganglionic blockade, arterial pressor responsiveness to adrenergic and nonadrenergic vasoconstrictor agents was similar for both groups (see Figure 2).

Autonomic neural mechanisms are essential to development of NaCl-induced hypertension in DS. Sympathetic ablation prevents or abolishes this hypertension. A number of neural alterations have been implicated. On the high NaCl diet, DS but not DR demonstrate enhanced vasoconstrictor response to sympathetic nerve stimulation without augmented vascular reactivity to NE. DS also display augmented responses to central pressor stimuli. We have not yet investigated effects of supplemental dietary calcium on these abnormalities.

<table>
<thead>
<tr>
<th>Element of plasma</th>
<th>Normal</th>
<th>High</th>
</tr>
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<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.9 ± 0.3</td>
<td>9.7 ± 0.5*</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>11.0 ± 0.9</td>
<td>6.9 ± 0.7*</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.4 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.27 ± 0.11</td>
<td>0.34 ± 0.05</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>153.5 ± 1.3</td>
<td>152.9 ± 2.1</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.2 ± 0.5</td>
<td>4.1 ± 0.5</td>
</tr>
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Entries are means ± SEM for 7 to 8 rats per value. *p < 0.05, high vs normal calcium diet.
ionized fraction of calcium. Plasma concentration of phosphate (phosphorus) in our DS on the normal calcium diet was notably higher (see Table 1) than that reported for Sprague-Dawley and other rats on normal calcium diets. This may be due to the hypophosphatemia that accompanies hypercalcemia in DS. In our study, high calcium intake appeared to restore normal plasma phosphate concentration (see Table 1), presumably by reducing intestinal absorption of phosphate. Thus, dietary calcium supplementation with an increased ratio of calcium to phosphate intake may be necessary to normalize ionized extracellular calcium concentration completely in the DS.

Conceivably, hyperphosphoremia in our DS on normal calcium diet may be due to renal glomerular insufficiency secondary to hypertension after 6 to 7 weeks of high NaCl intake. Indeed, histological evidence of renal lesions has been reported to appear as early as 2 weeks into regimens of high NaCl feeding. However, indices of renal function, such as inulin and p-aminohippurate clearance and plasma creatinine concentration have all been reported within normal limits in DS after 5 to 7 weeks of high NaCl feeding. In our study, plasma creatinine levels in both groups of DS on the high NaCl diet (see Table 1) were clearly not elevated in comparison to normal values reported for DS or DR on the low NaCl diet. Thus, it seems more reasonable that hyperphosphoremia as well as hypercalcemia in our DS on high NaCl and normal calcium diet were due to hypophosphatemia and hypercalcemia associated with intrinsic reduction in renal responsiveness to parathyroid hormone rather than to decreased glomerular filtration.

We can only speculate on mechanisms connecting hypocalcemia to autonomic neural dysfunction or, conversely, mechanisms whereby normalization of calcium homeostasis by high calcium intake may correct neural dysfunction. For example, certain neural membranes in DS may be particularly sensitive to destabilization in an environment of reduced calcium. However, we are unaware of any experimental evidence in support of this notion. A more plausible explanation is that altered hormonal activity, such as elevated plasma parathyroid hormone secondary to chronic hypocalcemia, might facilitate calcium influx in sympathetic nerve endings (as may occur in aortic and renal tissue) and thereby facilitate NE release despite the direct dependence of NE release on extracellular calcium concentration. Indeed, facilitation of calcium influx and NE release could be the reason for the enhanced vasoconstrictor response to sympathetic nerve stimulation reported in DS but not DR fed a high NaCl diet. Normalization of parathyroid hormone levels secondary to normalization of extracellular calcium concentration by dietary calcium supplementation would then restore normal NE release.

A recent report suggested that phosphorus deficiency mediated the antihypertensive action of a high calcium diet in the SHR, presumably by reducing the availability of high energy phosphate for myocardial and vascular smooth muscle performance. Phosphorus deficiency due to dietary phosphate restriction was found to lower both cardiac output and blood pressure in Sprague-Dawley rats. However, this was associated with reduced pressor responsiveness to NE and angiotensin II and with increased sympathetic tone. Such vascular and neural actions were the exact opposite of what we observed in DS on high calcium diet, and the plasma phosphorus reduction we observed (see Table 1) did not reach a level indicative of deficiency. Nonetheless, reduced plasma phosphorus may have neural and even vascular effects in DS that are distinct from effects in Sprague-Dawley rats.

High calcium intake reduced body weight in the young DS we studied. At 10 weeks of age, their food consumption was not reduced, although it may have been before 10 weeks. This raises the possibility that the antihypertensive action of high dietary calcium is related to less weight gain prior to 10 weeks of age. Less weight gain during a high calcium diet is also reported to occur in young SHR. In the SHR, this effect of a high calcium diet evidently appears only in young, developing rats in which the antihypertensive action of calcium is either absent or minimal. It is not reported to occur in adult, mature SHR in which calcium-induced attenuation of hypertension is most evident. These observations suggest that the antihypertensive action of supplemental calcium in genetically hypertensive rats is independent of weight loss.

Similar high urinary sodium and residual fecal sodium in DS ingesting similar normal or high calcium diets indicated that the antihypertensive action of supplemental calcium could not be related to reduced sodium absorption from the gastrointestinal tract.

Other investigators have reported that supplemental dietary calcium carbonate does not reduce blood pressure in NaCl-fed DS, whereas calcium phosphate produces a small reduction in blood pressure. In the present study, we found that supplemental dietary calcium carbonate had a considerable antihypertensive effect. The reason for our different finding is not clear, but it may be related to the fact that we measured blood pressure in awake animals, whereas Tobian and co-workers measured it while the animals were under ether anesthesia.

The finding that cardiac hypertrophy and frequency of sudden death were less in DS fed a high calcium diet suggests that the antihypertensive action of supplemental calcium was appreciable and probably present throughout the 6 to 7 weeks of high calcium feeding in our study.

The major conclusion from this study is that high calcium intake attenuates the development of NaCl-induced hypertension in DS and appears to do so by reducing the neural contribution to hypertension rather than by reducing pressor responsiveness to vasoconstrictor stimuli. In addition, evidence from this study and from others suggests that altered calcium homeostasis that is exaggerated by high salt intake and accompanied by altered phosphorus homeostasis may contribute to development of salt-induced hypertension in DS.
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J D Peuler, D A Morgan and A L Mark

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