Dietary Calcium Supplementation Restores Pressure Natriuresis Responses in Dahl-S Rats

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Calcium supplementation prevents hypertension in Dahl S (DS) rats. Because abnormal pressure natriuresis may contribute to the development of hypertension, we examined the effect of calcium on pressure natriuresis. DS and Dahl R (DR) rats maintained on a 4% sodium diet containing either 0.5% or 2% calcium for 4 weeks were anesthetized; sodium excretion, renal blood flow, and inulin clearance were determined at perfusion pressures of 100, 125, and 156 mm Hg. Inulin clearance and renal blood flow were not different between groups. Sodium excretion increased with increasing renal perfusion pressure in all groups. The slope of the line relating renal perfusion pressure to sodium excretion was greater (P < .05) in DR rats than in DS rats on normal calcium intakes. High calcium intake normalized the slope of the line relating renal perfusion pressure to sodium excretion in DS rats, but had no effect on DR rats. Thus, dietary calcium supplementation normalizes the blunted pressure natriuresis response in the DS rat and may contribute to the prevention of hypertension. Am J Hypertens 1995;8:615-621

KEY WORDS: Diet, calcium, Dahl rat, pressure natriuresis, salt-sensitive hypertension.

Dietary calcium supplementation has been found to improve blood pressure in hypertensive humans as well as in animal models of hypertension.1-9 The mechanisms for this effect are unclear. However, both human and animal studies suggest that calcium supplementation may be most effective in those forms of hypertension that are salt sensitive.1,3,5,8 Many of these conditions are characterized by an abnormal relationship between renal perfusion pressure and sodium excretion such that at equivalent renal perfusion pressures, kidneys from hypertensive individuals excrete sodium chloride less well than do kidneys from salt-resistant individuals. In the Dahl salt-sensitive rat (DS), this abnormality is intrinsic to the kidney and predates the onset of the elevation in blood pressure.10 In addition, the observation that hypertension can be induced in normotensive salt-resistant rats by a transplant of a kidney from a hypertensive-prone salt-sensitive rat suggests that this renal abnormality may be etiologic in the development of hypertension.11 From these considerations, it seems reasonable to postulate that at least part of the antihypertensive effect of calcium may occur through an improvement in the blunted pressure natriuresis relationship found in salt-sensitive hypertension. The current study was designed to examine this hypothesis by determining pressure natriuresis responses in DS rats maintained on a 4% sodium diet containing either 0.5% (normal) or 2% (high) calcium concentrations. Dahl salt-resistant rats (DR) maintained on the same diets were examined for comparison.
METHODS

Male DS and DR Dahl-Rapp rats (Harlan Sprague-Dawley, Indianapolis, IN), 4 to 5 weeks of age, were maintained on tap water and standard rodent chow (20 mmol/L sodium/g chow) for 72 h. All animals were housed according to Institutional guidelines, and the protocol was approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Baseline systolic blood pressures were obtained by tail-cuff plethysmography. Rats were then placed on 4% sodium chow containing either 0.5% (normal) calcium or 2% (high) calcium (ICN Biomedicals, Costa Mesa, CA) and given tap water ad lib. This calcium intake was slightly more than that found to be efficacious at attenuating the rate of development of hypertension in spontaneously hypertensive rats on high sodium intakes.12 The dietary calcium content is in the middle range of dietary calcium supplements used by other investigators to study blood pressure effects of calcium.5,15,14 Systolic blood pressures were monitored weekly. After 4 weeks on the diet, a subset of the DS rats was placed in individual metabolic cages for determination of 24-h urinary calcium excretion. These rats were then killed for determination of serum-ionized calcium concentrations. The remainder of the rats were anesthetized with intraperitoneal injections of 80 mg/kg of 5-sec-butyl-5-ethyl-2-thiobarbituric acid (Inactin, Promonta, Hamburg, Germany). Animals were placed on a thermostatically controlled animal table and body temperatures maintained at 37°C with a servoactivated controller (Vestavia Scientific Co., Vestavia Hills, AL). After a tracheostomy, PE50 polyethylene catheters were placed into the jugular veins and femoral arteries to allow for continuous measurement of mean arterial pressure above and below the renal arteries. The arterial catheters were connected to transducers (model 4-327-I; Trans-America DeLaval, Pasadena, CA), and arterial pressure was continuously monitored on a polygraph (LaFayette Instruments, LaFayette, IN). A flanged PE50 polyethylene catheter was placed into the bladder for urine collection. The aorta was exposed through a midline abdominal incision and ultramicroclamps were placed above and below the renal arteries. Ligatures were then placed loosely around the superior mesenteric and celiac arteries. At the start of surgery, isotonic Ringer’s solution containing 5% polyfructosan (Inutest, Laevasan Gesellschaft, Linz, Austria) and 1% para-aminohippuric acid (PAH) (Sigma Chemical Co., St. Louis, MO) was administered through the right jugular venous catheter at a rate of 1.2 mL/h and maintained throughout the study. To compensate for the reduction in plasma volume attendant on abdominal surgery, rats received a 1.2 mL/100 g body weight infusion of 5% albumin Ringer’s solution during the surgical procedure. In addition, all rats received an infusion of 154 mmol/L sodium chloride containing 1% bovine serum albumin at a rate of 100 μL/min throughout the study. After completion of the surgical procedure, a 40-min surgical recovery period was allowed. Four groups of rats were examined: group 1 (n = 5), DS rats maintained on normal calcium chow; group 2 (n = 7), DS rats maintained on high calcium chow; group 3 (n = 7), DR rats maintained on normal calcium chow; and group 4 (n = 8), DR rats maintained on high calcium chow.

After the 40-min surgical recovery period, renal perfusion pressure was reduced 30 mm Hg below baseline values by tightening the aortic clamp above the renal arteries. Renal perfusion pressure was approximately 125 mm Hg in group 1, and 100 mm Hg in groups 2, 3, and 4. After a 15-min equilibration period, urine was collected over a 40-min experimental period for excretion rates of sodium, inulin, and PAH. Blood samples were obtained at the beginning and end of this experimental period for plasma concentrations of sodium, inulin, and PAH. The red cells from these samples were resuspended in a small amount (400 μL) of 154 mmol/L sodium chloride and returned to the animal. In group 1 animals, the aortic clamp was tightened to further reduce renal perfusion pressure to 100 mm Hg. After a 15-min equilibration period, a 40-min clearance period was again performed. Plasma samples were obtained as described. In all groups, the aortic clamps were then released, allowing renal perfusion pressure to return to baseline values. After a 15-min equilibration period, urine and blood samples were collected. In groups 2, 3, and 4, pressure was then increased approximately 25 mm Hg above baseline values by tightening the aortic clamp below the renal arteries, and the snares on the celiac and superior mesenteric arteries. These maneuvers increased renal perfusion pressures to approximately 159 ± 3 mm Hg in groups 2, 3, and 4. After a 15-min stabilization period, urine and blood were again collected over a 40-min interval. To assure that the order of collection did not influence the findings, the order of pressure adjustment was varied in some rats from each group. At the end of the final urine collection, rats were killed by exsanguination while still under anesthesia and the kidneys removed and weighed.

Analytical Techniques Urine flow rate was determined by change in weight of preweighed vials. Inulin concentration in urine and plasma was determined by the diphenylamine method of Walser et al.13 Sodium concentration in urine and plasma samples was determined using a flame photometer (model 943; Instrumentation Laboratories, Lexington, MA). Concentration of PAH in plasma and urine was
determined according to the method of Waugh and Beall.16

Analysis of Data  Determination of the concentration of inulin, PAH, and sodium in blood and urine and urine flow rate permitted calculation of glomerular filtration, PAH clearance, and urinary sodium excretion rates according to standard expressions.17 Renal blood flow was calculated from PAH clearance, renal PAH extraction, and hematocrit as described previously.17 A value for PAH extraction of 85% was assumed. This is consistent with values measured in Sprague-Dawley rats by several investigators and confirmed in our laboratory.17,18 Urinary excretion data, renal blood flow, and glomerular filtration rate were all expressed per gram kidney weight. Fractional urinary sodium excretion was calculated as the ratio of the clearance of sodium to the clearance of inulin.

Statistical significance between values determined at various perfusion pressures within a single animal was determined using analysis of variance for repeated measures followed by Bonferroni's t test.19 Statistical significance between groups was determined using one-way analysis of variance and Bonferroni's t test. All values are reported as the mean ± the standard error of the mean for each group.

RESULTS

Systolic Blood Pressures and Calcium Excretion Rates in Conscious Rats  Systolic pressures measured by impedance plethysmography were 131 ± 4 mm Hg in DS rats and 121 ± 4 mm Hg in DR rats (P < .05) before initiation of the high and normal calcium diets (Figure 1).

After 1 week on 4% sodium intake, systolic blood pressure had increased to 144 ± 6 mm Hg in group 1 rats maintained on normal calcium chow, and eventually rose to 153 ± 3 mm Hg after 4 weeks on the diet. In DS rats begun on the high calcium diet, blood pressure did not increase at week 1 (129 ± 7 mm Hg) and increased to only 133 ± 2 mm Hg at week 4. Blood pressure was unchanged in DR rats receiving either high or normal calcium diets over the 4-week interval.

Serum ionized calcium was not different between DS rats ingesting high calcium and normal calcium diets (Figure 2).

Twenty-four-hour urinary calcium excretion was fourfold greater (P < .05) in DS rats on high calcium than on normal calcium intake (Figure 2). This reflects the differing dietary calcium contents (0.5% v 2%). In addition this finding confirms that food intake and thus dietary sodium intake was approximately equivalent on both calcium diets.

Hemodynamic Parameters in Anesthetized Dahl Rats  Mean arterial pressure at the conclusion of the surgical recovery period was 156 ± 5 mm Hg in group 1 rats and 134 ± 3 mm Hg in group 2 rats (P < .05). Mean arterial pressures at the conclusion of the surgical recovery period were 129 ± 3 mm Hg in group 3 rats and 130 ± 3 mm Hg in group 4 rats. Mean arterial pressure did not change in any of these four groups during the 40-min stabilization period.

Table 1 shows the hemodynamic and sodium excretion data obtained in each of the four groups when examined at the renal perfusion pressure equivalent to their ambient renal perfusion pressure recorded in the postsurgical stabilization period.

Mean arterial pressures reported in Table 1 were those actually recorded during this urine collection interval, and thus differ slightly from the values reported above. There were no differences in renal blood flow between any of the experimental groups when examined at their postsurgical stabilization period renal perfusion pressure. Renal blood flow tended to be lower in DS rats ingesting a normal calcium diet when compared to the other groups. This difference, however, did not reach statistical significance at any perfusion pressure examined. Likewise, there were
TABLE 1. MEAN ARTERIAL PRESSURE, RENAL BLOOD FLOW, INULIN CLEARANCE, AND SODIUM EXCRETION IN DAHL RATS AT THEIR AMBIENT RENAL PERFUSION PRESSURES ON 0.5% OR 2.0% CALCIUM INTAKES

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<th>MAP (mm Hg)</th>
<th>RBF (ml/min/gkw)</th>
<th>C\textsubscript{in} (\textmu L/min/gkw)</th>
<th>UNaV (nmol/min/gkw)</th>
<th>PNa (mmol/L)</th>
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<tr>
<td>Dahl S (n = 5)</td>
<td>156 ± 2</td>
<td>4.73 ± 0.65</td>
<td>750 ± 29</td>
<td>4084 ± 301</td>
<td>154 ± 2</td>
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<tr>
<td>0.5% calcium</td>
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<td>Dahl S (n = 7)</td>
<td>129 ± 2*</td>
<td>6.09 ± 1.04</td>
<td>860 ± 112</td>
<td>7660 ± 1062</td>
<td>155 ± 3</td>
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<tr>
<td>2.0% calcium</td>
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<td>Dahl R (n = 7)</td>
<td>125 ± 2*</td>
<td>6.25 ± 0.71</td>
<td>930 ± 77</td>
<td>5274 ± 625</td>
<td>154 ± 2</td>
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<td>0.5% calcium</td>
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<tr>
<td>Dahl R (n = 8)</td>
<td>131 ± 3*</td>
<td>6.20 ± 0.59</td>
<td>951 ± 89</td>
<td>5852 ± 868</td>
<td>153 ± 2</td>
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<td>2.0% calcium</td>
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Values are mean ± SE.
\*P < .05 v Dahl S 0.5%.

MAP, mean arterial pressure; RBF, renal blood flow; C\textsubscript{in}, inulin clearance; UNaV, absolute urinary sodium excretion; PNa, plasma sodium concentration; gkw, gram kidney weight.

no significant changes in renal blood flow elicited during changes in renal perfusion pressure within any of the four groups (data not shown). Inulin clearances were not different between any of the experimental groups when examined at each group's post-stabilization period renal perfusion pressure (Table 1). Inulin clearances tended to be lower in DS rats ingesting the normal calcium diet when compared to the other rat groups. This difference, however, did not reach statistical significance. There were no significant changes in inulin clearance elicited in any of the experimental groups during alterations in renal perfusion pressure (data not shown).

Sodium Excretion Rate in Dahl Rats on Varying Calcium Intakes Absolute and fractional urinary excretion rates were not different between groups at their ambient renal perfusion pressures (Table 1). Absolute urinary sodium excretion increased (P < .05) during increases in renal perfusion pressure in all groups (Figure 3, top). Fractional urinary sodium excretion increased (P < .05) with increasing renal perfusion pressure in DS rats on normal and high calcium intakes (groups 1 and 2), in DR rats on normal calcium intake (group 3), and during increases in renal perfusion pressure between 100 and 131 mm Hg in DR rats on high calcium intake (group 4) (Figure 3, bottom). The increase in fractional sodium excretion that occurred when perfusion pressure was increased from 131 to 160 mm Hg in DR rats on high calcium intake (group 4) did not reach statistical significance. The slope of the line relating renal perfusion pressure to absolute urinary sodium excretion was significantly greater (P < .05) in DS rats on high calcium intake when compared to DS rats on normal calcium intake (216 ± 30 mmol/min/g kidney weight/mm Hg v 61 ± 8 mmol/min/g kidney weight/mm Hg). The slope of the line relating renal perfusion pressure to urinary sodium excretion in DR rats ingesting high calcium intake was not different from that found in DR rats ingesting a normal calcium in-
take. There was also no difference between the slope of the relationship relating renal perfusion pressure to urinary sodium excretion between high calcium and normal calcium diet DR rats.

**DISCUSSION**

The current study confirms previous reports that hypertension can be prevented in DS rats exposed to a high sodium intake by increasing the dietary calcium content. This study extends these observations by demonstrating that the prevention of hypertension during dietary calcium supplementation is associated with improvement in the blunted relationship between renal perfusion pressure and urinary sodium excretion that usually characterizes prehypertensive and hypertensive DS rats. As is demonstrated in Figure 3, the slope of the relationship relating renal perfusion pressure to urinary sodium excretion is greater in DS rats on a high calcium intakes than in DS rats on a normal calcium intake and not different from that observed in DR rats. There was no difference in the slope of the relationship relating renal perfusion pressure to urinary sodium excretion between DR rats on a high and normal calcium intake. Thus, the effect of dietary calcium supplement is specific to the DS strain.

A number of mechanisms have been proposed to account for the antihypertensive effects of dietary calcium supplementation. Dietary salt loading has been shown to induce abnormalities in calcium homeostasis both directly and through alterations in calcium-regulating hormones, whereas the severity of these abnormalities has been related to the dietary sodium content. Dietary calcium supplementation has been shown to protect against the development of salt-sensitive hypertension in both humans and animals. Thus dietary calcium supplementation may prevent the dysregulation of calcium homeostasis induced by dietary salt loading. On the other hand, dietary calcium also prevents the development of hypertension in spontaneously hypertensive rats and rats with renovascular hypertension. These models of hypertension do not depend on increased dietary sodium intake for their pathogenesis and thus sodium chloride-induced abnormalities in calcium homeostasis are unlikely to occur. Dietary calcium supplementation may have direct antihypertensive effects that are unrelated to dietary sodium chloride intake as well.

Increasing dietary calcium intake could alter the susceptibility to hypertension in several ways. Changes in dietary calcium could alter systemic vascular resistance either directly or through changes in calcitropic hormones such as 1,25 dihydroxy vitamin D and the circulating pressor substance parathyroid hypertensive factor. Increases in calcium intake could also alter activity of the sympathetic nervous system. The relative importance of these individual events to the overall reduction in blood pressure observed during dietary calcium supplementation in hypertensive subjects remains to be determined. However, the usual response to a reduction in renal perfusion pressure is an increase in renal sodium reabsorption mediated through the pressure natriuresis relationship. Thus, effects of dietary calcium on systemic factors that do not also change renal sodium handling should result in sodium retention, vascular volume expansion, and an eventual return of blood pressure to hypertensive levels. Consequently, for the antihypertensive effect of calcium to be sustained, any effect of calcium on systemic vascular resistance must also be associated with a change in the relationship between renal perfusion pressure and urinary sodium excretion. Whether this change in pressure natriuresis is the dominant event accounting for calcium's antihypertensive effect requires further study.

How dietary calcium supplementation improves pressure natriuresis is unclear. It is unlikely that the improvement in pressure natriuresis induced by dietary calcium supplementation is the result of the prevention of hypertension per se. Abnormal pressure natriuresis can be demonstrated in young DS rats before the development of hypertension. In addition long-term administration of a low sodium diet that prevents the development of hypertension does not improve abnormal sodium handling in these animals. Thus, the improved pressure natriuresis that follows dietary calcium supplementation must result from effects of calcium on some determinant of renal sodium handling.

Dietary calcium supplementation could alter pressure natriuresis through several mechanisms. Increases in dietary calcium intake could reduce renal vascular resistance and improve hemodynamic factors important for renal sodium excretion. Oparil and associates have demonstrated that calcium supplementation reduces the increase in sympathetic nervous system outflow that occurs in spontaneously hypertensive rats after an increase in dietary sodium intake. Dietary calcium supplementation could also have effects on renal vascular resistance through changes in parathyroid hormone hypertensive factor or 1,25 dihydroxy vitamin D. In the current study both renal blood flow and glomerular filtration rate were numerically, but not statistically, greater in DS rats on the high calcium diet compared with DS rats on the normal calcium diet. Consequently, the improvement in sodium excretion observed in the current study could be attributable to increases in filtered sodium load or changes in intrarenal hemodynamic factors controlling sodium excretion.

On the other hand, excessive tubular sodium chloride reabsorption has been reported at several nephron locations in DS rats. Increasing calcium in-
take has been reported to decrease sympathetic nervous outflow, reduce α1-adrenergic receptor activity, reduce expression of proximal tubular angiotensin II receptors, and stimulate atrial natriuretic peptide production.24,32-34 These events alone or in combination could directly decrease tubular sodium transport and, consequently, improve pressure natriuresis. Dietary calcium supplementation could also have direct effects on cellular transport. Resnick35 has suggested that changes in the intracellular concentration of sodium, calcium, and magnesium in tubular cells accounts for the altered renal tubular sodium handling in hypertension. To the extent that dysregulation of calcium homeostasis contributes to abnormal intracellular ion concentrations, this hypothesis could explain both the initial derangement in intracellular electrolyte concentration that leads to sodium retention as well as provide a mechanism for dietary calcium supplementation to restore tubular cellular transport to normal. Furthermore, such a hypothesis would be consistent with the observation that abnormal sodium chloride reabsorption is present in isolated perfused kidneys from prehypertensive DS rats, and in primary renal cell cultures from DS rats where extrarenal influences on sodium transport are not operative.36,37 Calcium supplementation could also improve pressure natriuresis through stimulation of nitric oxide production.38 Recently, we have shown that increasing nitric oxide production also normalizes pressure natriuresis in DS rats.39

Where along the nephron calcium exerts its effects remains to be determined. If changes in dietary calcium intake effect sodium-potassium-ATPase activity, sodium transport could be altered at almost any nephron location. Calcium has been clearly shown to alter sodium reabsorption in the proximal tubule, loop of Henle, and cortical collecting tubules.40-42 Abnormal sodium reabsorption has been documented to occur in the DS rats in the latter two locations.27,29,31,37 Additional studies determining both the mechanism and location of the effect of calcium on sodium reabsorption should be of great interest.

In summary, the current study demonstrates that a high calcium diet not only prevents hypertension, but also restores the blunted pressure natriuresis response that usually characterizes DS rats. The current data suggest that both hemodynamic and tubular mechanisms are improved in calcium-supplemented DS rats, but the contribution of each of these events to the overall improvement in sodium handling remains to be determined.

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