Aggravation of Salt-Induced Hypertension in Dahl Rats by 2% Supplemental Dietary Calcium

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There is considerable interest in the antihypertensive potential of supplemental dietary calcium in salt-sensitive hypertension. Previously we reported that very high dietary calcium (4.0% vs. 0.4%) lowers mean arterial pressure in Dahl salt-sensitive (DS) hypertensive rats. However, we have recently observed that more moderate calcium supplementation (2.0% vs. 0.4%) increases mean arterial pressure in DS rats. To further evaluate the pressor action of 2.0% versus 0.4% calcium, we tested for effects of 2.0% calcium in female DS rats fed low (0.2%), moderate (1.0%), and high (2.7%) sodium and in Dahl salt-resistant (DR) rats fed high sodium from 6 to 12 weeks old (n=10–13 rats per group). At 12 weeks, 2.0% calcium increased mean arterial pressure and the cardiac ventricular weight/body weight ratio in DS rats fed high sodium (p<0.05) but not in DS rats fed low or moderate sodium or in DR rats fed high sodium. Ganglionic blockade decreased mean arterial pressure in all groups but failed to abolish or attenuate the difference in mean arterial pressure between high sodium-fed DS rats on 2.0% and 0.4% calcium diets. In the same DS rats fed a high sodium diet, 2.0% calcium increased systemic pressor responsiveness to graded norepinephrine administration after ganglionic blockade. Thus, 2.0% supplemental calcium intake enhances salt-induced hypertension in DS rats. This prohypertensive action of 2.0% calcium is dependent on a critically high level of between 1.0% and 2.7% sodium in the diet. The mechanism of this prohypertensive action of 2.0% calcium does not appear to be either increased sympathetic neural activity or increased responsiveness to norepinephrine since it remains intact after ganglionic blockade. The present study provides evidence that dietary calcium supplementation has the potential for increasing arterial pressure even in a model of hypertension induced by high dietary salt. (Hypertension 1989;13:929-934)
this action was mediated by increased sympathetic neural support of arterial pressure since neurogenic mechanisms contribute significantly to development of hypertension during high sodium intake in DS rats. Third, we examined systemic pressor responsiveness to vasoconstrictor stimulation with exogenous norepinephrine (NE) since pressor responsiveness was slightly enhanced in DS rats by very high (4.0%) calcium intake in our previous experiment. Finally, we examined circulating levels of calcium (total and ionized), phosphorus, and magnesium to determine if the effects of 2.0% calcium on arterial pressure in the two strains with different sodium intakes would correlate with the changes in circulating levels of calcium, phosphorus, and magnesium.

Materials and Methods
Female DS rats (n=73) from the Brookhaven Laboratory (Upton, New York) were fed low (0.2%), moderate (1.0%), and high (2.7%) sodium diets (ICN Biochems., Cleveland, Ohio) from 6 to 12 weeks of age. An age-matched group of female DR rats (n=20) were fed high sodium diet (2.7%). All sodium was in the form of NaCl. Potassium was 0.5% in all diets and in the form of KCl and potassium phosphate. Half the rats in each group were fed 0.4% calcium, and half were fed 2.0% calcium. The calcium in the 0.4% diet was in the form of calcium phosphate, and the calcium in the 2.0% diet was in the form of calcium phosphate plus calcium carbonate. Total phosphorus was 0.9% and magnesium was 0.1% in all diets. These were fortified phosphorus and magnesium levels as recommended to minimize risk of their deficiency when rats were chronically fed diets containing more than 1.0% calcium. Before 6 weeks of age, all rats were fed a standard ICN rat diet containing 0.62% calcium and 0.16% sodium.

At 12 weeks old, each rat was individually housed to estimate 24-hour food intake and then anesthetized with methohexital sodium (Brevital, 40 mg/kg i.p.) and instrumented with an abdominal aortic catheter through the ventral tail artery for direct monitoring of arterial pressure and with a femoral venous catheter for injection of vasoactive agents. Three days later each rat was monitored for a number of hours to obtain an accurate, reliable estimate of MAP in the awake, resting state. Subsequently, a ganglionic blocking agent (Ecolid, 5 mg/kg i.v.) was administered to evaluate the degree of sympathetic neural support of MAP, followed by a series of bolus injections of NE to assess systemic pressor responsiveness. After 4 days of recovery from ganglionic blockade, precise body weights were obtained at 8:00 AM, and aortic blood samples (4 ml each) were obtained anaerobically under light methohexital (Brevital) anesthesia (10–20 mg/kg i.v.) to measure ionized calcium in heparinized blood and other chemistries later from stored plasma. Animals were killed with additional methohexital (200 mg/kg i.v.). Cardiac ventricles were removed, carefully trimmed of atria, and weighed for calculation of the ventricular weight/body weight ratio.

Heparinized blood samples were chilled and analyzed within 1–2 hours after withdrawal for ionized calcium (ion-selective electrode, NOVA 2, Newton, Massachusetts), pH (ion-selective electrode, pH/blood gas analyzer, Instrumentation Laboratory, Lexington, Massachusetts), and hematocrit. Plasma obtained by centrifugation of the remaining blood was stored (−70°C) and analyzed later for total calcium (CPC reaction), phosphorus (ammonium molybdate reaction), magnesium (calmagite reaction), creatinine (Jaffe end-point reaction), sodium, and potassium (ion-selective electrodes). Data are expressed as mean±SEM. Statistical evaluations were performed by analysis of variance and subsequent multiple mean comparisons, with emphasis directed toward differences related to the level of dietary calcium. Differences were considered significant at p<0.05.

Results
Initial and final body weights did not differ between groups fed 0.4% and 2.0% calcium (Table 1). Estimates of food intake yielded similar results. Ad libitum food consumption measured on the day before cannulation surgery at 12 weeks was similar for rats fed 0.4% versus 2.0% calcium diets: 12.9±1.0 versus 12.5±0.9 g/day for DS rats fed 0.2% sodium, 16.6±0.4 versus 16.8±0.4 g/day for DS rats fed 1.0% sodium, 13.7±0.9 versus 15.3±0.7 g/day for DS rats fed 2.7% sodium, and 13.5±0.9 versus 15.2±0.8 g/day for DR rats fed 2.7% sodium, respectively (n=9–10 rats per group).

The 2.0% calcium diet did not affect MAP in DR rats fed high (2.7%) sodium or in DS rats fed low (0.2%) or moderate (1.0%) sodium but significantly increased MAP in DS rats fed high (2.7%) sodium diets (Figure 1, left panel). Cardiac ventricular weight/body weight ratios were also significantly increased by the 2.0% calcium intake in DS rats fed high sodium (Figure 2). Ganglionic blockade decreased MAP in all groups but failed to abolish or attenuate the difference in MAP between high sodium–fed DS rats on 2.0% and 0.4% calcium diets (Figure 1, right panel).

Assessed after ganglionic blockade, pressor responses to graded intravenous injections of NE were significantly enhanced by 2.0% calcium intake in DS rats fed high sodium (Figure 3). This enhancement was significant (p<0.05) both in terms of the slope of the relation (dMAP divided by dlog NE dose: +73 vs. +60, 2.0 vs. 0.4% calcium diet) over the entire NE dose range and at the higher doses of NE that raised MAP levels to values approximating the resting levels recorded before ganglionic blockade. This effect of 2.0% calcium was not observed in any other groups of rats (Figure 3).
Neither ionized nor total circulating calcium was significantly increased by feeding 2.0% calcium for 6 weeks to DS and DR rats (Table 1). Blood pH and hematocrit were also unaffected by 2.0% calcium feeding. Plasma phosphorus was decreased significantly (12-16%) by 2.0% calcium, but the magnitude of this decrease did not differ in the four groups. Plasma magnesium was decreased significantly by 2.0% calcium intake only in DS and DR rats fed 2.7% dietary sodium. Plasma creatinine, sodium, and potassium were unaffected by 2.0% calcium feeding.

**Discussion**

This study demonstrates that dietary calcium supplementation can increase arterial pressure even in an animal model of salt-induced hypertension. There were three features of this observation. First, the increase in MAP produced by 2.0% calcium occurred only in DS rats fed high (2.7%) sodium and not in DS rats fed moderate (1.0%) or low (0.2%) sodium and not in DR rats fed high (2.7%) sodium diets. Second, the calcium-induced increase in MAP remained intact after interruption of sympathetic neural support of arterial pressure. Third, the calcium-induced increase in MAP was accompanied by enhanced systemic pressor responsiveness to NE.

The increase in MAP induced by 2.0% calcium intake in DS rats fed high sodium was accompanied by an increase in cardiac ventricular weight, a measure that normally relates directly to the degree of hypertension in DS rats. Therefore the increase in MAP at 12 weeks most likely reflected a chronic and not a transient increase.

This prohypertensive action of 2.0% calcium intake in DS rats fed high sodium diet was neither abolished nor attenuated by interruption of sympa-
hypertension support of arterial pressure with ganglionic blockade. Therefore, this prohypertensive action cannot be attributed to either increased sympathetic neural activity or to increased pressor responsiveness to NE. Pressor responsiveness to NE was significantly enhanced by 2.0% versus 0.4% calcium intake in the DS fed high sodium. Thus, although the mechanism of the prohypertensive action of 2% calcium does not appear to be noradrenergic in origin, it may nonetheless increase responsiveness to noradrenergic stimulation. Since these rats were areflexic under ganglionic blockade, this increased responsiveness cannot be due to impaired reflex buffering. In our previous study with DS rats, 4.0% versus 0.4% calcium tended to increase systemic pressor responsiveness to angiotensin II, as well as to phenylephrine and NE, in rats under ganglionic blockade. Together these studies raise the possibility that high calcium intake may increase responsiveness to vasoconstrictor stimuli in general in DS rats fed high sodium. This increased responsiveness may underlie the mechanism of the prohypertensive action of 2.0% calcium intake in the present study although it has yet to be examined in terms of the role of other endogenous vasoconstrictor stimuli.

If we find the vasculature of high sodium-fed DS rats on 2.0% calcium is indeed more reactive, in general, then we will need to consider whether the effects we have already observed (increased MAP after ganglionic blockade and increased responsiveness to NE) are merely consequences of more vascular hypertrophy secondary to more hypertension. We know there is evidence of more cardiac hypertrophy in the same rats (Figure 2). However, in this study a number of rats with intermediate levels of hypertension had correspondingly intermediate levels of heart weight. Careful inspection of Figures 1 and 3 reveal no such direct multilevel relation between hypertension and baseline pressures after ganglionic blockade or between hypertension and responsiveness to NE. In fact, although there were graded levels of sodium-dependent hypertension in DS rats fed 1.0% and 2.7% sodium at 0.4% calcium, there were no corresponding increases in MAP after ganglionic blockade or in NE responsiveness. This is not surprising since there is evidence based on maximal hindquarter vasoconstrictor responses that despite development of hypertension, functionally significant vascular muscle hypertrophy is delayed in DS rats for at least 8 weeks after they start a diet of even higher salt content than that in this 6-week study. In addition, there is evidence based on maximal hindquarter vasodilator responses that significant structural vascular changes are delayed in hypertensive DS rats for at least 4 weeks after starting a higher salt intake. Finally, our previous report revealed evidence of...
dissociation between effects of high calcium intake on responsiveness to vasoconstrictor stimulation and on blood pressure. An intake of 4.0% versus 0.4% calcium for 6–7 weeks tended to increase, not decrease, pressor responsiveness to NE and angiotensin II in DS rats despite a marked attenuation of hypertension. Therefore, it seems unlikely that the prohypertensive effects of 2% calcium are simply consequences of hypertensive hypertrophy of vascular muscle.

Increased systemic calcium levels may increase blood pressure in hypertensive subjects. Cytosolic free calcium and serum total calcium levels correlate with blood pressure levels in hypertensive subjects. Calcium infusions that raise circulating calcium concentrations within a physiological range also raise blood pressure. In this study, 2.0% calcium intake did not significantly increase circulating levels of total or ionized calcium. Thus, the increase in MAP produced by a 2.0% calcium diet cannot be explained by increased systemic calcium levels. We did not measure circulating levels of calcium-regulating factors (e.g., parathormone; calcitonin; 1,25-dihydroxyvitamin D$_3$) that are known to exert cardiovascular actions. The adjustments of these calcium-regulating factors to supplemental calcium intake may contribute to the prohypertensive action of 2.0% calcium in DS rats fed high sodium diet.

An increased dietary concentration of calcium may decrease availability of other nutrients including phosphorus and magnesium, which are thought to exert cardiovascular effects. All diets in the present study were fortified with these and other minerals as recommended to prevent frank deficiencies. Nonetheless, the 2.0% calcium intake reduced plasma levels of both phosphorus and magnesium in DS rats fed high sodium diet. However, these reductions were not specific to DS rats fed high sodium diet, and therefore, may not relate to the prohypertensive effect of 2.0% calcium.

In our previous study, 4.0% calcium intake obviously decreased sympathetic neural activity in high sodium-fed DS rats more than enough to offset any opposing prohypertensive influence since MAP was substantially decreased. In our present study, we speculate 2.0% calcium intake might have slightly decreased sympathetic neural activity in high sodium-fed DS rats, enough to compensate for increased pressor responsiveness to NE. Otherwise, we would have expected ganglionic blockade to at least attenuate the difference in MAP between high sodium-fed DS rats on 2.0% versus 0.4% calcium diets. However, since MAP was increased, 2.0% calcium intake obviously did not decrease sympathetic neural activity enough to offset the nonadrenergic prohypertensive action. Together these observations suggest that the effect of supplemental calcium on MAP in DS rats depends on the balance between antihypertensive (sympathoinhibitory) and prohypertensive (nonadrenergic) actions that are not equally effective at the levels of 2.0% and 4.0% calcium in the diet.

An unusual phenomenon in the present study was the sodium dependence of the prohypertensive action of calcium supplementation. The increase in MAP during 2.0% calcium supplementation occurred only when DS rats were fed high (2.7%) sodium. It is known that dietary calcium supplementation can either increase or decrease arterial pressure. However, previous information has suggested that a high sodium diet promotes an antihypertensive response to supplemental dietary calcium. For example, Chen and colleagues reported that the antihypertensive action of 2.0% calcium intake in spontaneously hypertensive rats (SHR) is dependent on high sodium intake, and McCarron et al observed that excess sodium intake amplifies the antihypertensive action of 2.0% calcium intake in SHR.

In conclusion, moderate dietary calcium supplementation (2.0% vs. 0.4%) enhances salt-induced hypertension in DS rats but does not increase MAP in DS rats fed a low salt diet or in DR rats fed a high salt diet. This prohypertensive action of 2.0% supplemental calcium intake does not appear dependent on either increased sympathetic neural activity or on increased pressor responsiveness to NE since the prohypertensive action remains intact after ganglionic blockade.

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