Neurohumoral and Hemodynamic Responses to Dietary Calcium Supplementation in Deoxycorticosterone-Salt Hypertensive Dogs

Yo Kageyama and Emmanuel L. Bravo

SUMMARY We determined whether dietary calcium supplementation can influence the development and maintenance of hypertension in deoxycorticosterone (DOC)-salt-treated dogs. Dogs on normal dietary calcium (0.4%) had significant increases in mean arterial pressure (from 92 ± 2 to 131 ± 3 mm Hg, p<0.01); those given high dietary calcium (1.7%) had attenuated but significant increases in mean arterial pressure (from 90 ± 2 to 107 ± 1, p<0.01). The elevation of blood pressure in dogs on normal dietary calcium was primarily due to increased calculated total peripheral resistance, which was prevented by the high calcium diet. The increases in blood pressure could not be attributed to any changes in cardiac output, blood volume, or plasma norepinephrine. These results suggest that mineralocorticoid hypertension in the dog is associated with abnormalities not only in sodium, but also in calcium metabolism. Further, they suggest a direct link between sodium and calcium metabolism and may thus have implications for the pathogenesis and management of salt-dependent hypertension. (Hypertension 9 [Suppl III]: III-166-III-170, 1987)

KEY WORDS • calcium supplementation • mineralocorticoid hypertension • vascular resistance

The role of calcium in the initiation and maintenance of hypertension has recently received increasing attention. Some clinical and animal studies suggest that calcium deficiency is associated with elevations of blood pressure1-3 and that dietary calcium supplementation reduces blood pressure to normotensive levels.4-8 Resnick and co-workers8 have also reported that levels of plasma ionized calcium are lower in patients with low renin essential hypertension and that dietary calcium supplementation reduces blood pressure in these patients.

On the basis of these observations, we have assessed the effect of dietary calcium supplementation in a model of low renin hypertension — deoxycorticosterone (DOC)-salt–induced hypertension in the dog.9,10 Our primary objective was to determine whether dietary calcium supplementation can influence the development and maintenance of hypertension in this model, and if so, to define the hemodynamic and neurohumoral changes associated with the cardiovascular responses.

From the Department of Heart and Hypertension, Cleveland Clinic Research Institute, Cleveland, Ohio.

Address for reprints: Emmanuel L. Bravo, M.D., Research Division, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106.
valerate [Percorten], CIBA, Summit, NJ, USA) and served as the time-control group. The second and third groups received DOC, 20 mg/kg, i.m., and served as the experimental groups.

Studies were performed over a 15-day period. Food intake was monitored daily. The cumulative sodium and potassium intake, calculated from daily food consumption, was not different among the three groups. Arterial pressure was recorded directly through arterial catheters on Days 0, 3, 7, 10, and 14. Hemodynamic studies, serum electrolytes, serum calcium and phosphorus, and plasma catecholamines were measured before and on the 7th and 14th days of the study. Plasma ionized calcium was measured on Day 14.

Pressor responses to norepinephrine and angiotensin II were assessed on Day 15 of the experimental period. Norepinephrine was given in increasing doses of 50, 100, 200, and 500 ng/kg/min. Angiotensin II was given in increasing doses of 4, 8, 16, and 40 ng/kg/min. Each infusion was given for 3 minutes in ascending order, and blood pressure was recorded at the end of each infusion before the next higher dose was given.

### Hemodynamic Measurements

All investigations were performed in a quiet laboratory in the morning, with dogs in the fasting state and resting comfortably on a padded laboratory bench. Blood pressure was measured with a pressure transducer (Model P23Db, Statham, Oxnard, CA, USA). Cardiac output was determined in triplicate using indocyanine green dye (5 mg) as previously described.9 Variation in the measurement of cardiac output was calculated by the Stewart Hamilton method, and total peripheral resistance was obtained by dividing mean arterial pressure (MAP) by cardiac output times 1000. Hemodynamic responses are summarized in Table 1. Dogs on DOC-salt and normal dietary calcium had significant increases of blood pressure; by the 14th day, the mean blood pressure for the group averaged 131 mm Hg. Untreated dogs on identical dietary sodium and calcium had no changes in blood pressure. The dogs that received high dietary calcium had a significant but markedly attenuated rise in blood pressure; by the 14th day, the mean blood pressure for the group averaged only 107 mm Hg. For both DOC-salt–treated groups, cardiac output tended to increase; however, these increases did not achieve statistical significance when compared with the control group.

### Table 1. Hemodynamic and Metabolic Responses to Variations in Dietary Calcium and DOC-Salt

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control (normal Ca diet)</th>
<th>Normal Ca diet + DOC-salt</th>
<th>High Ca diet + DOC-salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>19.6±0.6</td>
<td>19.8±0.6</td>
<td>19.9±0.6</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>90±2</td>
<td>90±2</td>
<td>91±1</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>3594±129</td>
<td>3693±177</td>
<td>3628±176</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>80±2</td>
<td>78±3</td>
<td>83±3</td>
</tr>
<tr>
<td>TPR (U·M³)</td>
<td>26.7±0.9</td>
<td>24.8±0.9</td>
<td>25.8±1.1</td>
</tr>
<tr>
<td>TBV (ml)</td>
<td>2718±93</td>
<td>2666±97</td>
<td>2799±123</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>143±1</td>
<td>143±1</td>
<td>141±1</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.3±0.1</td>
<td>4.4±0.1</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>Total Ca (mg/dl)</td>
<td>9.7±0.3</td>
<td>9.5±0.3</td>
<td>9.4±0.3</td>
</tr>
<tr>
<td>PO₄ (mg/dl)</td>
<td>5.0±0.3</td>
<td>5.1±0.4</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>NE (ppm)</td>
<td>107±9</td>
<td>104±7</td>
<td>101±7</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SEM; 0, 7, and 14 indicate days of the study; MAP = mean arterial pressure; CO = cardiac output; HR = heart rate; TPR = total peripheral resistance; TBV = total blood volume; PO₄ = phosphate; NE = norepinephrine.

### Analytical Methods

Total blood volume was measured as the distribution of Evans blue dye after a 10-minute equilibration, as previously described.9 Variation in the measurement was 6%. The concentration of plasma catecholamines was measured by radioenzymatic assay.11 Serum calcium, phosphate, and electrolytes were measured by autoanalyzer. Plasma ionized calcium was measured by a calcium-specific ion electrode.

### Statistical Analysis

Data computation was accomplished using PROPHET, a national computer resource supported in part by Biotechnology Resource Program, Division of Research Resource, National Institutes of Health. Comparisons between the groups were evaluated by BMDP2V (analysis of variance and covariance, including repeated measurements), then the significance was obtained using one-way analysis of variance followed by the Newman-Keuls multiple-range test. All values are expressed as means ± SE.
Heart rates were significantly lower in DOC-salt-treated dogs than in nontreated dogs during the development and maintenance phases of hypertension. However, the degree of blood pressure rise influenced heart rate responses. Thus, DOC-treated dogs on the normal calcium diet whose blood pressure increased markedly (ΔMAP = 39 mm Hg) demonstrated significant decreases in heart rate compared to pretreatment values (80 ± 3 vs 68 ± 3 beats/min, *p < 0.01). On the other hand, DOC-salt dogs on the high calcium diet had attenuated blood pressure responses (ΔMAP = 17 mm Hg), and heart rate remained essentially unchanged (74 ± 2 vs 71 ± 3 beats/min).

The elevation of blood pressure in the DOC-salt-treated, normal calcium group was primarily a function of increased peripheral vascular resistance. The DOC-salt-treated, high calcium group had unaltered peripheral vascular resistance that was identical to that of the control group. Thus, it appears that oral calcium supplementation prevented the DOC-salt-induced rise in blood pressure by inhibiting the expected rise in vascular resistance.

Total blood volume values rose and fell similarly in the DOC-salt-treated dogs, although the attenuation of blood pressure was evident only in dogs that received high dietary calcium. Similarly, changes in body weight were not different between the DOC-salt-treated dogs.

**Metabolic and Neurohumoral Responses**

Serum concentrations of sodium increased and potassium decreased similarly in the DOC-salt-treated groups; however, no differences were noted between these groups (see Table 1). Total calcium was unaltered in the control and DOC-salt, normal calcium groups and increased slightly, but significantly, in the DOC-salt, high calcium group. However, on the 14th day of the study, plasma ionized calcium was distinctly reduced in the DOC-salt, normal calcium group when compared with the DOC-salt, high calcium group (1.18 ± 0.04 mmol/L vs 1.38 ± 0.05, *p < 0.01). Nontreated dogs on normal dietary calcium had plasma ionized calcium values that averaged 1.37 ± 0.02 mmol/L — a value that was not different from that obtained from DOC-salt dogs on the high calcium diet. Serum phosphate decreased in both DOC-salt-treated groups; however, these changes were not different between the normal calcium and high calcium groups.

Plasma norepinephrine values decreased significantly in DOC-salt-treated dogs regardless of dietary calcium intake and blood pressure response. Plasma epinephrine values were essentially unchanged in all three groups during the period of study.

**Pressor Responses to Norepinephrine and Angiotensin II**

Pressor responses to various doses of norepinephrine and angiotensin II are shown in Figure 1. High dietary calcium normalized pressor responses to both vasoactive agents.

**Discussion**

The present study has shown 1) that high dietary calcium attenuates the elevation of arterial pressure induced by DOC-salt in trained, conscious dogs, primarily because of a failure of the expected rise in peripheral vascular resistance to develop; and 2) that high dietary calcium normalizes reduced plasma ionized calcium values. These findings suggest that calcium supplementation may be beneficial in the treatment of hypertension and that dietary calcium intake should be considered in the management of hypertensive patients.
ized calcium and enhanced pressor sensitivity to both norepinephrine and angiotensin II associated with DOC-salt hypertension.

It is well known that sodium retention exacerbates\(^9\) and sodium depletion prevents the development of mineralocorticoid hypertension.\(^10\) Therefore, it is possible that high dietary calcium, which has been found to be natriuretic in some studies,\(^6\) exerted an antihypertensive action through its effect on salt and water balance. However, other studies conducted in the SHR,\(^7\) hypertensive patients,\(^5\) and normotensive subjects\(^5-12\) have been unable to demonstrate any significant natriuresis in response to oral calcium supplementation. This discrepancy is in all probability related not only to the degree of extracellular fluid expansion at the time of study, but also to the amount of oral calcium supplementation. Our animals received only moderate amounts of salt (150 mEq of sodium per day), and calcium supplementation only amounted to 1.7% of the diet.

Our results also provide no evidence of significant alterations in salt and water balance. First, the changes in total blood volume were similar in both DOC-salt-treated groups; however, only the dogs receiving high dietary calcium had attenuation of blood pressure. Second, there were no differences in body weight between the DOC-salt–treated groups throughout the period of study. Third, the almost identical levels of serum potassium concentrations achieved in both DOC-salt–treated groups suggest similarities in salt balance. Since the degree to which potassium is secreted depends on the amount of sodium delivered to distal tubular sodium–for–potassium exchange sites, serum potassium in the dogs on the high calcium diet would have been lower had they had a much higher sodium excretion.

Enhanced activity of the sympathetic nervous system is unlikely to explain the observed cardiovascular responses. Plasma norepinephrine fell similarly in DOC-salt–treated dogs, and this response depended neither on the amount of dietary calcium nor on the blood pressure response. This is consistent with our previous findings, which showed that, unlike the rat, DOC-treated dogs have decreases, rather than increases, in circulating catecholamines during the development and maintenance of hypertension.\(^10\)

The question arises, how does mineralocorticoid excess plus salt lower serum ionized calcium? The decrease in ionized calcium could have resulted from the renal hypercalcuria induced by the volume-expanding effects of DOC-salt.\(^13\) Alternatively, the low ionized calcium level may reflect a sodium-induced shift in the disposition of calcium from extracellular sites.\(^14\) Such intracellular shift of calcium, coupled with abnormal membrane calcium binding,\(^15,16\) could lead to significant accumulation of intracellular calcium and hence to enhanced contractility of vascular smooth muscle, increased peripheral vascular resistance, and finally, increased arterial pressure.

The above construction is supported by some recent clinical observations. Dietary sodium loading in patients with low renin essential hypertension is reported to result in consistent suppression of serum ionized calcium levels.\(^4\) These sodium-induced decreases in serum calcium levels were in direct proportion to the increases in blood pressure. Patients with primary aldosteronism (a salt-dependent form of hypertension) have low-normal ionized calcium levels. Resnick and Laragh reported that removal of a unilateral adrenal adenoma normalized the levels of ionized calcium and at the same time lowered the elevated arterial pressure.\(^17\)

Two important questions need to be addressed. First, is the low level of plasma ionized calcium directly responsible for the rise in peripheral vascular resistance? Second, how does calcium supplementation lower elevated peripheral vascular resistance?

Our results provide evidence, albeit indirectly, that the low levels of ionized calcium in plasma contributed importantly to the rise in vascular resistance. First, normalization of plasma ionized calcium prevented the rise of total peripheral resistance and corrected the enhanced pressor responsiveness to infusions of exogenous vasoactive substances. Second, the vascular changes occurred without significant alterations in salt and water balance and independent of the activity of the sympathetic nervous system. There may be, however, other contributory factors that are as yet undefined.

These findings do not permit delineation of the cellular mechanisms involved in the beneficial effects of calcium supplementation, but some clinical and experimental observations may provide some insight. High dietary sodium intake exacerbates high blood pressure in those patients in whom serum calcium is most suppressed. It has been proposed that this response might be mediated through 1,25-dihydroxycholecalciferol (1,25(OH)\(_2\)D), since the greater the induced fall in serum calcium on the reciprocal rise in 1,25(OH)\(_2\)D, the greater the elevation of blood pressure with salt loading.\(^8\) In these same patients, calcium supplementation normalized serum calcium, decreased 1,25(OH)\(_2\)D, and reduced blood pressure to normotensive levels.

Insight into another possible mechanism can be derived from studies about the effect of calcium on cell membrane dynamics. Holloway and Bohr\(^14\) and Jones and Hart\(^15\) have provided evidence that suggests that vessels from deoxycorticosterone acetate–salt rats have cells with fewer calcium binding sites on the plasma membrane. This leads to destabilization of the membrane, increasing its permeability to sodium and causing accumulation of intracellular calcium and enhanced contractility of vascular smooth muscle. Increasing concentrations of calcium have been shown to stabilize the plasma membranes of vascular smooth muscle cells.\(^19\) However, the calcium concentrations used in these studies were very high and, therefore, the physiological relevance of such observations is open to question.
Whatever the mechanisms involved, it is clear that mineralocorticoid hypertension in the dog is associated with abnormalities not only of sodium, but also of calcium metabolism. These findings provide a direct link between sodium and calcium metabolism and may thus have implications for the pathogenesis and management of salt-dependent hypertension.

References
6. Ayachi S. Increased dietary calcium lowers blood pressure in the spontaneously hypertensive rat. Metabolism 1979;28:1234-1238
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Y Kageyama and E L Bravo

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