Hypertension

Oral Calcium Treatment Lowers Blood Pressure in Renovascular Hypertensive Rats by Suppressing the Renin-Angiotensin System

YO KAGEYAMA, HIROMICHI SUZUKI, KOICHIRO ARIMA, AND TAKAO SARUTA

SUMMARY  The effects of calcium supplementation on blood pressure and its mechanisms were investigated in two-kidney, one clip renovascular hypertensive rats. Two series of experiments were performed: one was begun just after renal artery constriction, the other after the onset of hypertension. Calcium supplementation significantly attenuated the development of hypertension (systolic blood pressure: 183 ± 8 vs 130 ± 2 mm Hg) and was found to abate existing renovascular hypertension (systolic blood pressure: from 183 ± 8 to 151 ± 4 mm Hg). Calcium treatment did not cause significant alterations in fluid intake, urine volume, or urinary sodium excretion in either study. However, increased plasma renin activity and plasma aldosterone concentration were suppressed to the basal levels at the end of 3 weeks of calcium treatment (14 ± 3 vs 8 ± 2 ng angiotensin I/ml/hr; 530 ± 50 vs 380 ± 40 pg/ml). Blood pressure of calcium-treated renovascular hypertensive rats responded poorly to blockade of the renin-angiotensin system with captopril injection and angiotensin II analogue (saralasin) infusion. Further, in rats with chronic established renovascular hypertension, calcium treatment attenuated the enhanced pressor response to norepinephrine, but not to angiotensin II. These results suggest that the blood pressure–lowering actions of calcium supplementation are related primarily to suppression of renin secretion and secondarily to alteration of pressor response to norepinephrine in two-kidney, one clip renovascular hypertensive rats.

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KEY WORDS  • calcium • renin-angiotensin system • pressor response • renovascular hypertension

Accumulated epidemiological evidence has shown an association between lower dietary calcium consumption and higher blood pressure in adults.1,2 Further, several recent studies have demonstrated that oral calcium supplementation reduces the blood pressure of both patients with essential hypertension6-12 and experimental hypertensive animals,6-8 although this kind of treatment is still controversial.9-10 We have investigated calcium’s blood pressure–lowering effects in spontaneously hypertensive rats11 and have shown that the lowering actions are related to the attenuation of vascular reactivity to vasoactive substances. These findings are supported by the latest study by Bukoski and McCarron,12 demonstrating that calcium supplementation favorably alters vascular smooth muscle function in spontaneously hypertensive rats concurrently with its beneficial effects on blood pressure. In the present study, we examined the effects of calcium supplementation on blood pressure and its mode of action in two-kidney, one clip renovascular hypertensive (2K1C RVH) rats, in which increased renin has a primary role in the pathogenesis of the hypertension.13-15

Materials and Methods

Male Wistar rats (purchased from Nippon Rat Company of Japan) weighing 150 to 160 g were used throughout. They were kept in temperature-controlled and humidity-controlled quarters, and lights were on from 0800 to 2100. Rats were fed ad libitum a diet containing Na, 0.39 g/dl; K, 0.98 g/dl; and Ca, 0.98 g/dl (Nihon Krea Company, Tokyo, Japan). Renovascular hypertension was produced by the method previously reported, in which a 0.2-mm-wide silver clip was placed on the left renal artery, while the right
artery was left untouched. All surgical procedures were performed with the rats under ether anesthesia. All drugs administered were dissolved in 5% dextrose in water.

**Effects of Calcium Treatment on Systolic Blood Pressure**

Experiment 1 examined the effects of calcium treatment on systolic blood pressure during the developmental phase of 2K1C renovascular hypertension. One day after operation, rats were divided into four groups. Group 1 contained 30 calcium-treated 2K1C RVH rats in the developmental phase of hypertension given 1.5% CaCl₂ solution as drinking water. Group 2 contained non-calcium-treated 2K1C RVH rats in the developmental phase of hypertension given tap water to drink. In addition, the effects of calcium treatment were investigated in normotensive sham-operated rats, in which the left renal artery was exposed and a silver clip was placed in the perinephric fat. Group 3 contained calcium-treated normotensive rats given 1.5% CaCl₂ solution as drinking water. Group 4 contained non-calcium-treated normotensive rats given tap water. During 3 weeks of calcium treatment, blood pressure was measured every 3 days by the tail-cuff method (Model KW210-1; Natsume, Tokyo, Japan). At least six recordings were averaged in each measurement day.

Experiment 2 measured the effects of calcium treatment on systolic blood pressure during the established phase of 2K1C renovascular hypertension. Sixty established phase 2K1C RVH rats were used. A 0.2-mm silver clip had been placed on the left renal artery of each rat 3 weeks previously. Hypertension, defined as systolic blood pressure above 160 mm Hg, was confirmed by the tail-cuff method. The rats were divided into two groups. Group 5 contained 30 calcium-treated 2K1C RVH rats with established hypertension given 1.5% CaCl₂ solution as drinking water. Group 6 contained non-calcium-treated 2K1C RVH rats given tap water. During 3 weeks of calcium treatment, blood pressure was measured every 3 days as in Experiment 1.

**Metabolic Study**

Experiment 3 measured water-electrolyte balances during 3 weeks of dietary intervention. Rats from all groups (Group 1, n = 6; Group 2, n = 6; Group 3, n = 4; Group 4, n = 4; Group 5, n = 6; Group 6, n = 6) were kept in the metabolic cages to examine the effects of calcium treatment on water-sodium balances as well as blood pressure. Their daily water intake, urine volume, and urinary excretion of sodium were measured.

Experiment 4 assessed hormonal and serum electrolyte changes in the rats used in Experiment 3. Polyethylene catheters (PE-50 Intramedic; Becton Dickinson, Rutherford, NJ, USA) were placed in the left carotid artery and vein (PE 10) were cannulated in twelve 2K1C RVH rats of each group (Groups 1 and 2: calcium treated or nontreated in the developmental phase; Groups 5 and 6: calcium treated or nontreated in the established phase) and eight normotensive rats of each group (Groups 3 and 4: calcium treated or nontreated). These rats were randomly divided into two groups of equal number for Experiments 5 and 6. At least 24 hours was allowed for recovery from operation. The arterial catheter was connected to a pressure transducer (RM-25 recorder; Nihon Kohden, Tokyo, Japan), and mean arterial pressure was measured and recorded as described elsewhere.

Experiment 5 measured the effects of an analogue of angiotensin II (ANG II) and captopril administration. ANG II analogue ([Sar¹,Val³,Ala⁸]ANG II; Sigma Chemical, St. Louis, MO, USA), 5 μg/kg/min, was infused for 20 minutes. Then, at least 4 hours later, captopril (a gift of Sankyo Pharmaceutical, Tokyo, Japan), 10 mg/kg, was injected as a bolus. In each experiment, blood pressure was compared before and after drug administration.

Experiment 6 measured the pressor responses to ANG II and norepinephrine infusion. ANG II (Sigma) was infused at 50, 200, and 700 ng/kg/min (15 minutes each) with a Harvard infusion pump (Millis, MA, USA). The pressor response to norepinephrine was examined 24 hours later. Norepinephrine (Sigma) was infused at 0.5, 2, and 7 μg/kg/min (15 minutes each). In each experiment, the total volume of the infusate was less than 1 ml.

**Hormonal and Biochemical Measurements**

PRA and PAC were measured by radioimmunoassay. Epinephrine and norepinephrine were measured by radioenzymatic assay. Ionized calcium was measured by ion-specific electrodes. Total calcium was measured by atomic absorption spectrophotometry. Serum sodium, potassium, chloride, inorganic phosphate, total protein, creatinine, and blood urea nitrogen were measured by autoanalyzer.

**Statistics**

The results are expressed as means ± SE. The data were analyzed statistically using nonparametric methods. Slopes of the linear portions of the dose-response curves obtained from the whole-body pressor responses to norepinephrine and ANG II were determined for each animal by the least-squares method. Individual slopes and correlation coefficients were averaged for each group of rats and compared by nonparametric methods. All p values less than 0.05 were considered significant.
Results

Attenuation and Reduction of Blood Pressure with Calcium Treatment

Figure 1 (upper panel) shows the time course of the blood pressure response to calcium treatment in 2K1C RVH rats and their respective controls. Calcium treatment attenuated the blood pressure elevation, and this effect appeared 1 week after calcium administration was initiated and lasted for the next 2 weeks. On the 21st day of calcium treatment, systolic blood pressure was 130 ± 2 mm Hg, representing only a 5% increase (compared with the marked elevation of systolic blood pressure seen in the control group). Calcium treatment was without effect in normal rats.

Figure 1 (lower panel) shows that calcium treatment in established hypertension reduced systolic blood pressure from 183 ± 8 to 151 ± 4 mm Hg in 3 weeks. The maximum drop in systolic blood pressure was observed on the 14th day, and systolic blood pressure was stable for the following week.

Endocrine and Electrolyte Response to Calcium Treatment

PRA and PAC of 2K1C RVH rats were significantly suppressed with calcium treatment (p < 0.005; Figure 2, upper panel). Calcium treatment also reduced PRA and PAC in established 2K1C renovascular hypertension (Figure 2, lower panel). Calcium treatment did not influence PRA or PAC in normotensive rats (see Figure 2, upper panel).

The basal values of norepinephrine and epinephrine were not significantly different between 2K1C RVH and normotensive rats, and they did not change with calcium treatment (Table 1). Similarly, there were no significant changes in serum electrolytes and other biochemical indices in either 2K1C RVH or normotensive rats with calcium treatment (Figure 3; see Table 1).

Pressor Responses After Calcium Treatment

Calcium treatment did not induce any significant changes in pressor response to either norepinephrine or ANG II in normotensive rats (Figures 4 and 5). In 2K1C RVH rats, the pressor response to norepinephrine was similar to that in the normotensive rats (see Figure 4). Calcium treatment of existing renovascular hypertension, but not developing hypertension, attenuated the pressor responses to norepinephrine (see Figure 4). In contrast to the response to norepinephrine, the response curve to ANG II was shifted to the right in developing renovascular hypertension in relation to that of the normotensive animals (see Figure 5, upper panel). Calcium treatment restored the reduced pressor responses to ANG II in existing renovascular hypertension (see Figure 5, lower panel).
FIGURE 2. Effects of calcium treatment on PRA and plasma aldosterone concentration (PAC) in the developmental (upper panel) and established (lower panel) phases of renovascular hypertensive (RVH) rats. Values are means ± SE. Asterisks denote values that were statistically significant compared with those for RVH rats without calcium treatment.

Blockade of the Renin-Angiotensin System

Blockade of the renin-angiotensin system with the ANG II analogue or with captopril resulted in marked reduction of blood pressure in both developing and established renovascular hypertension (Figure 6). However, these procedures were ineffective in calcium-treated RVH animals (see Figure 6). Normotensive animals exhibited a mild, nonsignificant reduction of blood pressure, and calcium treatment did not influence this response.

Discussion

In the present study, oral calcium treatment attenuated the development of hypertension and reduced the elevated blood pressure in 2K1C RVH rats. To our knowledge, this is the first report demonstrating that

<table>
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<th>Control rats</th>
<th>Developmental RVH rats</th>
<th>Established RVH rats</th>
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<tr>
<td></td>
<td>Ca(−) (n = 6)</td>
<td>Ca(+) (n = 6)</td>
<td>Ca(−) (n = 6)</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>230 ± 16</td>
<td>216 ± 12</td>
<td>242 ± 15</td>
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<td>Epinephrine (pg/ml)</td>
<td>200 ± 20</td>
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<td>175 ± 18</td>
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<td>Na (mEq/L)</td>
<td>142.0 ± 0.5</td>
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<td>K (mEq/L)</td>
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<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Ca (mg/L)</td>
<td>10.0 ± 0.4</td>
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<tr>
<td>P(1) (mg/dl)</td>
<td>6.7 ± 0.3</td>
<td>6.8 ± 0.2</td>
<td>6.6 ± 0.4</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>28.6 ± 2.3</td>
<td>27.9 ± 1.9</td>
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<tr>
<td>Cr (mg/dl)</td>
<td>0.61 ± 0.01</td>
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<td>TP (g/dl)</td>
<td>6.1 ± 0.1</td>
<td>6.1 ± 0.2</td>
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</table>

Values are means ± SE.
Ca(−) = normal diet; Ca(+) = 1.5% CaCl2 diet; RVH = renovascular hypertensive; P(1) = inorganic phosphorus; BUN = blood urea nitrogen; Cr = creatinine; TP = total protein.
oral calcium treatment lowers blood pressure in rats with renin-dependent renovascular hypertension.

Among the hormonal responses, one of the most striking findings in the present study was marked suppression of PRA with calcium treatment. Elevated circulating ANG II generally is thought to play an important role in the pathogenesis of 2K1C renovascular hypertension.\textsuperscript{13-15} Thus, it seems likely that suppression of PRA is one of the major contributing factors in the attenuation and reduction of high blood pressure of 2K1C RVH rats. This assumption is supported by the results of pharmacological blockade with the renin-angiotensin system using captopril and saralasin. In the non-calcium-treated RVH animals, these two drugs markedly reduced blood pressure; however, calcium treatment abolished their blocking of the renin-angiotensin system. In contrast to calcium's blood pressure-lowering effects in 2K1C RVH rats, the blood pressure of the normotensive rats was not altered.

The mechanisms by which calcium suppresses PRA have been suggested by several investigators.\textsuperscript{16-23} Previously, Kotchen et al.\textsuperscript{21} reported that oral calcium loading decreased renin release and reduced renal renin content in sodium-depleted rats. They hypothesized that inhibition of renin release is related to an increased delivery of sodium to the macula densa because of a significant increase in sodium excretion. However, Watkins et al.\textsuperscript{22} demonstrated that intrarenal calcium infusion inhibits renin release in sodium-depleted dogs. In their study, both calcium chloride and calcium gluconate decreased renin release and no significant changes in renin release were observed between filtering and nonfiltering kidneys. These results indicate that calcium acts directly on the juxtaglomerular cells to inhibit renin secretion. In the present study, calcium supplementation failed to increase sodium excretion in spite of a marked increase in calcium excretion. Thus, it appears likely that calcium supplementation in 2K1C RVH rats decreased PRA and then caused a fall in blood pressure.

We have already shown that both pressor responses and vascular reactivity to norepinephrine were attenuated in calcium-treated spontaneously hypertensive rats, and this attenuation of vascular reactivity was one of the mechanisms of the antihypertensive action of calcium treatment.\textsuperscript{15} In the present study, pressor response to ANG II was suppressed in the non-calcium-treated groups in both the developmental and the established phases of hypertension. Judging from PRA

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Effects of calcium treatment or nontreatment on fluid intake, urine volume, and urinary excretion of sodium ($U_{\text{NaV}}$) in the developmental (left side) and established (right side) phases of renovascular hypertensive (RVH) rats. Values are means ± SE.}
\end{figure}
levels, circulating ANG II in the non-calcium-treated groups was much higher than that in the other groups. Therefore, these results were considered to show a down-regulation of ANG II receptors or prior occupancy of receptor sites by endogenous hormones in the vascular smooth muscle cells.

Recently, Resnick and Laragh have reported that the higher PRA is, the higher serum calcium concen-
Depressor effects of captopril (upper panel) and saralasin (lower panel) in renovascular hypertensive (RVH) rats and normotensive control rats with or without calcium treatment. Values are means ± SE. Brackets indicate values that were statistically significant compared with those for non-calcium-treated RVH rats.

The results of the pressor response to norepinephrine infusion were different from the results of the response to ANG II. There was neither attenuation nor augmentation in 2K1C RVH rats 3 weeks after renal artery constriction as compared with the normotensive rats. Moreover, calcium treatment did not induce any significant response in either normotensive or 2K1C RVH rats. However, in established renovascular hypertension, calcium treatment attenuated the pressor responses to norepinephrine infusion. This finding suggests that attenuation of pressor response as well as suppression of renin plays a role in the reduction of blood pressure by calcium treatment in renovascular hypertension.

Finally, calcium treatment induced no significant changes in food intake, urine volume, or urinary excretion of sodium in either the developmental or established phases of renovascular hypertension. Studies of the effects of calcium treatment on the electrolyte balance in hypertensive animals have been reported by several investigators, and the results are conflicting; some studies reported that a high calcium diet induced natriuresis. In the present study, calcium treatment did not cause natriuresis. It seems unlikely, therefore, that the calcium-induced attenuation and re-
duction of blood pressure in 2K1C RVH rats were caused by natriuresis or diuresis.

In conclusion, these results suggest that the blood pressure–lowering actions of calcium supplementation are related primarily to suppression of renin secretion and secondarily to alteration of the pressor response to norepinephrine in 2K1C renovascular hypertension in rats.

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