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Role of Renal Hemodynamics in the Antihypertensive Effect of Calcium Supplementation in Salt-Loaded Spontaneously Hypertensive Rats

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ABSTRACT

To clarify the mechanisms for the antihypertensive action of calcium (Ca) supplementation, we studied the effect of dietary Ca supplementation (4.07% Ca) for 4 weeks on mean arterial pressure (MAP) and renal hemodynamics in 6-week old spontaneously hypertensive rat (SHR) fed on a high salt (3.15% Na) diet as compared with SHR on normal salt (0.26% Na) diet. Salt loading accelerated the development of hypertension (214 ± 6 vs. 158 ± 2 mmHg, $P<0.01$) associated with elevated renal vascular resistance (RVR) (26.3 ± 2.3 vs. 17.7 ± 1.1 U, $P<0.01$). Ca supplementation markedly suppressed the pressor effect of salt loading (158 ± 4 vs. 214 ± 6 mmHg, $P<0.01$), but did not significantly affect MAP in salt-unloaded SHR (148 ± 3 vs. 158 ± 2 mmHg, n.s.). Moreover, the elevated RVR was reversed by dietary Ca supplementation in salt-loaded SHR (17.2 ± 1.8 vs. 26.3 ± 2.3 U, $P<0.01$), whereas RVR was not changed by dietary Ca in salt-unloaded SHR (16.3 ± 1.3 vs. 17.7 ± 1.1 U, n.s.). As a result, there was a positive correlation between RVR and MAP ($r = 0.6590$, $n = 30$, $P<0.001$). Dietary Na and/or Ca supplementation did not significantly affect glomerular filtration rate. In conclusion, dietary Ca supplementation attenuated the increased salt sensitivity of blood pressure, probably through the normalization of the elevated RVR in salt-loaded SHR.

INTRODUCTION

There is a growing body of evidence indicating that dietary Ca supplementation exhibits antihypertensive effect in humans (McCarron et al., 1982) and animals (Ayachi, 1979), but the mechanism for the antihypertensive effect of Ca remains to be determined. Interestingly, the antihypertensive effect of Ca is more apparent in salt-sensitive hypertensive humans (Resnick et al., 1986; Saito et al., 1989) and animal models, e.g. Dahl salt-sensitive rats (Peuler et al., 1987), DOCA-salt rats (DiPette et al., 1989), salt-loaded spontaneously hypertensive rats (SHR) (McCarron et al., 1985; Oparil et al., 1990) and angiotensin II-salt loaded rats (Ando et al., 1991). In contrast, dietary Ca supplementation did not affect blood pressure (BP) in salt-unloaded hypertensive animals (Liard, 1981; Resnick et al., 1986; Ando et al., 1991). It is well known that the impaired renal function for sodium excretion is involved in patients with salt-sensitive hypertension (Fujita et al., 1980; Ando and Fujita, 1985; Lawton et al., 1988; Fujita et al., 1990). Salt loading increased BP in young SHR, possible model of human essential hypertension, associated with increased renal vascular resistance (RVR), which is intimately related to sodium (Na) excretion (Sato et al., 1991). Several investigators have demonstrated that Ca supplementation decreased BP, associated with natriuresis in humans (Saito et al., 1991) and animals (Ayachi, 1979; Stern et al., 1984; Jirakulsomchok et al., 1990). Therefore, to clarify the participation of renal hemodynamic changes in the antihypertensive effect of Ca, the effect of dietary Ca supplementation on BP and renal hemodynamics was examined in salt-loaded young SHR, salt-sensitive hypertensive model (Sato et al., 1991).

METHODS

Male SHR were purchased from Charles River Japan at 5 week of age. All rats were maintained at constant humidity (60 ± 5%), temperature (23 ± 1°C) and light cycle (0600–1800). Five days after arrival,

the rats were placed on a sodium chloride and/or calcium carbonate containing diet for 4 weeks. All the diets were made according to our specifications by Oriental Yeast, Tokyo, Japan. The control group (n = 8) were placed on a normal rat chow (0.26% Na and 1.17% Ca). The Ca group (n = 7) were placed on a 0.26% Na and 4.07% Ca diet. The Na group (n = 8) were placed on 3.15% Na and 1.17% Ca diet. The Na + Ca group (n = 7) were placed on a 3.15% Na and 4.07% Ca diet. Food and tap water were supplied ad libitum during the entire period of the study.

After 4 weeks of each regimen, rats were anaesthetized with ether and the femoral artery and vein were cannulated with tip-tapered PE-50 and PE-20 polyethylene tube, respectively. The venous and arterial catheters were tunnelled to the back of the neck, filled with heparinized saline (50 U/ml) and plugged with stainless steel pins. The bladder was exposed through a supra pubic incision and was connected with urinary catheter (PE-160 tube). Then the catheter was secured by suturing to adjunct muscle, subcutaneous tissue and skin (Sato et al., 1991). After removal of ether, rats were placed in a Lucite restraining chamber (Natsume, Tokyo, Japan), which permitted forward and backward movement, to equilibrate for at least 90 min. After that, an isotonic saline containing 4% inulin and 2% sodium para-amino-hippurate (PAH) was infused through the venous catheter at 24.5 for 90 min to equilibrate before clearance periods begun. Femoral artery pressure was measured as mean arterial pressure (MAP) in conscious SHR by use of a pressure transducer (model TP-200T; Nihon Kohden, Tokyo, Japan) connected to thermal artery recorder (model WS-641G; Nihon Kohden). The urinary catheter was led to a collection beaker. For clearance experiment, urine was collected for two 20-min periods and venous samples (300 μl) were taken at the mid point of the clearance period.

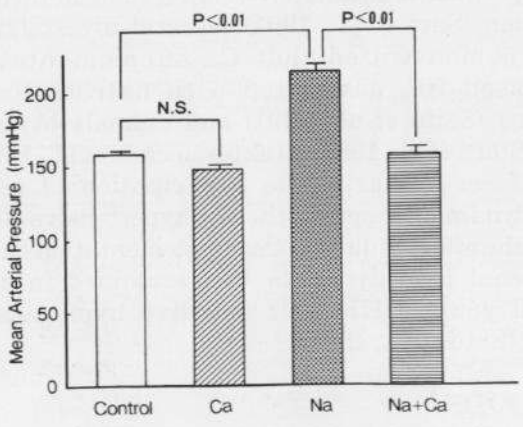

Fig. 1. Effect of salt loading (3.15% Na diet) and/or Ca supplementation (4.07% Ca diet) on mean arterial pressure (MAP) in young spontaneously hypertensive rats (SHR). Ca: Ca supplemented SHR; Na: salt-loaded SHR; Na+Ca: salt-loaded SHR with Ca supplementation.

Urine volume was determined gravimetrically. Haematocrit was measured in heparinized capillary tubes. Inulin levels in serum and urine were determined by anthron method (Fuehr et al., 1955). PAH levels in serum and urine were determined by p-aminobenzaldehyde method (Waugh and Beall, 1974). Measured values were not corrected for the extraction of PAH. Glomerular filtration rate (GFR) and renal plasma flow (RPF) was calculated standard formulas. Effective renal blood flow (ERBF) was calculated as RPF/(1-Ht). Renal vascular resistance (RVR) were calculated as MAP/ERBF. The values for GFR, ERBF and RVR represent the means for two clearance periods in each animal.

Statistical analysis

Data are presented as means ± SE. Statistical analyses were performed by use of one way analysis of variance, followed by Tukey method for comparisons among individual means (Wallenstein et al., 1980). Regression analyses were carried out by the least squares method. A value of $P<0.05$ was considered statistically significant.

RESULTS

Body weight was reduced by Ca supplementation in either salt-loaded or unloaded SHR. (Control: 275 ± 4 vs. Ca: 238 ± 6 g, $P<0.01$; Na: 261 ± 5 vs. Na + Ca: 239 ± 4 g, $P<0.01$). Salt loading did not affect body weight (Control vs. Na, NS).

As shown in Fig. 1, salt loading significantly ($P<0.01$) enhanced the spontaneous increase of MAP in SHR. However, Ca supplementation significantly ($P<0.01$) attenuated the acceleration of MAP rise in salt-loaded SHR. In contrast, MAP was not significantly changed by Ca supplementation in salt-unloaded SHR. As the result, the slope of the salt intake–MAP relationship function curve was steeper in Ca-supplemented SHR than in non-Ca-supplemented SHR (Fig. 2).

As shown in Table 1 and Fig. 3, salt loading significantly ($P<0.01$) increased RVR in SHR. Ca supplementation significantly ($P<0.01$) attenuated the increment in RVR of SHR. However, RVR was not significantly changed by Ca supplementation in salt-unloaded SHR. There were no significant changes in urine volume, GFR, RPF, haematocrit and ERBF by Na and/or Ca supplementation in SHR.

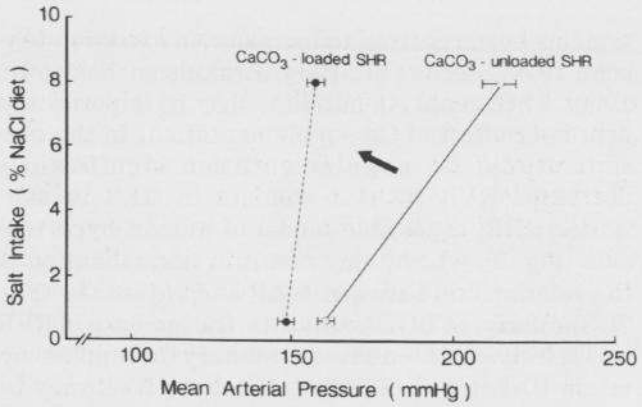

Fig. 2. Effect of Ca supplementation on relationship between MAP and salt intake in young SHR. Data are plotted as mean ± SE for MAP of rats in each group. Slope of this relationship represents salt sensitivity of BP. Ca supplementation ameliorated the decreased slope of renal function curve of young SHR.

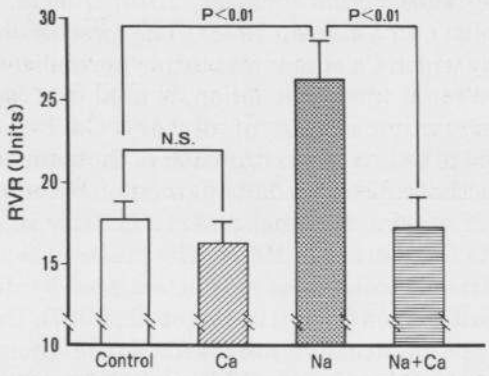

Fig. 3. Effect of salt loading (3.15% Na diet) and/or Ca supplementation (4.07% Ca diet) on renal vascular resistance (RVR) in young SHR. Ca: Ca supplemented SHR; Na: salt-loaded SHR; Na+Ca: salt-loaded SHR with Ca supplementation.

DISCUSSION

In the present study, salt loading augmented MAP increase associated with elevated RVR in young SHR. Ca supplementation attenuated the accelerated MAP increase in salt-loaded SHR, whereas it did not affect MAP in salt-unloaded SHR. Correspondingly, the elevated RVR was reversed by Ca supplementation in salt-loaded SHR, but RVR was not changed by dietary Ca in salt-unloaded SHR. Thus, it is suggested that changes in RVR may play an important role in both increased salt sensitivity of young SHR and antihypertensive effect of dietary Ca supplementation.

Although Ca supplementation reduced body weight in young SHR, the antihypertensive effect of dietary Ca may not be through body weight loss: less weight gain induced by Ca supplementation was observed not only in salt-loaded SHR, in which dietary Ca lowered MAP, but also in salt-unloaded SHR, in which it did not. In the previous reports, Ca supplementation decreased BP without body weight loss in 18-week old SHR (Ayachi, 1979), but hardly decreased BP with apparent body weight loss in 6-week old SHR (Saito et al., 1989). Moreover, less weight gain during high Ca diet occurred without reduced food intake in Dahl salt sensitive rats (Peuler et al., 1987) or in SHR (Stern et al., 1984). Thus the antihypertensive effect of Ca supplementation in young SHR may be independent of weight loss.

The accelerated MAP increase in salt-loaded SHR was associated with the increased RVR and there was a positive correlation between RVR and MAP (Fig. 4). This result is consistent with previous studies in humans and animals. For example, salt load-

TABLE 1

Effects of dietary Ca supplementation on renal function in conscious SHR with and without salt loading

Group	Control	Ca	Na	Na+Ca
n	8	7	8	7
Urine volume (μ l/min·g kidney wt)	32.8±3.1	41.4±4.2	40.2±5.8	37.1±6.3
Glomerular filtration rate (ml/min·g kidney wt)	1.30±0.16	1.49±0.07	1.38±0.17	1.45±0.09
Renal plasma flow (ml/min·g kidney wt)	4.83±0.32	5.29±0.50	4.33±0.36	5.43±0.43
Effective renal blood flow (ml/min·g kidney wt)	9.23±0.55	9.54±0.85	8.56±0.66	9.81±0.99
Renal vascular resistance (units)	17.7±1.1	16.3±1.3	26.3±2.3*	17.2±1.8#

Values are means ± SE. Ca: Ca-supplemented SHR; Na: salt-loaded SHR; Na+Ca: salt-loaded SHR with Ca supplementation; U: units of $mmHg \cdot ml^{-1} \cdot min \cdot g$ kidney wt.
*P<0 01, compared with Control group.#P<0.01 compared with Na group.

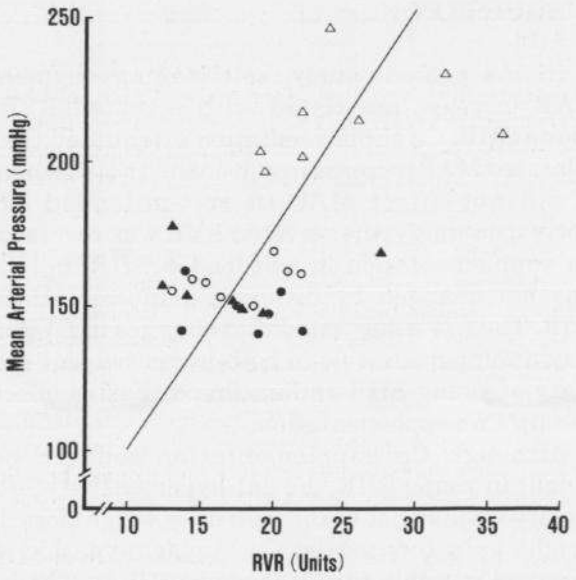

Fig. 4. Relationship between RVR and MAP in Na and/or Ca-loaded SHR. MAP was significantly correlated with RVR in all rats ($r = 0.659$, $n = 30$, $P<0.001$). Hollow circles: control rats; filled circles: Ca supplemented SHR; hollow triangles: salt-loaded SHR; solid triangles: salt-loaded SHR with Ca supplementation.

ing caused an exaggerated renal vasoconstriction and antinatriuretic response to standing in young patients with essential hypertension (Lawton et al., 1988). In our recent study, salt-sensitive hypertensive patients had increased BP associated with greater increase in RVR during high sodium diet than non-salt-sensitive patients (Fujita et al., 1990). In addition, the basal value of RVR was positively correlated with change in BP with salt loading (Ando and Fujita, 1985). Similarly, salt loading caused increased vascular resistance in the kidney in dogs with renal mass reduction (Liard, 1981) and in rats with DOCA-salt hypertension (Yates and Hiley, 1979). Increase in RVR has been reported to enhance Na reabsorption in peritubular capillaries through increased plasma oncotic pressure and decreased hydrostatic pressure (Brown et al., 1974: Hall et al., 1980). Thus, changes in RVR may be intimately related to salt sensitive hypertension through Na excretion.

Dietary Ca effectively prevented the accelerated MAP increase induced by high salt diet in SHR, but it did not affect spontaneous development of hypertension (Fig. 1). These findings are consisted with the previous reports that antihypertensive effect of Ca supplementation was shown exclusively in salt-loaded hypertensive humans and animals (McCarron et al., 1985; Resnick et al., 1986; Saito et al., 1989; Oparil et al., 1990). Dietary Ca supplementation has been reported to increase Na excretion (Ayachi, 1979; Stern et al., 1984; Jirakulsomchok et al., 1990). Then renal Na handling may be important in depressor effect of Ca supplementation. In the present study, Ca supplementation significantly decreased RVR without changes in GFR in salt-loaded SHR, a possible model of human hypertension (Fig. 3), which, may result in normalization of the relationship between MAP and Na intake (Fig. 2). Similarly, in DOCA-salt rats, the increase in RVR was selectively attenuated by dietary Ca supplementation (DiPette et al., 1989). Because RVR may be intimately related to Na excretion (Hall et al., 1980; Bohr and Webb, 1984), Ca supplementation may promote Na excretion through the normalization of increased RVR in salt-loaded young SHR.

The previous studies suggest that Na excretion are mediated by the sympathetic nervous system via both arterial vasoconstriction and alterations in tubular reabsorption (Schrier, 1974; Dibona, 1977; Pronsnitz and Dibona, 1978). The precise mechanism, by which Ca supplementation normalized salt-induced renal vasoconstriction, is unknown, but antihypertensive action of dietary Ca has been reported to be linked to alteration in the sympathetic nerve activity. High Ca diet augmented baroreceptor reflex of renal sympathetic nerve activity in Dahl-salt rats (Peuler et al., 1987). The presser responses to electrical shock stress was attenuated by dietary Ca in salt-loaded SHR (Hatton et al., 1987). Dietary Ca supplementation normalized the increased plasma norepinephrine (NE) and the altered NE turnover in the hypothalamus in salt-loaded SHR, but did not affect in salt-unloaded SHR (Oparil et al., 1990). In our recent study, Ca supplementation decreased BP associated attenuation of the increase of plasma catecholamine in salt-loaded rats with angiotensin II administration, but did not affect in salt-unloaded rats with angiotensin II (Ando et al., 1991). Because sympathetic activity affects renal vasculature, Ca supplementation may decrease RVR possibly through normalization of enhanced sympathetic nerve activity in salt-loaded SHR.

There are some other possible mechanisms that might account for the decreased RVR by Ca supplementation. First, the direct effect of Ca on vascular smooth muscle cells has been proposed. Ca ion inhibited Ca influx into the cells (Hurwitz et al., 1982). Dietary Ca and 1α,25-dihydroxyvitamine D3 ($1,25(OH)_2 D_3$) induced high-affinity Ca binding protein, which was decreased before the development of high blood pressure in the cell membrane of several tissues of SHR (Kowarski et al., 1986). Ca supplementation might exhibit the membrane-stabilizing effect, reversed membrane permeability and re-

duced conductance of ions, resulting in decreased vascular contractility (Bohr and Webb, 1984). Thus, Ca supplementation might directly dilate the vasculature of several tissues of hypertension. The concept might not clearly explain specific vasodilator effect of Ca loading on renal vasculature because it has not been demonstrated that the direct vascular action of Ca is greater in kidney than in the other organs. Second, prostaglandins may be involved in the vasodilating effect on renal vasculature of dietary Ca. Ca supplementation increased the production of prostacyclin, which may cause renal vasodilation, in vascular tissue in young SHR (Shuler et al., 1988). However, Urinary 6-keto-PGF 1α was increased by intrarenal Ca infusion without renal hemodynamic alterations (Lahera et al., 1990). The participation of prostaglandins on renal hemodynamics in the antihypertensive effect of dietary Ca remains to be determined. Third, Ca-regulating hormone might be the other candidate. It has been demonstrated that parathyroid hormone (PTH) (Bukoski et al., 1988) and $1,25(OH)_2$ D_3 (Baran and Milne, 1986) may have a direct effect on calcium regulation in vascular smooth muscle. PTH has a vasodilating effect (Pang et al., 1982), but was decreased with Ca loading so it hardly contribute to renal vasodilating effect. In contrast, $1,25(OH)_2$ D_3, which cause vasoconstriction, was decreased with Ca loading (Resnick et al., 1986). Then, $1,25(OH)_2$ D_3 might participate in vasodilating effect of Ca in renal vasculature, but the further study should be required.

CONCLUSIONS

Dietary Ca supplementation attenuated salt-induced hypertension and elevation of RVR in young SHR. These findings suggests the normalization of the elevated RVR may play an important in the antihypertensive effect of Ca supplementation in salt-loaded young SHR.

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