Hypertension in Rats: Interactions among Chloride, Sodium, and Calcium

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We examined whether supplementation of diets with calcium in excess of the requirement level could moderate the effects of supplemental NaCl on the blood pressure of rats. Rats were fed diets based on either unsalted cottage cheese or regular salted cottage cheese (which provided an additional ~0.4 mmol of Na and Cl/g of diet) with either adequate (~4.4 mg of Ca/g of diet) or supplemental (~9.9 mg of Ca/g of diet) Ca for 34 days. Rats fed the salted rather than the unsalted cheese had higher blood pressures only if their diets were not supplemented with calcium. The effect of calcium supplementation could not be attributed to the effects of dietary calcium on growth of animals, plasma renin activity, or decreased retention of sodium or chloride. Ingestion of excess NaCl adversely affected both potassium and magnesium retention; calcium supplementation tended to have a compensatory effect on potassium utilization.

A number of investigators have demonstrated that the ingestion of supplemental calcium delayed the development of hypertension in the spontaneously hypertensive rat (SHR) (Luft et al., 1988; McCarron et al., 1985; Porsti, 1992). The applicability of these data to other hypertension models and humans is limited by the uniqueness of the SHR model (MacGregor, 1985). Calcium supplementation of the diet has been reported to depress hypertension in rats treated with deoxycorticosterone (Resnick et al., 1986), to have no effect on hypertension in rats treated with lead (Bogden et al., 1991), and to elevate blood pressure in Goldblatt rat models (clips are placed on the renal artery of rats) (Resnick et al., 1986).

We hypothesized that the ingestion of supplemental calcium could affect the blood pressure of rats fed excess chloride and sodium in one of two ways. In the first scenario, we hypothesized that the ingestion of supplemental calcium would lower the blood pressures of young Sprague-Dawley rats fed excess chloride in a manner similar to that observed among rats treated with deoxycorticosterone, not like Goldblatt rat models, for two reasons. (1) Sprague-Dawley rats fed excess chloride excrete elevated amounts of calcium in urine (Greger and Tseng, 1993; Greger et al., 1991; Kaup et al., 1991b) as do rats treated with deoxycorticosterone (Resnick et al., 1986). Thus, both models potentially have at least transient reductions in ionized calcium concentrations in serum. (2) Plasma renin activities of Sprague-Dawley rats fed excess chloride are not elevated (Greger and Tseng, 1993). Renin activities are elevated in Goldblatt rat models and suppressed in rats treated with deoxycorticosterone. Resnick et al. (1986) hypothesized that ingestion of excess calcium stimulated renin activity only in renin-dependent hypertension (Goldblatt models).

In the alternate scenario, we recognize that the ingestion of excess calcium could exacerbate the hypertension. Ingestion of supplemental calcium, especially as a phosphate salt, has been found in some situations to depress magnesium absorption and retention and to exacerbate nephrocalcinosis (Greger, 1989; Ritskes-Hoitinga et al., 1991; Woodward and Jee, 1984). Ingestion of excess chloride appears to induce hypertension in Sprague-Dawley rats through its impact on kidney function (Greger and Tseng, 1993). Thus, we hypothesized that if calcium supplementation of the diet resulted in nephrocalcinosis and kidney hypertrophy, calcium supplementation would exacerbate the development of the hypertension.

To demonstrate that the dietary levels of chloride needed to induce hypertension are not extreme, we fed cottage cheese as the source of sodium chloride. This product is generally not considered to taste salty but contains 1162 mg of NaCl in a half-cup serving (Posati and Orr, 1976). Moreover, the calcium as well as the sodium and chloride content of this product can be adjusted relatively easily (Kaup et al., 1991a).

PROCEDURES

Rats were fed diets based on either unsalted cottage cheese or regular salted cottage cheese (which provided an additional ~0.4 mmol of Na and Cl/g of diet) with either adequate or supplemental calcium as calcium carbonate (Mallinckrodt Inc., Paris, KY) for 34 days. The resulting diets contained (by analyses) the following levels of chloride, sodium, and calcium: *diet control*, 1.28 mg of Na, 2.03 mg of Cl, and 4.62 mg of Ca/g of diet; *diet NaCl*, 11.35 mg of Na, 16.36 mg of Cl, and 4.22 mg of Ca/g of diet; *diet Ca suppl*, 1.09 mg of Na, 1.94 mg of Cl, and 10.00 mg of Ca/g of diet; *diet Ca suppl/NaCl*, 11.92 mg of Na, 15.75 mg of Cl, and 9.77 mg of Ca/g of diet.

Cheese and Diet Preparation. One large batch of cottage cheese curds was prepared at the university dairy plant and split into two parts. Half was mixed with a dressing that was prepared according to a commercial formula using reagent grade sodium chloride (Mallinckrodt); the other half was mixed with a similar dressing that contained no added NaCl. Both the salted and unsalted cottage cheeses were prepared to be 39.8% dressing and 60.2% curds, by weight, with a final fat content of 1%.

Both types of cottage cheese were freeze-dried (Freeze Mobile Consol with Quick Defrost, Virtis Co., Inc., Gardiner, NY) and ground to a fine powder.

The diets were formulated to contain 40.4% dried cottage cheese, 30% sucrose, 5% cellulose (Teklad Test Diets, Madison, WI), 5% corn oil (ADM Packaged Oils, Decatur, IL), 3.5% mineral mixture, 1% AIN-76 vitamin mixture (Teklad Test Diets), 0.3% *dl*-methionine (Teklad Test Diets), 0.2% choline dihydrogen citrate (Teklad Test Diets), and 14.2–14.6% cornstarch. When calcium carbonate was added to diet Ca suppl and diet Ca suppl/NaCl, the amount of cornstarch used was accordingly reduced.

On the basis of analyses of the freeze-dried cottage cheese, the composition of the mineral mixture was altered from the standard formulation so that the control diet contained levels of minerals (calcium, sodium, chloride, magnesium, phosphorous) approximating the AIN-76 diet recommendations (American Institute of Nutrition, 1977). The mineral mixture contained (in grams

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per kilogram of mineral mixture) the following: $CaHPO_4$, 257; NaCl, 25; $K_3C_6H_5O_7 \cdot H_2O$, 220; K_2SO_4, 52; MgO, 12.6; $MnCO_3$, 3.5; ferric citrate (16.7% Fe), 6.0; $ZnCO_3$ (52% Zn), 0.41; $CuSO_4 \cdot 5H_2O$ (53–55% Cu), 0.24; KIO_3, 0.01; $Na_2SeO_3 \cdot 5H_2O$, 0.01; $CrK(SO_4)_2 \cdot 12H_2O$, 0.55.

The diets provided 24% protein. The diets by analyses contained 0.55 mg of Mg, 5.15 mg of P, 5.22 mg of K, 50 μg of Fe, and 18 μg of Zn/g diet.

Animals. Male weanling Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) (n = 8/treatment, except for treatment Ca suppl/NaCl, then n = 7) were housed individually in stainless steel, wire-bottom cages in rooms maintained at 23–24 °C with a 12-h light/dark cycle. The facilities and protocols met the requirements of an Institutional Animal Care and Use Committee.

Deionized water was offer ad libitum. Feed consumption was monitored daily.

Sample Collection. Systolic blood pressure measurements were made 3 days prior to initiation of dietary treatments on days 28 and 33 on unanesthetized rats using an indirect rat tail cuff blood pressure system (Harvard Apparatus, South Natick, MA) as described previously (Kaup et al., 1991b). To increase reliability of blood pressure measurements, rats were familiarized with the blood pressure apparatus prior to measurement and a heating pad maintained animals at an ambient temperature of 35–40 °C. At least five readings were averaged for each rat.

Rats were weighed twice weekly. Two-day urine and fecal composites were collected during days 12 and 13. Rats were placed in Nalgene metabolic cages during this time. Urine was acidified with 50% nitric acid (Fisher Scientific, Fair Lawn, NJ) for a final concentration of 0.5% to prevent mineral precipitation, diluted, and frozen. Feces were dried to a constant weight, cleaned of foreign adhering matter, and ground to a fine powder for analysis.

At the conclusion of the study, rats were fasted overnight and killed by exsanguination. Blood was collected in prechilled plastic tubes containing 1.0 mg of EDTA/mL (Sigma Chemical Co., St. Louis, MO) and 2×10^{-5} M phenylmethanesulfonyl fluoride (Sigma). Plasma was stored at −70 °C. Tissues were excised, cleansed, weighed, and frozen in acid-washed plastic containers.

Analyses. Samples were analyzed for sodium, potassium, calcium, and magnesium by atomic absorption spectroscopy (Perkin-Elmer Corp., Model 3100, Norwalk, CT) (Greger and Snedeker, 1980) and for chloride (Jeffery and Hutchinson, 1981) and phosphorus (Fiske and Subbarow, 1985) colorimetrically. Aliquots of a milk standard (SRM 1549), obtained from the National Institute of Standards and Technology (Gaithersburg, MD), were analyzed with each batch of experimental samples. Agreement with certified values was acceptable: 97 ± 2% (n = 16) for calcium, 93 ± 1% (n = 24) for sodium, 90 ± 2% (n = 18) for potassium, 108 ± 2% (n = 11) for chloride, and 98 ± 2% (n = 14) for phosphorus.

Urine creatinine concentrations were determined (Peters, 1992). Radioimmunoassays were used to monitor plasma renin activity (Du Pont Co., Billerica, MA). Samples of pooled rat plasma were measured with each assay.

The effects of dietary treatments were assessed within the framework of general linear models for analysis of variance (SAS Institute, 1985). Tests for least significant differences (lsd) were applied when differences among treatments were significant ($p < 0.05$) as determined by analysis of variance.

RESULTS AND DISCUSSION

After both 28 and 33 days of dietary treatment, rats fed the salted cottage cheese without supplemental calcium had higher blood pressures than rats fed the unsalted cottage cheese without supplemental calcium (Table I). This observation is consistent with our previous observation that ingestion of excess chloride elevated blood pressures in young Sprague-Dawley rats fed semipurified diets (Kaup et al., 1991b; Greger and Tseng, 1993). Moreover, in previous studies, these small but significant differences in blood pressure induced by ingestion of excess chloride at 4 weeks were consistently maintained in longer (7–17 weeks) studies.

Table I. Blood Pressure and Body Weights of Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	blood pressure, mmHg: 3 days prior to treatment	blood pressure, mmHg: day 28	blood pressure, mmHg: day 33	body wt day 32, g
control	132 ± 4	143 ± 4[b]	146 ± 1[b]	269 ± 6
NaCl	132 ± 4	153 ± 2[a]	151 ± 2[a]	262 ± 6
Ca suppl	132 ± 4	140 ± 4[b]	148 ± 2[a,b]	262 ± 5
Ca suppl/NaCl	132 ± 3	138 ± 3[b]	149 ± 2[a,b]	270 ± 8

[a,b] Mean ± SEM (n = 8, except Ca suppl/NaCl, n = 7). Means in columns without common superscript letters differ significantly ($p < 0.05$) as determined by lsd tests.

No difference in blood pressure was noted at day 28 or day 33 among rats fed salted and unsalted cottage cheese when the rats were fed supplemental calcium. Previously, supplementation of diets with levels of calcium in excess of those usually considered to be adequate has been shown to slow the development of hypertension in spontaneously hypertensive rats (SHR) (Luft et al., 1988; McCarron et al., 1985; Porsti, 1992) and in rats treated with deoxycorticosterone (Resnick et al., 1986).

Blood pressures of rats fed the adequate level of calcium (regardless of salt intake) were similar at days 28 and 33; they differed ±3 mmHg. Blood pressures of rats fed supplemental calcium (regardless of salt intake) increased on average 10 mmHg between days 28 and 33. This suggests that long-term calcium supplementation may eventually elevate blood pressures of rats fed excess chloride. Resnick et al. (1986) noted that calcium supplementation for 1 month elevated blood pressures of rats with renin-sensitive hypertension (Goldblatt model). Thus, the response of Sprague-Dawley rats fed excess salt appeared to be intermediate between the responses of Goldblatt model rats and deoxycorticosterone-treated rats (Resnick et al., 1986).

The differences in blood pressure responses to calcium supplementation among these models may reflect differences in plasma renin activity. Plasma renin activities of the rats in this study did not differ among treatments. The rats fed the control diet, NaCl diet, Ca suppl diet, and Ca suppl/NaCl diet gave 5.91 ± 0.65 (mean ± SEM), 7.90 ± 2.44, 9.47 ± 2.39, and 7.89 ± 2.00 ng of angiotensin I generated mL^{-1} h^{-1}, respectively. These plasma renin concentrations are somewhat higher than those observed in the renin-suppressed rats treated with deoxycorticosterone (i.e., 1.89–2.73 ng of angiotensin I mL^{-1} h^{-1}) but much lower than those observed in the Goldblatt model rats (i.e., 34–46 ng of angiotensin I mL^{-1} h^{-1}) (Kotchen et al., 1987; Resnick et al., 1986). We do not know the cause for the larger variance in plasma renin activity among rats fed supplemental salt or calcium compared with that among rats fed the control diet in this study.

Lau et al. (1986) observed that ingestion of high levels of calcium prevented hypertension in SHR rats by depressing growth. However, in this study, the body and organ weights of rats did not differ among treatments. The average daily food intake of rats was 15.5 ± 0.4 g/day (mean ± pooled SE).

The rats fed supplemental calcium did not experience kidney hypertrophy or nephrocalcinosis. The weights of kidneys did not vary among treatments at sacrifice [1.05 ± 0.03 g/kidney (mean ± pooled SE)]. Thus, calcium supplementation would not be expected to elevate blood pressure through renal damage. However, the rats fed the salted cheese with calcium supplementation had higher

Table II. Calcium Utilization of Kidney Function by Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	apparent absorption of Ca, % of intake	urine Ca, mg/day	apparent retention of Ca,[a] mg/day	tibia Ca, mg/g of wet wt	kidney Ca, μg/g of wet wt	urine creatinine, mg/day
control	88 ± 1[c]	1.4 ± 0.4[d]	55 ± 2[c]	130 ± 2[c]	35 ± 1[b,c]	4.25 ± 0.18[c,d]
NaCl	94 ± 1[b]	1.8 ± 0.8[c,d]	49 ± 1[c]	131 ± 2[c]	30 ± 2[c]	4.03 ± 0.16[d]
Ca suppl	55 ± 1[d]	4.0 ± 0.6[c]	74 ± 3[b]	129 ± 2[c]	29 ± 1[c]	4.73 ± 0.26[b,c]
Ca suppl/NaCl	57 ± 3[d]	7.7 ± 1.6[b]	70 ± 6[b]	140 ± 2[b]	42 ± 6[b]	5.13 ± 0.21[b]

[a] Apparent retention = intake of Ca − fecal Ca − urine Ca. [b–d] Mean ± SEM (n = 8, except Ca suppl/NaCl, n = 7). Means in columns without common superscript letter differ significantly ($p < 0.05$) as determined by lsd tests.

Table III. Sodium Utilization by Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	apparent absorption of Na, % of intake	urine Na, % of intake	apparent retention of Na,[a] mg/day	tibia Na, mg/g of wet wt	kidney Na, mg/g of wet wt
control	97.0 ± 0.4[c]	31 ± 2[d]	12 ± 1[d]	3.30 ± 0.02[c]	1.80 ± 0.05[b,c]
NaCl	98.5 ± 0.2[b]	78 ± 2[b]	31 ± 3[c]	3.28 ± 0.04[c]	1.75 ± 0.04[b,c]
Ca suppl	97.0 ± 0.3[c]	36 ± 2[d]	10 ± 1[d]	3.29 ± 0.03[c]	1.38 ± 0.06[c]
Ca suppl/NaCl	98.9 ± 0.1[b]	69 ± 3[c]	48 ± 4[b]	3.41 ± 0.03[b]	1.93 ± 0.08[b]

[a] Apparent retention = intake of Na − fecal Na − urine Na. [b–d] Mean ± SEM (n = 8, except Ca suppl/NaCl, n = 7). Means in columns without common superscript letters differ significantly ($p < 0.05$) as determined by lsd tests.

Table IV. Chloride Utilization by Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	apparent absorption of Cl, % of intake	urine Cl, % of intake	apparent retention of Cl,[a] mg/day
control	98.6 ± 0.2[c,d]	68 ± 2[c]	8 ± 1[b]
NaCl	98.9 ± 0.2[b,c]	97 ± 2[b]	4 ± 5[b,c]
Ca suppl	98.3 ± 0.1[d]	61 ± 3[c]	10 ± 1[b]
Ca suppl/NaCl	99.3 ± 0.1[b]	100 ± 2[b]	−2 ± 5[c]

[a] Apparent retentions = intake of Cl − fecal Cl − urine Cl. [b–d] Mean ± SEM (n = 8, except Ca suppl/NaCl, n = 7). Means in columns without common superscript letter differ significantly ($p < 0.05$) as determined by lsd tests.

Table V. Potassium Utilization by Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	apparent absorption of K, % of intake	urine K, % of intake	apparent retention of K,[a] mg/day	kidney K, mg/g of wet wt
control	98.0 ± 0.3[b]	58 ± 1[c]	32 ± 2[c]	1.63 ± 0.09[b]
NaCl	97.2 ± 0.3[b]	64 ± 2[b]	20 ± 1[d]	1.09 ± 0.14[c]
Ca suppl	96.8 ± 0.3[b,c]	52 ± 2[d]	38 ± 2[b]	1.67 ± 0.11[b]
Ca suppl/NaCl	96.4 ± 0.5[c]	59 ± 2[c]	24 ± 1[d]	1.42 ± 0.12[b,c]

[a] Apparent retention = intake of K − fecal K − urine K. [b–d] Mean ± SEM (n = 8, except Ca Suppl/NaCl, n = 7). Means in columns without common superscript letters differ significantly ($p < 0.05$) as determined by lsd tests.

concentrations of calcium in their kidneys and excreted more creatinine in urine than other rats (Table II). In a longer study, calcium supplementation might eventually result in nephrocalcinosis that would exacerbate the effects of NaCl or the blood pressures of Sprague-Dawley rats. Moreover, by day 33, the blood pressures of the calcium-supplemented groups did not differ significantly from those of rats fed salted cheese without calcium supplementation.

We hypothesized that the observed effects of NaCl and of calcium on blood pressure partially reflected interactions among these elements. These interactions were complex.

The rats fed the supplemental calcium absorbed calcium less efficiently, excreted more calcium in urine, and retained more calcium during the balance period (Table II). Ingestion of the high rather than moderate levels of sodium and chloride in the cheese increased urinary calcium losses but increased tibia and kidney calcium concentrations among rats fed the supplemental calcium. Several investigators have observed previously that ingestion of supplemental salt increased urinary calcium excretion (Castenmiller et al., 1985; Greger et al., 1991; Whiting and Cole, 1986).

Those rats fed the salted rather than the unsalted cheese absorbed sodium less efficiently and lost more sodium in urine but still retained more sodium during the balance period (Table III). Calcium supplementation of the diets of rats fed the salted cheese decreased urinary sodium excretion, increased apparent retention of sodium during the balance period, and resulted in higher concentrations of sodium in tibias and kidneys.

Calcium intake had no effect on chloride utilization (Table IV). Those rats fed the elevated level of chloride in the salted cheese tended to absorb chloride more efficiently, lost more chloride in urine, and overall tended to retain less chloride during the balance period than rats fed the lower level of chloride in the unsalted cheese. The dietary treatments had no effect on chloride concentrations in bone [1.26 ± 0.2 (mean ± pooled SE) mg of Cl/g of wet weight] or kidney (2.12 ± 0.04 mg of Cl/g of wet weight).

Luft et al. (1988) noted that ingestion of supplemental calcium reduced urinary sodium excretion by SHR rats but did not affect urinary sodium excretion by Wistar-Kyoto rats. Overall, these and our data suggest that calcium supplementation does not affect the blood pressure by reducing sodium or chloride retention.

Potassium utilization was affected by both salt and calcium intakes (Table V). Ingestion of the salted rather than the unsalted cheese increased urinary excretion and reduced apparent retention of potassium during the balance period. Rats fed the salted cheese tended to retain less potassium in their kidneys than those fed the unsalted cheese. Tibia potassium concentrations [1.53 ± 0.04 (mean ± pooled SE) mg of K/g of wet weight] were unaffected by the dietary treatments.

Porsti et al. (1992) suggested that supplemental calcium lowered the blood pressure of SHR rats, not by changing sodium metabolism but by elevating serum potassium concentrations and by increasing smooth muscle Na^+, K^+ ATPase activity. Our observation is consistent with this suggestion. Calcium supplementation reduced urinary excretion of potassium. Among rats fed unsalted cheese, calcium supplementation promoted increased retention of potassium during the balance period.

Nephrocalcinosis, hypokalemia, and depressed tissue magnesium concentrations are symptoms of magnesium deficiency (Charlton and Armstrong, 1989; Shils, 1988).

Table VI. Magnesium Utilization by Rats Fed Cottage Cheese-Based Diets with Various Levels of NaCl and Calcium

diet treatment	apparent absorption of Mg, % of intake	urine Mg, mg/day	apparent retention of Mg,[a] mg/day	tibia Mg, μg/g of wet wt
control	82 ± 1[b]	0.9 ± 0.6[b]	5.6 ± 0.7[b]	2.35 ± 0.02[b]
NaCl	84 ± 2[b]	1.0 ± 0.6[b]	4.8 ± 0.6[b]	2.14 ± 0.04[c]
Ca suppl	70 ± 2[c]	1.2 ± 0.3[b]	4.6 ± 0.2[b]	2.18 ± 0.02[c]
Ca suppl/NaCl	72 ± 2[c]	2.3 ± 0.5[c]	3.0 ± 0.5[c]	2.13 ± 0.08[c]

[a] Apparent retention = intake of Mg − fecal Mg − urine Mg. [b,c] Mean ± SEM (n = 8, except Ca suppl/NaCl, n = 7). Means in columns without common superscript letter differ significantly ($p < 0.05$) as determined by lsd tests.

Rats fed excess NaCl, supplemental calcium, and the two in combination had depressed bone magnesium concentrations in this study (Table VI). This could be considered an indication of magnesium depletion and has been observed consistently in studies in which rats were fed supplemental NaCl or KCl (Charlton and Armstrong, 1989; Greger et al., 1991; Greger and Tseng, 1993).

The mechanisms of magnesium loss appeared to differ. Rats fed supplemental calcium absorbed magnesium less efficiently. Those rats fed salted cheese retained less magnesium during the balance period when their diets were supplemented with calcium largely because of increased urinary losses of magnesium.

We have demonstrated that the ingestion of supplemental salt will induce elevated blood pressure in young Sprague-Dawley rats. This animal model has relevance to humans because the amount of supplemental chloride we fed was roughly equivalent to the amount (0.2–0.3 mmol of Cl/g of dry diet) of chloride found in human metabolic diets (Greger et al., 1989). In this short-term study, calcium supplementation decreased the difference in blood pressures between animals fed salted and unsalted cheese. However, blood pressures of rats fed supplemental calcium increased between days 28 and 33. This suggests longer studies are needed to evaluate the potential long-term effects of calcium supplementation.

The beneficial effect of the supplemental calcium could not be attributed to differences among treatments in terms of growth of animals, plasma renin activity, or decreased retention of sodium or chloride. However, calcium supplementation appeared to lessen the effects of supplemental salt on potassium retention in kidneys.

Ingestion of both supplemental calcium and salt tended to reduce magnesium retention in bone. Rats fed supplemental levels of both NaCl and calcium tended to have elevated concentrations of calcium in their kidneys. Thus, the long-term effects of calcium supplementation on magnesium status, nephrocalcinosis, and blood pressure in this animal model need to be studied further.

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