High Calcium Diet Prevents Baroreflex Impairment in Salt-Loaded Spontaneously Hypertensive Rats

Ayumu Ono, Tomoyuki Kuwaki, Wei-Hua Cao, Mamoru Kumada, Toshiro Fujita

Abstract To investigate the role of the sympathetic control mechanism in the antihypertensive effect of dietary calcium supplementation, we examined whether a high calcium diet affected mean arterial pressure, renal sympathetic nerve activity, heart rate, and overall and central properties of the arterial baroreceptor reflex in salt-loaded young spontaneously hypertensive rats (SHR). Six-week-old SHR were fed either a normal (0.66%) or high (8.00%) salt diet with either a normal (1.17%) or high (4.07%) calcium content for 4 weeks. The arterial baroreceptor reflex was elicited with rats under halothane anesthesia by altering mean arterial pressure with nitroprusside or phenylephrine. The overall property of the arterial baroreceptor reflex was assessed by the median mean arterial pressure (MAPmed) and maximal gain (Gmax) of the two relations and an attenuation of reflex inhibition of renal sympathetic nerve activity by aortic depressor nerve stimulation. There were no significant differences in mean arterial pressure, renal sympathetic nerve activity, or the overall and central properties of the arterial baroreceptor reflex among the control, high salt/high calcium, and normal salt/high calcium groups. In conclusion, dietary calcium supplementation prevented accelerated hypertension with sympathetic overactivity as well as impairment of the arterial baroreceptor reflex in salt-loaded young SHR. It is suggested that normalization of both tonic and reflex control of sympathetic discharges underlies the antihypertensive effect of a high calcium diet on salt-induced hypertension. (Hypertension. 1994;24:83-90.)

Key Words calcium, dietary • sodium • central nervous system • pressoreceptors • sympathetic nervous system • rats, inbred SHR

Although epidemiologic studies have shown an inverse relation between daily oral calcium intake and the level of arterial pressure,1,2 clinical3,4 and experimental5-7 studies do not ubiquitously support the hypothesis that dietary calcium has an antihypertensive effect. With respect to hypertensive rat models, however, dietary calcium supplementation has been shown to consistently prevent salt-induced hypertension in Dahl salt-sensitive rats,8 young spontaneously hypertensive rats (SHR),5,6,9 and angiotensin II-treated rats.8 Moreover, evidence suggests that alteration of sympathetic activity may be involved in the antihypertensive effect of dietary calcium in these models.5-8

The arterial baroreceptor reflex (ABR) is a major mechanism in the central control of arterial pressure, heart rate (HR), and sympathetic vasomotor activity.10 The ABR has been demonstrated to be impaired in certain salt-sensitive animal models11-14 and patients with hypertension.15 Moreover, a high calcium diet in salt-induced hypertensive Dahl salt-sensitive rats augmented the baroreflex response of renal sympathetic nerve activity (RSNA) to alterations of arterial pressure.8 These results suggest that impairment of the ABR contributes to the pathogenesis of salt-induced hypertension and that normalization of the impaired ABR underlies the antihypertensive effect of a high calcium diet.

The present study, conducted in young salt-sensitive SHR,6,16 attempted to examine the effects of salt loading and simultaneous calcium supplementation on the sympathetic control mechanism with special attention paid to the ABR. We compared, in addition to basal levels of mean arterial pressure (MAP), HR, and RSNA, the overall and central properties of the ABR among four groups of SHR who were fed either a normal or high salt diet with either a normal or high calcium content. In examining the overall and central properties of the ABR, we made use of the fact that the aortic depressor nerve in the rat consists exclusively of baroreceptor afferents17 and that RSNA reflects sympathetic vasomotor fiber activity.18 Thus, the overall property of the ABR was assessed by the relation between MAP and RSNA and between MAP and HR, and the central property of the ABR was assessed by reflex inhibition of RSNA and HR, which was elicited by electrical stimulation of the aortic depressor nerve.
Methods

Preparation of Animals

Male SHR, confirmed to be salt sensitive by us\textsuperscript{16,19} and others,\textsuperscript{20} were purchased from Charles River Japan at 5 weeks of age. The following procedures were all in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences Recommended by the Physiological Society of Japan. The animals were maintained at constant humidity (60±5%), constant temperature (23±1°C) and regular light/dark cycle (light period from 6 AM to 6 PM). Beginning on the fifth day after arrival, rats were fed chow containing one of four combinations of NaCl and calcium carbonate for 4 weeks: 0.66% NaCl and 1.17% calcium (control group, n=13), 8.00% NaCl and 1.17% calcium (high salt/normal calcium group, n=14), 8.00% NaCl and 4.07% calcium (high salt/high calcium group, n=12), and 0.66% NaCl and 4.07% calcium (normal salt/high calcium group, n=8). Food and tap water were supplied ad libitum during the entire period of the study.

At the end of the dietary period, the rats were anesthetized initially with ether. After incision of tracheal, arterial, and venous cannu
as, the rats were immobilized by 0.5% of sodium nitroprusside (initially, 0.1 mg per rat IV; thereafter, 0.1 mg/h IV) and artificially ventilated with halothane in oxygen-enriched room air. The halothane concentration was kept at 1.0% to 1.5% during surgery and was then reduced to 0.6% to 0.8% during the recording period. Adequacy of anesthesia was evaluated by the absence of increases in arterial pressure, HR, and RSNA to hindlimb toe pinch. End-tidal PCO\textsubscript{2} was monitored continuously (Respina H26, Sanei-NEC) and maintained between 3% and 4.5%. Body temperature was maintained at 37±0.5°C by means of a ventral heat source.

Recording of Cardiovascular Variables and RSNA

Arterial pressure was recorded from the abdominal aorta by a polyethylene catheter (0.5-mm inner diameter) inserted through the femoral artery. MAP was recorded by passing the arterial pressure signal through a low-pass filter (corner frequency 0.2 Hz). HR was computed from the arterial pressure pulse by a tachometer (AT-601G, Nihon Kohden). The left renal nerve was approached retroperitoneally through a left flank incision and prepared for efferent discharge recording near the renal artery. Multifiber discharges were converted into a train of standard pulses by a window discriminator whose threshold was set slightly above the area above or below the prestimulus control level of each variable. The area calculated was normalized by dividing it by the prestimulus control level integrated over the 1-second interval. The area above or below the prestimulus control level of each variable was calculated by integrating the PSTH between the onset and end of the evoked response. A negative sign was used to indicate that the response decreased below that of the control. The area calculated was normalized by dividing it by the prestimulus control level integrated over the 1-second period. Since aortic depressor nerve stimulation elicited reflex inhibition of RSNA and HR, the response was expressed as a percent decrease in these variables.

Statistical Analysis

Data are presented as mean±SEM. Comparison of the data among the four groups of SHR was performed by one-way ANOVA followed by Duncan’s method for multiple comparisons among individual means. A value of P<.05 was considered statistically significant.

Results

Basal Hemodynamic Parameters and Body Weight

We compared basal levels of MAP, RSNA, HR, and body weight among the four groups of young SHR fed either a normal or high salt diet with either normal or high calcium intake for 4 weeks (Table 1). MAP and RSNA were significantly greater in the high salt/normal...
**TABLE 1. Effect of Salt Loading and/or Calcium Supplementation on Body Weight and Basal Mean Arterial Pressure, Heart Rate, and Renal Sympathetic Nerve Activity in Young Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>High Salt</th>
<th>High Salt+Ca</th>
<th>High Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>BW, g</td>
<td>254±7</td>
<td>242±3</td>
<td>233±3</td>
<td>240±8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>120±3</td>
<td>142±4*</td>
<td>119±3†</td>
<td>119±4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>335±11</td>
<td>340±19</td>
<td>322±10</td>
<td>356±9</td>
</tr>
<tr>
<td>RSNA, pulses/s</td>
<td>177±14</td>
<td>336±26*</td>
<td>185±19†</td>
<td>148±26</td>
</tr>
</tbody>
</table>

BW indicates body weight; MAP, mean arterial pressure; HR, heart rate; and RSNA, renal sympathetic nerve activity. Young spontaneously hypertensive rats (SHR) were divided into four groups: control, SHR fed a diet containing 0.66% NaCl and 1.17% calcium; high salt, high salt/normal calcium group fed a diet containing 8.00% NaCl and 1.17% calcium; high salt/high calcium group fed a diet containing 8.00% NaCl and 4.07% calcium; and high Ca, normal salt/high calcium group fed a diet containing 0.66% NaCl and 4.07% calcium. Values are mean±SEM.

*P<.01 compared with control.
†P<.01 compared with high salt.

calcium group compared with the control group. By contrast, in the high salt/high calcium group as well as in the normal salt/high calcium group, MAP and RSNA were not significantly different from those of the control group. Furthermore, the high salt/high calcium group had significantly lower MAP and RSNA compared with the high salt/normal calcium group. These results indicate that simultaneous calcium supplementation prevented salt-induced increases in MAP and RSNA. There was no significant difference in HR or body weight among the four groups.

**Relation Between MAP and RSNA**

The MAP-RSNA relation obtained from individual rats was fitted to a logistic function curve to determine $G_{\text{max}}$ and MAP$_{50}$ (see "Methods"). The two parameters were subsequently compared among the four groups (Table 2). In the high salt/normal calcium group, $G_{\text{max}}$ was significantly lower and MAP$_{50}$ was significantly higher than in the control group. Consequently, in the high salt/normal calcium group, the curve was blunted and shifted toward the higher pressure range (Fig 1). By contrast, in the high salt/high calcium group, as in the normal salt/high calcium group, $G_{\text{max}}$ and MAP$_{50}$ were not significantly different from those of the control group, and the MAP-RSNA relation was not apparently changed (Fig 1). Furthermore, the high salt/high calcium group had significantly greater $G_{\text{max}}$ and lower MAP$_{50}$ compared with the high salt/normal calcium group.

**Relation Between MAP and HR**

The MAP-HR relation obtained from the four groups was analyzed in the same manner as the MAP-RSNA relation (Table 2). In the high salt/normal calcium group, $G_{\text{max}}$ was significantly lower and MAP$_{50}$ was significantly higher than in the control group. Consequently, in the high salt/normal calcium group, the MAP-HR relation was blunted and shifted to the right (Fig 2). By contrast, in the high salt/high calcium group, as in the normal salt/high calcium group, $G_{\text{max}}$ and MAP$_{50}$ in the MAP-HR relation were not significantly different from those of the control group. Furthermore, the high salt/high calcium group had significantly

**TABLE 2. Effect of Salt Loading and/or Calcium Supplementation on MAP$_{50}$ and $G_{\text{max}}$ of the Relation Between Mean Arterial Pressure and Renal Sympathetic Nerve Activity or Heart Rate in Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>High Salt</th>
<th>High Salt+Ca</th>
<th>High Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>MAP-RSNA relation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP$_{50}$, mm Hg</td>
<td>145±7</td>
<td>196±13*</td>
<td>163±7†</td>
<td>158±5</td>
</tr>
<tr>
<td>$G_{\text{max}}$, mm Hg$^{-1}$</td>
<td>1.02±0.06</td>
<td>0.60±0.06*</td>
<td>0.95±0.12†</td>
<td>1.11±0.13</td>
</tr>
<tr>
<td>MAP-HR relation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP$_{50}$, mm Hg</td>
<td>136±5</td>
<td>169±13*</td>
<td>126±7†</td>
<td>142±4</td>
</tr>
<tr>
<td>$G_{\text{max}}$, mm Hg$^{-1}$</td>
<td>1.07±0.12</td>
<td>0.44±0.12*</td>
<td>0.99±0.09†</td>
<td>0.89±0.05</td>
</tr>
<tr>
<td>HR range, bpm</td>
<td>51±11</td>
<td>46±9</td>
<td>49±9</td>
<td>40±5</td>
</tr>
<tr>
<td>Maximum HR, bpm</td>
<td>359±12</td>
<td>380±17</td>
<td>362±10</td>
<td>352±11</td>
</tr>
<tr>
<td>Minimum HR, bpm</td>
<td>309±15</td>
<td>334±12</td>
<td>312±13</td>
<td>312±9</td>
</tr>
</tbody>
</table>

MAP$_{50}$ indicates median mean arterial pressure; $G_{\text{max}}$, maximal gain; MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity; and HR, heart rate. Values are mean±SEM. Rat groups are as in Table 1.

*P<.01 compared with control.
†P<.05, ‡P<.01 compared with high salt.
Reflex Changes in RSNA and HR by Electrical Stimulation of the Aortic Depressor Nerve

One-second tetanic stimulation of the aortic depressor nerve reflexly elicited transient inhibition of RSNA with bradycardia (Fig 3), which vanished well before the next cycle of aortic depressor nerve stimulation. At a stimulus frequency of 25 Hz, intended to elicit the ABR originating from unmyelinated baroreceptor afferents, there was no significant difference among the four groups in the percent decrease of RSNA (Fig 4B). Salt loading in young SHR did not appear to have significant central modulatory effects on the ABR originating from unmyelinated aortic depressor nerve fibers. With respect to the reflex HR response, there were no significant differences among the four groups at a stimulus frequency of either 25 or 100 Hz (Fig 5).

Discussion

This study was intended to clarify the role of the sympathetic control mechanism in the development of salt-induced hypertension and the antihypertensive effect of a high calcium diet. For that purpose we examined whether high dietary intake of salt and/or calcium affected MAP, RSNA, HR, and the ABR in young SHR. In keeping with previous reports, high salt intake without calcium supplementation resulted in accelerated hypertension with renal sympathetic overactivity in young SHR. Furthermore, we obtained the following two major results: (1) A high salt diet impaired both overall and central properties of the ABR. Only the reflex arising from myelinated baroreceptor afferents was affected centrally. (2) A high calcium diet...
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Fig 3. Peristimulus time histogram records of renal sympathetic nerve activity in response to electrical stimulation of the aortic depressor nerve. Records represent each of four groups of spontaneously hypertensive rats as defined in Fig 1. Histograms were constructed by superimposing 32 responses to tetanic stimulation of the aortic depressor nerve by a 1-second train of square-wave pulses having an intensity of 1 V and frequency of 100 Hz (left) or 5 V and 25 Hz (right). Horizontal bar above each tracing indicates duration of electrical stimulation. Abscissa is the number of renal nerve discharges recorded over 10 milliseconds. Prevented salt-induced accelerated hypertension and renal sympathetic overactivity. It also prevented the impairment of the overall and central properties of the ABR.

In the present study, salt loading attenuated the reflex responses of RSNA and HR to stimulation of arterial baroreceptors in young SHR. This result is consistent with previous studies in hypertensive animal models and humans, with the exception of one report by Calhoun et al. They demonstrated that salt loading in salt-sensitive SHR for 2 to 3 weeks enhanced rather than attenuated the response of lumbar sympathetic nerve activity (LSNA) to stimulation of arterial baroreceptors with rats under conscious, unrestrained conditions. We suspect that the apparent discrepancy between their results and ours is primarily due to the following two differences in experimental conditions.

First, we selected RSNA, not LSNA, to monitor the response of the ABR. Although efferent renal sympathetic postganglionic fibers innervate vascular and nonvascular elements of the kidney, both groups of fibers behave in an identical manner so that RSNA can be considered to reflect the activity of sympathetic vasoconstrictor fibers. The lumbar sympathetic nerve on the other hand is composed of postganglionic muscle and skin fibers of various modalities such as vasomotor, sudomotor, and piloereector fibers. As demonstrated in single-fiber preparations in laboratory animals and microneurograms in humans, these fibers behave differently with respect to their rate and temporal pattern of spontaneous discharges as well as their responses to various inputs such as baroreceptor, chemoreceptor, or thermal stimulation. For this reason, these two sympathetic nerves may respond differently to baroreceptor stimulation in normotensive and/or hypertensive animals. In fact, the reflex change in RSNA to baroreceptor stimulation was found to be attenuated in SHR and renal hypertensive rabbits, whereas in LSNA it was not.

Fig 4. Bar graphs show percent decrease in renal sympathetic nerve activity (RSNA) in response to electrical stimulation of the aortic depressor nerve. Bars correspond to four groups of spontaneously hypertensive rats as defined in Fig 1. Stimulation was a 1-second square-wave pulse with intensity of 0.5, 1, 2, 5, or 10 V and frequency of 100 Hz (A) or 25 Hz (B). Responses of the four rat groups were compared by means of one-way ANOVA. *P<.05, **P<.01 compared with the control group; †P<.05, ††P<.01 compared with the high salt/normal calcium group. Since aortic depressor nerve stimulation activates the reflex pathway without involving arterial baroreceptors, the RSNA response characterizes the central property of the arterial baroreceptor-RSNA reflex. Note that at a fixed stimulus frequency of 100 Hz with stimulus intensities of 1, 2, 5, and 10 V (intended to elicit arterial baroreceptor reflex arising from aortic depressor nerve myelinated fibers), reflex decrease in RSNA was significantly attenuated in the high salt/normal calcium group compared to the control or high salt/high calcium groups. This result indicates that salt loading impaired the central property of the arterial baroreceptor-RSNA reflex and that simultaneous calcium supplementation prevented this impairment. Difference in RSNA response was not present when the aortic depressor nerve was stimulated at a fixed frequency of 25 Hz (intended to elicit arterial baroreceptor reflex arising from aortic depressor nerve unmyelinated fibers).
Second, the observed discrepancy may be due to the effects of the halothane anesthesia used in our study. Halothane has an inhibitory effect on the ABR in laboratory animals and humans, so it might have preferentially attenuated the arterial baroreceptor–renal sympathetic reflex of salt-loaded rats without calcium supplementation. However, this is unlikely for the following reasons: (1) In our experiments, during the recording period after the initial surgical procedure, the halothane concentration was reduced and maintained at 0.6% to 0.8%. At this level of halothane anesthesia, the reflex response of sympathetic fibers should be comparable to that of the conscious state. Actually, $G_{\text{max}}$ of the MAP-RSNA relation in the present study was similar to that in conscious SHR as reported by Kumagai et al. (2) In another series of experiments, we observed that salt loading significantly enhanced $G_{\text{max}}$ of the MAP-RSNA relation in halothane-anesthetized young Wistar-Kyoto rats.

Evidence suggests that salt loading modifies the central neural control of sympathetic activity in SHR. For example, in SHR but not in Wistar-Kyoto rats, a high salt intake resulted in a greater increase in RSNA induced by stressful environmental stimulation (air jet to the face). Furthermore, a recent study we conducted demonstrated that both hypothalamic and renal norepinephrine turnover rates were augmented in salt-loaded young SHR. Therefore, we investigated whether salt loading altered the central property of the arterial baroreceptor–RSNA reflex. This was demonstrated by salt-induced attenuation of the RSNA reflex response to electrical stimulation of myelinated fibers of the aortic depressor nerve, which consists exclusively of baroreceptor afferents. Interestingly, in both SHR and renal hypertensive rabbits, a resetting of the stimulus–response curve (a functional representation of the baroreceptor–afferent fiber complex) was greater in myelinated fibers than unmyelinated fibers. We should stress that the duration of electrical stimulation of the aortic depressor nerve to obtain the PSTH was intentionally set short (1 second) in the present study. As a result, repeated stimulation of the aortic depressor nerve caused minimal reflex changes in HR. This had little effect on MAP and did not distort the aortic depressor nerve–RSNA reflex. Therefore, lack of a significant alteration of the aortic depressor nerve–HR reflex in salt-loaded SHR does not automatically imply that this reflex is not impaired centrally.

As previously demonstrated, dietary calcium supplementation exerts an antihypertensive effect in hypertensive patients and animals and the sympathetic nervous system participates in this process. The present study examined whether simultaneous dietary calcium supplementation affected MAP, RSNA, HR, and the ABR in salt-loaded young SHR. A high calcium diet prevented salt-induced hypertension with sympathetic overactivity and impairment of the overall and central properties of the ABR. These findings are consistent with the report by Peuler et al. in Dahl salt-sensitive rats, although there was no central change observed on the ABR function.

Evidence suggests a direct action of dietary calcium on the central nervous system. In angiotensin II–induced hypertensive rats to which calcium was given orally, calcium was transported into the central nervous system, caused a decrease in MAP. In addition, changes in calcium concentration in the lateral ventricle and in certain central nervous system regions such as the nucleus tractus solitarius in Dahl salt-sensitive rats, although there was no central change observed on the ABR function. The possibility does remain, however, that a high calcium diet initially prevents salt-induced hypertension and then secondarily prevents deterioration of the ABR. Hypertension per se can also cause a resetting of the ABR, which may be reversed by antihypertensive drugs.

There are some possibilities that dietary calcium may lower arterial pressure in part because of the peripheral sympathetic mechanism. Hatton et al. demonstrated that SHR fed a low calcium diet had larger pressor...
responses to environmental stress compared with those fed a high calcium diet, although both rat groups showed similar plasma norepinephrine release during the stress. Moreover, a high calcium diet could attenuate development of hypertension and α1-adrenergic receptor-mediated responses relative to a low calcium diet in young SHR.47 These results suggest that altered post synaptic responses to adrenergic agonists are involved in the antihypertensive effect of dietary calcium.

We are aware that the contents of NaCl and calcium in the diet used in this study are high and do not necessarily reflect clinically relevant values. We had three reasons for using diets containing 8.00% NaCl and/or 4.07% calcium. First, diets containing 8% NaCl have been widely used in SHR to increase arterial pressure without undesirable side effects.6,16,19,26,44 Second, diets containing 4% calcium have been consistently shown to reduce arterial pressure and/or sympathetic activity in hypertensive animal models.7,8,41,49 By contrast, there does not seem to be general agreement as to whether diets containing less than 4% calcium lower arterial pressure in hypertensive animal models. For example, Karanja et al9 demonstrated that a diet containing 0.98% to 1.7% calcium could temper the rate of NaCl-induced hypertension in SHR. In SHR loaded with 8% NaCl, diets containing 2% calcium attenuated the increases in arterial pressure and plasma norepinephrine.4 In Dahl salt-sensitive rats, however, 2% calcium diets accelerated salt-induced hypertension.40 In any case, it should be pointed out that the dose dependency of the effect of dietary NaCl and/or calcium on the ABR is an important issue that needs to be clarified.

In conclusion, salt loading in young SHR resulted in increases in MAP and RSNA and impairment of both overall and central properties of the arterial baroreceptor–RSNA reflex, all of which were prevented by simultaneous dietary calcium supplementation. These results suggest that an unimpaired baroreceptor reflex with intact peripheral and central mechanisms underlies the antihypertensive effect of a high calcium diet.

Acknowledgments

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