In Vivo Effect of Calcitriol on Calcium Transport and Calcium Binding Proteins in the Spontaneously Hypertensive Rat

Chantal M. Roullet, Jean-Baptiste Roullet, Anne-Sophie Martin, David A. McCarron

Abstract The abnormal intestinal Ca\textsuperscript{2+} transport reported in spontaneously hypertensive rats (SHR) has been attributed to decreased responsiveness to calcitriol. We reexamined this hypothesis by studying the calcitriol regulation of SHR duodenal calbindin-D\textsubscript{9K} and calmodulin and the relation of calciotriol to Ca\textsuperscript{2+} uptake by isolated enterocytes. SHR and normotensive Wistar-Kyoto (WKY) rats were injected with either 50 ng/d calcitriol (vit-D) or vehicle alone (control) for 3 days. Decreased calbindin-D\textsubscript{9K} (P<.001) and cellular Ca\textsuperscript{2+} content\textsuperscript{7} were observed in control SHR. Calcitriol increased total cell and brush border calbindin-D\textsubscript{9K} (P<.0001); this variation paralleled plasma calcium levels in both strains. In contrast, Ca\textsuperscript{2+} flux, which increased in vit-D animals, remained lower in SHR for plasma calcium levels similar to those in WKY rats. Immunoreactive calmodulin was similar in both strains whether assayed in total cell or brush border membranes. In contrast, when measured by ligand blotting (\textsuperscript{45}Ca), calmodulin was lower in SHR than in WKY rats (P<.01), suggesting the existence of a calmodulin pool with reduced Ca\textsuperscript{2+} binding capacity in the hypertensive strain. Calcitriol had no effect on calmodulin in either strain. In conclusion, Ca\textsuperscript{2+} binding protein regulation by calcitriol is normal in the SHR, and decreased hormone responsiveness cannot account for the defective duodenal calcium transport of this experimental model of hypertension. 

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Impaired calcium homeostasis is a well-recognized feature of human hypertension\textsuperscript{1-3} and has also been characterized in several animal models of essential hypertension, including the spontaneously hypertensive rat (SHR).\textsuperscript{4-6} Calcium abnormalities include decreased plasma ionized Ca\textsuperscript{2+46} and bone Ca\textsuperscript{2+46} content,\textsuperscript{7} elevated intracellular free Ca\textsuperscript{2+46} and parathyroid hormone levels,\textsuperscript{4,6} a urinary Ca\textsuperscript{2+} leak,\textsuperscript{1,2} and impaired intestinal Ca\textsuperscript{2+} transport. The existence of a defect of intestinal calcium transport has been characterized in several animal models of hypertension,\textsuperscript{5,6} and has also been described in several animal models of human hypertension.\textsuperscript{2,3}

Calcium transport across the intestine involves a saturable calciotriol-regulated transcellular process and an independent paracellular mechanism that is nonsaturable. The transcellular process involves translocation across the brush border via a facilitated diffusion along a chemical gradient, a second phase transiting the cytosol, and finally an extrusion across the basolateral plasma membrane mediated by Ca\textsuperscript{2+46}-ATPase or Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange.\textsuperscript{11} Calcium binding proteins, including calbindin-D\textsubscript{9K} and calmodulin, play a significant role in the transcellular process. The exact mechanism of action of calbindin-D\textsubscript{9K} remains uncertain; however, its role in intestinal calcium transport has been inferred from reports showing a parallel increase of its cellular levels and activity of Ca\textsuperscript{2+} uptake\textsuperscript{12} as well as direct regulation by calcitriol. Calmodulin activates Ca\textsuperscript{2+46}-ATPase and facilitates cellular Ca\textsuperscript{2+} extrusion.\textsuperscript{13} Studies have shown that blocking the activity of calmodulin with specific antagonists (trifluoperazine) inhibits Ca\textsuperscript{2+} transport in duodenum.\textsuperscript{14} Calmodulin may also be dependent on calcitriol, as the hormone can modify calmodulin cellular distribution.\textsuperscript{15,16}

The entire transport system is defective in the SHR, as documented by several reports using different techniques: everted gut sac,\textsuperscript{17} in vivo gut perfusion,\textsuperscript{18} modified Ussing chamber,\textsuperscript{19} and Ca\textsuperscript{2+} uptake in isolated duodenal cells.\textsuperscript{20} In an earlier study,\textsuperscript{21} we observed that abnormal enterocyte Ca\textsuperscript{2+} handling was associated with decreased levels of calbindin-D\textsubscript{9K} and calmodulin.

A causal role of calcitriol in defective calcium transport has been postulated but not established. A primary disturbance in calcitriol metabolism has been suggested by studies showing an abnormal age-dependent variation of plasma calcitriol in the SHR compared with normotensive controls: higher at 3.5 weeks of age\textsuperscript{22,23} and similar\textsuperscript{24,25} or lower\textsuperscript{26} at 12 weeks of age. Other studies have shown decreased production or abnormal clearance of the hormone.\textsuperscript{24} Altered end-organ sensitivity to 1,25(OH)\textsubscript{2}D\textsubscript{3} may also be contributory, in addition to impaired calcitriol metabolism. Toraason and Wright\textsuperscript{25} and Schedl et al\textsuperscript{28} reported that addition of calcitriol to the intestine had no effect on calcium transport in the SHR as measured by the everted sac technique. In contrast, Gaffer et al\textsuperscript{29} and Lucas et al\textsuperscript{7} reported that 1,25(OH)\textsubscript{2}D\textsubscript{3} significantly increased mucosal to serosal Ca\textsuperscript{2+} flux in the SHR. However, the...
study by Lucas et al did not compare the effect of the hormone on SHR intestinal calcium transport to that in controls, thereby weakening the conclusion of normal calcitriol sensitivity in their model.

We hypothesized that abnormal levels of calcium binding proteins in the SHR were the consequence of intestinal resistance to calcitriol. We therefore measured duodenal calbindin-D9K and calmodulin after short-term treatment with exogenous calcitriol with parallel measurements of Ca\(^{2+}\) uptake by isolated enterocytes. Our findings do not support a disturbance in SHR sensitivity to calcitriol and provide evidence that a functional abnormality of calmodulin is the primary cause of defective calcium transport in this model of essential hypertension.

### Methods

#### Animals

Male SHR and Wistar-Kyoto (WKY) rats were obtained from Charles River Breeding Laboratories (Boston, Mass) at 10 to 12 weeks of age and weighed approximately 260 g. They were housed in stainless steel cages in groups of three, maintained under a 12-hour day/night cycle, and given free access to food (Purina lab chow containing 1.0% calcium) and water. All procedures were in accordance with institutional guidelines. Rats were randomly allocated to either a control group or calcitriol (vit-D)-treated group. Vit-D rats were injected with 50 ng/d IP calcitriol for 3 days; controls received vehicle only (propylene glycol). Rats were fasted for 16 to 18 hours and killed between 9 and 11 AM 24 hours after the last calcitriol injection. They were anesthetized with ether, and blood was collected through the abdominal aorta.

#### Enterocyte Isolation

The first 10 cm of proximal duodenum was removed, rinsed, and everted on a glass spiral. Enterocytes were isolated by mechanical vibration and suspended in 20 mmol/L Tris-HCl buffer, pH 7.4, as described elsewhere. The cells were then used immediately (Ca\(^{2+}\) uptake experiments) or frozen at \(-70^\circ\text{C}\) (calcium binding protein experiments).

#### Ca\(^{2+}\) Uptake Experiments

Eighteen SHR and 18 WKY rats (9 control and 9 vit-D animals per strain) were used. Calcium uptake was measured on a freshly isolated cell suspension using \(^{45}\text{Ca}\) as previously detailed. The Ca\(^{2+}\) uptake curve was fitted to a double exponential equation, a model for a three-compartment series system. This allowed the determination of membrane (S\(_m\)) and cellular (S\(_c\)) calcium pools as well as membrane (J\(_m\), flux from the extracellular medium to the membrane) and cellular (J\(_c\), flux from the membrane to the cellular pool) fluxes.

#### Calcium Binding Protein Experiments

In one set of experiments, 24 SHR and 24 WKY rats (12 control and 12 vit-D animals per strain) were used. Duodenal cells were collected as described above and frozen. After solubilization, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% to 27% gradient). Calbindin-D9K and calmodulin concentrations were determined by ligand blotting. Briefly, after electrophoretic transfer to 0.1-\(\mu\)m nitrocellulose, calcium binding proteins were labeled with radioactive calcium (\(\text{Ca}^{45}\)). The dried nitrocellulose was then exposed to Kodak X-OMAT film for autoradiography. Films were developed and scanned with a laser densitometer. Calmodulin was also measured using a radioimmunoassay (IgG Corp). Bovine brain calmodulin was used as the standard in both assays, and calibration curves were parallel (data not shown).

In a second set of experiments, 48 SHR and 48 WKY rats (24 control and 24 vit-D animals per strain) were killed at the age of 12 weeks. The first 10 cm of duodenum was removed, rinsed with saline, and opened. Cells were collected by scraping. The cells from 3 rats of the same group were pooled, and brush border membranes were isolated according to the calcium precipitation method described by Ling et al. Protein concentration was determined by the method of Peterson. Calbindin-D9K and calmodulin were then measured by ligand blotting, and calmodulin was also assayed by radioimmunoassay. Preliminary experiments were conducted in which the degree of purification of the brush border preparations was determined by measuring the activity of sucrase, a brush border-specific marker.

#### Blood Chemistry

Ionized calcium concentrations and pH were measured with ion-selective electrodes (Labyte, Beckman) on fresh whole blood. Plasma levels of total calcium, phosphorus, and magnesium were determined in frozen plasma samples using standard laboratory methods. Plasma calcitriol was determined using a radioreceptor assay with an interassay variation of 6%.

#### Statistical Analysis

All results are given as mean±SEM. Two-way ANOVA was used to assess strain differences, treatment effect, and interactions between strain and calcitriol treatment.

### Results

#### Preliminary Experiments

Duodenal cell isolation yielded identical amounts of protein and DNA in the four experimental groups (Table 1), with no difference in the protein-to-DNA ratio (not shown). The quality of the brush border preparation was evaluated by measuring the enrichment in sucrase activity (Table 2). The factor of enrichment was similar for both strains (15±2.5 versus 21±3.2) and in agreement with previous reports.

#### Ca\(^{2+}\) Uptake

Cellular calcium flux was significantly lower in SHR than in WKY rats (\(P<.0001\), Table 3). Membrane flux, membrane pool, and cellular calcium pool were not different. Calcitriol injection induced a significant increase in membrane pool (\(P<.015\)), cellular calcium pool (\(P<.002\)), and cellular calcium flux (\(P<.0003\)) in both strains. However, calcitriol administration did not correct the initial strain difference in cellular calcium flux: the flux of SHR remained significantly lower than that of WKY rats in the vit-D group. There was no effect

| Table 1. Protein and DNA Yield by Duodenum From Control and Calcitriol-Treated SHR and WKY Rats |
|-----------------|-----------------|-----------------|
|                 | Protein, \(\mu\)g (n=11) | DNA, \(\mu\)g (n=11) |
| WKY rats        |                  |                 |
| Control         | 4.65±0.35        | 504±46          |
| Vit-D           | 4.38±0.48        | 405±39          |
| SHR             |                  |                 |
| Control         | 4.67±0.33        | 493±37          |
| Vit-D           | 4.60±0.49        | 532±42          |

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; and vit-D, injected with 50 ng/d IP calcitriol.
of the hormone on membrane flux. Plasma calcitriol levels were similar in control SHR and WKY rats and reached comparable levels in vit-D groups (Table 3).

### Calcium Binding Proteins

Analysis of the whole-cell duodenal extract showed a decreased calbindin-D9K level in SHR compared with WKY rats \( (P<.03, \text{Table 4}) \). The strain difference was still observed in the vit-D group. Calcitriol induced a significant increase of the protein \( (P<.0001) \) in both strains (81% for WKY rats and 107% for SHR). Fig 1 shows the results for duodenal calmodulin. The ligand-blotting method revealed that SHR had lower levels of calmodulin than WKY rats \( (P<.01) \) and showed no effect of calcitriol injection. With the radioimmunoassay (Fig 1), no strain difference or effect of the hormone was observed.

In the second set of experiments, preparations of brush border membranes were analyzed. Calbindin-D9K levels were similar in SHR and WKY rats (Table 4). Calcitriol injection significantly increased calcium binding protein level in both strains \( (P<.015) \). For calmodulin (Table 5), there was no strain difference in brush border content or significant effect of calcitriol injection. These results were not influenced by assay type. Table 6 shows the plasma levels of calcitriol, ionized calcium, phosphorus, and magnesium in both control and calcitriol-treated WKY rats and SHR. Plasma calcitriol, ionized \( \text{Ca}^{2+} \), and pH were significantly lower in SHR than in WKY rats. Calcitriol injection induced a significant increase in the hormone level as well as in ionized \( \text{Ca}^{2+} \) in both strains. However, there was a much smaller increase of ionized \( \text{Ca}^{2+} \) after calcitriol treatment in SHR than in WKY rats (interaction of strain and treatment significant, \( P<.0001 \)). Total \( \text{Ca}^{2+} \) was identical in both strains and increased significantly after calcitriol treatment.

Phosphorus levels were lower in control SHR than in their WKY counterparts. Calcitriol injection significantly increased phosphorus level in both strains. However, this increase was higher in SHR than in WKY rats, leading to an absence of difference between the two strains in the treated group. Finally, Mg\( ^{2+} \) plasma levels were similar in both strains and significantly decreased by calcitriol injection.

### Discussion

The present study tested the hypothesis that impaired intestinal calcium transport in the SHR is related to an altered sensitivity of duodenum calcium binding pro-

### Table 2. Sucrase Activity In Whole Duodenal Cell Extract and Brush Border Preparation In SHR and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY Rats (n=7)</th>
<th>SHR (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-cell</td>
<td>25.9±4.8</td>
<td>25.7±4.7</td>
</tr>
<tr>
<td>Brush border</td>
<td>399.6±97.0</td>
<td>523.9±128.0</td>
</tr>
<tr>
<td>Enrichment</td>
<td>15.0±2.5</td>
<td>21.0±3.2</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto. Values are expressed in nanomoles glucose per milligram protein per hour. \( P=NS \) by strain; \( P<.0001 \) by localization, ANOVA.

### Table 3. Calcium Flux, Pools, and Plasma Levels of Calcitriol In Control and Calcitriol-Treated SHR and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>( J_m )</th>
<th>( S_m )</th>
<th>( J_o )</th>
<th>( S_o )</th>
<th>Calcitriol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WKY rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ( (n=8) )</td>
<td>7.6±0.9</td>
<td>3.0±0.3</td>
<td>0.74±0.05</td>
<td>9.5±0.7</td>
<td>49±4</td>
</tr>
<tr>
<td>Vit-D ( (n=10) )</td>
<td>9.7±1.6</td>
<td>3.5±0.2</td>
<td>0.98±0.09</td>
<td>16.6±1.8</td>
<td>173±23</td>
</tr>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ( (n=9) )</td>
<td>6.2±0.6</td>
<td>3.6±0.3</td>
<td>0.41±0.04</td>
<td>8.6±0.8</td>
<td>39±4</td>
</tr>
<tr>
<td>Vit-D ( (n=9) )</td>
<td>7.8±0.9</td>
<td>4.1±0.3</td>
<td>0.64±0.05</td>
<td>13.8±1.3</td>
<td>189±25</td>
</tr>
</tbody>
</table>

\( P (\text{ANOVA}) \)

| Strain          | NS       | NS       | <.0001   | NS       | NS       |
| Drug            | <.015    | <.0003   | <.002    | <.001    |

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; \( J_m \), membrane flux; \( S_m \), membrane pool; \( J_o \), cellular flux; \( S_o \), cellular pool; and vit-D, injected with 50 ng/d IP calcitriol. Flux values are expressed as nanomoles \( \text{Ca}^{2+} \) per milligram protein per minute; pool values as nanomoles \( \text{Ca}^{2+} \) per milligram protein; and calcitriol levels as picograms per milliliter. \( n \) is number of rats.
However, the results do not support the initial hypothesis of a decreased responsiveness to calcitriol. These results confirm previously reported abnormalities of duodenum calcium transport in the SHR: decreased intestinal calcium transport, decreased calcium flux in isolated enterocytes, and decreased levels of both calbindin-D9K and calmodulin. However, the results do not support the initial hypothesis of a decreased responsiveness to calcitriol.

Experiments were conducted in 10- to 12-week-old animals. At this age, calcium transport abnormalities are well established in the SHR. We chose a short (3-day) injection schedule because of previous reports showing that the adaptive response of calbindin-D9K to calcitriol was rapid and certainly maximal after 24 to 48 hours. As shown in Tables 3 and 6, this schedule, together with a 50-ng/d calcitriol dose, increased the plasma levels of the hormone to high (two to four times higher than in control animals) but still physiological values. Duodenal cells were chosen because of their major role in active calcium absorption.

Sensitivity to calcitriol was investigated using three different approaches: (1) measurement of the calcium flux in isolated cells as an index of Ca$^{2+}$ transport, (2) determination of calbindin-D9K and calmodulin in both whole-cell and brush border membrane preparations to detect any influence of calcitriol on the cellular partitioning of calcium binding proteins, and (3) use of both a radioimmunoassay and ligand-blotting assay to reveal hormone-induced changes of functional and immunoreactive calmodulin. Calbindin-D9K was determined only by ligand blotting as we previously demonstrated that there is excellent correlation between the radioimmunoassay and ligand-blotting methods in both strains.

Calcium flux, calbindin-D9K, and plasma calcitriol levels increased in response to injection with calcitriol as previously reported. The increase in calbindin-D9K levels was proportional to the increase of plasma calcitriol in both WKY rats and SHR; as shown in Fig 2, for identical calcitriol plasma levels, the calbindin-D9K level is expected to be similar in both strains. In contrast, for identical calcitriol plasma levels, Ca$^{2+}$ influx remains lower in the SHR than the WKY rat (Fig 3). These findings strongly suggest that the responsiveness to the hormone is preserved in the hypertensive animals, without excluding the participation of a decrease in calbindin-D9K to the Ca$^{2+}$ transport defect of the SHR. In our earlier report, we observed decreased duodenal levels of calbindin-D9K and an absence of correlation between calbindin-D9K level and calcitriol plasma level in the SHR. As opposed to the present study, which examines the short-term effect of exogenous calcitriol, our earlier study focused on the consequences of long-term dietary calcium manipulations and associated induced chronic changes of calbindin plasma level on duodenal calcium binding proteins.

Singh and Bronner reported that high calcium intake acts directly on the intestinal cells so as to repress plasma level on duodenal calcium binding proteins. The coexistence in the SHR of an abnormal regulation by dietary calcium (our previous study) and of a normal regulation by calcitriol (present data) would therefore explain the apparent discrepancy between our two reports.

The fact that calcitriol had an effect on cellular calcium flux but not on membrane calcium flux is in agreement with the observations of Takito et al. Using calcitriol-supplemented chicks, these authors observed a significant increase in the second (slower) phase of calcium uptake in brush border membrane vesicles, whereas in the initial phase of uptake, which represents the binding of calcium to the membrane, no effect was observed.

Our data also demonstrate the presence of calcitriol-regulated calbindin-D9K at the brush border level. The finding of detectable calbindin-D9K in the brush border is in agreement with Shimura and Wasserman but contrasts with some earlier reports of exclusive cytосolic...
Table 6. Plasma Calcitriol, Ionized Calcium, pH, Total Calcium, Phosphorus, and Magnesium in Control and Calcitriol-Treated SHR and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY rats</th>
<th>SHR</th>
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<tbody>
<tr>
<td></td>
<td>Control (n=35)</td>
<td>Vdt-D (n=35)</td>
</tr>
<tr>
<td>Calcitriol, pg/mL</td>
<td>53±3</td>
<td>128±7</td>
</tr>
<tr>
<td>Ionized Calcium, mmol/L</td>
<td>1.37±0.01</td>
<td>1.52±0.01</td>
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<tr>
<td>pH</td>
<td>7.43±0.01</td>
<td>7.45±0.01</td>
</tr>
<tr>
<td>Total Calcium, mg/dL</td>
<td>9.3±0.12</td>
<td>9.79±0.12</td>
</tr>
<tr>
<td>Phosphorus, mg/dL</td>
<td>6.0±0.08</td>
<td>6.41±0.10</td>
</tr>
<tr>
<td>Magnesium, mg/dL</td>
<td>1.48±0.03</td>
<td>1.30±0.02</td>
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P (ANOVA)

<table>
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<tr>
<th></th>
<th>Strain</th>
<th>Drug</th>
<th>Interaction</th>
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<tr>
<td></td>
<td>&lt;.0003</td>
<td>&lt;.0001</td>
<td>NS</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>&lt;.01</td>
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Definitions are as in Table 1. n is number of rats.

Localization. The likelihood that calbindin-D9K is also present in the brush border is supported by our demonstration that calcitriol supplementation significantly increases calbindin-D9K content of the brush border in both strains. Thus, calbindin-D9K may participate in calcium transport at this level. Ghishan et al. in the rat and Takito et al. in the chicken reported that calcitriol administration enhanced calcium entry in the brush border membrane vesicles by increasing the maximum transport capacity (Vmax) but not the affinity (Km). An increase in the amount of calbindin-D9K at the brush border could explain these earlier observations. The percentage of calbindin-D9K in the brush border membrane of the SHR tends to be higher than in controls (10% versus 7%) though not significantly. However, the presence of a decreased whole-cell level in association with a similar concentration in the brush border membrane indicates the existence of abnormal cell partitioning of calbindin-D9K in the SHR. This abnormality is not vitamin D dependent but may play a role in the defective Ca2+ transport observed in SHR.

We were not able to demonstrate an effect of calcitriol on functional or immunoreactive calmodulin. The hormone did not even affect the cell localization of the protein. Conflicting reports exist in the literature about calmodulin regulation by 1,25(OH)2D3. Thomasset et al. reported an absence of a significant effect of calcitriol on calmodulin as opposed to calbindin-D9K. In chick duodenal epithelial cells, Bikle et al. reported an increase at the brush border level and Long et al. an increase at the basolateral membrane. In both cases, there were no changes in total cellular calmodulin content. In contrast, in chick embryo proliferating myoblast, calcitriol induced an increase of calmodulin content together with increased calmodulin mRNA. Differences among the models used in these studies may account for the discrepancies in the findings. Overall, the absence of calcitriol-dependent regulation of duodenal calmodulin in the SHR eliminates the possibility that impaired calcitriol responsiveness accounts for decreased Ca2+ transport in this model of hypertension.

Calmodulin levels were lower in the whole-cell extract of the SHR compared with that of the WKY rat. The difference was detected with the ligand-blotting method but not with the radioimmunoassay. Using the radioimmunoassay only, others have reported decreased calmodulin levels in various SHR tissues (aorta, kidney, and brain), in contrast to our findings. Relatively high cell turnover in the duodenum compared with other tissue, together with potential higher cell proliferation.

Fig 2. Plot shows relation between plasma calcitriol and calbindin-D9K level in control (●) or calcitriol-treated (◆) spontaneously hypertensive rats (SHR) and control (●) or calcitriol-treated (◆) Wistar-Kyoto (WKY) rats.

Fig 3. Plot shows relation between plasma calcitriol and cellular calcium flux in control (●) or calcitriol-treated (◆) spontaneously hypertensive rats (SHR) and control (●) or calcitriol-treated (◆) Wistar-Kyoto (WKY) rats.
rates in the SHR than in the WKY rat,44 may explain this discrepancy, as cellular calmodulin content doubles immediately before cell division.45 However, the presence of a normal DNA-to-protein ratio in the SHR (Table 1 of the present study as well as our earlier report46) does not support this hypothesis. The radioimmunoassay provides quantitative results based on the presence of immunoreactive calmodulin, whereas the ligand-blotting assay gives both quantitative and qualitative (ability to bind calcium) data. Thus, our results indicate the existence of an anomaly at the calcium binding site or sites of the molecule. This finding is supported by Nojima et al,47 who reported an anomaly of the SHR calmodulin gene in the region coding for the calcium binding site. The strain difference and the method discrepancy were not noted in the brush border, suggesting that immunoreactive calmodulin binds Ca2+ normally at this cellular site. To reconcile the brush border and whole-cell data, one could postulate the coexistence in the same cell of two different forms of immunoreactive calmodulin, one with normal Ca2+ binding functions and one (inactive) partially binding Ca2+. This possibility is supported by two other studies conducted in different experimental systems: one reports the existence of two mRNAs for calmodulin,48 the other shows the presence of two forms of protein differing by their intracellular location.49

The functional consequences of a less “active” calmodulin were not directly explored in the present study. Abnormal calmodulin in the cytosol of SHR enterocytes could very well impair calcium transport by decreasing the activity of the Ca2+-Mg2+-ATPase of the basolateral membrane and subsequently decreasing Ca2+ efflux, as has been previously reported.60,61-62 However, it must be pointed out that decreased calmodulin does not necessarily result in decreased activation of calmodulin-dependent enzymes. Huang et al59 reported the presence of a calmodulin activator in the SHR together with increased phosphodiesterase activity and decreased calmodulin content. Future experiments are therefore needed to address this issue in the context of SHR abnormal duodenal calcium transport.

Our plasma chemistry profile confirms previous findings of anomalies in the electrolyte metabolism of the SHR: lower ionized calcium with normal total calcium, lower pH, and hypophosphatemia compared with the WKY rat.22 Interestingly, calcitriol was less effective in increasing plasma ionized Ca2+ and decreasing plasma phosphorus in the SHR than in the WKY rat, suggesting decreased responsiveness.

In summary, the regulation of intestinal calbindin-D9K by calcitriol is normal in the SHR. Thus, we conclude that a resistance to the action of calcitriol on calcium binding proteins cannot account for the abnormal intestinal calcium transport in essential hypertension. Our findings also provide indirect evidence for the existence of a decreased pool of cytosolic, functional calmodulin in the SHR enterocyte. Elucidation of these alterations in the functional properties of calmodulin in the SHR may be critical to understanding the pathophysiological basis for the diffuse abnormalities of divalent cation metabolism in this genetic model of experimental hypertension.

References


In vivo effect of calcitriol on calcium transport and calcium binding proteins in the spontaneously hypertensive rat.

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