Calcium Transport and Vitamin D in Three Breeds of Spontaneously Hypertensive Rats

HAROLD P. SCHEL, HELEN D. WILSON, AND RONALD L. HORST

SUMMARY Conflicting results have been published by different laboratories comparing the rate of intestinal calcium transport and concentration of circulating 1,25-dihydroxy vitamin D (1,25-[OH]2D) in the spontaneously hypertensive rat (SHR) and control Wistar-Kyoto rat (WKY): They have been reported to be greater, the same, or lower in the SHR than in the WKY. We tested the possibility that the conflicting results might be breeder-related by measuring 1) the rate of intestinal mucosal calcium transport, 2) the concentration of circulating 25-hydroxy vitamin D (25-OH-D) and 1,25-(OH)2D, and 3) the concentration of intestinal mucosal receptor for 1,25-(OH)2D in the two strains of animals from three different breeders. Sodium and water transport were also measured because of their relevance to hypertension. Blood pressure was always higher and calcium, as well as mean sodium and water transport, was always lower in the SHR than in the WKY. The concentration of 1,25-(OH)2D was the same, higher, or lower in the SHR than in the WKY and was age- and breeder-dependent. Mean mucosal 1,25-(OH)2D receptor concentration was higher in the SHR and was variable, depending on breeder. We conclude that 1) the rate of calcium transport is lower in the SHR than in the WKY and independent of breeder and concentration of 1,25-(OH)2D in serum, 2) the variability in 1,25-(OH)2D concentration among investigators may be breeder-dependent, and 3) the higher receptor concentration in the intestinal mucosa of the SHR could be a compensatory response to the decreased rate of calcium transport. These differences in calcium and sodium transport may be an expression in the enterocyte of factors etiological for hypertension.

KEY WORDS • calcium transport • intestinal mucosa • 1,25-dihydroxy vitamin D • 1,25-(OH)2 receptor

BECUASE of lower serum ionized calcium, differences in calcium homeostasis between the spontaneously hypertensive rat (SHR) and its normotensive control, the Wistar-Kyoto rat (WKY), and the possible relationship of membrane calcium transport to hypertension, calcium transport by the alimentary tract has been studied in this animal model of essential hypertension. No clear concept of the relationship between rates of calcium transport in the SHR and WKY has emerged from these studies. Calcium transport in the SHR has been found to be higher, lower, and the same as in the WKY, using techniques that assess transport directly. Less direct methods of measuring transport, such as balance studies, have also given conflicting results: no difference, lower in SHR, and greater in SHR. Rats were obtained from different breeders, but blood pressure was always elevated in the SHR independently of calcium transport. These conflicting findings raised the possibility that calcium transport and its regulation might be independent of hypertension. Thus, studies of intestinal calcium transport might not evaluate etiological factors for hypertension. To test this hypothesis, we compared calcium transport in animals from three different breeders. Because of conflicting data on vitamin D metabolites and the possible role of vitamin D in these differences, we measured the concentration of circulating 1,25-dihydroxy vitamin D (1,25-[OH]2D) and of its mucosal receptor in the animals. In most experiments, mucosal-to-serosal flux of sodium was also measured.

Materials and Methods

Animals

Weanling male WKY and SHR were obtained from the University of Iowa colony (Iowa City, IA, USA), Charles River (COBS; Wilmington, MA, USA), and Taconic Farms (MBU,C; Germantown, IA, USA).
Transport Studies

Calcium transport was measured at 6 or 12 weeks of age by the everted duodenal sac technique. The identical solution was used for the mucosal and serosal media: 0.4 mM calcium chloride containing tracer calcium-45 (15 μCi/L; New England Nuclear, Boston, MA, USA), 151 mM sodium chloride, 20 mM glucose, and 4 mM NaHCO3, adjusted to pH 7.2. In some experiments mucosal-to-serosal flux of sodium was measured using tracer sodium-22 (50 μCi/L; NaCl, 638 μCi/mg; New England Nuclear), added to the mucosal solution only. The sac was prepared from the segment of small intestine immediately distal to the pylorus, 8 cm in 6-week-old animals and 10 cm in 12-week-old animals. The bile duct was ligated, the segment was stripped from the mesentery, everted, tied at one end, and the other end was tied to a cannula. With the use of a syringe calibrated by weighing, a known amount of solution was added to the serosal side of the sac (approximately 0.5 ml for 5- to 6-week-old animals, or 0.75 ml for 12-week-old animals). After 1 hour of incubation, the sac was removed from the incubation medium, blotted, and drained into a tared tube. After removal from the cannula, the sac was weighed and dried in a vacuum oven overnight to obtain dry weight. 45Ca was measured in mucosal and serosal media by liquid scintillation counting. Calcium was measured by atomic absorption. 23Na was counted in a gamma counter (Model 5500, Beckman Scientific Instruments, Irvine, CA, USA). Net water movement was calculated as the difference between initial and final sac volumes. Calcium transport was expressed as the serosal-to-mucosal concentration ratio of 45Ca or calcium analyzed by atomic absorption. Transport was also calculated using the following equations, where the subscripts i and f refer to initial and final values. Net serosal 45Ca transport (μmol/g wet weight of sac/hr) =

\[
\frac{[(C_f \times V_f) - (C_i \times V_i)]}{SA} \times \text{g wet weight of sac/hr}
\]

where C is 45Ca concentration (cpm/ml in the serosal medium), V is serosal volume (ml), and SA is mean specific activity of the mucosal medium (cpm/μmol) = [(cpm/μmol) + (cpm/μmol)]/2. Net serosal 40Ca transport (μmol/g wet weight of sac/hr) =

\[
\frac{[(C_r \times V_r) - (C_i \times V_i)]}{SA} \times \text{g wet weight of sac/hr}
\]

where C is calcium concentration (μmol/ml) and V is volume (ml); all data are for serosal medium.

Sodium transport was expressed as net flux of sodium into the serosal medium: mucosal-to-serosal sodium flux (μmol/g wet weight of sac/hr) =

\[
\frac{[(cpm_i \times V_{\text{serosal}}) - (cpm_f \times V_{\text{mucosal}})]}{SA} \times \text{g wet weight of sac/hr}
\]

Statistical analysis was by a mixed-mode analysis of variance (ANOVA) and two fixed sources of variation (age and strain) and with breeder as the random source of variation. We included all two-way interactions of these sources and the three-way interaction. Any interaction that included breeder colony was also considered a random source of variation. In this ANOVA model the fixed source of variation for age is tested against the overall variation. The fixed source of variation for age, for strain, and for the age-by-strain interaction are each tested against the overall variation for age, for strain, and for the age-by-strain interaction. Any interaction that included breeder colony was also considered a random source of variation. In this ANOVA model the fixed source of variation for age is tested against the overall variation. The fixed source of variation for strain is tested against the colony-by-strain source, which measures the random variation among colonies. The fixed source of variation for strain is tested against the colony-by-strain source, which measures the random variation among colonies in the strain difference. The fixed age-by-strain interaction source is tested against the three-way interaction source of variation, which measures the random variation among colonies in the relationship between age and strain.

For each measure, we also examined a second, simpler mixed-mode ANOVA, one that excluded the interaction sources involving breeder colony. In this model the random source of variation among breeder colonies is restricted to an influence on the mean value of the dependent responses, independent from other sources. The fixed sources of variation for age, for strain, and for the age-by-strain interaction are each tested against the overall residual variation in the measure. The decision of which ANOVA to use for inferences about the fixed sources of variation was made by comparing the standardized R² values of the ANOVAs. When that of the more complex ANOVA is not at least 10% greater than the R² of the simpler ANOVA, the simpler is the analysis used; otherwise, the more complex ANOVA is used.
complex ANOVA is used for inferences. A fixed source of variation was considered statistically significant if its F value is associated with a probability less than or equal to the 0.05 α level.

When, with the use of this decision process, the more complex ANOVA was selected, in addition post hoc multiple-comparison t tests were obtained between strains at each colony-by-age combination. Each such comparison is considered statistically significant if the t statistic is associated with a probability less than or equal to a Bonferroni-adjusted α level of 0.0083 for six comparisons or 0.01 for five comparisons. The overall residual from the complex ANOVA was used as the "error" term for these t tests.

### Results

Table 1 shows sources of rats studied, their age, weight, and blood pressure. The weaning rats from the Iowa colony were weighed the day after weaning and transferred to an adjoining room in the university animal care facility. The animals from the other suppliers were shipped after weaning, and weights shown were obtained the morning after they arrived. Initial and final body weights demonstrate differences in growth patterns of animals from different suppliers. Compared with WKY, the initial weights tended to be greater in the SHR from Charles River, the same in the SHR and WKY from Taconic Farms, and lower for SHR from the Iowa colony. Final body weight tended to be similar for the SHR and WKY strains from the Charles River and Iowa colonies, whereas for the strains from Taconic Farms, the SHR tended to weigh less than the WKY. Between strains, the weight of SHR and WKY did not differ at 6 weeks, but weight was greater in WKY at 12 weeks (p < 0.001). Mean systolic blood pressure was greater in the SHR than in the WKY at the same age (p < 0.0001). While both strains showed elevation of blood pressure with age, the increase at 12 weeks above that at 6 weeks was greater in the SHR (p < 0.0003). For both strains, animals from Taconic Farms tended to show higher blood pressure than those from other breeders.

Data for calcium, water, and sodium transport are shown in Table 2. The net increase in serosal medium calcium is shown. This increase was calculated from 1) the net increase in serosal medium 43Ca during the incubation period and specific activity (mean of initial and final values) of the mucosal medium (4Ca transport) and 2) the net increase in serosal medium calcium, considered as the difference between initial and final amounts of calcium measured by atomic absorption (4Ca transport). Both measures of net serosal calcium transport gave the same result: for the SHR a lower rate of transport, with similar absolute value, independent of breeder (p < 0.0001). The higher rate of 4Ca transport in the WKY was variable and dependent on breeder. The decline in rate with age was significant (p < 0.0001) and occurred in both SHR and WKY. These calcium transport data are based on initial wet weight of the sac. Basing data on final dry weight of the sac gave the same results.

Mean net transport of water into the serosal medium was lower in the SHR than in the WKY in all experiments, and the difference was significant (p < 0.0001; see Table 2). The data for calcium transport by the everted duodenal sac are usually presented as serosal-to-mucosal (S/M) concentration ratios. For example, S/M ratios for 4Ca were 3.7 ± 0.3 and 5.6 ± 0.4 and for 43Ca were 3.0 ± 0.3 and 4.6 ± 0.2 for the SHR and WKY, respectively, of the second Charles River group, 6 weeks of age (SHR < WKY, p < 0.05). The difference between the SHR and WKY in water movement caused the estimate of calcium transport based on the S/M ratio to be biased. Lower water movement in the SHR caused calcium transport based on the S/M ratio to be overestimated, whereas the converse was true for the WKY. Thus, although S/M concentration ratios showed the same pattern as net transport data (see Table 2), they did not give a quantitative estimate of transport and are not presented.

Mean mucosal-to-serosal flux of sodium was lower in the SHR than in the WKY (p < 0.0001). Thus, sodium and water transport showed the same pattern as calcium transport (i.e., lower in SHR).

Concentrations of 25-OH-D and 1,25-(OH)2D in the serum and of the 1,25-(OH)2D receptor in the

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td><strong>6 weeks of age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charles River</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>38 ± 1</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>WKY (n = 8)</td>
<td>33 ± 1</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>SHR (n = 5)</td>
<td>47 ± 2</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>35 ± 1</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>Taconic Farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>59 ± 2</td>
<td>126 ± 3</td>
</tr>
<tr>
<td>WKY (n = 7)</td>
<td>62 ± 2</td>
<td>158 ± 6</td>
</tr>
<tr>
<td>Iowa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>62 ± 2</td>
<td>130 ± 3</td>
</tr>
<tr>
<td>WKY (n = 7)</td>
<td>70 ± 2</td>
<td>123 ± 6</td>
</tr>
<tr>
<td><strong>12 weeks of age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charles River</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 8)</td>
<td>52 ± 3</td>
<td>245 ± 3</td>
</tr>
<tr>
<td>WKY (n = 8)</td>
<td>30 ± 1</td>
<td>242 ± 5</td>
</tr>
<tr>
<td>Taconic Farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 5)</td>
<td>53 ± 2</td>
<td>277 ± 4</td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>53 ± 2</td>
<td>368 ± 14</td>
</tr>
<tr>
<td>Iowa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 9)</td>
<td>40 ± 2</td>
<td>320 ± 9</td>
</tr>
<tr>
<td>WKY (n = 8)</td>
<td>46 ± 3</td>
<td>319 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE for age at the time of the transport study. Initial body weight was measured the day after weaning for animals from the Iowa Colony. For Charles River and Taconic Farms rats, initial body weight was measured the day after receipt. Final body weight is that at the time of the absorption study.

**Differences in blood pressure significant (p ≤ 0.003) for all effects (strain, age, and age-by-strain).**

**tP ≤ 0.05, compared with respective values in WKY.**

**Data from a separate shipment of Charles River animals.**

---

HYPERTENSION VOL 12, No 3, SEPTEMBER 1988
mucosa are shown in Table 3. There were no statistically significant differences in serum 25-OH-D concentrations for age, strain, or age-by-strain. For the Iowa colony at 6 weeks, 25-OH-D concentration was greater in the SHR than in the WKY (p < 0.0001). For the Iowa and Charles River colonies at 12 weeks and Taconic Farms colony at 6 and 12 weeks, 25-OH-D concentration was the same in SHR and WKY. When tested against the random variation in age difference among colonies, serum concentration of 1,25-(OH)₂D₃ was lower at 12 than at 6 weeks (p < 0.03). At 6 weeks, 1,25-(OH)₂D₃ concentration was greater in the SHR than in the WKY animals from the Charles River colony (p < 0.003), whereas the reverse was true for Taconic Farms colony animals (p < 0.001), and there was no difference between the SHR and WKY animals from the Charles River colony (p < 0.003), and there was no difference between the SHR and WKY animals from the Iowa colony animals. At 12 weeks, 1,25-(OH)₂D₃ concentration was lower in the SHR than in the WKY for Charles River animals (p < 0.004), but there was no difference between SHR and WKY for animals from the other two breeders. The random variation in age difference between strains among breeders caused no significant difference between strains or for age-by-strain. The random pattern of 25-OH-D and, in particular, 1,25-(OH)₂D data prevented demonstration of strain differences for these data taken as a whole. The pattern of these data was variable, in comparison with the consistency of the transport data. Thus, the breeder-independent lower calcium transport in the SHR as compared with the WKY cannot be explained by 1,25-(OH)₂D concentrations.

The 1,25-(OH)₂D receptor concentration varied depending on breeder and did not correlate with 1,25-(OH)₂D concentration or calcium transport. With one exception, mean concentration of unoccupied receptors was higher in the SHR than in the WKY from the same breeder, but the difference was not significant (p = 0.09). The difference was significant in the 6-week-old Iowa colony (p < 0.04) and the 12-week-old Taconic Farms colony (p < 0.0001). As for unoccupied receptors, occupied receptor concentration showed a strong trend to be increased in SHR as compared with WKY, but the difference was not significant (p = 0.10). Occupied receptor concentration increased with age (p < 0.0001).

### Table 3. Concentrations of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D in Serum and 1,25-Dihydroxyvitamin D Receptor in Intestinal Mucosa

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum 25-OH-D (ng/ml)</th>
<th>Serum 1,25-(OH)₂D (pg/ml)</th>
<th>Mucosal 1,25-(OH)₂D receptors (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>6 weeks of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charles River</td>
<td>—</td>
<td>—</td>
<td>67±3</td>
</tr>
<tr>
<td>Taconic Farms</td>
<td>23±1</td>
<td>23±1</td>
<td>65±4*</td>
</tr>
<tr>
<td>Iowa</td>
<td>37±1t</td>
<td>25±1t</td>
<td>63±8</td>
</tr>
<tr>
<td>12 weeks of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charles River</td>
<td>35±2</td>
<td>33±2</td>
<td>30±4*</td>
</tr>
<tr>
<td>Taconic Farms</td>
<td>26±1</td>
<td>25±3</td>
<td>38±2</td>
</tr>
<tr>
<td>Iowa</td>
<td>21±1</td>
<td>24±1</td>
<td>33±1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Six animals used for each data set. 25-OH-D = 25-hydroxyvitamin D; 1,25-(OH)₂D = 1,25-dihydroxyvitamin D.

* p < 0.05, compared with respective value in WKY.
Discussion

How are calcium transport and circulating 1,25-(OH)2D and its mucosal receptor related in the SHR and WKY, and are they relevant to the hypertension in this animal model? Intestinal mucosa has an intrinsic calcium transport activity regulated by circulating 1,25-(OH)2D concentration and the concentration of its mucosal receptor. Are the differing transport rates determined by the concentration of 1,25-(OH)2D and its mucosal receptor (i.e., vitamin D regulation)? Conversely, are these regulatory factors responding to different intrinsic calcium transport rates inherent in the SHR and WKY? Calcium transport was uniformly lower in the SHR than in the WKY from all breeders. This lower rate was present 1) in SHR from Charles River, in which 1,25-(OH)2D concentration was greater (6 weeks) or lower (12 weeks) in the SHR; 2) in SHR from Taconic Farms, in which 1,25-(OH)2D concentration was lower in the SHR (6 weeks) or the same in both SHR and WKY (12 weeks); 3) in SHR from the Iowa colony, in which 1,25-(OH)2D was the same in both strains. With one exception, mean receptor concentration was greater in the SHR with the lower transport rate. Thus, the hypothesis that the different transport rates are determined by vitamin D regulation is not tenable. The observed transport rates could not be secondary to the effects of 1,25-(OH)2D and its receptor.

The alternative hypothesis is that intrinsic calcium transport rate is primary and that concentrations of 1,25-(OH)2D and its receptor are secondary responses to achieve homeostasis under the experimental conditions. If a higher intrinsic calcium transport rate for the SHR is assumed, the observed lower transport rate should be associated with a decreased concentration of 1,25-(OH)2D or mucosal receptor (or both). If the intrinsic transport rate is assumed to be the same in the two strains, the observed lower transport rate should also be associated with a decreased concentration of 1,25-(OH)2D or receptor (or both). If the intrinsic calcium transport rate is assumed to be lower in the SHR, compensatory increases in the concentration of 1,25-(OH)2D or its receptor (or both) should be observed. In fact, mean unoccupied receptor concentration was elevated in the SHR, and the increase was significant in two comparisons, although 1,25-(OH)2D concentrations and occupied receptor concentrations were the same in these comparisons. Occupied receptor showed a strong trend to be increased in SHR. An inherently low rate of calcium transport in the SHR, with the compensatory response of an increase in 1,25-(OH)2D receptor concentration, can be hypothesized. However, the pattern of 1,25-(OH)2D concentration is not consistent with an inherently low rate of calcium transport in the SHR as compared with the WKY. This state should have determined elevated 1,25-(OH)2D concentration in the SHR. Regulatory factors determining 1,25-(OH)2D concentration appear to differ between strains and are breeder-dependent. Thus, these data do not establish a consistent pattern for calcium transport and 1,25-(OH)2D and its receptor. The hypothesis that calcium transport is primary is not supported by the data. This conclusion is also true when other factors that affect calcium requirements, such as growth rate, are considered. Thus, in animals from Taconic Farms, if the greater growth rate in the WKY as compared with SHR were the primary stimulus, vitamin D regulation of transport should be adjusted upward. However, the response pattern of 1,25-(OH)2D and its receptor do not support this scenario.

We have previously demonstrated higher concentrations of 25-OH-D in the SHR than in the WKY from the Iowa colony at 12 weeks in animals fed Purina chow,7 as well as normal and low calcium semisynthetic diets.8 The 25-OH-D concentration decreased with the feeding of a low calcium diet in both strains, but remained greater in SHR than in WKY.8 In studies by others, 25-OH-D was found to be elevated20 or the same10, 21, 22 in the SHR as compared with the WKY. Thus, the findings of the present study with animals from different breeders reflect these published data: 25-OH-D concentration is dependent on breeder and age of animal. This is also true for 1,25-(OH)2D: In five studies using young male rats aged 5 to 13 weeks, similar plasma concentrations were found in the SHR and WKY,7, 11, 21, 23, 24 and in one study,8 1,25-(OH)2D concentration was the same at 5 weeks but lower in the SHR at 12 weeks, when intake of calcium was adequate. The 1,25-(OH)2D concentration was lower in the SHR than in the WKY in two studies10, 20 and was greater in the SHR than in the WKY in two studies.22, 23 Our present results reflect this heterogeneity, particularly for the Charles River animals: Values in SHR were greater than those in WKY at 6 weeks and less than those in WKY at 12 weeks. There is no doubt that vitamin D metabolism differs between the SHR and WKY. In addition, there are differences dependent on breeder.

Mucosal receptors for 1,25-(OH)2D for both the SHR and WKY showed breeder-dependent variation. In both the SHR and WKY, the greater rate of calcium transport in the younger animals was associated with higher serum 1,25-(OH)2D concentrations. Mucosal receptors for 1,25-(OH)2D, however, were the same for both the younger and older animals, and mean values were higher with one exception in the SHR that showed the lower transport rate. Measurement of receptors for both strains from a given breeder was performed on the same day, but data from different breeders were obtained on separate days. Concentrations of receptors are determined in part by the conditions of the individual experiment in which receptors were measured. This may explain in part the breeder dependence of receptor concentration that involves both SHR and WKY.
The postulated low intrinsic calcium transport rate in the SHR is in accord with findings of low serum ionized calcium and elevated serum immunoreactive parathyroid hormone under experimental conditions comparable to those of the present study (i.e., 1% calcium diet). The SHR adjusts circulating 1,25-(OH)\textsubscript{2}D and calcium transport to stimuli such as calcium depletion. In control albino rats fed a 1% calcium diet, homeostatic responses for adjusting calcium metabolism are probably minimally activated. In the SHR regulatory mechanisms are activated in comparison with the WKY, but homeostasis is achieved, at least in terms of growth rate. In animals fed a 1% calcium diet, we found no evidence that the decreased calcium absorption defined by transport studies is associated with systemic deficiency of calcium. Calcium intake compensates for decreased transport, and calcium retention by both strains is appropriate for growth rate (unpublished studies). The differences in serum ionized calcium can be explained by differing distribution of calcium between extracellular and intracellular compartments. Although the older (12 weeks) SHR is limited as compared with the WKY in the capacity to increase 1,25-(OH)\textsubscript{2}D in response to a maximal stimulus such as infusion of parathyroid hormone, this is not relevant to conditions of adequate calcium intake, under which these animals are usually studied. Thus, the differences in calcium transport between the SHR and WKY appear to be unrelated to the vitamin D–endocrine system. This regulatory system seems to have attempted to adjust to a primary transport difference rather than be etiological for the differences in transport between the two strains.

In the present experiment, the everted duodenal sac was used to study calcium transport. This model provides a sensitive and specific index for comparing calcium transport in differing physiological states (e.g., age, vitamin D effects). The duodenum is the most active site for the development of transmucosal concentration gradients, and the everted sac method best expresses this activity, which is a measure of basolateral membrane function. In the present experiment, the everted sac consistently demonstrated a lower transmucosal calcium transport rate in the SHR. This lower rate can be considered an expression of cellular function for calcium distribution between the interior and exterior of the cell. It cannot be equated to overall calcium transport by the gastrointestinal tract and in no way demonstrates calcium deficiency from calcium malabsorption.

The SHR and WKY strains were developed by inbreeding rats of the Wistar strain in Kyoto, Japan. Genes associated with hypertension were segregated in the SHR, while the WKY remained normotensive. Colonies of these animals established in various parts of the world are subject to genetic drift as well as environmental impact, which will alter their behavior. For example, a recent study of body growth and blood pressure demonstrated differences between the WKY groups from two breeders, whereas these variables were similar in the SHR groups. It is clear that this animal model has evolved subsequent to its initial establishment at Kyoto and that growth pattern evolved differently among breeders. In all the colonies that we studied, blood pressure continued to be elevated in the SHR as compared with the WKY. Although other characteristics differ, all suppliers provide an animal model of hypertension. The association of the hypertension of the SHR and the difference in calcium transport between the SHR and WKY supports continuing studies of intestinal calcium transport. The consistently lower calcium transport that we found has not been uniformly shown in prior studies with the everted sac, the S/M ratio of \textsuperscript{44}Ca (not adjusted for water movement) was the same in SHR and WKY at 5 weeks and greater in SHR at 12 weeks. Intestinal calcium transport was greater in SHR than WKY in older female rats (24–26 weeks and 1 year of age) studied by the in vivo isolated loop technique and by calcium balance. In the balance study, 10-week-old male rats demonstrated mild calcium malabsorption. Studies of urinary calcium excretion after oral calcium loading were interpreted as showing greater calcium absorption in the SHR than in the WKY. In any case, localization of the difference in calcium transport to a specific site in the enteroocyte and elucidation of the mechanism continue to be priority problems in relation to understanding calcium transport mechanism and regulation and, possibly, the pathogenesis of at least one syndrome of essential hypertension.

Sodium and water absorption per se affect calcium transport. Greater transport rates of calcium, sodium, and water in the WKY may express this interrelation. In a prior study of in vivo transport by the most proximal 20 cm of small intestine, we found greater transport of sodium in the SHR than in the WKY (i.e., in the strain with lower calcium transport). The reason for the difference between current in vitro and previous in vivo findings on sodium transport are unknown, but they could be the result of differences in methodology and segment length (i.e., proximal 8 or 10 cm in the present study). It is possible that sodium transport is determined to a greater extent by basolateral membrane function in the everted sac as compared with the in vivo studies.

Finally, how is transport function in the enteroocyte related to increased vascular resistance? The enteroocyte shares calcium and sodium transport functions with the smooth muscle cell. Specifically, the basolateral membrane of the enteroocyte and the plasma membrane of smooth muscle cells use the same mechanisms for pumping calcium and sodium out of the cell as well as for entry of these ions into the cell. A strain-dependent difference in the handling of calcium and sodium would be expressed in all cells from that strain. Calcium is pumped out of the cell by calcium adenosine triphosphatase and by...
sodium-calcium exchange. Decreased transcellular transport of calcium and sodium in the enterocyte is compatible with decreased pumping of these ions out of the cell. Such a difference between SHR and WKY is a possible mechanism for increased vascular resistance.

The brush border membrane, through which luminal calcium enters the enterocyte, has no analogue in the smooth muscle cell. Calcium influx measured under initial rate conditions in intact segments and in brush border membrane vesicles does not differ in the SHR and WKY. In subsequent studies with brush border membrane vesicles, we characterized the kinetics of saturable and nonsaturable calcium uptake at two temperatures and in the presence and absence of inhibitor and showed no difference between the two strains (S.E. Thomas and H.P. Schedl, unpublished observations). Thus, transport differences between the two strains are not localized to the brush border. In contrast, adenosine triphosphatase-dependent uptake of calcium by basolateral membrane vesicles, which is analogous to extrusion of calcium from the enterocyte, is lower in the SHR than in the WKY. Thus, decreased calcium transport in the enterocyte may express the same functions that cause increased vascular resistance.

Acknowledgment

The statistical consultation of Kice Brown has been invaluable in preparation of the manuscript.

References

22. Bindels RJM, van den Brock LAM, Jongen MJM, Hackeng WHL, Löwik CWGM, van Os CH. Increased plasma calcium levels in young spontaneously hypertensive rats: role in disturbed phosphate homeostasis. Pflugers Arch 1987;408:395-400
31. Walters JRF. Reduced calcium transport in duodenal basolateral membrane vesicles prepared from the spontaneously hypertensive rat [Abstract]. Clin Res 1986;34:446A
Calcium transport and vitamin D in three breeds of spontaneously hypertensive rats.
H P Schedl, H D Wilson and R L Horst

Hypertension. 1988;12:310-316
doi: 10.1161/01.HYP.12.3.310
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/12/3/310

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/