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Calcium Transport in the Spontaneously Hypertensive Rat Is Responsive to Vitamin D[1] (42789)

H. D. WILSON, H. P. SCHEDL, AND R. L. HORST

Medical Service, VA Medical Center, Iowa City, Iowa, 52240; National Animal Disease Laboratory, Ames, Iowa, 50010; and Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

Abstract. Intestinal calcium transport rate and response to treatment with a vitamin D agonist [24,24-difluoro-1,25-dihydroxycholecalciferol, F_2-1,25-$(OH)_2D_3$)] were studied in the spontaneously hypertensive (SH) rat–Wistar Kyoto (WKy) rat model of hypertension. We used the everted duodenal sac to study untreated, orally treated, and parenterally treated groups of SH and WKy rats. In untreated groups, net calcium transport was lower ($P < 0.05$) in the SH rat than in the WKy rat (0.46–0.66 vs 0.81–1.04, all data μmole/g segment wet wt per hr). Both groups responded to treatment (SH vs WKy; 0.84–0.90 vs 1.56–1.57, $P < 0.05$), but even in treated groups net calcium transport by the SH rat was lower than that by the WKy rat ($P < 0.05$). Net water transport increased 3- to 8-fold in response to treatment in the WKy but not in the SH rat. The increased water transport prevented demonstration of treatment-mediated increased calcium transport based on serosal/mucosal concentration ratio in the WKy rat. We conclude that (i) both the SH and the WKy rat have the capability to increase calcium transport when treated with an agonist having vitamin D activity; (ii) the unstimulated and stimulated transport rate is lower in the SH rat than in the WKy rat; and (iii) water transport responds to treatment in the WKy rat but not in the SH rat.

Calcium transport by the gut has been extensively studied in the spontaneously hypertensive (SH)–Wistar Kyoto (WKy) rat model of hypertension with conflicting results (1). Both responsiveness and unresponsiveness to stimulation of calcium transport by 1,25-$(OH)_2D_3$ have been demonstrated. In addition, comparison of rates of intestinal calcium transport by the two strains have shown both higher and lower transport in the SH rat than in the WKy rat, as well as the same rate in both strains. Calcium transport studies from our laboratory using animals from the Iowa Colony had consistently shown (i) lower transport in the SH than in the WKy rat; and (ii) calcium transport responsiveness to vitamin D by the criterion of increased transport in response to calcium depletion by both strains (1). In the present study we used animals from a commercial breeder, Taconic Farms, to test for (i) transport differences in the untreated SH and WKy rat; and (ii) responsiveness to direct stimulation by a vitamin D agonist in animals from this source. We also considered the possibility that conflicting data on calcium transport responsiveness were secondary to differences between strains in sensitivity to 1,25-$(OH)_2D_3$, since we had shown responsiveness of transport to calcium depletion by both SH and WKy *in vivo,* but only by SH *in vitro* (1). Therefore, in the present study we used 24,24-difluoro-1,25-dihydroxycholecalciferol, which has several times the potency of the parent compound. Because differences between strains in intestinal absorption of the vitamin D agonist might explain conflicting results, we also tested both oral and parenteral treatment.

Methods. Male WKy and SH rats were purchased from Taconic Farms, Germantown, New York. They were housed in the Animal Care Unit under controlled temperature and humidity and a dark cycle from 6:00 PM to 6:00 AM. Rats were fed Purina laboratory chow 5012 (1% calcium, vitamin D_3 3.3 IU/g) *ad libitum* and weighed three times per week. At specific ages, calcium transport by the everted duodenal sac was measured as previously described (2). The transport solution contained 0.4 m*M* calcium chloride and tracer ^{45}Ca (15 μCi/liter, New England Nuclear Corp., Boston, MA), 151 m*M* NaCl, 20 m*M* glucose, 4 m*M* Na_2HPO_4 adjusted to pH 7.2. After the bile duct was tied off, the 10-cm segment just

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distal to the pylorus was removed, flushed, weighed, and everted. The solution (0.75 ml) was delivered to the inside of the sac (serosal solution) with a calibrated 1-ml syringe and PE 20 tubing. The sac was then incubated for 1 hr in 15 ml of the same solution while bubbling with 95% O_2 and 5% CO_2. After 1 hr, the serosal contents were emptied into a tared tube and weighed. The sac was slit open, the segment was placed on a glass plate cooled on ice, and mucosa was scraped from the underlying tissue using a microscope slide. Wet and dry weights of the mucosa were obtained. Blood was drawn for measurement of calcium and magnesium in serum by atomic absorption spectrometry immediately after removal of the gut segment.

Methods for measurement of water movement and analysis of calcium and ^{45}Ca have been previously described (3). Calcium transport was expressed as net increase in serosal calcium analyzed by atomic absorption (net ^{40}Ca transport) or based on mucosal ^{45}Ca specific activity (net ^{45}Ca transport), and as serosal to mucosal (S/M) concentration ratio.

Calculations. In the equations below, the subscripts i and f refer to initial and final values.

$$\text{Net } ^{40}\text{Ca transport, } \mu\text{mole/g sac dry wt per hr} = \frac{(C_f \times V_f) - (C_i \times V_i)}{\text{sac wt, g} \times \text{hr}}$$

where C is calcium concentration, μmole/ml; and V is volume; ml; all data are for serosal medium.

$$\text{Net } ^{45}\text{Ca transport, } \mu\text{mole/g dry wt of sac per hr} = \frac{[(C_f \times V_f) - (C_i \times V_i)]/\overline{SA} \text{ of } ^{45}Ca}{\text{sac wt, g} \times \text{hr}}$$

where C is ^{45}Ca concentration, cpm/ml in the serosal medium; V is serosal volume, ml; $\overline{SA}$ is mean specific activity of the mucosal medium, cpm/μmol = [(cpm/μmol)$_i$ + (cpm/μmole)$_f$]/2.

Net calcium transport measured as the net increase in serosal medium calcium analyzed by atomic absorption gives the highest rate of any of the indices of transport by the everted sac technique. This is the most sensitive index of movement of calcium from the enterocyte into the serosal medium. Net calcium transport measured from the net increase in serosal medium ^{45}Ca and expressed as micromoles per unit weight of tissue, based on mucosal medium ^{45}Ca specific activity, gives a slightly lower rate. Transport expressed as S/M concentration ratio of calcium, measured by atomic absorption or counting ^{45}Ca does not consider the weight of the sac or the effect of differences in net water movement.

Weanling animals were obtained and studied at 7 weeks of age in Studies 1 and 2 and at 6 weeks in Study 3. Study 1 animals were untreated. For treating animals of Studies 2 and 3, 24,24-difluoro-1,25-dihydroxycholecalciferol [F_2-1,25-$(OH)_2D_3$] (4) obtained from Hoffmann LaRoche, Inc. was dissolved in ethanol to which propylene glycol was subsequently added to give a solution consisting of 30% ethanol and 70% propylene glycol containing 12.5 ng of F_2-1,25-$(OH)_2D_3$ per 0.1 ml. In Study 2, 0.1 ml of this solution was given by gavage daily for 3 days prior to the transport study. In Study 3, this dose was given parenterally for 4 days. For both studies, untreated groups received vehicle by the treatment route.

In a separate set of WKy and SH rats, the effect of treatment with F_2-1,25-$(OH)_2D_3$ on blood pressure was studied. During the period from 6 to 12 weeks of age animals were weighed twice a week and blood pressure was measured two to five times per week. Animals were gavaged with either 25 ng of F_2-1,25-$(OH)_2D_3$ three times a week (treated) or vehicle (untreated) during the 6-week study period.

Statistics. Data were compared by Student's unpaired t test. Differences between groups were considered significant at $P < 0.05$ level.

Results. The groups studied are described in Table I. Study 1 comprised a set of untreated WKy and SH groups. Sets of untreated and treated WKy and SH groups were compared in Studies 2 (oral treatment) and 3 (parenteral treatment). Body weight was lower in the SH than in the WKy group for both untreated and treated sets. Sac weight did not differ among strains and was not affected by treatment. In Study 2, serum

TABLE I. GROUPS STUDIED (MEANS ± SEM)

	WKy		SH	
	Untreated	Treated	Untreated	Treated
		Study 1, no treatment		
N	9		7	
Age (weeks)	7		7	
Body wt (g)				
Final	168 ± 6		153 ± 1[a]	
Sac wet wt (g)	0.767 ± 0.020		0.830 ± 0.025	
		Study 2, oral treatment		
N	10	10	10	10
Age (weeks)	7	7	7	7
Body wt (g)				
Initial	66 ± 1	66 ± 1	59 ± 1[a]	59 ± 1[a]
Final	179 ± 5	183 ± 3	151 ± 5[a]	149 ± 2[a]
Sac wet wt (g)	0.779 ± 0.029	0.811 ± 0.025	0.835 ± 0.030	0.842 ± 0.014
Serum (μM)				
Calcium	2546 ± 15	2726 ± 33[b]	2406 ± 17[a]	2662 ± 26[b]
Magnesium	836 ± 14	852 ± 16	950 ± 30[a]	917 ± 14[a]
		Study 3, parenteral treatment		
N	6	6	6	7
Age (weeks)	6	6	6	6
Body wt (g)				
Initial	58 ± 2	58 ± 1	50 ± 2[a]	49 ± 2[a]
Final	143 ± 3	131 ± 7	117 ± 4[a]	100 ± 3[a]
Sac wet wt (g)	0.829 ± 0.051	0.766 ± 0.067	0.870 ± 0.022	0.805 ± 0.025
Serum (μM)				
Calcium	2516 ± 34	3148 ± 31[b]	2473 ± 57	3284 ± 56[b]
Magnesium	864 ± 51	796 ± 28	859 ± 29	868 ± 44

Note. *N* = number in group.
[a] SH differs from WKy in same set, $P < 0.05$.
[b] Treated group differs from control, $P < 0.05$.

calcium was lower in the SH than in the WKy group in the untreated set, and increased with treatment in both strains. Serum magnesium was higher in the SH than in the WKy group in both untreated and treated sets. In Study 3, serum calcium did not differ between strains in either the untreated or the treated set, and increased in both strains with treatment. Serum magnesium was the same in both strains and did not change with treatment.

Calcium transport data are presented in the Fig. 1 and Table II. Study 1 compared calcium transport by the untreated WKy and SH rat. Calcium transport was lower in the SH than in the WKy rat expressed both as net transport data (Fig. 1) and as S/M concentration ratio (Table II).

In Study 2, calcium transport was lower in the SH than in the WKy rat in both treated and untreated groups by all criteria for expressing transport (Fig. 1). Treatment increased net calcium transport measured by calcium analysis for both the WKy and the SH rat. The small treatment-induced increases in net transport measured by counting ^{45}Ca were not significant for either strain. Treatment increased net water transport in the WKy but not in the SH rat (Table II). This difference between the WKy and the SH rat in water transport in response to treatment must be considered in the interpretation of concentration ratio data for Study 2 (Table II). Increased water transport dilutes serosal medium calcium concentration, causing underestimation of transport

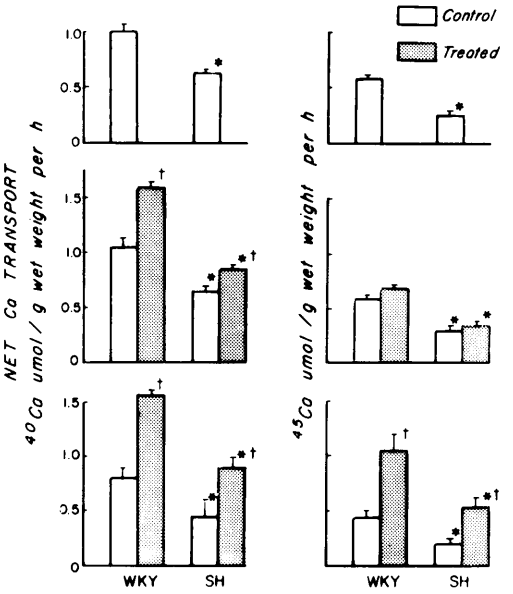

FIG. 1. All data are means ± SEM. Study 1 (top). Net transport of calcium into the serosal medium by the everted duodenal sac: calcium analyzed by atomic absorption (^{40}Ca) and by counting ^{45}Ca specific activity (^{45}Ca). *Transport is lower in the SH than the WKy rat in untreated animals. Study 2 (middle). Net transport of calcium by the everted duodenal sac: effect of oral treatment with 24,24-difluoro-1,25-$(OH)_2D_3$. †Transport of calcium measured as ^{40}Ca increased in both the WKy and the SH rat in response to treatment. *Transport is lower in the SH than in the corresponding WKy group in both untreated and treated sets. Study 3 (bottom). Net calcium transport by the everted duodenal sac: effect of parenteral treatment with 24,24-difluoro-1,25-$(OH)_2D_3$. †Transport based on ^{40}Ca and ^{45}Ca increased in response to treatment in both the WKy and the SH rat. *Transport is lower in the SH than in the corresponding WKy group in both treated and untreated sets.

based on S/M ratio in the WKy group. Therefore, the treated WKy rat did not show a significant increase in transport based on concentration ratio. The SH rat responded to treatment by the criterion of S/M ratio of calcium, but the small increase in serosal medium ^{45}Ca in response to treatment was not significant.

In Study 3, net calcium transport responded to treatment in both the SH and the WKy rat, and was lower in the SH than in the WKy rat for both untreated and treated groups (Fig. 1). The marked treatment response of the WKy group was associated with the concurrent increase in net water movement (Table II), whereas water movement was unaffected by treatment in the SH group. These differences in water movement biased transport data expressed as concentration ratio: (i) the SH rat increased S/M ratio with treatment, whereas the WKy did not; and (ii) after treatment S/M ratio did not differ between SH and WKy.

Net transport expressed per gram dry weight of mucosa gave relationships identical to those shown for initial wet weight of sac, and hence are not presented.

In the experiment examining the effect of F_2-1,25-$(OH)_2D_3$ on blood pressure, all animals grew. Blood pressure (means ± SE for the 6-week period) was unaffected by treatment: WKy untreated, 137 ± 3, n = 5; treated, 148 ± 4, n = 6; SH, untreated, 192 ± 5, n = 6; treated, 191 ± 3, n = 6. Blood pressure was higher in the SH group ($P < 0.001$).

Discussion. The primary purpose of these experiments was to examine responsiveness of intestinal calcium transport of the WKy and SH rat to vitamin D treatment. Toraason and Wright (5), using the everted sac technique, showed that 1,25-$(OH)_2D_3$ treat-

TABLE II. SEROSAL/MUCOSAL (S/M) CONCENTRATION RATIO OF CALCIUM AND NET WATER TRANSPORT (MEANS ± SEM)

	Wky		SH	
Study	Untreated	Treated	Untreated	Treated
	S/M concentration ratio, calcium			
1	3.6 ± 0.2	—	2.7 ± 0.2[a]	—
2	4.1 ± 0.3	4.7 ± 0.3	2.8 ± 0.1[a]	3.4 ± 0.1[a,b]
3	4.6 ± 0.4	4.6 ± 0.3	2.7 ± 0.2[a]	4.2 ± 0.4[b]
	S/M concentration ratio, ^{45}Ca			
1	2.6 ± 0.1	—	1.9 ± 0.2[a]	—
2	2.9 ± 0.2	3.0 ± 0.2	1.9 ± 0.1[a]	2.2 ± 0.1[a]
3	3.3 ± 0.3	3.7 ± 0.2	1.8 ± 0.2[a]	3.1 ± 0.3[b]
	H_2O transport, mmole/g wet wt			
1	4 ± 2	—	1 ± 2	—
2	2 ± 2	7 ± 1[b]	2 ± 1	3 ± 1
3	2 ± 3	16 ± 2[b]	0 ± 1	0 ± 1[a]

[a] SH < WKy, $P < 0.05$ or less.
[b] Treated group > untreated group, $P < 0.05$.

ment increased S/M concentration ratio in the WKy rat but not in the SH rat. In the present experiment we found the S/M concentration ratio to have limitations as an index of calcium transport response to treatment. The potent agonist of vitamin D action, F_2-1,25-$(OH)_2D_3$, caused water to increase in the sac from the WKy but not the SH rat. Increased water transport caused underestimation of transport based on S/M concentration ratio, by dilution of the serosal fluid. Net transport of calcium into the serosal fluid was the most sensitive index of transport, and demonstrated responsiveness in both the SH and the WKy rat.

24,24-F_2-1,25-$(OH)_2D_3$ selectively increased water transport in the WKy rat but not in the SH rat. Water transport is secondary to movement of electrolytes, primarily sodium and anions, chiefly chloride. Thus, the difference in water transport response to treatment is consistent with a difference in sodium transport between the two strains. Interrelationships between sodium and calcium transport in gut and kidney are well established. Therefore, the difference in calcium transport between the SH and the WKy rat might be related to the differential response of water transport to treatment with 24,24-F_2-1,25-$(OH)_2D_3$.

Treatment with 1,25-$(OH)_2D_3$ also increased duodenal and colonic calcium transport measured as fluxes by the Ussing chamber technique in both the SH and the WKy rat (6) and in the SH rat (no treated WKy group) (7). Treatment of parathyroidectomized animals with 1,25-$(OH)_2D_3$ also increased calcium transport measured by the *in situ* duodenal loop technique in both the SH and the WKy rat (8). Calcium transport measured by the balance technique was also increased by 1,25-$(OH)_2D_3$ treatment in both the SH and the WKy rat (8).

In addition to the conflicting data on responsiveness, comparisons between rates of transport by the WKy with the SH rat are also conflicting.

In vitro studies. With the everted sac, transport measured as S/M concentration ratio was the same in the SH and WKy strains at 5 weeks and greater in the SH strain at 12 weeks in one study (5). In another study, S/M ratio was lower in the SH than in the WKy at both 5 and 12 weeks of age (9). With the Ussing chamber technique, duodenal mucosal-to-serosal flux of calcium was the same in both strains (6). Studies with the Ussing technique by another group showed lower flux of calcium in the SH than in the WKy rat (7, 10).

In vivo studies. Calcium transport measured directly by *in vivo* perfusion has been shown to be greater in the SH than in the WKy rat (5), as well as lower in the SH rat (9, 11). By the isolated loop technique, calcium uptake was also greater in the SH than in the WKy rat (12).

Thus both for responsiveness to 1,25-$(OH)_2D_3$ treatment and for relative rates of transport for the WKy and SH rat, data are contradictory. Reasons for the discrepancies are not evident at this time. These studies with rats from Taconic Farms showed a transport pattern consistent with our prior studies using animals from the University of Iowa colony (9, 11). Further work is required to determine whether breeder, sex, or age conributes in part to the conflicting results. Treatment with F_2-1,25-$(OH)_2$ did not affect blood pressure in either the SH or the WKy strain in this study.

Alan Reitz performed blood pressure measurements. Dr. Milan R. Uskokovic supplied 24,24-F_2-1,25-$(OH)_2D_3$.

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