Calcium and Inorganic Phosphate Transport in Rat Colon

DISSOCIATED RESPONSE TO 1,25-DIHYDROXYVITAMIN D_3

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ABSTRACT In the small intestine, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] stimulates both calcium (Ca) and inorganic phosphate (Pi) absorption. This is mediated through an increase in mucosal-to-serosal flux (Jms) whereas the serosal-to-mucosal flux (Jsm) remains unchanged. We now report that in rat proximal colon, 1,25$(OH)_2D_3$ produces active Ca absorption without affecting Pi transport, and that this induced active Ca absorption is associated with alterations in kinetics of both Jms and Jsm so that both processes demonstrate saturable components. Vitamin D-deficient rats were given daily injections of solvent (−D) or 270 ng 1,25$(OH)_2D_3$ (+D) for 3 d. ^{45}Ca and [^{32}P]phosphate fluxes were measured employing the Ussing technique using a modified Krebs-Ringer-HCO_3 buffer ([Ca] 1.25, [Pi] 1.18, [glucose] 11 mM). In −D rats there was no net flux (Jnet) of either Ca or Pi. In +D rats net active Ca absorption was observed (−D = 3.3 nmol/cm² per h ±3.4 (SEM); +D = 27.3 ±3.8, $n = 11$, $P < 0.001$) whereas Pi transport was unchanged, i.e., still no Jnet. Pi Jms was not different from Pi Jsm measured at the following buffer [Pi]: 0.0118, 0.118, 1.18, and 2.36 mM. Ca saturation kinetics were estimated using buffer [Ca] from 0.0125 to 5.0 mM. Saturable processes were demonstrated for both Jms and Jsm. Jnet for Ca across colon from +D rats exhibited saturation at [Ca] > 3 mM, with an estimated V_{max} of 44.0 nmol/cm² per h and a K_m of 0.9 mM. This colonic model may provide a useful system for studying 1,25$(OH)_2D_3$-induced molecular events related to Ca but not Pi transport. The apparent action of 1,25$(OH)_2D_3$ on Ca secretory process may furnish new insights into the mechanism of action of vitamin D.

INTRODUCTION

In the small intestine 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$][1] stimulates absorption of both calcium (Ca) and inorganic phosphate (Pi) (1). Consequently, the issue of whether this hormone induces a coupled or separate active transport processes for Ca and Pi has not been completely resolved (1, 2). Ca transport by the colon has been investigated using everted sacs of intestine (3) and the transepithelial transfer of Ca across perfused *in situ* loops (4). Studies with everted sacs have demonstrated no evidence for active ^{45}Ca transport in colon from vitamin D-deficient animals, whereas repletion with the vitamin produced results indicative of active Ca absorption (3). This study also showed that decreasing the Na concentration from 145 to 50 mM increased ^{45}Ca serosal-to-mucosal compartment concentration ratio in both vitamin D-deficient and D-replete animals. Subsequent in vivo experiments have shown that dietary Ca restriction of otherwise normal rats, increases colonic absorption of Ca (4). This dietary maneuver increases the renal production of 1,25$(OH)_2D_3$ which presumably produces the elevation in Ca absorption. Data on 1,25$(OH)_2D_3$-induced changes on Ca transport kinetics as well as the influence of this hormone on Pi transport in colon has not been reported.

In the present study we have examined the kinetics of colonic Ca and Pi transport in vitro with electrochemical gradients eliminated by the Ussing technique (5). Treatment with 1,25$(OH)_2D_3$ was used to stimulate

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[1] *Abbreviations used in this paper*: −D, vitamin D-deficient rats; +D, 1,25-dihydroxyvitamin D_3-treated rats; Jms, mucosal-to-serosal flux; Jnet, net flux; Jsm, serosal-to-mucosal flux; Pi, inorganic phosphate; SCC, short-circuit current.

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Ca transport. The results indicate that $1,25(OH)_2D_3$ induces an active carrier-mediated Ca absorptive process without altering the transport of Pi.

METHODS

Weanling male Holtzman rats were raised on a vitamin D-deficient diet containing adequate Ca(0.5%) and Pi(0.4%) in a room free from UV light for 6–8 wk (1, 6). Animals then received daily subcutaneous injections of 0.2 ml propanediol (−D) or 270 ng $1,25(OH)_2D_3$ in 0.2 ml propanediol (+D) for 3 d. Chemically synthesized $1,25(OH)_2D_3$ was generously provided by Dr. M. Uskokovic of Hoffmann-La Roche Inc., Nutley, N. J., courtesy of Dr. A. W. Norman. The rats were sacrificed by decapitation and a 5–7-cm segment of colon immediately distal to the cecum was removed and mounted across Lucite chambers which expose a circular area of epithelium of 0.67 cm^2.

Transmural Ca and Pi fluxes were studied in vitro employing the modified Ussing technique described in detail in prior publications (1, 7–9). A modified Krebs-Ringer-HCO_3 buffer, containing in millimoles per liter: 1.25 Ca, 1.18 Pi, and 11 D-glucose, was used unless indicated otherwise (10). ^{45}Ca as $CaCl_2$ and [^{32}P]Pi as carrier-free orthosphosphoric acid were obtained from New England Nuclear, Boston, Mass. Before use the [^{32}P]H_3PO_4 was neutralized and converted to a sodium salt. The radioisotopes were counted in a Beckman LS 250 dual-window liquid scintillation spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) (9), and ion fluxes were calculated as described (10). The transmural potential difference was abolished using automatic voltage clamps (Netronics, Inc., Hudson, Mass.) that passed a short-circuit current (SCC) across the epithelium after a correction for the resistance of the buffer between the agar bridge tips (10). Conductance was monitored at 15-min intervals by recording the current required for the voltage clamp to produce an increase in potential difference of 10 mV. Net flux is calculated as the difference between unidirectional mucosal-to-serosal flux (Jms) and serosal-to-mucosal flux (Jsm) across pieces of adjacent colon that had conductances that matched within 75%. About 10% of the tissues studied failed to meet the matching criterion and these data were eliminated from the study.

Statistical comparisons of independent variables within treatment groups were made using one-way analyses of variance (9). When the analysis of variance indicated a difference between means, Dunnett's format for multiple t test comparisons was used to obtain probability estimates (11).

RESULTS

Figs. 1 and 2 illustrate unidirectional Ca fluxes, measured at 15-min intervals over a 2-h period, in −D and +D colon, respectively. In both −D and +D colon, unidirectional ^{45}Ca fluxes reached steady rates in ~60–75 min and remained stable through the subsequent 60 min. During the same period, unidirectional Pi fluxes reached steady state (Fig. 3), and SCC and tissue conductance were stable throughout (Fig. 4). The results presented for both unidirectional fluxes and electrical parameters represent the average of at least three to four steady-state measurements for each experiment. Fig. 1 clearly demonstrates that Jms was not different from Jsm in −D colon at all time intervals

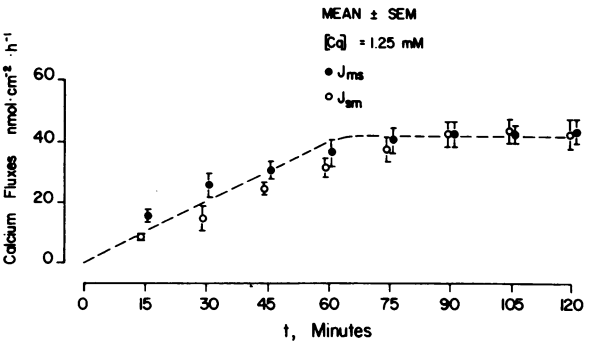

FIGURE 1 Unidirectional Ca fluxes across −D colon determined at 15-min intervals over a 120-min period (n = 12).

studied, and a single curve adequately represents either of the two unidirectional fluxes of Ca. On the other hand, in +D colon (Fig. 2), Ca Jms was consistently higher than Jsm at all intervals. The interrupted line represents the curve obtained for Jms and Jsm from −D colon (Fig. 1); it is similar to the curve for Jsm of the +D colon suggesting that $1,25(OH)_2D_3$ treatment has no apparent effect on Jsm at this Ca concentration (1.25 mM). There were no differences between the unidirectional fluxes of Pi in either −D or +D colon (Fig. 3, Table I).

Table I summarizes the effect of three daily doses of 270 ng $1,25(OH)_2D_3$ on colonic transport of Ca and Pi. In colon from −D rats there was no Jnet of Ca or Pi, while net active Ca absorption was observed in colon from $1,25(OH)_2D_3$-treated rats (−D Jnet vs. +D Jnet, $P < 0.001$). The $1,25(OH)_2D_3$-stimulated net Ca flux occurred through increased Jms (−D Jms vs. +D Jms $P < 0.001$) whereas Jsm was unchanged (Table I). Treatment with $1,25(OH)_2D_3$ did not alter Pi transport; i.e., neither Pi Jms nor Pi Jsm was changed, and there was no net Pi flux. Because Pi fluxes in this group of studies were measured at an ambient [Pi] of 1.18 mM, further measurements of Pi fluxes were performed

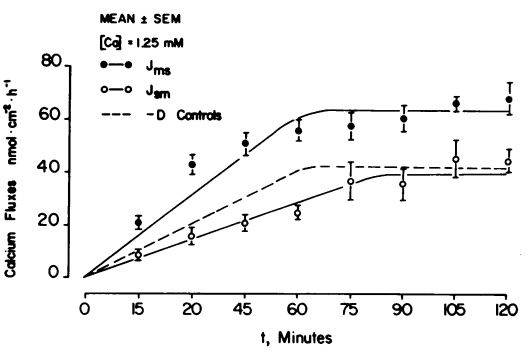

FIGURE 2 Unidirectional Ca fluxes across +D colon determined at 15-min intervals over a 120-min period (n = 7). Interrupted curve representing both Jms and Jsm for Ca across −D colon is included for comparison.

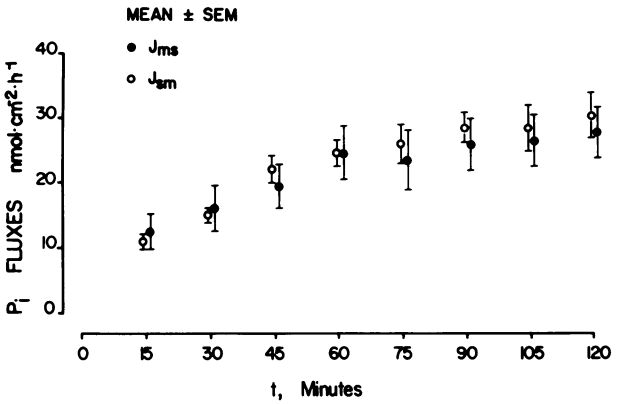

FIGURE 3 Unidirectional Pi fluxes across −D colon at 15-min intervals over a 120-min period (n = 12).

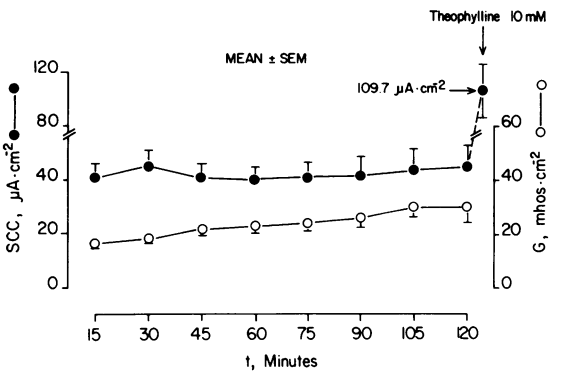

FIGURE 4 SCC and tissue conductance (G) across +D colon (n = 12).

using buffer [Pi] of 0.0118, 0.118, and 2.36 mM (Table II). Pi Jms was not different from Pi Jsm, and Pi Jnet was not different from zero in all instances studied.

The proximal colon from these rats had higher conductances than values reported from normal animals (12). To estimate the influence of edge damage on conductance, this parameter was measured in proximal colon of normal adult rat (400–500 g) using Lucite chambers of different diameters to vary the edge to surface ratio. A linear, inverse rather than a positive correlation was observed between tissue conductance and the ratio of serosal circumference/serosal area (Fig. 5) indicating that edge damage does not account for the higher values for tissue conductance. Tissue conductance and SCC were not significantly different between the −D and +D groups (Table I). However, in a variety of studies conducted over a range of buffer Ca concentrations from 0.0125 to 5.0 mM, slightly higher tissue conductance values were consistently observed in 1,25$(OH)_2D_3$-treated rats than in −D animals, but the difference never achieved statistical significance. The transmural potential difference was ~1.5–2.0 mV during the period of steady-state fluxes (this can be derived from Fig. 4). To test for viability of the epithelium, we added 10 mM theophylline to the mucosal and serosal buffers at 120 min and observed an average increase in SCC of 150% caused by the inhibition of phosphodiesterase and a presumed increase in the levels of cyclic AMP (Fig. 4).

Saturation kinetics. Fig. 6 illustrates unidirectional Ca fluxes measured in −D colon using buffer with calcium concentrations of 0.0125, 0.25, 1.25, and 5.0 mM, respectively. Jms approximated Jsm at all buffer calcium concentrations and the data fit a straight line (r = 0.99). Fig. 7 illustrates the unidirectional Ca fluxes measured in +D colon using buffers with different Ca concentrations. Unlike −D colon, saturation kinetics were observed for Jms and Jsm. This resulted in net Ca flux across colon from +D rats that exhibited saturation at [Ca] > 3 mM. Estimation of kinetic constants from Fig. 8 indicates that 1,25$(OH)_2$-D_3-stimulated Ca absorption is a saturable transport process with an approximate V_{max} of 44.0 nmol/cm² per h and a K_m of 0.9 mM. We wanted to test whether or not the increasing Ca concentration might cause the saturation of the unidirectional fluxes by increasing paracellular resistance, i.e., decreasing paracellular conductance. This was tested in normal adult rat colon by incubating two adjacent pieces of proximal colon in two identical chambers containing buffer solution with 0.125 mM [Ca]. Measurements were made 90–120 min after mounting the tissue (Fig. 9). At zero

TABLE I
Effect of 1,25$(OH)_2D_3$ on Ca and Pi Transport in Rat Proximal Colon

Condition	n	Ca			Pi			G	SCC
		Jms	Jsm	Jnet	Jms	Jsm	Jnet		
		nmol/cm²/h			*nmol/cm²/h*			*mmhos/cm²*	*μA/cm²*
−D	12	40.6±3.7	37.3±3.0	3.3±3.4	26.5±3.8	27.4±3.1	−0.9±2.6	24.9±1.8	43.5±7.0
+1,25$(OH)_2D_3$	11	70.5±5.0	43.2±4.4	27.3±3.8	27.0±2.3	31.4±2.5	−4.4±3.3	29.1±2.9	46.7±8.0

Values are mean±1 SEM. G, tissue conductance.

TABLE II

Unidirectional and Net Pi Fluxes across Proximal Colon from 1,25(OH)$_2$D$_3$-treated Rats Measured at Different [Pi] in Media

Medium [Pi]*	n	Jms	Jsm	Jnet‡	Jms vs. Jsm
mM/liter			*nmol/cm²/h*		
0.0118	8	0.50±0.07	0.46±0.07	0.04±0.06	NS
0.118	10	5.77±0.94	5.07±0.54	0.70±0.90	NS
1.18	11	27.0±2.3	31.4±2.5	−4.4±3.3	NS
2.36	11	274.7±34.2	234.9±44.0	39.8±43.9	NS

* Medium [Ca] 1.25 mM/liter in all groups.

‡ Jnet not different from zero at each medium [Pi].

time 0.9 ml of 55 mM $CaCl_2$ was added to the serosal as well as the mucosal bath of one chamber, and 0.9 ml of 0.125 mM $CaCl_2$ was added to both bath of the other chamber containing the paired tissue. Because each bath originally contained 10 ml of buffer with 0.125 mM [Ca] the final buffer [Ca] in one chamber was increased to 5 mM whereas the [Ca] in the other chamber remained unchanged at 0.125 mM. Increasing [Ca] produced a small but significant increase in resistance (decrease in conductance), which could not produce the saturation kinetics observed in unidirectional Ca fluxes depicted in Fig. 7, unless the change in resistance is highly specific for Ca rather than monovalent ions.

DISCUSSION

This study demonstrates that neither Ca nor Pi are actively transported in ascending colon of vitamin D-deficient rats. Transmural movement of these ions in the D-depleted state is concentration related and appears to occur by some type of passive diffusion. Treatment with 1,25(OH)$_2$D$_3$ selectively increases Ca Jms without altering Jsm at low Ca concentrations, resulting in net active absorption of Ca (Fig. 2, Table I). Interestingly, 1,25(OH)$_2$D$_3$ was without effect on either Jms or Jsm for Pi measured over a range of ambient [Pi] varying from 0.0118 to 2.36 mM. The lower [Pi] were used because several investigators have found in small intestine carrier-mediated Pi transport processes with K_m substantially lower than 1.0 mM (13, 14). This observation suggests that the colon contains cells which are capable only of a Ca transport response and it provides the first clear example of a total separation of Ca and Pi absorptive processes in the intestine. In contrast, in rat small intestine, 1,25(OH)$_2$D$_3$ increases the active absorp-

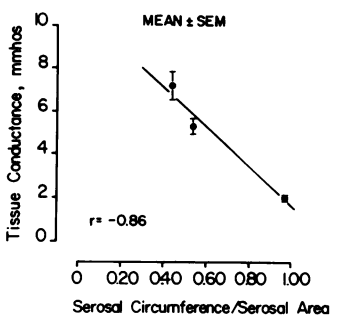

FIGURE 5 The influence of edge damage on tissue conductance. Lucite chambers of different diameters were used so that conductance could be measured at different edge to surface ratio. The linear inverse (rather than a positive) correlation observed suggests that edge damage does not significantly influence the measured conductance (n = 8).

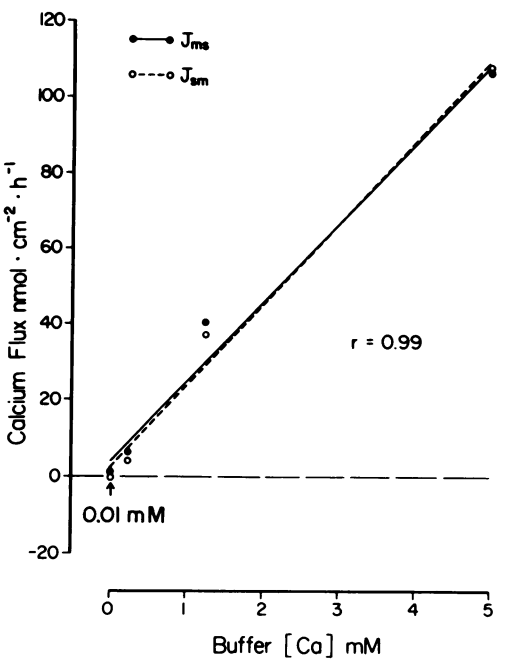

FIGURE 6 Unidirectional Ca fluxes measured in −D colon using buffer with Ca concentrations [Ca] of 0.0125 (n = 5), 0.25 (n = 12), 1.25 (n = 12), and 5.0 (n = 8) mM, respectively.

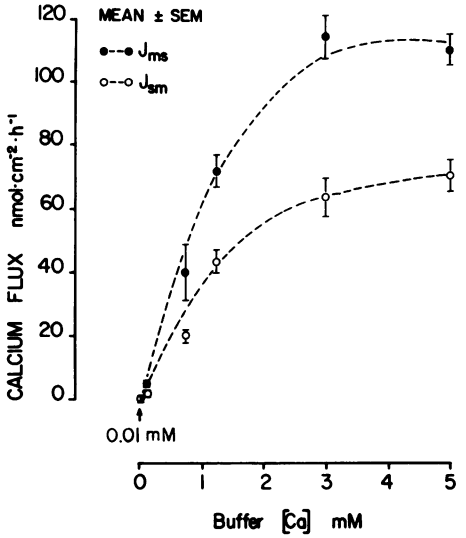

FIGURE 7 Unidirectional Ca fluxes measured in +D colon using buffers with Ca concentrations [Ca] of 0.0125 (n = 7), 0.125 (n = 7), 0.75 (n = 4), 1.25 (n = 11), 3.0 (n = 4), and 5.0 (n = 5) mM.

tion of both Ca and Pi (1). Our data show that 1,25-$(OH)_2D_3$ induces a saturable active absorptive process for Ca in colon with an apparent K_m of 0.9 mM, which is similar to those estimated for rat duodenum, (7) and ileum (15). This suggests that the colonic cells which transport Ca may have the same 1,25$(OH)_2D_3$-stimulated process as their small intestinal counterparts and provides a persuasive argument for the existence of Pi-independent Ca-transporting cells in the small intestine (7, 16). Also, unlike the small intestine, Ca Jsm of the colon appears to saturate under

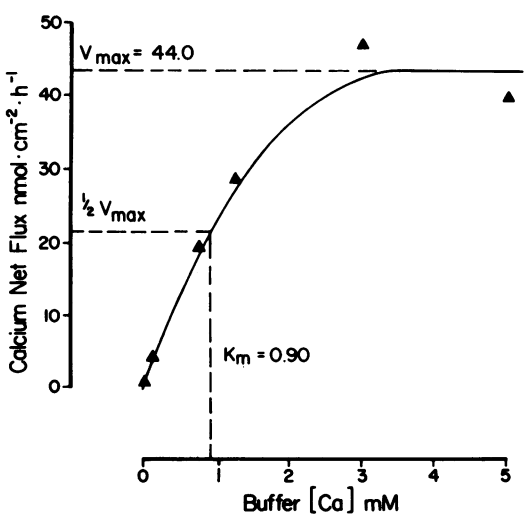

FIGURE 8 Ca net flux measured in +D colon using buffer with different Ca concentrations [Ca].

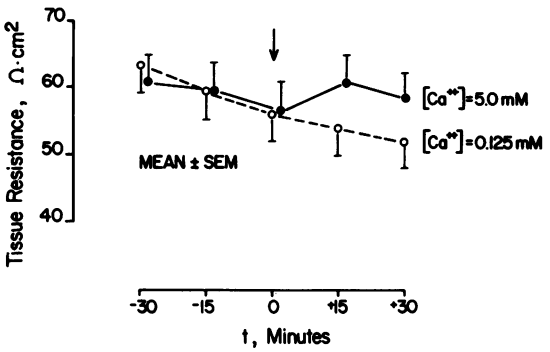

FIGURE 9 Effect of ambient calcium concentration [Ca] on electrical resistance of adult rat proximal colon in vitro. At 0 min (arrow) [Ca] was either changed from 0.125 to 5 mM or maintained unchanged at 0.125 mM (see text). Paired differences at 0 min, +15, and +30 min were 0.7±2.1 (P = NS), 6.9±2.1 ($P < 0.02$), and 6.4±2.6 ($P < 0.05$), respectively, n = 8 for each pair.

the influence of 1,25$(OH)_2D_3$, whereas under similar conditions Ca Jsm in the duodenum and ileum is a linear function of [Ca] as it is in −D colon (7, 10, 15, 17).

The present study also provides unequivocal confirmation of the presence of active Ca transport process in rat colon noted by other workers (3, 4). The physiological significance of this distal intestinal mechanism is not fully explored. It may be important in the full expression of intestinal adaptation to dietary Ca restriction, since under such conditions the conservation of Ca by ileum is incomplete (18). This distal Ca reabsorptive process may also provide the mechanism accounting for the virtual disappearance of fecal Ca in rapidly growing rats (19, 20).

The clear dissociation between Ca and Pi transport activities in colon suggests that this intestinal segment may provide a useful model system for studying molecular events, e.g., induction of proteins and enzymes, related to Ca but not Pi transport activities; whereas the apparent influence of 1,25$(OH)_2D_3$ on the Ca secretory process may furnish further insight into the mechanism of action of vitamin D.

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