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Calcium competes with zinc for a channel mechanism on the brush border membrane of piglet intestine

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Abstract

Interactions between Ca^{+2} and Zn^{+2} at the intestinal brush border membrane occur via unclear mechanisms. We hypothesized that Zn^{+2} and Ca^{+2} are transported across the brush border membrane via a multidivalent metal channel. Using brush border membrane vesicles (BBMV) prepared from intestines of 8 sow-fed piglets, we sought to determine whether Ca^{+2} competes with Zn^{+2} for uptake. Extravesicular Zn^{+2} was removed with ethylenediamine-tetraacetic acid. Time curves of Zn^{+2} and Ca^{+2} uptake by BBMV were conducted with increasing concentrations of Ca^{+2} and Zn^{+2}, respectively. Saturation curves compared kinetic parameters of Zn^{+2} uptake with and without Ca^{+2}. In addition, Zn^{+2} uptake was measured in the presence of various classical Ca^{+2} channel modulators. Over 20 min, a 0.4× concentration of Zn^{+2} lowered Ca^{+2} uptake by vesicles, whereas a 30× concentration of Ca^{+2} was necessary to lower Zn^{+2} uptake. These data suggest that Ca^{+2} has lower affinity than Zn^{+2} for a brush border membrane transport protein. Kinetic parameters showed higher K_m values with 4 or 15 mM Ca^{+2} but unchanged J_{max}, suggesting competitive inhibition. The Ca^{+2} channel blocking agents, La^{+3}, Ba^{+2}, verapamil, and diltiazem, inhibited Zn^{+2} uptake, whereas calcitriol, *trans* 1,2 cyclohexanediol, *cis/trans* 1,3 cyclohexanediol, and the L-type Ca^{+2} channel agonist, Bay K8644, induced Zn^{+2} uptake. These data were consistent with competition for a common transport mechanism on the brush border membrane, possibly a novel multimetal channel.

Keywords: calcium; zinc; competition; channel; brush border membrane; piglet

1. Introduction

Interactions between Ca and Zn are of relevance to infant nutrition due to the highly variable Ca:Zn ratios found in infant feedings [1]. In vivo, Ca lowers Zn absorption in the absence of phytic acid in animals [2,3], adult humans [4], and infants [1]. In vitro, Ca^{+2} and Zn^{+2} have been shown to compete for uptake in rat intestinal brush border membrane vesicles (BBMV) [5,6].

Divalent metal interactions due to similar coordination chemistry were first described by Hill and Matrone [7]. Consistent with this concept, Zn^{+2} can replace Ca^{+2} in Ca^{+2}-binding sites of various transport proteins such as mitochondrial Ca^{+2} transporter and Ca^{+2} channels of excitable membranes [8]. Furthermore, multidivalent metal channels have been described in hepatic cell membranes [9], Caco-2 cells [10], and epithelia from several tissues, particularly the duodenum [11]. The competition between Ca^{+2} and Zn^{+2} at the protein level has been documented; however, such competition at the brush border membrane has yet to be described.

We have observed various metal:Zn^{+2} interactions at the brush border membrane level [12].[1] We hypothesize that such metal–metal interactions are due to competition for a common transporter; the most likely type of transporter with such a broad specificity for divalent metals is probably a channel mechanism. Of the metals studied (Ca^{+2}, Mg^{+2}, Fe^{+2}, Cu^{+2}, Mn^{+2}, and Zn^{+2}), Ca^{+2} has the largest ionic radius and, thus, we hypothesized that a nonspecific type of Ca^{+2} channel may be responsible for our observations. By analyzing Zn^{+2} uptake in the pres-

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ence of Ca^{+2} and Ca^{+2} channel inhibitors and agonists, we intend to support our hypothesis that Ca^{+2} and Zn^{+2} compete at the brush border membrane for a multidivalent metal channel.

2. Methods and materials

2.1. Piglets

Eight 20–24-day-old male Yorkshire piglets (4.5–6.1 kg) were removed from sows at the Arkell Research Farm (Guelph, ON Canada) and brought to the McMaster University Central Animal Facility. The piglets were not littermates and were sow fed. Handling of piglets conformed with the *Guide to the Care and Use of Experimental Animals* [13]. Upon arrival, the piglets were killed by euthanyl injection and the proximal and medial jejuna were removed. We have previously shown that no differences in Zn^{+2} uptake by BBMV exist between the proximal, medial, or distal small intestine [14]. Intestinal mucosa was obtained as described by Wang et al. [14].

2.2. BBMV preparation and purity

Vesicles were obtained by a Mg precipitation/differential centrifugation method [15,16] and the final pellet was resuspended in an incubation buffer (112 mM NaCl, 100 mM D-mannitol, 10 mM Tris-HEPES, pH 6.7). The extent of brush border purification was determined by measuring changes in the specific activity of sucrase (EC 3.2.1.48) [17]. The BBMV suspension was used in $^{65}Zn^{+2}$ transport studies the same day to avoid vesicle damage caused by freezing and thawing.

2.3. Ca^{+2}/Zn^{+2} uptake time curves

For all time curves, a BBMV solution in incubation buffer was added to the appropriate stock solution (see below) at 37°C. A 60-μL aliquot of this mixture ($\sim$40 μg vesicular protein) was removed in triplicate at various time points (0.5 to 20 min) and applied to 0.45-μm filters (Millipore, Groton, CT USA) under vacuum. The filter wells were rinsed twice with 200 μL of an ethylenediaminetetraacetic acid (EDTA) buffer (incubation buffer, 5 mM EDTA, pH 6.7) to remove nonspecific extravesicular binding of Zn^{+2}. The triplicate filters at each time point were collected and assayed for radioactivity by a gamma counter (Beckman Gamma 5500, Fullerton, CA USA) for $^{65}Zn^{+2}$ or a scintillation counter (Beckman LS-330, Fullerton, CA USA) for $^{45}Ca^{+2}$. Samples were prepared in triplicate with a blank of nonspecific binding (no BBMV) included to correct for background radioactivity. Uptake rates (J) were expressed as nmol Zn^{+2} per mg total vesicular protein per min. Total protein was measured using Bradford's assay [18].

For Zn^{+2} uptake stock solutions, the Ca^{+2} concentrations of interest were so great that the osmolar contribution of $CaCl_2$ needed to be considered. For Zn^{+2} uptake time curves, 0.2 mM $ZnCl_2$ and 15 kBq $^{65}Zn^{+2}$ (New England Nuclear Corp., Dupont, Boston, MA USA) were added to a stock solution containing only incubation buffer or various amounts of $CaCl_2$ in an incubation buffer that was adjusted by removal of D-mannitol (and NaCl for 40 mM Ca^{+2}) to maintain constant osmolarity (0.32 Osm/L). Final Ca^{+2} concentrations of 2 mM (100 mM mannitol, 100 mM NaCl), 6 mM (54 mM D-mannitol, 100 mM NaCl), and 40 mM (0 D-mannitol, 0 NaCl) were used in Zn^{+2} uptake time curves. For Ca^{+2} uptake time curves, 0.5 mM $CaCl_2$ and 550 kBq $^{45}Ca^{+2}$ (Amersham, Mississauga, ON Canada) were added to stock solutions of incubation buffer with final concentrations of 0, 0.05, 0.2, or 0.5 mM $ZnCl_2$. Aliquoting, filtration, rinsing, and counting were conducted as above.

2.4. Zn^{+2} uptake saturation curves

A modification of the technique by Wang et al. [14] was used to perform Zn^{+2} saturation curves. Approximately 120 μg vesicular protein in 40 μL were added to 160 μL of a solution containing the incubation buffer with 3.7 kBq $^{65}Zn^{+2}$ and final $ZnCl_2$ concentrations of 0.1 to 0.5 mM. These solutions also contained $CaCl_2$ at increasing concentrations such that (1) the Ca^{+2}:Zn^{+2} molar ratios were maintained at 0, 30:1, or 200:1; and (2) Ca^{+2} concentrations were maintained at 0, 4, or 15 mM. BBMV were incubated for 1 min at 25°C, whereupon 100 μL of the solutions were removed and filtered, rinsed, and counted as described above. In addition to the conditions with Ca^{+2}, Zn^{+2} uptake in two buffers containing 10 mM Tris-HEPES, 3.7 kBq $^{65}Zn^{+2}$, $ZnCl_2$ concentrations of 0.1 to 0.5 mM, and either 300 mM D-mannitol or 112 mM NaCl and 100 mM D-mannitol were compared (pH 6.7).

2.5. Perturbations and Zn^{+2} uptake

Approximately 120 μg vesicular protein were added to the following solutions (final concentrations in 200 μL): Ca^{+2} channel inhibitors/agonists included incubation buffer alone (control), incubation buffer with 10 mM verapamil, 10 mM diltiazem, 10 μM 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS), 1 mM $LaCl_3$, 1 mM $BaCl_2$, or 1 μM of the L-type Ca^{+2} channel agonist, Bay K8644; vitamin D metabolites included incubation buffer with 0.001% ethanol (control), at most 0.001% ethanol with either various concentrations (0.1, 1, 10, 100, 500 nM) of 1,25 $(OH)_2$ cholecalciferol (calcitriol), or 100 nM of cholecalciferol, *cis* 1,2 cyclohexanediol, *trans* 1,2 cyclohexanediol, or *cis/trans* 1,3 cyclohexanediol. All data were expressed as percentage of respective controls. All perturbation solutions were incubated for 1, 10, and 30 min at 25°C with 3.7 kBq $^{65}Zn^{+2}$ and 0.2 mM $ZnCl_2$, whereupon 100 μL were filtered, rinsed, and counted as above.

2.6. Statistics

Due to the high variation among animals, time curve data were corrected by setting the 5-min point for the control curve of each piglet at 100%. All other time points were expressed as a percentage of this point; mean percentages were calculated and plotted. For time and saturation curves, the total area under each piglet's curve was calculated, and treatment groups were compared using Student-Newman-Keuls multiple comparisons (SigmaStat, Jandel Scientific, San Rafael, CA USA). To obtain kinetic parameters, Eadie-Hofstee plots of saturation curve data used linear regression. Group parameters were compared using two-factor analysis of variance (ANOVA; with block as one factor) and Student-Newman-Keuls multiple comparisons. For perturbation experiments, data for each piglet were corrected and expressed as percentage of control uptake. Because control data were assigned a SEM of 0, data were considered significantly different if SEM bars did not overlap with control data (100% line). Data are presented as mean ± SEM.

3. Results

3.1. Membrane purity

Sucrase assays before and after the BBMV preparation produced specific activity increases of 13.8 ± 1.4-fold, consistent with previous studies using this BBMV preparation method [14,16,19].

3.2. Time and saturation curves

Zn^{+2} uptake over 20 min was not influenced when 2 mM Ca^{+2} (10:1 Ca^{+2}:Zn^{+2} ratio) was present (*Figure 1a*). However, at both 6 and 40 mM Ca^{+2}, Zn^{+2} uptake by BBMV was significantly suppressed ($P < 0.05$). Ca^{+2} uptake over 20 min was significantly lowered at Zn^{+2}:Ca^{+2} molar ratios of 0.4:1 and 1:1 (*Figure 1b*; $P < 0.05$), but not at 0.1:1. Furthermore, Ca^{+2} uptake was lower with 1:1 Zn^{+2}:Ca^{+2} compared to 0.4:1 Zn^{+2}:Ca^{+2} ($P < 0.05$). The absence of measurable Ca^{+2} uptake from 1 to 10 min was curious despite its repeatability and low variability between samples. The two-phase uptake of Ca^{+2} by BBMV may reflect the involvement of multiple transporters or may be an anomaly of the methods employed for Ca^{+2} uptake. Nevertheless, with respect to the objectives of this study, it is clear that Zn^{+2} can lower Ca^{+2} uptake by BBMV as time proceeds.

Zn^{+2} uptake saturation curves without Ca^{+2}, with 30:1 Ca^{+2}:Zn^{+2} (molar), or with 200:1 Ca^{+2}:Zn^{+2} were also analyzed (*Figure 2a*). Initial Zn^{+2} uptake over increasing Zn^{+2} concentrations was suppressed with Ca^{+2} present at constant Ca^{+2}:Zn^{+2} ratios ($P < 0.05$). Initial Zn^{+2} uptake was also analyzed in the presence of 0, 4, or 15 mM Ca^{+2} (*Figure 2b*); under these conditions, initial Zn^{+2} uptake was lower with Ca^{+2} present at either 4 or 15 mM ($P < 0.05$).

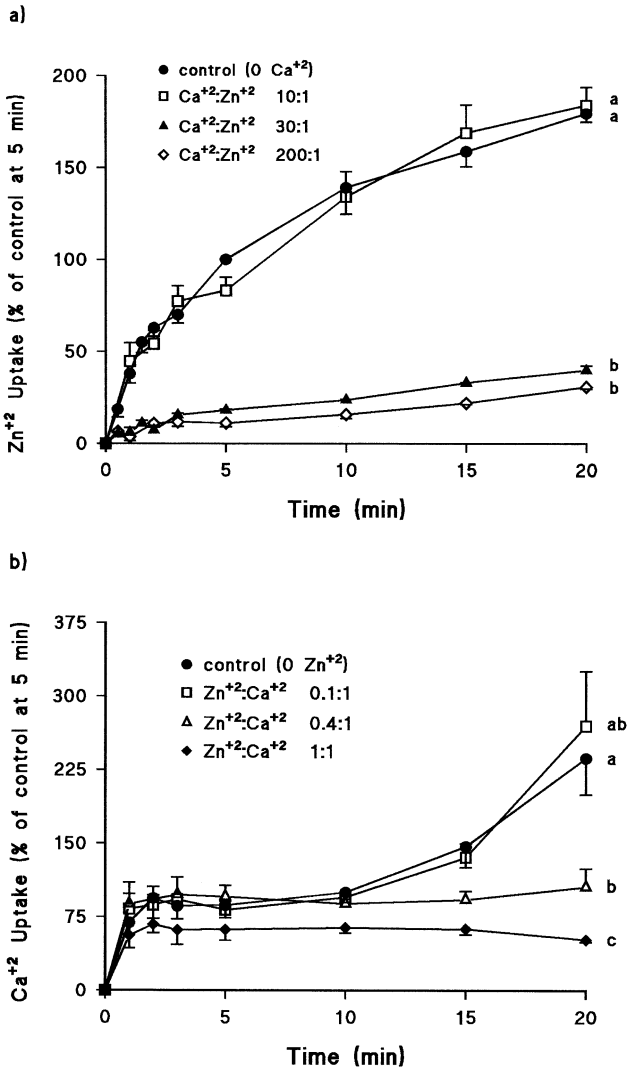

Figure 1 (a) Zn^{+2} (0.2 mM) uptake over time by brush border membrane vesicles (BBMV) in the presence of 2, 6, or 20 mM Ca^{+2} (37°C). Isoosmolarity was maintained by removing D-mannitol from the buffer. (b) Ca^{+2} (0.5 mM) uptake over time by BBMV in the presence of 0.05, 0.2, or 0.5 mM Zn^{+2} (37°C). In each piglet, the 5-min value for the control (0 Ca^{+2} or 0 Zn^{+2}) was set as 100% and all other data for each piglet were expressed as a percentage of control. Data represent mean ± SEM for 4 piglets. Curves not sharing a letter are significantly different ($P < 0.05$).

3.3. Inhibitors of Zn^{+2} uptake

Zn^{+2} uptake in the presence of classical transporter inhibitors and agonists is shown in *Figures 3a and 3b*. La^{+3} and Ba^{+2} are classically used as general Ca^{+2} channel blockers and both inhibited Zn^{+2} uptake at a 1-mM concentration at all time points (*Figure 3a*). By 30 min, Zn^{+2} uptake was reduced by 85% and 65% for La^{+3} and Ba^{+2}, respectively. The anion antiporter inhibitor, DIDS, only slightly reduced Zn^{+2} uptake by BBMV at 10 and 30 min.

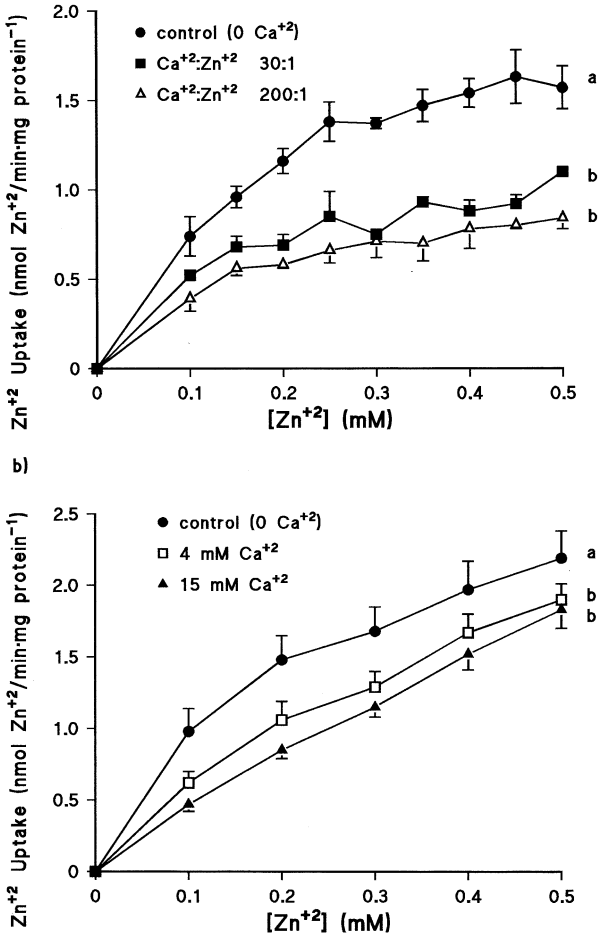

Figure 2 Zn^{+2} uptake saturation curves analyzing Zn^{+2} uptake (1 min at 25°C) by brush border membrane vesicles (BBMV) over Zn^{+2} concentrations of 0.1 to 0.5 mM. $CaCl_2$ was included at increasing concentrations such that (a) Ca^{+2}:Zn^{+2} molar ratios were maintained at 0, 30:1, or 200:1; and (b) Ca^{+2} concentrations were kept at 0, 4, and 15 mM. Data represent mean ± SEM for 3 piglets and uptake rates were expressed as nmol Zn^{+2}/min/mg total protein. Curves not sharing a letter are significantly different ($P < 0.05$).

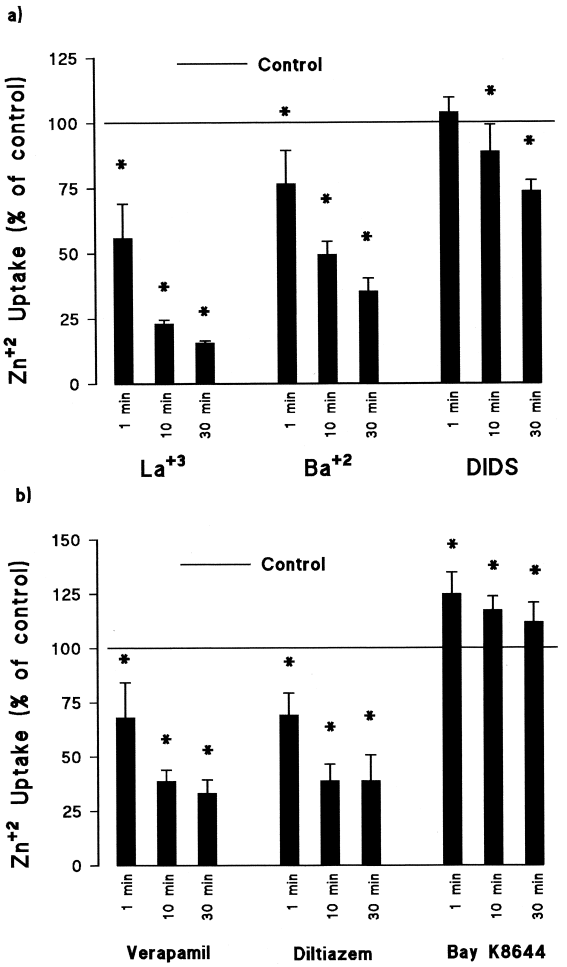

Figure 3 Zn^{+2} (0.2 mM) uptake in the presence of various transporter agents (25°C): (a) Ca^{+2} channel antagonists (1 mM La^{+3}, Ba^{+2}; $n = 4$) and anion exchanger inhibitor (10 μM DIDS; $n = 4$); and (b) L-type Ca^{+2} channel antagonists (10 mM verapamil, diltiazem; $n = 3$) and L-type Ca^{+2} channel agonist (10 μM Bay K8644; $n = 6$). Bars represent mean + SEM. An asterisk indicates data were different than control uptake (100% line).

In *Figure 3b*, the L-type Ca^{+2} channel inhibitors, verapamil and diltiazem (10 mM), suppressed Zn^{+2} uptake at all time points with approximately 60% inhibition by 10 min ($n = 3$). A 100-μM concentration of these agents had no effect (not shown). The L-type Ca^{+2} channel agonist, Bay K8644, increased Zn^{+2} uptake to 125% of control at 1 min ($n = 6$).

3.4. Calcitriol and Zn^{+2} uptake

The effects of vitamin D metabolites and analogues on Zn^{+2} uptake were compared in *Figures 4a* and *4b*. There were no differences for Zn^{+2} uptake in 0.001% ethanol versus incubation buffer alone control (not shown). No effect on Zn^{+2} uptake was shown with 0.1 nM calcitriol. In *Figure 4a*, Zn^{+2} uptake increased at all time points in the presence of 1 or 10 nM calcitriol, ranging from 125% to 146%. Lesser increases of Zn^{+2} uptake were observed in the presence of 100 or 500 nM calcitriol (111–137%; not shown). The lower calcitriol concentrations (139–146%) were especially more effective than higher concentrations (111–118%) at inducing Zn^{+2} uptake at 1 min. Zn^{+2} uptake was slightly higher with 100 nM cholecalciferol only at 30 min. In *Figure 4b*, *trans* 1,2 cyclohexanediol induced Zn^{+2} uptake at all times to 139–148% of control. Zn^{+2} uptake in the presence of *cis,trans* 1,3 cyclohexanediol was 110–130% of control. No effect on Zn^{+2} uptake was observed with *cis* 1,2 cyclohexanediol.

4. Discussion

The brush border membrane transporter for Zn^{+2} has not been identified. Other research from this laboratory showed that several divalent metals of similar chemistry reduced Zn^{+2} uptake by BBMV [12], perhaps as a consequence of

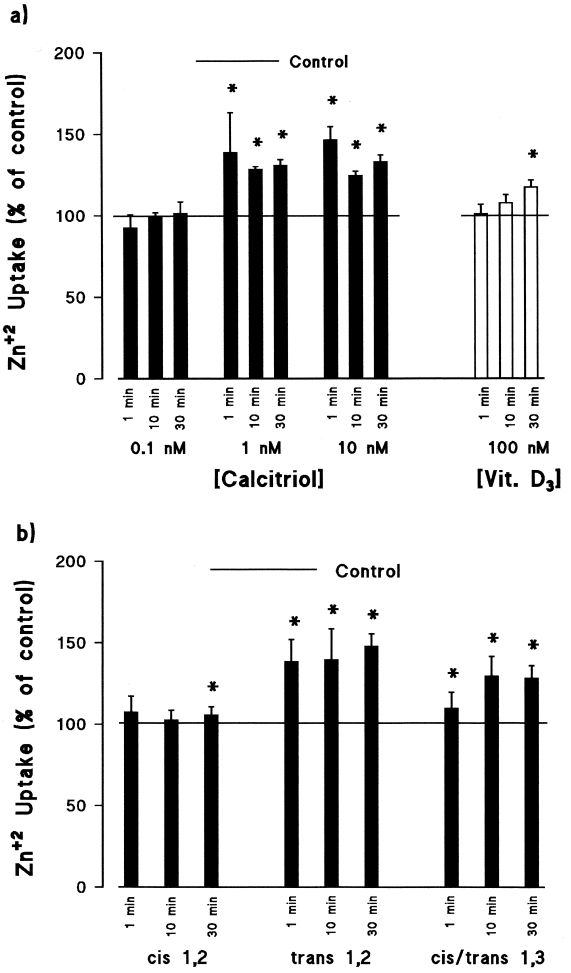

Figure 4 Zn^{+2} (0.2 mM) uptake was measured in the presence of (a) various concentrations of calcitriol or 100 nM cholecalciferol (25°C), and (b) 100 nM of *cis* 1,2 cyclohexanediol, *trans* 1,2 cyclohexanediol, or *cis/trans* 1,3 cyclohexanediol. Bars represent mean + SEM for 6 pigs (except for 1, 10, and 500 nM calcitriol, where $n = 3$). An asterisk indicates data were different than control uptake (100% line).

competition for a multidivalent metal channel that can transport Ca^{+2}, Mg^{+2}, Fe^{+2}, Cd^{+2}, Cu^{+2}, Mn^{+2}, and Zn^{+2}. In the present study, we attempted to characterize the interaction between Ca^{+2} and Zn^{+2}.

It is evident that Ca^{+2} and Zn^{+2} compete for a common transport pathway into BBMV. As Zn^{+2} concentrations increased, Ca^{+2} inhibited initial Zn^{+2} uptake when introduced at constant Ca^{+2}:Zn^{+2} ratios or at constant Ca^{+2} concentrations, consistent with data in BBMV from adult rats [6]. In addition, Ca^{+2} suppressed Zn^{+2} uptake by BBMV over time. Competition for a common transporter was further supported by observations that Zn^{+2} moderately suppressed Ca^{+2} uptake over time, similar to results in rat BBMV using a Zn^{+2}:Ca^{+2} molar ratio of 0.57:1 [5]. The transporter affinity for Zn^{+2} is higher than its affinity for Ca^{+2} because only a 0.4:1 Zn^{+2}:Ca^{+2} molar ratio was needed to lower Ca^{+2} uptake, whereas greater than a 10:1 Ca^{+2}:Zn^{+2} molar ratio was needed to lower Zn^{+2} uptake. These findings were consistent with our previous study that showed that the dissociation constant of Ca^{+2} (K_i) was 20-fold larger than that calculated for Zn^{+2} (K_m) [12]. These data suggest that Ca^{+2} and Zn^{+2} compete for a common transport pathway into BBMV.

Evidence for a common channel mechanism in BBMV for Ca^{+2}/Zn^{+2} uptake was provided by the response of Zn^{+2} uptake in the presence of classical Ca^{+2} channel antagonists. La^{+3} is consistently the most potent ion blocker of Ca^{+2} channels in a variety of tissues [20]. Of the agents tested, we found that La^{+3} had the greatest inhibitory effect on Zn^{+2} uptake. Other researchers have shown that La^{+3} blocks Ca^{+2} uptake by BBMV [21,22] and inhibits a cation (Ca^{+2} and Mn^{+2}) influx pathway described in Caco-2 cells [10]. Another classical blocker of Ca^{+2} channels is Ba^{+2}. Ba^{+2} is considered less potent as a blocker than La^{+3} because Ba^{+2} is more mobile through Ca^{+2} channels [23]. In our study, Ba^{+2} suppressed Zn^{+2} uptake to a lesser extent than did La^{+3}. Also, in BBMV, others have shown Ba^{+2} inhibition of Zn^{+2} uptake [6] and Ca^{+2} uptake [21].

To determine whether brush border transport of Zn^{+2} occurs via voltage-sensitive Ca^{+2} channels analogous to those found in excitable tissues, we tested Zn^{+2} uptake by BBMV in the presence of classical L-type Ca^{+2} channel agents. A 1-μM concentration of the L-type Ca^{+2} channel agonist, Bay K8644, induced Zn^{+2} uptake by BBMV. A comparable dose enhanced Ca^{+2} transport from lumen to blood in chick intestine [24]; however, others have shown no effect of Bay K8644 on Ca^{+2} uptake in Caco-2 cells [10]. In addition, the L-type Ca^{+2} channel blockers, verapamil and diltiazem (at 10 mM, but not at 100 μM), both reduced Zn^{+2} uptake. Other studies have also shown inhibition of Ca^{+2} uptake by large concentrations of verapamil (>1 mM) in intestinal segments [25] and in BBMV [22,26]. Millimolar concentrations of these agents are much greater than the concentrations reported to antagonize Ca^{+2} transport in other tissues (10^{-9}–10^{-5} M) [27]. It is possible that the intestinal membrane is more resistant to these pharmacological drugs, or that verapamil may exert its effect on intestinal Ca^{+2} or Zn^{+2} uptake via nonspecific mechanisms. The channel of interest in this study seems to have distinct properties that are not common to classical voltage-sensitive L-type Ca^{+2} channels of other tissues.

Zn^{+2} uptake by BBMV was higher in the presence of calcitriol at physiological concentrations. If the effects of calcitriol on Zn^{+2} uptake were due to nonspecific mechanisms, higher concentrations of calcitriol should be more potent. However, the inductive capacity of calcitriol did not increase with concentration; indeed, at 1 min, 500 and 100 nM of calcitriol were less potent than 10 or 1 nM. The increased potency of calcitriol at lower concentrations has been reported in studies on intestinal Ca^{+2} transport via transcaltachia [28]. In contrast, equimolar cholecalciferol had minimal effect on Zn^{+2} uptake in BBMV, which is comparable to results for Ca transport in perfused chick intestine exposed to micromolar concentrations of cholecalciferol [29]. Enhanced Zn^{+2} transport in the presence of

calcitriol has also been observed in Caco-2 cells by Fleet et al. [30]. These actions occurred at the brush border membrane, but only after at least a 24-hr incubation, suggesting a genomic role for calcitriol. The direct rapid action of calcitriol on brush border membrane transport of Ca^{+2} has been reported also in Caco-2 cells [10]. In that study, a 1-min exposure to calcitriol and $^{45}Ca^{+2}$ resulted in a rapid Ca^{+2} influx that was inhibitable by La^{+3}. Our findings support such a nongenomic role for calcitriol at the brush border membrane, perhaps by modifying the membrane transporter, thereby increasing ion permeability. Such a mechanism has been suggested for the effects of calcitriol on Ca^{+2} uptake by Caco-2 cells [31]. This mechanism has physiological relevance because intracellular calcitriol of vascular or biliary [32] origin may modify membrane proteins within minutes during transcaltachia.

To further describe calcitriol's effects on Zn^{+2} uptake, we employed simple analogue stereoisomers. The active ring of calcitriol (with *trans* 1, 3-hydroxylation) was approximated by cyclohexanediols. We were able to obtain only a *cis/trans* mix of 1,3 cyclohexanediol, but pure stereoisomers were available for 1,2 cyclohexanediol. The induction of Zn^{+2} uptake by *trans* 1,2 cyclohexanediol and the negligible effect by *cis* 1,2 cyclohexanediol suggested that transporter modification may be stereospecific. Furthermore, we observed a moderate induction of Zn^{+2} uptake by *cis/trans* 1,3 cyclohexanediol, perhaps limited by the *cis* isomers; the separate stereoisomers for the more analogous 1,3 cyclohexanediol should be analyzed.

In red blood cells, Zn^{+2} is transported primarily by DIDS-sensitive anion-dependent exchangers [33]. Given the ineffectiveness of DIDS to lower Zn^{+2} uptake by BBMV in this study and another [34], it is concluded that an anion exchange pathway for intestinal Zn^{+2} transport is unlikely.

Although the evidence presented provides only indirect support for the existence of a multidivalent metal channel, the inhibition of Zn^{+2} uptake observed with some of the Ca^{+2} transport inhibitors and agonists suggests that competition between Ca^{+2} and Zn^{+2} does occur at a specific site. Furthermore, metals that block Ca^{+2} channels generally do so by competing for binding to the channel with a higher affinity, but with lower mobility through the channel [20]. This follows from our previous study that suggested Mg^{+2}, Fe^{+2}, Cu^{+2}, and Mn^{+2} may also be transported via the channel. Multication channels have been described in cell membranes of Caco-2 cells [10], hepatocytes [9], fibroblasts [35], red blood cells [36], and epithelia of kidney, thymus, and duodenum [11]. The relative affinities of the divalent metals in these studies were consistent, with Cu^{+2} having the highest affinity, followed by Zn^{+2}, Fe^{+2}, Cd^{+2}, and Mn^{+2}; the lowest belonging to Ca^{+2} and Mg^{+2}. Such findings are consistent with the data of our previous study in which relative affinities indicated $Cu^{+2} >> Zn^{+2} > Fe^{+2} > Mn^{+2} >> Ca^{+2} > Mg^{+2}$ [12]. Our data do not preclude the possibility that Zn^{+2} is transported across the brush border membrane via multiple mechanisms. It is feasible that in addition to a specific Zn^{+2} carrier, Zn^{+2} is also transported via Ca^{+2} channels in the absence of Ca^{+2}. The question is, which pathway is most important physiologically? In the present study, we utilized physiologically relevant Ca^{+2} and Zn^{+2} concentrations, which suggests that both pathways are important depending on dietary mineral concentrations and ratios. Alternatively, instead of Zn^{+2} having a unique brush border membrane transporter, it is possible that Zn^{+2} only uses a Ca^{+2}-type channel, which can also be used by other divalent metals.

In conclusion, Ca^{+2} and Zn^{+2} appear to compete for a nontypical Ca^{+2} (or Zn^{+2}) channel in BBMV that is sensitive to La^{+3} and Ba^{+2}, and transport through the channel may be stimulated by calcitriol, *trans* 1,2 cyclohexanediol, *cis/trans* 1,3 cyclohexanediol, and Bay K8644. In addition, our previous study suggests that such a channel may also transport Mg^{+2}, Fe^{+2}, Cu^{+2}, and Mn^{+2}, and may be stimulated by lactose [12]. More research using direct methods is necessary to confirm the existence of such a channel.

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