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Studies on calcium binding to brush-border membranes from rabbit small intestine

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A study was made of the uptake of Ca^{2+} by brush-border membrane vesicles prepared from rabbit small intestine. The process was found to be time, temperature and substrate concentration dependent, displayed saturability, did not depend on added energy sources and occurred optimally in a pH range of 7.5–8.0. Although the transport of D-glucose by these membrane vesicles responded to changes in osmotic pressure as modified by adding cellobiose to the medium, the uptake of Ca^{2+} was found not to be osmotically-sensitive. Moreover, the equilibrium uptake value obtained when vesicles were exposed to 0.36 mM Ca^{2+} was some 60-fold higher than the amount that could have been accommodated by the intravesicular space, calculated from the equilibrium uptake of D-glucose. It was concluded from these results that the uptake involved complete binding of the Ca^{2+} to the membrane. The ionophore A23187 enhanced the rates of uptake and efflux of Ca^{2+} without affecting equilibrium values, which suggests that the binding of Ca^{2+} measured under our conditions was to interior sites of the membrane. The binding capacity was decreased in the presence of 10 mM lidocaine as indicated by a diminution of the equilibrium binding values. Generating an electrochemical potential (negative inside) by addition of valinomycin to vesicles pre-equilibrated with K_2SO_4, enhanced the rate of uptake of Ca^{2+}. Addition of metal ions, on the other hand, inhibited the uptake, La^{3+} and Tb^{3+} being most effective followed by Mn^{2+}, Ba^{2+} and Mg^{2+}. Na^+ and K^+ were the least inhibitory. The properties of the Ca^{2+} uptake process found in rabbit brush-border membranes were compared to those of similar processes occurring in other species.

Introduction

The mechanism whereby Ca^{2+} is transported across the brush border of small intestine is not well understood [1]. Studies performed mainly with duodenal brush-border membrane vesicles from chick and rat species have revealed that the uptake of Ca^{2+} in these membranes is saturable and involves mostly binding [2–4] much of which appears to be to interior sites of the membranes [5,6]. Both high- and low-affinity sites were characterized from Scatchard plot analysis, the latter sites being composed of phospholipids [6].

Although the brush-border membrane is equipped with an ATPase-alkaline phosphatase complex, it is unlikely that ATPase activity is involved as a Ca^{2+} pump at this site since the affinity of this enzyme for Ca^{2+} is low [7]. Moreover, Ca^{2+} uptake in these vesicles can proceed without added energy sources [2–6]. It must be pointed out, however, that the conditions for uptake have not been varied extensively and the

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involvement of an active transport system for Ca^{2+} cannot be precluded at this stage.

Although a number of studies have been described regarding Ca^{2+} uptake by brush-border membrane vesicles prepared from chick and rat intestine, relatively few such investigations have been made with rabbit brush-border membranes [8] in which the process remains ill-defined. Attempts to further characterize this process in rabbit-derived brush-border membranes are described presently.

Materials and Methods

$^{45}CaCl_2$ and D-[1-^{14}C]glucose were purchased from new England Nuclear Corp. (Boston, MA). Ionophore A23187 and D(+)-cellobiose and the sodium salts of 2-(*N*-morpholino)ethanesulfonic acid (Mes), *N'*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 3-(cyclohexylamino)-1-propanesulfonic acid (Caps), ethylene glycol bis(*β*-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of brush-border membranes. Brush-border membranes were routinely isolated from small intestine of female New Zealand white rabbits by the method of Selhub and Rosenberg [9] which makes use of $CaCl_2$ as precipitant. Isolations substituting $MgCl_2$ [10] or $MgCl_2$ + EGTA [11] for Ca^{2+} were also tested but found to yield fractions which were more heavily contaminated by basolateral membranes and which were characterized by a lysophosphoglyceride content not very much lower than that found in Ca^{2+}-prepared membranes [12]. Furthermore, the rates of uptake and the equilibrium uptakes of $^{45}Ca^{2+}$ were found to be similar in both types of fractions [13]. For these various reasons the procedure making use of $CaCl_2$ precipitation [9] was retained for the present study.

Ca^{2+} and D-glucose uptake. The procedure of Miller and Bronner [5] was followed essentially. Briefly, the uptake was initiated by the addition of 10 μl of brush-border membrane vesicles, i.e., 30 μg protein determined by the method of Lowry et al. [14], to 25 μl of $^{45}CaCl_2$, 0.36 mM final concentration. Incubations maintained at 25°C were terminated after 5 min, or after other specified times, by addition of 40 volumes of ice-cold stop solution containing 100 mM mannitol, 10 mM Hepes-Tris buffer (pH 7.5), 5 mM EDTA, 20 μM $LaCl_3$, pH 7.5. Following filtration of the suspension through 0.45 μm nitrocellulose filters under low vacuum, the trapped brush-border membranes were washed twice with 2.5 ml of ice-cold stop solution. Since EDTA removes the exchangeable, superficially-bound Ca^{2+} [5,6] the remaining radioactivity after washing represented mainly interiorly-located Ca^{2+}. Samples were counted in a Beckman LS233 spectrometer by placing the filters in vials containing toluene-PCS (Amersham Corp., Oakville, Ont.) 1 : 1 (v/v). Under these conditions, using 5 min incubation periods, linear uptakes were obtained with protein concentrations of 1.7 mg/ml or less.

D-Glucose uptake was measured in a similar manner. Brush-border membranes (110 μg protein) were incubated together with 100 μM D-[1-^{14}C]glucose, 100 μM $MgCl_2$, 150 mM NaSCN in 10 mM Hepes-Tris buffer, pH 7.5 (final volume 40 μl) at 25°C for up to 10 min or for longer periods when equilibrium uptake values were estimated. The incubations were terminated by a 40-fold dilution with stop solution consisting of 100 mM mannitol, 150 mM NaCl, 0.2 mM phlorizin, 55 mM $MgCl_2$ in 10 mM Hepes-Tris buffer (pH 7.5). The suspension was filtered through 0.45 μ nitrocellulose filters. The latter were washed twice with ice-cold stop solution and counted as indicated for Ca^{2+} uptake. Under these conditions D-glucose uptake displayed a typical overshoot phenomenon and proved to be an osmotically-sensitive phenomenon as determined by the use of high concentrations of cellobiose in the incubation medium [15].

Free Ca^{2+} concentrations were measured with an Orion Ionalyzer model 407 A/L equipped with an ion-selective electrode sensitive to 10^{-7} M calcium.

All values reported in this study are the means ± S.E. from at least four determinations with membranes obtained from two rabbits or more.

Results

Fig. 1 shows the uptake of Ca^{2+} with time and the effect of temperature. Linearity was usually

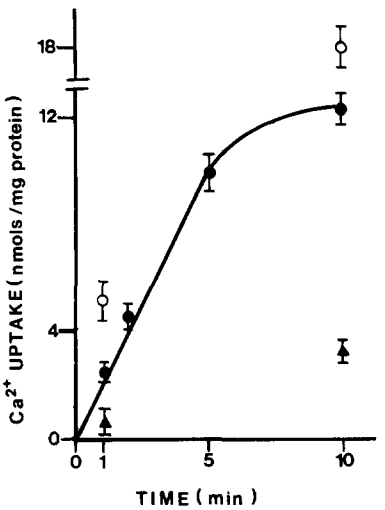

Fig. 1. The effect of time and temperature on Ca^{2+} uptake. The uptake was initiated by the addition of 25 μl of 0.5 mM $^{45}CaCl_2$ to 10 μl (30 μg protein) of brush-border membranes and pursued at 5°C (▲), 25°C (●) and 37°C (○) for various times. 100 mM mannitol in 10 mM Hepes-Tris buffer (pH 7.5) was used to prepare the Ca^{2+} solution or membrane suspension used for the incubation mixture. The uptake was terminated by dilution and rapid filtration.

seen up to 5 min at 25°C under the conditions of incubation stated. The rate of uptake increased with Ca^{2+} concentration (Fig. 2) and displayed

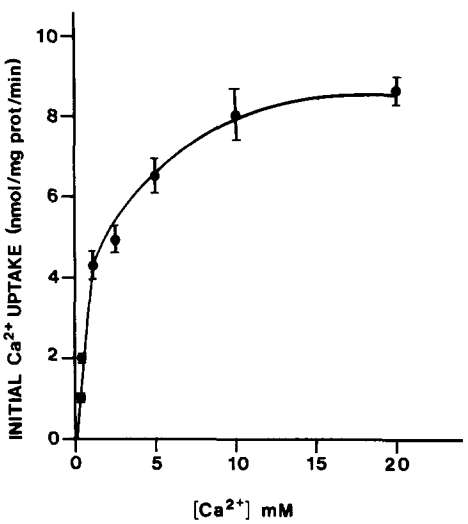

Fig. 2. The effect of Ca^{2+} concentration on uptake. The conditions were as stated for Fig. 1 except that the final Ca^{2+} concentration was varied as indicated, the incubation time was 5 min, and the temperature was 25°C.

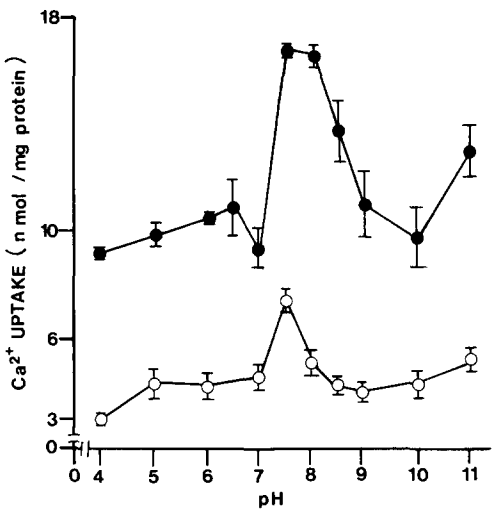

Fig. 3. The effect of pH on calcium ion uptake. The conditions were as stated for Fig. 1 except that the pH was varied by using the following buffers prepared in 100 mM mannitol: pH 4.0–6.0, 10 mM Mes-Tris; pH 6.5–9.0, 10 mM Hepes-Tris; pH 9.5–11.0, 10 mM Caps-Hepes and the time of incubation was 5 min (○) or 120 min (●).

saturability when measured for 5 min. However at concentrations of Ca^{2+} above 5 mM the rate of uptake was not as precisely linear with time per-

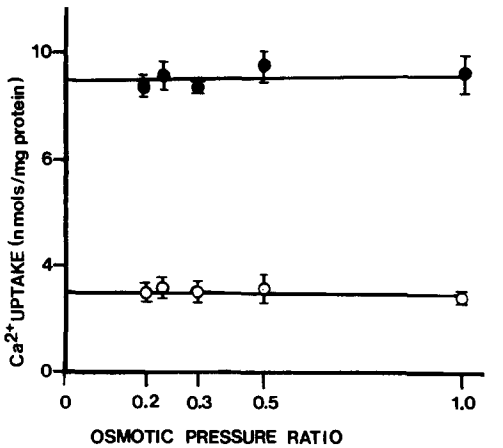

Fig. 4. The effect of osmolarity on uptake. Brush-border membranes were prepared in the presence of 50 mM D-cellobiose to entrap this saccharide. The Ca^{2+} uptake was then measured at 25°C as stated for Fig. 1 except that the incubation mixture contained various concentrations of D-cellobiose. The osmotic ratio was taken to be that of the concentration of cellobiose inside/outside. Uptake was measured for 1 min (○) or for 10 min (●).

haps because of Ca^{2+}- and time-dependent conformational changes in the membrane. Nonetheless, when measured for the minimum possible time of 20 s, the rates at the higher Ca^{2+} concentrations were not significantly different. Under these conditions, 10 mM and 20 mM Ca^{2+} gave rates of 7.74 ± 1.16 and 8.10 ± 0.97 nmol/mg per min, respectively. Rates obtained at concentrations below 10 mM when measured for 1 min were identical. It may be added that the free Ca^{2+} concentration did not change appreciably in the course of these incubations. Under the usual incubation conditions (0.36 mM Ca^{2+} and 0.85 mg protein/ml) the fall in free Ca^{2+} concentration due to total uptake including unspecific adsorption was no greater than approx. 7% when studied over a 120 min period as measured with an ion-selective electrode.

Rates of uptakes measured for 5 min were not greatly affected by pH although an optimum at about pH 7.5 was noticed. The uptake measured at 120 min, representing equilibrium binding rather than rate, displayed an optimum in the pH range of 7.5–8.0 (Fig. 3).

Cellobiose which is not absorbed by the vesicles has been used to increase osmotic pressure and decrease the intravesicular space [15] which in turn decreases the uptake of substances not bound by the membrane and normally accumulated within this space. Accordingly in the case of D-glucose, this was reported to occur previously [15] and was verified with our own vesicle preparations as indicated in Materials and Methods. Results given in Fig. 4, on the other hand, reveal a complete lack of effect of cellobiose on uptake of Ca^{2+} which suggests that the process represents mainly binding to the membrane. This conclusion is also substantiated by the fact that the intravesicular space, calculated to be 0.9 μl/mg protein on the basis of the equilibrium uptake of D-glucose, can accommodate, in the presence of 0.36 mM Ca^{2+} no more than 0.30–0.35 nmol/mg protein of this cation at equilibrium. However, the equilibrium uptake noted under these conditions was approx. 18 ± 4 nmol/mg protein.

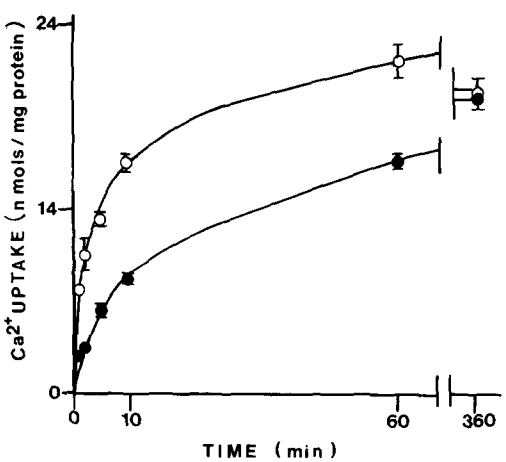

Fig. 5. Effect of ionophore A23187 on uptake. Incubations were at 25°C under conditions stated for Fig. 1. (●) no ionophore added; (○) 15 μM A23187 added.

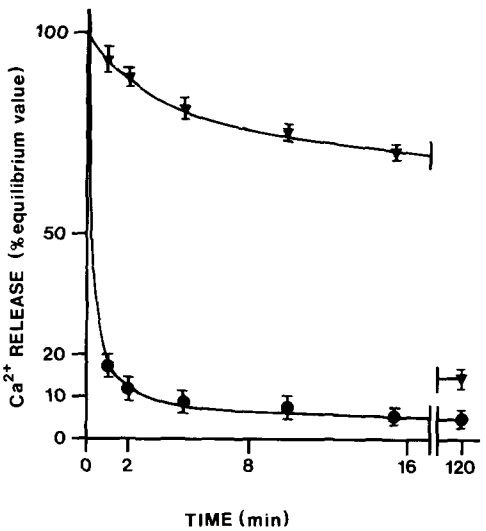

Fig. 6. Effect of ionophore A23187 on efflux. Membrane vesicles (30 μg protein) were preloaded to equilibrium by incubation for 120 min at 25°C with 0.36 mM $CaCl_2$. The membranes were then diluted 40-fold with 10 mM Hepes-Tris buffer (pH 7.5) containing 0.1 mM EGTA (▼) or containing 0.1 mM EGTA + 15 μM A23187 (●) and filtered after the times indicated.

Results illustrated in Fig. 5 indicate a marked effect of the ionophore A23187 on the rate of uptake but not on the equilibrium uptake of Ca^{2+}. Fig. 6 reveals that efflux from vesicles equilibrated in the presence of 0.36 mM Ca^{2+} and diluted 40-fold in media containing EGTA or EGTA + A23187 was greatly accelerated by the presence of the ionophore. After 1 min, an increase of 75% in efflux due to ionophore was seen which corre-

TABLE I

THE EFFECT OF WASHING WITH CHELATING AGENTS ON Ca^{2+} UPTAKE

The uptake was measured at 25°C for 5, 60 and 120 min under conditions stated for Fig. 1. The reaction was stopped by addition of ice-cold 100 mM mannitol, 10 mM Hepes-Tris (pH 7.5) solution containing either no chelating agent, 5 mM EDTA or 5 mM EGTA as indicated. The suspension was then rapidly filtered and collected membranes washed.

Wash conditions	Uptake (nmol/mg protein)		
	5 min	60 min	120 min
Buffer	9.13±0.44	22.3±1.6	22.7±0.7
Buffer+EDTA	8.13±0.20	13.6±1.4	15.8±1.8
Buffer+EGTA	7.38±0.37	14.8±1.3	16.8±1.4

sponds to an increased loss of approx. 13–14 nmol/mg protein. Since the effect of the ionophore can not be easily rationalized on a basis other than an increased rate of translocation of Ca^{2+} from an internal pool to the exterior of the vesicle, the value obtained for enhanced loss most likely represents interior Ca^{2+}. Results summarized in Table I indicate that brief washing (20 s) of the vesicle preparations, previously equilibrated with 0.36 mM Ca^{2+}, with EDTA- or EGTA-containing stop solutions removes about 30–35% of the Ca^{2+} that is bound after brief washing with Hepes-Tris buffer only. Losses, however, were less (about 10–20%) when uptakes were studied for 5 min only, which indicates that binding to internal sites probably proceeds at a rate greater than that of unspecific adsorption to exterior sites. During such a short washing time the chelating agents would be expected to remove mainly reversibly-bound Ca^{2+} located at the exterior surface of the membrane [5,6]. It will be noted that when equilibrium conditions prevailed, the pool of internally-bound Ca^{2+}, revealed by washing with chelators, is similar in size to the one found with the use of ionophores.

TABLE II

EFFECT OF METAL CATIONS ON Ca^{2+} UPTAKE

The incubations were performed for 5 and 120 min at 25°C. The other conditions were as specified for Fig. 1 except that the incubation mixtures contained 0.10 mM $^{45}CaCl_2$ together with chloride salts (0.5 and 2.5 mM) of other metal ions as indicated. In the case of Ca^{2+}, the labelled Ca^{2+} was diluted to 0.6 mM or 2.6 mM with unlabelled $CaCl_2$. The values represent averages of 4–8 determinations performed with membranes from two or more rabbits. The standard deviations were within 12% of the mean. Control values in the absence of any competing cations were 3.2 and 12.1 nmol/mg protein for the rate of Ca^{2+} uptake and the equilibrium value, respectively.

Cation	Rate of Ca^{2+} uptake (nmol/mg for 5 min)		Equilibrium uptake (nmol/mg for 120 min)	
	0.50 mM	2.5 mM	0.50 mM	2.5 mM
La^{3+}	0.3	–	1.7	–
Tb^{3+}	0.7	–	3.0	–
Ca^{2+}	1.2	0.4	4.1	1.4
Mn^{2+}	1.8	0.8	5.6	2.3
Mg^{2+}	2.8	2.2	6.8	4.0
Ba^{2+}	3.1	1.7	6.5	3.6
K^+	3.0	3.0	9.7	8.9
Na^+	3.2	3.2	11.9	8.5

Results summarized in Table II reveal the effect of adding various metal cations to the incubation mixture. The trivalent cations, La^{3+} and Tb^{3+} were the most effective in reducing rate of uptake and equilibrium uptake. Of the divalent cations tested, Mn^{2+} was the most effective in this respect after unlabelled Ca^{2+} itself. Mg^{2+} and Ba^{2+} affected the rate of uptake only at the higher concentrations of 2.5 mM. The monovalent cations, K^+ and Na^+, produced the least marked effects. At high concentrations (100 mM) these have been shown to have inhibitory effects on uptake which were explained on the basis of increased ionic strength [5,6].

Because Tb^{3+} is effectively bound by Ca^{2+} binding proteins and since it displays characteristic fluorescence when bound in the vicinity of tyrosine and/or tryptophane residues, this cation is currently used to report on Ca^{2+} binding sites involving proteins [16]. We decided therefore to investigate further the inhibitory effect of Tb^{3+} on Ca^{2+} uptake in the hope that this trivalent cation could be used as fluorescent probe for at least some of the interior Ca^{2+} binding sites of brush-border membranes. Results illustrated in Fig. 7 indicate that when vesicles are preincubated with Ca^{2+}, washed with stop solution to eliminate externally-bound Ca^{2+}, and treated with increasing concentrations of Tb^{3+}, there is relatively little loss of Ca^{2+} from the vesicles that results. On the other hand, preincubation of the vesicles with Tb^{3+} almost completely blocked the subsequent

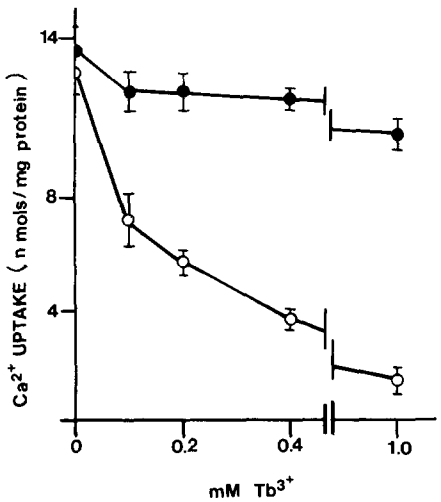

Fig. 7. Effect of Tb^{3+} on Ca^{2+} uptake. Membranes were incubated at 25°C for 120 min with 0.36 mM $^{45}CaCl_2$ as stated for Fig. 1 after which time various concentrations of Tb^{3+} were added and incubations were pursued for another 120 min (●). Membranes were incubated for 120 min at 25°C with various concentrations of Tb^{3+} after which time 0.36 mM $^{45}CaCl_2$ was added and incubations were pursued for another 120 min (○).

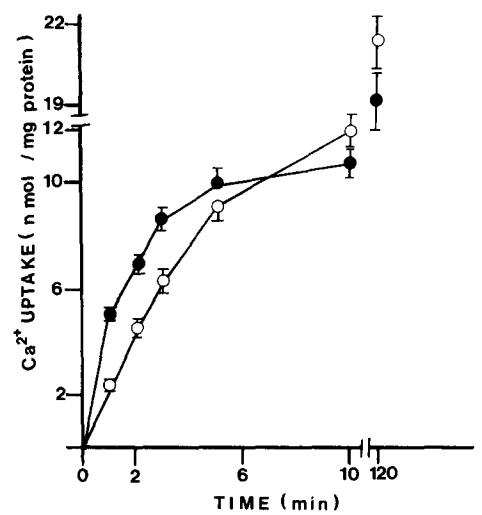

Fig. 8. The effects on Ca^{2+} uptake of a valinomycin-induced electrochemical potential (inside negative). Brush-border vesicles were prepared in the presence of 50 mM K_2SO_4 to entrap this salt. Control brush-border membranes were prepared in the same manner but in the absence of K_2SO_4. 10 μl of control and experimental brush-border membranes (100 μg protein) were diluted 20-fold at time 0 with 190 μl of a solution containing $^{45}CaCl_2$ (0.379 mM), mannitol (100 mM) and Hepes-Tris buffer (10 mM, pH 7.5). The uptake was pursued for up to 120 min at 25°C and terminated with the addition of 2 ml ice-cold stop solution. (●) K_2SO_4; (○) no K_2SO_4.

uptake of Ca^{2+}. The results suggest that Tb^{3+} mimicks La^{3+} in that little of this cation penetrates through the membrane [4,17] and consequently is unable to dislodge Ca^{2+} from its interior binding sites. Once bound to the surface of the membrane, however, Tb^{3+} prevents translocation of Ca^{2+} to interior binding sites.

Results illustrated in Fig. 8 indicate that in vesicles preloaded with 50 mM K_2SO_4 and then exposed to buffer containing valinomycin and no K_2SO_4 there is initially a greatly enhanced rate of Ca^{2+} uptake compared to results obtained with vesicles exposed to similar conditions but not preloaded with K_2SO_4. These differences are not likely due to any decrease in free Ca^{2+} concentration resulting from interaction with SO_4^{2-}, the product of the concentrations of these ions ($1.80 \cdot 10^{-5}$) being sizeably lower than the solubility product of $CaSO_4$ at 25° ($2.45 \cdot 10^{-5}$). Since the effect of valinomycin is to transport K^+ outwardly while leaving SO_4^{2-} inside, the results indicate that an increase in negative charge within, promotes the rate of translocation of Ca^{2+} and its binding to membrane.

Lidocaine (Fig. 9), known to decrease binding of Ca^{2+} to phospholipids [18], had a slight stimu-

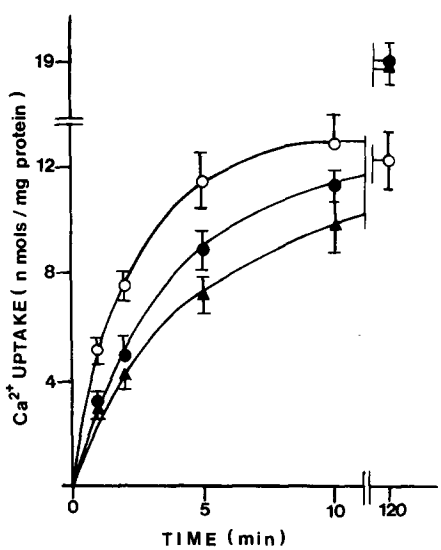

Fig. 9. The effect of lidocaine on uptake. The final Ca^{2+} concentration was 0.36 mM and other conditions were as stated for Fig. 1. (▲) No lidocaine added (●) 1 mM; (○) 10 mM lidocaine added to incubation mixture as an ethanolic solution (final alcohol concentration, 2%).

lating effect on the rate of uptake but moderately decreased the equilibrium uptake (by about 35% when 10 mM lidocaine was used). This result would suggest that a fair portion of the interior binding sites are proteins rather than phospholipids. Supporting this conclusion to some extent is the fact that pretreatment of the vesicles with 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide, an agent used to block carboxyl and other reactive groups of proteins [19], caused a marked reduction in the equilibrium uptake of Ca^{2+} (results not shown). The effect of this agent, however, depends a great deal on the conditions of the reaction which could not be varied extensively because of the lability of the membranes. At any rate its effects were quite unspecific since a complete inhibition of D-glucose uptake also resulted from this treatment.

Discussion

Most of the knowledge concerning the transport of Ca^{2+} by brush-border membranes of the small intestine comes from studies performed with either chick or rat tissue [4–6,20,21]. Relatively little remained known about this process as it occurs in other species [1,8]. Our results show that the uptake of Ca^{2+} by rabbit membranes is a process similar to that found in the membranes derived from chick and rat. Accordingly, the uptake involves a time, temperature and substrate concentration-dependent process, the kinetics of which reveal saturability. Also, increasing the negative charge inside the rabbit membrane vesicles enhances the uptake as was reported for rat-derived membranes [5,6].

The uptake of Ca^{2+} by rabbit vesicles differs, however, from the systems characterized for rat and chick in that it is not an osmotically sensitive phenomenon and consequently involves mostly if not complete binding of the cations to the membrane. The uptake process in rabbit membranes measured under conditions results from translocation across the membrane nonetheless since most of the Ca^{2+} is bound to interior sites of the membrane. This conclusion is based on the fact that chelating agents such as EDTA and EGTA, expected to act mainly on externally-bound Ca^{2+} during the stop and wash steps of the uptake procedure [5,6], removed no more than approximately a third of the Ca^{2+} taken up under equilibrium conditions. Furthermore, it was shown that both the influx and efflux of Ca^{2+} were enhanced by ionophore and that the efflux enhancement involved a pool, the size of which after equilibrium was similar to the one resistent to the action of chelating agents added externally.

The pH study was carried out mainly to arrive at optimal standard conditions for Ca^{2+} uptake. The effects of this variable on transport and binding are difficult to interpret at this stage and would best be studied with purified preparations of the calcium-binding protein involved.

Although the brush-border membrane of several species [1], including rabbit [12], contains an ATPase-alkaline phosphatase complex it is unlikely that this enzyme is involved in the actual transport of Ca^{2+}. Previous reports, however, have described the enhancement of either influx or efflux of Ca^{2+} by ATP added internally or externally to the brush-border vesicles of different species [22–24]. One such report dealing with kidney brush-border membrane [25] attributed the enhancing effect of the nucleotide to the formation of phosphatidic acid from diacylglycerol, this phosphoglyceride having known Ca^{2+} ionophoric properties. Our own results (not shown) with rabbit membranes used immediately after isolation revealed no enhancing effect of ATP on either influx or efflux of Ca^{2+} whether or not diacylglycerol was also added or whether the nucleotide was entrapped in the vesicles or added to the exterior as described in other reports [22–24]. However, the significance of these results would greatly depend on the rate at which the added nucleotide is hydrolysed by ATPase and alkaline phosphatase. Preliminary results have indicated that the rate of ATP hydrolysis under conditions reported previously [22–24] and used in our laboratory is quite rapid. Consequently further work is required to establish whether suitable inhibitors of these hydrolytic enzymes can be used for such studies. Our results do reveal nonetheless that uptake can proceed in the absence of added energy sources and in this respect as well as in the quantitative aspects of the uptake, the system resembles that characterized in other species [4–6,19,20]. The possibility that ATP-requiring

mechanisms may intervene in processes that are very labile or lost in some manner during membrane isolation and consequently not measurable under our conditions, cannot be precluded either. Again the results of this study do not preclude the existence of other energy-requiring Ca^{2+} transport systems in isolated brush-border membranes which are not ATP dependent; however, there is at present no evidence indicating that natural metabolic routes exist in the isolated membranes which could generate either a pH gradient or a chemical gradient of some sort. Such gradients might well be generated at the level of the brush-border membrane in the intact cell and results reported presently and elsewhere [6] reveal that electrochemical gradients (inside negative) generated by preloading vesicles with K_2SO_4 followed by their suspension in buffer containing valinomycin greatly enhanced uptake of Ca^{2+}.

The uptake of Ca^{2+} was inhibited to various degrees by the presence of other cations. Least inhibitory were the monovalent cations which over a concentration range of 0.5 mM to 2.5 mM had no marked effects either on equilibrium uptake or the rate of uptake. La^{3+} and Tb^{3+} which have ionic radii similar to that of Ca^{2+} had the greater inhibitory effects followed in order by Mn^{2+}, Ba^{2+} and Mg^{2+}. At lower concentration the divalent cations inhibited the equilibrium uptakes but did not decrease the rates of uptake, whereas at higher concentration (2.5 mM) both these parameters were affected. One could suggest from this that the interior binding sites have less affinity for Ca^{2+} than the sites used for the translocation of this cation. In the case of the lanthanide cations, both the rate of uptake and the equilibrium binding are affected even at the lower concentration. One possibility explaining this effect is that these cations bind avidly to the translocation sites and are not readily transported across the membrane. In this manner they block the entry of Ca^{2+} and thus affect primarily the rate of translocation and secondarily the extent of interior binding. In support of this possibility is the fact that Tb^{3+} cannot displace Ca^{2+} once it is bound internally whereas vesicles preincubated with Tb^{3+} are impaired in their ability to take up Ca^{2+}.

Results of the present study have partially characterized an energy-independent Ca^{2+} uptake process occurring in isolated brush-border membrane vesicles of rabbit small intestine. In the isolated membrane the process is a composite of two interlinked phenomena, i.e., a translocation of Ca^{2+} across the membrane and a subsequent apparently complete binding to its interior. Our experimental protocol was designed to eliminate uptake due to external binding and consequently the significance of this phenomenon in the overall uptake process remains unknown. The interior binding appears to involve high- and low-affinity sites as could be surmised from Scatchard plot analyses of our equilibrium uptake data [13] and in this, as well as in other aspects already mentioned, resembles the rat and the chick uptake processes [4,6]. It has been suggested on the basis of pH effects on binding that the low-affinity sites are constituted by phospholipids and this might well explain the decrease in equilibrium binding obtained with lidocaine [6]. Studies involved with the isolation of the binding and translocation elements from brush-border membranes and their reconstitution into liposomes would no doubt be very useful for the further characterization of this Ca^{2+} uptake process.

Acknowledgement

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