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Ricinoleate and Deoxycholate are Calcium Ionophores in Jejunal Brush Border Vesicles

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Summary. The intestinal secretagogues ricinoleate and deoxycholate have been tested for a capacity to form complexes with Ca^{2+} ions and to affect the passive equilibration of Ca^{2+} ions across the jejunal brush border membrane. Both of these agents formed butanol-soluble Ca^{2+} complexes in a model phase distribution system. They also promote the passive uptake and efflux of Ca^{2+} across brush border vesicles in a concentration-dependent manner. The levels of ricinoleate and deoxycholate that increase the rate of transvesicular Ca^{2+} movement are in the 100 to 300 μM range. Concentrations as high as 1.0 mM had no significant detergent effects in vesicles as measured by release of entrapped sorbitol. The kinetics of Ca^{2+} uptake and efflux are similar in brush border vesicles treated with A23187, ricinoleate, or deoxycholate. The influx rates observed in this study were high enough to cause the collapse of a Ca^{2+} gradient, which had been generated by Ca–Mg ATPase enzyme activity in the brush border membrane. Ricinoleate did not affect Ca–Mg ATPase activity at concentrations used in this study, but deoxycholate was inhibitory, indicating two potential modes for elevation of intracellular Ca^{2+} content by deoxycholate. When compared with the effects of the Ca^{2+} ionophore, A23187, it appears that both ricinoleate and deoxycholate could have significant intestinal secretory activity due to this Ca^{2+} ionophore property. It is also noteworthy that, at least in this model system, potential secretory effects are expressed at concentrations significantly below levels that have been associated with detergent effects or altered epithelial morphology.

Key Words intestinal secretion · calcium · calcium ionophore · deoxycholate · ricinoleate

Introduction

Intestinal secretory agents may act in different ways to induce a net accumulation of fluid and electrolytes in the intestinal lumen. The two most common secretory mechanisms are an increase in the permeability of the mucosal barrier to passive ion movement, and the triggering of an active ion secretory process. Agents that induce active secretion increase the mucosal cell levels of intracellular messenger compounds such as cyclic AMP, cyclic GMP or Ca^{2+} [13]. The process used to increase intracellular messenger levels, and the relative contribution of each messenger to the secretory process, is poorly understood for some secretagogues.

Ricinoleic acid can induce fluid and electrolyte accumulation in several species [3, 7, 10]. Increases in mucosal permeability may be an important factor in this fluid accumulation [11, 15]. In addition, Rascusen and Binder [28] have demonstrated that ricinoleate can induce active secretion of anions across the rat colon. This effect occurred along with an increase in mucosal cell cyclic AMP levels [28]; however, ricinoleate has been shown to be incapable of direct activation of adenylate cyclase [16]. Exposure of rat colon to ricinoleate causes the release of prostaglandin E, which may be responsible for increased cyclic AMP concentrations [5]. Calcium is required for the process of prostaglandin synthesis and release as a cofactor for phospholipase activity [29]. However, the mechanisms for production of prostaglandin E by ricinoleate and the contribution of this process to the induction of active secretion is not known.

Deoxycholate has similar functional groups to ricinoleate and similar concentration requirements for induction of fluid and electrolyte accumulation [5]. Deoxycholate has also been shown to increase mucosal cyclic AMP levels [7], but to be incapable of direct activation of adenylate cyclase [16]. The induction of secretion by deoxycholate does not correlate well with prostaglandin E release [5]. Deoxycholate has, however, been shown to act as a Ca^{2+} ionophore when incorporated into black lipid membranes [1].

The Ca^{2+} ionophore, A23187, is a potent stimulator of active ion secretion, acting independently of cyclic AMP concentration, but showing a dependence on extracellular Ca^{2+} ion [8, 14]. Increases in cytosol Ca^{2+} concentration can produce changes in ion transport identical to those induced by cyclic AMP [8, 14].

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The related secretory actions of ricinoleate and deoxycholate led us to test the effects of these two agents on Ca^{2+} movement across the intestinal brush border. The significance of any actions of the test compounds was evaluated by comparison to the effects of the reference Ca^{2+} ionophore A23187. The initial experiments made use of the phase distribution model of Pressman [27] to measure Ca^{2+} binding by the test compounds in a nonpolar phase. Ca^{2+} movement across biological membranes was evaluated by measuring the Ca^{2+} content of intestinal brush border vesicles formed from pig jejunal mucosal scrapings.

Materials and Methods

The phase distribution experiments involved an evaluation of the phase distribution activity of ricinoleic acid, deoxycholic acid, and A23187. One ml of a solution of the test compound in butanol was mixed with 1 ml of an aqueous solution (5 mM tris·HCl at pH 7.2 + 10 μM $CaCl_2$ + 1.0 nCi $^{45}Ca^{2+}$). The mixture was shaken for 2 min, which was found to be sufficient for equilibration to occur, centrifuged, and samples were taken from each of the resultant phases. The Ca^{2+} content of the samples was determined by liquid scintillation counting.

The second series of experiments involved a measure of passive Ca^{2+} uptake and efflux across brush border vesicles (BBV) prepared from the proximal jejunum of the weanling pig. The pigs were obtained from a local commercial source, were of mixed breeds, and averaged 15 kg in weight. The procedure used to form the vesicles represents an adaptation of several established procedures [19, 21, 24]. Fifty-cm segments of jejunum were surgically removed, cut open, and washed in ice-cold saline, and the mucosal layer was scraped off with a glass slide. These scrapings were placed in 30 ml of solution A (250 mM sorbitol, 5 mM tris·HCl at pH 7.4, 240 units/ml penicillin and 125 units/ml streptomycin) and frozen at −70 °C. On the day of use a sample of scrapings was thawed, homogenized using a Polytron homogenizer (Brinkman Instruments) for 4 pulses of 30 sec and then centrifuged at 4,500 × *g* for 10 min. The supernatant was kept on ice while the pellet was resuspended in 15 ml of solution A and centrifuged again for 10 min. The two supernatants were then combined and centrifuged at 45,000 × *g* for 1 hr. The resultant pellet was resuspended in 30 ml of solution A and homogenized in a motor driven glass Teflon homogenizer (1 stroke 1,000 rpm) to further separate fibrous from membranous material [21]. $MgCl_2$ was then added to this solution to give a final concentration of 10 mM. The solution was left on ice for 20 min to allow for complete precipitation of non-brush border components [24]. This precipitate was pelleted by centrifugation at 3,000 × *g* for 15 min. The supernatant was centrifuged at 27,000 × *g* for 30 min. The BBV pellet was resuspended in 5 ml of solution A and assayed for protein concentration [9]. This solution was then diluted to give the final concentration of BBV required for the particular experiment.

The purity and recovery of the brush border fraction was determined by measuring marker enzyme activities. Alkaline phosphatase and K^+-stimulated ATPase were assayed according to Parkinson et al. [26]. Cytochrome C oxidase was assayed following the procedure of Cooperstein and Lazarow [12]. NADPH cytochrome C reductase was assayed by the method of Sottocasa et al. [31].

The passive uptake of Ca^{2+} into the vesicles was initiated by mixing a required volume of BBV solution (0.80 mg BBV protein/ml) with an equal volume of solution B (solution A + 0.30 mM $CaCl_2$ + 0.50 μCi $^{45}Ca^{2+}$).

Duplicate samples were taken at the indicated times by transferring 500 μl of the mixture to a cellulose acetate filter (0.45 μm pore size) and washing with 3 × 3 ml volumes of ice-cold solution A. The filters were then placed directly in vials and the Ca^{2+} content determined by liquid scintillation counting. Calcium actually held by the vesicles was calculated by correcting for $^{45}Ca^{2+}$ cpm retained on the filters in the absence of vesicles.

The passive efflux of Ca^{2+} from BBV was studied by diluting a solution of Ca^{2+} preloaded vesicles with Ca^{2+}-free solution A and then sampling the mixture to monitor the decrease in vesicle Ca^{2+} levels. Vesicles were preloaded with Ca^{2+} by adding a $CaCl_2$ + $^{45}Ca^{2+}$ solution to give a final concentration of 10 mg BBV protein/ml + 0.150 mM $CaCl_2$ and then allowing Ca^{2+} to equilibrate during a 2-hr incubation at 0 °C. After the incubation, the vesicle solution was transferred to a 37 °C water bath and after 5 min aliquots were taken, diluted 25 × with solution A (37 °C) and samples were immediately taken as previously described to determine the 0 time vesicle Ca^{2+} content. The remaining vesicles were used to follow Ca^{2+} efflux in the presence and absence of test compounds. Effluxes were initiated by diluting a volume of BBV solution 25 × with solution A supplemented with control or test additions. Duplicate samples were then taken at the indicated times to monitor Ca^{2+} efflux from the vesicles.

Nonspecific leakiness of the BBV was studied by measuring the efflux of sorbitol during a 2-hr incubation period. Vesicles prepared in the presence of 250 mM sorbitol were resuspended in 2.0 ml of solution C (100 mM NaCl, 50 mM tris·HCl, pH 7.4) and passed through Sephadex G-25 to remove extravesicular sorbitol. Void volume material was assayed for protein and diluted to 0.40 mg BBV protein/ml with solution C. Samples were removed at zero time and after 2 hr of incubation at 37° with solution C or with solution C plus deoxycholate or ricinoleate. The sorbitol content in the suspending medium was determined and the total vesicular sorbitol content was assayed after ultrasonic disruption of the vesicles. Sorbitol concentration was measured by the reduction of NAD^+ in the presence of sorbitol dehydrogenase [32]. Increased absorbance at 340 nm was measured in an assay system containing 80 μg BBV protein, 5 units of sheep liver sorbitol dehydrogenase (Sigma Chemical Co.) and 0.7 μmol NAD^+ in 400 μl of solution C.

The effects of A23187, ricinoleate, and deoxycholate on the Ca^{2+} levels of BBV containing ATP was studied by adding ATP (final concentration 2 mM) to the mucosal scrapings prior to starting the vesicle preparation procedure. In this study the final BBV pellet was resuspended and diluted to 0.8 mg BBV protein/ml using solution A supplemented with 2.5 mM $MgCl_2$. Solution B (with added Ca^{2+}) was then mixed with an equal volume of BBV solution and the Ca^{2+} was allowed to equilibrate across the vesicles by incubating the mixture at 0° for 2 hr. After the incubation, 0° samples were taken for Ca^{2+} content determination as previously indicated. The solution was then transferred to a 37° bath and after 5 min samples were taken. The remaining BBV solution was subdivided and at 10 min from the start of the 37° incubation, control and test solutions were added. After a further 5 min incubation samples were taken to determine the effect of the additions on the Ca^{2+} content of BBV preloaded with ATP.

The effects of ricinoleate and deoxycholate on the Ca − Mg ATPase activity of the brush border was determined by measuring the disappearance of ATP. BBV were prepared and diluted using solution A to a concentration of 0.40 mg BBV protein/ml.

Test compounds (0.20 mM ricinoleate or 0.25 mM deoxycholate) were preincubated for 5 min at 37° with a vesicle suspension which was disrupted by ultrasonic treatment. ATP addition (to 2.0 mM) was followed by a further 15-min incubation. The reaction was stopped by mixing with an equal volume of an ice cold 12% trichloroacetic acid solution. Soluble ATP was then assayed using the Sigma ATP assay kit following the procedure of Adams [2]. ATPase activity was expressed as μmol ATP consumed/hr/mg protein. Ca−Mg dependent ATPase was calculated by subtracting basal ATPase activity from activity measured in the presence of 1.25 mM $MgCl_2$ and 0.150 mM $CaCl_2$.

Calcium ionophore A23187 was purchased from Calbiochem (LaJolla, Calif.). Ricinoleate and deoxycholate were purchased from the Sigma Chemical Co. and were judged as 99% pure as indicated by the supplier.

Results

The Ca^{2+} ionophore A23187, ricinoleic acid, and deoxycholic acid all had substantial Ca^{2+} phase distribution activity in the water-butanol system used in this study. In the absence of any test compound, very little Ca^{2+} could be detected in the butanol phase. When 0.20 mM A23187 was incorporated into the system, a 21.4% transfer of Ca^{2+} to the butanol phase resulted. At a concentration of 5.0 mM, ricinoleate and deoxycholate are known to promote intestinal fluid accumulation [5], and this concentration was chosen to test for phase distribution activity. Both compounds had substantial activity with ricinoleate promoting 14.9% Ca^{2+} transfer to butanol and deoxycholate causing 11.7% Ca^{2+} transfer (Fig. 1). These indications of Ca^{2+} complexing by ricinoleate and deoxycholate were pursued by extending the study to examine the effects of these Ca^{2+} complexes on passive Ca^{2+} movement across pig jejunal brush border membranes.

The purity of the brush border preparation was determined by marker enzyme assays on the initial homogenate and final BBV solution. Using alkaline phosphatase as the marker for brush border membrane, a 32% recovery and a 15-fold increase in specific activity was obtained. These results are comparable to those obtained by Kessler et al. [24] and indicate a higher degree of purification than obtained by Hearn and Russell [11]. There is little contamination by mitochondria or microsomes as determined by cytochrome C oxidase (0.3% recovery) and NADPH cytochrome C reductase (0.7% recovery) assays. As reported by other workers [24], some contamination by basal lateral membrane was present in the final preparation as indicated by the 5.8% recovery and 3.8-fold increase in specific activity obtained for the assay of $Na^+ - K^+$-stimulated ATPase activity.

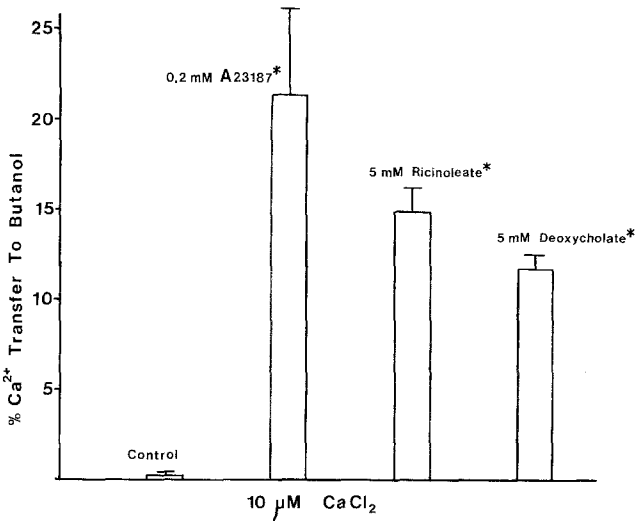

Fig. 1. Ca^{2+} phase distribution activities of A23187, Na-ricinoleate, Na-deoxycholate. The test compounds, dissolved in methanol, (methanol alone in the control) were added to butanol to give the indicated concentrations. Each bar indicates the mean and SD obtained for 10 distributions with 10 μM $^{45}CaCl_2$ (1.0 nCi per assay) originally present in the aqueous solution. * Indicates a significant difference compared to the control distributions ($P<0.5$)

These isolated BBV were used to measure the effects of A23187, ricinoleate, and deoxycholate on passive Ca^{2+} uptake into BBV. Figure 2 indicates the results obtained from this experiment. In the control state it took the full 60 min to reach equilibrated Ca^{2+} concentrations and the time required to reach one half of the equilibrated Ca^{2+} level ($t\frac{1}{2}$) was 7.2 min. In the presence of 20 μM A23187, Ca^{2+} uptake was markedly accelerated, reaching completion at 20 min and $t\frac{1}{2}$ value of 0.6 min. At a concentration of 0.20 mM ricinoleate also facilitated Ca^{2+} entry into the vesicles. In this case the equilibration process was completed between 30 and 40 min and the $t\frac{1}{2}$ was 1.5 min. Deoxycholate at a concentration of 0.25 mM enhanced Ca^{2+} uptake as indicated by completion of the uptake within 40 min and the $t\frac{1}{2}$ of 1.3 min.

The process of Ca^{2+} efflux from BBV is slower than that of Ca^{2+} uptake (Fig. 3). In the control state it took a full 90 min for the efflux to be completed and 18 min for the vesicle Ca^{2+} content to drop by 50% ($t\frac{1}{2}$) toward the final 90-min level. As was the case for Ca^{2+} uptakes, all three test agents facilitated passive Ca^{2+} movement down the concentration gradient as indicated by the lower $t\frac{1}{2}$ values and time periods required for the efflux to reach completion.

The effects of various concentrations of ricinoleate and deoxycholate on the Ca^{2+} content of BBV at 2 and 60 min from the initiation of Ca^{2+}

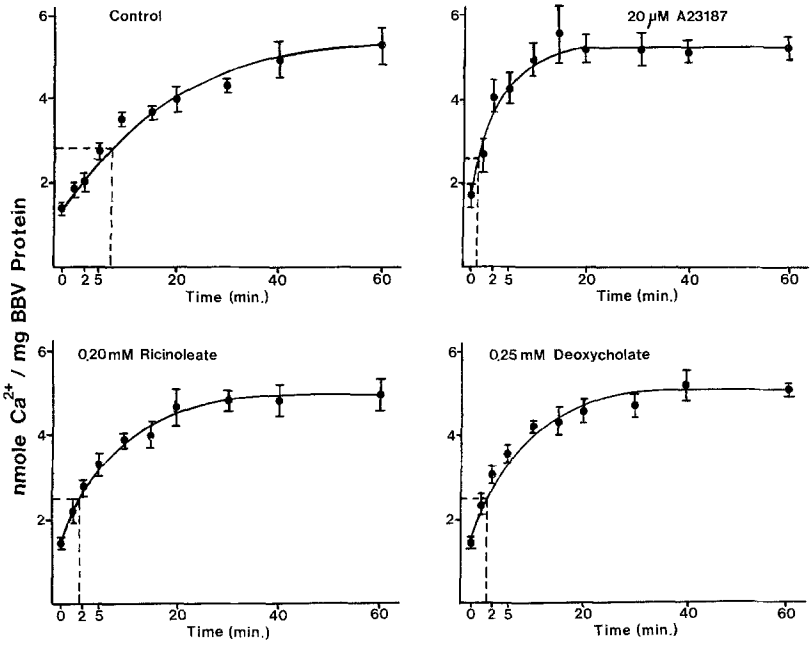

Fig. 2. Effect of A23187, ricinoleate, and deoxycholate on the passive uptake of Ca^{2+} across BBV. A23187 (20 μM), Na-ricinoleate (200 μM), and Na-deoxycholate (250 μM) were all added from concentrated solutions in methanol 5 min prior to initiation of the uptake to give the indicated final concentrations. Equal amounts of methanol were added to the control and test solutions used for each uptake. All uptakes were performed in triplicate with duplicate samples at each of the indicated time, and the points indicate the mean and SD obtained. At less than 2 min the time scale on each graph has been extended to accommodate the data. The hatched line indicates the estimated time at which the vesicle Ca^{2+} content was 50% of the final equilibrated Ca^{2+} content

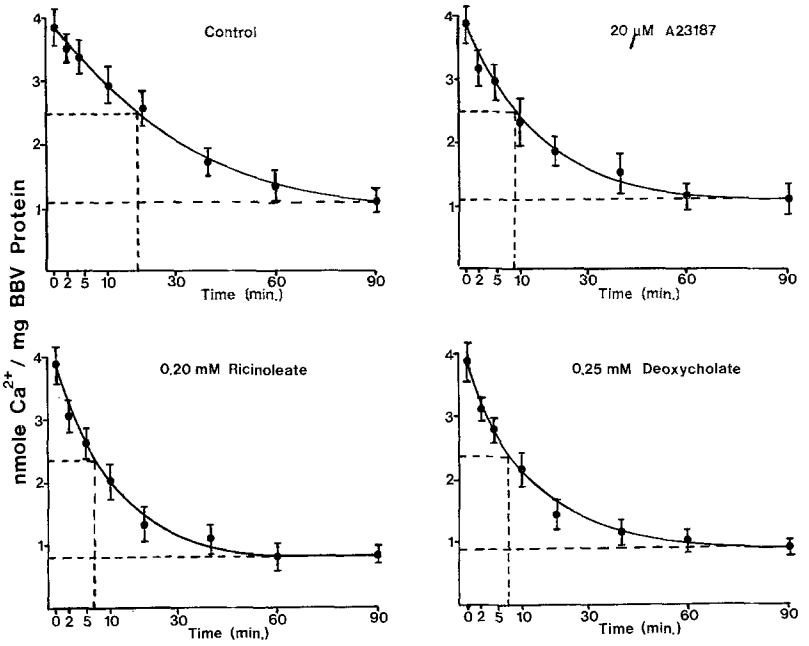

Fig. 3. Effect of A23187, ricinoleate, and deoxycholate on the passive efflux of Ca^{2+} across BBV. Effluxes were initiated by diluting a solution of Ca^{2+} preloaded vesicles 25 × with solution A supplemented with test or control additions. For each condition three separate effluxes were performed with duplicate samples taken at the indicated times and the points represent the mean and SD obtained. The verticle hatched line indicates the estimated times at which the vesicle Ca^{2+} content had decreased by 50% toward the final equilibrated level

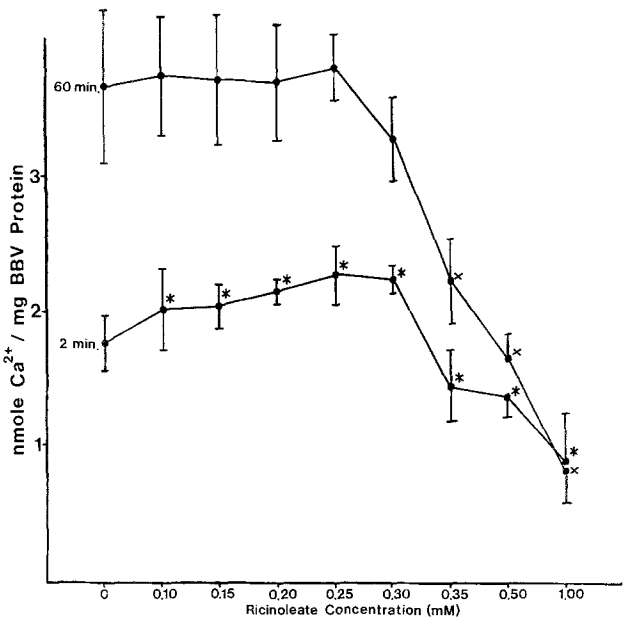

Fig. 4. Effect of ricinoleate concentration on passive Ca^{2+} uptake by vesicles at 2 and 60 min. Ricinoleate was added to the BBV suspension 5 min before the initiation of Ca^{2+} uptake. (*See* text for experimental conditions.) Points represent mean ± SD for duplicate determinations on three separate replicates of the experiment. Significant differences ($P<0.05$) from the corresponding zero concentration uptakes are indicated by * for the 2 min value and X for the 60-min value

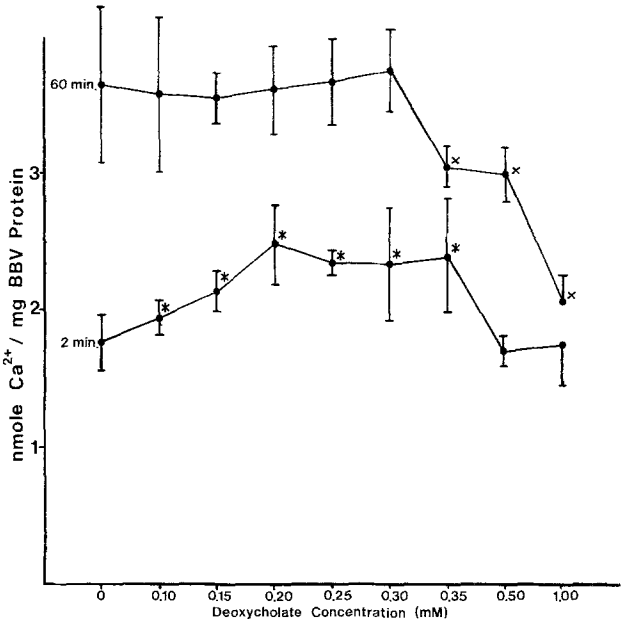

Fig. 5. Effect of deoxycholate concentration on passive Ca^{2+} uptake by vesicles at 2 and 60 min. The experimental detail and representation of data are the same as described in Fig. 4

uptake are indicated in Figs. 4 and 5. At a concentration of 0.10 mM both ricinoleate and deoxycholate significantly increased the 2 min Ca^{2+} content of the vesicles when compared to controls. Increasing the concentration of ricinoleate and deoxycholate to 0.20 mM resulted in a further elevation of the 2-min Ca^{2+} content. Further increases in the concentration of test compound up to 0.30 mM did not cause any additional change in the 2-min BBV Ca^{2+} levels; however, when the concentration of ricinoleate was increased to 0.50 and 1.0 mM, the 2 min Ca^{2+} content was significantly less than controls. In the case of deoxycholate at 0.5 and 1.0 mM, the 2-min Ca^{2+} content was found to be similar to controls. In the concentration range from 0.10 to 0.30 mM, neither ricinoleate nor deoxycholate had any significant effects on the final, 60-min, equilibrated Ca^{2+} content of the vesicles. At the higher concentrations of 0.5 and 1.0 mM, both compounds caused substantial decreases in the 60-min Ca^{2+} levels when compared to controls. In this case the effect of ricinoleate on decreasing BBV Ca^{2+} was found to be greater than that of deoxycholate.

The effect of ricinoleate and deoxycholate on BBV integrity was measured by assaying sorbitol leakage during a 2-hr incubation period with these agents. Initially a 0 time control and a sonicated sample of BBV were assayed for free sorbitol. Sonication of the BBV solution prior to assay increased the free sorbitol concentration from 1.2 ± 0.1 mM (control 0 time) to 2.6 ± 0.1 mM (sonicated), indicating that 54% of the total sorbitol was contained within the vesicles. The release of entrapped sorbitol was not increased above control values in BBV incubated for 2 hr with 1 mM ricinoleate or deoxycholate (Fig. 6).

Steady-state Ca^{2+} concentrations in cells must depend on the relative rates of Ca^{2+} influx and efflux. We measured the effects of the influx rates caused by our test compounds on the steady-state Ca^{2+} concentration by a combined treatment with test compounds and a supply of ATP to power the brush border Ca−Mg ATPase. This ATPase should control the normal Ca^{2+} efflux, at least from the luminal side of the cell.

The effect of preincorporation of ATP and subsequent addition of the test compounds on the equilibrated Ca^{2+} levels of BBV is indicated in Fig. 7. After allowing the Ca^{2+} to equilibrate across the vesicles via preincubation in ice, the Ca^{2+} content of the BBV containing ATP was found to be less than that of control vesicles (*compare* 0 time value in *A* with 0 time value for *B*, *C*, *D*). When the vesicle incubation temperature was increased to 37 °C, the Ca^{2+} content dropped in both control and ATP-containing vesicles. The

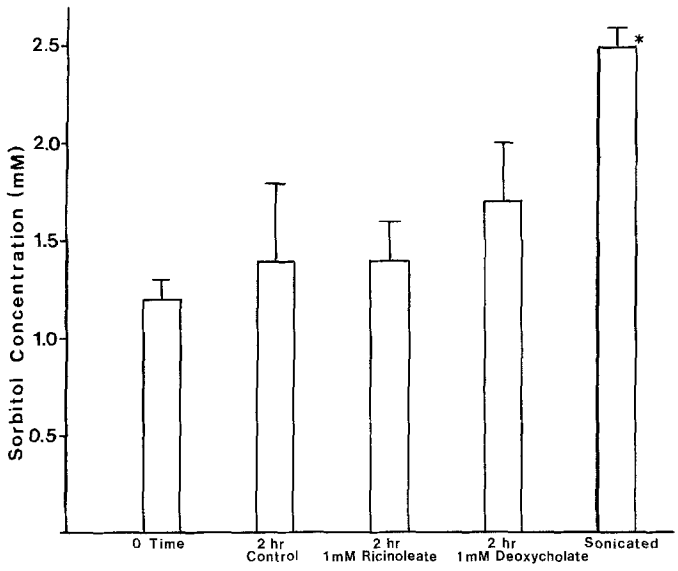

Fig. 6. Effect of ricinoleate and deoxycholate on sorbitol efflux from vesicles. BBV prepared with 250 mM sorbitol were washed on Sephadex G-25 to remove excess external sorbitol, suspended in iso-osmotic NaCl-Tris HCl, pH 7.5, and incubated for 2 hr at 37° with the indicated additions. Sorbitol concentrations correspond to the sorbitol associated with 80 μg BBV protein suspended in 200 μl of the iso-osmotic NaCl-Tris HCl. The total sorbitol content available for exchange was measured after ultrasonic disruption of vesicles. Mean ± SD from triplicate assays with significant differences ($P<0.05$) from control incubation represented as*

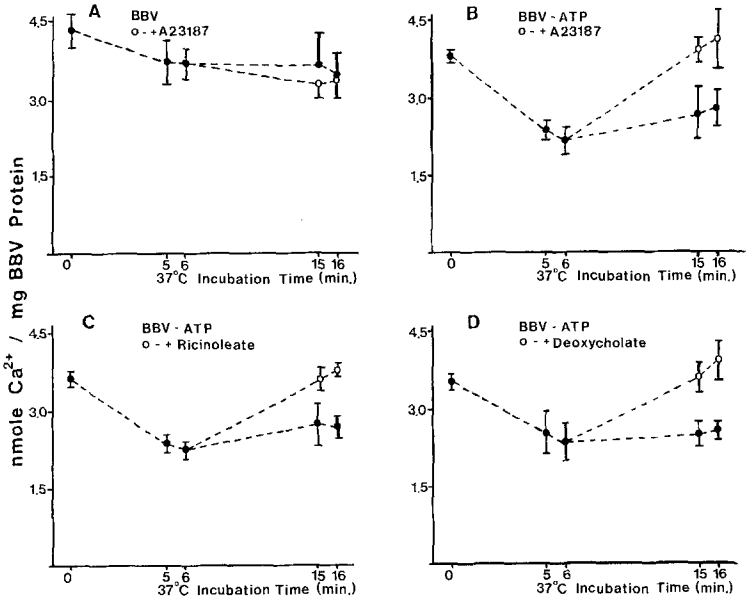

Fig. 7. Steady-state BBV Ca^{2+} content in the presence of ATP and ionophores. Control vesicles or vesicles loaded with 2.0 mM ATP during preparation were equilibrated with 0.30 mM $CaCl_2$ plus $^{45}Ca^{2+}$ by incubating for 2 hr at 0°. The incubation medium also contained 2.5 mM $MgCl_2$. Zero time levels of equilibrated Ca^{2+} were measured in the 0° preparations before warming to 37°. Sampling at 5 and 6 min indicates the Ca^{2+} remaining after Ca−Mg ATPase is activated by heating. Addition of ionophores at 10 min followed by sampling at 15 and 16 min shows the steady-state Ca^{2+} concentration resulting from ATPase catalyzed efflux and ionophore facilitated entry. Vesicles in panel A were prepared without ATP. Points represent mean ± SD for duplicate determinations from three replicates of each condition

drop in Ca^{2+} in the ATP vesicles averaged 1.21 nmol Ca^{2+}/mg protein and in each case was significantly greater ($P<0.05$) than the 0.59 nmol Ca^{2+}/mg protein drop occurring in the control vesicles. When a methanol solution of A23187 was added to a fraction of the control vesicles at 37 °C there was no significant difference in the BBV Ca^{2+} content when compared to a second vesicle fraction with only methanol added (Fig. 7*A*). In comparison when A23187 was added to BBV containing ATP the Ca^{2+} content of the vesicles increased up to the 0 °C equilibrated level, with only a slight increase occurring in the vesicle fraction with only solvent additions (Fig. 7*B*). The effects of ricinoleate and deoxycholate on BBV containing ATP were found to be similar to the effects of A23187 (Fig. 7*C* and *D*). At concentrations of the test compound known to facilitate passive Ca^{2+} movement across the vesicles, both agents acted to increase BBV Ca^{2+} to initial 0 °C levels. In comparison, BBV maintained at 37 °C for the same period of time with control additions displayed only a slight elevation in Ca^{2+} content.

The effect of ricinoleate and deoxycholate on the Ca−Mg dependent ATPase activity of the brush border is shown in Table 1. Neither compound affected the capacity of the assay system to measure ATP as indicated by the ATP concen-

Table 1. The effect of ricinoleate and deoxycholate on brush border Ca – Mg ATPase activity

Additions	ATP standard without BBV[a] (μmol ATP/80 μl)	Ca^{2+}-dependent ATP disappearance[b] (μmol/hr/mg protein)
Control	0.14 ± 0.003	1.9 ± 0.16
0.2 mM ricinoleate	0.14 ± 0.006	1.9 ± 0.18
0.25 mM deoxycholate	0.14 ± 0.007	0.53 ± 0.17[c]

[a] Ricinoleate or deoxycholate added to 2.0 mM ATP solution to determine effect of these compounds on enzymatic ATP assay [2].

[b] Disappearance of 2.0 mM ATP from incubation systems containing 33 μg protein from BBV treated with ultrasonic radiation. Means and standard deviations from four independent assays.

[c] Significantly different from control ($P < 0.05$).

trations found in the absence of brush border. At a concentration of 0.25 mM, ricinoleate was found to have no effect on brush border Ca – Mg ATPase. In contrast, 0.25 mM deoxycholate had a marked inhibitory effect on the enzyme as indicated by the 73% decrease in activity.

Discussion

The finding that ricinoleate, deoxycholate, and A23187 have the capacity to bind Ca^{2+} in the butanol phase of a water-butanol phase distribution system supports the hypothesis that ricinoleate and deoxycholate have the potential to act as membrane Ca^{2+} ionophores. All three compounds displayed a high affinity for Ca^{2+} as indicated by the 10 μM $CaCl_2$ concentration used in the experiment, and thus the compounds could act as ionophores even at low $CaCl_2$ concentrations.

Actual Ca^{2+} ionophore activity of ricinoleate and deoxycholate was indicated by their capacity to accelerate the passive uptake and efflux of Ca^{2+} across intestinal BBV. Other studies have indicated that a significant amount of the total Ca^{2+} taken up by BBV is bound to the surface of the vesicles [33]. In this study the effect of ricinoleate and deoxycholate on elevating the Ca^{2+} content of the vesicles after the initiation of Ca^{2+} uptake was not a result of increased surface binding, since there was no difference in the final equilibrated Ca^{2+} content of control and test vesicles. In addition the rapid efflux of Ca^{2+} from preloaded vesicles in the presence of the test compounds could not occur if the agents acted only to sequester Ca^{2+} to the vesicles. Thus the increase in initial rates of Ca^{2+} uptake and efflux is likely a result of facilitated transport of Ca^{2+} down a concentration gradient across the vesicles.

The finding that 1.0 mM ricinoleate or deoxycholate had no effect on sorbitol leakage from BBV indicates that the compounds did not disrupt vesicle integrity under the conditions used in this study. In agreement, Helenius et al. [20] have shown that breakdown of membrane protein-phospholipid associations does not occur at deoxycholate concentrations less than 1.5 mM. In this study the drop in the equilibrated Ca^{2+} content of the vesicles occurring in the presence of 0.5 and 1.0 mM ricinoleate or deoxycholate may result from a complexing effect of the compounds which would make Ca^{2+} unavailable for uptake. The 60-min Ca^{2+} content of the vesicles was less with 1.0 mM ricinoleate than in the presence of 1.0 mM deoxycholate. This data could reflect a higher Ca^{2+} binding capacity of ricinoleate. In support of this hypothesis, the Ca^{2+} phase distribution activity of ricinoleate was found to be greater than that of deoxycholate.

The Ca^{2+} ionophore A23187, ricinoleate, and deoxycholate all interfere with the process by which ATP acts to lower BBV Ca^{2+} levels. This and other studies [17, 25] have demonstrated that Ca – Mg ATPase activity is present in the intestinal brush border. In addition, Hearn and Russell [19] were able to demonstrate Ca^{2+} pumping out of BBV in the presence of ATP. In our study ATP preincorporation into the vesicles also resulted in a distinct reduction in the equilibrated Ca^{2+} levels. Subsequent addition of A23187 resulted in a substantial increase in Ca^{2+} levels. Since addition of A23187 to BBV with no ATP did not effect equilibrated Ca^{2+} levels, the effect of A23187 on ATP-containing vesicles was most likely due to a collapse in the ATP-generated Ca^{2+} gradient, rather than Ca^{2+} sequestration by the ionophore. Ricinoleate and deoxycholate at concentrations previously established to facilitate passive Ca^{2+} transport also acted to elevate vesicular Ca^{2+} concentration in the presence of ATP. This effect could be a result of a collapse in the Ca^{2+} gradient due to ionophore activity or a direct inhibition of Ca^{2+} pump activity. Ricinoleate did not effect Ca – Mg ATPase activity under the conditions of this study, and thus its effects may be due solely to ionophore activity. In contrast, deoxycholate was found to markedly inhibit Ca – Mg ATPase activity. Deoxycholate, at a concentration range of 0.1 to 1.0 mM, has been shown to either stimulate or inhibit the activity of Ca-ATPase, depending on the particular set of conditions used [25]. The inhibitory effect of deoxycholate seen in this study indicates that

the elevation in Ca^{2+} levels of ATP-containing vesicles could be due to an inhibition of Ca^{2+} pumping, an ionophore activity dependent collapse in the Ca^{2+} gradient or a combination of these two mechanisms.

The mechanism by which bile acids and fatty acids induce intestinal fluid accumulation appears to be relatively complex. Both ricinoleate and deoxycholate act to increase mucosal permeability [15, 18, 30]. However, there is evidence that an increase in mucosal permeability is not the only process involved in fluid and electrolyte accumulation. Administration of propranolol or indomethacin has been shown in a number of studies to inhibit the fluid and electrolyte accumulation induced by ricinoleate or deoxycholate [13, 14]. There is also direct evidence that ricinoleate and dihydroxy bile acids can inhibit or reverse the absorption of Na^+ and Cl^- by mechanisms independent of changes in mucosal permeability [7, 28]. These observations have lead to the recent speculation of Binder [6], that ricinoleate and dihydroxy bile acids act to induce an active anion secretion simultaneous to the increase in mucosal permeability, and that it is the active secretory process which is primarily responsible for the net fluid and electrolyte accumulation. In addition, Kelly et al. [23] have demonstrated that ricinoleate can induce secretion in the absence of hormonal or nervous stimulation, indicating a local mechanism for the secretory effect of the compound.

This induction of active ion secretion is likely dependent on an increase in mucosal cell levels of a second messenger such as Ca^{2+} or cAMP. Ricinoleate and deoxycholate have been shown to be associated with an increase in cAMP levels [7, 28], but neither compound is capable of direct activation of adenyl cyclase [16]. Ricinoleate can stimulate the release of prostaglandin E from the intestine, and this is thought to account for part of the secretory process [4, 5]. Furthermore, ricinoleate has been shown to stimulate the release of arachidonic acid and its metabolites from perfused rabbit vascular tissue in a Ca^{2+}-dependent manner analogous to the effects of A23187 in the same system [22]. The current study also indicates that at low concentrations ricinoleate and deoxycholate act as Ca^{2+} ionophores in the brush border membrane of pig jejunal epithelial cells and that deoxycholate can inhibit the activity of the Ca^{2+} pump. These effects could elevate mucosal cell Ca^{2+} levels which could then lead to the other observed effects of these compounds such as prostaglandin release, cAMP accumulation, and the activation of a secretory process.

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