Sodium Caprate Elicits Dilatations in Human Intestinal Tight Junctions and Enhances Drug Absorption by the Paracellular Route

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The effects of the absorption enhancer sodium caprate on human intestinal epithelial cells were investigated using Caco-2 cell monolayers. The effects on epithelial integrity and drug transport are dependent on time and concentration and are decreased by Ca^{2+}, most likely through the formation of Ca^{2+} soaps. Morphological data indicate that exposure to sodium caprate results in cytoskeletal changes and in structural alterations of the tight junctions in the form of dilatations, while the effects on the apical cell membranes are limited. We conclude that sodium caprate increases the absorption of drugs mainly by the paracellular route.

KEY WORDS: absorption enhancer; tight junction; drug absorption; intestinal epithelium; Caco-2; fatty acid; sodium caprate.

INTRODUCTION

Passive absorption of drugs and peptides across the intestinal epithelium involves two pathways—transcellular and paracellular. Absorption via these pathways is selective: only lipophilic compounds partition into the membrane lipids of the epithelial cells to be absorbed by the transcellular route (1). Absorption of hydrophilic drugs is therefore restricted to the paracellular pathway (2). However, the paracellular pathway occupies <0.1% of the total surface area of the intestinal epithelium (3). This, together with restricted permeation through the tight junctions, often limits the paracellular absorption of hydrophilic drugs and peptides (4).

Consequently, various compounds that increase the permeability of the intestinal epithelium have been used to facilitate the absorption of incompletely absorbed drugs and peptides (5). Many natural and synthetic surfactants have been investigated. The mechanisms of action of most of these compounds are relatively nonspecific and seem to involve both the transcellular and the paracellular pathways (6). So far, most studies have focused on the membrane solubilization properties of the surfactants. As a result, it is generally assumed that surfactants increase the permeability of epithelia via the transcellular route. However, in some studies, clear effects on the tight junctions have also been observed (e.g., Ref. 7).

A particularly interesting absorption enhancer is the sodium salt of the saturated fatty acid decanoic acid (sodium caprate; C10), which is used in Japan to enhance the absorption of drugs in humans (8). C10 is relatively well studied in comparison to many other absorption enhancers and has been suggested to enhance absorption by paracellular as well as transcellular pathways (9,10). Studies published during the course of this investigation suggest that C10 increases the permeability of the rat colon mainly by the paracellular pathway (11).

We recently developed a cell culture model for studying the effects of pharmaceutical additives and absorption enhancers on the human intestinal epithelium (7,12). The model, which is based on monolayers of the human intestinal epithelial Caco-2 cell line, has been used to study the absorption enhancing effects of common wetting agents used in solid oral dosage forms (12). In this study, investigation of surfactants has been extended to the absorption enhancing agent C10. The results show that C10 has a more specific mechanism of action than the wetting agents and that it reversibly enhances the absorption of drugs mainly via the paracellular pathway.

MATERIALS AND METHODS

Materials

^{14}C-Mannitol (MW 182; sp radioact, 271 mCi/g) and ^{3}H-mannitol (MW 182; sp radioact, 165 Ci/g) were obtained from New England Nuclear, Boston, MA, through Du Pont Scandinavia AB, Kista. The sodium salt of capric acid (99–100% purity) was obtained from Sigma Chemical Co, St. Louis, MO. Potassium fenoximethylpenicillin (penicillin V; PcV; MW 388) was a gift from Astra Läkemedel AB, Södertälje, Sweden. Propidium iodide and rhodamine phalloidin were purchased from Molecular Probes Inc., Eugene, OR.

Cells

Caco-2 cells originating from a human colorectal carcinoma (13) were obtained from the American Type Culture Collection, Rockville, MD. The cells were cultivated on polycarbonate filters for transport experiments (Transwell cell culture inserts with a mean pore diameter of 0.45 μm; Costar, Badhoevedorp, The Netherlands) and on polyethyleneterephtalate filters (Falcon cell culture inserts with a mean pore diameter of 0.45 μm; Becton Dickinson, Oxford, England) for fluorescence microscopy: 0.4–0.6 × 10^6 cells/cm^2 were seeded onto filters and allowed to grow and differentiate for 21–35 days. The cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1% nonessential amino acids, and benzylpenicillin (10 IU/mL) and streptomycin (10 μg/mL) as described previously (14). Cells of passages 90–100 were used.

Determination of the Critical Micelle Concentration (CMC)

A DuNouy interfacial tensiometer (A. Kruss Optische-Mechanische Werkstätten, Hamburg, Germany) was used to determine the critical micelle concentration (CMC) of C10 in Hank's balanced salt solution containing 25 m*M* *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid buffer (HBSS) without Ca^{2+}. Measurements were performed at room temperature, 22 ± 1°C. The CMC value, obtained from

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a plot of surface tension versus surfactant concentration, was 13 m*M*.

Osmolality

Osmolalities were measured with a 5500 Vapor Pressure Osmometer (Wescor Inc., Logan, UT).

Transport Studies

The transport of ^{14}C- or ^{3}H-mannitol and PcV across Caco-2 cell monolayers was studied as described previously (14). The transport studies were performed in air at 37°C and 95% relative humidity in HBSS. Solutions were added to the donor chamber and samples were taken at regular time intervals from the basolateral side. The apparent permeability coefficient (P_{app}) was determined according to the following equation:

$$P_{app} = dQ/dt\ 1/AC_0$$

where dQ/dt is the permeability rate (steady state flux, mol/sec), C_0 is the initial concentration in the donor chamber (mol/mL), and A is the surface area of the membrane (cm^2).

Absorption Enhancement. The effects of C10 (10–24 m*M*) on the transport rate of ^{3}H- and ^{14}C-mannitol (0.20–24 μ*M*) and PcV (10 m*M*) were studied for 60 min. In general, HBSS without Ca^{2+} was used in the apical chamber, while the basolateral chamber always contained Ca^{2+}. The osmolalities of the C10 solutions never exceeded 350 mOsm. Control experiments with HBSS to which mannitol had been added to obtain an osmolality of 350 mOsm showed that this slightly hyperosmotic solution did not affect the integrity of the cell monolayers. The transport of PcV across the monolayers was found to be a passive process (not shown).

Recovery Experiments. Initially, monolayers were exposed to HBSS for 20 min. The medium was then changed to HBSS containing 10 m*M* C10 and ^{14}C-mannitol and the cells were incubated for another 20 min. Finally, the C10 solution was replaced by Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1% nonessential amino acids, and ^{14}C-mannitol. This complete medium was needed in order to obtain complete recovery of the monolayers. The transport rates for ^{14}C-mannitol were measured as described above. Similar experiments, excluding C10, were used as controls. The absorption enhancement was quantified by calculating the area under the curve (AUC) with the trapezoidal rule:

$$\text{AUC} = P_{app}t$$

where AUC is the fraction absorbed of the applied dose (cm), P_{app} is the apparent permeability coefficient (cm sec^{-1}), and t is the time (sec).

Transmission Electron Microscopy

Glutaraldehyde-fixed specimens were immersed consecutively in 1% osmium tetroxide and 1% uranyl acetate, dehydrated, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 420 electron microscope operated at 60 kV.

Transepithelial Electrical Resistance Measurements

The transepithelial electrical resistance (TEER) was measured, as described previously (15). The resistance of the control monolayers was 385 ± 19 Ω cm^2 (n = 4).

Fluorescence Microscopy

Membrane Permeability. The intercalating dye, propidium iodide (PI), was used to discern cells with damaged membranes (16). Propidium iodide does not permeate intact cell membranes. The monolayers were rinsed twice with PBS and incubated with 30 μg/mL propidium iodide in phosphate-buffered saline (PBS) for exactly 3 min. They were rinsed twice with PBS and fixed for 10 min in 4% formaldehyde in PBS on ice before being rinsed again in PBS (four times). Finally, the filters were mounted on glass slides in a 1:1 solution of PBS and glycerol.

Actin. F-actin was stained with rhodamine-labeled phalloidin according to the manufacturers' specification. Ten microliters of a stock solution (200 U/mL rhodamine phalloidin in methanol) was evaporated under nitrogen gas. The residue was redissolved in 400 μL PBS. The monolayers were rinsed three times with PBS, fixed for 10 min in 4% formaldehyde in PBS on ice, rinsed with PBS three times, and extracted over 5 min with 1% Triton X-100 on ice. They were then rinsed with PBS twice, air-dried, and stained with rhodamine phalloidin for 20 min in the dark. The monolayers were again rinsed with PBS three times and the filters were then mounted on glass slides as described above.

All preparations were examined with a fluorescence microscope (Zeiss Axioskop, Oberkochen, Germany) fitted with a ×40 objective for oil immersion. The photographs were taken with a microscope camera (MC 100, Zeiss, Oberkochen, Germany) and Kodak T-Max 400 film.

Sample Analysis

The radioactive markers were analyzed in a liquid scintillator and PcV was analyzed by an HPLC method at ASTRA Läkemedel AB, Södertälje, Sweden.

Statistics

All figures are expressed as means ± SD. The AUCs and the effects of calcium were analyzed using the unpaired, two-tailed Students t test. The concentration- and time-dependent effects on mannitol and PcV transport were analyzed using the one-factor ANOVA.

RESULTS

Effects of Ca^{2+}

Initial experiments showed that the addition of C10 to the buffer used in the transport experiments resulted in precipitation. No precipitation occurred after omitting Ca^{2+} from the buffer (not shown). Therefore, the effect of Ca^{2+} on the absorption enhancing effect of C10 was investigated. The depletion of apical Ca^{2+} did not affect the integrity of the cell monolayers. The P_{app} values for ^{3}H-mannitol in the presence and absence of apical Ca^{2+} were 3.67 ± 1.07 × 10^{-7} and 3.06 ± 0.47 × 10^{-7} cm/sec, respectively (P >

0.05). The enhancement of ^{3}H-mannitol transport by C10 was concentration dependent in both the presence and absence of apical Ca^{2+} (Fig. 1, inset). However, the absorption enhancing effect was higher when Ca^{2+} was excluded from the apical medium. This finding, together with the potential difficulties in controlling the Ca^{2+}-mediated precipitation of C10, motivated the exclusion of Ca^{2+} from the apical medium in further studies.

Time- and Concentration-Dependent Effects

Exposure to different concentrations of C10 for 20, 40, and 60 min revealed that the transport of mannitol was dependent on concentration and time (Fig. 1). A clear concentration dependence was observed at 10–24 m*M*. The time dependence was studied by determining P_{app} values at consecutive time intervals during the exposure to C10. At C10 concentrations of 10 m*M* the time-dependent effect was small and nonsignificant, but at 13 m*M* and higher concentrations significant time dependent effects were observed ($P < 0.05$). Thus, after the first 20 min of exposure to 13 m*M* C10, the P_{app} value was $9.22 \pm 5.55 \times 10^{-7}$ cm/sec, or four times higher than in control monolayers exposed to buffer only ($P_{app} = 2.36 \pm 0.54 \times 10^{-7}$ cm/sec), while the corresponding P_{app} value after the last 20 min of exposure was $3.82 \pm 1.37 \times 10^{-6}$ cm/sec, or 15 times higher than the control ($P_{app} = 2.62 \pm 0.20 \times 10^{-7}$ cm/sec). Experiments with PcV as a hydrophilic marker drug gave similar results (Fig. 2).

The effect of C10 (13 m*M*) on the structure of the tight junctions was markedly dependent on time (Fig. 3). After a 20-min exposure, less than 5% of the tight junctions were slightly dilated (Fig. 3b). After a 40-min exposure, approx. 10% of the tight junctions were dilated, and the dilatations were more pronounced than after the shorter exposure time (Fig. 3c). After exposure for 1 hr, more than one-third of the tight junctions displayed large dilatations (Fig. 3d). The junctional structure was, however, partly preserved, with the cell membranes of adjacent cells remaining in close association at the most apical and basal parts of the junctions.

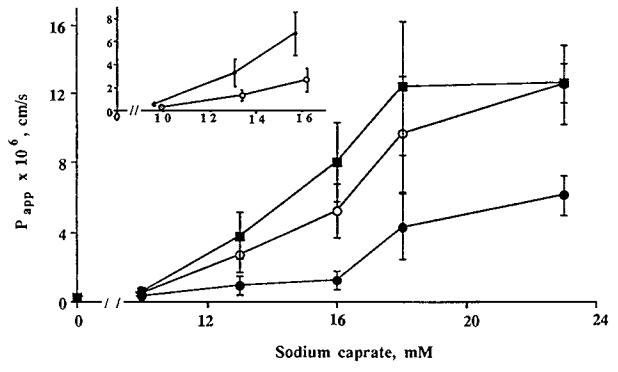


Fig. 1. Concentration- and time-dependent effects of sodium caprate on the transport of ^{3}H-mannitol. The graphs represent P_{app} values determined during different time intervals after the addition of sodium caprate: (●) 0–20 min, (○) 20–40 min, and (■) 40–60 min, respectively. Mean values ± SD ($n = 3$). The inset shows the concentration-dependent effects of sodium caprate on the transport of ^{3}H-mannitol in the presence (○) and absence (●) of Ca^{2+}. The P_{app} values are mean values ± SD from 60-min experiments ($n = 3$).

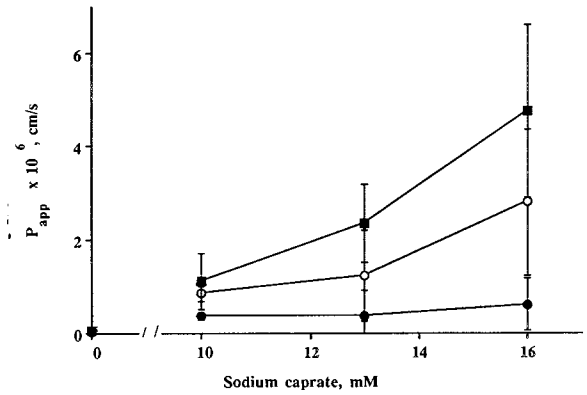


Fig. 2. Concentration- and time-dependent effects of sodium caprate on the transport of PcV. The graphs represent P_{app} values determined during different time intervals after the addition of sodium caprate: (●) 0–20 min, (○) 20–40 min, and (■) 40–60 min, respectively. Mean values ± SD ($n = 3$).

Moreover, the adherence junctions were unaffected by the C10 exposure. Similar dilatations were found in the presence of Ca^{2+} in the apical medium (Fig. 3e). The morphology of the apical cell membranes after exposure to C10 was generally indistinguishable from control cells (not shown).

The effect of C10 on TEER was immediate (Fig. 4). Exposure to 10, 13, and 16 m*M* C10 for 1 min reduced the resistance to approximately 75, 50, and 30% of the initial value, respectively. At the lowest concentration (10 m*M*) of C10, no further decrease with time could be observed after the first min. After exposure to 13 and 16 m*M* C10, the initial rapid decrease was followed by a second phase during which the resistance continued to decrease slowly. However, no further decrease in the resistance was observed after the initial 10 min. Thus, the resistance values after exposure to C10 for 10 and 60 min were comparable (data not shown).

Exposure to C10 also resulted in an increase in the permeability of the apical cell membrane, as detected with the fluorescent probe propidium iodide (Fig. 5). The untreated control monolayers had intact cell membranes and were not stained by propidium iodide (Fig. 5a). After exposure to 13 m*M* C10 for 1 min, patches of stained cells could be observed, indicating that C10 rapidly increased the permeability of the apical cell membranes of these cells (Fig. 5b). The number of stained cells increased slightly during the first 20 min of exposure (Fig. 5c). No further increase in the number of stained cells could be observed after longer exposure times (40 and 60 min; not shown).

Reversibility of the Absorption Enhancement

The highest concentration of C10 to enhance the transport of mannitol in a reversible manner after 20 min of exposure was 10 m*M* (Fig. 6). The recovery could be divided into two phases, one initial rapid phase which lasted for approx. 40 min and a second slower recovery phase (Fig. 6). The area under the curve (AUC) after exposure to C10 was approximately 10 times higher than for the untreated control ($P < 0.001$). Thus, the absorption enhancement was larger than that obtained in previous experiments (see Fig. 1). This can be explained partly by the change from ^{3}H-mannitol in the previous experiments to ^{14}C-mannitol in the recovery

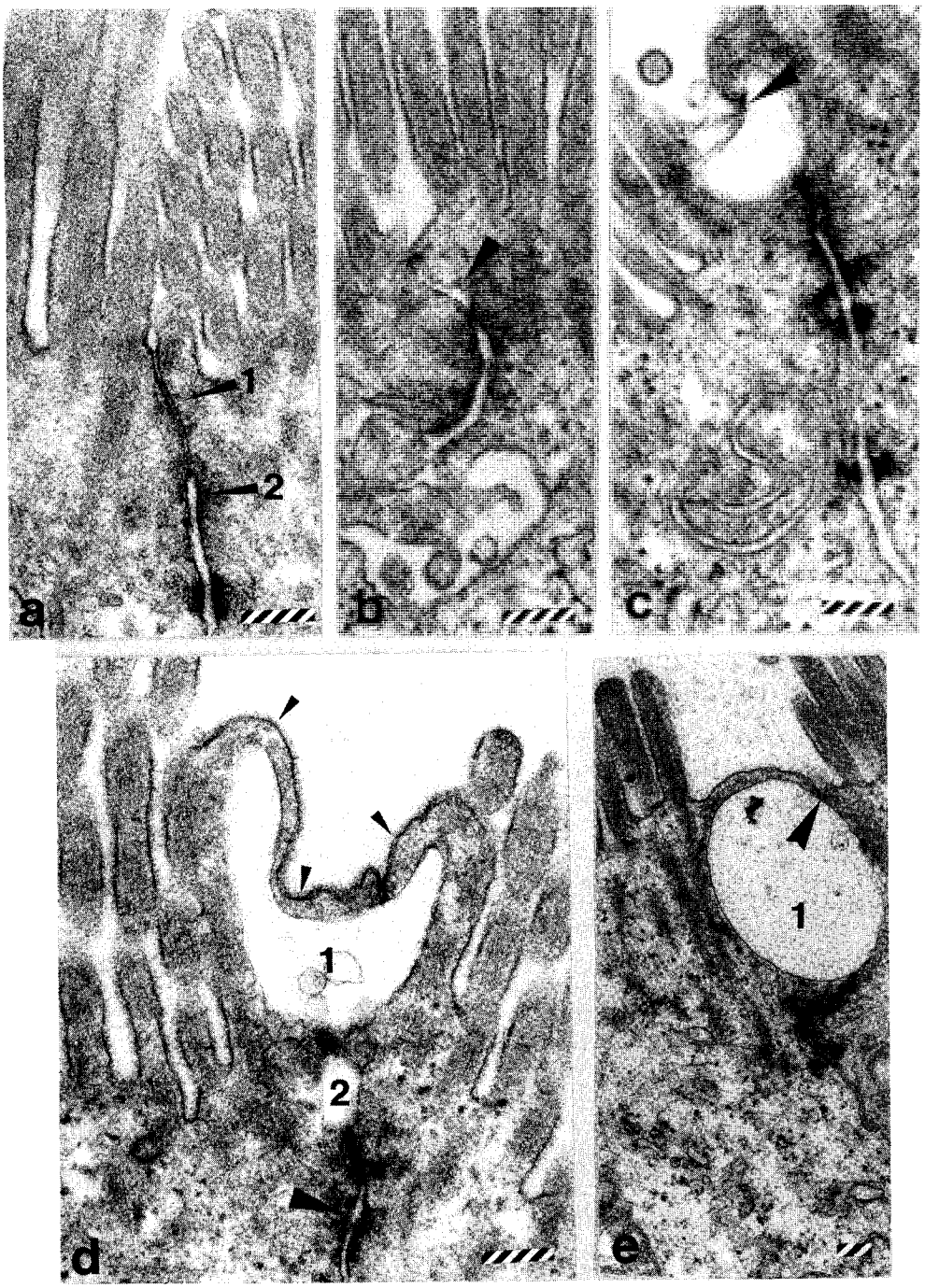


Fig. 3. Time-dependent effects of 13 m*M* sodium caprate on the structure of the tight junctions. The bars indicate 0.2 μm. (a) Control cells with tight junction (1) and adherence junction (2). (b) Cells exposed to sodium caprate for 20 min. Note the structural deformation of the tight junctions in the form of a dilatation (arrowhead). (c) Cells exposed to sodium caprate for 40 min. A large dilatation can be seen in the tight junction. Note that the most apical part of the tight junction remains attached (arrowhead). (d) Cells exposed to sodium caprate for 60 min. One large (1) and one small (2) dilatation in the tight junction can be seen. Note the dense staining of the apical cell membrane surrounding the larger dilatation, indicating an intact cell membrane (small arrowheads). The structure of the adherence junction is normal (large arrowhead). (e) Cells exposed to 13 m*M* sodium caprate for 60 min in medium containing Ca^{2+}. A dilatation can be seen in the tight junction (1). Note that the most apical part of the tight junction remains attached (arrowhead).

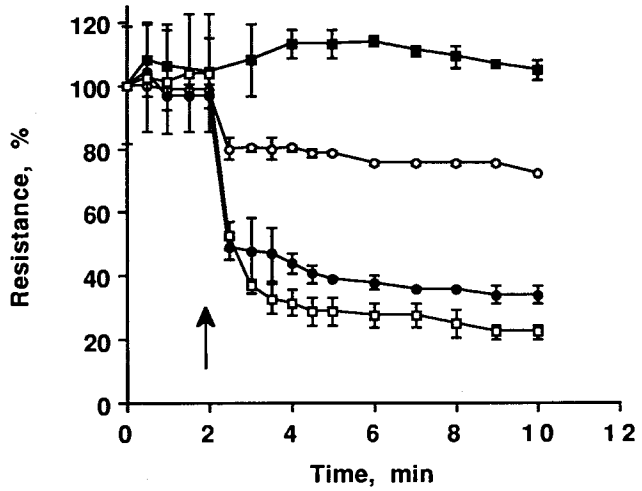


Fig. 4. Effect of 10 m*M* (○), 13 m*M* (●), and 16 m*M* (□) sodium caprate on TEER. (■) Control cells exposed to buffer only. Mean values ± SD (n = 3).

experiments. Since different batches of ^{3}H-mannitol were found to have different permeability coefficients, despite removal of tritium-labeled water by evaporation, we substituted ^{3}H-mannitol for ^{14}C-mannitol. The latter had a P_{app} value approximately three times lower than that of ^{3}H-mannitol, and consequently, in Fig. 6, the P_{app} value of the control is at least three times lower than those presented in the earlier experiments. Another explanation for the difference may be related to the different design of the experiments. In the recovery experiment, P_{app} continued to increase rapidly for at least 20 min after the removal of C10. The increase was faster than that observed without removal of C10 (Fig. 1). It is possible that the change of medium in the recovery experiment diluted essential repair factors produced by the monolayers with delayed recovery as a result.

The reversible effects on mannitol transport coincided with reversible effects on the cellular distribution of F-actin (Figs. 6a–d). After exposure to C10 (10 m*M*) for 20 min, areas with markedly diminished or disbanded actin staining were found, and the F actin appeared more aggregated than in untreated controls (Figs. 6a and b). Forty minutes after exposure, cells with disbanded actin could still be observed. In some cases the perijunctional F actin rings of adjacent cells were clearly separated (Fig. 6c). Ninety minutes after exposure, the intensity of the actin staining was almost restored but the actin was still more aggregated than in controls (Fig. 6d).

The effects of a 20-min exposure to C10 (10 m*M*) on the cellular morphology were similar to but less pronounced than those produced by 13 m*M* C10 (Fig. 3). Twenty minutes after exposure, clear dilatations were observed in approx. 5% of the tight junctions (transmission electron microscopy; data not shown). After the initial (40-min) recovery period, the dilatations were fewer but persisted in some cells. After 90 min of recovery no dilatations were observed.

DISCUSSION

The effects of C10 on epithelial integrity and drug absorption are complicated by its ability to complex with Ca^{2+} (17,18). This is not unique for C10; many lipids, including bile acids and other fatty acids, complex with Ca^{2+} (19,20). The formation of biologically inert Ca^{2+} soaps is believed to be an important mechanism for the protection of the intestinal mucosa against the irritating effects of the dietary lipids (17,19,20). Indeed, in studies on the effects of C10 on the intestinal epithelium, Ca^{2+} reduces the absorption enhancing effect of C10 to a minimum (21).

Similar results were found in this study: a clear reduction of the absorption enhancement was observed in the presence of Ca^{2+}. The magnitude of the reduction is consistent with the Ca^{2+} binding capacity of C10 reported previously (18): approximately 3.7 m*M* C10 is required to complex all of the Ca^{2+} in the buffer in this study. Thus, if we assume that C10 is inactive as a Ca^{2+} complex, a 3.7 m*M* excess of C10 would be required in the presence of apical Ca^{2+} to produce the same response as in the absence of apical Ca^{2+}. This is in excellent agreement with our experimental results (see inset in Fig. 1). This shows that in the present study as well as in the *in vivo* situation (where the extracellular Ca^{2+} concentration is of the same magnitude as in our buffer), only a fraction of the C10 will be "neutralized" by Ca^{2+} complexation. Thus the removal of Ca^{2+} from the apical side of the monolayers will have only a limited effect on the results. Indeed, 13 m*M* (0.25%) of C10 was recently used to enhance drug absorption across the rat colon, suggesting that C10 functions as absorption enhancer at the same concentrations in the rat colon as in Caco-2 monolayers (11). Since the integrity of the intercellular junctions is dependent on Ca^{2+}, cellular access to Ca^{2+} was achieved by keeping the Ca^{2+} at normal physiological levels on the basolateral side of the cells. In this way, the structural integrity of the Caco-2 cell monolayers could be preserved.

The kinetics of the effect of C10 on the TEER and the integrity of the cell membranes were comparable to those previously observed for the anionic synthetic surfactant sodium dodecyl sulfate (7). In both cases, a distinct effect was observed within 1 min of addition of the surfactant. However, while transmission electron microscopy showed that sodium dodecyl sulfate incubation resulted in severe damage to the apical cell membranes of the Caco-2 cells, the effects of C10 were less pronounced and no wounds could be observed, despite the tests with propidium iodide which indicated that C10 increased the membrane permeability. We conclude that the propidium iodide assay is a more sensitive marker of membrane permeability than conventional transmission electron microscopy.

Tomita *et al.* have shown that C10 interacts with membrane proteins and lipids of brush border membrane vesicles from rat colon at concentrations as low as 0.02% (10). This concentration is approx. 10 times lower than the lowest concentration to produce significant absorption enhancement in this study (10 m*M* = 0.19%). Thus, the results of Tomita *et al.* suggest that membrane perturbation may occur at concentrations that are substantially lower than those resulting in absorption enhancement. Consequently, membrane perturbation may not be an ideal marker for C10-mediated absorption enhancement.

In contrast to the findings with the other anionic surfactants (12), exposure to C10 resulted in an increased transport rate with time. The increase was not specific for the drug

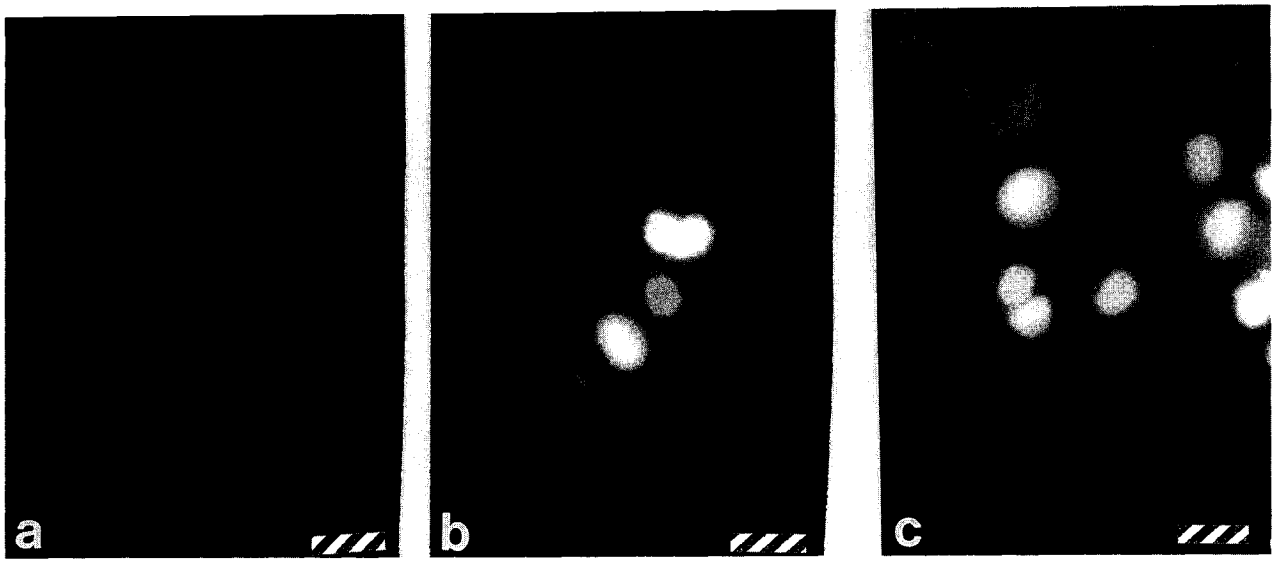


Fig. 5. Cells stained with propidium iodide. The bars indicate 20 μm. (a) Control cells exclude propidium iodide. Cells exposed to 13 m*M* sodium caprate for (b) 1 min and (c) 20 min.

since similar increases were obtained when the transport of two markers, mannitol and PcV, was studied. PcV was used as an alternative marker molecule since it is an example of a commonly used hydrophilic drug associated with problems of absorption and since incompletely absorbed antibiotics have previously been used extensively in studies on absorption enhancement (9,22,23).

Exposure to C10 resulted in structural deformations of the tight junctions in the form of dilatations. The number and size of the dilatations increased with time, indicating that the observed time-dependent increase in permeability involves the paracellular route. Several alternative explanations can be advanced to account for the changes in the junctional structure. Lack of Ca^{2+} is known to affect the junctional integrity, especially during the formation of tight junctions (24). However, it is unlikely that the effect of C10 on the tight junctions is caused by the depletion of Ca^{2+} since Ca^{2+} was absent in the apical medium of the control monolayers without any effects on junctional integrity. In addition, dilatations in the tight junctions caused by C10 were independent of the Ca^{2+} concentration. Further, the structure of the adherence junctions, which are situated below the tight junctions (24), was preserved during the exposure to C10.

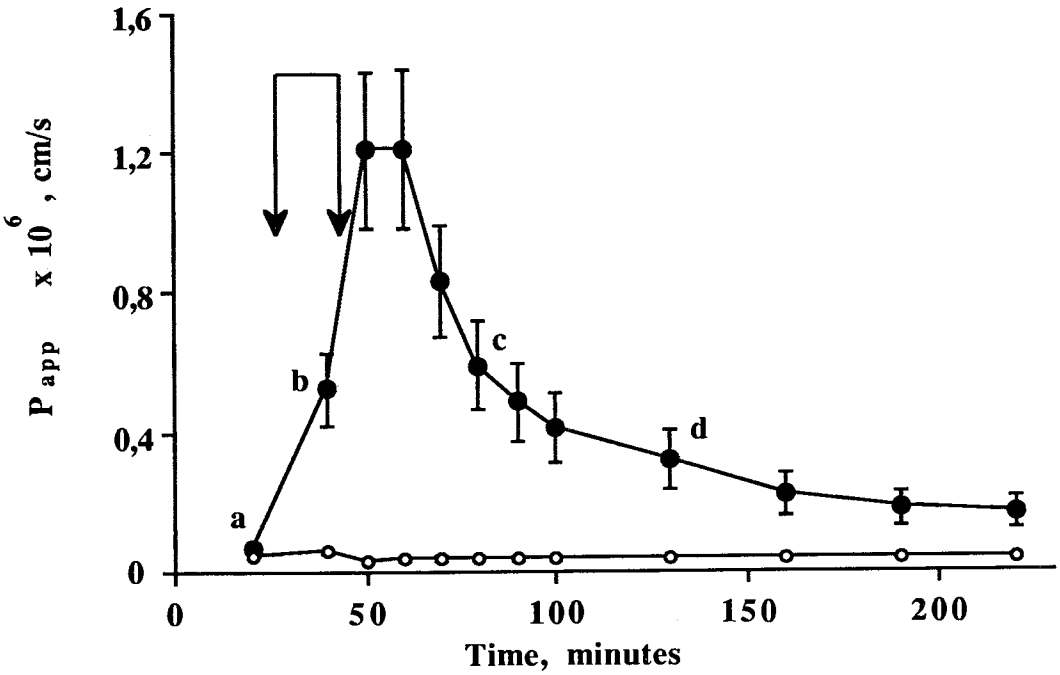
Another and perhaps more likely explanation for the dilatations in the tight junctions could be that the interaction between C10 and the cell membrane results in intracellular responses, such as mobilization of the intracellular Ca^{2+} stores and contraction of the perijunctional actin ring. Such changes have previously been suggested to alter the permeability of tight junctions (7,25–28). However, in those studies, no structural changes in the tight junctions were observed or, alternatively, the structural changes were more subtle than those obtained after exposure to C10.

It has recently been established that high luminal concentrations of nutrients in the form of amino acids and/or sugars induce structural changes in the tight junctions and enhance the absorption of hydrophilic solutes via the paracellular pathway (3,29). Interestingly, the structural changes induced by the nutrients are similar to those obtained with C10 in this study. It has been suggested that activation of Na^+-nutrient cotransporters is the triggering event that eventually causes dilatations of, and increased absorption across, the tight junctions (3,29). This suggestion is consistent with preliminary results in our laboratory indicating that the effects of C10 are sodium dependent and with the results obtained by Shiga *et al.* showing that the effect of C10 is inhibited by ouabain, an inhibitor of Na^+ transport (30). It can therefore be hypothesized that not only nutrients in the form of amino acids and sugars, but also lipids in the form of fatty acids may regulate epithelial permeability.

The reversible effect of C10 on mannitol transport was consistent with reversible effects on F actin distribution and the tight junctions. The redistribution of F actin was comparable to that observed after exposure to, e.g., cytochalasin D, *Chlostridium difficile* toxin A, and sodium dodecyl sulfate (7,25,26), agents known to affect the permeability of the tight junctions. However, the structural alterations of tight junctions induced by C10 were more pronounced than those observed in the previous studies.

Interestingly, the effects of C10 on the intestinal epithelium are more selective than those of other, previously investigated, synthetic and natural anionic surfactants (7,12). However, whether C10 has all the properties required of a selective absorption enhancer remains to be seen. As with other anionic surfactants, the effects of C10 are concentration dependent. At higher concentrations than those used in the present investigation, the effects on the intestinal epithelium include membrane solubilization, cell extrusion, and cell death (data not shown).

The recovery of the Caco-2 cell monolayers from the effects of C10 was as rapid as the recovery of denuded villus tips after exposure to emulsified lipids (31). Thus, in the different models, the intestinal epithelium recovered within the first few hours. These results may be relevant to studies on absorption enhancers. In such studies, the morphological effects of the enhancers are often examined after completion



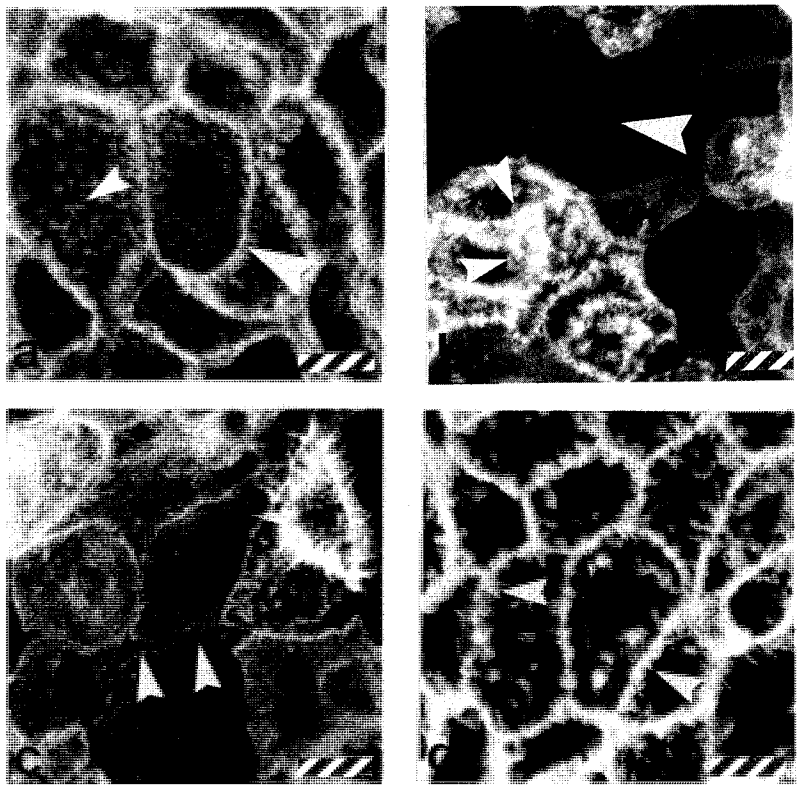


Fig. 6. Top: Reversible effect of 20-min exposure (arrows) to sodium caprate (10 m*M*) on ^{14}C-mannitol transport (●). No absorption enhancement was observed in the control monolayers (○). Mean values ± SD (n = 3–6). The letters a, b, c, and d refer to the photomicrographs below. Bottom: (a–d) Cells stained for F actin with rhodamine-phalloidin before and at different times after 20-min exposure to 10 m*M* sodium caprate; cf. the letters a–d in the graph above. The bars indicate 20 μm. (a) Control cells with typical perijunctional F actin rings (large arrowhead) and microvilli (small arrowhead). (b) Directly after the exposure to sodium caprate, cells with disbanded perijunctional rings appeared (large arrowhead). The actin was generally more aggregated than in the control cells (small arrowheads). (c) Forty minutes after the exposure, cells with disbanded actin could still be observed. In some cases, the normally closely apposed perijunctional F actin rings of adjacent cells were clearly separated (arrowheads). (d) Ninety minutes after the exposure the actin distribution was normalized, but the actin was generally more aggregated than in the control cells (arrowheads).

of the absorption experiments. Since absorption experiments often last for several hours, the epithelium may have recovered even from complete denudation by this time. Thus, morphological examinations performed several hours after the administration of an absorption enhancer may be misleading.

In summary, we conclude that C10 mainly increases the absorption of drugs by the tight junctions. We also conclude that C10 is a more selective absorption enhancer than other synthetic (sodium dodecyl sulfate, docusate sodium, sodium taurodihydrofusidate) and natural (bile acids) anionic surfactants that we have investigated in the Caco-2 model (7,12).

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REFERENCES

1. A. Helenius and K. Simons. Solubilization of membranes by detergents. *Biochim. Biophys. Acta* 415:29–79 (1975).
2. T. Y. Ma, D. Hollander, R. A. Erickson, H. Truong, and P. Krugliak. Is the small intestinal epithelium truly 'tight' to inulin permeation. *Am. J. Physiol.* 260:G669–G676 (1991).
3. J. R. Pappenheimer. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. *J. Membr. Biol.* 100:137–148 (1987).
4. V. H. L. Lee. Enzymatic barriers to peptide and protein absorption and the use of penetration enhancers to modify absorption. In *Proceedings, NATO Advanced Research Workshop on Advanced Drug Delivery Systems for Peptides and Proteins, Copenhangen, Denmark,* Plenum Press, New York, 1986, pp. 87–104.
5. E. J. van Hoogdalem, A. G. de Boer, and D. D. Breimer. Intestinal drug absorption enhancement, an overview. *Pharmacol. Ther.* 44:407–443 (1989).
6. V. H. L. Lee and A. Yamamoto. Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Deliv. Rev.* 4:171–207 (1990).
7. E. K. Anderberg and P. Artursson. Epithelial transport of drugs in cell culture. VIII. The effects of the pharmaceutical surfactant excipient sodium dodecyl sulfate on cell membrane and tight junction permeability in human intestinal epithelial (Caco-2) cells. *J. Pharm. Sci.* 82:392–398 (1993).
8. T. Murakami, H. Kawakita, M. Kishimoto, Y. Higashi, H. Amagase, T. Hayashi, N. Nojima, T. Fuwa, and N. Yata. Intravenous and subcutaneous pharmacokinetics and rectal bioavailability of human epidermal growth factor in the presence of absorption promoter in rats. *Int. J. Pharm.* 46:9–17 (1988).
9. M. Tomita, M. Shiga, M. Hayashi, and S. Awazu. Enhancement of colonic drug absorption by the paracellular permeation route. *Pharm. Res.* 5:341–346 (1988).
10. M. Tomita, M. Hayashi, T. Horie, T. Ishizawa, and S. Awazu. Enhancement of colonic drug absorption by the transcellular permeation route. *Pharm. Res.* 5:786–789 (1988).
11. T. Sawada, T. Ogawa, M. Tomita, M. Hayashi, and S. Awazu. Role of paracellular pathway in nonelectrolyte permeation across rat colon epithelium enhanced by sodium caprate and sodium caprylate. *Pharm. Res.* 8:1365–1371 (1991).
12. E. K. Anderberg, C. Nyström, and P. Artursson. Epithelial transport of drugs in cell culture. VII. Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cells. *J. Pharm. Sci.* 81:879–887 (1992).
13. I. Chantret, A. Barbat, E. Dussalaux, M. G. Brattain, and A. Zweibaum. Epithelial polarity, villin expression, and enterocytic differentation of human colon carcinoma cells: A survey of twenty cell lines. *Cancer Res.* 48:1936–1942 (1988).
14. P. Artursson. Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.* 79:476–482 (1990).
15. P. Artursson and C. Magnusson. Epithelial transport of drugs in cell culture. II. Effect of extracellular calcium concentration on the paracellular transport of drugs of different lipophilicities across monolayers of intestinal epithelial (Caco-2) cells. *J. Pharm. Sci.* 79:595–600 (1990).
16. K. H. Jones and J. A. Senft. An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. *J. Histochem. Cytochem.* 33:77–79 (1985).
17. M. J. Wargovich, V. W. S. Eng, and H. L. Newmark. Calcium inhibits the damaging and compensatory proliferative effects of fatty acids on mouse colon epithelium. *Cancer Lett.* 23:253–258 (1984).
18. M. Mishima, Y. Wakita, and M. Nakano. Studies on the promoting effects of medium chain fatty acid salts on the nasal absorption of insulin in rats. *J. Pharmacobio-Dyn.* 10:624–631 (1987).
19. M. J. Wargovich, W. S. Eng, H. L. Newmark, and W. R. Bruce. Calcium ameliorates the toxic effect of deoxycholic acid on colonic epithelium. *Carcinogenesis* 4:1205–1207 (1983).
20. J. J. Rafter, V. W. S. Eng, R. Furrer, A. Medline, and W. R. Bruce. Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. *Gut* 27:1320–1329 (1986).
21. N. Yata, Y. Higashi, T. Murakami, R. Yamajo, W. M. Wu, K. Taku, Y. Sasaki, and Y. Hideshima. A possible mechanism of absorption promoters. *J. Pharm. Dyn.* 6:78 (1983).
22. N. Kakeya. Antibiotic suppositories. In *Proceedings of a Workshop Held at the 14th International Conference of Chemotherapy,* University of Tokyo Press, Tokyo, 1985, pp. 15–22.
23. K.-I. Nishimura, Y. Nozaki, A. Yoshimi, S. Nakamura, M. Kitagawa, N. Kakeya, and K. Kitao. Studies on the promoting effects of carboxylic acid derivatives on the rectal absorption of β-lactam antibiotics in rats. *Chem. Pharm. Bull.* 33:282–291 (1985).
24. B. Gumbiner. Structure, biochemistry and assembly of epithelial tight junctions. *Am. J. Physiol.* 253:C749–C758 (1987).
25. J. L. Madara, D. Barenberg, and S. Carlson. Effects of cytochalasin D on occluding junctions of intestinal absorptive cell: Further evidence that the cytoskeleton may influence paracellular permeability and junctional charge selectivity. *J. Cell Biol.* 102:2125–2136 (1986).
26. G. Hecht, J. Pothoulakis, J. T. LaMont, and J. L. Madara. Clostridium difficile Toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. *J. Clin. Invest.* 82:1516–1524 (1988).
27. J. L. Madara, J. Stafford, D. Barenberg, and S. Carlson. Functional coupling of tight junctions and microfilaments in T84 monolayers. *Am. J. Physiol.* 254:G416–G423 (1988).
28. J. Llopis, G. E. N. Kass, S. K. Duddy, G. C. Farrel, A. Gahm, and S. Orrenius. Mobilization of the hormone-sensitive calcium pool increases hepatocyte tight junctional permeability in the perfused rat liver. *FEBS Lett.* 280:84–86 (1991).
29. K. Atisook, S. Carlson, and J. L. Madara. Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. *Am. J. Physiol.* 258:C77–C85 (1990).
30. M. Shiga, M. Hayashi, T. Horie, and S. Awazu. Promotion of drug rectal absorption related to water absorption. *Chem. Pharm. Bull.* 34:2254–2256 (1986).
31. P. R. Kvietys, R. D. Specian, M. B. Grisham, and P. Tso. Jejunal mucosal injury and restitution: Role of hydrolytic products of food digestion. *Am. J. Physiol.* 261:G384–G391 (1991).