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Report

Enhancement of Colonic Drug Absorption by the Transcellular Permeation Route

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The effects of sodium caprate and sodium caprylate on transcellular permeation routes were examined in rats. The release of membrane phospholipids was significantly increased only by caprate, while protein release did not change from the control in the presence of caprate or caprylate, indicating that the extent of membrane disruption was insufficient to account for enhanced permeation. Using brush border membrane (BBM) vesicles prepared from colon, with their protein and lipid component labeled by fluorescent probes, the perturbing actions of caprate and caprylate toward the membrane were examined by fluorescence polarization. Caprate interacted with membrane protein and lipids, and caprylate mainly with protein, causing perturbation to the membrane. The release of 5(6)-carboxyfluorescein previously included in BBM vesicles was increased by caprate but not by caprylate. These results suggest that caprate enhances permeability via the transcellular route through membrane perturbation.

KEY WORDS: colonic absorption; absorption promoter; transcellular route; fluorescence polarization; membrane perturbation; sodium caprate.

INTRODUCTION

There are two permeation routes in epithelial cell membranes (1), the transcellular route through lipoidal membranes and the paracellular route to lateral intercellular space through tight junctions. In the previous paper (2), sodium caprate, sodium laurate, and mixed micelles, composed of sodium oleate and sodium taurocholate, were found effectively to promote colonic absorption of the poorly absorbed drug, cefmetazole, when present at a concentration of 0.25%. However, sodium caprylate and sodium taurocholate were much less effective. To study the routes for this promotion, the change in water channels was examined according to the equivalent pore concept of Solomon (3). It was found that the equivalent pore radius was increased by the above promoters to allow the permeation of inulin, a paracellular permeant, and hence, the pore radius (~8 Å), under control conditions, increased to 13–15 Å. Therefore, the paracellular route is part of the mechanism by which the absorption of celmetazole is promoted.

The promotion mechanism for the transcellular route has also been reported by Nishihata *et al.*, who found sodium salicylate to be effective for enhancing rectal absorption of water-soluble drugs such as β-lactam antibiotics (4,5) through interaction of salicylate and membrane protein (5,6). Kajii *et al.* also found sodium salicylate and sodium caprylate to interact mainly with membrane protein, causing membrane perturbation and enhanced permeability (7,8).

In this study, differences in caprate and caprylate for promoting the colonic absorption of cefmetazole (2) were examined on the basis of changes in the transcellular permeation route. Fluorescence polarization was determined in colonic BBM[4] vesicles labeled with the same fluorescent probes used by Kajii *et al.* (7,8). Membrane permeability was evaluated in terms of the release ratio of CF from BBM vesicles in the presence of the two promoters.

MATERIALS AND METHODS

Chemicals. Sodium caprate and sodium caprylate were purchased from Tokyo Kasei Kogyo, Tokyo. The fluorescent probes and their sources are as follows: 2-AS from Molecular Probes Inc., Junction City, Ore.; DPH from Tokyo Kasei Co. Ltd., Tokyo; and DNS-Cl from Sigma Chemical Co., St. Louis, Mo. CF was purchased from Eastman Kodak Co., Rochester, N.Y. Hyaluronidase (Type 1-S) for preparation of colonic BBM vesicles and bovine serum albumin (Fraction V) for protein determination were from Sigma Chemical Co., St. Louis, Mo. All other reagents were of analytical grade or better.

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[4] Abbreviations used: BBM, brush border membrane; DPH, 1,6-diphenyl-1,3,5-hexatriene; 2-AS, 2-(9-anthroyloxy)stearic acid; DNS-Cl, 1-dimethylaminonaphthalene-5-sulfonyl chloride; CF, 5(6)-carboxyfluorescein; AUC, area under the plasma concentration–time curve.

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In Situ *Loop Technique for Determination of Released Membrane Protein and Phospholipid Content.* Male Wistar rats, (200 ± 20 g) fasted overnight, were used under anesthesia of ethyl carbamate (1.1 mg/kg) administered intraperitoneally. The proximal and distal ends of the colon (about 8 cm length) were cannulated according to Doluisio *et al.* (9), as described in a previous paper (10). After flushing the cannulated loop with 0.9% NaCl at 37°C until the eluted solution was clear, a 10-ml plastic syringe was attached to each cannula. Immediately following the addition of 5 ml of 50 m*M* isotonic phosphate buffer solution (Na_2HPO_4 + KH_2PO_4, pH 6.5) containing 0.25% sodium caprate or sodium caprylate to the lumen previously warmed to 37°C, 2 ml was taken, as a sample, at zero time and then 1 ml each at 30 and 60 min. The amount of protein released into the luminal solution was determined by the method of Lowry *et al.*, using bovine serum albumin as the standard (11). The amount of phospholipids was estimated by the kit, Phospholipids B-Test Wako (Wako Pure Chemical Industries, Ltd.), for assaying serum phospholipids.

Preparation of BBM Vesicles from Rat Colon. BBM vesicles were prepared by the method of Kessler *et al.* (7,12) from the colon (about a 10-cm length) of a male Wistar rat (200 ± 20g) fasted overnight. The enzyme activity of marker enzymes, alkaline phosphatase for BBM (12) and Na^+–K^+ ATPase for the basolateral membrane enzyme (13), was determined in the manner reported by Kajii *et al.* (7). The activity of alkaline phosphatase in the final preparation was made about 10 times that of the original homogenates during purification. No Na^+–K^+ ATPase activity could be detected in the vesicles. The protein concentration in the vesicles was determined according to Lowry *et al.* (11).

Fluorescent Probe Labeling of BBM Vesicles and Fluorescence Measurements. The labeling of BBM vesicles by fluorescent probes, the separation of labeled from unlabeled vesicles, and fluorescence polarization measurements were conducted according to Kajii *et al.* (7). Vesicles containing 4–6 mg of protein, prepared from 30–40 rats, were used for each experiment which was conducted with one promoter and one fluorescent probe. The excitation and emission wavelengths were 380 and 455 nm for DPH, 390 and 445 nm for 2-AS, and 328 and 510 nm for DNS-Cl, respectively. The emitted light was passed through a 430-nm cutoff filter for all the probes. Fluorescence polarization, P, was determined by

$$P = \frac{I_1 - I_2}{I_1 + I_2} \quad (1)$$

where I_1 and I_2 are fluorescence intensities parallel and perpendicular to the polarized exciting light, respectively.

Preparation of Colonic BBM Vesicles Containing CF and Release of CF from the Vesicles. BBM vesicles containing CF were prepared according to Kajii *et al.* (7). After adding 3 ml of a vesicle suspension solution, 100 m*M* mannitol–20 m*M* Tris-HCl buffer solution (pH 7.4) containing 0.6–0.9 mg of BBM protein, to a cuvette, the permeability of BBM in the presence and absence of the promoters was examined by measuring directly the fluorescence intensity of CF released from the vesicles since CF in the vesicles hardly emits fluorescence by self-quenching. The excitation and emission wavelengths were 490 and 520 nm, respectively. The emitted light was passed through a 510-nm cutoff filter. These experiments were performed at 25°C to minimize spontaneous leakage of CF from BBM vesicles. The percentage of CF released was determined as follows:

$$\% \text{ CF release} = \frac{I_t - I_0}{I_\infty - I_0} \times 100 \quad (2)$$

where I_0 is the fluorescence intensity of the vesicle suspension containing CF in the absence of promoters, I_∞ is the fluorescence intensity of CF following complete membrane disruption due to the addition of Triton X-100 (Final concentration, 0.005%) to the vesicle suspension, and I_t is the fluorescence intensity of CF at time t after adding a promoter to the suspension.

RESULTS

The amounts of membrane protein and phospholipids released during 60 min in the presence of 0.25% caprate or caprylate are shown in Table I, along with the AUC for colonic absorption of cefmetazole in each case (2). The results using taurocholate and laurate are also listed. The pro-

Table I. Amount of Membrane Protein and Phospholipids Released into Colonic Lumen and AUC for Colonic Absorption of Cefmetazole in the Presence of 0.25% Promoters

	Amount released (mg)[a]		AUC
	Protein	Phospholipids	(μg min/ml)[b]
Control	1.16 ± 0.02	0.070 ± 0.024	26.2 ± 4.1
Sodium caprate	0.93 ± 0.02	0.229 ± 0.044**	263 ± 21***
Sodium caprylate	0.96 ± 0.08	0.101 ± 0.027	46.5 ± 0.3**
Sodium taurocholate	0.78 ± 0.05	0.239 ± 0.081*	62.6 ± 1.0***
Sodium laurate	0.61 ± 0.06	0.106 ± 0.037	178 ± 11***

[a] Cumulative amount released for 60 min. Each value represents the mean ± SE of four to six rats.

[b] Calculated by trapezoidal method using plasma concentration data in Fig. 2 of the previous paper (2). Each value represents the mean ± SE of four to six rats.

* Significantly greater than the control value at $0.05 < P < 0.10$.

** Significantly greater than the control value at $0.01 < P < 0.05$.

*** Significantly greater than the control value at $P < 0.01$.

moting effect of caprate on cefmetazole absorption was greatest and the AUC value was five times that in the presence of caprylate. No promoter caused protein release to exceed significantly that of the control level. However, the release of phospholipids increased to three times the control level by either caprate or taurocholate. The action of caprylate and laurate for bringing about phospholipid release was too small to cause significant variation from the control level.

Fluorescence polarization in colonic BBM vesicles with their lipids labeled by DPH and 2-AS was monitored in the presence of caprate (Fig. 1). The fluorescence polarization of both probes decreased gradually with an increase in caprate concentration. When membrane protein was labeled by DNS-Cl, the fluorescence polarization of the probe decreased with an increase in caprate concentration, in the same manner as that noted in the case of membrane lipids (Fig. 2).

Figure 3 and 4 show change in fluorescence polarization due to caprylate in membrane lipid and protein regions labeled by the fluorescent probes, respectively. No change in the lipid region was found (Fig. 3). Fluorescence polarization of the protein region decreased with an increase in caprylate concentration but the decrease was less than that in response to caprate (Fig. 4).

The cumulative amount of CF released from BBM vesicles in the presence of caprate or caprylate is shown in Fig. 5. No leakage of trapped CF from the vesicles was observed for 5 min until a promoter was added to the medium. The release of CF was dependent on time and promoter concentration. Caprate at 0.12 and 0.21% increased the release ratio of CF to 22 and 33% for 20 min, respectively. Caprylate failed to have any effect at 0.12%, while at 0.21%, the release was only 6% for 20 min.

DISCUSSION

A comparison of caprate and caprylate on the basis of AUC for promoting cefmetazole absorption indicated the former to be five times stronger than the latter (Table I).

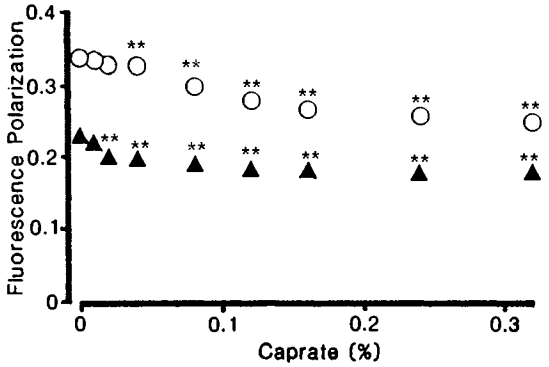

Fig. 1. Effects of caprate on the fluorescence polarization of DPH-labeled (○) and 2-AS-labeled (▲) BBM vesicles from rat colon. SE values were so small as to be included in the symbols. (**) Fluorescence polarizations of DPH- and 2-AS-labeled vesicles in the presence of caprate were significantly less than those in its absence ($P < 0.01$). The minimum effective concentrations of caprate were 0.04% for DPH and 0.02% for 2-AS.

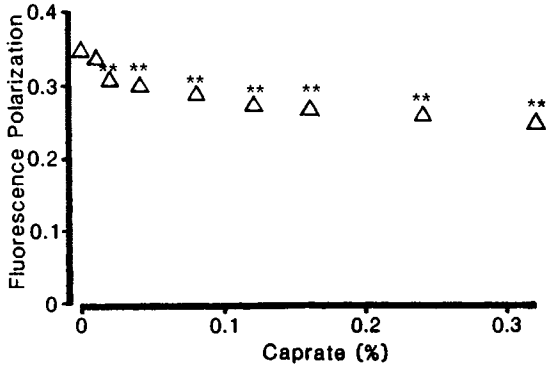

Fig. 2. Effects of caprate on the fluorescence polarization of DNS-Cl-labeled BBM vesicles from rat colon. SE values were so small as to be included in the symbols. (**) Caprate at a concentration of more than 0.02% caused the fluorescence polarization to be significantly less than that in its absence ($P < 0.01$).

Damage to colonic membranes by highly effective (caprate and laurate) and slightly effective promoters (caprylate and taurocholate) of cefmetazole absorption was assessed on the basis of the amounts of membrane protein and phospholipids released into the lumen (Table I). The increase in these amounts over the control level was noted only for phospholipids in the presence of either caprate or taurocholate, and not with caprylate or laurate. A comparison of the effects between caprate and caprylate indicated that the amounts of phospholipids released were related to their promoting effects on cefmetazole absorption. However, the results with laurate and taurocholate indicated highly effective promoters not necessarily to cause the release of greater amounts of phospholipids than less effective promoters. Thus, membrane damage as determined from the extent of phospholipid release was not considered seriously to affect promoting effects on cefmetazole absorption.

DPH and 2-AS are often used to monitor membrane lipid status (7,8,13,14). DPH is considered to label the interior of the lipid layer (13), and 2-AS the exterior of the lipid bilayer situated closer to the aqueous interface (14). The fluorescent probes DNS-Cl and fluorescein isothiocyanate, for reaction with amino groups (15,16), and eosine-

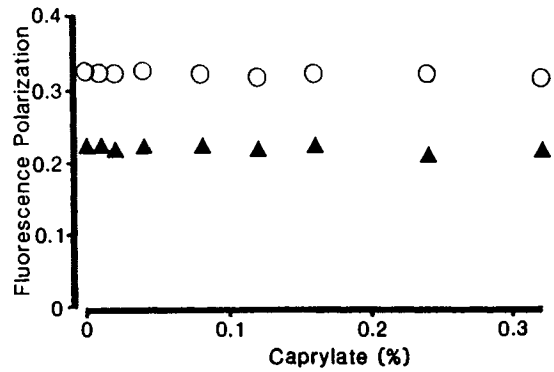

Fig. 3. Effects of caprylate on the fluorescence polarization of DPH-labeled (○) and 2-AS-labeled (▲) BBM vesicles from rat colon. SE values were so small as to be included in the symbols. No change in fluorescence polarization occurred from 0 to 0.32% caprylate.

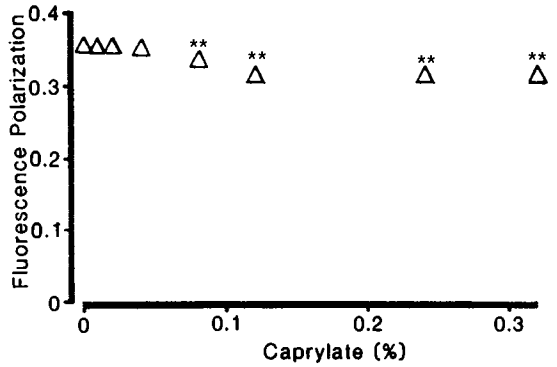

Fig. 4. Effects of caprylate on the fluorescence polarization of DNS-Cl-labeled BBM vesicles from rat colon. SE values were so small as to be included in the symbols. Caprylate at a concentration of more than 0.08% caused the fluorescence polarization to be significantly less than that in its absence ($P < 0.01$).

5-maleimide, for binding with sulfhydryl groups (17) have been used to monitor membrane proteins. In our previous study, when these probes were used to label membrane protein in jejunal BBM vesicles (7,8), decreased fluorescence polarization in the presence of caprylate was observed for all the probes. In the case of the lipid component, labeled by DPH and 2-AS, no decrease was noted to occur. Consequently, in the present study, DNS-Cl was used as the representative probe for membrane protein labeling.

As shown in Figs. 1 and 2, caprate at a concentration of less than 0.1% decreased the fluorescence polarization of DPH, 2-AS, and DNS-Cl by which membrane lipids and protein in BBM vesicles were labeled. Thus it is evident that caprate interacts with membrane lipids and protein, causing membrane perturbation. Caprylate caused a small decrease in the fluorescence polarization of DNS-Cl in BBM vesicles but not in that of DPH or 2-AS (Figs. 3 and 4). The interaction of caprylate with membrane protein is possibly weaker than that of caprate, and the interaction with membrane lipids may be too slight to affect fluorescence polarization.

The effects of caprate and caprylate for promoting colonic absorption of cefmetazole *in situ* were proportional to the membrane perturbation. The relation between the latter and membrane permeability was examined using colonic BBM vesicles (Fig. 5). Kajii *et al.* reported that caprylate at a concentration of 0.1–0.3% releases CF, a poorly permeable compound, to the extent of 10–20% of the trapped amount from rat jejunal BBM vesicles (8). The release ratio of CF from the colonic BBM vesicles was 22–33% in the presence of 0.12–0.21% caprate. There was no release with 0.12% caprylate, although the release ratio by 0.21% caprylate was only 6%. Thus, caprate, which causes extensive membrane perturbation, enhances the membrane permeability of CF to a greater degree than caprylate, which causes very slight perturbation. Also, from a comparison of the present results on the colon with those on the jejunum as reported by Kajii *et al.* (8), differences in caprylate effects on jejunal and colonic BBM permeabilities indicate colonic BBM possibly to be less sensitive to promoters than jejunal BBM.

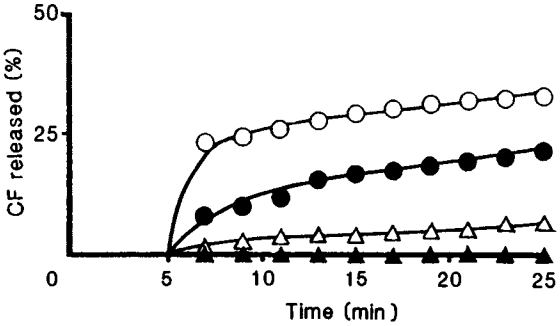

Fig. 5. Amount of CF released from BBM vesicles of rat colon by caprate and caprylate: (●) 0.12% caprate; (○) 0.21% caprate; (▲) 0.12% caprylate; (△) 0.21% caprylate.

Muranishi *et al.* found by the ESR method that interactions of fusogenic lipid in mixed micelles and membrane lipids cause change in membrane fluidity (18) and have reported fusogenic lipid to interact with membrane protein (19). Therefore the mechanism for the promoting action of caprate in transcellular routes is essentially the same as that of mixed micelles.

In summary, caprate is not only a paracellular promoter which increases membrane pore radius (2) but also, on the basis of the present results, a transcellular promoter causing membrane perturbation.

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