Role of lysophosphatidylcholine in brush-border intestinal alkaline phosphatase release and restoration

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Nakano T, Inoue I, Alpers DH, Akiba Y, Katayama S, Shinozaki R, Kaunitz JD, Ohshima S, Akita M, Takahashi S, Koyama I, Matsushita M, Komoda T. Role of lysophosphatidylcholine in brush-border intestinal alkaline phosphatase release and restoration. Am J Physiol Gastrointest Liver Physiol 297: G207–G214, 2009. First published April 30, 2009; doi:10.1152/ajpgi.90590.2008.—Intestinal alkaline phosphatase (IAP) is a brush-border membrane ectoenzyme (BBM-IAP) that is released into the lumen (L-IAP) after a high-fat diet. We examined the effects of oil feeding and the addition of mixed-lipid micelles on the formation of L-IAP in oil-fed rat intestine, Caco-2 cell monolayers, and mouse intestinal loops. We localized IAP in the duodenum of rats fed corn oil using fluorescence microscopy with enzyme-labeled fluorescence-97 as substrate. Four hours after oil feeding, L-IAP increased ~10-fold accompanied by the loss of BBM-IAP, consistent with BBM-IAP release. Rat IAP isozyme mRNAs progressively increased 4–6 h after oil feeding, followed by the increase of IAP activity in the subapical location at 6 h, consistent with the restoration of IAP protein. Postprandial lipid-micelle components, sodium taurocholate with or without oleic acid, mono-oleylglycerol, cholesterol, or lysophosphatidylcholine (lysoPC) were applied singly or as mixed-lipid micelles to the apical surface of polarized Caco-2 cell monolayers. LysoPC increased L-IAP >10-fold over basal release. LysoPC released IAP into the apical medium more than other intestinal brush-border enzymes, 5’-nucleotidase, sucrase, aminopeptidase N, and lactase, without comparable lactate dehydrogenase release or cell injury. LysoPC increased human IAP mRNA levels by 1.5-fold in Caco-2 cells. Luminally applied lysoPC also increased release of IAP preferentially in mouse intestinal loops. These data show that lysoPC accelerates the formation of L-IAP from BBM-IAP, followed by enhanced IAP synthesis, suggesting the role that lysoPC might play in the turnover of brush-border proteins.

Caco-2 cells; lipid absorption; small intestine; enzyme-labeled fluorescence-97

MAMMALIAN ALKALINE PHOSPHATASES (APs; orthophosphoric monooester phosphohydrolase, EC 3.1.3.1) are ectoenzymes anchored in the outer leaflet of the plasma membrane by glycosylphosphatidylinositol (GPI) (15). Human intestine expresses one intestinal type of AP (IAP) encoded by the IAPI gene, whereas rats have two IAP isoenzymes, named IAP-I and IAP-II, which are encoded by the ALPi and ALPii genes, respectively (18). IAP expression is largely restricted to the gut, especially to the brush border of the enterocytes, and its expression and activity are highest in the duodenum (4).

The nonhydrolytic functions of IAP still remain speculative. There has been emerging evidence for the functional role of IAP as a detoxifying enzyme for lipopolysaccharide (LPS) (3, 9). IAP activity is associated with the reduction of inflammatory conditions in the bowel (9): exposure of the intestinal wall to LPS induces IAP gene expression (3). IAP detoxifies LPS by removing its phosphate esters in vitro (30). IAP deficiency is associated with increased LPS toxicity in zebrafish and in Caco-2 cells (3, 9). In vivo, however, the localization of IAP and LPS is mismatched because IAP is expressed mostly in the upper small intestine, whereas the exposure of LPS produced by the microflora to the intestinal mucosa occurs in the ileum and colon.

Many studies suggest that IAP plays a role in the process of lipid absorption and transport. First, an IAP inhibitor l-phenylalanine inhibits fat absorption in the small intestine (14, 19). Second, after ingestion of a high-fat diet, IAP activity in the intestinal luminal content and in the enterocytes increases by more than 10-fold and by two- to threefold, respectively (12, 32). Moreover, there is a parallel increase of IAP activity and triacylglycerol concentration in the thoracic duct during lipid transport (8). Third, alimentary fatty acids may mediate changes in the activity and localization of IAP in the small intestine because the effect of a high-fat diet on the increase of IAP in the luminal contents of the small intestine (L-IAP) is also induced by ingestion of edible oils (12). In addition, oleic acid enhanced rat ALPi gene promoter-mediated expression of luciferase (31). The mechanism by which fatty acids regulate an increase of L-IAP has been studied over the past few decades (12, 16). The process by which ingested fatty acids mediate the IAP release into the intestinal lumen involves production of lipoprotein particles secreted from the enterocytes (5, 16). However, this mechanism does not explain all the changes that occur after ingestion of a fat meal. Such a meal stimulates the secretion of bile and pancreatic juice containing taurocholate and lipases, leading to lipid digestion and micelle formation to enhance fat absorption. Lysophosphatidylcholine (lysoPC) concentration is also increased in the intestinal lumen after fat feeding attributable to the rapid hydrolysis of bile phosphatidylcholine (PC) by pancreatic phospholipase A2 (7). Although lysoPC is a potent detergent molecule, its role in fat absorption or IAP release is unclear.

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The aims of the present study were 1) to evaluate the cellular distribution of IAP after corn oil feeding in vivo, 2) to elucidate in Caco-2 cell monolayer the mechanism(s) regulating the dynamics of IAP activity and localization after fat feeding, and 3) to examine the applicability of the IAP release to mouse intestinal loop model. Our results suggest that lysoPC enhances the release of IAP on the brush-border membrane (BBM-IAP) into the intestinal lumen followed by increased IAP synthesis, presumably for BBM-IAP restoration. The roles of lysoPC in the release and restoration of BBM-IAP may be implicated in the turnover of other brush-border proteins as well.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats (150–200 g) and 8–10-wk-old C57BL male mice were purchased from Clea Japan (Tokyo, Japan). The rodents were kept at 23°C under a 12-h light-dark cycle with free access to water and standard chow. All experiments were approved by the Animal Care Committee of Saitama Medical University.

Detection of alkaline phosphatase activity in situ. Rats were fasted overnight and fed 2 ml of corn oil by gavage. Then the rats were euthanized by terminal exsanguination under pentobarbital sodium anesthesia (115 mg/kg) at 0, 2, 4, and 6 h after corn oil gavage. The whole small intestine was rapidly excised and cut into the duodenum, jejunum, and ileum. The luminal side of the ileum segments was washed with 10 ml of cold PBS (pH 7.4) (12), and the luminal washings were assayed for AP activity and protein concentration as described below. A duodenal portion was fixed in 4% paraformaldehyde and embedded in optimal cutting temperature compound (Sakura Finetek, Tokyo, Japan). Frozen sections were cut with a cryostat at 4-μm thickness. To visualize AP activity, the serial sections were incubated with a fluorogenic AP substrate, 2-(5-chloro-2-nitrophenyl)-6-chloro-4(3H)-quinazolinone (enzyme-labeled fluorochrome-97, ELF-97; Invitrogen, Carlsbad, CA) as previously described (1). The sections were counterstained by AlexaFluor 488-phalloidin (Invitrogen) for brush border and 7-aminoactinomycin D (Invitrogen) for nuclei. Briefly, the sections were first stained with phallolidin for 20 min, followed by the incubation with 125 μM of ELF-97 for 1 min, and then treated with 7-aminoactinomycin D for 1 h. All reactions were performed at room temperature in a dark room. All fluorescence detections with ELF-97 were performed with an exposure time of 100 ms. The images of sections were observed with a fluorescent microscope (Zeiss Axioplan 2 imaging; MOT, Carl Zeiss, Jena, Germany) and captured using AxioVision (Carl Zeiss). Densitometric analysis was performed for ELF-97 and phalloidin staining with an image analyzing software Image-J (http://rsb.info.nih.gov/ij/).

Caco-2 cell culture. Caco-2 cells were obtained from Dr. Terrence Riehl (Washington University School of Medicine), which were originally cloned by Dr. Jeffrey Field (Department of Internal Medicine, University of Iowa). This cell line possesses a marked induction of IAP expression after confluence (28). Cell culture reagents were purchased from Invitrogen (Tokyo, Japan), FCS from Biowest (Nu-aille, France), and microporous PET membrane inserts (1-m pore size, 23-mm diameter) from BD Biosciences (Franklin Lakes, NJ).

Caco-2 cells (between passages 37 and 42) were seeded at a density of 5 × 10^4 cells/cm² on a porous filter membrane and grown in DMEM containing 25 mM glucose and GlutaMax (Invitrogen) containing 20% heat-inactivated FCS under a humidified atmosphere (5% CO₂-15% atmosphere) at 37°C. Cells were grown to confluence for 1 wk and then cultured in the same medium containing 5 mM glucose in the upper compartment and 20% FCS in the lower compartment for the following 4 days. Serum-free low-glucose medium containing 1% insulin, transferrin, and selenium-G (Invitrogen) was used for the last 3 days. All media were changed daily. Penicillin/streptomycin (100 IU/ml and 100 μg/ml, respectively) and 1% non-essential amino acids were added to all the media. Transepithelial resistance (TER) of Caco-2 cells grown on filters was measured by using a Millicell-ERS instrument (Millipore, Billerica, MA).

Preparation of mixed-lipid micelles. Oleic acid, mono-oleylglycerol, cholesteryl, and lysoPC (Sigma Chemical, St. Louis, MO) were dried under nitrogen gas individually. The residue obtained was dissolved in 2 mM taurocholate (Nacalai Tesque, Kyoto, Japan) in serum-free medium and mixed to generate micelles. Final lipid concentrations in the Caco-2 culture media (in mM) were 0.6 for oleic acid, 0.05 for cholesteryl, 0.2 for oleylglycerol, and 0.2 for lysoPC. Each freshly prepared and sterile preparation of single lipid component (e.g., lysoPC plus taurocholate) or mixed-lipid micelles were added to the apical side of cell monolayer to mimic luminal contents in the small intestine after ingestion of a meal.

**AP activity assay.** We measured AP activity at 37°C using 1 M ethylaminoisobutanol buffer (pH 10.5) containing 15 mM p-nitrophenyl-phosphate and 5 mM MgCl₂ as previously described (17). Absorbance for p-nitrophenol was measured at 405 nm with a microplate reader (Sunrise; Tecan Group, Männedorf, Switzerland). IAP activity was expressed in units (U), which defines the enzyme activity that catalyzes the hydrolysis of 1 μmol of p-nitrophenyl-phosphate/min. BBM-IAP activity was estimated as described previously in the cell monolayer (21). Briefly, the cells grown on filters were fixed with 1% glutaraldehyde in 10 mM Tris·HCl buffer (pH 8.0) for 15 min at 4°C and washed with cold Tris-buffered saline. BBM-IAP activity was then detected at 37°C using 0.2 M carbonate buffer (pH 10.5) containing 10 mM p-nitrophenyl-phosphate and 5 mM MgCl₂. The latter method was also used to measure AP activity in the luminal washings.

Caco-2 cell preparation and toluidine blue staining. Caco-2 cell monolayers were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for an hour and postfixed with 1% OsO₄ solution buffered with Sorensen’s phosphate at pH 7.4 for another hour. Then the monolayers were dehydrated in alcohol series and embedded in epoxy resin (Epok 821;OKEN Shoji, Tokyo, Japan). Semi-thin sections were cut and stained with 0.3% toluidine blue.

Cytotoxicity assays for lysoPC. To examine the effect of lysoPC on cell viability, lactate dehydrogenase (LDH) release and propidium iodide (PI) staining were examined in Caco-2 cell monolayers. Apical side of Caco-2 cell monolayers was washed with 2 ml Krebs buffer, and we added 1 ml Krebs buffer containing 5 mM glucose. LysoPC (0.2 or 2 mM) was added to the apical solution, and the monolayers were incubated for 1 h at 37°C. LDH activity in the apical solution and whole cell lysate was measured with LDH cytotoxicity detection kit (Roche Applied Science, Mannheim, Germany). For PI staining to detect the damaged cells, PI (1 μM; Molecular Probes, Eugene, OR) was added to the apical Krebs buffer with or without lysoPC. After 1 h of incubation, PI-positive damaged cells were visualized at 535-nm excitation and 590-nm emission using a Zeiss microscope with a ×10 objective lens. Images were recorded with a cooled charge-coupled device video camera (Hamamatsu Orca-ER; Hamamatsu USA, Bridgewater, NJ), captured, and digitized using an image analyzing software (OpenLab; Improvision, Lexington, MA).

The cell monolayers fixed with 4% parafomaldehyde overnight at 4°C were used to count the total number of the cells in the observed area. The number of PI-positive cells in each image was counted and expressed as a percentage of total cell number.

**Enzyme release experiments.** The jejunal was removed from mice after an overnight fasting, and the luminal contents were flushed out with 5 ml cold saline. After the proximal end was ligated, the lumen was filled with PBS with or without 4 mM lysoPC. After the other end was ligated, the loops were bathed in warm PBS, incubated for 20 min at 37°C, and then washed with 2 ml PBS. All of the fluid in the lumen was collected. After the segments were opened longitudinally, the mucosal surface was scraped lightly with Whatman no. 3 filter papers, and then the papers were immediately soaked in cold 10 mM Tris·HCl buffer containing 1% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride, and 2 mM benzamidine (16) and were sonicated.
for 1 min. The luminal washings and the scrapings were assayed for enzyme activities.

We assayed aminopeptidase N, sucrase, and lactase as previously described (6) with some modifications. Aminopeptidase N (EC 3.4.11.2) activity was assayed with a substrate buffer containing 50 mM Na₂HPO₄ and 18 mM L-alanine- p-nitroanilide hydrochloride (pH 7.0). The sample solutions obtained from the mouse jejunal loops or from Caco-2 cells treated with or without lysoPC were added to the buffer and incubated at 37°C for 20 min. The reactions were stopped by heating at 98°C for 2 min. The absorbance for p-nitroaniline was measured at 405 nm. Sucrose and lactose (300 mM each) were used as substrates for sucrase (EC 3.2.1.48) and lactase (EC 3.2.1.23–62), respectively. Each enzyme activity was measured in 50 mM sodium maleate buffer (pH 6.0). The same assay condition for aminopeptidase N was used.

![Fig. 1. Effect of corn oil feeding on dynamics of intestinal alkaline phosphatase (IAP) in the rat duodenum. A: fluorescence microscopic images of the rat duodenal villi after corn oil feeding up to 6 h. Rats (n = 3 for each period of time) were fed with 2 ml of corn oil by gavage, and the duodenum was removed at the time point indicated. Duodenal frozen sections were stained with enzyme-labeled fluorescence (ELF)-97 for IAP activity (green), phalloidin for F-actin (red), and 7-aminoactinomycin D (7-AAD) for nuclei (blue). Left: lower magnification of ELF-97 and 7-AAD staining. IAP activity (white arrow heads) was gradually decreased up to 4 h after oil feeding but was recovered at 6 h. Right: high magnification of the corresponding images in the left panels with phalloidin staining. IAP activity (purple arrow heads) was observed in the surface of the villi above phalloidin at 0 h. The surface IAP activity was decreased at 2 and 4 h. At 6 h, IAP activity was detected beneath phalloidin. The distribution of IAP activity became short and narrow up to 4 h with its peak positioned to the right of the red peak at 6 h (black arrow head). B: densitometric analysis of ELF-97 and 7-AAD staining. IAP activity (white arrow heads) was gradually decreased up to 4 h after oil feeding but was recovered at 6 h. C: time course changes of IAP activity (top) and protein concentration (bottom) in the luminal contents of the rat ileum after oil feeding. Each plot indicates means ± SE (n = 3). D: time course changes of rat IAP (ALPi and ALPii) mRNA levels in the rat small intestine after oil feeding. Each plot indicates means ± SE (n = 3).
The end product of both enzyme reactions, D-glucose, was measured using a Glucose C2 kit (mutarotase-GOD method; Wako, Osaka, Japan). 5′-Nucleotidase was assayed with a commercial kit (Diazyme Laboratories, San Diego, CA).

The percentage of enzyme released from the Caco-2 cell culture was calculated by means of the following formula: enzyme release (%) = enzyme activity in the apical media/total enzyme activity (cell extract and media) × 100.

**Real-time RT-PCR.** Total RNA was extracted from the cells or intestinal segments (duodenum, jejunum, and ileum) with ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer’s instructions. The samples were treated with RNase-free DNase I (Invitrogen) for 30 min at 37°C. First-strand cDNA synthesis was performed with a Superscript-II reverse transcription kit (Qiagen, Tokyo, Japan) according to the manufacturer’s instructions. The samples were treated with RNase-free DNase I (Nippon Gene, Toyama, Japan) according to the manufacturer’s instructions. The samples were then denatured with total RNA (10 μg, 15 min, 65°C). Reactions were performed in a total volume of 25 μl containing 2.5 μl of cDNA solution, 12.5 μl of qPCR Master Mix (Applied Biosystems, Tokyo, Japan), and 1.25 μl of TaqMan probe. Reactions were run on an ABI PRISM 7900 Sequence Detector (Applied Biosystems). The cycle threshold (Ct), which corresponds to the number of cycles after which the target-DNA concentration increase becomes exponential, was monitored. Results were analyzed using SDS 2.1 Software (Applied Biosystems). Each expression level was normalized against a housekeeping gene, the gene coding ribosomal protein L19. All reactions were done in duplicate.

**Statistical analysis.** Data are shown as means ± SE. For parametric data, means were compared with Student’s t-test. For nonparametric data, Mann-Whitney U-test was used. The statistical analyses were performed with StatView (version 5.0 for Win software) (SAS Institute).

**RESULTS**

**Dynamics in the activity and localization of alkaline phosphatase in rat duodenum.** A newly developed fluorescence microscopy technique for AP activity detection (1) was used to better observe the dynamics of IAP activity and localization in enterocytes in rats that were fed 2 ml of corn oil as previously described (34). Fluorescence microscopy analysis with ELF-97 showed that, at t = 0, all IAP activity was located external to the internal margin of the brush border, identified with phalloidin binding to F-actin (Fig. 1A, right). IAP staining on brush border was gradually decreased by 4 h (Fig. 1A), corresponding to the increase of IAP activity in the luminal washings in the ileum, where L-IAP was gradually increased by ~10-fold at...
6 h after the oil feeding compared with those at $t = 0$ (Fig. 1C). The BBM-IAP activity became nearly undetectable at 4 h and 6 h (Fig. 1A). Intracellular IAP activity was detected close to the brush border at 4 h after oil feeding as seen by the orange band containing both green IAP and red phalloidin fluorescence, and it became stronger and more obvious at 6 h (Fig. 1A), suggesting the increase of IAP activity in the subapical pool. At 6 h, IAP was also located external to the band of phalloidin staining, representing IAP restoration in the BBM. Densitometric analysis, however, showed that most of the IAP activity was still localized within the cytosol at 6 h (Fig. 1B).

The rat duodenum showed high mRNA expression levels of both IAP isoenzymes, as expected (Fig. 1D). In particular, mRNA coding ALPi is dominantly expressed in the duodenum. The mRNA level of the gene encoding IAP-I ($ALPi$) increased gradually more than twice after oil feeding, whereas the mRNA encoding IAP-II ($ALPii$) increased by approximately threefold.

IAP activity after adding mixed-lipid micelles and lysoPC to Caco-2 cell cultures. Human ALPI mRNA expression, IAP secretion, and cellular IAP activity were progressively increased in the Caco-2 cells cultured with serum-containing medium (Fig. 2, A and B). After 2 wk of culture (serum-free medium in the last 4 days), Caco-2 cells demonstrated a complete monolayer with morphological differentiation as manifested by polarized IAP localization (data not shown). Thus the culture conditions as described in MATERIALS AND METHODS were used in the following studies. Most IAP secretion was apically directed in all cultures, and basolateral secretion was either undetectable in the presence of serum (Fig. 2A) or limited when induced by the addition of mixed-lipid micelles (Fig. 2C).

Feeding a meal rich in triacylglycerol to animals increases IAP activity in the luminal contents of the small intestine as much as $>10$-fold (12). Because fatty acids may mediate the IAP release in the small intestine after fat feeding, we examined the effect of fatty acid-containing mixed micelles on IAP activity in Caco-2 cell monolayers. Mixed-lipid micelles resembling the lipid composition of the postprandial period were added to the apical medium of Caco-2 cell monolayers. The addition of mixed-lipid micelles rapidly increased IAP activity in the apical medium (Fig. 2C). By contrast, little IAP activity was detected in the basolateral medium at 2 h although after that time basolateral secretion did increase somewhat.

![Figure 3. Effect of lysoPC application in the apical medium on the integrity of Caco-2 cell monolayers.](http://ajpgi.physiology.org/)
analysis confirmed that the IAP protein concentration increased two- to threefold, consistent with increased IAP activity (data not shown). Choline moieties and lysoPC were reported to affect BBM-AP activity in the cultured cells (11, 29), but we found that neither PC nor lysoPC had any direct effect on AP enzyme activity (data not shown). These findings suggested that the increased IAP activity in the apical medium after mixed-lipid micelle treatment was attributable to IAP release derived from BBM-IAP.

Next, we attempted to identify which component in the mixed-lipid micelles increased IAP release into the apical medium from the Caco-2 cell monolayer. LysoPC alone increased IAP activity in the apical medium, ~14-fold greater than control, and twofold more than the mixed-lipid micelles, whereas other components had no effect (Fig. 2D). The addition of lysoPC rapidly reduced BBM-IAP activity by about half within 30 min (Fig. 2E). Because fatty acids increase IAP activity in the intestinal tissues after fat feeding (20, 31), we also compared the effects of oleic acid and lysoPC on IAP gene (ALPI) expression in Caco-2 cells. Real-time PCR showed that oleic acid had no significant effect on the level of IAP gene expression (Fig. 2F). In contrast, lysoPC significantly increased IAP gene expression ~1.5-fold at 2 h after the treatment.

The detergent-like action of lysoPC suggested that the release of IAP from the BBM could be attributable to the nonspecific shedding of the membranes or cells and/or attributable to a cytotoxic effect of lysoPC. LysoPC (0.2 mM) at the concentration used in Caco-2 cell study had no effect on TER, whereas a higher concentration (2 mM) decreased TER (Fig. 3A). The polarized monolayers were morphologically intact with or without exposure to 0.2 mM lysoPC (Fig. 3, B and C). LysoPC (0.2 mM) had a little effect on LDH release into the apical medium (Fig. 3D), suggesting that the large increase of IAP release (~50%) induced by lysoPC was attributable to an effect on the apical membrane distinct from cell lysis. In addition, exposure of Caco-2 cells to 0.2 mM lysoPC had no significant effect on the number of damaged cells as determined by PI staining (Fig. 3E).

Release of brush-border enzymes from Caco-2 cells and mouse jejunum. The effect of lysoPC for the release of IAP and other brush-border enzymes was examined in Caco-2 cells and mouse jejunal loops. The IAP release into the apical medium from Caco-2 cells (10-fold increase vs. untreated control) and into the luminal contents from the mouse jejunum (24-fold increase) was the greatest of the enzymes tested (Fig. 4, A and B). LysoPC also increased the release of other enzymes both from Caco-2 cells and mouse jejunum but to a lesser extent than the release of IAP. Thus lysoPC has a nonselective effect on increasing brush-border enzyme release, but IAP release predominates. There was no significant change in any of the enzyme levels measured in the light scrapings from mouse jejunum (Fig. 4C). The effect of lysoPC thus appeared to be restricted to the BBM and did not affect the overlying surfactant-like particles that are found in the light scrapings (5, 16).

DISCUSSION

The concentration of lysoPC is rapidly increased after a meal intake and reaches 2–3 mM in the upper small intestine (22) because of the rapid hydrolysis of bile PC (11–12 g/day) by pancreatic phospholipase A2 (7). In the present study, we have demonstrated that lysoPC mediated the release of IAP from the BBMs of Caco-2 cells and increased mRNA encoding IAP. These data are consistent with the increase of IAP release into the small intestinal lumen after corn oil feeding, accompanied by the rapid loss of BBM-IAP with the increased IAP mRNA expression, followed by BBM-IAP restoration demonstrated in rat duodenum. Our results suggest that lysoPC is a major factor that mediates the release of BBM-IAP and its restoration in the upper small intestine after corn oil feeding. Surfactant-like particles (SLPs) provide one mechanism whereby an IAP-enriched membrane is secreted to the luminal surface of the enterocytes following corn oil feeding (5). Since lysoPC, but not oleic acid, increased IAP mRNA expression, lysoPC may be an additional factor in inducing secretion of SLPs in the intestinal mucosa during the postprandial period (28), a process associated with increased expression of IAP. The direct action...
of lysoPC on the BBM appears to be a separate mechanism from the effect of corn oil stimulating SLP production after fat feeding because lysoPC does not lead to an enrichment in IAP over other brush-border enzymes in the light scrapings of the intestinal mucosa, the compartment in which SLP are concentrated (Fig. 4C).

Cholecystokinin-induced increases of L-IAP as well as other brush-border enzymes (5, 10) can be explained by this effect of lysoPC because cholecystokinin stimulates the secretion of pancreatic juice and gallbladder contraction, producing luminal contents containing phospholipid lipases and lecithin as substrate. LysoPC had little cytotoxicity and had no obvious effect on TER or cellular morphology at the concentration used (0.2 mM), inconsistent with IAP release by detergent-based shedding or cell lysis. Hung and Melnykovych (11) showed that lysoPC at 2 mM (calculated as palmitoyl) solubilizes the cellular membranes and releases membrane-bound tissue-nonspecific AP from HeLa cells. However, the predominant lysoPC-mediated IAP release compared with other hydrolases indicates that the enzyme release from the BBM is not attributable solely to nonspecific membrane shedding. Absorption of long-chain fatty acids and larger lipids is limited by diffusion through the unstirred water layer adjacent to the apical enterocyte membrane (27). Thus the effective lysoPC concentration at the surface of the BBM may not be predicted by the total concentration in luminal fluid (2–3 mM) and may be low enough to release IAP in preference to other hydrolases.

The preferential release of IAP may be related to the fact that it is a GPI-anchored and lipid raft-associated protein. Interestingly, Staneva et al. (24, 25) show that lysoPC induces the fission and budding of lipid-raft domains from artificial membranes. We have observed that methyl-


References


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