Mechanisms of lysophosphatidic acid (LPA) mediated stimulation of intestinal apical Cl-/OH- exchange

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Singla A, Dwivedi A, Sakseña S, Gill RK, Alrefai WA, Ramaswamy K, Dudeja PK. Mechanisms of lysophosphatidic acid (LPA) mediated stimulation of intestinal apical Cl-/OH- exchange. Am J Physiol Gastrointest Liver Physiol 298: G182–G189, 2010. First published November 12, 2009; doi:10.1152/ajpgi.00345.2009.—Lysophosphatidic acid (LPA), a potent bioactive phospholipid, is a natural component of food products like soy and egg yolk. LPA modulates a number of epithelial functions and has shown to inhibit cholera toxin-induced diarrhea. Antidiarrheal effects of LPA are known to be mediated by inhibiting chloride secretion. However, the effects of LPA on chloride absorption in the mammalian intestine are not known. The present studies examined the effects of LPA on apical Cl-/OH- exchangers known to be involved in chloride absorption in intestinal epithelial cells. Caco-2 cells were treated with LPA, and Cl-/OH- exchange activity was measured as DIDS-sensitive 36Cl uptake. Cell surface biotinylation studies were performed to evaluate the effect of LPA on cell surface levels of apical Cl-/OH- exchangers, downregulated in adenoma (DRA) (SLC26A3), and putative anion transporter-1 (SLC26A6). Treatment of Caco-2 cells with LPA (100 μM) significantly stimulated Cl-/OH- exchange activity. Specific agonist for LPA2 receptor mimicked the effects of LPA. LPA-mediated stimulation of Cl-/OH- exchange activity was dependent on activation of phosphatidylinositol 3-kinase/Akt signaling pathway. Consistent with the functional activity, LPA treatment resulted in increased levels of DRA on the apical membrane. Our results demonstrate that LPA stimulates apical Cl-/OH- exchange activity and surface levels of DRA in intestinal epithelial cells. This increase in Cl-/OH- exchange may contribute to the antidiarrheal effects of LPA.

downregulated in adenoma; chloride absorption; human intestine; LPA receptor 2; phosphatidylinositol 3-kinase/Akt

LYSOPHOSPHATIDIC ACID (LPA) is a naturally occurring bioactive glycerophospholipid involved in a broad range of biological processes including platelet aggregation, smooth muscle cell contraction, cell differentiation, and cytoskeletal rearrangements (10, 24, 26). LPA is highly abundant in foods such as soybean and egg yolk (29, 41). Previous studies have suggested that LPA generation is affected by the composition of the diet because high dietary α-linolenic acid has been shown to suppress the formation of LPA in activated platelets (25). LPA is also produced from the plasma membrane lipids in cells and biological fluids by the action of phospholipase A1 or A2 as well as lysophospholipase D (5, 24). Blood platelets are the major source of LPA; however, epithelial cells, macrophages, neuronal cells, and some tumor cells also produce LPA (6, 13, 36). The normal concentration of LPA in the blood ranges from 0.5–50 μM, which increases to high concentrations ranging from 80–100 μM in pathological conditions such as ovarian cancer and inflammation (6, 13, 36, 42).

LPA mediates its effects predominantly by its interaction with specific G protein-coupled receptors (GPCRs) via activation of intracellular signal transduction mechanisms (2–4, 7, 17, 27, 45). To date, seven LPA receptor subtypes have been identified and designated as LPA1–7 receptors (2–4, 7, 17, 21, 30–31, 40). In the gastrointestinal tract, LPA1, 2, and 3 receptor subtypes have been shown to be expressed in colon and in various colonic epithelial cell lines derived from human and rabbit (22, 45). Also, LPA 5 receptor has been shown to be expressed in the mouse small intestine and colon with a moderate level of its expression in stomach (19, 21).

In the gastrointestinal tract, LPA has been implicated to enhance intestinal restitution and wound healing (16, 38). Previous studies have suggested that LPA protects intestinal epithelial cells against radiation- and chemotherapy-induced apoptosis (11). Studies utilizing a rat model of colitis showed that LPA can reduce the degree of inflammation and necrosis in the distal colon compared with control rats (39). Additionally, LPA has been implicated as a potential therapeutic agent in cholera toxin-induced secretory diarrhea in mice by inhibiting CFTR-dependent Cl- channel activity via LPA2 receptor-mediated pathway (22). Diarrhea could result from increased secretion and/or decreased absorption. Previous studies from our laboratories and others have shown NaCl absorption to occur predominantly via an electroneutral process involving the operation of Cl-/OH- exchangers coupled to Na+/H+ exchangers (NHEs) (12, 23, 32, 37). Interestingly, a recent study indicated that LPA stimulates NHE isoform 3 (NHE3) activity in opossum kidney cell line by increasing exocytic trafficking of NHE3 protein in the plasma membrane by activation of phosphatidylinositol 3 (PI3) kinase (20). However, the effects of LPA on intestinal Cl- absorption have not yet been investigated. Recent studies have characterized two members of SLC26 gene family, SLC26A3 or downregulated in adenoma (DRA) and SLC26A6 or putative anion transporter-1 (PAT-1), as the potential apical membrane Cl-/OH- exchangers involved in electroneutral Cl- absorption along the length of the human intestine (28). Because DRA has been shown to be functionally coupled to NHE3, it was critical to examine the effects of LPA on intestinal apical membrane anion exchangers.

The present studies were undertaken to examine the effects of LPA on apical Cl-/OH- exchangers and to elucidate the signaling pathways and the membrane events involved. Our studies demonstrate that LPA stimulates apical Cl-/OH- ex-
change activity by increasing the surface levels of DRA on the apical membrane via a LPA2 receptor-mediated process and activation of PI3 kinase/Akt pathways in human intestinal Caco-2 monolayers. These results indicate that an increase in Cl⁻/HCO₃⁻ exchange activity via increased surface DRA levels may contribute to the antidiarrheal effects of LPA.

MATERIALS AND METHODS

Materials. Caco-2 cells and MEM were obtained from American Type Culture Collection (ATCC, Manassas, VA). Radionuclide ³⁶Cl⁻ was obtained from American Radiolabeled Chemicals (ARC, St. Louis, MO). DIDS and niflumic acid (NFA) were obtained from Sigma Aldrich (St. Louis, MO). 1-Oleoyl-sn-glycerol 3-phosphate sodium salt (LPA) was purchased from Sigma-Aldrich or Avanti Polar Lipids (Alabaster, AL). LPA was prepared in PBS containing 0.1% BSA (vol/vol) and was sonicated before use. Pharmacological inhibitor LY294002 was purchased from Biomol (Plymouth Meeting, PA), and triciribine was procured from Calbiochem (San Diego, CA). Sulfo-NH-SS-biotin was obtained from Pierce Biotechnology (Rockford, IL). All other chemicals were of at least reagent grade and were purchased from Sigma or Fisher Scientific (Pittsburgh, PA).

Cell culture. Caco-2 cells were grown at 37°C in a 5% CO₂ environment in T-75-cm² plastic flasks. Cells were cultured in MEM medium with high glucose, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mg/l gentamycin, and 20% fetal bovine serum. For the uptake studies, cells between passages 25 and 45 were plated on 24-well plates at a density of 2 × 10⁴ cells/well. To study the effect of basolateral LPA on Cl⁻/OH⁺ (HCO₃⁻) exchange activity, Caco-2 cells were plated on Transwell inserts (Costar, Corning, NY) at a density of 1 × 10⁴ cells/Transwell. Uptake studies were performed using fully differentiated cells at day 10–14 postplating. To study the effect of LPA on apical Cl⁻/OH⁺ (HCO₃⁻) exchange activity, cells were exposed to 100 µM LPA in cell culture medium for 30 min from either the luminal or basolateral side. In a separate set of experiments, cells were pretreated with PI3 kinase inhibitor, LY294002 (50 µM) or Akt inhibitor, triciribine (1 µM), for 1 h before the addition of 100 µM LPA for 30 min. These inhibitors were also coincubated with LPA for another 30 min.

Assessment of Cl⁻/OH⁺ exchange activity. Cl⁻/OH⁺ exchange activity was measured as described previously by our laboratory (35).

Fig. 1. Lysophosphatidic acid (LPA) stimulates Cl⁻/OH⁺ exchange activity in Caco-2 cells. Serum-starved Caco-2 cells were treated with different doses of LPA (50–150 µM) for 30 min (A) or 100 µM LPA for 15–60 min (B). Cl⁻/OH⁺ exchange activity was measured as DIDS-sensitive (600 µM) ³⁶Cl⁻ uptake for 5 min in base-loaded cells. Results are expressed as percentages of control and represent means ± SE of 6 separate experiments performed in triplicate. Control values in nmol/mg protein/5 min are as follows: 11.13 ± 1.19 (A) and 8.6 ± 1.62 (B). *P < 0.05 or less compared with control.

Fig. 2. LPA2 receptor is involved. Caco-2 cells were treated with LPA2 receptor agonist dodecyl phosphate (350–1,000 nM) for 30 min (A) or LPA1/3 receptor agonist VPC31143 (100–750 nM) for 30 min (B). Cl⁻/OH⁺ exchange activity was measured as DIDS-sensitive (600 µM) ³⁶Cl⁻ uptake for 5 min in base-loaded cells. Results are expressed as percentages of control and represent means ± SE of 4 separate experiments performed in triplicate. Control values in nmol/mg protein/5 min are: 5.66 ± 1.3 (A) and 5.8 ± 0.56 (B). *P < 0.05 or less compared with control.
ice-cold 1× PBS two times and lysed in 20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, and 1× protease cocktail inhibitor mixture. The cells were lysed by sonication, and the lysate was centrifuged at 7,000 revolution/min for 7 min at 4°C. Protein concentration was determined by the Bradford assay (9). To monitor Akt phosphorylation in treated Caco-2 cells, equal amounts (75 μg) of cell lysates were solubilized in gel loading buffer and boiled for 5 min. Samples were loaded on 10% SDS-polyacrylamide gels and transblotted to nitrocellulose membranes. The membranes were incubated in blocking buffer containing 1× TBS and 5% nonfat dry milk for 1 h followed by incubation with rabbit anti-phospho-Akt antibody (1:100 dilution) in 1× TBS and 3% BSA overnight at 4°C. The membranes were washed four times with the wash buffer containing 1× TBS and 0.1% Tween-20 for 5 min. Finally, the membranes were probed with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:2,000 dilution), and the bands were visualized with enhanced chemiluminescence detection reagents.

**Cell surface biotinylation and immunoblotting.** Cell surface biotinylation studies were performed in Caco-2 monolayers utilizing Sulfo-NHS-Biotin (1.5 mg/ml; Pierce) in borate buffer (in mM: 154 NaCl, 7.2 KCl, 1.8 CaCl₂, 10 H₂BO₃, pH 9.0) as described previously (8). Labeling was allowed to proceed at 4°C to prevent endocytosis

**Western blotting.** Differentiated Caco-2 cells were treated with 100 μM LPA for indicated times. After treatment, cells were washed with

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**Fig. 3.** Both luminal and serosal LPA increased Cl⁻/OH⁻ exchange activity. Caco-2 cells grown on Transwells were treated with 100 μM LPA (A) or 700 nM LPA2 receptor agonist (B) from both the apical and basolateral sides. Apical Cl⁻/OH⁻ exchange activity was measured as niflumic acid-sensitive (100 μM) ³⁶Cl⁻ uptake for 5 min in base-loaded cells. Results are expressed as percentages of control and represent means ± SE of 6 independent wells. Control values in nmol/mg protein/5 min are as follows: apical treatment 1.5 ± 0.29 and basolateral treatment 3.8 ± 0.73 (A); apical treatment 4.7 ± 0.96 and basolateral treatment 3.4 ± 0.46 (B). *P < 0.05 or less compared with control.

**Fig. 4.** LPA-mediated stimulation of Cl⁻/OH⁻ exchange activity is phosphatidylinositol 3 kinase (PI3K)-Akt dependent. Caco-2 cells were preincubated with specific PI3K inhibitor, LY294002 (50 μM) (A), or Akt inhibitor, triciribine (1 μM) (B), in the cell culture medium for 60 min. Cells were then coincubated in the presence of LPA (100 μM) for 30 min. Cl⁻/OH⁻ exchange activity was measured as DIDS-sensitive (600 μM) ³⁶Cl⁻ uptake for 5 min. Results are expressed as percentages of control and represent means ± SE of 4 separate experiments performed in triplicate. Control values in nmol/mg protein/5 min are as follows: 7.19 ± 1.8 (A) and 6.95 ± 1.69 (B). *P < 0.05 or less compared with control.
and internalization of antigens for 60 min. The biotinylated antigens were immunoprecipitated utilizing streptavidin agarose beads, and the biotinylated proteins were released by boiling in Laemmli buffer containing 100 μM dithiothreitol. Proteins were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Membranes were then labeled with either anti-DRA (custom synthesized against peptide corresponding to COOH-terminal sequence) or anti-PAT-1-purified antibody. The surface DRA or PAT-1 levels were compared with total cell antigen as determined by immunoblotting in solubilized cell extract and with the amount of DRA or PAT-1 not removed by the avidin-precipitation method (intracellular pool).

Statistical analysis. Results are expressed as means ± SE. Each independent set represents the mean ± SE of data from four to five independent experiments. One-way ANOVA with Dunnett’s multiple comparison test was used for statistical analysis. Differences between control and treated groups were considered significant at $P < 0.05$.

RESULTS

LPA treatment stimulates Cl⁻/OH⁻ exchange activity. Previous studies have shown that LPA decreases Cl⁻ secretion via inhibition of CFTR chloride channels in intestinal epithelial cells (22). To determine the effects of LPA on Cl⁻ absorption, we examined the effects of LPA on apical Cl⁻/OH⁻ exchange activity in human intestinal Caco-2 monolayers. Caco-2 cells were serum starved overnight and were treated with different doses of LPA ranging from 50 nM to 100 μM in serum-free media for 30 min, and DIDS-sensitive $^{36}$Cl⁻ uptake was assessed in base-loaded differentiated Caco-2 cells. LPA treatment for 30 min resulted in a dose-dependent increase (~60% at 100 μM) in Cl⁻/OH⁻ exchange activity (Fig. 1A). A time course for LPA treatment (100 μM) was then assessed in Caco-2 cells for different time periods ranging from 15–60 min. LPA-mediated stimulation of Cl⁻/OH⁻ exchange activity occurred at 30–60 min with maximal stimulation at 30 min (Fig. 1B). Therefore, for subsequent experiments, 100 μM dose of LPA was used for 30 min.

LPA 2 receptor is involved in LPA-mediated effects on Cl⁻/OH⁻ exchange activity. LPA is known to mediate its effects via seven LPA receptors (2–4, 7, 17, 21, 30). To identify the receptor subtype(s) involved in mediating the stimulatory effects of LPA on Cl⁻/OH⁻ exchange activity, we used specific LPA2 receptor and LPA1/3 receptor agonists. Serum-starved Caco-2 monolayers were treated with different doses of dodecyl phosphate (LPA2 receptor agonist) and VPC 31143 (LPA1/3 receptor agonist) for 30 min. Incubation with LPA2 receptor agonist (350–1,000 nM) significantly increased DIDS-sensitive $^{36}$Cl⁻ uptake in Caco-2 cells (Fig. 2A), whereas LPA1/3 receptor agonist (100–700 nM) did not show any effect (Fig. 2B). These results were further confirmed by pretreating the Caco-2 cells with LPA1/3 receptor antagonist VPC 32183 for 1 h followed by treatment with LPA for 30 min. VPC 32183 at 300 nM failed to block the LPA-mediated stimulation of Cl⁻/OH⁻ exchange activity in Caco-2 cells (data not shown). These studies suggest that LPA2 but not LPA1 and LPA3 receptors mediate the effects of LPA on Cl⁻/OH⁻ exchange activity.

Fig. 5. LPA induces Akt phosphorylation in Caco-2 cells. Overnight serum-deprived Caco-2 cells were treated with 100 μM LPA for 5–15 min (A and B), Akt inhibitor, triciribine (Tri) (1 μM) for 60 min and then coincubated with 100 μM LPA for 10 min (C), or LPA2 receptor agonist dodecyl phosphate (700 nM) for 5–15 min (D). Cells were lysed, and extracted proteins were subjected to 10% SDS-polyacrylamide gel for Western blot analysis utilizing phospho-specific Akt antibody. The blots were stripped and reprobed with the anti-Akt antibody to normalize for equal loading of protein in each lane. Quantification of phospho-Akt was performed by densitometric analysis and expressed as arbitrary units and represents means ± SE of 3 different experiments. *$P < 0.05$ or less compared with control (0 min).

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Luminal and basolateral LPA stimulate Cl⁻/OH⁻ exchange activity on the brush-border membrane of Caco-2 cells. We next examined whether exposure to LPA from basolateral compartment also affected the apical Cl⁻ absorption. For these studies, Caco-2 cells grown on Transwell inserts were exposed to LPA either from the apical or basolateral side, and the effect of another conventional anion exchange inhibitor niflumic acid (NFA) on 36Cl⁻ uptake was assessed in base-loaded differentiated cells. As shown in Fig. 3A, exposure to LPA from both apical and basolateral side (100 μM) for 30 min significantly increased the apical Cl⁻/OH⁻ exchange activity. Similarly, incubation with LPA2 receptor agonist from either the apical or basolateral side (700 nM) for 30 min significantly increased the NFA-sensitive Cl⁻ uptake across the apical membrane of Caco-2 cells (Fig. 3B). In contrast, treatment with 750 nM of LPA1/3 receptor agonist from either side did not show any effect (data not shown). These data suggest that exposure to LPA from either apical or basolateral side increases the luminal Cl⁻/OH⁻ exchange activity via LPA2 receptor.

Role of PI3 kinase/Akt in LPA-mediated stimulation on Cl⁻/OH⁻ exchange activity. LPA2 receptor has been shown previously to couple to Gi proteins and stimulate PI3-Akt cascade in Caco-2 cells (45). Also, studies have demonstrated the involvement of PI3 kinase in the stimulation of NHE3 by LPA in opossum kidney cell line (20). We thus examined the role of PI3 kinase and Akt pathway in effects of LPA on Cl⁻/OH⁻ exchange activity by utilizing LY294002, a specific inhibitor for PI3 kinase, and triciribine (Akt inhibitor). For these studies, serum-starved Caco-2 cells were pretreated with LY294002 (50 μM) or triciribine (1 μM) for 1 h followed by coincubation in the presence of LPA (100 μM) for 30 min. The stimulation of Cl⁻/OH⁻ exchange activity in response to LPA treatment was completely abrogated in the presence of LY294002 or triciribine as shown in Fig. 4, A and B. These results indicate that PI3 kinase and Akt are involved in the stimulation of Cl⁻/OH⁻ exchange activity by LPA.

Akt phosphorylation by LPA. As a direct measure of Akt activation, we determined the phosphorylation of Akt in response to LPA (100 μM) by treating Caco-2 cells with LPA for different time points. Western blot analysis of the total lysates showed that LPA stimulated Akt phosphorylation in Caco-2 cells as early as 5 min, which persisted for at least 10 min (Fig. 5, A and B). This increase in Akt phosphorylation was completely blocked in the presence of Akt inhibitor triciribine (Fig. 5C). Because LPA2 receptors are involved in mediating the effects of LPA on Cl⁻/OH⁻ exchange activity, we further determined the phospho-Akt levels in response to LPA2 receptor agonist dodecyl phosphate at 700 nM. LPA2 receptor agonist also stimulated Akt phosphorylation in Caco-2 cells at 10 min (Fig. 5D). Also, Akt phosphorylation was attenuated in the presence of PI3 kinase inhibitor LY294002 (data not shown), indicating that Akt is downstream of PI3K.

![Image](http://ajpgi.physiology.org/)

**Fig. 6.** LPA increases surface expression of downregulated in adenoma (DRA) but not putative anion transporter-1 (PAT-1) in Caco-2 cells. Cells were treated with LPA (100 μM) for 30 min in a cell culture medium. Cells were washed with 1× PBS and were subjected to biotinylation at 4°C utilizing sulfo-NH-SS-biotin. Biotinylated proteins were extracted with streptavidin-agarose, and surface and total fractions were run on 10% SDS-polyacrylamide gel. The blot was immunostained with rabbit anti-DRA antibody (A and B) or rabbit anti-PAT-1 antibody (C and D). Representative blots of 3 separate experiments are shown. Results of densitometric analysis are expressed as surface DRA/total DRA or surface PAT-1/total PAT-1. Values represent means ± SE of 3 different experiments. *P < 0.05 or less compared with control.
LPA increases surface DRA expression. Two members of SLC26 gene family DRA and PAT-1 have been implicated in apical Cl\(^{-}/\)OH\(^{-}\) exchange activity in the human intestine (28). Recently, we have shown that acute modulation of DRA in Caco-2 cells involves alterations in DRA surface levels (8, 14). To examine the effects of LPA on surface levels of Cl\(^{-}/\)OH\(^{-}\) exchangers, cell surface biotinylation studies were performed. Our results showed that LPA treatment significantly increased the surface levels of DRA, whereas the total cellular DRA levels did not change (Fig. 6, A and B). These data are in parallel with an increase in Cl\(^{-}/\)OH\(^{-}\) exchange activity. Densitometric analysis of the protein bands suggested that LPA treatment increased surface DRA levels by 70–80% compared with control. In contrast, as shown in Fig. 6, C and D, the surface levels of PAT-1 in response to LPA treatment were not significantly altered compared with control. Densitometric analysis showed no quantitative difference in surface PAT-1 levels in LPA-treated cells compared with control.

DISCUSSION

Diarrhea is the most common symptom associated with various gastrointestinal disorders such as inflammatory bowel diseases and bacterial infections and causes high morbidity and mortality. Diarrhea occurs either because of decreased absorption or increased secretion or both of water and electrolytes such as sodium and chloride. Earlier studies have shown that LPA inhibits CFTR-dependent Cl\(^{-}\) secretion and stimulates NHE3 activity, which is well known to play a central role in the diarrheal process (22). Therefore, it was of interest to examine the effects of LPA on Cl\(^{-}/\)OH\(^{-}\) exchange activity in the mammalian intestine. Cl\(^{-}/\)OH\(^{-}\) exchangers or anion exchangers are known to play a pivotal role in the vectorial transport of Cl\(^{-}\) across the plasma membrane of polarized epithelial cells. Previously, we have shown that apical Cl\(^{-}/\)OH\(^{-}\) exchange activity is inhibited by various inflammatory mediators, such as nitric oxide, serotonin, and phorbol esters, and also by infection with enteropathogenetic Escherichia coli in Caco-2 cells and is stimulated by probiotic bacteria, Lactobacillus acidophilus (8, 14, 33–35). In the present studies, our data for the first time demonstrate that LPA significantly stimulates Cl\(^{-}/\)OH\(^{-}\) exchange activity in Caco-2 cells via activation of PI3 kinase/Akt pathways, suggesting that this effect of LPA could contribute to potential antidiarrheal effects of LPA.

LPA is a biologically active phospholipid that is mainly generated in blood platelets but is also highly abundant in foods such as soybean and egg yolk (13, 29, 41). The effects of LPA are mediated via its interaction with LPA receptors designated as LPA 1–7 (2–4, 7, 17, 21, 30). The LPA1, LPA2, and LPA3 receptors belong to the endothelial differentiation gene family of GPCRs and show about 50% sequence homology to one another (27, 43), whereas, LPA4, LPA5, GPR87 (LPA6), and P2Y5 (LPA7) belong to purinergic receptors and share 35% sequence homology (21, 30–31, 40). LPA receptors differ with respect to their distribution in normal tissues. Earlier studies have shown that LPA1, LPA2, and LPA3 receptors are expressed in colonic epithelial cells and colonic tissues (22, 45). These studies also showed that the expression level of LPA2 is more in colon cancer cell lines (Caco-2, T84, and HT-29) compared with normal colon and noncancerous cells (IEC6) (22, 45). LPA5 is also shown to be expressed at higher levels in small intestine and colon (19, 21, 30). With regard to LPA effects on intestinal Cl\(^{-}/\)OH\(^{-}\) exchange activity, our studies clearly showed the involvement of LPA2 and ruled out the role of LPA1 and 3 receptors utilizing LPA2 receptor agonists and LPA1/3 receptor agonists and antagonists, respectively. These findings are similar to previous studies showing the involvement of LPA2 receptors in inhibiting the cholera toxin-induced chloride secretion (22). Our results also showed that both luminal and serosal LPA stimulate apical anion exchange activity via LPA2 receptor. Consistent with previous studies (22), these data indicate the expression of LPA2 receptors at both the apical and basolateral membranes of the intestinal epithelial cells. These data suggest that both the luminal (dietary) and serosal (platelets) pools of LPA exert stimulatory effects on apical Cl\(^{-}/\)OH\(^{-}\) exchange activity. Further studies need to be done to determine the signaling mechanisms involved in the stimulation of Cl\(^{-}/\)OH\(^{-}\) exchange activity by LPA from the basolateral side.

LPA receptors are known to signal through multiple G proteins, which include Gi12/13, Gq, Gi, and Gs and trigger distinct intracellular signal transduction mechanisms in mediating the effects (15, 26). Studies have shown that LPA stimulates the PI3 kinase-Akt pathway via Gi in differentiated Caco-2 cells, which predominantly express LPA2 receptor (45). Our data showed that the effects of LPA on Cl\(^{-}/\)OH\(^{-}\) exchange activity were blocked in the presence of PI3 kinase and Akt inhibitors, indicating the involvement of PI3 kinase-Akt pathway. The involvement of PI3 kinase-dependent pathway is in agreement with other previous studies from our laboratory that have shown the involvement of PI3 kinase in the regulation of Cl\(^{-}/\)OH\(^{-}\) exchange activity by Lactobacillus acidophilus, taurodeoxycholic acid, and PMA (1, 8, 33). Furthermore, PI3 kinase has been shown to be involved in medi-
atting the effects of LPA on NHE3 in opossum kidney cell line (20). Our data also show the activation of Akt (Akt phosphorylation) by 100 μM LPA and by LPA2 receptor agonist (700 nM) in Caco-2 cells, further confirming the fact that PI3 kinase-Akt activation is downstream of LPA2 receptor.

Previous studies have suggested that PI3 kinase is involved in regulating the amount of transporter on the plasma membrane by translocating the transporters from the intracellular storage sites to the plasma membrane or retrieval of the transporters from the plasma membrane (18). Studies have shown that the stimulation of glucose uptake by interleukin-8 was a PI3 kinase-dependent process and that it involved the membrane trafficking of glucose transporter-1 to the membrane surface mediated via PI3 kinase/Akt pathway (44). In the same manner, our present data clearly demonstrate that LPA treatment significantly increased the surface levels of DRA with no change in the total cellular DRA level. These results are in agreement with our previous studies showing the involvement of PI3 kinase in regulating the surface levels of DRA by "Lactobacillus acidophilus" (8).

In summary, our results for the first time showed that LPA stimulates Cl−/OH− exchange activity in Caco-2 cells. On the basis of our results, we propose a model (Fig. 7) that LPA through LPA2 receptor and activation of PI3 kinase/Akt pathway increases Cl−/OH− exchange activity. In parallel to increased Cl−/OH− exchange activity, LPA increased the surface levels of DRA but not PAT-1. The results from these studies further enhance our understanding of the mechanisms regulating chloride absorption in the human intestine and also define the mechanisms underlying the beneficial effects of LPA as a potential anti diarrheal and therapeutic agent.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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