Modulation of ileal apical $\text{Na}^+$-dependent bile acid transporter ASBT by protein kinase C

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Sarwar Z, Annaba F, Dwivedi A, Saksena S, Gill RK, Alrefai WA. Modulation of ileal apical $\text{Na}^+$-dependent bile acid transporter ASBT by protein kinase C. Am J Physiol Gastrointest Liver Physiol 297: G532–G538, 2009. First published July 1, 2009; doi:10.1152/ajpgi.00052.2009.—Ileal apical $\text{Na}^+$-dependent bile acid transporter (ASBT) is responsible for reabsorbing the majority of bile acids from the intestinal lumen. Rapid adaptation of ASBT function in response to physiological and pathophysiological stimuli is essential for the maintenance of bile acid homeostasis. However, not much is known about molecular mechanisms responsible for acute posttranscriptional regulation of ileal ASBT. The protein kinase C (PKC)-dependent pathway represents a major cell signaling mechanism influencing intestinal epithelial functions. The present studies were, therefore, undertaken to investigate ASBT regulation in intestinal Caco-2 monolayers by the well-known PKC activator phorbol 12-myristate 13-acetate (PMA). Our results showed that $\text{Na}^+$-dependent $[^{3}\text{H}]$taurocholic acid uptake in Caco-2 cells was significantly inhibited in response to 2 h incubation with 100 nM PMA compared with incubation with 4s-PM (inactive form). The inhibitory effect of PMA was blocked in the presence of 5 $\mu$M bisindolylmaleimide I (PKC inhibitor) but not 1,2-bis(2-aminophenoxy)ethane-$N,N,N,N'$-tetraacetic acid-AM (Ca$^{2+}$ chelator) or LY-294002 (phosphatidylinositol 3-kinase inhibitor). PMA inhibition of ASBT function was also abrogated in the presence of myristoylated PKC$\zeta$ pseudosubstrate peptide, indicating involvement of the atypical PKC$\zeta$ isoform. The inhibition by PMA was associated with a significant decrease in the maximal velocity ($V_{\text{max}}$) of ASBT activity and a decrease in ASBT surface expression. Dysregulation of ASBT surface expression on the apical membrane of cholangiocytes was decreased by the hormone secretin, via a mitogen-activated protein kinase-dependent pathway (1). Because intestinal epithelial cells are exposed to constantly changing milieu, rapid posttranscriptional modifications by cellular intermediates is essential to adjust ASBT function in response to various stimuli. However, very little is known about the mechanisms involving posttranscriptional regulation of ASBT in intestinal epithelial cells.

In this regard, activation of signaling intermediates such as protein kinase C (PKC) has been previously shown to influence epithelial functions underlying diarrhea associated with intestinal disorders such as inflammatory bowel disease (8, 42). Previous studies have shown that short-term exposure to the PKC activator, phorbol 12-myristate 13-acetate (PMA), resulted in a decrease in intestinal $\text{Na}^+$, chloride absorption, and epithelial barrier function (14, 19, 33). The current studies were undertaken to investigate whether ASBT function is acutely regulated by PKC-dependent pathway in a posttranscriptional manner.

Acute regulation of ASBT function was examined in human intestinal Caco-2 monolayers by the DAG analog, phorbol ester PMA. Our studies showed that short-term incubation with PMA significantly decreased ASBT activity by a reduction in the maximal velocity ($V_{\text{max}}$) of the transporter. The inhibition by PMA appears to be mediated by PKC$\zeta$ and a decrease in ASBT surface expression on the plasma membrane. Dysregulation of ASBT function by the PKC-dependent pathway may underlie the pathophysiology of diseases associated with disturbances in bile acid homeostasis.

MATERIALS AND METHODS

Materials. Radionuclide $[^{3}\text{H}]$taurocholic acid (TC) was obtained from Perkin-Elmer (Boston, MA). Caco-2 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). PMA, 4s-PM (inactive form), 1,2-bis(2-aminophenoxy)ethane-$N,N,N,N'$-tetraacetic acid-AM (BAPTA-AM), LY-294002, bisindolylmaleimide I (BIM), and myristoylated PKC pseudosubstrate peptide inhibitors were obtained from Calbiochem (San Diego, CA).

Bile acids are secreted in the intestine to promote lipid and cholesterol absorption (2, 18). The majority of bile acids is avidly reabsorbed in the distal ileum and recycles back to the liver (2, 18). The apical $\text{Na}^+$-dependent bile acid transporter (ASBT) is responsible for the reabsorption of secreted bile acids in the distal ileum (2, 18). ASBT function represents a rate-limiting step in bile acid circulation from the intestine to the liver and thus plays a critical role in the maintenance of bile acid and cholesterol homeostasis (2, 15). A decrease in ASBT function causes bile acid malabsorption and increases the bile acid concentrations in the lumen (15). High decrease in ASBT function causes bile acid malabsorption and increase of bile acid and cholesterol homeostasis (2, 15). ASBT plays an important role in the maintenance of bile acid homeostasis and optimal intestinal function (2).

Dysregulation of ASBT function and expression has been implicated in the pathophysiology of diseases associated with bile acid malabsorption and diarrhea such as intestinal inflammation (35, 38). ASBT expression and promoter activity have been previously shown to be downregulated by inflammatory cytokines in intestinal epithelial cells (22). Interestingly, ASBT function has also been shown to be modulated via posttranscriptional mechanisms in cholangiocytes (1, 43). Recent studies demonstrated a decrease in ASBT expression by short-term exposure of cholangiocytes to cytokines through a ubiquitin-mediated mechanism (43). Also, ASBT surface expression on the apical membrane of cholangiocytes was decreased by the hormone secretin, via a mitogen-activated protein kinase-dependent pathway (1). Because intestinal epithelial cells are exposed to constantly changing milieu, rapid posttranscriptional modifications by cellular intermediates is essential to adjust ASBT function in response to various stimuli. However, very little is known about the mechanisms involving posttranscriptional regulation of ASBT in intestinal epithelial cells.

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were obtained from Biomol (Plymouth Meeting, PA). Affinity-purified rabbit polyclonal antibody against PKC and goat-anti-rabbit antibody conjugated to horseradish peroxidase (HRP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were of at least reagent grade and were obtained from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

**Cell culture.** Caco-2 cells were routinely grown in T-75 cm² plastic flasks in minimal essential medium (Eagle) supplemented with 20% FBS in a 5% CO₂-95% O₂ air environment at 37°C. For the uptake experiments, cells from between passages 20 and 25 were plated in 24-well plates at a density of 2 × 10⁴ cells/ml. Confluent monolayers were then used for transport experiments on the 14th day after plating (i.e., 6–8 days after confluence). To study the effect of phorbol esters on TC uptake, cells were rendered quiescent by overnight serum removal. Initial studies demonstrated that results generated in cells with serum removal for 4–6 h were similar to those generated utilizing overnight serum-starved cells. Na⁺/K⁺-ATPase activity was essentially measured as previously described (23).

**[³H]TC uptake.** Na⁺-dependent TC transport in Caco-2 cells was measured as previously described by us (5). Briefly, cells were equilibrated in preuptake solutions by incubation for 15 min at 25°C with buffer containing (in mM): 110 NaCl (with Na⁺) or choline chloride (without Na⁺), 4 KCl, 1 MgSO₄, 1 CaCl₂, 50 mannitol, and 10 HEPES, pH 7.4. Cells were then incubated with the same buffer containing the indicated concentration of TC along with 1 µCi/ml of [³H]TC for the designated period of time. Transport was then terminated by rapid washes with ice-cold PBS. Cells were then solubilized with 0.5 N NaOH for at least 4 h. The protein concentration was measured by the method of Bradford (6), and the radioactivity was counted by a Packard liquid scintillation analyzer (Tri-CARB 1600-TR; Packard Instrument, Downers Grove, IL). The uptake was measured at 5 min and was expressed as picomoles per 10⁴ cells. For the kinetic experiments, the uptake values were analyzed for simple Michaelis-Menten kinetics utilizing a nonlinear regression data analysis from a computerized model (GraphPad; PRISM, San Diego, CA).

**Subcellular fractionation.** Membrane and cytosolic fractions were prepared from Caco-2 cells treated with PMA or 4α-PMAM, as described by us (33). Caco-2 cells grown to confluence in 25-cm² flasks (Corning Costar) were washed with ice-cold PBS three times and scraped into 400 µl of the cold homogenization buffer (HB) containing (in mM) 20 Tris-HCl, pH 7.5, 250 sucrose, 4 EDTA, and 2 EGTA with complete protease inhibitor cocktail tablets (Boehringer Mannheim). The cells were homogenized on ice with 25 strokes of a glass homogenizer. The resulting homogenate was ultracentrifuged at 59,000 rpm for 50 min at 4°C (Optima TLX ultracentrifuge; Beckman). The supernatant was designated the cytosolic fraction. The pellet was resuspended in 150 µl of the HB containing 0.5% (vol/vol) Triton X-100 by brief sonication and incubated on ice for 30 min. At the end of the incubation period, the samples were centrifuged at 14,000 rpm for 20 min at 4°C. The resulting supernatant was designated the membrane fraction.

**Transfection experiments.** For transfection studies, Caco-2 cells were transfected utilizing the Amaxa Nucleofector System (Amaza) according to the manufacturer’s instructions. Briefly, ~2 X 10⁶ cells were harvested and then were electroporated in 100 µl of solution T (supplied by Amaza) along with 10 µg of vector containing ASBT-V5 fusion protein (5). The cells were then transferred to culture medium and plated on six wells of a 24-well plate.

**Cell surface biotinylation.** Cell surface biotinylation was performed using sulfo-NHS-SS-biotin (0.5 mg/ml; Pierce, Rockford, IL) in borate buffer (in mM: 154 NaCl, 7.2 KCl, 1.8 CaCl₂, and 10 H₂BO₃, pH 9.0), as previously described (5), with labeling for 60 min at 4°C to stop endocytosis and internalization of antigens. After immunoprecipitation of biotinylated antigens with streptavidin agarose, biotinylated proteins were released by incubation in 100 mM dithiothreitol, reconstituted in Laemmli buffer. The immunoprecipitates were subjected to SDS-PAGE, and blots were then probed with anti-V5 antibodies. To determine the specificity of biotinylation, the blots were stripped and reprobed with anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:1,000 dilution for 1 h).

**Cell surface biotinylation.** Equal amounts (~75 µg/sample) of protein were combined with Laemmli’s sample buffer containing 5% (vol/vol) β-mercaptoethanol and boiled for 5 min. Proteins were separated by electrophoresis on 10% SDS-PAGE and blotted to nitrocellulose membranes. The protein-bound nitrocellulose membranes were then incubated with the primary antibody in the blocking buffer containing 1× PBS, 0.1% Tween 20, and 5% nonfat dry milk. Nitrocellulose membranes were then incubated with HRP-conjugated goat anti-rabbit IgG antibody (1:2,000 dilution) for 1 h at room temperature and washed for 45 min with agitation, during which the wash buffer was changed every 5 min. Bands were visualized with enhanced chemiluminescence detection reagents.

**Results.** Results are expressed as means ± SE. Each independent set represents means ± SE of data collected on at least three separate occasions. One-way ANOVA was used for statistical analysis. P < 0.05 was considered statistically significant.

**RESULTS**

**Effects of phorbol ester PMA on ASBT function in Caco-2 cells.** To investigate the acute regulation of ASBT, we examined the effect of the conventional PKC activator phorbol ester, PMA, on Na⁺-dependent [³H]TC transport in human intestinal Caco-2 monolayers. Na⁺-dependent [³H]TC uptake in Caco-2 cells was significantly inhibited compared with incubation with 4α-PMMA (inactive form) in a time (Fig. 1A) and dose (Fig. 1B)-dependent manner. The maximal inhibition by PMA was attained by 2 h incubation with 100 nM concentration of PMA. These data clearly indicate the downregulation of ASBT activity by PMA.

Because ASBT activity is a Na⁺-dependent process, a decrease in its activity by PMA may be secondary to a decline in Na⁺ gradient across the apical plasma membrane. To examine whether the effect of PMA on TC uptake was specific and not secondary to inhibition of other processes, we examined the PMA effect on the Na⁺-dependent D-[¹⁴C]glucose uptake in Caco-2 cells. Figure 2 shows that incubation with 100 nM PMA had no effect on Na⁺-dependent D-[¹⁴C]glucose uptake compared with 4α-PMAM. Moreover, the activity of Na⁺/K⁺-ATPase was not significantly altered in response to 2 h incubation of 100 nM PMA in Caco-2 cells (170.3 ± 43 vs. 125 ± 41 µmol·mg protein⁻¹·min⁻¹ in 4α-PMAM- and PMA-treated cells, respectively, n = 3). Collectively, these findings demonstrate a specific inhibition of ASBT function by phorbol ester PMA.

**Phosphatidylinositol 3-kinase and intracellular Ca²⁺ are not involved in the inhibition of [³H]TC uptake in Caco-2 cells.** We have previously shown the activation of Ca²⁺-, and phosphatidylinositol 3-kinase (PI 3-kinase)-dependent pathways by PMA in Caco-2 monolayers (33). Therefore, we investigated the possible role of these pathways in the inhibition of TC uptake by PMA. Caco-2 monolayers were incubated with PMA in the presence and the absence of the Ca²⁺ chelator BAPTA-AM or the PI 3-kinase inhibitor LY-294002. The inhibition of TC uptake was unaltered in the presence of...
BAPTA-AM (Fig. 3A) and LY-294002 (Fig. 3B), ruling out the involvement of intracellular Ca\(^{2+}\) and PI 3-kinase in PMA-mediated inhibition of ASBT activity.

**Role of PKC.** Phorbol ester PMA is a potent activator of PKC (33). To test the involvement of PKC in PMA-mediated inhibition of ASBT activity, Caco-2 cells were incubated with 100 nM PMA for 2 h in the presence of BIM, which is known to inhibit PKC isoforms at different concentrations. As shown in Fig. 4, the presence of BIM at concentrations of 50 and 500 nM sufficient to inhibit conventional and novel isoforms of PKC, respectively, did not affect PMA-induced inhibition of TC uptake in Caco-2 cells. However, incubation with 5 \(\mu\)M concentration of BIM (adequate to inhibit the atypical \(\alpha\)PKC\(\zeta\) isoform) completely abrogated the inhibition of TC uptake by PMA in Caco-2 cells. These data suggest that activation of \(\alpha\)PKC\(\zeta\), but not conventional or novel isoforms of PKC, is responsible for the inhibition of ASBT by PMA in Caco-2 cells. Furthermore, the decrease in ASBT activity by PMA was blocked in the presence of myristoylated \(\alpha\)PKC\(\zeta\) peptide (Fig. 5A) but not myristoylated cPKC\(\alpha\) inhibitory peptide (Fig. 5B), further confirming the involvement of \(\alpha\)PKC\(\zeta\) in PMA-mediated inhibition of TC uptake in Caco-2 cells.

**PKC\(\zeta\) activation by PMA in Caco-2 cells.** To examine the activation of \(\alpha\)PKC\(\zeta\) in Caco-2 cells in response to 2 h incubation with 100 nM PMA, translocation of \(\alpha\)PKC\(\zeta\) from cytosolic membrane fractions was assessed by Western blotting. Figure 6 shows a representative Western blot demonstrating an increase in the association of \(\alpha\)PKC\(\zeta\) with membrane fractions of Caco-2 cells after 2 h incubation with 100 nM PMA that was blocked in the presence of 5 \(\mu\)M but not 50 nM concentration of BIM. These observations demonstrate the activation of \(\alpha\)PKC\(\zeta\) by PMA in Caco-2 cells and further confirm the notion that activation of \(\alpha\)PKC\(\zeta\) is involved in PMA-induced inhibition of \(^3\)H[TC]uptake.

**Effects of phorbol ester PMA on the kinetics of TC uptake in Caco-2 cells.** To further characterize PMA-induced inhibition of ASBT function in Caco-2 cells, we determined the effect of PMA treatment on the kinetic parameters of the uptake. Figure 7 demonstrates Na\(^{+}\)-dependent \(^3\)H[T]C uptake in the presence of increasing concentrations of TC and reveals a significant decrease in the \(V_{\text{max}}\) of the uptake in response to PMA treatment (160 ± 20 vs. 68 ± 24 pmol-mg protein\(^{-1}\)-5 min\(^{-1}\)) in 4α-PMA- and PMA-treated cells, respectively) with no alteration in the apparent Michaelis constant. These results indicate that exposure to PMA may result in a decrease in the number of active transporters on the surface of the apical membrane of Caco-2 cells.

**Phorbol ester PMA decreases ASBT membrane expression in Caco-2 cells.** Because the decrease in ASBT activity by PMA is associated with a reduction in the \(V_{\text{max}}\) of the transporter, we next examined alterations in ASBT expression on the plasma membrane. For these experiments, Caco-2 cells were transiently transfected with ASBT-V5 fusion protein by electroporation utilizing Amaza technology. Transfected cells
were treated with PMA or 4α-PMA for 2 h, and surface plasma membrane proteins were labeled with biotin. The distribution of ASBT-V5 expression in biotinylated (surface) and nonbiotinylated (intracellular) fractions of Caco-2 cells was examined by Western blotting utilizing anti-V5 antibodies, and ASBT level on plasma membrane was expressed as a ratio of surface/(surface/intracellular). ASBT is endogenously expressed in Caco-2 cells; however, cells transfected with ASBT-V5 fusion protein were used for a better detection of ASBT surface expression by V5 antibodies. As depicted in Fig. 8, ASBT-V5 fusion protein (~40 kDa) is detected by Western blot as two bands representing glycocylated and nonglycocy- lated forms of the transporter (5). Also, ASBT surface expression was decreased significantly by PMA compared with 4α-PMA in response to 2 h treatment with 100 nM phorbol ester, PMA. The decrease in ASBT activity was associated with a reduction in the V_max of the transporter and ASBT membrane level. Additionally, our findings showed the involvement of PKCα in the inhibition of TC uptake by PMA.

The human intestinal Caco-2 cell line was used in the current studies as a model for the small intestinal epithelial cells. Although Caco-2 cells are colonic in origin, they differentiate in culture as a function of time and exhibit a phenotype that resembles small intestinal epithelial cells (increased expression of alkaline phosphatase, sucrase isomaltase, and glucose and amino acid transporters) (25). Furthermore, Caco-2 cells have been previously used as an excellent model to investigate a number of intestinal transporters, including ASBT (4, 5, 7, 30, 31, 39, 41). Also, previous studies showed that observations obtained using Caco-2 cells were validated in an in vivo animal model demonstrating that Caco-2 cells are suitable as a model of small intestinal epithelial cells (12).

Recent studies by Sun et al. (37) indicated the presence of a potential serine-threonine phosphorylation site in the COOH terminal of rat ASBT, suggesting that the transporter could be a target for phosphorylation by protein kinases. Indeed, our data demonstrated the inhibition of ASBT function in response to PMA incubation of Caco-2 cells. This observed inhibition of thereby decreasing active ASBT molecules on the apical membrane of epithelial cells.

**DISCUSSION**

The current studies were designed to examine the acute regulation of ASBT by the PKC activator, phorbol ester PMA. The human intestinal Caco-2 cell line has been previously used as a model to study various small intestinal and colonic electrolyte and nutrient transport processes (5, 9, 12, 13, 16). We have previously shown that postconfluent Caco-2 monolayers express functional ASBT, representing an excellent model to investigate its regulation (4, 5). Our data demonstrated a significant inhibition of Na^+-dependent TC uptake in Caco-2 cells in response to 2 h treatment with 100 nM phorbol ester, PMA. The decrease in ASBT activity was associated with a reduction in the V_max of the transporter and ASBT membrane level. Additionally, our findings showed the involvement of PKCα in the inhibition of TC uptake by PMA.
TC uptake could be a result of decreasing the inwardly directed Na$^+$ gradient across the apical membrane of Caco-2 cells. However, the finding that the Na$^+$/H$^+$-dependent glucose cotransport process was unaffected in the presence of PMA negates this possibility and demonstrates the specificity of the effect of PMA on the function of ASBT.

Interestingly, PMA-mediated inhibition of TC uptake in Caco-2 cells was blocked in the presence of the PKC inhibitor BIM, indicating the involvement of PKC activation in the regulation of ASBT. In this regard, PKC family members are classified into the following three subfamilies (17): the conventional, Ca$^{2+}$/H$^+$- and DAG-dependent isoforms of PKC ($\alpha$, $\beta$, $\beta'$, $\gamma$, and $\delta$), the novel PKC isoforms that are DAG dependent but Ca$^{2+}$-independent (including $\epsilon$, $\eta$, and $\theta$), and the atypical Ca$^{2+}$- and DAG-independent isoforms of PKC that includes PKC$\zeta$ (17, 34). In the current study, the Ca$^{2+}$ chelator BAPTA-AM failed to ablate PMA-induced alteration of ASBT, ruling out the possible participation of the conventional, Ca$^{2+}$-dependent isoforms of PKC in the observed inhibition of ASBT by PMA. This notion was further supported by the failure of specific PKC$\alpha$ pseudosubstrate inhibitor to block BIM, indicating the involvement of PKC activation in the regulation of ASBT. In this regard, PKC family members are classified into the following three subfamilies (17): the conventional, Ca$^{2+}$- and DAG-dependent isoforms of PKC ($\alpha$, $\beta$, $\beta'$, and $\gamma$), the novel PKC isoforms that are DAG dependent but Ca$^{2+}$-independent (including $\delta$, $\epsilon$, $\eta$, and $\theta$), and the atypical Ca$^{2+}$ and DAG-independent isoforms of PKC that includes PKC$\zeta$ (17, 34). In the current study, the Ca$^{2+}$ chelator BAPTA-AM failed to ablate PMA-induced alteration of ASBT, ruling out the possible participation of the conventional, Ca$^{2+}$-dependent isoforms of PKC in the observed inhibition of ASBT by PMA. This notion was further supported by the failure of specific PKC$\alpha$ pseudosubstrate inhibitor to block
the effect of PMA on TC uptake. Furthermore, 500 nM concentration of BIM, which is sufficient to inhibit both conventional and novel isoforms of PKC (20), failed to abrogate the inhibition of ASBT by PMA, further ruling out the role of conventional and novel PKC isoforms.

The failure of PMA to inhibit TC uptake in the presence of 5 μM concentration of BIM (sufficient to inhibit atypical PKC isoforms) (20) indicated the possible involvement of the atypical PKCζ isoform. The possible role of the PKA-dependent pathway (which is also inhibited by 5 μM concentration of BIM) (40) was excluded utilizing PKA antagonist (Rp-cAMP) that failed to block the PMA-induced effect on ASBT (data not shown). Furthermore, the blockade of PMA effect on ASBT function in the presence of the pseudosubstrate inhibitor Myr-PKCζ provides compelling evidence for the involvement of PKCζ activation in the inhibition of ASBT in response to PMA treatment in Caco-2 cells. In parallel to the functional data, the activation of PKCζ by PMA was revealed in the current study by assessing its translocation from the cytosolic to membrane fraction of Caco-2 cells.

The atypical PKCζ isoform has been widely considered to be insensitive to phorbol ester (10, 27). However, this notion was recently challenged by many studies demonstrating PKCζ activation by PMA in various cell types, including Caco-2 cells (21, 24, 32, 36). We have recently shown the involvement of PKCζ activation by PMA in the modulation of monocarboxylate transporter MCT-1 (32). Similarly, our current studies strongly suggest an important role of PKCζ in the acute regulation of ASBT by PMA in the human intestinal Caco-2 cell line. Interestingly, the inhibition of PKCζ isoform has been recently demonstrated to increase ASBT expression and promoter activity (11). Collectively, these data demonstrate that PKCζ isoform mediates the regulation of ASBT both at the transcriptional and posttranscriptional levels.

ASBT function has been shown to be regulated by recycling of the transporter between the apical membrane and subapical intracellular compartment in cholangiocytes (1). In the current studies, PMA treatment of human intestinal Caco-2 monolayers led to a decrease in ASBT surface level as well as the Vmax of Na+-dependent TC uptake. The reduced level of ASBT on the apical membranes suggests a regulation via PKCζ-dependent mechanisms. It will be interesting to demonstrate future studies whether ASBT transporter is directly phosphorylated by PKCζ.

In conclusion, our studies demonstrated the inhibition of ASBT in response to phorbol ester treatment of Caco-2 cells via the activation of PKCζ. The inhibition of ASBT activity is associated with a reduction in the Vmax of the transporter along with a decrease in membrane content. Because intestinal epithelial cells are exposed to a rapidly changing milieu both from the luminal and serosal sites, this acute modulation via the PKCζ-dependent signaling pathway may represent a prompt response altering ASBT function and membrane expression subsequent to varying external stimuli. Dysregulation of this pathway may contribute to disturbances in bile acid homeostasis.

REFERENCES


GRANTS

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