Bile acids inhibit duodenal secretin expression via orphan nuclear receptor small heterodimer partner (SHP)

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Lam IPY, Lee LTO, Choi H-S, Alpini G, Chow BKC. Bile acids inhibit duodenal secretin expression via orphan nuclear receptor small heterodimer partner (SHP). Am J Physiol Gastrointest Liver Physiol 297: G90–G97, 2009. First published April 16, 2009; doi:10.1152/ajpgi.00094.2009.—Small heterodimer partner (SHP) is an orphan nuclear receptor in which gene expression can be upregulated by bile acids. It regulates its target genes by repressing the transcriptional activities of other nuclear receptors including NeuroD, which has been shown to regulate secretin gene expression. Here, we evaluated the regulation on duodenal secretin gene expression by SHP and selected bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA). In vitro treatment of CDCA or fexaramine elevated the SHP transcript level and occupancy on secretin promoter. The increase in the SHP level, induced by bile acid treatment or overexpression, reduced secretin gene expression, whereas this gene inhibitory effect was reversed by silencing of endogenous SHP. In in vivo studies, double-immunofluorescence staining demonstrated the coexpression of secretin and SHP in mouse duodenum. Feeding mice with 1% CA-enriched rodent chow resulted in upregulation of SHP and a concomitant decrease in secretin transcript and protein levels in duodenum compared with the control group fed with normal chow. A diet enriched with 5% cholestyramine led to a decrease in SHP level and a corresponding increase in secretin expression. Overall, this study showed that bile acids via SHP inhibit duodenal secretin gene expression. Because secretin is a key hormone that stimulates bile flow in cholangiocytes, this pathway thus provides a novel means to modulate secretin-stimulated choleresis in response to intraduodenal bile acids.

bile flow; choleresis; cholangiocyte; liver

Bile acids are synthesized from cholesterol in the liver (22). The two primary bile acids are cholic acid (CA) and chenodeoxycholic acid (CDCA) (22). Upon stimulation, bile acids in the bile are released from liver and gallbladder into the duodenum (4, 12). Bile acids emulsify oil into droplets to facilitate the digestion of fat and fat-soluble vitamins (4, 12). The secreted bile acids are reabsorbed at the terminal ileum and returned to the liver, thus completing their enterohepatic circulation (4, 22). Apart from facilitating fat digestion, bile acids also serve as signaling agents that activate bile acid receptors [e.g., farnesoid X receptor (FXR)] and thus control various physiological functions, such as cholesterol and bile acid homeostasis (8).

Bile acid homeostasis is a tightly controlled process involving small heterodimer partner (SHP, NR0B2), an atypical member of orphan nuclear receptors that lacks a conventional DNA-binding domain (27). SHP is highly expressed in liver, heart, brain, pancreas, and small intestine (19, 24, 27), and the SHP gene is regulated by several nuclear receptors, including FXR (NR1H4), liver receptor homolog-1 (LRH-1) (NR5A2), estrogen-related receptor-γ (NR3B3), estrogen receptor-α (NR3A1), liver X receptor (NR1H3), and steroidogenic factor-1 (NR5A1) (8, 19, 20, 27). Recently, the bile acid-activated SHP expression was found to be mediated by the binding of the heterodimer-consisting FXR with retinoid X receptor onto the mouse and human SHP promoters (14, 30). SHP itself is also an important modulator of nuclear signaling pathways by acting as a repressor antagonizing the activities of many nuclear receptors via a two-step mechanism. In the first step, SHP displaces coactivators, such as p300 (also known as cAMP response element-binding protein) and steroid receptor coactivator-1, by competing with the activation function-2 domain of nuclear receptors. In the second step, the transrepressor domain of SHP represses target genes by recruiting a corepressor histone deacetylase, mSin3A, and E1A-like inhibitor of differentiation-1 (17, 26, 27, 34).

Previously, SHP was demonstrated in vitro and in vivo to repress the activator activities of NeuroD, a cell-specific basic helix-loop-helix transcription factor, by inhibiting the interactions of NeuroD and p300 (19). The heterodimer of NeuroD and ubiquitously expressed transcription factors, E47 and E42, binds on the E-box within the secretin gene core promoter to regulate secretin gene expression (25, 31). However, there was no in vitro and in vivo evidence indicating a possible link between bile acids and SHP with secretin gene expression. The objective of this study is to find the missing link in this novel signaling pathway.

The roles of secretin as a gastrointestinal hormone are well established (23). One of the major known physiological activities of secretin is to stimulate bicarbonate-rich bile flow in intrahepatic bile duct (18). We proposed here a regulatory cascade that is mediated by bile acids and, hence, activation of SHP gene expression, which subsequently represses NeuroD/p300 interactions to inhibit secretin gene expression. This hypothetical pathway may be responsible for secretin-induced choleresis in intrahepatic bile duct via the control of secretin gene expression in the duodenal S cells.

MATERIAL AND METHODS

Cell culture. The human duodenal adenocarcinoma cell line, HuTu-80, was purchased from American Type Culture Collection (ATCC) and cultured in MEM (Invitrogen, Carlsbad, CA) with nonessential amino acids, 10% FBS (Hyclone, Logan, UT), 100 U/ml penicillin G,
and 100 μg/ml streptomycin (Invitrogen) at 37°C with 5% CO₂.

CDCA, cholestyramine (CY) (Sigma, St. Louis, MO), and fexaramine (Tocris, Ellisville, MO) were included in culture medium at the indicated concentrations for 24 h before further examination.

**Promoter constructs and shRNA-expressing vectors.** Constructions of the human secretin core promoter vector (p341; from −11 bp to −341 bp, relative to the start codon) and expression vectors of SHP, NeuroD, and E47 (SHP-pcDNA3.1, NeuroD-pCMV, and E47-pCMX) were previously described (19, 25). The mouse secretin core promoter construct (from −27 to −399 bp) was produced by PCR amplification from mouse genomic DNA and subcloning into the pGL2-Basic vector. Two shRNAs (siSHP-1 and siSHP-2, Table 1) were designed, and the annealed oligos were inserted into the ApaI and EcoRI sites of the pSilencer shRNA expression vector (Ambion, Austin, TX). As a negative control, siControl (Ambion) with a sequence that shares no significant identity to any known gene sequence was used. Sequences of all the oligos used in this study were shown in Table 1.

**Transient transfection and luciferase assay.** HuTu-80 cells were plated at a density 1.5 × 10⁵ cells/35-mm well (6-well plate; Costar, San Diego, CA). After 2 days of incubation, 2 μg promoter-luciferase construct, 0.5 μg pCMV-β-gal, and various amounts (0.5–2.0 μg) of Table 1.

**Table 1. Nucleotide sequences of siRNA oligos and primers for real-time PCR assay**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>siSHP-1</td>
<td>5′GATGCCTGACCTTTGGATCGACCTGGAGATCTTTTTT3′</td>
</tr>
<tr>
<td>Sense</td>
<td>5′ATTAAAATATTTCTGCGGAGGACGTTGTACAGCATCTTTTTT3′</td>
</tr>
<tr>
<td>Antisense</td>
<td>5′TTCTTCGAGACGTGACGAGAATTTTTT3′</td>
</tr>
<tr>
<td>siSHP-2</td>
<td>5′ATGATGACCTTTGGAGATCTTTTTT3′</td>
</tr>
<tr>
<td>Sense</td>
<td>5′ATTAAAATATTTCTGCGGAGGACGTTGTACAGCATCTTTTTT3′</td>
</tr>
<tr>
<td>Antisense</td>
<td>5′TTCTTCGAGACGTGACGAGAATTTTTT3′</td>
</tr>
<tr>
<td>hGAPDH-F</td>
<td>5′CATGAACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>hGAPDH-R</td>
<td>5′GTTCGAGACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>hSHP-F</td>
<td>5′CATGAACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>hSHP-R</td>
<td>5′GTTCGAGACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>mGAPDH-F</td>
<td>5′CATGAACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>mGAPDH-R</td>
<td>5′GTTCGAGACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>mSHP-F</td>
<td>5′CATGAACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>mSHP-R</td>
<td>5′GTTCGAGACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>NS</td>
<td>5′CATGAACGAGAGTTGTTCGCTC3′</td>
</tr>
<tr>
<td>NAS</td>
<td>5′GTTCGAGACGAGAGTTGTTCGCTC3′</td>
</tr>
</tbody>
</table>

h, human; m, mouse; SHP, small heterodimer partner; F, forward; R, reverse; NS, normal sense; NAS, normal antisense.

Fig. 1. Relationship between small heterodimer partner (SHP) and secretin gene expression in human duodenal HuTu-80 cells. A: effects of over-expressing SHP on secretin gene promoter. p341 (2.0 μg), and various amounts of SHP-pcDNA3.1 (0, 0.5, 1.0, and 2.0 μg) were cotransfected into HuTu-80 cells. B: real-time quantitative PCR to show the relative mRNA levels of secretin and S14 in HuTu-80 cells transiently transfected with the indicated amount of SHP vector. C: effects of silencing endogenous SHP on secretin promoter activity. p341 (2.0 μg) was cotransfected with various amounts of siSHP-1 (1.0 and 2.0 μg), siSHP-2 (1.0 and 2.0 μg), and siControl. D: Top: Western blot analysis of SHP protein in HuTu-80 cells transfected with pSilencer (1), siSHP-1 (2), siSHP-2 (3), and siControl (4). Bottom: Coomassie blue staining of a SDS-PAGE ran in parallel as a loading control; *P < 0.001; **P < 0.05 vs. p341 2.0 μg.
expression or silencing vector (SHP-pcDNA3.1, NeuroD-pCMV, and E47/pCMX) were cotransfected into the cells with GeneJuice (Novagen; EMD Biosciences, Darmstadt, Germany) according to the manufacturer’s protocol. Cell extracts were harvested 48 h after transfection and used for luciferase and β-galactosidase assays as described previously (25).

Western blotting. Western blotting was performed as described earlier (6). HuTu-80 cells were transfected with 2 μg SHP-1, SHP-2, siControl, or siSHP vector. Two days after transfection, the cells were lysed in RIP buffer (50 mM Tris, pH 7.4, 0.25% Na-deoxycholate, 1% NP-40, 150 mM NaCl, and 1 mM EDTA). For immunoprecipitation, 100 μg of cell lysate was incubated with 1 μg goat anti-NeuroD antibody (Santa Cruz Biotechnology, Santa Cruz, CA), followed by protein A/G-agarose. The nuclear extracts and precipitated protein were resolved by 10% SDS-PAGE and transferred onto the Hybond-C extra membrane (Amersham Biosciences, Piscataway, NJ). SHP and FXR were detected by goat anti-SHP and goat anti-FXR antibodies, anti-goat horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology), and the ECL reagent (Piscataway, NJ). SHP and FXR were detected by goat anti-SHP and goat anti-FXR antibodies, anti-goat horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology), and the ECL system (PerkinElmer Life Sciences, Boston, MA).

ChIP assay. The chromatin immunoprecipitation (ChIP) assays were performed essentially according to Ref. 33. In brief, HuTu-80 cells were cross linked with 1.42% formaldehyde and harvested in the IP buffer [1% 150 mM NaCl, 50 mM Tris·HCl (pH 7.5), 5 mM EDTA, 0.5% NP-40, 1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin]. After sonication, DNA-protein complex was precipitated with 2 μg antibody (goat anti-SHP and goat anti-NeuroD antibodies) followed by 20 μl protein A-agarose. DNA was isolated in Chelex 100 (BioRad, Hercules, CA) slurry, and the protein was removed by proteinase K digestion (0.2 μg/ml). The purified DNA was used for real-time quantitative PCR with the normal sense and normal antisense primers (Table 1).

Animal models and treatment. All animal handling procedures were approved by the Committee on the Use of Live Animal in Teaching and Research (CULATR) of the University of Hong Kong. Age-matched C57/BL6 mice (8–10 wk old) were fasted for 24 h and fed ad libitum with either standard rodent chow (No. 5010; Test Diet, Richmond, IN) or rodent chow enriched with 1% CA (Sigma) for 1 day. A separate group of mice were fed with normal diet or 5% CY-enriched diet for 10 days. After treatment, duodenums (1 cm) were isolated from the mice (more than 6 for each experimental group) for RNA and protein extraction.

Real-time quantitative PCR analysis. Total RNA from mouse duodenum or transfected cells was isolated by the TriPure isolation reagent (Roche Molecular Biochemicals, Basel, Switzerland), and 5 μg of total RNA was reverse transcribed with oligo-dT primer and Superscript III reverse-transcriptase (Invitrogen). One-tenth of the
first strand cDNAs was used as the template in quantitative real-time PCR. Human secretin transcript levels were measured by an Assay on Demand System (assay ID: Hs00360814_g1; Applied Biosystems, Foster City, CA) with the TaqMan Universal Master Mix (Applied Biosystems). SHP, GAPDH, S14, and mouse secretin transcript levels were monitored by the SYBR Green PCR Master Mix kit (Applied Biosystems) with specific primers (Table 1). The fluorescence signals were measured in real time during the extension step by the 7300 Real-Time PCR System (Applied Biosystems). The specificity of the SYBR Green PCR signal was confirmed by melting curve analysis and agarose gel electrophoresis. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence signal reaches 10-fold standard deviation of the baseline (from cycle 2 to 10). The ratio change in target gene relative to the GAPDH control gene was determined by the $2^{-\Delta\Delta Ct}$ method (29).

**Enzyme immunoassay.** Mouse duodenums were homogenized in isolation buffer (250 mM sucrose, 25 mM imidazole, 10 mM triethanolamine hydrochloride, 1 μg/ml leupeptin, and 1 mg/ml phenylmethylsulfonyl). Protein extracts were collected after centrifugation, and secretin levels were measured by enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, Belmont, CA) according to the manufacturer’s manual.

**Double immunofluorescence staining.** Duodenum from C57/BL6 mouse was fixed in 3.7% formalin, embedded in paraffin, and sectioned (7 μm). After deparaffinization, rehydration, and permeabilization in PBS supplemented with 2% bovine serum albumin and 0.5% Triton X-100, sections were blocked in a 10% chicken serum solution and incubated overnight with rabbit anti-human SHP antibody (1:300 dilution; MBL International, Woburn, MA), followed by Alexa Fluor 594 chicken anti-rabbit IgG (1:500 dilution; Molecular Probes, Eugene, OR). Sections were blocked again by donkey serum (10%) and incubated at 4°C overnight with goat anti-mouse secretin antibody (1:150, Santa Cruz Biotechnology), followed by Alexa Fluor 488 chicken anti-rabbit IgG (1:500 dilution, Molecular Probes). Images were captured with the Zeiss LSM 510 Meta computerized image analysis system.

**Data analysis.** Data from the transfection assays are shown as the means ± SE of triplicate assays in at least three independent experiments. Data from the quantitative PCR analysis and EIA are shown as the means ± SE of duplicate assays in at least three independent experiments. Data were analyzed by either one-way ANOVA followed by Dunnett’s test, or unpaired t-test using the computer software PRISM (version 3.0; GraphPad Software, San Diego, CA).

**RESULTS**

**SHP inhibits secretin gene expression.** To evaluate the regulatory effect of SHP on secretin gene expression, the human secretin core promoter-luciferase construct was cotransfected with SHP-pcDNA3.1 into the duodenal HuTu-80 cells. Overexpression of SHP dose dependently inhibited secretin promoter activity (Fig. 1A; 75% reduction with 2.0 μg SHP).
pcDNA3.1) as well as endogenous secretin transcript level (Fig. 1B; 58% reduction with 2.0 μg SHP-pcDNA3.1). The specificity of SHP inhibition on secretin gene expression was confirmed by a negative control measuring the transcript level of S14 (Fig. 1B). To further support the inhibitory activities of SHP on secretin gene expression, an approach using shRNA to silence endogenous SHP was employed. Two shRNA expression vectors (siSHP-1 and siSHP-2) were constructed and transfected into HuTu-80 cells. Consistently, silencing of SHP significantly elevated secretin promoter activity by 2.4- and 1.8-fold with 2.0 μg siSHP-1 and 2.0 μg siSHP-2, respectively (Fig. 1C), whereas transfection of the control vectors pSilencer and siControl had no effects on the core promoter. The down-regulation of endogenous SHP was confirmed by Western blotting (Fig. 1D), and reduced expressions of SHP were observed in both siSHP-1- and siSHP-2-transfected cells (lanes 2 and 3) when compared with the controls (pSilencer and siControl, lanes 1 and 4, respectively). Taken together, these data suggest that changes in SHP levels negatively modulate the expression of secretin and subsequently the expression of secretin in duodenal HuTu-80 cells.

SHP suppresses secretin promoter activity by inhibiting NeuroD transcriptional activity. One possible mechanism by which SHP suppresses secretin promoter activity is via inhibition of NeuroD, an activator that had previously shown to interact and regulate both human and rat secretin genes (25, 31). Since the mechanism through which SHP inhibits NeuroD was thoroughly studied (19), the following experiments were designed to confirm the putative relationship between SHP and NeuroD on regulating secretin gene expression. Initially, the physical interaction between SHP and NeuroD in HuTu-80 cells was confirmed by coimmunoprecipitation (Fig. 2A). Next, we demonstrated the function of NeuroD by cotransfecting NeuroD expression vector with the secretin core promoter-luciferase construct into HuTu-80 cells. Overexpression of either NeuroD alone or together with E47 markedly stimulated secretin promoter activity, whereas overexpression of E47 alone had no significant effect (Fig. 2B). Consistently, coexpression of SHP suppressed the activator functions of NeuroD or NeuroD/E47 significantly (P < 0.001).

In summary, these in vitro data confirmed the effects of SHP and NeuroD on the E-box motif in regulating the human secretin gene. Because it is well known that secretin is a bile secretagogue (23) and bile acids in bile stimulate SHP synthesis (8, 14, 30), we therefore hypothesized that bile acids could regulate secretin gene expression via the cascade involving SHP. Before substantiating this hypothesis in the in vivo mouse model, we reconfirmed previous observations in the mouse secretin gene. A mouse secretin promoter-luciferase was constructed and cotransfected with SHP-pcDNA3.1 into HuTu-80 cells. Consistently, overexpression of SHP reduced (44% reduction with 2.0 μg SHP-pcDNA3.1; Fig. 3A, P < 0.001), while silencing of endogenous SHP by siSHP-1 and siSHP-2 upregulated, the mouse secretin promoter (126% and 75%, respectively; Fig. 3B, P < 0.001 and P < 0.05, respectively). These data thus confirmed that SHP could repress both human and mouse secretin genes. By our comparison of the promoter sequences of human and mouse secretin gene (Fig. 3C), both promoters were found to include an E-box and two GC-boxes, which are the binding sequences for NeuroD and Sp proteins, respectively, and further suggested that these two genes are potentially controlled by similar regulatory mechanisms.

![Double fluorescence immunohistochemistry of secretin and SHP in mouse duodenum. Secretin was detected by Alexa Fluor 594 green fluorescent secondary antibody, whereas SHP was stained by Alexa Fluor 488 red fluorescent secondary antibody. The merged image shows the colocalization of secretin and SHP, indicated by the arrow. In the control, PBS was used instead of the primary antibodies. Scale bar = 20 μm.](http://ajpgi.physiology.org)
Bile acids increase SHP expression and decrease secretin expression. Before examining whether bile acids could upregulate SHP in duodenal cells as previously described in the liver (5, 8, 14, 30), the presence of FXR in mouse duodenum was confirmed by Western blotting (Fig. 4A). Bile acid treatment was performed in HuTu-80 cells with CDCA (50, 100, and 200 μM) and fexaramine (5, 25, and 50 μM) for 24 h. Both bile acid and FXR agonist were able to upregulate SHP mRNA levels in HuTu-80 cells, and CDCA (200 μM) and fexaramine (50 μM) increased SHP transcript levels by 1.9- and 2.1-fold, respectively (Fig. 4B). In consistency with the upregulation of SHP gene by bile acids, ChIP assay demonstrated that CDCA treatment resulted in increased SHP binding on the human secretin promoter (Fig. 4C). However, no significant effect on NeuroD occupancy was detected. Meanwhile, the cells were treated with various concentrations of CDCA and fexaramine, and the endogenous secretin transcript levels were significantly reduced (48% in 200 μM CDCA and 33% in 50 μM fexaramine; Fig. 4D). In addition, CDCA and fexaramine treatment also significantly suppressed secretin promoter activity by 30% and 32%, respectively (Fig. 4E). To further confirm that bile acid-induced reduction on secretin expression was mediated via SHP, endogenous SHP was silenced by shRNA (2.0 μg siSHP-1), followed by CDCA and fexaramine treatment on the cells for 24 h. The knockdown of SHP function in HuTu-80 cells was associated with the loss of inhibitory effect of bile acids on secretin promoter activity (Fig. 4F). These in vitro data clearly indicate a link between bile acids, SHP, and secretin gene expression in duodenal cells.

In vivo effects of bile acids on SHP and secretin levels in mouse duodenum. We first demonstrated the colocalization of SHP and secretin in mouse duodenum by double-immunofluorescence staining. Representative microscopy images revealed that SHP and secretin were coexpressed in the epithelial cells of villi (Fig. 5). In the other cell layers, including mucosa, submucosa, muscle, and serosa, no positive signals were found (personal observations). This suggested the potential of SHP to regulate secretin expression in the duodenal cells.

To provide in vivo evidence for the effects of duodenal bile acids on SHP and secretin expression, mice were fed with CA-enriched diet (1%) for 1 day following fast of 24 h, which ensured that a significant amount of the diet was consumed in...
the provided time and prevented the side effects of chronic feeding of bile acid on animals. The CA diet caused a 33% increase in SHP (Fig. 6A), 21% reduction in secretin transcript levels (Fig. 6B), and 0.5-fold decrease in protein level (Fig. 6C) compared with the control group fed with normal diet.

Aside from charging the mice with bile acid, we also demonstrated the in vivo effects of duodenal bile acids by feeding the mice with a bile acid chelator, CY, that can reduce biliary bile acids concentration by twofold after the rat was fed with 5% CY for 10 days (21). After 10 days of CY-enriched rodent chow (5%) diet treatment, the transcript levels of SHP and secretin decreased 23% (Fig. 6D) and increased 43% (Fig. 6E), respectively. In parallel, CY diet upregulated secretin protein levels by 7.2-fold compared with the controls (Fig. 6F).

**DISCUSSION**

The present study provided in vitro and in vivo evidence demonstrating that intestinal expression of secretin gene is controlled by the bile acid-SHP inhibitory cascade. A previous finding on the inhibitory effect of SHP on NeuroD transcriptional activity was substantiated in human duodenal cells that NeuroD-driven secretin gene expression was suppressed by SHP. In mouse, the CA diet increased the bile acid content in the duodenum, leading to the upregulation of SHP gene and its transcriptional inhibitory activity. Despite the fact that structures of primary bile acids are modified by bacterial enzymes in the intestine (15) and their taurine-conjugated forms are the most potent activators for FXR (36), administration of a single form of bile acid would be insufficient to represent the effect of a pool of bile acids on duodenal secretin gene expression. Moreover, not all bile acids activate FXR; for instance, ursodeoxycholic acid is a FXR antagonist and is used in bile acid displacement therapy in some liver diseases to reduce the cytotoxicity of circulating bile acids (16). Therefore, an experimental approach using CY to reduce biliary bile acid content was adopted. Correspondingly, the CY diet diminished the bile acid activation effect on SHP gene transcription and eventually upregulated secretin gene expression. In addition, the immunofluorescence staining demonstrated the colocalization of SHP- and secretin-immunoreactive cells in the mouse duodenum (Fig. 5), suggesting the potential of SHP to regulate secretin gene in duodenum S cells in an in vivo environment.

Aside from secretin gene regulation, the bile acid-SHP regulatory cascade was demonstrated to regulate enzymes and transporters involved in bile acid synthesis and transport, thus conferring a pivotal role on SHP in bile acid homeostasis (8). The transporters involved in bile acid synthesis and transport, thus regulatory cascade was demonstrated to regulate enzymes and num (Fig. 5), suggesting the potential of SHP to regulate SHP- and secretin-immunoreactive cells in the mouse duodenum. Correspondingly, the CY diet diminished the bile acid concentration by twofold after the rat was fed with 5% CY for 10 days (21). After 10 days of CY-enriched rodent chow (5%) diet treatment, the transcript levels of SHP and secretin decreased 23% (Fig. 6D) and increased 43% (Fig. 6E), respectively. In parallel, CY diet upregulated secretin protein levels by 7.2-fold compared with the controls (Fig. 6F).

**REFERENCES**


