INTRODUCTION

Systemic bile acids (BAs) fluctuate postprandially and are involved in the emulsification and absorption of dietary fats and lipid-soluble vitamins. Primary BAs, Chenodeoxycholic acid (CDCA), and cholic acid (CA; in humans) are the product of hepatocyte cholesterol metabolism. They are readily absorbed (~95%) across the terminal ileum via the apical sodium-dependent bile acid transporter, SLC10A2 (ASBT,
also known as the ileal BA transporter, IBAT), while the organic solute transporter (Ostα/Ostβ) is responsible for BA movement across basolateral membranes, delivering conjugated BAs into the lamina propria. BAs that remain in the lumen (~5%) can be deconjugated, oxidized, and dehydroxylated by microbial enzymes. The resultant secondary BAs (eg, deoxycholic acid, DCA; and lithocholic acid, LCA) are thought to be absorbed passively across the colonic epithelial lining.2

Conjugated and deconjugated BAs differentially alter motility, contributing to “ileal brake” that enhances nutrient absorption in the upper GI tract, while in the colon, they increase motility, cause defecation,3 and are laxative. The prokinetic effect is probably linked to increased epithelial electrolyte and fluid secretion4,5 most likely via sensory enteric neurons activated by 5-hydroxytryptamine (5-HT).6 In contrast, the mucosal effects of selective G protein-coupled BA receptor (GPBA) agonism are not well understood. Abnormalities in BA delivery to the distal bowel are known to result in GI dysfunction. Increased colonic BA delivery, for example, following bariatric surgery or reduced ileal absorption as a consequence of inflammatory bowel disease, is associated with diarrhea,6,7 while reduced colonic BA delivery (eg, as a consequence of BA sequestrants to treat lipid disorders) is associated with constipation.7,8

Recently, BAs have been identified as chemosensory molecules with important roles in regulating lipid, glucose, and energy metabolism.9 They exhibit affinity for two receptors, the membrane-located GPBA (previously known as TGR510) and the nuclear farnesoid X receptor (FXR11). Both receptors are expressed by nutrient-sensing enteroendocrine L cells, which predominate in mammalian distal ileum and colon,12 although GPBAR1 expression appears to be consistent along the length of the murine and human GI tract.13 L cells in the distal ileum and colon release the satiety hormone, peptide YY (PYY) and the incretin, glucagon-like peptide 1 (GLP-1). Another incretin, glucose-dependent insulinotropic peptide (GIP) is a major product with important roles in regulating lipid, glucose, and energy metabolism.9 They exhibit affinity for two receptors, the membrane-located GPBA (previously known as TGR510) and the nuclear farnesoid X receptor (FXR11). Both receptors are expressed by nutrient-sensing enteroendocrine L cells, which predominate in mammalian distal ileum and colon,12 although GPBAR1 expression appears to be consistent along the length of the murine and human GI tract.13 L cells in the distal ileum and colon release the satiety hormone, peptide YY (PYY) and the incretin, glucagon-like peptide 1 (GLP-1). Another incretin, glucose-dependent insulinotropic peptide (GIP) is a major product secreeted by proximal small intestinal K and L cells in response to nutrient metabolites and BAs.14-16 Synthetic, high-affinity GPBA agonists have been shown to stimulate GLP-1,17,18 PYY, and neurotensin release from colonic organoids.19 They also lower blood glucose in rodent models,20,21 and GPBA remains a target with therapeutic potential for treating metabolic diseases.9

Unexpectedly, GPBA is trafficked predominantly to basolateral epithelial membranes,17,21-23 and therefore, BA or drug absorption is a necessary step for efficacy at this receptor. Consequently, lumen-restricted GPBA ligands are likely to be less efficacious than readily absorbed ligands. Systemic BA signaling, for example, to taurodeoxycholate (TDCA), increases L cell cAMP, and Ca2+, notably to a greater extent than either selective GPBA agonist (GP-A) or FXR agonist, GW4064.17 GP-A causes GLP-1 release preferentially via basolateral GPBA,21 and in preliminary studies, we observed more rapid PYY-mediated responses when GP-A was applied to mucosa basolaterally.22 BA-mediated PYY release has been widely demonstrated in rodent GI tract14,21,24-26 and human colon following luminal DCA.23,27,28 The elevation in circulating PYY and GLP-1 following ileal BA absorption indicates that L cell GPBA signaling has antiobesity (PYY-mediated) and type 2 anti diabetic (GLP-1-mediated) potential.

Thus, our initial aim was to establish the similarities and differences in mucosal mechanisms stimulated by a GPBA-selective agonist compared with a BA such as TDCA, focusing on ileal and colonic mucosa from the mouse and including human colonic mucosa. The time dependence of luminal vs basolateral TDCA responses (at a low concentration, 100 µmol/L) was performed using two proven GPBA synthetic agonists (GP-A,17 and Merck V19), each administered to ileal or colonic preparations with intact submucosal innervation, from wild-type (WT), PYY−/− or GPBA−/− mice. Further aims were to establish (a) whether luminal GPBA responses required ASBT in ileal and colonic mucosa, and (b) to determine whether the GPBA response was glucose-dependent, as is the case for other L cell mechanisms.29-33 Finally, we investigated the neuronal and non-neuronal mediators of GPBA-specific mucosal signaling and motility in vitro, in WT, PYY−/−, and GPBA−/− colon in order to better understand the paracrine consequences of selective GPBA stimulation.

2 | MATERIALS AND METHODS

2.1 | Materials

BIBO3304, BIE0246, zamifenacin fumarate, and nifedipine were purchased from Tocris, and GSK2330672 was from BioVision, while TTX, RS39604, and tropisetron were from Abcam Biochemicals. PYY, extendin-4, and extendin (9-39) were purchased from Generon, and VIP and ω-conotoxin GVIA were from AnaSpec. Merck V was a generous donation to TWS from Merck & Co and is the same agonist utilized by Hauge et al.18 All other chemicals were purchased from Sigma-Aldrich. BIBO3304, BIE0246, RS39604, tropisetron, and the GPBA small molecule agonists were each dissolved in neat DMSO at 1 mmol/L. BAs were dissolved in water except for LCA, which was
soluble in 95% ethanol. All peptide stocks were in aqueous solution, frozen, and stored at -20°C, undergoing one freeze-thaw cycle only.

2.2 | Methods

2.2.1 | Ethical approval

Animal experimentation and care were compliant with UK Home Office regulations (Animals Scientific Procedures Act 1986) and were approved by the local KCL Ethics Committee. Mice were housed in open-top cages under a 12-hour light-dark cycle (0700-1900 hours) at 20-24°C and 55 ± 10% humidity, with free access to standard chow and tap water. WT tissues (10 weeks or older, males and females) were obtained from C57BL/6 mice (from Charles River) or from knockouts with a mixed C57BL/6-129SvJ background. Germine PYY−/− mice were on the same mixed background, as were GPBA+/+ and their germline GPBA−/− littermates. Animals were killed by cervical dislocation and the GI tract excised for in vitro studies.

Human colonic tissue was gained from eight consenting patients (six males and two females, mean age: 68.5 ± 4.2 years) undergoing surgery for primary colon carcinoma (as approved by the Research Ethics Committee of Guy’s and St Thomas’s National Health Service Foundation Trust).

2.2.2 | Measurement of short-circuit current (Isc) and transepithelial resistance (TER)

Mucosal sheets were prepared from mouse duodenum, jejunum, terminal ileum, proximal and distal colons, or normal human colon, by removing the smooth muscle and myenteric plexus, leaving the submucosal innervation and the mucosal layer intact. Dissected preparations were mounted in Ussing chambers, bathed in Kreb’s-Henseleit (KH) buffer (constituents in mmol/L: NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, and glucose 11.1) and voltage-clamped at 0 mV, recording the resultant short-circuit current (Isc, in µA) continuously as described previously. Preparations were equilibrated for 20 minutes prior to experimentation. Some mucosae were exposed to vasoactive intestinal polypeptide (VIP; 10 nmol/L) to cause cAMP-dependent anion secretion, some were not, in order to establish GPBA agonism in basal conditions. The changes in Isc are primarily due to altered electrogenic Cl⁻ secretion as indicated by previous studies using rat and mouse mucosae. Trans-epithelial resistance (TER) was monitored throughout experiments by delivering a 1 mV pulse every 250 seconds, recording the resultant Isc deflection, and applying Ohm’s law. Initially, we tested several BAs (cholic acid [CA], deoxycholic acid [DCA], hyodeoxycholate [HDCA], TDCA, and lithocholic acid [LCA] each administered apically at 2 mmol/L), based upon data from rat intestine showing significant BA-mediated PYY release. TDCA was chosen as the BA for further investigation in WT and knockout tissues, and its activity was compared with two synthetic GPBA agonists, Merck V and GP-A (utilized previously). Throughout these experiments, we recorded absolute changes in Isc from the point of drug addition.

To study the underlying mechanisms of GPBA agonism, pharmacological investigations in WT colon utilized optimized pretreatments with the ASBT inhibitor, GSK2330672 (10 µmol/L, apically), or basolateral exposure to cholinergic antagonists, hexamethonium (Hex, 100 µmol/L) and atropine (Atr, 1 µmol/L), the neurotoxin, tetrodotoxin (TTX, 100 nmol/L, basolateral), or the dipeptidyl peptidase IV (DPPIV) inhibitor, sitagliptin (1 µmol/L), the 5-HT₄ antagonist, tropisetron (100 nmol/L, 5-HT₄ antagonist, RS39604 (RS, 1 µmol/L), the GLP-1 agonist, exendin(9-39) (Ex(9-39), 1 µmol/L), or the PYY Y₄ receptor agonist, BIBO3304 (BIBO, 300 nmol/L) alone or in combination with the Y₂ receptor antagonist, BIIE0246 (BIIE, 1 µmol/L). Ca²⁺ channel blockers, CdCl₂ (100 µmol/L, blocks most Ca²⁺ channels), L-type inhibitor nifedipine (100 µmol/L), and P/Q-type blocker ω-conotoxin GVIA (1 µmol/L), were used to interrogate TDCAs responses in GPBA−/− tissue. Each pretreatment lasted 20 minutes prior to the agonist of choice. When glucose sensitivity was tested, glucose was replaced with mannitol (at 11.1 nmol/L) on either the apical or basolateral surface. The SGLT1 inhibitor, phlorizidin (50 µmol/L, apically), was used as a control to show loss of electrogenic transport in the presence of apical mannitol.

2.2.3 | Natural fecal pellet transit

The movement of natural fecal pellets along the colon was performed in vitro as described previously. Briefly, the entire colon was excised from WT, PYY−/−, or GPBA−/− mice. The colonic length was measured from cecum to rectum and photographed to provide the initial positions of fecal pellets (t = 0 minute). Colons were placed in KH buffer with either vehicle (0.1% DMSO or KH) or Merck V (1 µmol/L) or TDCA (at 100 µmol/L or 1 mmol/L) for 20 minutes before taking a second photograph (t = 20 minutes) to establish fecal pellet transit. In PYY−/− colons, the mediation by GLP-1 or nitric oxide (NO) was tested using Ex(9-39) (1 µmol/L, to block GLP-1 receptors) or the NOS inhibitor L-NNA (1 mmol/L, respectively. Here, colons were incubated with vehicle or drugs for 20 minutes prior to addition of Merck V (1 µmol/L) and exposure continued for a further 20 minutes (t = 20-40 minutes) before the position of pellets was recorded.

2.2.4 | Statistical analyses

Responses were expressed as the mean ± 1 SEM from pooled individual preparations taken from different animals. Quoted means were the responses observed at either a given time point for time-course studies or were the peak responses within 5-15 minutes following drug administration. Data from in vitro colonic motility studies were expressed as a percentage of total colon length. GraphPad Prism...
(version 7.03, GraphPad Software) was used to establish statistical differences, using one-way ANOVA with either Tukey’s or Dunnett’s post-tests as appropriate for multiple data groups, and Student’s t test for single comparisons.

3 | RESULTS

3.1 | Basal electrical parameters of mucosae from different murine GI areas and human colon

Basal levels of Isc and TER values in different intestinal tissues are compared in Table S1. WT terminal ileum mucosa exhibited significantly higher Isc values than WT jejunum or distal colon. WT proximal colon Isc levels were higher than those of the distal colon. Terminal ileum exhibited significantly lower TER than WT jejunum, proximal, and distal colon mucosae. There were no statistical differences between WT and PYY−/− jejunal or distal colonic Isc or TER values, but PYY−/− jejunal basal Isc was higher (and TER lower) than mucosa from the PYY−/− distal colon. There were no significant differences in either parameter between GPBA+/+ and GPBA−/− colon, or between GPBA+/+ and WT colon mucosae. Human colon mucosa exhibited basal Isc values of 82.4 ± 7.8 μA cm⁻² (n = 23) and TERs of 26.7 ± 7.6 Ω cm² (n = 23).

3.2 | Tissue survey, sidedness, and time courses of Isc responses to Merck V or TDCA in WT and GPBA−/− tissues

An initial survey of responses to the synthetic GPBA agonist Merck V or to TDCA was undertaken using pairs of adjacent preparations from the duodenum, jejunum, terminal ileum, proximal, and distal colon. In tissues exposed to vehicle (0.1% DMSO was the vehicle for Merck V), the Isc changed transiently after apical addition (by 0.8 ± 2.0 μA/cm² (n = 5) and, by −0.5 ± 0.3 μA/cm² (n = 3) after VIP in descending colon), returning to the original Isc level within 10 minutes. Water was the vehicle for bile salts, and it had no effect on Isc (Figure S1I). Apical Merck V-induced significant monophasic reductions in Isc (i.e., antisecretory responses) that were larger in the terminal ileum, proximal, and distal colon (Figure 1A). Apical TDCA also exerted monophasic antisecretory effects at 100 μmol/L, with a similar pattern of sensitivity (Figure 1B). Time-course analysis of apical agonism showed Merck V responses were delayed in WT descending colon and absent in GPBA−/− colon (Figure 1C). Apical TDCA (at 100 μmol/L) also reduced Isc levels, rapidly in WT colon, but the BA had no effect on GPBA−/− colon at this low concentration (Figure 1D). Basolateral Merck V responses were quicker than apical agonism in WT colon, and GPBA−/− tissue was insensitive to basolateral ligand (Figure 1E). However, in GPBA−/− colon, basolateral TDCA increased Isc rapidly, reaching a maximum 2 minutes after BA application (Figure 1F). Clearly, this activity was not GPBA-mediated. Mucosal responses to an unrelated GPR119 agonist, PSN632408 (10 μmol/L), were similar in WT and GPBA+/− descending colon (WT: -11.1 ± 2.3 μA/cm² (n = 5), GPBA−/−: -13.9 ± 3.3 μA/cm² (n = 5). P > .05, NS), exhibiting the same response observed previously in WT mucosae, indicating normal L cell Gα₁₅-mediated signaling. Additionally, PYY (10 nmol/L) responses were the same in mucosae from WT and GPBA−/− tissues (WT: -27.0 ± 5.9 μA/cm² (n = 5), GPBA−/−: -41.7 ± 11.3 μA/cm² (n = 5), P > .05, NS). Because basolateral Merck V and TDCA responses in WT tissue exhibited a fast initial, transient increase in Isc (termed the 1° response), followed by a longer-lasting reduction in Isc (termed the 2°, antisecretory response, Figure 1E,F), we analyzed these components separately.

Concentration dependence was observed for responses to apical and basolateral Merck V, GP-A, and TDCA (Figure 1G,H). The synthetic agonists were similarly efficacious on either mucosal surface (Figure 1G). Basolateral TDCA activity, however, was biphasic, in contrast to monophasic apical TDCA responses, at 0.3 and 1.0 mmol/L, where the increases in Isc were rapid and large (Figure 1H). No maxima were discerned for these 1° responses so pEC50 values were not calculated for this component. However, pEC50s for basolateral (bl) 2° responses were the same as those for apical (ap) addition of Merck V pEC50 values (6.8 ± 0.2 [bl] vs 6.8 ± 0.2 [ap]). GP-A pEC50 values were similar (6.3 ± 0.3 [bl] vs 6.3 ± 0.2 [ap]) as was TDCA potency (4.1 ± 0.1 [bl] vs. 4.0 ± 0.1 [ap]). Apical TDCA reduced Isc levels slightly in GPBA−/− colon, but this change in Isc was significantly smaller across the concentration range compared with apical TDCA responses in WT colon (Figure 1H).

A comparison of TERs prior to and 5–6 minutes after agonist addition was not significantly altered by TDCA (100 μmol/L). TER values were 42.2 ± 5.7 Ω cm² before and 45.1 ± 5.5 Ω cm² (n = 5) 5–6 minutes after apical administration; and 34.1 ± 2.5 Ω cm² before and 40.2 ± 3.6 Ω cm² (n = 6) 5–6 minutes after basolateral addition of this BA to WT colon. These values were consistent with previous observations pre- and post-Merck V addition to either mucosal surface (see supplemental data in 22).

The ability of four other natural BAs and TDCA was tested (apically at 2 mmol/L) in jejunal and distal colon mucosae from WT and PYY−/− mice. Mucosae were initially pretreated with VIP (10 nmol/L) to raise intracellular epithelial Gα₁₅-mediated cAMP levels and thus amplify subsequent Gα₁₅-coupled (PYY-mediated) epithelial effects. Biphase responses were observed with each BA added to WT jejunum and colon, with an immediate increase in Isc (1° response) followed by a slower (2°) decrease in Isc (Figure S1A,B). The most consistent difference between mucosae from these genotypes was a loss of the reductions in Isc (the 2° response) in PYY−/− tissues. As a consequence, the 1° increases in Isc were amplified to DCA, hyodeoxycholic acid (HDCA), and TDCA in PYY−/− tissues (Figure S1B). In addition, DCA, CA, and HDCA decreased basal Isc levels in human colon (Figure S1C). Since TDCA exhibited significant efficacy at 100 μmol/L in mouse colon, where L cell storage of PYY and enterocyte Y1 receptor signaling is most robust, this concentration of BA was used in subsequent experiments.


3.3 | Apical TDCA responses require ASBT activity in mouse distal ileum and colon

ASBT is expressed predominantly in the terminal ileum on apical surfaces, and we used the inhibitor GSK2330672 to test whether apical TDCA or GPBA agonism was dependent on transporter activity. TDCA responses were investigated in mucosae under basal or secretagogue-stimulated (ie, with VIP) conditions. In both situations, GSK2330672 significantly inhibited apical TDCA responses in terminal ileum (Figure 2A) and distal colon mucosa (Figure 2B). GSK2330672 had no effect on basolateral TDCA responses in either region (Figure 2C,D). This indicates that GPBA is located predominantly on basolateral surfaces and that apical BAs are absorbed rapidly across the epithelium via ASBT, before activating basolateral GPBA. In contrast, responses to apical Merck V or GP-A were unaffected by GSK2330672 (Figure 2E). In human colon mucosa,
basolateral TDCA responses were biphasic (as seen in mouse tissue) while apical activity was monophasic, with partial sensitivity to the ASBT inhibitor, although this was not statistically significant ($P = .15$; Figure 2F).

### 3.4 | GPBA-induced reductions in Isc are PYY-Y$_1$ mediated, whereas the initial increases in Isc are partially cholinergic and 5-HT mediated in colon mucosa

Since BAs can activate L cells to cause the release of PYY and GLP-1, we postulated that the 2° antisecretory response may be due to local PYY-Y$_1$ receptor activation (as observed for other L cell nutrient receptor activators). We included the Na$^+$ channel blocker, TTX in this study in order to inhibit all submucosal neurotransmission. TTX had no effect on the Merck V or TDCA antisecretory responses in mouse (Figure S2A) or human (Figure 3B) colon mucosa, indicating a post-junctional, non-neuronal GPBA mechanism in tissue from both species. This was true of Merck V responses along the length of the mouse gut (Figure S2A). In WT colon, the antisecretory Merck V response was abolished by the Y$_1$ antagonist BIBO3304 (± Y$_2$ antagonist, BIIE0246; Figure 3A). The combination of these antagonists blocked both Merck V and TDCA responses in human distal colon (Figure 3B) indicating involvement of endogenous PYY in GPBA-induced antisecretory effects, again consistent with observations in mouse colon. The DPPIV inhibitor sitagliptin, which prolongs GLP-1 and PYY half-lives, did not alter Merck V responses significantly in mucosae from different GI regions (Figure S2B).

Basolateral TDCA 2° antisecretory responses were also abolished by Y$_1$ blockade, whereas the transient 1° increases in Isc were potentiated compared with vehicle controls (Figure 3C). TTX had no significant effect on either phase, nor did the GLP-1 receptor antagonist, Ex(9-39) (Figure 3D), discounting neural and GLP-1 mechanisms in these basolateral BA responses. However, the cholinergic antagonists, hexamethonium and atropine, or the 5-HT$_4$ antagonist RS39604, partially inhibited the 1° TDCA response (Figure 3D). Taken together, this indicates that TDCA co-stimulates enterochromaffin cell 5-HT secretion, and specifically, cholinergic neurotransmission, in addition to L cell PYY release in WT mouse distal colon mucosa with intact submucosal innervation.

Because Y$_1$/Y$_2$ antagonists abolished the 2° response to basolateral TDCA in WT mucosa, we next used PYY$^{-/-}$ tissues to focus on the 1° component aiming to confirm 5-HT and cholinergic neuron mediation. First, we observed that basolateral Merck V was inactive in PYY$^{-/-}$ colon (Figure 3E) as shown previously.

![Figure 2](image-url) **Figure 2.** ASBT involvement in apical BA-mediated but not Merck V responses in terminal ileum and descending colon. In (A) TDCA (100 µmol/L, Ap), responses in mouse terminal ileum (TI) and in (B) the descending colon (DC) were inhibited significantly by GSK2330672 (±GSK233, 10 µmol/L) with or without VIP (10 nmol/L), whereas in (C, D), the biphasic effects (1° and 2° changes in Isc) following basolateral (Bl) TDCA were unaltered under basal conditions (no VIP). In (E), apical ASBT blockade with GSK2330672 (10 µmol/L) had no effect on apical (Ap) Merck V (300 nmol/L) or GP-A (1 µmol/L) responses in mouse DC. In (F) comparison of TDCA (100 µmol/L) responses after basolateral (bl) or apical (ap) addition under baseline conditions (±GSK2330672 (10 µmol/L, or vehicle DMSO, 0.1%) in human descending colon ($P = .15$). Bars are pooled from n values as indicated, and data are expressed as mean ± 1 SEM. $P < .05$, **$P < .01$, and ***$P < .001$, using Student’s $t$-test.
(Figure 1E). The transient increase in Isc to basolateral TDCA was, however, inhibited significantly by 5-HT antagonists and inclusion of cholinergic blockers abolished these basolateral BA responses in PYY−/− colon (Figure 3F). Subsequent 5-HT (1 µmol/L) responses were abolished by the combination of 5-HT3 and 5-HT4 antagonists (Control: 29.3 ± 8.5 µA/cm² (n = 4), plus RS & Trop: 1.0 ± 1.0 µA/cm² (n = 4) * P < .02).

To investigate the non-GPBA mechanisms underpinning basolateral TDCA responses, we pretreated GPBA−/− colon with different Ca²⁺ channel antagonists (ie, CdCl₂, a non-selective blocker; nifedipine, the L-type inhibitor; or the P/Q-type blocker, ω-conotoxin). Nifedipine or CdCl₂ reduced TDCA responses significantly, while ω-conotoxin had no effect (Figure 3G). Proven blockers of endogenous 5-HT (RS39604 and tropisetron), or cholinergic mechanisms (Figure 3H) had no significant effect on GPBA−/− colon responses to basolateral TDCA, indicating an alternate target for this secretory aspect of low concentration (100 µmol/L) BA signaling.

3.5 | Glucose dependence of Merck V and TDCA colonic Isc responses

Following substitution of apical glucose by mannitol, Merck V and TDCA responses were attenuated (Figure 4A-D). The SGLT inhibitor, phloridzin’s electrogenic response, was inhibited by mannitol replacement of apical glucose (confirming glucose removal), whereas exogenous PYY responses were unaffected. The 2° component of
the basolateral TDCA response (Figure 4C) and the apical BA antisecretory response (Figure 4D) were each inhibited significantly by apical mannitol, indicating the glucose dependence of these signals. However, the 1° response to basolateral TDCA was not significantly reduced following removal of apical glucose. As expected, removal of basolateral glucose had no effect on Merck V or TDCA responses, or upon phloridzin’s electrogenic effect, as SGLT1 is located on apical surfaces.

3.6 | Merck V slows natural fecal pellet progression and requires GLP-1

Fecal pellet movement was unaltered by Merck V in WT colon (Figure 5A), but it was reduced in PYY−/− colons (Figure 5B) where the basal (control) rate of pellet movement was higher compared with WT transit (as seen previously for PYY−/− transit). To investigate whether endogenous GLP-1 or NO mediated the inhibitory GPBA effect (in the absence of PYY), we utilized optimal concentrations of the blockers, Ex(9-39) (1 µmol/L) or LNNA (1 mmol/L), neither of which altered motility significantly (Figure 5C). Each inhibitor reversed the antimotile Merck V response, indicating the involvement of endogenous GLP-1 and NO in GPBA agonism in PYY−/− colon (Figure 5D). In contrast with the inhibitory GPBA-mediated effect, TDCA (at 100 µmol/L) was pro-motile in WT colon, significantly so (Figure 5E). The faster transit rate of PYY−/− colons was unaltered by TDCA (at 100 µmol/L or 1 mmol/L; Figure 5F). Finally, in GPBA+/+ colons Merck V reduced, and TDCA increased transit (Figure 5G; as seen in the WT cohort; Figure 5A,E) although not statistically significant (P = .07, one-way ANOVA). Control GPBA−/− transit was slightly faster than WT transit (not significantly; Figure 5H), and Merck V or TDCA were inactive (Figure 5H) in these knockout colons. The only significant difference was between the transit observed for Merck V in WT vs GPBA−/− colon, indicating Merck V’s requirement for GPBA expression.

4 | DISCUSSION

BAs stimulate incretin hormone GLP-1 and GIP secretion. Plasma taurine- and glycine-conjugated BAs peak at concentrations (~10 µmol/L) after a test meal and correlate positively with plasma GLP-1 and PYY levels. Taurine-conjugated BA, TCA (at 5-50 mmol/L), stimulates PYY, alongside GLP-1 and GIP release in rat small intestine. Recently, Christiansen et al observed that a physiologically relevant BA mixture caused PYY and GLP-1 co-release from normal rat and mouse colon in vivo, but neither hormone was released from GPBA−/− colon when stimulated with BAs. Given the robust endogenous PYY−Y1 receptor epithelial signaling present in mouse and human colon, we focussed primarily on this peptide's mediation of mucosal GPBA responses. PYY and GLP-1, however, alter epithelial ion transport via discrete mucosal pathways, and this mechanistic understanding permitted the pharmacological investigation utilizing WT, GPBA−/−, and PYY−/− tissues and normal human colon mucosa. In addition, appreciation that neural 5-HT3 and epithelial 5-HT4 receptors mediate endogenous 5-HT-induced ion

**FIGURE 4** Glucose sensitivity of Merck V and TDCA decreases in Isc in mouse distal colon mucosa. Merck V added basolaterally (in A) or apically (B) reduced Isc levels in colonic mucosa. Only after substitution of apical glucose (Glc) by mannitol (Mann) were Merck V responses attenuated. Similarly, SGLT1 inhibitor, phloridzin (Phlor, 50 µmol/L ap. an internal control), effects were absent with apical mannitol. The 2° effects of basolateral (C) or apical TDCA (100 µmol/L; D) were inhibited when apical glucose was replaced by mannitol. Responses to phloridzin (in C and D) were absent when apical glucose was replaced by mannitol. In (A-D), PYY (10 nmol/L) responses were unaltered by glucose replacement on either side. Bars are the mean ± 1 SEM from n values as shown; *P < .05, **P < .01, and ***P < .001 using one-way ANOVA with Dunnett’s post-test.
secretion (44 and Cox et al unpublished) allowed inclusion of these mechanisms in our study. Ligand specificity was important, so the synthetic GPBA agonist Merck V was included, as previous studies had shown it causes PYY and GLP-1 secretion from colonic crypts.19

**FIGURE 5** Merck V reduces colonic motility independent of PYY, but dependent on GPBA. Merck V (1 µmol/L) effects on natural fecal pellet transit in isolated colons from (A) WT and (B) PYY−/− mice, over a 20-minute period compared with vehicle control (DMSO, 0.1% throughout except for E). In (C), pretreatment of PYY−/− colons with either vehicle (control), Ex(9-39) (1 µmol/L), or LNNA (1 mmol/L; Control, t = 0-20 minutes) and in (D) each blocker reversed the effect of subsequent Merck V (t = 20-40 minutes), displaying a similar transit to that observed with PYY−/− controls in B (dashed lines). TDCA increased WT colonic transit significantly (in E) compared with water control, and this effect (F) was absent in PYY−/− colon. Comparison of Merck V (1 µmol/L) and TDCA (100 µmol/L) activities in GPBA+/+ (G), and GPBA−/− colon (in H). Bars are the mean ± 1 SEM from 5 specimens throughout. *P < .05, **P < .01 using Student’s t test (A, B and E) or one-way ANOVA compared with controls (in C and G), Merck V (D), or TDCA (in F). ** P < .01 (in H) compares the Merck V response in GPBA−/− colon with that in WT tissue (in G; Student’s t test)

The mucosal GPBA antisecretory response observed in WT tissues with Merck V (and another synthetic ligand, GP-A17,22) or, with TDCA added to either mucosal surface, was both found to be PYY-mediated, and this was consistent in mouse and human mucosae. However, basolateral TDCA exerted additional mucosal activities in mouse tissue (discussed below, and Figure 6). Antisecretory responses were observed with 10 µmol/L TDCA, which is in the region of postprandial total plasma BA levels12 and thus is physiologically relevant. Higher TDCA concentrations (>300 µmol/L) elevated Isc levels in a GPBA-independent manner, so we selected ~ EC50 concentrations (ie, 100 µmol/L TDCA or 300 nmol/L Merck V) in subsequent investigations. Interestingly, although the potencies of Merck V and GP-A were similar, their efficacies were blunted compared with the less potent TDCA, added to either surface. This difference in efficacy may be linked to the selectivity of the former ligands relative to several co-incident BA activities3,4,5,6, some of which may amplify epithelial signaling and/or directly activate the apical cystic fibrosis transmembrane conductance regulator.67 The latter activity may contribute to the increase in Isc observed after apical application of a high concentration (2 mmol/L) of TDCA or other BAs that was more prominent in PYY−/− compared with WT colonic and jejunal mouse mucosae. It is not clear why monophasic (rather than biphasic) Isc responses were observed in human colon to apical DCA, CA, or HDCA, but it should be noted that only three specimens contributed to this dataset.

Establishing the sidedness of mucosal BA signaling was important. Comparison of Merck V (or GP-A) with TDCA responses added to either surface revealed marked differences in agonist kinetics. Apical Merck V responses were slower to peak than apical TDCA responses, and both activities were slower than basolateral Merck V activity, indicating that GPBAs are preferentially basolateral and the synthetic agonist’s passage across the epithelium was slower than that of the BA. The greater efficacy of basolateral Merck V and TDCA antisecretory responses also indicates preferential localization of GPBA on basolateral cell surfaces, as suggested previously.17,21,22,48 Basolateral GPBA agonism was not only more rapid, it was also biphasic, particularly for TDCA (>300 µmol/L) and at the highest concentration used (10 µmol/L) of Merck V or GP-A. Furthermore, apical ileal and colonic TDCA responses required transport via ASBT (Figure 6). We showed that ASBT inhibition (with GSK2336702) attenuated the apical TDCA response but had no effect on Merck V or GP-A activity as these small molecules do not utilize ASBT but can diffuse passively across mucosae. Our findings concur with the basolateral signal preference of GPBA-induced GLP-1 release from mouse distal ileum and perfused rat intestine.17 Christiansen et al28 observed ASBT-dependent and independent absorption of a mixture of conjugated BAs across rat colon, while unconjugated BAs appeared to diffuse passively. Our mouse studies showed the ASBT inhibitor blocked apical TDCA responses by 74%-92% in ileum (depending on the basal or VIP-stimulated conditions), and in mouse colon, BA responses were inhibited by 60%-71%. Using the conjugated BA, TDCA, or a number of other unconjugated BAs (CA, DCA,
and LCA), we observed consistent endogenous PYY involvement. These antisecretory responses were absent from PYY−/− colon. The ASBT-insensitive activity of TDCA-induced PYY responses may be similar to those described by Christiansen et al.28 It is clear, however, that conjugated BAs are absorbed swiftly via ASBT across ileal and colonic mucosae.

Responses to Merck V or GP-A (added to either surface) or apical TDCA were absent from GPBA−/− tissues, indicating a common GPBA-specific antisecretory mechanism in WT tissues. This response was also absent in PYY−/− colon, and from WT and importantly human colon mucosa, pretreated with PYY-Y1 and Y2 antagonists. Thus in WT mouse and human colon, endogenous PYY mediates GPBA antisecretory signaling (Figure 6). In contrast, the rapid Isc increase after basolateral TDCA administration to GPBA−/− colon was GPBA-independent. This nifedipine-sensitive BA signal appears to utilize L-type Ca2+ channels, plus a small component of P/Q-type Ca2+ channel activity. This broader BA pharmacology matches that seen in ileal organoids, where TDCA exerted significant effect on L-type and P/Q-type Ca2+ channels causing GLP-1 release.48 Together, these studies show that TDCA-induced Ca2+ channel signaling is significant in intact colonic GPBA−/− mucosa. This channel activity is presumably ongoing but is masked in WT tissue by the coincident effects of endogenous PYY and other mediators of GPBA mucosal signaling (see below).

While the L cell GPBA signaling was consistent in WT mouse and human mucosae, basolateral TDCA activity involved coincident mechanisms that included epithelial 5-HT3 and enteric cholinergic responses.45,48,49 Both 5-HT and cholinergic secretory effects were more evident in PYY−/− colon (where the peptide’s antisecretory effects were absent). Notably, Merck V was inactive in PYY−/− mucosa, indicating that no other electrogenic epithelial mechanisms are stimulated by this GPBA agonist. TDCA, on the other hand, is reported to be a partial agonist at M3 receptors50 (present on basolateral surfaces) and when stimulated would elevate Isc levels transiently via a Gαq-Ca2+ mechanism. While we found that muscarinic signaling contributed to TDCA’s initial secretory action in WT colon; in GPBA−/− tissue, this component of the BA response was insensitive to several cholinergic blockers including atropine, and the M3 antagonist, zanifenacin. Thus, it is unlikely that TDCA acts directly as a partial M3 agonist in mouse colon. TDCA’s capacity to activate L-type Ca2+ channels may contribute to the 5-HT3-mediated and cholinergic secretory effects that we observed in PYY−/− and GPBA−/− colon.

The GPBA-PYY response was glucose-sensitive, irrespective of whether Merck V or TDCA was used and this requirement for glucose complements studies with primary cultures.37 Interestingly, the initial increases in Isc to basolateral TDCA were not significantly glucose-sensitive, implicating an alternative non-L cell mechanism. Our mechanistic findings also complement those in diabetic human
volunteers who exhibited significantly raised levels of PYY, GLP-1, and insulin, alongside reduced food intake and plasma glucose levels following intrarectal administration of taurocholate. We also observed the antisercretory GPBA response was not amplified by the DPPIV inhibition indicating that endogenous PYY and consequent Y1 signaling is potentially maximal. The basolateral BA response was also insensitive to GLP-1 receptor antagonism, indicating limited GLP-1 mediation in the mouse distal colon. This may however be due to the GPBA-1 response in descending colon being much smaller than that of the proximal colon.

The GLP-1 mediation of Merck V’s inhibitory effect upon fecal pellet movement was clear and complements studies showing GPBA-mediated GLP-1 secretion, the peptide’s well described antimotile GI effects and inhibition of gastric emptying. In WT and PYY+/– colons, we found Merck V slowed fecal pellet transit, which was reversed by blockers of GLP-1 and NO signaling. A significant degree of co-expression between GPBA and NOS occurs in myenteric neurons (90% of NOS-positive cells co-express GPBA), and the marked inhibition of spontaneous phasic contractions of proximal colon by DCA complements our observation of slower fecal pellet progression following GPBA stimulation. Notably, we found the slightly higher rate of fecal pellet transit in GPBA+/– colon was unaltered by Merck V, or TDCA (at 100 μmol/L) showing that at this concentration the BA’s antimotile effect was GPBA-dependent.

Enhanced delivery of BA to the colon as observed following ASBT inhibitor administration can result in diarrhoea, and this pathophysiological prokinetic effect (when luminal BA levels can reach high, mmol/L concentrations) most likely involves 5-HT release from EC cells, and possibly also neurons, CGRP release from intrinsic sensory neurons (both submucosal and myenteric IPANs) resulting in the stimulation of peristalsis, alongside coincident electrolyte secretion (for the mucosal GPBA mechanisms, see Figure 6). 5-HT, CGRP, and cholinergic activity exert robust epithelial anion secretion, the former via 5-HT3 and CGRP via epithelial CGRP receptors. We established that a combination of 5-HT3 and cholinergic activities was responsible for the mucosal secretory response to basolateral TDCA administration in the mouse distal colon. Therefore, the EC cell (5-HT3-mediated) and L cell (PYY, with GLP-1 mechanisms occur coincidentally upon GPBA stimulation in the colon, an area where the two cell types account for the overwhelming majority of colonic enteroeendocrine cells.

In conclusion, our results highlight the complexity of acute GPBA signaling within the ileal and colonic mucosa; luminal BAs require ASBT-mediated absorption in both GI regions prior to receptor activation (Figure 6). GPBA therefore senses predominantly systemic, rather than luminal BAs, alongside which deconjugated BAs are able to diffuse passively across the epithelium (not shown in Figure 6) and thus access basolateral GPBA. GPBA agonism results in L cell, EC cell hormone, and neurotransmitter release, with consequent local antisercretory (PYY-mediated), antimotility (GLP-1-mediated), and even transient pro-sercretory (5-HT-mediated) activities. The resolution of these coincident pathways contributes to our understanding of GPBA signaling and cautions against future luminally restricted GPBA agonists (with fewer systemic side effects) that may lack ef-ficacy because they cannot readily reach their basolateral receptor targets when the epithelium is intact.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

HC designed the research with input from TWS. IRT performed the experiments, and all three authors contributed to the writing of the paper. All authors reviewed the final manuscript.

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REFERENCES

1. Lieu T, Jayaweera G, Bunnett NW. GPBA: a GPCR for bile acids and an emerging therapeutic target for disorders of digestion and sensation. Br J Pharmacol. 2014;171:1156-1166.
2. Hegyi P, Maleth J, Walters JR, Hofmann AF, Keely SJ. Guts and gall: bile acids in regulation of intestinal epithelial function in health and disease. Physiol Rev. 2018;98:1983-2023.
3. Alemi F, Poole DP, Chiu J, et al. The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. Gastroenterology. 2013;144:145-154.
4. Moschetta A, Portincasa P, Debellis L, et al. Basolateral Ca2+-dependent K+ channels play a key role in Cl– secretion induced by taurodeoxycholate from colon mucosa. Biol Cell. 2003;95:115-122.
5. Bajor A, Gillberg PG, Abrahamsson H. Bile acids: short and long-term effects in the intestine. Scand J Gastroenterol. 2010;45:645-664.
6. Arow FL, Dekovich AA, Priest RJ, Beher WT. Bile acid-mediated postcholecystectomy diarrhoea. Arch Intern Med. 1987;147:1327-1329.
7. Keely SJ, Walters JRF. The Farnesoid X receptor: good for BAD. Cell Mol Gastroenterol Hepatol. 2016;2:725-732.
8. Knodel LC, Talbert RL. Adverse effects of hypolipidaemic drugs. Med Toxicol. 1987;2:10-32.
9. McGlone ER, Bloom SR. Bile acids and the metabolic syndrome. Mol Gastroenterol Hepatol. 2016;2:725-732.
10. Kawamata Y, Fujiy R, Hosoya M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003;278:9435-9440.
11. Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. Science. 1999;284:1362-1365.
12. Sundler F, Ekblad E, Håkanson R. Localisation and colocalisation of gastrointestinal peptides. In: Brown DR, ed. Gastrointestinal Regulatory Peptides, vol. 106. Springer Verlag; 1993:1-28.
13. Symonds EL, Peiris M, Page AJ, et al. Mechanisms of activation of mouse and human enteroendocrine cells by nutrients. Gut. 2015;64:618-626.

14. Mace OJ, Schindler M, Patel S. The regulation of K- and L-cell activity by GLUT2 and the calcium-sensing receptor CasR in rat small intestine. J Physiol. 2012;590(12):2917-2936.

15. Egerod KL, Engelstoft MS, Grunddal KV, et al. A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1 PYY and neurotensin but not somatostatin. Endocrinology. 2012;152:5782-5795.

16. Habib AM, Richards P, Cairns LS, et al. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. Endocrinology. 2012;153:3054-3065.

17. Brighton C, Rievaj J, Kuhre RE, et al. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. Endocrinology. 2015;156:3961-3970.

18. Hauge M, Ekberg JP, Engelstoft MS, et al. Gq and Gs signaling acting in synergy to control GLP-1 secretion. Mol Cell Endocrinol. 2017;449:64-73.

19. Grunddal KV, Ratner CF, Svendsen B, et al. Neurotensin is co-expressed, co-released and acts together with GLP-1 and PYY in enteroendocrine control of metabolism. Endocrinology. 2016;157:176-194.

20. Thomas C, Gioiello A, Noriega L, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. 2009;10:167-177.

21. Ullmer C, Alvarez Sanchez R, Sprecher U, et al. Systemic bile acid sensing by G protein-coupled bile acid receptor 1 (GPRAR1) promotes PYY and GLP-1 release. Br J Pharmacol. 2013;169:671-684.

22. Tough IR, Forbes S, Herzog H, et al. Bidirectional GPR119 agonism requires PYY and glucose for activity in mouse and human colon mucosa. Endocrinology. 2018;159:1704-1717.

23. Kuhre RE, Wewer Albrechtsen NJ, Larsen O, et al. Bile acids are important direct and indirect regulators of the secretion of appetite- and metabolism-regulating hormones from the gut and pancreas. Mol Metab. 2018;11:84-95.

24. Ballantyne GH, Longo WE, Savoca PE, et al. Deoxycyclate-stimulated release of peptide YY from the isolated perfused rabbit left colon. Am J Physiol. 1989;257:G715-G724.

25. Plaisancié P, Dumoulin V, Chaïvialle J-A, et al. Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. J Endocrinol. 1996;151:421-429.

26. Dumoulin V, Moro F, Barcelo A, et al. Peptide YY, glucagon-like peptide-1 and neurotensin responses to luminal factors in the isolated vascularly perfused rat ileum. Endocrinology. 1998;139:3780-3786.

27. Adrian TE, Ballantyne GH, Longo WE, et al. Deoxycyclate is an important releaser of peptide YY and enteroglucagon from the human colon. Gut. 1993;34:1219-1224.

28. Christiansen CB, Trammell S, Albrechtsen NJW, et al. Bile acids drive colonic secretion of GLP-1 and PYY in rodents. Am J Physiol. 2019;316:G574-G584.

29. Cox HM, Tough IR, Woolston A-M, et al. Peptide YY is critical for acetylcholinesterase receptor Gpr119-induced activation of gastrointestinal mucosal responses. Cell Metab. 2010;11:532-542.

30. Patel S, Mace OJ, Tough IR, et al. Gastrointestinal hormonal responses upon GPR119 activation in lean and diseased rodent models of type 2 diabetes. Int J Obesity. 2018;34:1365-1373.

31. Forbes S, Stafford S, Coope G, et al. Selective FFA2 agonism appears to act via intestinal PYY to reduce transit and food intake but does not improve glucose tolerance in mouse models. Diabetes. 2015;64:3763-3771.

32. Cox HM. Neuroendocrine peptide mechanisms controlling intestinal epithelial function. Curr Opin Pharmacol. 2016;31:50-56.

33. Moodaley R, Smith DM, Tough IR, Schindler M, Cox HM. Agonism of free fatty acid receptors 1 and 4 generates peptide YY-mediated inhibitory responses in mouse colon. Br J Pharmacol. 2017;174:4508-4522.

34. Boey D, Lin S, Karl T, et al. Peptide YY ablation in mice leads to the development of hyperinsulinaemia and obesity. Diabetologia. 2006;49:1360-1370.

35. Velazquez-Villegas LA, Perino A, Lemos V, et al. TGR5 signaling promotes mitochondrial fission and beige remodelling of white adipose tissue. Nat Comms. 2018;9:245-258.

36. Tough IR, Forbes S, Cox HM. Signaling of free fatty acid receptors 2 and 3 differs in colon mucosa following selective agonism or coagonism by luminal propionate. Neurogastroenterol Motil. 2018;30(12):e13454. https://doi.org/10.1111/nem.13454

37. Cox HM, Pollock EL, Tough IR, Herzog H. Multiple Y receptors mediate pancreatic polypeptide responses in mouse colon mucosa. Peptides. 2001;22:445-452.

38. Tough IR, Forbes S, Herzog H, et al. Endogenous peptide YY and neuropeptide Y inhibit colonic ion transport, contractility and transit differentially via Y1 and Y2 receptors. Br J Pharmacol. 2011;164:66-79.

39. Cox HM, Cuthbert AW, Håkanson R, Wahlstedt C. The effect of neuropeptide Y and peptide YY on electrogenic ion transport in rat intestinal epithelia. J Physiol. 1988;398:65-80.

40. Joshi S, Tough IR, Cox HM. Endogenous PYY and GLP-1 mediate L-glutamine responses in intestinal mucosa. Br J Pharmacol. 2013;170:1092-1101.

41. Panaro BL, Tough IR, Engelstoft MS, et al. The melanocortin-4 receptor is expressed in enteroendocrine L cells and can regulate the release of peptide YY and glucagon-like peptide 1 in vivo. Cell Metab. 2014;20:1018-1029.

42. Roberts RE, Glicksman C, Alaghaa-Zadeh J, et al. The relationship between postprandial bile acid concentration, GLP-1, YY and ghrelin. Clin Endocrinol. 2011;74:67-72.

43. Tough IR, Moodaley R, Cox HM. Mucosal GLP-1 responses are mediated by CGRP in the mouse colon and both peptide responses are area-specific. Neurogastroenterol Motil. 2018;30(1):e13149. https://doi.org/10.1111/nem.13149

44. Mawe G, Hoffman JM. Serotonin signaling in the gut—functions, dysfunctions and therapeutic targets. Nature Rev Gastroenterol Hepatol. 2013;10:473-486.

45. Poole DP, Godfrey C, Cattaruzza F, et al. Expression and function of the bile acid receptor GpBAR1 (TGR5) in the murine enteric nervous system. Neurogastroenterol Motil. 2010;22:814-825.

46. Goldspink DA, Lu VB, Billing LJ, et al. Mechanistic insights into the detection of free fatty and bile acids by ileal GLP-1 secreting cells. Mol Metab. 2018;7:90-101.

47. Bijvelds MJC, Jorna H, Verkade HJ, et al. Activation of CFTR by ASBT-mediated bile salt absorption. Am J Physiol Gastro Liver Physiol. 2005;289:G870-G879.

48. Ward JBJ, Mroz MS, Keely SJ. The bile acid receptor, TGR5, regulates basal and cholinergic-secreted secretory responses in rat colon. Neurogastroenterol Motil. 2013;25:708-711.

49. Duboc H, Tolstanova G, Yuan PQ, et al. Reduction of epithelial secretion in male rat distal colonic mucosa by bile acid receptor TGR5 agonist, INT777: role of submucosal neurons. Neurogastroenterol Motil. 2016;28:1663-1676.

50. Cheng K, Kaufman JP. Bile acid-induced proliferation of a human colon cancer cell line is mediated by transactivation of epidermal growth factor receptors. Biochem Pharmacol. 2005;70:1035-1047.

51. Adrian TE, Gariballa S, Parekh KA, et al. Rectal taurocholate increases L cell and insulin secretion and decreases blood glucose and food intake in obese type 2 diabetic volunteers. Diabetologia. 2012;55:2343-2347.

52. Parker HE, Wallis K, Le Roux CW, et al. Molecular mechanisms underlying bile acid-stimulated glucagon-like peptide-1 secretion. Br J Pharmacol. 2012;165:414-423.
53. Nauck MA, Niedereichholz U, Ettler R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol*. 1997;273:E981-E988.

54. Maljaars PW, Peters HP, Mela D1, Masclee AA. Ileal brake: a sensible food target for appetite control: a review. *Physiol Behav*. 2008;95:271-281.

55. Camilleri M. Pharmacology of the new treatments for lower gastrointestinal motility disorders and irritable bowel syndrome. *Clin Pharmacol Therap*. 2012;91:44-59.

56. Kidd M, Modlin IM, Gustafsson BI, et al. Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants and olfactants. *Am J Physiol*. 2008;295:G260-G272.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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