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Abstract: Bile acids are becoming increasingly appreciated as enteric hormones that regulate many aspects of intestinal physiology. The bile acid receptor, TGR5, has been recently shown to be expressed on enteric nerves and enterochromaffin cells, where its activation regulates small intestinal and colonic motility. Here, we show that TGR5 is also expressed on colonic epithelial cells and that its activation decreases basal secretory tone and inhibits cholinergic-induced secretory responses. Our data demonstrate a new role for TGR5 in regulating colonic fluid and electrolyte transport and suggest the receptor represents a good therapeutic target for intestinal transport disorders.

Introduction

Although classically known for their roles in facilitating fat digestion and absorption, bile acids (BAs) are now emerging as important hormone-like molecules that regulate many aspects of intestinal physiology. The importance of the enteric nervous system (ENS) in mediating BA actions on intestinal motility and transport is well-established, and a major breakthrough in our understanding of how they recruit the ENS came with the discovery of TGR5 expression on enteric neurons [1,2]. TGR5 is a G-protein coupled receptor expressed on inhibitory motor neurons of the small intestine, where its activation by BAs slows small intestinal motility. Thus, it has been proposed that TGR5 may contribute to the “ileal brake”, a mechanism that slows intestinal transit during digestion, thereby facilitating nutrient absorption [2]. A subsequent study has shown TGR5 to be also expressed on myenteric neurons and enterochromaffin cells (ECs) in the colon, where it mediates prokinetic actions of BAs [3]. Furthermore, studies in TGR5 (-/-) mice have also shown the receptor to be important in maintenance of mucosal barrier function and protection against colitis [4]. Thus, TGR5 is emerging as an important mediator of bile acid actions in the intestine and as a potentially important therapeutic target for intestinal diseases. However, although fluid and electrolyte transport is a critical aspect of intestinal function that can be regulated by bile acids [5,6], there have been no reported studies of a possible role for TGR5 in its regulation. Here, we begin to address this gap in our knowledge by investigating a potential role for TGR5 in regulating basal and cholinergic-stimulated colonic secretory responses.

Results

TGR5 expression was detected in isolated rat colonic crypt preparations at both the mRNA (Fig. 1A, upper panel) (n = 3) and protein levels (Fig. 1A, lower panel) (n = 3), with the receptor being expressed both at the apical and basolateral membranes and within subcellular compartments. To examine the effects of TGR5 activation on epithelial ion transport, nerve-free, submucosally-stripped colonic tissues were mounted in Ussing chambers and challenged with a specific TGR5 agonist, INT-777 [7]. This tissue preparation was used as it allows investigation of the role of TGR5 activation in basal and agonist-induced secretion, without the influence of intrinsic submucosal neurons. Interestingly, under these conditions, INT-777 (100 μM) induced a significant decrease in basal I_{sc} of 13 ± 4 $\mu A/cm^2$ (n = 9, $p \leq 0.001$ compared to untreated control tissues; Fig 1B). INT-777-induced decreases in basal I_{sc} were rapid in onset and transient, being maximal within 4 min and sustained for 10 min. Responses to the TGR5 agonist were observed at concentrations as low as 10 μM (n = 5, $p \leq 0.05$, data not shown). Reductions in basal I_{sc} were observed when INT-777 was applied bilaterally or basolaterally, but not apically (n = 6; Fig. 1C). Furthermore, effects of INT-777 were abolished in Cl^- free bathing solution (Fig. 1C; n = 3), confirming them to be due to inhibition of basal Cl^- secretion, the primary driving force for fluid secretion in the intestine. Pretreatment of the tissues with TTX (TTX; 100 nM) did not alter basal I_{sc} or responses to INT-777, confirming the tissue to be nerve-free and that the effects of TGR5 activation are independent of the ENS (data not shown). Similar to INT-777, the natural TGR5 agonist, lithocholic acid (LCA; 50 μM), also rapidly reduced basal I_{sc} (Fig. 1E). Finally, since cholinergic secretomotor neurons are critical in regulating intestinal secretory responses, we examined the effects of TGR5 activation on responses to the

cholinomimetic, carbachol (CCh). Responses to CCh (100 μM) were significantly attenuated by pretreatment with either INT-777 (100 μM) or LCA (50 μM) to 64 ± 10% (n = 3, $p \leq 0.05$; Fig. 1D) and 45 ± 18% (n = 5, $p \leq 0.05$; Fig 1F) of control responses, respectively, while responses to the cAMP-dependent secretagogue, forskolin (FSK; 10 μM), were not altered (100.7 ± 13.2% of controls; n = 5).

Discussion

The bile acid receptor, TGR5, is emerging as an important regulator of intestinal physiology and an exciting new target for treating intestinal diseases. Here, we identify a new role for TGR5 in regulating intestinal epithelial transport function. We show that TGR5 is expressed in rat colonic epithelium and that its activation, either by a semi-synthetic agonist, INT-777, or its naturally-occurring ligand, LCA, rapidly decreases basal Cl^- secretion and attenuates Cl^- secretory responses to the cholinergic agonist, CCh. In contrast to its effects on intestinal motility, which are mediated by nerves [2, 3], the antisecretory effect of TGR5 activation is non-neuronally mediated. It is interesting to note that even though TGR5 is expressed towards the apical and basolateral poles of T_{84} cells, only basolateral addition of INT-777 was effective. Further studies are required to determine why this is the case but it could be due to differential expression of accessory signaling proteins, or bile acid transporters, within the apical and basolateral domains of colonic epithelial cells. Whatever the case, our data suggest that bile acids, normally present in the lumen, would have to cross the epithelial layer in order to exert their effects at the basolateral membrane. Indeed, this is quite likely to occur under physiological conditions *in vivo*, since the natural ligand for TGR5, LCA, is a lipophillic monohydroxy bile acid that can easily cross lipid bilayers. How TGR5 activation regulates epithelial secretion remains to be determined, but it is clear that the mechanism involved is specific for Ca^{2+}-dependent secretagogues, since responses to the cAMP-dependent agonist, FSK, were unaltered by INT-777. This suggests that TGR5 specifically targets a molecular component of the Ca^{2+}-dependent secretory pathway, but further studies are required to elucidate this important aspect of epithelial responses to TGR5 activation.

At present we can only speculate as to the potential physiological significance of the antisecretory actions of TGR5 activation. However, one possibility could be that attenuated basal secretory tone induced by TGR5 in response to its natural ligands may serve to promote colonic absorptive function under normal conditions. Interestingly, recent studies have shown that bile acids acting at TGR5 on enterochromaffin cells promote colonic motility by a neurally-mediated pathway involving 5-HT and CGRP, and that overactivation of TGR5 can lead to the onset of diarrhea in mice [3]. In this context, our data suggest that activation of epithelial TGR5 by lipophillic bile acids present in the luminal contents, such as DCA or LCA, may serve to limit fluid secretion into the lumen, thereby preventing excessive loss in the feces.

In conclusion, our data reveal a new role for epithelial TGR5 in regulating basal and cholinergic-stimulated secretion in the colon. These findings increase our understanding of the important role of TGR5 in regulating intestinal physiology and underline the potential for targeting the receptor in treatment of intestinal disease.

Fig. 1 Effects of TGR5 activation on Cl^- secretion in rat distal colon. A) Submucosally-stripped rat distal colon was analyzed for TGR5 mRNA expression (upper panel) (n = 3). Rat colonic crypts were isolated and TGR5 was visualized by confocal microscopy (lower panel). TGR5 is represented in green, nuclei in blue, and F-actin in red (n = 3). **B)** Submucosally-stripped rat colonic tissues were mounted in Ussing chambers and apical amiloride (10 μM) was added to inhibit electrogenic Na^+ absorption. Under these conditions, addition of INT-777 (100 μM) induced a rapid decrease in basal I_{sc} (n = 9), which was apparent only when the agonist was present on basolateral side (n = 3 – 11). The INT-777 (100 μM)-induced decrease in I_{sc} was not observed when tissues were bathed in Cl^--free Ringer's solution (n = 3). **D)** Tissues were treated with INT-777 (100 μM) and after 4 min secretory responses to CCh (100 μM) were measured as changes in I_{sc} (n = 3; * $p \leq 0.05$). **E)** Similar to INT-777, the endogenous ligand of TGR5, LCA (50 μM), also reduced basal I_{sc} in rat colonic tissues. The upper panel shows the time course of responses to LCA and the lower panel shows the mean maximal decrease in I_{sc} (n = 6). **F)** After treatment with LCA (50 μM) secretory responses to CCh (100 μM) were measured and expressed as changes in I_{sc} (n = 8; * $p \leq 0.05$). All data are expressed as mean ± sem from a series of *n* individual animals.

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Author Contributions:

JW – Conducted experiments, analyzed and interpretation of data, drafted manuscript

MM - Conducted experiments, analyzed and interpretation of data, drafted manuscript

SK – Study conception and design, analyzed and interpretation of data, drafted manuscript

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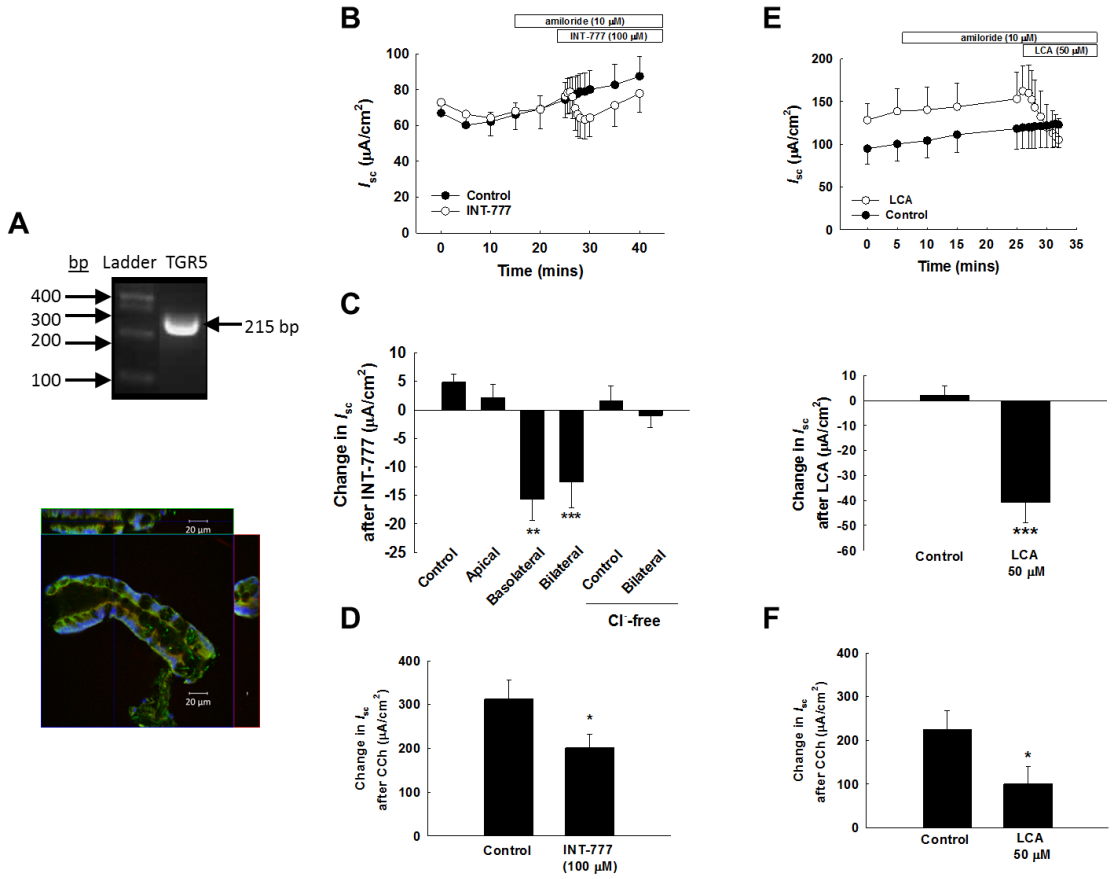
Figure 1


A
bp Ladder TGR5
400
300
200
100
215 bp
20 µm
20 µm
B
amiloride (10 µM)
INT-777 (100 µM)
I_{sc} (µA/cm^2)
Control
INT-777
Time (mins)
C
Change in I_{sc} after INT-777 (µA/cm^2)
Control
Apical
Basolateral
Bilateral
Control
Bilateral
Cl$^-$-free
D
Change in I_{sc} after CCh (µA/cm^2)
Control
INT-777 (100 µM)
E
amiloride (10 µM)
LCA (50 µM)
I_{sc} (µA/cm^2)
LCA
Control
Time (mins)
Change in I_{sc} after LCA (µA/cm^2)
Control
LCA 50 µM
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Change in I_{sc} after CCh (µA/cm^2)
Control
LCA 50 µM