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Epithelial-Neuronal Communication in the Colon: Implications for Visceral Pain

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Abstract

Visceral hypersensitivity and pain result, at least in part, from increased excitability of primary afferents that innervate the colon. In addition to intrinsic changes in these neurons, emerging evidence indicates that changes in lining epithelial cells may also contribute to increased excitability. Here we review recent studies on how colon epithelial cells communicate directly with colon afferents. Specifically, anatomical studies revealed specialized synaptic connections between epithelial cells and nerve fibers, and studies using optogenetic activation of the epithelium showed initiation of pain-like responses. We review the possible mechanisms of epithelial-neuronal communication and provide an overview of the possible neurotransmitters and receptors involved. Understanding of the biology of this interface and how it changes in pathological conditions may provide new treatments for visceral pain conditions.

Keywords

visceral pain; sensory neurons; colon epithelium; gut-brain axis

Epithelial-neuronal signaling and visceral pain

Visceral hypersensitivity and pain are common debilitating symptoms of inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS). These disorders represent widespread health problems, estimated to affect up to 20% of the population [1]. Effective treatments to control pain and resolve hypersensitivity are lacking. Pathophysiological changes associated with IBS and IBD are thought to cause sensitization of spinal afferent neurons that innervate the colon, which transmit noxious stimuli to the spinal cord and central nervous system (CNS) [2, 3]. Pain research has largely focused on injury-evoked changes in spinal afferent and CNS neuron activity, but recent studies of skin, bladder, and colon have revealed new regulatory roles for epithelial cells in sensory transduction. In skin, epithelial cells (keratinocytes) have been shown to have direct communication with

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epidermal nerve fibers that is sufficient to drive action potential firing and nociceptive responses [4–6]. Augmentation of keratinocyte-neural signaling may also occur under inflammatory conditions [7–9]. The bladder epithelium (urothelium) not only serves a barrier function but also has an active role in mechanotransduction via release of neuroactive substances such as ATP, acetylcholine, and nitric oxide [10]. This release is increased in models of bladder inflammation and cystitis [11].

In the context of the colon, changes in epithelial structure and permeability are common in IBD (i.e., ulcerative colitis, Crohn's disease). In active disease a compromised epithelium allows infiltration of bacteria, which provokes inflammatory immune cell responses [12, 13]. Activated immune cells (e.g., macrophages and mast cells) release cytokines and neuroactivators that can affect primary afferent function and result in pain [14, 15]. Biopsies of colon from IBD patients with pain often exhibit inflammation [14]. However, pain is reported by some patients with no evidence of inflammation or epithelial damage [16]. This finding and other preclinical studies suggest that epithelial regulation of sensory afferent activity may have a more significant role in pain signaling than previously thought and that subtle changes in epithelial function could lead to persistent pain. In support of this concept, several studies have shown that the intestinal epithelium produces and releases neurotransmitters such as ATP [17], glutamate [18], serotonin (5-HT) [19] and acetylcholine (ACh) [20], which act on colon nociceptive neurons. Additionally, channelrhodopsin-mediated selective activation of the colon epithelium is sufficient to evoke action potential firing in colon afferents and a pain-related visceromotor response [21]. Thus, the epithelium alone, in the absence of applied mechanical, chemical or thermal stimuli, can initiate sensory neuron activity and pain-like behavior. At the anatomical level, studies have indicated direct synaptic communication between specialized colon epithelial cells and surrounding neurons, providing a direct means of communication [22, 23]. Taken together, these observations indicate that with respect to structure and function, epithelial cells share many of the properties associated with neurons.

In this review, we summarize what is known about sensory afferents that innervate the colon and how they convey sensory information. We describe the different cell types that make up the colon epithelium, with a focus on enteroendocrine cells (EECs) and their role in sensory signaling. The chemical mechanisms of epithelial-neuronal communication in the colon are also discussed, with emphases on how these neurotransmitter systems are altered in response to colon inflammation.

Anatomical and functional organization of colon epithelial-neuronal communication

Sensory afferents that innervate the colon

Colon function is coordinated by intrinsic and extrinsic subpopulations of neurons. The colon receives extrinsic innervation from autonomic pathways (not discussed in the current article) and sensory pathways [24]. Extrinsic primary afferent neurons (hereafter referred to as "colon afferents") convey sensory information from the colon to the central nervous system. They are the first in a chain of neurons that give rise to conscious sensations of pain,

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bloating, fullness and urgency [24]. Colon afferent somata are in dorsal root ganglia (DRG) at thoracolumbar and lumbosacral spinal levels. Thoracolumbar afferents project to the colon via the splanchnic nerve and lumbosacral afferents project via the pelvic nerve [25]. The vagus nerve is a third source of sensory innervation; vagal afferents that project to the colon have cell bodies in the nodose ganglion [25]. Single-unit electrophysiological recordings made in rodent *ex vivo* preparations have revealed five main classes of colon afferents, defined by their functional properties: muscular afferents, which respond to stretching of the colon, mucosal afferents, which respond to light distortion of the mucosa, muscular/mucosal afferents, which respond to stretch as well as light distortion of the mucosa, and vascular afferents, which respond to focal probing of the colon wall (serosal afferents) or mesentery (mesenteric afferents) [25, 26]. Muscular afferents include both low-threshold mechanosensors with a slowly adapting response to low-intensity stretch stimuli and high threshold fibers with response properties associated with dedicated nociceptors [26–28]. The low-threshold fibers are thought to respond to physiological distension induced by the passing of fecal matter. Mucosal afferents are also low-threshold responders and thought to participate in detecting luminal contents as part of defecatory reflexes [24]. In contrast, vascular afferents are high-threshold mechanosensors and display an adapting response to noxious stimuli, making them likely to transmit mechanically-induced pain stimuli [26, 29]. Mechanically insensitive afferents or “silent afferents” represent a fifth class of colon afferents that respond to chemical stimuli and, after activation by inflammatory mediators, to mechanical stimuli [30]. These five major classes of colon afferents have been demonstrated in both rodent and human [31].

The classification of colon afferents continues to be updated as more advanced techniques become available. A recent study using single-cell RNAseq analyses proposed distinct classes of extrinsic colon afferents based on molecular profiles [32]. These included peptidergic, non-peptidergic, and neurofilament-positive populations at both thoracolumbar and lumbosacral levels. This study also identified neurotransmitter receptors expressed across these populations. Ongoing molecular and functional studies aim to reveal the signaling mechanisms of these colon afferent classes and their role in visceral pain.

Colon epithelial cell types

Epithelial cells that line the small and large intestine form a simple columnar epithelium with two major functions: absorbing nutrients and water and forming a protective barrier (Figure 1). Epithelial morphology (i.e., number of villi vs. crypts) and secretory cell populations vary along the GI tract [33]. This review focuses on the colon epithelium, partly because most studies of IBD and IBS in humans examine colon biopsies. Additionally, measures and outcomes of most animal models of visceral pain target the colon epithelium and colon afferents. Most colon epithelial cells are absorptive enterocytes, with remaining types having secretory functions. Secretory cell types include goblet cells that secrete mucus for lubrication, tuft cells (also known as brush cells) that secrete opioids and immune mediators [34], and enteroendocrine cells (EECs) that secrete hormones and peptides [35]. There are over 10 types of EECs. Most prevalent in the human and mouse colon are L-cells, which express peptide YY (PYY) and glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), and enterochromaffin (EC) cells, which release serotonin (5-HT) [36]. The mouse colon also

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contains a small number of I-cells that express cholecystokinin (CCK) [37] and the human colon contains a small proportion of somatostatin-expressing D-cells [38].

Although EECs comprise about 1% of the epithelium, they are the most likely cell type to participate in epithelial-neuronal communication in the colon. EECs function as endocrine regulators; they sense luminal content and release hormones that regulate secretion, motility and satiety signals [39]. EECs express many receptors typically found on afferent neurons, e.g., taste receptors that sense glucose, amino acids and fatty acids [40]. EECs also express receptors implicated in mechanotransduction and nociceptive signaling, such as Piezo2 and transient receptor potential ankyrin 1 (TRPA1) [41–45]. These epithelial cells are electrically excitable and can form synaptic structures with neurons [22, 23]. Using EEC-specific fluorescent reporter lines and transcriptional profiling, axon-like basal processes (a.k.a. neuropods) and presynaptic vesicle proteins were identified [46]. Other studies targeted rabies virus to EECs in the colon and demonstrated synaptic connectivity between EECs (specifically, PYY-expressing cells) and surrounding neurons [23], which include extrinsic primary afferents with cell bodies in lumbar DRG [47]. This EEC-colon afferent connection provides at least one potential cellular substrate for visceral pain transmission.

Transmitters involved in epithelial-primary afferent communication in the colon

Diverse cell types within the colon express numerous signaling molecules and receptors that are likely to facilitate epithelial-neuronal communication. Some of these are highlighted in Figure 1.

Adenosine triphosphate (ATP)—One mechanism of colon mechanosensory transduction was proposed by Geoffrey Burnstock, a pioneer of the purinergic signaling field, who posited that colon distension leads to ATP release from epithelial cells, which then acts on purinergic receptors expressed on primary afferents [17]. This hypothesis was supported by studies showing that mechanical stimulation of the mucosa resulted in ATP release and that afferent responses to mechanical stimulation were attenuated in the presence of purinergic receptor antagonists [48]. Distension-evoked release of ATP may be mediated, at least in part, by activation of transient receptor potential channel V4 (TRPV4), which is present in human and mouse epithelial cells [49–51]. TRPV3 channel activation is also linked to ATP release; one study showed that addition of the TRPV3 agonist carvacrol to cultured colon epithelial cells increased ATP in culture supernatants [52].

The mechanisms that underlie ATP release from epithelial cells are only partially known. As shown for neurons and adrenal chromaffin cells [53, 54]. ATP may be released in combination with other neurotransmitters from enterochromaffin cells or other EECs [55]. ATP can also be co-released with hormones from EECs, e.g., L-cells release ATP along with GLP-1 and PYY [56]. ATP is also likely released on its own from diverse epithelial cell types. Ubiquitous expression of vesicular nucleotide transporter (VNUT) in the mouse colon epithelium and in human intestinal epithelium cell lines suggest all epithelial types are capable of ATP release. In addition, VNUT-mediated exocytosis of ATP-containing vesicles may be dependent on TRPV4 activation [51]. Other mechanisms of ATP release from colon

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epithelial cells, including ATP-permeable channels (e.g., pannexins), have not been ruled out [57].

Targets of ATP release on colon afferent endings include ionotropic $P2X_3$ and $P2X_{2/3}$ receptors [48, 58] as well as metabotropic $P2Y_1$ and $P2Y_2$ receptors [59] expressed by muscular and vascular colon afferents [58, 59]. Recent findings from our group support these targets, as epithelial-neuronal signaling was blocked using a cocktail of $P2X_3$, $P2X_{2/3}$, and $P2Y_2$ antagonists [21]. Additionally, ATP released during distension has been shown to activate and sensitize TRPV1 channels on colon afferents [60].

Models of visceral hypersensitivity suggest inflammation-induced changes occur in purinergic signaling. In the trinitro benzene sulfonic acid (TNBS) model of IBD, increased distension-evoked ATP release augmented colon afferent firing in response to ATP application and this was correlated with upregulation of $P2X_3$ receptors in lumbosacral dorsal root ganglia [61]. ATP signaling is also implicated in colon hypersensitivity resulting from intracolonic infusion of zymosan (a model of post-infectious IBS), based in part on the absence of hypersensitivity in $P2X_3^{-/-}$ mice [58]. In addition, $P2X_3$ protein level is higher in colon biopsies from IBD subjects (which is often accompanied by persistent pain) compared with control tissue [62]. The authors hypothesized that both submucosal enteric neurons and primary afferent nerve endings contributed to the increase in ATP. Together, these studies suggest that inflammation-induced changes in ATP release (possibly from epithelial cells) and ATP receptor activity (on colon sensory neurons) contribute to hypersensitivity.

Serotonin (5-hydroxytryptophan)—Serotonin (5-HT) in the gut regulates peristalsis, secretion and nociceptive signaling. Enterochromaffin (EC) cells make up less than 1% of the epithelium, yet they synthesize and release over 95% of the body's 5-HT [19]. The 5-HT release machinery, located at the basal surface of EC cells, is in close apposition to nerve fibers [22, 63]. Secretory granules at the apical surface may facilitate release of 5-HT into the gut lumen [64]. EC cells are the major source of 5-HT whereas virtually all epithelial cells in the gut express the serotonin-selective reuptake transporter (SERT) and thus take part in controlling 5-HT levels [65].

The excitatory ionotropic 5-HT_3 receptor (5-HT_3R) is widely expressed on colon afferents [32] and has been implicated in visceral pain signaling. In the mouse model of dextran sulfate sodium (DSS)-induced colitis, an increase in 5-HT_3R-positive nerve fibers in the colon mucosa was measured [66]. *In vivo* studies of colon sensitivity, measured by responses to colorectal distension (CRD), have shown that i.v. administration of alosetron, a 5-HT_3R antagonist, diminishes these responses. Alosetron administration also decreased c-Fos positive neurons in the spinal cord dorsal horn in response to CRD [67]. Importantly, alosetron has been used to treat IBS-D (diarrhea-predominant) and randomized controlled trials showed pain relief. However, due to side effects of constipation and colitis, alosetron was withdrawn from the market [68].

A study examining both intestinal cryosections and organoids derived from small intestine tissue showed that EC cells form synapse-like contacts with 5-HT_3R-expressing nerve endings [22]. In the same study, functional connectivity between EC cells and nerve fibers

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was revealed using a colon-nerve *ex vivo* preparation in which selective pharmacological activation of EC cells caused action potential firing in 5-HT_3 positive colon mucosal afferents, presumably via the release of 5-HT [22]. Communication between nerves and EC cells could be initiated via EC expressed molecules that transduce sensory stimuli. For example, studies show that some EC cells express the mechanosensitive Piezo2 receptor, localized adjacent to 5-HT vesicles [45, 69]. Stretching of the colon tissue can activate Piezo2, providing a link between sensations associated with colon distension and 5-HT signaling. However, it should be noted that colon mechanotransduction is likely to be a shared responsibility of EC cells and colon afferents; colon afferents also express Piezo2, most frequently in neurofilament-positive populations [32]. TRPA1 is another potential stimulus-transducing molecule expressed by EC cells. In the context of gustatory sensation, this ligand-gated receptor is responsible for the oral cavity's ability to detect molecules like allyl isothiocyanate (found in mustard oil, wasabi, and horseradish) and cinnamaldehyde (found in cinnamon). The presence of TRPA1 in colon EC cells supports the concept that these cells can act as "taste buds" of the colon [70] and as sensors of chemical irritants. Like Piezo2, TRPA1 is expressed in colon afferents and its deletion degrades, but does not eliminates mechanosensory function [71], indicating mechanistic redundancy for this sensory modality that could include a role for the epithelium.

Several studies have examined the role of 5-HT in visceral pain disorders. One study reported that inflamed colon tissue samples from IBD patients have decreased 5-HT compared to controls, decreased SERT levels and fewer 5-HT-immunoreactive EC cells per crypt in the mucosa [72]. In contrast, animal models of colitis (TNBS and DSS) display increases in EC cell count and mucosal 5-HT content [73, 74]. The discrepancy in EC cell count and 5-HT levels could be due to limitations of the models, where acute colitis does not directly compare to the chronic inflammation in human IBD [63].

Studies of 5-HT signaling in IBS have also shown conflicting results. One study compared patients with IBS-D (diarrhea-predominant) and IBS-C (constipation-predominant). Both groups had decreased mucosal SERT expression and no change in EC density or in tissue agitation-evoked 5-HT release [72]. Another study showed that colon samples from post-infectious (PI) IBS patients had significantly more 5-HT-immunoreactive EC cells per imaging field compared to healthy controls, whereas non-PI-IBS samples showed no difference in EC cell count [75]. In an analysis of samples from IBS patients of all phenotypes, no difference in EC cell count or 5-HT content was found between patients with and without hypersensitivity [76]. Thus, 5-HT signaling may be altered in IBS, but its role in colon hypersensitivity and pain remain unclear.

Glutamate—Glutamate is another epithelial-derived transmitter involved in sensory signaling. PYY- and CCK-expressing EECs have been shown to release glutamate [47] and the vesicular glutamate transporter 2 (VGLUT2) colocalizes with PYY and GLP-1 throughout the intestines [77]. Additional culture studies using a neuroendocrine cell line (GLUTag) showed that glutamate is co-released with GLP-1 in response to KCl or glucose [18].

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Glutamate receptors are expressed by colon afferents and are implicated in visceral hypersensitivity [78]. Single cell RNA sequencing indicates several types of glutamate receptors are expressed across subtypes of colon afferents, including the ionotropic AMPA and NMDA receptors, and the metabotropic receptors mGluR1-5 [32]. Studies in rat showed mGluR5 antagonists diminish visceromotor responses to CRD and, in an *ex vivo* colon-nerve preparation, decrease muscular and serosal colon afferent responses to mechanical stimulation [79]. Changes in NMDA receptors (NMDAR) are also associated with IBS. Specifically, IBS patients were shown to have upregulation of NMDAR, which correlated with abdominal pain scores, whereas mice showed increased sensitivity to colorectal distension in response to intracolonic administration of NMDA [80]. These findings complemented previous reports showing that NMDAR antagonists prevented hypersensitivity in a mouse model of TNBS-induced colitis [81].

A recent study employed rabies virus retrograde tracing in mice to show that PYY- and CCK-positive EECs synapse with DRG afferent fibers [47]. Many EECs were shown to co-express VGLUT2 and CCK. Optogenetic activation *in vitro* confirmed that CCK-positive EECs release glutamate, causing activation of vagal neurons. These studies support the idea that EECs use glutamate to signal to colon afferents.

Acetylcholine (ACh)—The ACh synthesizing enzyme choline acetyltransferase (ChAT) is one of the commonly used indicators of ACh production. Expression of ChAT in the intestinal epithelium has been recognized for decades [82]. ACh may be an important transmitter between tuft cells, which express ChAT, and surrounding neurons [83]. The vesicular acetylcholine transporter (VAChT), another indicator of ACh production, has been detected via immunohistochemistry in both human [84] and mouse [85] intestinal epithelium. In mice, VAChT is expressed in only about 20% of ChAT-expressing tuft cells in the proximal colon and not detected in distal colon or small intestine [85]. In VAChT+ tuft cells, ACh may be released through synaptic vesicles, but in VAChT− cells, ACh may be released via organic cation transporters [86], or gap junctions [87].

Nicotinic ACh receptors (nAChR) are expressed on colon afferents, but their role in visceral pain is unclear. Single cell RNAseq analysis has shown high levels of nAChRα subunits 3-7 in colon afferents [32]. Another study showed nAChRα3 expression in about 50% of peptidergic afferents that innervate visceral organs. This study also showed that in skin, mechanically insensitive afferents (MIAs), also known as “silent” nociceptors, can be defined by expression of nAChRα3 [88], raising the possibility that MIAs that innervate the colon also express nAChRα3.

Although tuft cells represent only 0.4% of intestinal epithelial cells (in the mouse), they have an important role in mediating immune responses [34]. They are a primary source of interleukin-25, which recruits helper T cells and innate lymphoid cells during infection [89]. They also express cyclooxygenase enzymes (COX1 and COX2), which produce inflammatory prostaglandins [90]. Recent evidence suggests tuft cells also initiate a type 2 immune circuit in response to succinate, a metabolite secreted by parasitic helminths [91]. Visceral hypersensitivity, which often occurs with infection, is thought to result from an inflammatory response to the parasite. As suggested by this study, this response may be

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initiated by succinate activation of tuft cells and subsequent activation of sensory signaling pathways. The role of tuft cells in prolonged inflammatory conditions such as IBD is unclear, but alteration in the gut's non-neuronal cholinergic system is a possible link. Evidence consistent with this idea comes from immunohistochemical and mRNA analyses of human colon tissue, which show that patients with ulcerative colitis expressed significantly lower levels of ChAT and VAChT in the colon epithelium [84].

Proteases—Proteases released by colon epithelial cells are likely regulators of colon afferent hypersensitivity. Supernatants obtained from IBS colon biopsies increase the excitability of sensory neurons in culture, an effect that is blocked by protease inhibitors [92]. These supernatants also induce visceral hypersensitivity in mice, but not in the presence of a protease-activated receptor-2 (PAR_2) antagonist or in PAR_2-deficient mice [93]. In addition, application of a PAR_2 agonist (SLIGRL-NH_2) into the colon lumen results in hypersensitivity to colorectal distension and increased Fos expression in the spinal cord dorsal horn [94]. Electrophysiology studies have shown PAR_2 sensitizes serosal afferents via a TRPV4-dependent mechanism [95]. Colon epithelial cells, enterocytes in particular, are a source of proteases; addition of lipopolysaccharide (LPS) to Caco-2 cells in culture caused release of the protease trypsin-3 [96]. Trypsin-3 has been shown to induce colorectal hypersensitivity in a PAR_2-dependent manner and is increased in epithelium of colon biopsies of human IBS patients and rat IBS models [96].

Efforts to identify proteases active in IBS and IBD patients are ongoing. Functional proteomic assays of colon tissue and supernatants from IBD patients (both ulcerative colitis and Crohn's disease), showed increased trypsin-like activity as well as overactive cathepsin G and thrombin activity in IBD patients [97]. Flow human colon afferents respond to proteases is also unclear; although PAR_2 is expressed in 40% of human sensory afferent neurons, neuronal responses to supernatants from IBS patients are mediated by PAR_1 [98].

Cyclic guanosine-3',5'-monophosphate (cGMP)—Signaling molecules released from colon epithelial cells may also inhibit neural activity. One such molecule is cGMP, which is released upon activation of the epithelial guanylate cyclase C (GC-C) receptor [99]. Linaclotide, a drug prescribed for IBS-C for relief of pain and constipation, is a peptide agonist of GC-C, a transmembrane receptor expressed on the luminal aspect of the intestinal epithelium that also binds bacterial enterotoxins (responsible for traveler's diarrhea) and peptide hormones (e.g., guanylin) [100]. In randomized controlled trials, linaclotide reduced abdominal pain in over 60% of patients [101]. Linaclotide's binding to GC-C stimulates the synthesis and release of cGMP from epithelial cells, which has effects that are twofold: stimulation of fluid production in the intestinal lumen and inhibition of colon afferent activity [102]. The increase in epithelial cGMP initiates a protein kinase-dependent pathway which activates the cystic fibrosis transmembrane regulator (CFTR), increasing secretion of bicarbonate and chloride into the lumen. These secretions inhibit the sodium/hydrogen exchanger, leading to fluid secretion into the lumen [103]. The increased production of cGMP in the epithelial cells leads to more extracellular cGMP, which has been shown to inhibit colon afferent firing via a membrane receptor target, though little is known about the

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identity of this target [104]. Linaclotide itself does not reduce afferent excitability; only cGMP released from the epithelium has this effect.

In rodent *ex vivo* preparations, cGMP applied to the colon lumen decreased colon afferent firing rates [101, 105]. This effect was more robust in a mouse model of TNBS-induced colon inflammation [104]. Patch clamp analysis of cultured human DRG neurons also showed greater effectiveness of cGMP in the presence of inflammatory mediators [104]. Although the mechanisms involved in cGMP-induced inhibition remain unclear, further investigation of cGMP targets is warranted as this approach has significant therapeutic potential.

Concluding remarks and future perspectives

Colon epithelial cells, EECs in particular, are likely to have significant influence on the activity of colon afferents and visceral pain signaling. The neuroactivators released by epithelial cells that are most likely to be involved in this epithelial-neuronal communication are summarized in Table 1. This table shows each neurotransmitter and its epithelial cell sources, as well as the model systems and techniques that have been used to identify the sources of each transmitter.

As summarized in Figure 1, colon epithelial cells express some of the same sensory receptors that are found in colon afferents, indicating that they have a role in monitoring the environment in the gut lumen. This is supported by studies showing that colon afferent responses to mechanical stimuli are diminished when epithelium-released neurotransmitters are blocked [21, 48]. In many cases, transmission from epithelial cells may be diffuse and have slow or indirect actions on colon afferent terminals. However, communication from some EECs to colon afferents may be achieved through direct synaptic transmission. Enterochromaffin cells (ECs) are one type of EEC that show evidence of these synapses and they contain the receptors TRPA1 and Piezo2, which are critical in mechanosensation and pain signaling [22, 69]. This receptor expression profile and connectivity with surrounding colon afferents may indicate that ECs have a more salient role in sensory signaling than other cell types.

Data discussed in the previous sections support the hypothesis that the combination of all epithelial cell types is necessary for normal sensation in the colon, but further studies are needed for confirmation (see Outstanding Questions). To determine the contribution of each cell type to sensory signaling, it would be imperative to develop cell type-specific manipulation paradigms applicable to the colon physiology, for instance via optogenetics and chemogenetics. More comprehensive studies are also needed to identify all sensory receptors expressed in each colon epithelial cell type, similar to the single-cell RNAseq analysis of small intestine epithelium [106]. Molecular techniques should also be employed to determine whether other epithelial-released molecules, such as neuropeptides, play a role in modulation of colon afferent activity. Functional analysis of specific colon afferent receptors is also needed to determine which receptors are important in epithelial-neural signaling.

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Acute and chronic inflammation likely affect epithelial-neuronal signaling in the colon, and more studies are required to examine how alterations at this interface affect disease processes and symptoms. One possibility is that, like neurons, epithelial cells become hypersensitive with inflammation, thus amplifying the sensory signaling pathways. This could occur via inflammation-induced changes in voltage-sensitive channels that regulate electrically excitable epithelial cell types (such as ECs) and/or via changes in proteins that regulate exocytosis/release of neuroactive substances. In addition, appropriate animal models that elicit the hallmarks of colon diseases with different etiology should be employed (e.g., DSS or TNBS (IBD), zymosan or repeated stress (IBS), and parasite infection models) to assess the full range of epithelial responses to different disease challenges. A thorough analysis of epithelial changes will require multimodal strategies including calcium imaging and electrophysiology techniques, high resolution anatomical analysis (e.g., 3D electron microscopic reconstruction) as well as neurotransmitter release assays. This comprehensive approach is required to fully understand the complexity of epithelial-nerve interactions.

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Highlights:

- Colon epithelial cells express some of the same mechano- and chemosensors expressed on extrinsic colon afferents, and they release neurotransmitters that can act on these neurons.
- Specific activation of colon epithelial cells can initiate action potential firing in colon afferents and pain-like responses, suggesting a significant role for these cells in sensory transduction and pain signaling.
- Mediators of colon epithelial cell-colon afferent communication include ATP, serotonin, glutamate, acetylcholine, proteases, and cGMP.
- Alterations in the colon epithelium are common in inflammatory bowel disease and irritable bowel syndrome disorders. It is likely, therefore, that changes in the colon epithelium contribute to the pain associated with these disorders.

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Outstanding Questions

- Can visceral pain be triggered by a single epithelial cell type, or does this signaling depend on a combination of cell types?
- Several sensory receptors have been reported as expressed by specific colon epithelial cell types. Are there other such sensory receptors in addition to those already identified?
- What other epithelial-released neuroactive molecules influence colon afferent activity?
- How is the release of neurotransmitters from epithelial cells affected in inflammatory conditions such as IBD and IBS?
- Can epithelial cells be a therapeutic target for colon associated pain?

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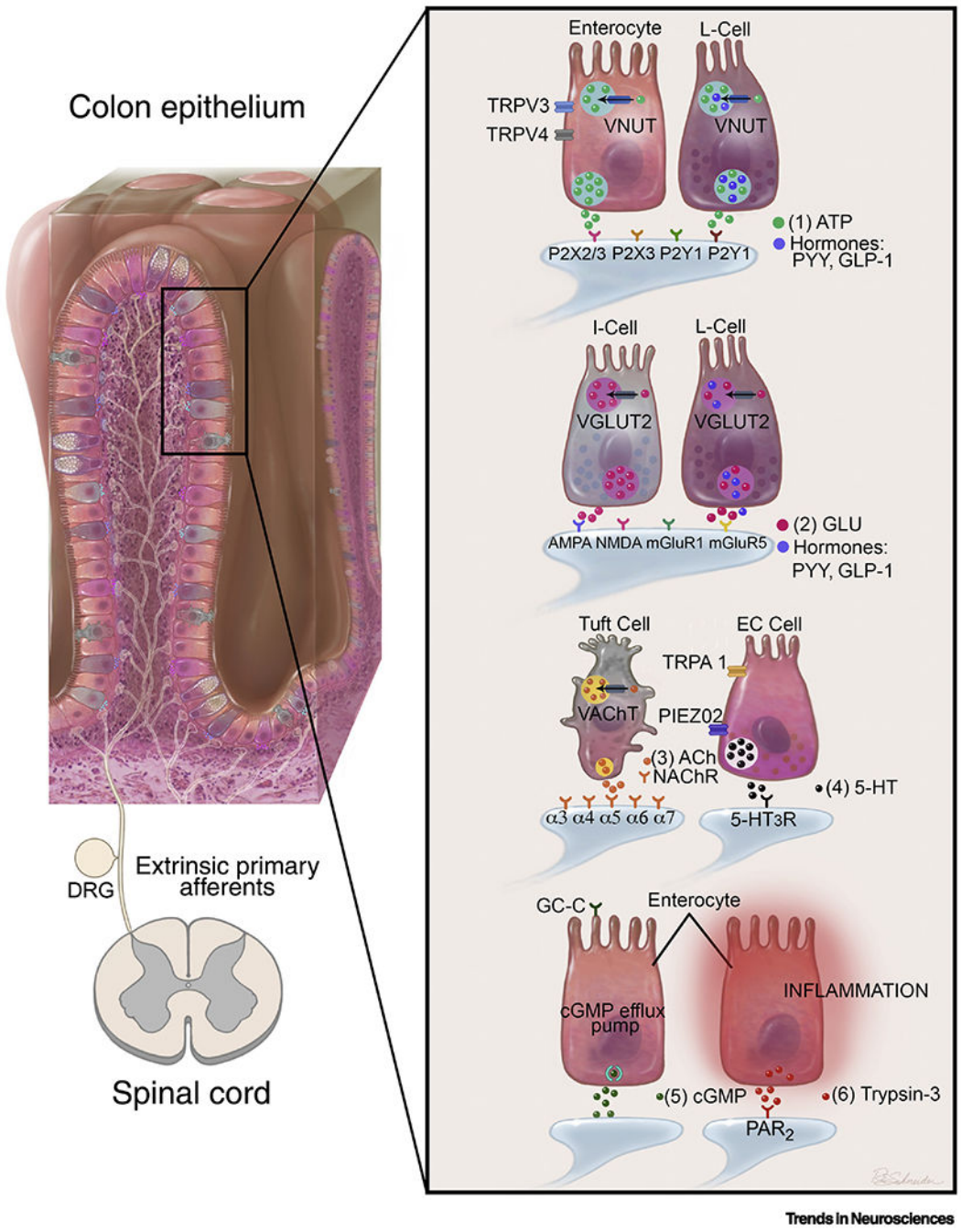

Figure 1. The mechanisms of communication between colon epithelial cells and colon afferents.

This graphic summarizes some of the key mechanisms of epithelial-neuronal communication, as discussed in this review. Neuroactive substances released by specific epithelial cell types are illustrated, along with the corresponding receptors that are found on colon afferent terminals. In some cells, mechanisms of release are also illustrated, including the ion channels and vesicular transporters involved. 1) ATP: Both enterocytes and EECs release ATP (along with other cell types) and VNUT expression is ubiquitous in the epithelium. ATP is also co-released with hormones from L-cells [49–51, 56]. 2) Glu:

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VGLUT2 is expressed in I-cells and L-cells, and glutamate is co-released with hormones from L-cells [18, 47, 77]. 3) ACh: VAChT is specifically expressed in tuft cells [85]. 4) 5-HT: EC cells are a primary source of 5-HT and express the sensory receptors TRPA1 and Piezo2. Evidence shows that 5-HT may be released upon activation of these receptors [22, 41, 45, 69]. 5) cGMP: Activation of the GC-C receptor on enterocytes causes release of cGMP [100, 101]. 6) Trypsin-3: Epithelial cells express trypsin-3 and release it in the presence of inflammation [96]. (Illustration credit: Roy Schneider)

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Table 1.

Major regulators of epithelial-neuronal communication in the small intestine and colon.

Transmitter	Epithelial Cell Source	Model System	Techniques Used to Identify Source of Transmitter	Ref
Acetylcholine (ACh)	Tuft cells	Mouse: small intestine and colon tissue	Immunohistochemistry (IHC), *in situ* hybridization, transgenic fluorescent reporter	[82]
Adenosine triphosphate (ATP)	All	Rat: colon tissue	Luciferin-luciferase ATP release assay	[48]
	All	Human: CCD 841 cell line	Luciferin-luciferase ATP release assay	[51]
	All	Mouse: colon epithelium primary culture	Luciferin-luciferase ATP release assay	[52]
	L-cells	GLUTag cell line Mouse: cultures of small intestine epithelium Human: culture of colon epithelium	Epithelial-neuronal co-cultures, luciferin-luciferase ATP release assay, sniffer patch IHC	[56]
	All	CCD 841 cell line Mouse: colon tissue	qRT-PCR, IHC, luciferin-luciferase assay qRT-PCR, IHC	[51]
Glutamate (Glu)	L-cells	Rat: small intestine tissue	IHC	[77]
	L-cells	GLUTag cell line	*In vitro* chemically evoked release	[18]
	I-cells	Mouse: small intestine tissue	IHC, *in vitro* optogenetics	[47]
Cyclic guanosine-3',5'-monophosphate (cGMP)	All	Human: T84 cell line	Enzyme immunoassay	[102]
Serotonin (5-HT)	EC cells	Rat: small intestine tissue	Immunoelectron microscopy	[64]
	EC cells	Mouse: small intestine, cultured organoids, *ex vivo* colon-nerve preparation	IHC, *in vitro* electrophysiology, 5-HT biosensors (sniffer patch)	[22]
	EC cells	Mouse: small intestine and colon, cultured organoids	IHC, *in vitro* calcium imaging, 5-HT biosensors (sniffer patch)	[69]
Trypsin-3	All	Rat: colon tissue Human: colon tissue, Caco-2 cell line	IHC, Western blot	[96]