—Review—

Chemosense for Luminal Environment in the Large Intestine

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Gut lumen is continually exposed to many agents, including noxious compounds. The intestinal epithelia form a barrier between the internal and luminal (external) environments. Chemical receptors that detect the luminal environment are thought to play an important role as sensors and as modulators of epithelial cell functions. The Molecular analysis of various epithelial cell membrane receptor proteins has elucidated the sensory role of these cells in the gut chemosensing system. Nutrient sensing systems by these receptors in the small intestinal epithelia are thought to influence nutrient metabolism and local physiological function. Much less is known, however, about the physiological roles of chemosensing in the large intestine. We have investigated the contractile and secretory effects of short-chain fatty acids (SCFAs), the primary products of commensal bacteria, and the expression of SCFA receptors in the large intestine. The findings indicate that the epithelia in the large intestine also detect and respond to luminal contents, particularly bacterial metabolites, for host defense. We recently reported that luminal bitter tastants and odorants affect transepithelial ion transport in human and rat colon, and that putative receptors are expressed in colonic mucosa. In this review, we describe the secretory effects of chemical stimuli on lumen associated with the expression pattern of sensory receptors, focusing on the large intestine.

Key words——luminal chemosensing; colonic secretion; short-chain fatty acid (SCFA); 6-propyl-2-n-thiouracil (6-PTU, PROP); thymol

INTRODUCTION

The intestinal lumen is an ambient environment. Similar to skin, the intestinal epithelia form a barrier between the internal and luminal (external) environments. In contrast to skin, however, the intestinal epithelium is a monolayer consisting of several types of cells, because it also acts as a gateway for nutrients and water. These cells, including absorptive columnar, goblet, M, enteroendocrine, and brush (tuft, caveolated) cells, form a sheet, with lymphocytes and dendritic cells present between epithelial cells but without direct contact with the lumen (Fig. 1). Together, these epithelial and lamina propria cells function to balance nutrient absorption with host defense. Recent molecular analysis of membrane receptor proteins has elucidated the sensory role of these epithelial cells in the gut chemosensing system.

Several types of receptors have been identified in the intestinal epithelia, including olfactory receptors (ORs),[1] sweet and umami receptors (T1Rs),[2] bitter receptors (T2Rs),[3–5] metabolic glutamate receptor (mGluR),[6] calcium sensing receptor (CaR),[7] and free fatty acid receptors (FFAs, GPR120).[8–11] Nutrient sensing through these receptors in the small intestinal epithelia is thought to influence nutrient metabolism and local physiological function.[12–14] Less is known, however, about the physiological roles of chemical sensing in the large intestine. We have investigated the contractile and secretory effects of

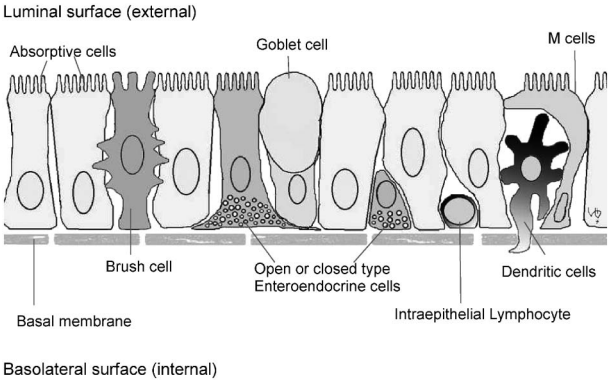

Fig. 1. Diagram of the Large Intestinal Epithelial Cells

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short-chain fatty acids (SCFAs), the main products of commensal bacteria, as well as the expression of SCFA receptors in the large intestine. These findings have indicated that the large intestinal epithelia also detect and respond to luminal contents, particularly various bacterial metabolites, for host defense. Host defense responses include transepithelial anion secretion followed by fluid secretion, which are regulated by diverse systems, including the enteric nervous system and a variety of gut hormones and cytokines.[15,16] This review therefore describes the secretory effect of chemical stimuli from lumen associated with the expression pattern of sensory receptors, focusing on the large intestine.

EFFECTS OF SHORT-CHAIN FATTY ACIDS (SCFAs) AND THEIR RECEPTORS

In the non-ruminant mammalian colon, SCFAs are produced by the bacterial fermentation of dietary fibers, such as indigestible carbohydrates, oligosaccharides, and resistant starch. The main components of SCFAs in the human colon include acetate (C2), propionate (C3), and butyrate (C4), at a ratio of about 3 : 1 : 1.[17,18] The physiological functions of these SCFAs investigated in rats are colonic contraction,[19–21] mucus secretion,[22] and anion secretion.[23] The luminal application of propionate and butyrate, but not acetate, in the rat distal colon was found to induce luminal electrogenic Cl^- secretion in a concentration dependent manner.[23] The enteric nervous system partly mediates these secretory responses, but the serosal application of SCFAs had no effect.[23] Most recently, luminal propionate was found to induce ACh release from colonic epithelial cells themselves.[24] This propionate- and butyrate-induced Cl^- secretion was also observed in the distal small intestine,[25] but not in the proximal colon.[23] Additionally, there are species differences in the secretory effect of SCFAs. For example, propionate induced rapid K^+ secretion in the guinea pig distal colon, independent of the neural pathway, as well as anion (Cl^- and HCO_3^-) secretion through neural reflexes (Fig. 2).[26] In the human colon, however, luminal acetate, as well as propionate and butyrate, induced sustained K^+ secretion in a concentration-dependent manner.[27] These observations have indicated that SCFAs are likely detected by epithelial cells through specific receptors.

In 2003, GPR41 and GPR43 were deorphanized as

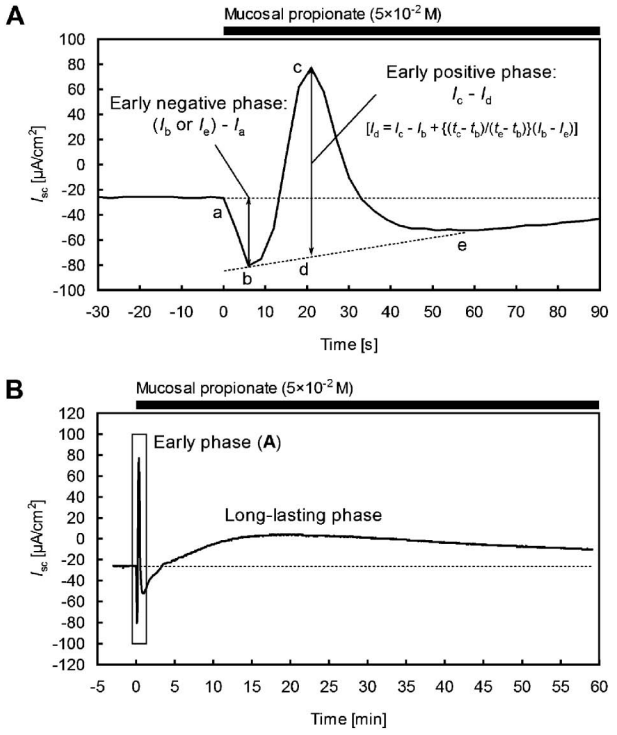

Fig. 2. Representative Traces of Luminal Propionate-induced Changes in Short-circuit Current (I_{sc}) in the Guinea Pig Distal Colon

The addition of propionate (at time 0) induced three-phase I_{sc} responses, including early negative (a↔b) and early positive (c↔d) phase responses (A) and a long-lasting phase response, calculated as the ΔI_{sc} from basal I_{sc} (*dotted line*) 15 min after the addition of propionate (B). The width and height of the box in (B) are equivalent to that in graph A. Delta I_{sc} values of the early phase responses were obtained using the formula described in (A). Reproduced from ref. 26.

SCFA receptors,[28–30] and renamed FFA3 and FFA2, respectively.[31] The ligand binding order and the coupled G-protein type of these receptors differ from each other (Table 1). Using immunohistological techniques, we reported that FFA2 is expressed in rat, guinea pig, and human colonic epithelia, with strong expression in PYY- and GLP-1-producing enteroendocrine cells, called L cells (Fig. 3).[10,26,32,33] FFA3 was also detected in human colonic L cells,[34] but it is still unclear whether these two receptors function in the same cells. Consistent with our findings, several *in vivo* studies have demonstrated that intraluminal injection of SCFAs induced plasma PYY and GLP-1

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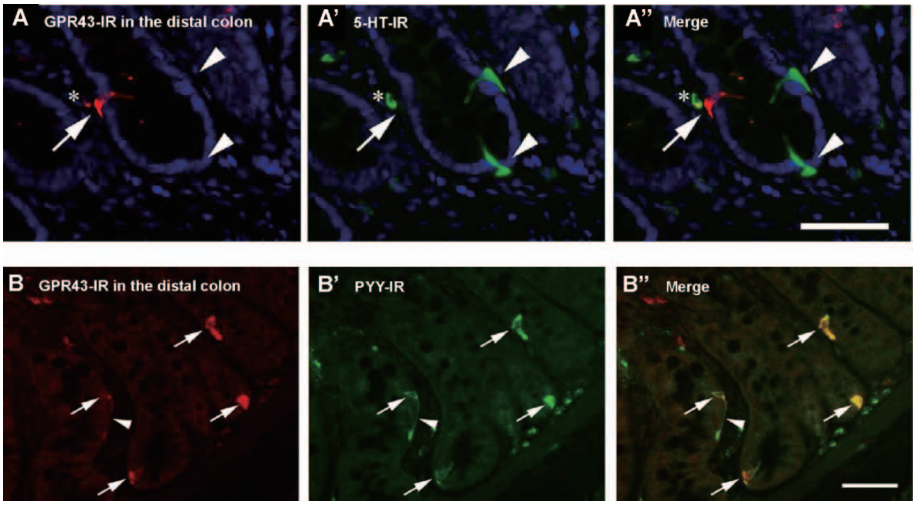

Fig. 3. Expression of FFA2 (GPR43) in the Rat Colon

Triple staining for FFA2 (*red*), 5-HT (A) or PYY (B) (*green*), and DAPI (4,6-diamidino-2-phyenylindole; *blue*) in the rat colonic mucosa. Colocalization appears in *yellow*. A: lack of colocalization of FFA2 and 5-HT in enteroendocrine cells. *Arrows* FFA2-IR enteroendocrine cells, *arrowheads* 5-HT-IR enteroendocrine cells, *asterisks* mucosal mast cells immunoreactive for both FFA2 and 5-HT in the lamina propria. B: colocalization of FFA2 and PYY in enteroendocrine cells (*arrows*). Processes are seen at the base of the cells (*arrowheads*). *Bar* 50 μm. Reproduced from ref. 10.

Table 1. Comparisons between FFA2 and FFA3

Receptor	Coupling	Ligand affinity
FFA2 (GPR43)	$G_{i/o}$	Butyrate=propionate=acetate
FFA3 (GPR41)	$G_{i/o}$, G_q	Butyrate=propionate≫acetate

release.[35,36] Luminal SCFAs may be involved in these gut hormone releases *via* FFA2 and/or FFA3.

LUMINAL COMPONENTS AFFECT COLONIC ENTEROENDOCRINE CELL POPULATIONS AND FFA2 EXPRESSION

Do changes in the luminal environment caused by dietary components influence epithelial sensory function? The bacterial fermentation of dietary fiber produces gases and SCFAs throughout the non-ruminant mammalian large intestine. Long-term ingestion of fermentable dietary fibers ensures high luminal concentrations of SCFAs. In particular, ingestion of the easily fermented soluble dietary fiber, fructo-oligosaccharide (fructo-OS, FOS), increased SCFA concentration in the cecum but not in fecal excretion,[37,38] indicating that the fermentation and absorption of fructo-OS are complete in the colon. Fructo-OS supplementation for 28 days induced an approximately two-fold increase in the numbers of L cells expressing FFA2 and GLP-1 in the upper large intestine (Fig. 4), but did not affect the fecal amount

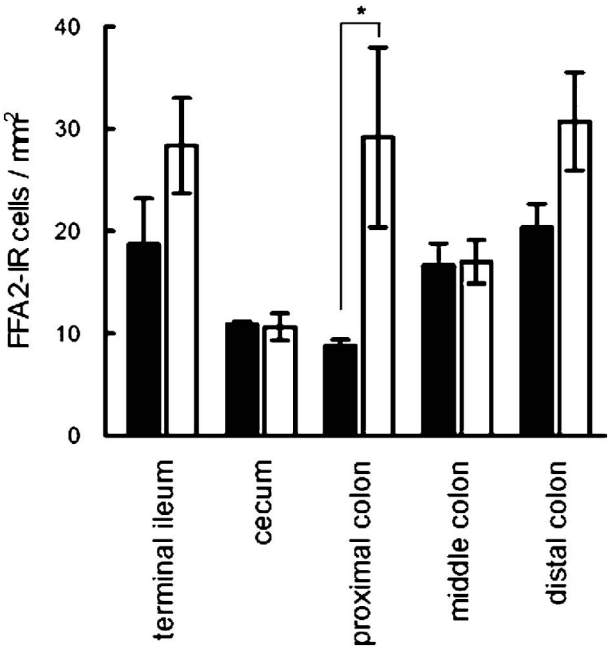

Fig. 4. Density of FFA2-positive Enteroendocrine Cells in the Lower Intestinal Mucosa of Rats Fed a Control or Fructo-OS-containing Diet[39]

The numbers of FFA2-immunoreactive enteroendocrine cells per square millimeter (mm^2) of mucosa in the control (filled bars) and Fructo-OS-fed (open bars) groups were counted. The values are expressed as mean±S.E. (n=3–4). ***$p<0.001$ by unpaired t test.

or the density of enterochromaffin (EC) cells, another type of enteroendocrine cell that produces 5-HT.[39] However, supplementation with cellulose, an insoluble dietary fiber, greatly increased the fecal amount and the density of EC cells in the rat colon

compared with those in rats fed a fiber-free diet.[40] These findings indicate that increased luminal concentrations of SCFA induce L cell proliferation, and that increased luminal content volume induces EC cell proliferation. Consequently, L cells may be chemosensors, while EC cells are likely mechanosensors in the gastrointestinal epithelia,[41,42] and the proliferation mechanisms of L cells and EC cells differ from each other.

EFFECT OF TASTE COMPOUNDS AND RECEPTOR EXPRESSION

In the taste transduction system, specialized taste buds consisting of taste cells play a role as sensors. The sour (H^+) and salt (Na^+) tastes are detected by individual channels, while sweet, bitter, and umami tastes are detected by G-protein coupled receptor (GPCR) families. The taste receptor type 1 (T1R) family includes three members, with the T1R1/T1R3 heterodimer being an umami receptor and the T1R2/T1R3 heterodimer being a sweet receptor. Using T1R3 knockout mice, intestinal taste signals were found to modulate expression of a glucose transporter SGLT1,[12] suggesting that luminal chemosensing affects nutrient absorption. In contrast to T1Rs, the bitter taste receptor (taste receptor type 2, T2R, TRB) family includes about 30 members in humans and rodents.[43,44] Each T2R member consists of a short extracellular domain, with relatively divergent amino acid sequences (25% to 90% identity).[45] This variability is thought to correspond with an ability to detect diverse ligands present in the external environment. Furthermore, each T2R functions as a monomer, suggesting low ligand specificity. Some bitter compounds activate one T2R member, whereas others activate multiple T2Rs.[46] Since T2R mRNA expression has been detected in human and rat large intestine,[4,47] these T2Rs may detect bacterial products. We have reported that a bitter tastant 6-*n*-propyl-2-thiouracil (6-PTU, PROP) induces electrogenic anion (Cl^- and HCO_3^-) secretion in human and rat colon (Fig. 5). The range of 6-PTU concentrations that could induce anion secretion was consistent with those with gustatory activity.[48–50] The secretory response in the colon was completely inhibited by the cyclooxygenase inhibitor piroxicam, but was insensitive to TTX.[47] Interestingly, 6-PTU-induced anion secretion was enhanced by exogenous prostaglandin E_2 (PGE_2) in a PGE_2-concentration dependent manner, and there were segmental differences in responses (Fig. 6). PGE_2 concentrations in the intestine are increased by mechanical stimulation[51] or inflammation,[52] with concentration $>10^{-7}$ M considered pathophysiological.[53] Our observations therefore suggest that these concentrations of tissue PGE_2 induce inflammation, potentiating a bitter stimuli-induced secretory response, which flushes out noxious substances from the colonic lumen. This finding supports our hypothesis that the concentration of tissue PGE_2 indicates the tissue 'alert level,'.[54] Thus, intestinal bitter sensing may play an important role in host defenses by interacting with PG, and may be involved in the interaction of luminal homeostasis with commensal bacteria.

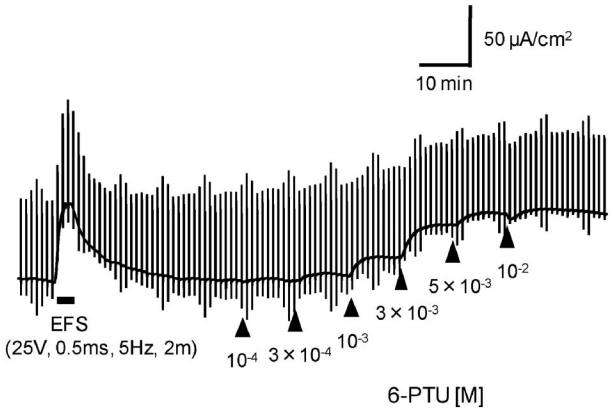

Fig. 5. Electrical Field Stimulation (EFS)- and 6-PTU-induced I_{sc} Increases in the Mucosal-submucosal Preparation of Human Rectum[47]

6-PTU (10^{-4} to 10^{-2} M) was added to the mucosal bathing solution every 10 min, and increases in I_{sc} and tissue conductance (G_t) were measured.

EFFECT OF ODORANTS AND RECEPTOR EXPRESSION

ORs are expressed by isolated duodenal EC cells, EC cell lines, and the small intestine of humans and rats, and luminal odorants may induce serotonin (5-HT) secretion.[1,55] In porcine small intestine, thymol, a classical odorant extracted from herbs, has been reported to induce luminal anion secretion through a cholinergic neural pathway.[56] Volatile odorants, including terpenoids derived from five carbon isoprene units, are produced by many plants, insects, and bacteria, including gut commensal bacteria.[57] Therefore, odorants synthesized in the colonic lumen may be detected by mucosal chemosensors. We have demonstrated several activities of thymol, an agonist of OR1G1, in colonic lumen.[33]

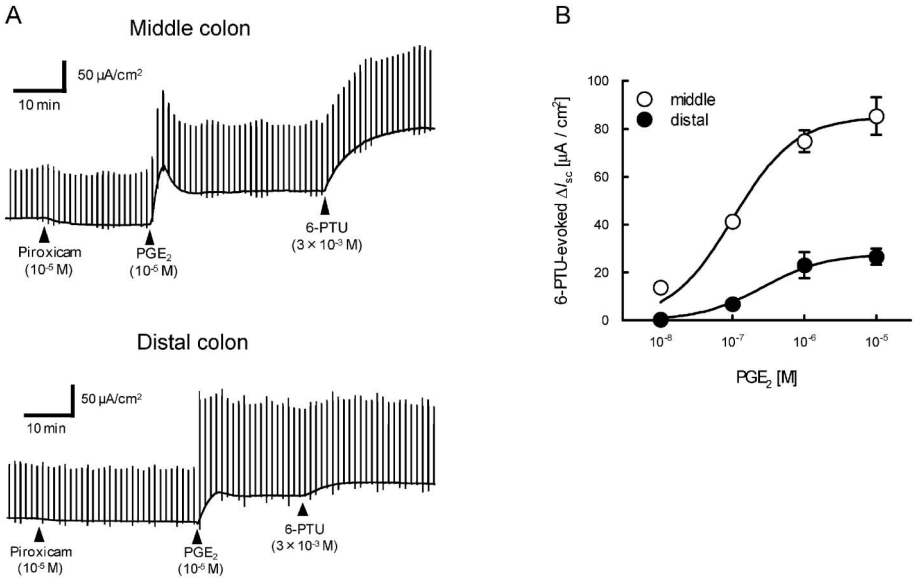

Fig. 6. Effects of PGE_2 and Further Addition of 6-PTU on I_{sc} in the Presence of Piroxicam in Rat Middle and Distal Colon[47]

Various concentrations (10^{-8} to 10^{-5} M) of PGE_2 were added to the serosal bathing solution, and 6-PTU (3×10^{-3} M) was added to mucosal bathing solution after stabilization of basal I_{sc}. All tissues were pretreated with piroxicam (10^{-5} M). A: representative traces showing the effects of piroxicam, PGE_2 and further addition of 6-PTU to the rat middle and distal colon. B: concentration-response curve of 6-PTU-evoked increases in I_{sc}. Values are expressed as means±S.E., $n=3$–5. Significant segmental differences ($p<0.01$) were obtained by two-way ANOVA.

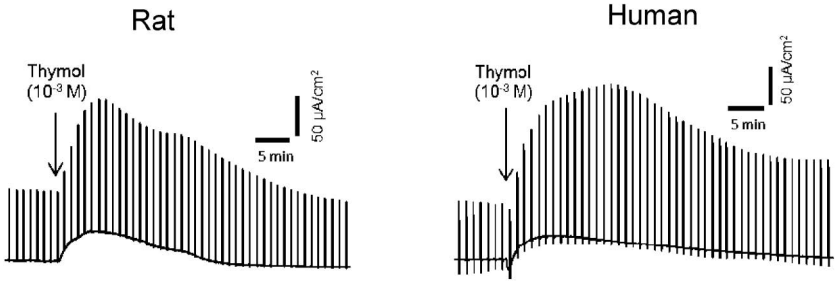

Fig. 7. Effect of Thymol on I_{sc} and G_t in Mucosa-submucosal Preparations of Human and Rat colon[33]

Representative traces showing that mucosal thymol (10^{-3} M) increased I_{sc} and G_t in human ascending colon and rat distal colon.

Thymol concentration-dependently induced anion secretion in both human and rat colon (Fig. 7), and greatly increased both tissue conductance and nonelectrolyte transepithelial permeability in rat distal colon.[33] However, thymol-induced anion secretion was not inhibited by 5-HT receptor antagonists or TTX, but required extracellular Ca^{2+}.[33] These results suggest species and segmental differences in luminal chemosensing mechanisms. Several odorants, especially those in spices, are agonists not only of ORs, but also of transient receptor potential (TRP) cation channels. Thymol has been reported to activate TRPV3 and TRPA1 in cell-expression systems,[58–60] and TRPV3 is expressed in the mouse colonic epithelia.[61] We observed involvement of the TRPA1 channel in thymol-induced electrogenic anion secretion.[33] TRPA1 mRNA was detected in isolated mucosa from human and rat colon, suggesting that TRPA1 functions in colonic mucosa. Furthermore, thymol affected luminal SCFA-induced ion secretion. Thymol concentration-dependently inhibited propionate-induced ion secretion, and this inhibitory effect was reversible (Fig. 8), suggesting that it may function as a competitive antagonist for SCFA receptors FFA2 and/or FFA3. Thus, luminal odorants produced by microflora may modulate SCFA function in colonic lumen and bacterial metabolites may be detected by colonic mucosa and involved in colonic physiological function.

CONCLUSION

Bitter taste and odor signals may be indicators of

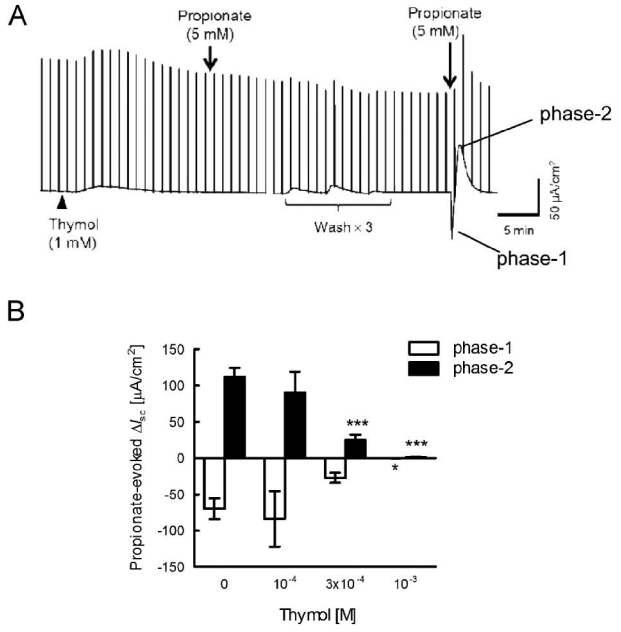

Fig. 8. Effect of Mucosal Thymol on the Response to EFS and SCFA Propionate in Rat Distal Colon[33]

A: representative traces showing that the response to propionate was restored after washing tissue. B: concentration-dependent inhibition by thymol on propionate-evoked I_{sc} decreasing (phase-1) and increasing (phase-2) responses. $^{*}p<0.05$, $^{***}p<0.001$ compared with non-treated tissue by Dunnet's test. Peak values are expressed as means±S.E., $n=4$–14.

toxic substances, thus avoiding injury to the central nervous system. Similarly, gut chemosensing may act as a repellent mechanism for poisons, preventing exposure of the large intestine to these substances. Distinct mechanisms in the colonic mucosa monitor luminal ambient chemicals. When present, these chemicals induce anion secretion, which in turn stimulates fluid secretion, an important host defense mechanism to flush out noxious agents from the colonic lumen. Although essential nutrients are received from the external environment, there is no 'physiologically perfect diet'. Therefore, intestinal epithelia are in continuous contact with unfamiliar agents and discern necessary from noxious substances. Moreover, the colonic epithelia may communicate with commensal bacteria through their products and chemical receptors, maintaining host-bacterial mutualism. Investigations of physiological phenomena of the intestinal epithelia and their mechanisms are important in revealing how homeostasis is maintained while interacting with the external environment.

Morphological and physiological studies have suggested the functional similarity of taste cells and gut enteroendocrine cells.[62] Enteroendocrine cells, both in the small intestine and as isolated populations, have been reported to express a variety of chemosensory receptors. However, the expression patterns of individual receptor proteins *in situ* remain unclear, particularly in the large intestine. We have also shown species and segmental differences in the response to luminal chemicals throughout the intestine, suggesting the need for further studies to identify sensor cells in each intestinal region.

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