Secretry effects of a luminal bitter transient and expressions of bitter taste receptors, T2Rs, in the human and rat large intestine

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Kaji I, Karaki SL, Fukami Y, Terasaki M, Kuwahara A. Secretry effects of a luminal bitter transient and expressions of bitter taste receptors, T2Rs, in the human and rat large intestine. Am J Physiol Gastrointest Liver Physiol 296: G971–G981, 2009. First published January 29, 2009; doi:10.1152/ajpgi.90514.2008.—Taste transduction molecules, such as Gaq, gust, and taste receptor families for bitter [taste receptor type 2 (T2R), sweet, and umami], have previously been identified in taste buds and the gastrointestinal (GI) tract; however, their physiological functions in GI tissues are still unclear. Here, we investigated the physiological function and expression of T2R in human and rat large intestine using various physiological and molecular biological techniques. To study the physiological function of T2R, the effect of a bitter compound, 6-n-propyl-2-thiouracil (6-PTU), on transepithelial ion transport was investigated using the Ussing chamber technique. Mucosal-submucosal preparations, mucosal 6-PTU evoked Cl− and HCO3− secretions in a concentration-dependent manner. In rat middle ileum, levels of 6-PTU-evoked anion secretion were higher than in distal colon, but there was no such difference in human large intestine. The response to 6-PTU was greatly reduced by piroxicam, but not by tetrodotoxin. Additionally, prostaglandin E2 concentration-dependently potentiated the response to 6-PTU. Transcripts of multiple T2Rs (putative 6-PTU receptors) were detected in both human and rat colonic mucosa by RT-PCR. In conclusion, these results suggest that the T2R ligand, 6-PTU, evokes intestinal anion secretion, and such response is regulated by prostaglandins. This luminal bitter sensing mechanism may be important for host defense in the GI tract.

intestinal chemo-sensing; taste receptor type 2; prostaglandin E2; transepithelial ion transport; 6-n-propyl-2-thiouracil

INTESTINAL TRANSEPITHELIAL ion transport is regulated by diverse systems, including the enteric nervous system (ENS), a variety of gut hormones and cytokines, responding to mechanical and chemical stimuli (10). One type of chemical stimuli at the intestinal lumen is short-chain fatty acids (SCFAs), including propionate and butyrate, which are bacterial metabolites, especially in the large intestine. SCFAs have been reported to evoke epithelial ion transport through mucosal stimulation and ENS activation (42). Although the sensing mechanism for SCFAs at mucosa is still unclear, we recently found that the SCFA receptor, G protein-coupled receptor 43, is expressed by enteroendocrine cells and mucosal mast cells in human and rat intestine (19, 22). Therefore, we speculate that other chemical receptors are expressed by sensory cells in the intestine.

Recently, the same taste transduction mechanism found in the taste buds of lingual papillae was also reported to be present in the intestine. The α-subunit of taste-specific G protein gustducin (29) was expressed in gastrointestinal (GI) mucosa of humans and rodents (14, 34, 38); mRNA expression of taste receptor type 1 (T1R) and type 2 (T2R) families in the human and rodent GI tract has also been reported (34, 40). These reports suggest that taste receptors are also employed for intestinal chemical sensing.

The T2R family has been identified as a specific receptor family for bitter tastants by genomic sequencing analysis and consists of ~30 members in human and rodents (1, 11, 28, 36). Until now, only ~10 substances have been identified as human T2R (hT2R) agonists, and it is known that each member of T2R family responds to multiple limiting substances. As taste transduction molecules are present in intestinal sensory cells that secrete gut hormones (e.g., glucagon like peptide-1, peptide YY, and serotonin), it has been speculated that the T2Rs function to regulate nutrient absorption and metabolism in the GI tract and may also affect diet behavior (34, 40, 41). Administration of T2R agonists to the lumen of the GI tract is reported to activate neurons in the nucleus tractus solitarii (13). However, to date, there is no physiological study documenting that any bitter compounds affect physiological function in the GI tract.

In the present study, we, therefore, examined the effect of bitter taste compounds, especially 6-n-propyl-2-thiouracil (6-PTU, PTU, or PROP), on ion transport in human and rat colonic epithelia to elucidate the physiological function of T2R. 6-PTU is an anti-hyperthyroid agent and has been used for researching the gene loci that control bitter detection in humans in behavioral studies because of its strong bitterness and relatively low toxicity (24). Multiple T2R family members, at least T2R-1, -4, and -38 in humans, are known to detect bitter [taste receptor type 1 (T1R) and type 2 (T2R) families in the human and rodent GI tract has also been reported (34, 40, 41)]. The expression of hT2R-1 in humans and rT2Rs in rat has not been confirmed in the large intestine. Therefore, these T2R expressions were also investigated in isolated mucosa.

MATERIALS AND METHODS

Human intestinal specimens and tissue preparation. This study was approved by the Institutional Review Board of Shizuoka Saiseikai General Hospital and the University of Shizuoka. Human intestinal surgical specimens were obtained (following informed consent) from patients undergoing extirpation for carcinoma. The age of patients was 71.4 ± 2.1 yr (mean ± SE, n = 19, including 7 women and 12 men). A nonpathological region was cut from the specimen, and this region was then placed in ice-cold Krebs-Ringer solution saturated with 95% O2-5% CO2 and transported to the laboratory. Krebs-Ringer solution contained (in mM) 117 NaCl, 4.7 KCl, 1.2 MgCl2, 1.2 NaH2PO4, 25 NaHCO3, 2.5 CaCl2, and 11 glucose.

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Tissues were pinned flat on a silicone filled Petri dish with mucosal surface down, and tissue preparations were constructed by using forceps and scissors. To obtain the mucosal-submucosal preparations, the smooth muscle layer was gently removed, leaving the blood vessels in the submucosal connective tissue intact. During preparation, tissues were bathed in ice-cold Krebs-Ringer solution (bathing solution) and continuously oxygenated with a gas mixture of 95% O₂ and 5% CO₂.

Animals and tissue preparation. The handling and death of animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka, and the study was approved by the University of Shizuoka Animal Usage Ethics Committee. Adult male Wistar rats (Chars River Laboratories Japan, Yokohama, Japan or SLC, Hamamatsu, Japan), weighing 180–220 g, were anesthetized with ether and decapitated by guillotine. Tissue preparations were constructed under stereomicroscope by using two pairs of fine forceps and microscissors.

Ussing flux chamber experiments. The mucosal-submucosal preparations of human and rat, each with a cross-sectional area of 0.64 cm², were mounted between halves of Ussing flux chambers. Mucosal arations of human and rat, each with a cross-sectional area of 0.64 cm², were prepared. By using a stable basal short-circuit current (Isc), transepithelial conductance (Gt) was calculated from Isc by Ohm’s law. The solution was bubbled with a gas mixture of 95% O₂ and 5% CO₂.

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Table 1. Primers for human and rat T2Rs and β-actin

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence</th>
<th>bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>hT2R-1</td>
<td>Forward</td>
<td>5’-GATCTGCTTCTTCTTTTCTGCTG-3’</td>
</tr>
<tr>
<td>hT2R-4</td>
<td>Reverse</td>
<td>5’-CTAGAATATGGAAGAATG-3’</td>
</tr>
<tr>
<td>hT2R-38</td>
<td>Forward</td>
<td>5’-GCCAACCTGGTGCAAAGCCG-3’</td>
</tr>
<tr>
<td>hβ-actin</td>
<td>Forward</td>
<td>5’-ATTTTGAATCTGATTCCGGATCT-3’</td>
</tr>
<tr>
<td>rT2R-1</td>
<td>Forward</td>
<td>5’-GCCACAGATGGGCGGGAAATC-3’</td>
</tr>
<tr>
<td>rT2R-16</td>
<td>Reverse</td>
<td>5’-ATGATTAGGATGAGCTAACG-3’</td>
</tr>
<tr>
<td>rT2R-26</td>
<td>Forward</td>
<td>5’-CGACACTCTTCTGAGGCTCCT-3’</td>
</tr>
<tr>
<td>rβ-actin</td>
<td>Reverse</td>
<td>5’-CAATGTACTGCTGCTGCTG-3’</td>
</tr>
</tbody>
</table>

T2R, taste receptor type 2; h, human; r, rat; bp, base pair. Primers for hT2Rs were as described in a previous study (50). hβ-Actin primer was purchased from a commercial source (Clontech).

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ing tissues (~20 mg) were then transferred to new 2.0-ml Eppendorf tubes and freeze-ground by using a grinding mill (SK-100; Tokken, Kashiwa, Chiba, Japan). Total RNAs were isolated by RNeasy Micro Kit (Qiagen). To remove the genome DNA, deoxyribonuclease (RT Grade) for heat stop (Nippon Gene, Tokyo, Japan) was used, and then cDNA was synthesized by using first-strand cDNA synthesis kit for RT-PCR (AMV) (Roche Applied Science, Mannheim, Germany). Synthesized cDNAs and primers were stored at −20°C until use.

Primers for hT2Rs were used, according to a previous study (34), and the primers for rT2Rs and rat β-actin were designed in the present Table 2.

### Table 2. Basal electrical properties of human and rat tissues

<table>
<thead>
<tr>
<th>Human tissue</th>
<th>n</th>
<th>Basal ( I_{sc} ), ( \mu A/cm^2 )</th>
<th>( G_t ), mS/cm²</th>
<th>PD, mV</th>
<th>5-Hz EFS-evoked ( \Delta I_{sc} ), ( \mu A/cm^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending colon</td>
<td>8</td>
<td>56.1±8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2±1.1</td>
<td>−7.9±1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.7±12.1</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>6</td>
<td>3.4±8.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.0±1.5</td>
<td>−0.5±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.2±14.6</td>
</tr>
<tr>
<td>Rectum</td>
<td>5</td>
<td>−3.3±13.6&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>10.8±1.9</td>
<td>−0.7±1.8&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>68.5±10.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rat tissues</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Middle colon</td>
<td>70</td>
<td>19.9±1.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.0±0.32</td>
<td>−1.8±0.10</td>
<td>106.3±4.29&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distal colon</td>
<td>41</td>
<td>15.6±0.91&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0±0.50</td>
<td>−1.5±0.12</td>
<td>87.0±4.84&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of tissues. \( I_{sc} \), short-circuit current; \( \Delta I_{sc} \), change in \( I_{sc} \); \( G_t \), tissue conductance; PD, potential difference; EFS, electrical field stimulation. \( ^aP < 0.001 \) and \( ^bP < 0.01 \) by ANOVA. \( ^cP < 0.01 \) and \( ^dP < 0.05 \) vs. ascending colon by Tukey-Kramer test. \( ^eP < 0.01 \) between segments by unpaired \( t \)-test.

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![Fig. 1](http://example.com/fig1.png)

**Fig. 1.** Effect of 6-n-propyl-2-thiouracil (6-PTU) on basal short-circuit current (\( I_{sc} \)) and tissue conductance (\( G_t \)) in human colorectal and rat colonic mucosal-submucosal preparations. 6-PTU (10<sup>−4</sup>-10<sup>−2</sup> M) was added to the mucosal bathing solution (human and rat) or serosal bathing solution (rat) every 10 min, and increases in \( I_{sc} \) and \( G_t \) were measured. A: representative trace to illustrate effects of electrical field stimulation (EFS) and 6-PTU on basal \( I_{sc} \) and \( G_t \) in the human rectum. B: concentration-dependent curves of 6-PTU-induced increases in \( I_{sc} \) and \( G_t \) in human tissues. The values of change in \( I_{sc} \) (\( \Delta I_{sc} \)) were normalized with \( I_{sc} \) of control tissues. C: concentration-dependent curves of 6-PTU-induced increases in \( I_{sc} \) and \( G_t \) in rat tissues. Basal \( I_{sc} \) was increased by the addition of 6-PTU in a concentration-dependent manner, but \( G_t \) was not changed in both human and rat tissues. Values are expressed as means ± SE; \( n = 3–5 \) for each segment. **\( P < 0.01 \) between middle and distal colon by two-way ANOVA followed by multiple comparison of Tukey.
study, based on the mRNA sequence (NCBI, rT2R1, AF227140, and NM_023993; rT2R16, NM_001024686, AY916510, and AY362740; rT2R26, AY362746, AY916512, and NM_001024685). The human β-actin primer was purchased from a commercial source (Clontech Laboratories, Mountain View, CA). All primer sequences used in the present study are shown in Table 1. The sequence specificities of primers for rT2Rs were tested by NCBI BLAST. RT-PCR with mRNA of tongue circumvallate papillae was performed to test primer reliability as a positive control. PCR was performed by using a RT-PCR kit (Ready-To-Go RT-PCR Beads; Amersham plc, Buckinghamshire, UK) and Takara PCR Thermal cycler MP (TP3000, Takara Bio, Otsu, Shiga, Japan). The reaction mixture was 25 µL, including 50 ng of cDNA and 0.4 µM of forward and reverse primers. Before PCR, reaction mixtures were incubated at 94°C for 5 min to completely denature the template. PCR cycles consisted of denaturing at 94°C for 1 min, annealing at 55–57°C for 1 min, and extension at 72°C for 1–1.5 min (35 cycles), followed by final extension at 72°C for 7 min; amplification products were stored at 4°C. PCR products and DNA size marker (OneSTEP Marker 5, Nippon Gene, Toyama, Japan) were separated by electrophoresis on 1.5% agarose gel and stained by SYBR Green I (Molecular Probes, Eugene, OR). Gel images were viewed by excitation of 488 nm and a band-pass filter of 530 nm in Molecular Imager Pharos FX (Bio-Rad Laboratories, Hercules, CA).

**Chemicals.** 6-PTU, DB, PTC, DMSO, bumetanide, and TTX was from Sigma (St. Louis, MO); piroxicam was from Biomol Research Laboratories (Plymouth Meeting, PA); CYX from Calbiochem (Merck KGaA, Darmstadt, Germany); and PGE2 was from Cayman Chemical (Ann Arbor, MI). TTX was dissolved in citrate buffer (pH 4.8), while other drugs were dissolved in DMSO. Volume of dissolved

![Fig. 2. Effect of 6-PTU on \( I_{sc} \) responses stimulated by EFS in rat middle colon.](image)

- **A**. representative trace to illustrate the enhancement of the \( I_{sc} \) responses to EFS by 6-PTU. **B**. EFS-evoked \( I_{sc} \) increases before (control) and after the addition of 6-PTU. Peak values are expressed as means ± SE; \( n = 5 \). ***P < 0.001, **P < 0.01, *P < 0.05 vs. control by paired t-test.

**Fig. 3. Effect of bumetanide on \( I_{sc} \) response to 6-PTU in rat middle colon.** Bumetanide (10⁻⁴ M) was added to serosal bathing solution 15 min before addition of 6-PTU (3 × 10⁻³ M). 6-PTU-evoked increases in \( I_{sc} \) were measured between 15 min after and just before the addition of 6-PTU. A: representative traces to illustrate effects of 6-PTU on \( I_{sc} \) in the presence or absence of bumetanide. B: time course of basal \( I_{sc} \) changes in the presence or absence of bumetanide. C: 6-PTU-evoked increases in \( I_{sc} \) between 15 min after and just before the addition of 6-PTU. Pretreatment of the tissues with bumetanide significantly decreased basal \( I_{sc} \) itself (††P ≤ 0.01 vs. 0 min by paired t-test; B), but not \( G_{j} \). 6-PTU-evoked increases in \( I_{sc} \) were significantly reduced by the pretreatment with bumetanide (***P < 0.01 vs. control by paired t-test; B and C). Values are expressed as means ± SE; \( n = 5 \).
drugs in H₂O or DMSO added to the bathing solutions did not exceed 100 μl (1% of bathing solution).

Data analysis and statistics. All data are expressed as means ± SE. The n values represent the numbers of human patients and animals. ANOVA followed by Tukey-Kramer’s test and unpaired Student’s t-test was used to determine whether there were significant differences in basal electrical parameters among the tissue segments. Concentration-response curve of 6-PTU-induced Iₑₑ responses were fitted to Michaelis-Menten binding curves by the nonlinear-square procedure using KyPlot, a data analysis and graph-creating software (KyensLab, Tokyo, Japan) and compared among the tissue segments using two-way ANOVA. Paired Student’s t-test was used to examine the effects of inhibitors on basal electrical parameters and to compare control and experimental conditions. P ≤ 0.05 was considered statistically significant.

RESULTS

Segmental differences of basal electrical parameters in human and rat large intestine. To examine the basal electrical parameters in the mucosal-submucosal preparations, the average PD, basal Iₑₑ and Gₑₑ after stabilization, and 5 Hz EFS-evoked change (Δ) in Iₑₑ were measured utilizing the Ussing flux chamber in human ascending colon, sigmoid colon, and rectum, as well as rat middle and distal colon (Table 2). These electrical parameters in human tissues are shown in Table 2 for each segment as the averages of patients’ tissues. Segmental heterogeneity of basal electrical parameters in human colon was reported previously (33), and we obtained similar results. Basal Iₑₑ (P < 0.01) and PD (P < 0.05) in the ascending colon were significantly higher than those in other regions (n = 5–8). Table 2 also shows the electrical parameters in rat tissues. In rat middle colon, the basal Iₑₑ (P < 0.01) and 5 Hz EFS-evoked ΔIₑₑ (P < 0.01) were significantly higher than those in the rat distal colon (n = 41–70).

Effect of T2R ligands on electrical activity in human and rat large intestine. The addition of 6-PTU to mucosal bathing solution evoked an increase in Iₑₑ in both human and rat large intestine without changing Gₑₑ. Neither the addition of DB, PTC, nor CYX affected basal electrical activity, even in high concentrations (3 × 10⁻³ M). After the addition of 6-PTU, Iₑₑ increased gradually for 10–15 min, achieved plateau, and then sustained for ≥20 min. To investigate the effects of 6-PTU on basal Iₑₑ and Gₑₑ in human and rat colon, 6-PTU (10⁻⁴–10⁻² M) was cumulatively added to mucosal bathing solution every 10 min, and changes in Iₑₑ and Gₑₑ were measured. In rat tissues, serosal addition of 6-PTU was also tested; 6-PTU to serosal bathing solution evoked an increase in Iₑₑ without changing Gₑₑ.
in rat colon, as well as mucosal addition. Concentration-dependent curves for 6-PTU in the various regions of the human and rat colon (human ascending and sigmoid colon and rectum; and rat middle and distal colon) were constructed. Figure 1 shows concentration-dependent increases in \( I_{sc} \) in human (Fig. 1, A and B) and rat colon (Fig. 1C). The 50% effective concentrations (EC50) in human ascending colon, sigmoid colon, and rectum were \(-2.7 \times 10^{-2}, 2.6 \times 10^{-2}, \) and \(3.2 \times 10^{-3} \) M, respectively. Since, in rat tissues, \(10^{-2} \) M of 6-PTU did not clearly achieve maximum response, EC50 in rat was not calculated in this study. Among the regions of human colon, there was no significant difference in the 6-PTU-induced increase in \( I_{sc} \) (as analyzed by two-way ANOVA). On the other hand, in rat middle colon, both the mucosal and serosal 6-PTU-induced \( \Delta I_{sc} \) were significantly higher than that found in distal colon (\( P < 0.01 \) by two-way ANOVA followed by multiple comparison of Tukey, \( n = 4 - 5 \)). The \(3 \times 10^{-3} \) M 6-PTU-evoked increases in \( I_{sc} \) were 14.7 ± 3.4 \( \mu \)A/cm² in the middle colon and 1.86 ± 1.5 \( \mu \)A/cm² in the distal colon. Since the present study focused on the mechanism of mucosal chemical sensing, the following experiments were performed to unravel the mucosal 6-PTU effects using rat middle colonic segments.

Effect of 6-PTU on EFS-evoked increases in \( I_{sc} \) in rat middle colon. To examine the effect of mucosal 6-PTU on enteric nerve-mediated secretory action, various frequencies of EFS (25 V, duration of 0.5 ms, 1, 2, 5, and 10 Hz, 2 min for each frequency) were applied before and 15 min after the addition of 6-PTU (\(3 \times 10^{-3} \) M). In the absence of 6-PTU, the EFS-evoked increases in \( I_{sc} \) did not differ from control responses (1 Hz; 15.4 ± 5.2 \( \mu \)A/cm², 2 Hz; 47.1 ± 14.0 \( \mu \)A/cm², 5 Hz; 95.7 ± 24.3 \( \mu \)A/cm², 10 Hz; 118.8 ± 33.0 \( \mu \)A/cm²). However, the presence of \(3 \times 10^{-3} \) M 6-PTU significantly potentiated the EFS-evoked \( I_{sc} \) responses from 24.2 ± 9.0, 54.6 ± 12.8, 108.9 ± 20.6, and 124.6 ± 12.7 \( \mu \)A/cm² to 37.8 ± 6.3, 83.3 ± 12.9, 127.8 ± 13.6, and 140.7 ± 15.5 \( \mu \)A/cm², by 1, 2, 5, and 10 Hz, respectively (Fig. 2, 1 Hz: \( P < 0.05 \), 2 Hz: \( P < 0.01 \), 5 and 10 Hz: \( P < 0.001 \) by paired \( t \)-test, \( n = 5 \)).

Effects of bumetanide and NPPB on 6-PTU-evoked increase in \( I_{sc} \) in rat middle colon. These experiments were designed to investigate the ion components of \( I_{sc} \) response induced by 6-PTU. Two preparations of middle colon from one rat were used for the control and experimental groups. An inhibitor of Na⁺-K⁺-2Cl⁻ cotransporter, bumetanide (\(10^{-4} \) M), or an inhibitor of cystic fibrosis transmembrane conductance regulator (CFTR), NPPB (\(10^{-4} \) M) was used. Bumetanide (\(10^{-4} \) M) was added to serosal bathing solution 15 min before the mucosal application of 6-PTU (\(3 \times 10^{-3} \) M). Bumetanide itself significantly decreased basal \( I_{sc} \) from 21.0 ± 3.2 to 16.0 ± 2.8 \( \mu \)A/cm² (\( P < 0.05 \) by paired \( t \)-test, \( n = 5 \), Fig. 3B) without changing \( G_{t} \). The 6-PTU-evoked \( \Delta I_{sc} \) was measured 15 min after the addition of 6-PTU. The 6-PTU-evoked \( \Delta I_{sc} \) was significantly reduced from 20.1 ± 4.3 \( \mu \)A/cm² (in control group) to 6.3 ± 2.4 \( \mu \)A/cm² (\( P < 0.01 \) by paired \( t \)-test, \( n = 5 \), Fig. 3C) in the experimental group.

NPPB (\(10^{-4} \) M) was added to mucosal bathing solution 10 min before the application of 6-PTU (\(3 \times 10^{-3} \) M). NPPB itself significantly reduced basal \( I_{sc} \) from 15.4 ± 2.1 to 11.7 ± 3.3 \( \mu \)A/cm² (\( P \leq 0.05 \) by paired \( t \)-test, \( n = 4 \)) and increased \( G_{t} \) from 9.11 ± 0.5 to 13.4 ± 1.7 mS/cm² (\( P = 0.077 \), \( n = 4 \)). When basal \( I_{sc} \) and \( G_{t} \) were stabilized, 6-PTU was added.

NPPB almost completely abolished the 6-PTU-induced \( I_{sc} \) increase (from 5.4 ± 1.6 to -0.07 ± 0.86 \( \mu \)A/cm², \( P < 0.05 \), \( n = 4 \)).

Effects of Cl⁻ and HCO₃⁻-free solution on 6-PTU-evoked increase in \( I_{sc} \) in rat middle colon. It has been reported that, in addition to Cl⁻, HCO₃⁻ can also pass through CFTR, and Cl⁻-independent HCO₃⁻ secretion is inhibited by NPPB (17, 39). To determine the ionic basis for the increases in \( I_{sc} \) evoked by 6-PTU, Cl⁻-free and Cl⁻/HCO₃⁻-free solutions were used. The mucosal 6-PTU-induced (\(3 \times 10^{-3} \) M) \( I_{sc} \) responses were measured in the absence of Cl⁻ and Cl⁻/HCO₃⁻ from Krebs-Ringer solutions. Both mucosal and serosal bathing solutions were replaced with Cl⁻-free solution or Cl⁻/HCO₃⁻-free solution, and EFS (25 V, 5 Hz, 0.5 ms-duration, for 2 min) and 6-PTU-induced increases in \( I_{sc} \) were measured (Fig. 4). In Cl⁻-free solution, basal \( I_{sc} \) and \( G_{t} \) decreased from 20.8 ± 4.2 to 13.3 ± 1.5 \( \mu \)A/cm² (\( P = 0.16 \) by paired \( t \)-test, \( n = 6 \)) and from 9.3 ± 1.1 to 5.4 ± 0.4 mS/cm² (\( P < 0.05 \) by paired \( t \)-test, \( n = 6 \)), respectively. In Cl⁻/HCO₃⁻-free solution, basal \( I_{sc} \) and \( G_{t} \) also significantly decreased from 18.0 ± 2.6 to 4.6 ± 0.5 \( \mu \)A/cm² (\( P < 0.05 \), \( n = 4 \)), from 11.6 ± 0.7 to 7.0 ± 0.8 mS/cm² (\( P < 0.01 \), \( n = 4 \)), respectively. The EFS-evoked increase in \( I_{sc} \) was significantly reduced by the absence of Cl⁻ from 92.2 ± 11.1 \( \mu \)A/cm² (\( \Delta I_{sc} \), control, \( n = 10 \)) to 30.3 ± 2.4 \( \mu \)A/cm² (\( \Delta I_{sc} \), Cl⁻/HCO₃⁻-free solution).
potentiation of EFS-evoked increases in \( I_{sc} \) by 6-PTU were dependent on PGE\(_2\). To examine endogenous PG involvement, a nonselective COX inhibitor, piroxicam (10\(^{-5}\) M), was added to serosal bathing solution 10 min before the application of 6-PTU (3 \( \times \) 10\(^{-3}\) to 10\(^{-2}\) M). Piroxicam itself significantly decreased \( I_{sc} \) from 17.3 \( \pm \) 2.3 to 10.8 \( \pm \) 2.1 \( \mu \)A/cm\(^2\) (\(-4.6 \pm 1.6 \, \mu \text{A/cm}^2\), \( \Delta I_{sc} \); \( P < 0.01 \) by paired \( t \)-test, \( n = 5 \), Fig. 6A, bottom trace). The 6-PTU-evoked increase in \( I_{sc} \) was greatly reduced by the serosal addition of piroxicam (from 17.1 \( \pm \) 1.9 to 4.7 \( \pm \) 1.5 \( \mu \)A/cm\(^2\) by 3 \( \times \) 10\(^{-3}\) M; \( P < 0.01 \) by paired \( t \)-test, \( n = 5 \), Fig. 6A, bottom trace). On the other hand, the anion secretion by EFS following addition of 6-PTU was not affected by piroxicam (Fig. 6A, bottom trace, and B). The mucosal addition of piroxicam (10\(^{-5}\) M) also decreased \( I_{sc} \) (from 17.8 \( \pm \) 1.6 to 6.9 \( \pm \) 1.6 \( \mu \)A/cm\(^2\); \( P < 0.01 \) by paired \( t \)-test, \( n = 5 \), Fig. 6C), as well as serosal piroxicam. These data indicate that 6-PTU-evoked Cl\(^-\)/HCO\(_3\)^- secretion was dependent on endogenous PG synthesis by COX.

**Effect of PGE\(_2\) on 6-PTU-evoked increases in \( I_{sc} \) in rat middle and distal colon.** To investigate whether \( I_{sc} \) response to 6-PTU requires the presence of PGE\(_2\), various concentrations (10\(^{-8}\) to 10\(^{-5}\) M) of PGE\(_2\) were added to serosal bathing solution in the presence of piroxicam (10\(^{-5}\) M). When \( I_{sc} \) achieved plateau by the addition of PGE\(_2\), 6-PTU (3 \( \times \) 10\(^{-3}\) M) was added to the mucosal bathing solution. Although the 3 \( \times \) 10\(^{-3}\) M 6-PTU evoked little increase in \( I_{sc} \) in basal conditions in the distal colon (Fig. 1B, \( n = 5 \)), PGE\(_2\) concen-
tation-dependently enhanced the 6-PTU-evoked $I_{sc}$ response in both the distal and middle colon. Figure 7A shows representative traces of the 6-PTU-evoked responses following the addition of PGE$_2$ ($10^{-5}$ M) in the presence of piroxicam in middle and distal colon. In both regions, pretreatment of the tissues with piroxicam significantly decreased basal $I_{sc}$ from $23.2 \pm 1.1$ to $12.6 \pm 1.4$ $\mu$A/cm$^2$ in the middle colon ($-10.6 \pm 1.3$ $\mu$A/cm$^2$, $\Delta I_{sc}$: $P < 0.0001$ by paired $t$-test, $n = 12$) and $12.0 \pm 1.3$ to $8.8 \pm 0.9$ $\mu$A/cm$^2$ in the distal colon ($-3.1 \pm 0.6$ $\mu$A/cm$^2$, $\Delta I_{sc}$: $P < 0.0001$ by paired $t$-test, $n = 15$), respectively. The effect of piroxicam on basal $I_{sc}$ of the middle colon was significantly larger than that of the distal colon (Fig. 7A). Serosal application of PGE$_2$-induced biphasic increases in $I_{sc}$ composed of transient and sustained phases in

![Diagram](http://ajpgi.physiology.org/)

**Fig. 7.** Effects of prostaglandin (PG) E$_2$ and further addition of 6-PTU on $I_{sc}$ in the presence of piroxicam in rat middle and distal colon. Various concentrations ($10^{-8}$ to $10^{-3}$ M) of PGE$_2$ were added to the serosal bathing solution, and 6-PTU ($3 \times 10^{-3}$ M) was added to mucosal bathing solution after stabilization of basal $I_{sc}$. All tissues were pretreated with piroxicam-HCl ($10^{-5}$ M to serosal bathing solution). A: representative traces to illustrate the effects of piroxicam, PGE$_2$, and further addition of 6-PTU in rat middle and distal colon. B and C: concentration-response curve of PGE$_2$ (B) and the further addition of 6-PTU-evoked (C) increases in $I_{sc}$. TP, transient phase; SP, sustain phase. Values are means $\pm$ SE; $n = 3-5$. ***$P < 0.01$ by two-way ANOVA.
a concentration-dependent manner (Fig. 7B). The 10^{-5} M PGE2-evoked transient phase responses were 47.3 ± 7.4 μA/cm^2 in the middle colon and 26.5 ± 3.3 μA/cm^2 in the distal colon and were maximally enhanced 6-PTU-evoked increases in I_{sc} (Fig. 7C, n = 3–5). The 6-PTU-evoked I_{sc} increases were dependent on the concentration of PGE2, and the EC_{50} was calculated to 1.0 × 10^{-7} or 2.4 × 10^{-7} M with estimated V_{max} of 85.2 or 27.4 μA/cm^2 in the middle and distal colon, respectively. The increase in I_{sc} by both PGE2 and 6-PTU in the middle colon were significantly larger than those in the distal colon (P < 0.001 by two-way ANOVA).

Expression of T2Rs in RT-PCR analysis. Previous studies suggest that many, but not all, members of the T2R family that detect 6-PTU are expressed in large intestinal epithelia, RT-PCR analysis was performed by using isolated mucosa of human and rat without submucosa and intestinal epithelia, RT-PCR analysis was performed by using the T2Rs thought to detect 6-PTU were expressed in large distal colon (Fig. 8A). In the rat middle colon, mRNA of rT2R-1, -16, and -26, orthologous with hT2R-1, -4, and -38, respectively, were also detected (Fig. 8B).

DISCUSSION

In the present study, we first demonstrated the action of a bitter taste receptor ligand, 6-PTU, on electrolyte transport in human and rat large intestine and the expressions of putative receptors for 6-PTU. This study suggests that some luminal bitter tastants in the large intestine induce secretory responses, probably through the T2R chemical-sensing mechanism, and the process is dependent on PGs, but not dependent on the neural pathway.

Species and region dependency of 6-PTU-evoked anion secretion in the large intestine. The mucosal application of 6-PTU at >10^{-4} M increased I_{sc} without changing G_{i} in both human and rat large intestine in a concentration-dependent manner (Fig. 1). A previous human taste-test study (24) has shown that the detectability threshold for 6-PTU is 0.88 × 10^{-4} M. Mouse brief-access studies have also showed that minimal and maximal concentrations for avoidance of 6-PTU were 10^{-4} M and 10^{-2} M, respectively (2, 30, 32). Thus the range of the 6-PTU concentrations in the present results in both human large intestine and rat middle colon were consistent with these previous studies related to the gustatory sense.

There was a segmental difference in the response to 6-PTU in rat colon; the 6-PTU-evoked increase in I_{sc} in middle colon was much higher than that in distal colon (Fig. 1C). Furthermore, the EFS-evoked increase in I_{sc} in middle colon was also higher than that in distal colon (Table 2). Therefore, such segmental difference in secretion responses may influence the difference of 6-PTU-evoked response between middle and distal colon in rat. Table 2 showed segmental differences in basal electrical activities of human and rat tissues. The same segmental difference in basal I_{sc} was observed in both human and rat, i.e., the values of basal I_{sc} in the upper region were larger than those in lower region. However, in contrast with rat colon, there was no segmental difference in EFS- and 6-PTU-evoked I_{sc} responses in human colorectal tissues. These may suggest the species difference.

Precipitation of the tissues with bumetanide decreased the 6-PTU-evoked ΔI_{sc} by 69%, and NPPB almost completely abolished 6-PTU-evoked ΔI_{sc}. Bumetanide-sensitive Na^{+}-K^{+}-2Cl^{-} cotransporter is known as the predominant transporter at basolateral membrane for Cl^{-} uptake into epithelial cells (18), and an NPPB-sensitive Cl^{-} channel, CFTR, is known as an apical membrane channel, releasing Cl^{-} from epithelial cells into the lumen (6). Therefore, it has been suggested that the 6-PTU-evoked increase in I_{sc} occurred by Cl^{-} secretion. However, in the present study, the 6-PTU-evoked increase in I_{sc} was reduced only 40% in Cl^{-}-free conditions (Fig. 4). The EFS-evoked increase in I_{sc} was also not completely abolished, remaining at ~20% of control response. This suggests that other ion components (not Cl^{-}) are involved in the 6-PTU- and EFS-evoked increased in I_{sc}. It has been reported that, in addition to Cl^{-}, HCO_{3}^{-} can also pass through CFTR (17). Furthermore, a previous study has shown that HCO_{3}^{-} secretion is Cl^{-} independent and inhibited by NPPB (39). We, therefore, tested the effect of absence of HCO_{3}^{-} in addition to Cl^{-} from the Krebs-Ringer solution. Our results show that 6-PTU- and EFS-evoked I_{sc} responses were almost completely abolished by both Cl^{-}/HCO_{3}^{-}-free solution (Fig. 4C). These observations suggest that anion secretions, including Cl^{-} and/or HCO_{3}^{-}, are involved in the I_{sc} responses to 6-PTU and EFS.

The role of PGs. The 6-PTU-evoked I_{sc} increase was greatly reduced by pretreatment with a nonselective COX inhibitor, piroxicam (Fig. 6), but was not affected by TTX (Fig. 5). Furthermore, the I_{sc} response induced by 6-PTU in the presence of piroxicam was enhanced by exogenous addition of

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Fig. 8. Expressions of taste receptor type 2 (T2R) in human and rat colorectal mucosa. RT-PCR was performed using the specific primers listed in Table 1 to detect the expressions of T2Rs thought to sense 6-PTU at the mucosa of human and rat colorectal tissues. A: expressions of human T2R (hT2R)-1, -4, and -38 in human ascending colon, sigmoid colon, and rectum. B: expressions of rat T2R (rT2R)-1, -16, and -26 in rat middle colon. RT-PCR for β-actin was performed as a positive control and verifies the absence of genome contaminations. N/C, PCR performed without DNA as a negative control.
PG E2 in a concentration-dependent manner (Fig. 7). In rat middle colon, $3 \times 10^{-3}$ M 6-PTU-evoked increase in $I_{sc}$ was 16.3 ± 1.9 $\mu$A/cm$^2$ under normal condition, but the response was reduced to 4.7 ± 1.5 $\mu$A/cm$^2$ by piroxicam ($\Delta I_{sc}$, Fig. 6). If PGE2 is present in serosal bathing solution, for example, the 10$^{-5}$ M PGE2 enhanced the response to $3 \times 10^{-3}$ M 6-PTU to 85.3 ± 7.4 $\mu$A/cm$^2$ ($\Delta I_{sc}$, Fig. 7), even in the presence of piroxicam. These results suggest that 6-PTU-evoked increase in $I_{sc}$ is able to increase when concentrations of PGE2 in the colon are increasing by some reasons: inflammation, etc. However, precise mechanisms of this enhancement are currently unknown (see Possible role of bitter sensing in large intestine below). The value of $3 \times 10^{-3}$ M 6-PTU-evoked increase in $I_{sc}$ under normal condition was similar to that of the response induced by $2.6 \times 10^{-8}$ M of PGE2 in the presence of piroxicam in rat middle colon. The concentration of exogenous PGE2 necessary to evoke the same effect in the presence of piroxicam can be calculated from the Michaelis-Menten equation by utilizing estimated $V_{max}$ and EC$_{50}$ values. Therefore, these results suggest that the 6-PTU-evoked increase in $I_{sc}$ is dependent on basal PG synthesis under normal conditions. However, it remains unclear which types of COX (COX-1 or COX-2) are activated by 6-PTU.

Although it is quite difficult to measure physiological concentrations of PGs directly, these results suggest that the concentration of PGs in rat middle colon might be $2.6 \times 10^{-8}$ M of PGE2 under normal conditions. In distal colon, the 6-PTU-evoked ($3 \times 10^{-3}$ M) increase in $I_{sc}$ was 1.9 ± 1.5 $\mu$A/cm$^2$ under the normal condition (Fig. 1C); therefore, basal concentration of PGE2 was calculated to be $1.8 \times 10^{-8}$ M, utilizing the Michaelis-Menten equation (Fig. 7D). This concentration of PGE2 is consistent with previous reports that noted PG concentrations estimated as nanomoles to $\sim 10^{-7}$ M level (12).

Basal $I_{sc}$ in rat middle colon was significantly higher than that in distal colon; however, the values of basal $I_{sc}$ in the presence of piroxicam ($10^{-5}$ M) were similar (Fig. 7B) among these segments. These results suggest that segmental differences of basal electrical parameters among rat colonic segments might be due to the basal PG level differences, and, therefore, that basal PG levels are involved in transepithelial anion secretion by luminal stimuli.

**Mechanism of 6-PTU detection.** Figure 1 showed that 6-PTU induced $I_{sc}$ increases in human and rat large intestinal epithelia concentration dependently. The mRNA expression of hT2R-1, -4, and -38 (Fig. 8A), and these orthologous rT2R-1, -16, and -26, were detected in colonic mucosa by RT-PCR analysis (Fig. 8B). Although it has not yet been determined which specific receptor detected 6-PTU in this study, all T2R family members known as receptors for 6-PTU were detected in large intestinal mucosa. These results suggest that 6-PTU may be detected at epithelial T2Rs. It has been reported that STC-1, a mouse enteroendocrine cell line, expresses mRNA of these T2Rs, and that $10^{-3}$ M 6-PTU evokes increases in intracellular $\text{Ca}^{2+}$ concentration ([Ca$^{2+}$]). (40). Others have reported that other putative T2R ligands also evoke a transient increase of [Ca$^{2+}$], in T2R positive (at the mRNA level) cell lines (34, 41). These observations raised the possibility of mechanisms for bitter tastants (including 6-PTU) that induce increases in [Ca$^{2+}$], of intestinal epithelia and that evoke anion secretion by interaction with PGs.

**Possible role of bitter sensing in large intestine.** The bitter taste signal is considered to be a “notifier” of toxic substances to avoid harm to the central nervous system (30). Similarly, it is thought that bitter sensing may act as a repellent mechanism for poisons to “prevent” exposure to poisons in the large intestine. This idea is supported by the present observation that 6-PTU-induced anion secretion evokes fluid secretion. Fluid secretion stimulated by anion secretion evoked by bitter tastants appears to be an important mechanism for host defense to flush out noxious agents in the colonic lumen. Furthermore, we showed that PGE2 ($>10^{-7}$ M) potentiated the 6-PTU-evoked anion secretion. Bitter compounds that generally come into large intestine under normal conditions are most frequently that of bile acids and its bacterial metabolites. As secondary bile acids have been reported to promote tumors (31), bitter sensing in the large intestine may be considered as a necessary mechanism for host defense. Normal concentrations of PGE2 are $\sim 2.6 \times 10^{-8}$ M in rat middle colon and $1.8 \times 10^{-8}$ M in rat distal colon. PGE2 levels in the intestine are increased by mechanical stimulation (7) or inflammation (35, 37), and concentrations of PGE2 are considered to be pathophysiological at $>10^{-7}$ M (12). The present observations, therefore, suggest that high levels ($>10^{-7}$ M) of tissue PGE2 induce inflammation, and these conditions enhance the bitter stimuli-evoked secretory response to flush out the noxious substance from the colonic lumen. Thus intestinal bitter sensing may play an important role in host defense mechanism by interacting with PG levels and may be involved in the interaction of luminal homeostasis with intestinal bacteria.

**Conclusion.** The present results indicate that the anion secretion induced by 6-PTU, a T2R ligand, in human and rat large intestine depends on PGs, irrespective of neural pathway. Furthermore, the expressions of putative 6-PTU receptors were detected in the colorectal mucosa. It is suggested that detection of bitter compounds via T2R takes a part in the luminal chemosensing mechanism in the large intestine to flush harmful compound by enhancing fluid secretion, and, therefore, the bitter sensing mechanism may even play a role in host defense.

**REFERENCES**


