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

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Kaji I, Karaki SI, Fukami Y, Terasaki M, Kuwahara A. Secretory effects of a luminal bitter tantant 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 Goast 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. In mucosal-submucosal preparations, mucosal 6-PTU evoked Cl− and HCO3− secretions in a concentration-dependent manner. In rat middle colon, 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 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 chemosensing; 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 tantants 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 6-PTU (3, 8, 28), and these genes are the most conserved between humans and rodents (41) [rat T2R (rT2R)-1, -16, and -26 are ortholog, respectively], but 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 between 280 and 380 g, were used for this study. Animals were fed pellet diet (type MF or C2) with water ad libitum. They were anesthetized with ether and decapitated by guillotine. Tissue preparation was as follows, according to a previous study (42). Segments of rat middle colon (6–9 cm proximal to anus) (around the small patchy area) was used in the present study. To construct the concentration-response curve, various concentrations of 6-PTU (1 × 10⁻⁶ to 1 × 10⁻³ M) were added to mucosal bathing solution. DB was dissolved in distilled water as 1 × 10⁻¹ M, while 6-PTU, PTC, and CYX were in dimethyl sulfoxide (DMSO) stock solution. It was confirmed that 100 µM DMSO did not affect Iac or G1 in human and rat tissues that was used in this study. To construct the concentration-response curve, various concentrations of 6-PTU (1 × 10⁻⁶ to 1 × 10⁻³ M) were added cumulatively to mucosal or serosal bathing solution, and changes in Iac were measured. Bumetanide (10⁻⁴ M), 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) (10⁻⁴ M), Cl⁻ free solution, and both Cl⁻/bicarbonate (HCO₃⁻) free solution were used to investigate the ion component of 6-PTU-evoked Iac changes. The Cl⁻/HCO₃⁻ free solution contained (in mM) 117 Na-gluconate, 4.7 K-gluconate, 8 Ca-(gluconate)₂, 1.2 Mg-(gluconate)₂, 1.2 Na H₂PO₄, 25 NaHCO₃, and 11 glucose. The solution was bubbled with 95% O₂ and 5% CO₂ and buffered at pH 7.2. The Cl⁻/HCO₃⁻ free solution was titrated to 7.2 by 1 N NaOH. The solution was bubbled with 100% O₂, and acetazolamide (5 × 10⁻⁴ M) was added to inhibit endogenous HCO₃⁻ synthesis. Tetrodotoxin (TTX) (10⁻⁶ M) and piroxicam (10⁻⁵ M) were used to test the influence of neural pathway and endogenous prostaglandin (PG) synthesis, respectively. In the studies of all inhibitors, two preparations of the rat middle colon were paired on the basis of similar conductance. To test whether 6-PTU responses required the presence of PGs, various concentrations of PGE₂ (10⁻⁶ to 10⁻⁵ M) were added to serosal bathing solution of piroxicam-treated (10⁻⁵ M) rat distal colon before the addition of 6-PTU (3 × 10⁻³ M). The concentration of inhibitors used was based on recent studies (16, 20).

**Reverse transcriptase-polymerase chain reaction analysis.** The reverse transcriptase-polymerase chain reaction (RT-PCR) protocol used was based on a previous study (21). Scrapped mucosa from human and rat colorectal segments were immersed immediately in the RNA stabilization regent (RNA later; Qiagen K.K., Tokyo, Japan). Remaining mRNA was extracted with RNA later (Qiagen K.K., Tokyo, Japan) and purified with an RNeasy mini kit (Qiagen K.K., Tokyo, Japan). Due to the small sample size, the reverse transcription was performed using the one-step assay (Clontech).

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′-CATCTGCTTCTTCTGCTG-3′</td>
</tr>
<tr>
<td>hT2R-4</td>
<td>Forward</td>
<td>5′-TAAATTAAGATGAGAGTG</td>
</tr>
<tr>
<td>hT2R-7</td>
<td>Reverse</td>
<td>5′-GAAAAGCTGCTAGCAGC-3′</td>
</tr>
<tr>
<td>hT2R-38</td>
<td>Reverse</td>
<td>5′-CATCTGCTTCTTCTGCTG-3′</td>
</tr>
<tr>
<td>hβ-actin</td>
<td>Forward</td>
<td>5′-GCAGCAGCAGTACGCAATTC</td>
</tr>
<tr>
<td>hβ-actin</td>
<td>Reverse</td>
<td>5′-GGGTAGATGCTTCTGCTG</td>
</tr>
<tr>
<td>rT2R-1</td>
<td>Forward</td>
<td>5′-ATGCTGCGACACACCTCTCATGGAGTGGG-3′</td>
</tr>
<tr>
<td>rT2R-16</td>
<td>Reverse</td>
<td>5′-GGGTAGATGCTTCTGCTG</td>
</tr>
<tr>
<td>rT2R-26</td>
<td>Forward</td>
<td>5′-CTGTCGATGCTGATGCTG</td>
</tr>
<tr>
<td>rβ-actin</td>
<td>Reverse</td>
<td>5′-CCGGTGTTATCTGCTG</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).
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></th>
<th>n</th>
<th>Basal I_{sc}, μA/cm²</th>
<th>G_{t}, mS/cm²</th>
<th>PD, mV</th>
<th>5-Hz EFS-evoked ΔI_{sc}, μA/cm²</th>
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<tbody>
<tr>
<td><strong>Human tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending colon</td>
<td>8</td>
<td>56.1 ± 8.7^{a}</td>
<td>9.2 ± 1.1</td>
<td>−7.9 ± 1.7^{b}</td>
<td>47.7 ± 12.1</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>6</td>
<td>3.4 ± 8.4^{c}</td>
<td>12.0 ± 1.5</td>
<td>−0.5 ± 0.7^{c}</td>
<td>63.2 ± 14.6</td>
</tr>
<tr>
<td>Rectum</td>
<td>5</td>
<td>−3.3 ± 13.6^{c}</td>
<td>10.8 ± 1.9</td>
<td>−0.7 ± 1.8^{b,d}</td>
<td>68.5 ± 10.6</td>
</tr>
<tr>
<td><strong>Rat tissues</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle colon</td>
<td>70</td>
<td>19.9 ± 1.02^{a}</td>
<td>12.0 ± 0.32</td>
<td>−1.8 ± 0.10</td>
<td>106.3 ± 4.29^{a}</td>
</tr>
<tr>
<td>Distal colon</td>
<td>41</td>
<td>15.6 ± 0.91^{c}</td>
<td>11.0 ± 0.50</td>
<td>−1.5 ± 0.12</td>
<td>87.0 ± 4.84^{c}</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of tissues. I_{sc}, short-circuit current; ΔI_{sc}, change in I_{sc}; G_{t}, tissue conductance; PD, potential difference; EFS, electrical field stimulation. ^{a}P < 0.001 and ^{b}P < 0.01 by ANOVA. ^{c}P < 0.01 and ^{d}P < 0.05 vs. ascending colon by Tukey-Kramer test. ^{e}P < 0.01 between segments by unpaired t-test.

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^{-4}–10^{-2} 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} (ΔI_{sc}) were normalized with ΔI_{sc} of control tissues. C: concentration-dependent curves of 6-PTU-induced increases in I_{sc} and G_{t} in rat colon. 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 (Clonthech 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

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**Fig. 2.** Effect of 6-PTU on \( I_{sc} \) responses stimulated by EFS in rat middle colon. EFS (25 V, duration of 0.5 ms, 2 m each for frequencies of 1, 2, 5, and 10 Hz) were performed before and after addition of 6-PTU (3 × 10^{-3} M) to mucosal bathing solution. The EFS-evoked increases in \( I_{sc} \) for all frequencies were significantly enhanced by the presence of 6-PTU. 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 < 0.01, ***P < 0.001 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 application 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 \( I_{sc} \). 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 $I_{sc}$ 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. $P \leq 0.05$ 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 $I_{sc}$ and $G_t$ after stabilization, and 5 Hz EFS-evoked change ($\Delta I_{sc}$) in $I_{sc}$ 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 $I_{sc}$ ($P < 0.01$) 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 $I_{sc}$ ($P < 0.01$) and 5 Hz EFS-evoked $\Delta I_{sc}$ ($P < 0.01$) 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 $I_{sc}$ in both human and rat large intestine without changing $G_t$. Neither the addition of DB, PTC, nor CYX affected basal electrical activity, even in high concentrations ($3 \times 10^{-3}$ M). After the addition of 6-PTU, $I_{sc}$ increased gradually for 10–15 min, achieved plateau, and then sustained for 20 min. To investigate the effects of 6-PTU on basal $I_{sc}$ and $G_t$ 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 $I_{sc}$ and $G_t$ were measured. In rat tissues, serosal addition of 6-PTU was also tested; 6-PTU to serosal bathing solution evoked an increase in $I_{sc}$ without changing $G_t$.

Fig. 4. Effects of Cl⁻-free solution and both Cl⁻ and bicarbonate (HCO₃⁻)-free solution on $I_{sc}$ responses to EFS and 6-PTU in rat middle colon. The bathing solution was replaced with Cl⁻-free solution or both Cl⁻ and HCO₃⁻-free solution after check of basal electrical parameters. After stabilization of baseline $I_{sc}$, EFS (25 V, duration of 0.5 ms, 5 Hz for 2 min) was applied to verify the absence of anions, and then 6-PTU ($3 \times 10^{-3}$ M) was added to mucosal bathing solution. A: representative traces to illustrate effects of EFS and 6-PTU on $I_{sc}$ in the presence or absence of Cl⁻ and/or HCO₃⁻. B and C: effects of Cl⁻-free and both Cl⁻/HCO₃⁻-free solution on EFS- (B) and further addition of 6-PTU-evoked (C) increase in $I_{sc}$. Values are expressed as means ± SE; $n = 4–6$. *$P < 0.05$, ***$P < 0.001$ by Tukey-Kramer’s test. n.s., Not significant.
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. 1A and B) and rat colon (Fig. 1C). The 50% effective concentrations (EC50) in human ascending colon, sigmoid colon, and rectum were $-2.7 \times 10^{-2}$, $2.6 \times 10^{-3}$, 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 $14.7 \pm 3.4 \mu A/cm^2$ in the middle colon and $18.6 \pm 1.5 \mu A/cm^2$ 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: $15.4 \pm 5.2 \mu A/cm^2$, 2 Hz: $47.1 \pm 14.0 \mu A/cm^2$, 5 Hz: $95.7 \pm 24.3 \mu A/cm^2$, 10 Hz: $118.8 \pm 33.0 \mu A/cm^2$). However, the presence of $3 \times 10^{-3}$ M 6-PTU significantly potentiated the EFS-evoked $I_{sc}$ responses from $24.2 \pm 9.0$, 54.6 $\pm 12.8$, 108.9 $\pm 20.6$, and 124.6 $\pm 12.7 \mu A/cm^2$ to 37.8 $\pm 6.3$, 83.3 $\pm 12.9$, 127.8 $\pm 13.6$, and 140.7 $\pm 15.5 \mu A/cm^2$, 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 $\pm 3.2$ to 16.0 $\pm 2.8 \mu A/cm^2$ ($P < 0.05$ 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 $20.1 \pm 4.3 \mu A/cm^2$ (in control group) to $6.3 \pm 2.4 \mu A/cm^2$ ($P < 0.01$ 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 $\pm 2.1$ to 11.7 $\pm 3.3 \mu A/cm^2$ ($P < 0.05$ by paired t-test, $n = 4$) and increased $G_t$ from 9.11 $\pm 0.5$ to 13.4 $\pm 1.7$ mS/cm$^2$ ($P = 0.077$, $n = 4$). 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 $5.4 \pm 1.6$ to $-0.07 \pm 0.86 \mu A/cm^2$, $P < 0.05$, $n = 4$).

**Effects of Cl$^-$ and HCO$_3^-$-free solution on 6-PTU-evoked increase in $I_{sc}$ in rat middle colon.** It has been reported that, in addition to Cl$^-$, HCO$_3^-$ can also pass through CFTR, and Cl$^-$-independent HCO$_3^-$ 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$^-$/HCO$_3^-$-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$^-$/HCO$_3^-$ from Krebs-Ringer solutions. Both mucosal and serosal bathing solutions were replaced with Cl$^-$-free solution or Cl$^-$/HCO$_3^-$-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 $\pm 4.2$ to 13.3 $\pm 1.5 \mu A/cm^2$ ($P = 0.16$ by paired t-test, $n = 6$) and from 9.3 $\pm 1.1$ to 5.4 $\pm 0.4$ mS/cm$^2$ ($P < 0.05$ by paired t-test, $n = 6$), respectively. In Cl$^-$/HCO$_3^-$-free solution, basal $I_{sc}$ and $G_t$ also significantly decreased from 18.0 $\pm 2.6$ to 4.6 $\pm 0.5 \mu A/cm^2$ ($P < 0.05$, $n = 4$), from 11.6 $\pm 0.7$ to 7.0 $\pm 0.8$ mS/cm$^2$ ($P < 0.01$, $n = 4$), respectively. The EFS-evoked increase in $I_{sc}$ was significantly reduced by the absence of Cl$^-$ from 9.22 $\pm 11.1 \mu A/cm^2$ ($\Delta I_{sc}$, control, $n = 10$) to 30.3 $\pm 2.4 \mu A/cm^2$ ($\Delta I_{sc}$, Cl$^-$/HCO$_3^-$-free solution, $n = 10$).
P < 0.001 vs. control by Tukey-Kramer’s test, n = 5), and almost completely abolished by the absence of both Cl\(^-\) and HCO\(_3\)\(^-\) (ΔI\(_{sc}\), P < 0.001 vs. control by Tukey-Kramer’s test, n = 5, Fig. 4B). The 6-PTU-evoked increase in I\(_{sc}\) measured at 15 min after addition was reduced by ~40% (from 14.5 ± 1.9 μA/cm\(^2\), ΔI\(_{sc}\), n = 6) in the absence of Cl\(^-\) and by 90% in the absence of both Cl\(^-\) and HCO\(_3\)\(^-\) (to 1.08 ± 0.49 μA/cm\(^2\), ΔI\(_{sc}\), P < 0.001 vs. control by Tukey-Kramer’s test, n = 4, Fig. 4C).

**Effect of TTX on the response to 6-PTU in rat middle colon.** The ENS plays an important role in the regulation of colonic epithelial ion transport (5). EFS-evoked Cl\(^-\) secretion has been reported to be almost completely abolished by the absence of both Cl\(^-\) and HCO\(_3\)\(^-\) (4, 9). PGE\(_2\) released in response to distention and mechanical stimulation and endogenous PG synthesis by COX. 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 × 10\(^{-3}\) to 10\(^{-2}\) M). Piroxicam significantly decreased basal I\(_{sc}\) from 17.3 ± 2.3 to 10.8 ± 2.1 μA/cm\(^2\) (ΔI\(_{sc}\)) (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 ± 1.9 to 4.7 ± 1.5 μA/cm\(^2\), P < 0.01 by paired t-test, n = 5, Fig. 6A, bottom trace, and B). 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 basal I\(_{sc}\) (from 7.6 ± 2.9 μA/cm\(^2\), ΔI\(_{sc}\), n = 5) and reduced 6-PTU-evoked ΔI\(_{sc}\) (from 17.8 ± 1.6 to 6.9 ± 1.6 μA/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 × 10\(^{-3}\) M) was added to the mucosal bathing solution. Although the 3 × 10\(^{-3}\) M 6-PTU evoked little increase in I\(_{sc}\) in basal conditions in the distal colon (Fig. 1B, n = 5), PGE\(_2\) concent-

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**Fig. 6. Effects of piroxicam on I\(_{sc}\) response to 6-PTU and EFS in rat middle colon.** Piroxicam-HCl (10\(^{-5}\) M) was added to serosal or mucosal bathing solution 10 min before addition of 6-PTU (3 × 10\(^{-3}\) to 10\(^{-2}\) M). Fifteen minutes after addition of 6-PTU (10\(^{-2}\) M), EFS (25 V, duration of 0.5 ms, 2 min for each frequency of 1, 2, 5, and 10 Hz) was applied. A: representative traces to illustrate effects of 6-PTU and EFS on I\(_{sc}\) in the presence or absence of piroxicam in serosal bathing solution. B and C: 6-PTU-evoked (3 × 10\(^{-3}\)) increase in I\(_{sc}\) in the presence or absence of piroxicam in serosal bath (B) or mucosal bath (C) between 10 min after and just before application of 6-PTU. Values are means ± SE; n = 5. **P < 0.01 vs. control by paired t-test.
istration-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

![Representative traces of 6-PTU-evoked responses](image)

**B**

**transient phase (TP)**

![Transient phase graph](image)

**C**

**sustain phase (SP)**

![Sustain phase graph](image)

![Concentration-response curve](image)

**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 $10^{-5}$ 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 ± SE; n = 3–5. ***$P < 0.01$ by two-way ANOVA.

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a concentration-dependent manner (Fig. 7B). The $10^{-5}$ M PGE2-evoked transient phase responses were 47.3 ± 7.4 μA/cm² in the middle colon and 26.5 ± 3.3 μA/cm² 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 PGE$_2$, and the EC$_{50}$ was calculated to $1.0 \times 10^{-7}$ or $2.4 \times 10^{-7}$ M with estimated $V_{max}$ of 85.2 or 27.4 μA/cm² 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 the colon (34). To confirm that the T2Rs thought to detect 6-PTU were expressed in large intestinal epithelia, RT-PCR analysis was performed by using isolated mucosa of human and rat without submucosa and smooth muscle. Specific bands of the same base pair sizes as the expected size (Table 1) of mRNA for hT2R-1, -4, and -38 were detected in the mucosa of human ascending and sigmoid colons and rectum (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 tastes 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 $G_{t}$ 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 $0.88 \times 10^{-4}$ 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 colons. 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.

Pretreatment of the tissues with bumetanide decreased the 6-PTU-evoked $\Delta I_{sc}$ by 69%, and NPPB almost completely abolished 6-PTU-evoked $\Delta I_{sc}$. 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...
PGE₂ in a concentration-dependent manner (Fig. 7). In rat middle colon, 3 × 10⁻³ M 6-PTU-evoked increase in \( I_{sc} \) was 16.3 ± 1.9 μA/cm² under normal condition, but the response was reduced to 4.7 ± 1.5 μA/cm² by piroxicam (Δ\( I_{sc} \), Fig. 6). If PGE₂ is present in serosal bathing solution, for example, the 10⁻⁵ M PGE₂ enhanced the response to 3 × 10⁻³ M 6-PTU to 85.3 ± 7.4 μA/cm² (Δ\( I_{sc} \), 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 × 10⁻³ M 6-PTU-evoked increase in \( I_{sc} \) under normal condition was similar to that of the response induced by 2.6 × 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 EC₅₀ 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 × 10⁻⁸ M of PGE₂ under normal conditions. In distal colon, the 6-PTU-evoked (3 × 10⁻³ M) increase in \( I_{sc} \) was 1.9 ± 1.5 μA/cm² under the normal condition (Fig. 1C); therefore, basal concentration of PGE₂ was calculated to be 1.8 × 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 ~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 epithelial 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 enterodocrine cell line, expresses mRNA of these T2Rs, and that 10⁻³ M 6-PTU evokes increases in intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) (40). Others have reported that other putative T2R ligands also evoke a transient increase of [Ca²⁺]ᵢ 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 [Ca²⁺]ᵢ 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 ~2.6 × 10⁻⁸ M in rat middle colon and 1.8 × 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**


