5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and Ca^{2+}-dependent signaling pathways and 5-HT_{4} receptors in mice

Bi-Guang Tuo, Zachary Sellers, Petra Paulus, Kim E. Barrett, and Jon I. Isenberg

Division of Gastroenterology, Department of Medicine, School of Medicine, University of California, San Diego, California 92103

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Tuo, Bi-Guang, Zachary Sellers, Petra Paulus, Kim E. Barrett, and Jon I. Isenberg. 5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and Ca^{2+}-dependent signaling pathways and 5-HT_{4} receptors in mice. Am J Physiol Gastrointest Liver Physiol 286: G444–G451, 2004. First published October 23, 2003; 10.1152/ajpgi.00105.2003.—In previous studies, we have found that 5-hydroxytryptamine (5-HT) is a potent stimulant of duodenal mucosal bicarbonate secretion (DMBS) in mice. The aim of the present study was to determine the intracellular signaling pathways and 5-HT receptor subtypes involved in 5-HT-induced DMBS. Bicarbonate secretion by murine duodenal mucosa was examined in vitro in Ussing chambers. 5-HT receptor involvement in DMBS was inferred from pharmacological studies by using selective 5-HT receptor antagonists and agonists. The expression of 5-HT_{4} receptor mRNA in duodenal mucosa and epithelial cells was analyzed by RT-PCR.

5-HT induces duodenal mucosal bicarbonate secretion in mice. The aim of the present study was to determine the intracellular signaling pathways and 5-HT receptor subtypes involved in 5-HT-induced DMBS. Bicarbonate secretion by murine duodenal mucosa was examined in vitro in Ussing chambers. 5-HT receptor involvement in DMBS was inferred from pharmacological studies by using selective 5-HT receptor antagonists and agonists. The expression of 5-HT_{4} receptor mRNA in duodenal mucosa and epithelial cells was analyzed by RT-PCR.

A number of neural and humoral factors [such as prostaglandin E_{2}, vasoactive intestinal peptide (48), dopamine (13), somatostatin (34), luminal acid (19), etc.] are known to be involved in regulation of this physiological process. 5-Hydroxytryptamine (5-HT) is widely distributed in the gastrointestinal tract. More than 90% of 5-HT is localized within the enterochromaffin (EC) cells of gastrointestinal mucosal epithelia and enteric neurons (15, 42). 5-HT is an important neurotransmitter and intercellular messenger. A variety of neural, humoral, and intraluminal stimuli have been shown to release 5-HT from EC cells (47). 5-HT has been shown to participate in the regulation of gastrointestinal motility (31), gastric acid secretion (27), pancreatic secretion (44), and intestinal chloride secretion (36). Moreover, we recently showed that 5-HT is a potent stimulant of duodenal bicarbonate secretion (46), but the signal transduction pathway(s) and the 5-HT receptor subtypes involved in the action of 5-HT on duodenal bicarbonate secretion were unknown. However, elucidation of such information is important given clinical usage of specific 5-HT receptor antagonists.

The 5-HT receptor population is comprised of several subtypes. Through pharmacological studies and molecular cloning (21, 22, 37), at least seven families of 5-HT receptor subtypes, including 5-HT_{1c}, 5-HT_{2a}, 5-HT_{3}, 5-HT_{4}, 5-HT_{5}, 5-HT_{6}, and 5-HT_{7}, have been discovered. 5-HT and its receptors are found both in the central and peripheral nervous systems, as well as in a number of nonneuronal tissues in the gastrointestinal tract, cardiovascular system, and blood. 5-HT_{5}, 5-HT_{6}, and 5-HT_{7} receptors are cloned novel receptors with as yet undefined physiological correlates. On the other hand, according to the current classification, four main subtypes of 5-HT receptors, 5-HT_{1c}, 5-HT_{2a}, 5-HT_{3}, and 5-HT_{4}, can be distinguished functionally (21), and these four 5-HT receptors are also recognized to exist in the gastrointestinal tract. Each subtype of 5-HT receptors is involved in various regulatory functions in different organs (21, 22).

The aim of the present study was to further characterize the signal transduction pathway(s) and 5-HT receptor(s) that mediate duodenal mucosal bicarbonate secretion in mice.

MATERIALS AND METHODS

Chemicals and solutions. 5-HT, 5-carboxamidotryptamine (5-CT), (α-methyl-5-HT, 5-methiothepine, ketanserin, ICS-205930, SB-204070, MDL-12330A, H-89, Rp-cAMP, verapamil, W-13, and KN-62 were purchased from Sigma (St. Louis, MO). 1-Phenylbiguanide and RS-67506 were from Tocris (Ellisville, MO). KT-5823 and NS-2028 were from Calbiochem (San Diego, CA). All other chemicals were obtained from Fisher Scientific (Santa Clara, CA). For Ussing chamber studies, the mucosal solution contained the following (in mM): 140 Na^{+}, 1.2 K^{+}, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 120 Cl^{−}, 25 gluconate, and 10 mannitol. The serosal solution contained (in mM) 140 Na^{+}, 5.4 K^{+}, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 120 Cl^{−}, 25 HCO_{3}^{−}, 2.4 HPO_{4}^{2−}, 2.4 H_{2}PO_{4}^{−}, 10 glucose, and 0.001 indomethacin. The osmolalities for both solutions were ~284 osmol/kg H_{2}O.

Animal preparation. Experiments were performed on White Swiss mice (6–10 wk of age). All studies were approved by the University of California-San Diego Committee on Investigations Involving Animals. The mice were housed in a standard animal care room with a 12:12-h light-dark cycle and were allowed free access to food and water.

Address for reprints and other correspondence: K. E. Barrett, Univ. of California, San Diego Medical Center, Div. of Gastroenterology, 8414, 200 W. Arbor Dr. San Diego, CA 92103-8414 (E-mail: kbarrett@ucsd.edu).

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water. Before experiments, the mice were deprived of food and water for at least 1 h. After anesthesia with a cocktail of hypnorm and midazolam (10 ml/kg ip; Janssen Pharmaceutica, Beerse, Belgium), the abdomen was opened by a midline incision. The proximal duodenum (a portion stretching approximately from 2 mm distal to the pylorus to the common bile duct ampulla) was removed and immediately placed in ice-cold isosmolar mannitol and indomethacin (1 μM) solution (to suppress trauma-induced prostaglandin release). The anesthetized mice were then killed by cervical dislocation. The duodenum was opened along the mesenteric border and stripped of external serosal and muscle layers by sharp dissection in the above-mentioned ice-cold isosmolar mannitol and indomethacin solution.

**Ussing chamber experiments.** Mucosa was mounted between two Lucite half-chambers with an exposed area of 0.1 cm² and placed in an Ussing chamber. Duodenal tissue from each animal was randomly divided among three or four chambers for experiments. The mucosal side was bathed with unbuffered bicarbonate-free modified Ringer solution circulated by a gas lift with 100% O₂. The serosal side was bathed with modified buffered Ringer solution (pH 7.4) containing 25 mM HCO₃⁻ and gassed with 95% O₂-5% CO₂. Each bath contained 3.0 ml of the respective solution maintained at 37°C by a heated water jacket. Experiments were performed under continuous short-circuited conditions (voltage-current clamp model VCC 600; Physiologic Instruments, San Diego, CA) to maintain electrical potential difference at zero, except for a brief period (<2 s) at each time point when the open-circuit potential difference was measured. Luminal pH was maintained at 7.40 by the continuous infusion of 5 mM HCl under the automatic control of a pH-stat system (model ETS 822; Radiometer America, Westlake, OH). The volume of the tranitirr affinit per unit time was used to quantify bicarbonate secretion. These measurements were recorded at 5-min intervals, and mean values for consecutive 10-min periods were calculated. The rate of luminal bicarbonate secretion is expressed as micromoles per square centimeter per hour. Short-circuit current (Iₘ) was measured in microamperes (μA) and converted into microequivalents per square centimeter per hour, and potential difference was measured in millivolts.

**Effect of cAMP-, Ca²⁺-, and cGMP-dependent signaling pathway inhibitors on 5-HT-stimulated duodenal mucosal bicarbonate secretion and Iₘ.** To explore the signaling pathways involved in the action of 5-HT, after a 20-min measurement of basal parameters, cAMP-dependent signaling pathway inhibitors MDL-12330A (10⁻⁵ M), Rp-cAMP (10⁻⁴ M), or H-89 (10⁻⁵ M); Ca²⁺-dependent signaling pathway inhibitors verapamil (5×10⁻⁵ M), W-13 (5×10⁻⁵ M), or KN-62 (10⁻⁵ M); or cGMP-dependent signaling pathway inhibitors NS-2028 (5×10⁻⁵ M) or KT-5823 (5×10⁻⁶ M) were added to the serosal side. Thirty minutes later, 5-HT was added to the serosal side. Duodenal bicarbonate secretion and Iₘ during the 60-min period after the addition of 5-HT were then determined.

**Effect of 5-HT receptor antagonists on 5-HT-stimulated duodenal mucosal bicarbonate secretion and Iₘ.** These studies were performed to determine 5-HT receptor subtype(s) involved in the action of 5-HT. After a 20-min measurement of basal parameters, one of the 5-HT receptor antagonists, methiothepine (10⁻⁵ M), methysergide (10⁻⁶ M), IC-205930 (10⁻⁵ M), 8-Cladenine (10⁻⁶ M), SCH-23390 (10⁻⁶ M), and 1-phenylpseudooxindole (10⁻⁷ M) were added to the serosal side. Recordings were then made over the subsequent 60-min test period as described above for 5-HT.

RNA extraction and RT-PCR. Expression of 5-HT₂A receptor mRNA in duodenal mucosa and epithelial cells was studied by RT-PCR. Segments of duodenal mucosae (~10 mg) were dissected free of seromucosal layers as described above for Ussing chamber experiments. For some studies, duodenal epithelial cells were further isolated according to a previously validated method (1). In brief, a 7-mm segment of proximal duodenum was excised. The lumen was rinsed to remove the mucus layer. The luminal surface was then exposed to an EDTA-containing solution and vortexed briefly to facilitate cell detachment. Duodenal mucosa and isolated duodenal epithelial cells were homogenized and lysed separately. Total RNA was extracted by using the RNeasy mini kit (Qiagen). RNA was treated with RNase-free DNase to remove any contaminating genomic DNA. Total RNA from duodenal mucosa or epithelial cells was converted into single-stranded cDNA by using Sensiscript reverse transcriptase (Qiagen). The primer pairs for 5-HT₄ receptor were sense 1 (5'-ATG GTC AAC AAG CCC TAT GC-3') and antisense 1 (AGG AAG GCA CGT CTG AAA GA-3'), corresponding, respectively, to bases 561–580 and 954–973 of the mus musculus 5-HT₄ receptor cDNA (GenBank accession no. NM_008313). The final concentration of the primers was 0.2 μM. After denaturation at 94°C for 3 min, 35 cycles of PCR amplification were performed (94°C, 40 s; 55°C, 60 s; 72°C, 80 s). The last cycle included 10 min of final extension at 72°C. Two types of negative control (without template DNA and where reverse transcriptase was omitted) were included in every experiment, in which no PCR product was detected. Ten microliters of each PCR product was electrophoresed on a 1.2% agarose gel containing ethidium bromide. Resulting gel bands were visualized in a UV transilluminator, and images were captured using a camera. Identification of 5-HT₄ receptor expression was based on observation of an RT-PCR product of appropriate size (~412 bp).

**Statistical analysis**. All results are expressed as means ± SE. Net peak bicarbonate and net peak Iₘ were also refer to stimulated peak responses minus basal levels. Data were analyzed by one-way ANOVA followed by Newman-Keuls post hoc test. P < 0.05 was considered statistically significant.

**RESULTS**

Effect of cAMP-, Ca²⁺-, and cGMP-dependent signaling pathway inhibitors on 5-HT-stimulated duodenal mucosal bicarbonate secretion and Iₘ. We first sought to identify signaling pathway(s) that might underlie the effect of 5-HT on duodenal mucosal bicarbonate secretion. To accomplish this, a pharmacological approach was taken. Thus the adenyl cyclase inhibitor MDL-12330A (10⁻⁵ M), the cAMP antagonist Rp-cAMP (10⁻⁴ M), and the cAMP-dependent PKA inhibitor H-89 (10⁻⁴ M) all markedly inhibited 5-HT-stimulated duodenal bicarbonate secretion and Iₘ (P < 0.001) (Fig. 1). Likewise, the Ca²⁺ channel blocker verapamil (5×10⁻⁵ M) and the calmodulin antagonist W-13 (5×10⁻⁵ M) also markedly inhibited 5-HT-stimulated duodenal bicarbonate secretion and Iₘ (P < 0.01), whereas the Ca²⁺/calmodulin-dependent protein kinase (CaM-PK) inhibitor KN-62 did not reduce 5-HT-stimulated duodenal bicarbonate secretion or Iₘ (Fig. 2). In contrast, neither the guanylyl cyclase inhibitor NS-2028 (10⁻⁵ M) nor the cGMP-dependent PKG inhibitor KT-5823 (5×10⁻⁶ M), which can effectively inhibit guanylyl cyclase and PKG, respectively, at these concentrations (16, 43), reduced 5-HT-stimulated duodenal bicarbonate secretion or Iₘ (Fig. 3).

**Effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal mucosal bicarbonate secretion and Iₘ.** The effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal bicarbonate and Iₘ are shown in Fig. 4. The 5-HT₂A receptor

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antagonist methiothepine (10⁻⁵ M), which can effectively inhibit 5-HT₁ receptors at this concentration (33), the 5-HT₂ receptor antagonist ketanserin (10⁻⁶ M), which can effectively inhibit 5-HT₂ receptors at this concentration (33), and a low concentration of ICS-205930 (10⁻⁷ M), which acts selectively as a 5-HT₃ receptor antagonist when employed at a final concentration of 10⁻⁷ M (15, 17), had no effect on 5-HT-stimulated duodenal bicarbonate secretion or Iₛ.c. On the other hand, a high concentration of ICS-205930 (10⁻⁴ M), at which this drug is known to antagonize the 5-HT₄ as well as the 5-HT₃ receptor (18), markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and Iₛ.c (P < 0.001). Similarly, SB-204070 (10⁻⁵ M), a highly selective 5-HT₄ receptor antagonist, also markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and Iₛ.c (P < 0.001). Moreover, the inhibitory effect of SB-204070 on 5-HT-stimulated duodenal bicarbonate secretion and Iₛ.c was concentration dependent (P < 0.0001) (Fig. 5). SB-204070 (10⁻⁶ M) produced a significant inhibitory effect on the action of 5-HT (P < 0.05). At the highest concentration of SB-204070 (10⁻⁴ M), it reduced 5-HT-stimulated duodenal bicarbonate secretion by 73.8% and Iₛ.c by 76.9%. The concentration of SB-204070 required to inhibit bicarbonate secretion and Iₛ.c was therefore essentially equivalent. The IC₅₀ for bicarbonate secretion and Iₛ.c were ~1.1 × 10⁻⁶ M and 0.23 × 10⁻⁶ M, respectively. In addition, none of the antagonists studied altered basal duodenal bicarbonate secretion or Iₛ.c by themselves (data not shown).

**Fig. 1.** Role of cAMP-dependent signaling pathways in 5-hydroxytryptamine (5-HT)-stimulated duodenal bicarbonate secretion (A) and short-circuit current (Iₛ.c) (B) in murine duodenum. Adenylyl cyclase inhibitor MDL-12330A (10⁻⁵ M), cAMP antagonist Rp-cAMP (10⁻⁴ M), PKA inhibitor H-89 (10⁻⁵ M), or control vehicle was added to the serosal side 30 min before 5-HT (10⁻⁴ M). Values are expressed as means ± SE; n ≥ 10 in each series. These drugs markedly inhibited 5-HT-stimulated bicarbonate secretion and Iₛ.c. * * * P < 0.001 (compared with control) by one-way ANOVA with Student-Newman-Keuls post hoc test.

Effect of 5-HT receptor agonists on duodenal mucosal bicarbonate secretion and Iₛ.c. To examine fully the receptors involved in mediating 5-HT-induced bicarbonate secretion, we studied the effect of 5-HT receptor agonists on duodenal...
bicarbonate secretion. The effect of 5-HT receptor agonists on duodenal bicarbonate secretion and $I_{sc}$ is shown in Fig. 6. The 5-HT$_1$ receptor agonist 5-CT ($10^{-4}$ M), 5-HT$_2$ receptor agonist $\alpha$-methyl-5-HT ($10^{-4}$ M), and 5-HT$_3$ receptor agonist phenylbiguanide ($10^{-4}$ M) did not significantly stimulate either duodenal bicarbonate secretion or $I_{sc}$ ($P > 0.05$, compared with control). These agonists have previously been shown to activate relevant 5-HT receptors when used at the concentrations employed here (33, 45). On the other hand, RS-67506 ($10^{-4}$ M), a partial agonist of 5-HT$_4$ receptor, markedly stimulated duodenal bicarbonate secretion and $I_{sc}$ ($P < 0.001$). The effect of RS-67506 on duodenal bicarbonate secretion and $I_{sc}$ was concentration dependent with EC$_{50}$ of $9.2 \times 10^{-6}$ M and $23.5 \times 10^{-6}$ M, respectively, for elevating bicarbonate secretion and $I_{sc}$, respectively (Fig. 7). RS-67506 was less efficacious than 5-HT. The net maximal increases in duodenal bicarbonate secretion and $I_{sc}$ induced by RS-67506 ($10^{-4}$ M) were equivalent to 67.4 and 49.6% of those evoked by the same concentration of 5-HT, respectively (Fig. 6).

Expression of 5-HT$_4$ receptor mRNA in murine duodenal mucosa and epithelial cells. The pharmacological studies described above indicated that 5-HT$_4$ receptors mediate the effect of 5-HT on both duodenal mucosal bicarbonate secretion and $I_{sc}$. To confirm that this receptor subtype is present in the murine duodenum, we assessed the expression of 5-HT$_4$ receptor mRNA in duodenal mucosa and epithelial cells by using RT-PCR. Figure 8 shows a typical ethidium bromide-stained gel for 5-HT$_4$ receptor RT-PCR products in duodenal mucosa and epithelial cells. Prominent bands are present for 5-HT$_4$ receptor mRNA.

Fig. 3. Role of cGMP-dependent signaling pathways in 5-HT-stimulated duodenal bicarbonate secretion (A) and $I_{sc}$ (B) in murine duodenum. The guanylyl cyclase inhibitor NS-2028 ($10^{-5}$ M), PKG inhibitor KT-5823 ($5 \times 10^{-5}$ M), or control vehicle was added to the serosal side 30 min before 5-HT ($10^{-4}$ M). Values are expressed as means ± SE; n = 10 in each series. Neither NS-2028 nor KT-5823 altered 5-HT-stimulated bicarbonate secretion and $I_{sc}$.

Fig. 4. Effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal bicarbonate secretion (A) and $I_{sc}$ (B) in murine duodenum. Methiothepine ($10^{-5}$ M), ketanserin ($10^{-6}$ M), ICS-205930 ($10^{-7}$ M or $10^{-5}$ M), or SB-204070 ($10^{-5}$ M) was added into the serosal side 30 min before 5-HT ($10^{-4}$ M). Values are expressed as means ± SE; n = 10 in each series. SB-204070 and ICS-205930 ($10^{-7}$ M) markedly reduced 5-HT-stimulated bicarbonate secretion and $I_{sc}$.

Fig. 5. Effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal bicarbonate secretion (A) and $I_{sc}$ (B) in murine duodenum. The guanylyl cyclase inhibitor NS-2028 ($10^{-5}$ M), PKG inhibitor KT-5823 ($5 \times 10^{-5}$ M), or control vehicle was added to the serosal side 30 min before 5-HT ($10^{-4}$ M). Values are expressed as means ± SE; n = 10 in each series. Neither NS-2028 nor KT-5823 altered 5-HT-stimulated bicarbonate secretion and $I_{sc}$.

Fig. 6. Effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal bicarbonate secretion (A) and $I_{sc}$ (B) in murine duodenum. Methiothepine ($10^{-5}$ M), ketanserin ($10^{-6}$ M), ICS-205930 ($10^{-7}$ M or $10^{-5}$ M), or SB-204070 ($10^{-5}$ M) was added into the serosal side 30 min before 5-HT ($10^{-4}$ M). Values are expressed as means ± SE; n = 10 in each series. SB-204070 and ICS-205930 ($10^{-7}$ M) markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and $I_{sc}$.

Fig. 7. Effects of 5-HT receptor antagonists on 5-HT-stimulated duodenal bicarbonate secretion (A) and $I_{sc}$ (B) in murine duodenum. The guanylyl cyclase inhibitor NS-2028 ($10^{-5}$ M), PKG inhibitor KT-5823 ($5 \times 10^{-5}$ M), or control vehicle was added to the serosal side 30 min before 5-HT ($10^{-4}$ M). Values are expressed as means ± SE; n = 10 in each series. Neither NS-2028 nor KT-5823 altered 5-HT-stimulated bicarbonate secretion and $I_{sc}$.

Fig. 8. Expression of 5-HT$_4$ receptor mRNA in murine duodenal mucosa and epithelial cells. The pharmacological studies described above indicated that 5-HT$_4$ receptors mediate the effect of 5-HT on both duodenal mucosal bicarbonate secretion and $I_{sc}$. To confirm that this receptor subtype is present in the murine duodenum, we assessed the expression of 5-HT$_4$ receptor mRNA in duodenal mucosa and epithelial cells by using RT-PCR. Figure 8 shows a typical ethidium bromide-stained gel for 5-HT$_4$ receptor RT-PCR products in duodenal mucosa and epithelial cells. Prominent bands are present for 5-HT$_4$ receptor mRNA.
receptors. The location of the bands for 5-HT<sub>4</sub> receptors corresponds to the expected amplified cDNA fragment size based on the choice of oligonucleotide primers. 5-HT<sub>4</sub> receptor RT-PCR product was detected, indicating that functional 5-HT<sub>4</sub> receptors likely exist in murine duodenal mucosa and epithelial cells.

**DISCUSSION**

In a previous study (46), we found that 5-HT is a potent stimulant of duodenal mucosal bicarbonate secretion. However, the intracellular signaling transduction pathways underlying the 5-HT-induced duodenal secretory response had not been investigated. Bicarbonate is secreted in response to a number of agonists via the activation of various signal transduction pathways. cAMP, Ca<sup>2+</sup>, and cGMP are three important intracellular modulators of duodenal mucosal bicarbonate secretion (12). We thus examined the role of cAMP-, Ca<sup>2+</sup>- and cGMP-dependent signaling pathways in 5-HT-induced duodenal bicarbonate secretion. Our results demonstrated that an adenylyl cyclase inhibitor, a cAMP antagonist, and a PKA inhibitor markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and I<sub>sc</sub>. Likewise, a Ca<sup>2+</sup>-channel blocker and a calmodulin inhibitor, but not a CaM-PK inhibitor, also markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and I<sub>sc</sub>. However, a guanylyl cyclase inhibitor and a PKG inhibitor failed to alter 5-HT-stimulated bicarbonate se-

![Graph A](image1.png)

**Fig. 5.** Effects of graded concentrations of the 5-HT<sub>4</sub> receptor antagonist SB-204070 on 5-HT-stimulated duodenal bicarbonate secretion (A) and I<sub>sc</sub> (B) in murine duodenum. Each concentration was tested independently in a separate tissue and added to the serosal side 30 min before 5-HT (10<sup>-4</sup> M). The control bar represents the response to 5-HT alone. Values are expressed as means ± SE; n = 10 in each series. SB-204070 reduced 5-HT-stimulated duodenal bicarbonate secretion and I<sub>sc</sub> in a concentration-dependent manner (P < 0.0001). SB-204070 (10<sup>-6</sup> M) produced a significant inhibitory effect on the action of 5-HT. *P < 0.05, **P < 0.01, ***P < 0.001 (compared with control) by one-way ANOVA with Student-Newman-Keuls post hoc test.

![Graph B](image2.png)

**Fig. 6.** Effects of 5-HT receptor agonists on basal duodenal bicarbonate secretion (A) and I<sub>sc</sub> (B) in murine duodenum. After a 20-min measurement of basal values, 5-carboxamidotryptamine (5-CT; 10<sup>-4</sup> M), α-methyl-5-HT (10<sup>-4</sup> M), phenylbiguanide (10<sup>-4</sup> M), RS-67506 (10<sup>-4</sup> M), 5-HT (10<sup>-4</sup> M), or the control vehicle was added to the serosal side of a separate tissue. Values are expressed as means ± SE; n = 10 in each series. RS-67506 markedly stimulated duodenal bicarbonate secretion and I<sub>sc</sub>, whereas 5-CT, α-methyl-5-HT, and phenylbiguanide did not significantly stimulate duodenal bicarbonate secretion and I<sub>sc</sub>. ***P < 0.001 (compared with control) by one-way ANOVA with Student-Newman-Keuls post hoc test.
creatin and \( I_{\text{sc}} \). These results indicated that 5-HT likely stimulates duodenal mucosal bicarbonate secretion via both cAMP- and \( \text{Ca}^{2+} \)-dependent signaling pathways, but the \( \text{Ca}^{2+} \)-signaling pathway is independent of CaM-PK. cAMP is a predominant signaling pathway in duodenal bicarbonate secretion, and indeed inhibition of this pathway had a greater effect on bicarbonate secretion than did modulation of calcium-related signaling. In a study of epididymal epithelial cells, Leung et al. (29) found that 5-HT increased intracellular cAMP concentrations and stimulated anion secretion via the activation of cAMP-dependent signal transduction pathways. 5-HT also increased cytosolic \( \text{Ca}^{2+} \) in rat heart endothelial cells (26) and stimulated net \( \text{Ca}^{2+} \) flux in the ventricular muscle of a mollusc (10). In addition, in our previous study in this model (46), we found that 5-HT stimulated the release of ACh from duodenal mucosa in mice. ACh is known to stimulate cell secretion mainly by increasing cellular \( \text{Ca}^{2+} \) levels (11, 32). We therefore conclude that 5-HT stimulates duodenal bicarbonate secretion via increases in duodenal cAMP and \( \text{Ca}^{2+} \) concentrations but acts independently of cGMP-dependent pathways. However, it remains unknown whether these signaling events occur at the level of the epithelium exclusively or also involve indirect pathways requiring additional cell types.

We further studied the 5-HT receptor subtypes involved in the action of 5-HT. This study demonstrated that the 5-HT_{1} receptor agonist methiothepine or the 5-HT_{2} receptor antagonist ketanserin failed to inhibit 5-HT-induced duodenal bicarbonate secretion or \( I_{\text{sc}} \). Likewise, a low concentration of ICS-205930 (10^{-7} \text{ M}), at which it is known to act as a 5-HT_{3} receptor antagonist (39, 41), had no effect on 5-HT-induced duodenal bicarbonate secretion or \( I_{\text{sc}} \). However, a high concentration of ICS-205930 (10^{-5} \text{ M}), at which it is known to antagonize 5-HT_{3} receptors as well as 5-HT_{3} receptors (18, 23), markedly reduced 5-HT-stimulated duodenal bicarbonate secretion and \( I_{\text{sc}} \). In addition, the 5-HT_{1} receptor agonist 5-CT, 5-HT_{2} receptor agonist \( \alpha \)-methyl-5-HT, and 5-HT_{3} receptor agonist phenylbiguanide did not significantly stimulate duodenal bicarbonate secretion or \( I_{\text{sc}} \). In contrast, a partial 5-HT_{4} receptor agonist, RS-67506, concentration-dependently stimulated duodenal bicarbonate secretion and \( I_{\text{sc}} \). These findings indicated that 5-HT_{4} receptor likely mediates 5-HT-induced duodenal mucosal bicarbonate secretion and \( I_{\text{sc}} \).

Specific mechanism(s) whereby 5-HT binding to 5-HT_{4} receptors activates duodenal mucosal bicarbonate secretion are...
not yet clear. In our previous study using the same model of murine duodenum in vitro (46), we found that 5-HT-stimulated duodenal bicarbonate secretion was partially inhibited by the neurotoxin TTX and cholinergic receptor antagonist atropine, indicating that 5-HT-stimulated duodenal bicarbonate secretion in mice involves both neural and nonneural pathways. For the case of intestinal chloride secretion, some studies showed that 5-HT\(_4\) receptors mediate chloride secretion via a nonneural pathway (5–7), whereas the 5-HT\(_3\) receptor appears to be the primary receptor mediating effects dependent on the enteric nervous system (5). However, in the guinea pig isolated ileal mucosal preparation, 5-HT\(_4\) receptor mediated chloride secretion via TTX-sensitive mechanism (28). These findings suggest that secretory responses to 5-HT\(_4\) receptor ligation are mediated both neurally and nonneurally. In this study, we demonstrated that the 5-HT\(_4\) receptor alone appears to mediate 5-HT-stimulated duodenal bicarbonate secretion. Considering our previous results in this model (46), we can speculate that the 5-HT\(_4\) receptor mediates 5-HT-stimulated duodenal bicarbonate secretion in mice by both neural and nonneural pathways.

Some studies have shown that the stimulation of 5-HT\(_4\) receptors facilitates the release of ACh from not only the central nervous system (8, 38) but also from enteric nerve terminals (24, 25). In our previous study in this model (46), we also found that 5-HT stimulates the release of ACh from duodenal mucosa in mice. ACh is an important neurotransmitter and plays an important role in the regulation of both duodenal bicarbonate secretion (20) and intestinal chloride secretion (9). Therefore, it is possible that the 5-HT\(_4\) receptor mediates duodenal mucosal bicarbonate by stimulating localized mucosal cholinergic neurons. On the other hand, the 5-HT\(_4\) receptor is a member of the superfamily of G protein-coupled receptors and is positively coupled to adenylate cyclase (4, 18). It has been demonstrated that the activation of 5-HT\(_4\) receptors augments adenylate cyclase activity and elevates cAMP levels in rat esophagus (14) and human colon (30). Likewise, in isolated mucosal cells from rat distal colon, the study of Albuquerque et al. (2) demonstrated that 5-HT acts at a 5-HT\(_4\) receptor to induce production of cAMP in rat distal crypt colonocytes. Our RT-PCR study demonstrated that 5-HT\(_4\) receptor mRNA are expressed not only in the duodenal mucosa as a whole but also likely in duodenal epithelial cells, indicating that functional 5-HT\(_4\) receptors may exist in duodenal mucosa and epithelial cells. Therefore, 5-HT may act directly at 5-HT\(_4\) receptors on epithelial cells to induce cAMP production and regulate duodenal bicarbonate secretion. Our signal transduction data are likewise consistent with a direct effect of 5-HT mediated by cAMP and by an indirect effect, involving the known Ca\(^{2+}\)-dependent secretagogue ACh, mediated by calcium.

5-HT\(_3\) receptors are found on neurons of both central and peripheral origin. In the periphery, they are located on pre- and postganglionic autonomic neurons and on neurons of the sensory nervous system (22). In addition to its pronounced effect on the cardiovascular system, the 5-HT\(_3\) receptor also mediates the regulation of gastrointestinal motility and secretion. In the rat, 5-HT\(_3\) receptors have been shown to mediate colonic chloride secretion via neural pathways (5, 41). However, in the present study, we were unable to observe that the 5-HT\(_3\) receptor antagonist, low-dose ICS-205930, influenced 5-HT-stimulated duodenal bicarbonate secretion or that the 5-HT\(_3\) receptor agonist phenylbiguanide had any stimulatory effect on duodenal bicarbonate secretion, suggesting that 5-HT\(_3\) receptors may not participate in regulating duodenal bicarbonate secretion in mice. Likewise, the 5-HT\(_2\) receptor antagonist ketanserin had no inhibitory effect on 5-HT-induced duodenal bicarbonate secretion and the 5-HT\(_2\) receptor agonist \(\alpha\)-methyl-5-HT had no stimulatory effect on duodenal bicarbonate secretion, suggesting that 5-HT\(_2\) receptor is not involved in duodenal bicarbonate secretion in mice either. Other reports showed that ketanserin inhibits 5-HT-induced secretion in rat colon (40) and rat jejunum (3). The differences among these results indicate that the function of 5-HT receptors depends on the species and anatomic region. In fact, the 5-HT\(_1\) receptor is comprised of five receptor subtypes. Some of these subtypes exist in the gastrointestinal tract (17, 35). However, at present, it has not been reported that the 5-HT\(_1\) receptor is involved in regulation of intestinal secretion. In this study, our results also demonstrated that the 5-HT\(_1\) receptor did not mediate duodenal bicarbonate secretion.

In conclusion, our results demonstrated that 5-HT stimulates duodenal bicarbonate secretion via a 5-HT\(_4\) receptor and both cAMP- and Ca\(^{2+}\)-dependent signal pathways. RT-PCR confirmed that 5-HT\(_4\) receptor mRNAs are expressed in duodenal mucosa and epithelial cells, indicating that functional 5-HT\(_4\) receptors may exist in epithelial and perhaps also subepithelial compartments.

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