Chinese Journal of Physiology 52(5 Supplement): 395-405, 2009
DOI: 10.4077/CJP.2009.AMK037

Effects of Serotonin on Acid Secretion in Isolated Rat Stomach: the Role of 5-HT$_3$ Receptors

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Abstract

Serotonin (5-hydroxytryptamin; 5-HT) content has been measured using high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The distributions of 5-HT-containing cells and 5-HT$_3$ receptors have been determined with specific antibodies against 5-HT and 5-HT$_3$ receptors, respectively. The effect of serotonin on acid secretion has been studied using an isolated rat stomach model. It has been shown that 5-HT concentrations in the fundus, mucosal layers of the corpus, remaining layer of the corpus and antrum are approximately 152, 498, 1494 and 972 nmol/mg protein, respectively. The distribution of 5-HT-containing cells is concentrated in the enteric plexus and enterochromaffin (EC) cells in the deep mucosal layer. Immunoreactivity to 5-HT$_3$ receptors is localized in numerous neurons of the myenteric and submucosal plexus and concentrated in the neuronal plasma membrane, submucosa, endocrine cells and lamina propria. In the present study, the effect of 5-HT on gastric acid secretion was investigated on an everted preparation of isolated rat stomach. 5-HT at 1~100 μM reduced acid secretion stimulated by oxotremorine while 10 μM 5-HT did not modify the basal secretion of gastric acid. We further showed that 10 μM 5-HT reduced acid secretion and pepsin output stimulated by oxotremorine, histamine and pentagastrin; among the 5-HT receptors agonists tested, 2-methyl-5-HT (1~10 μM), a 5-HT$_3$ receptor agonist, inhibited oxotremorine-, histamine- and pentagastrin-stimulated acid secretions, and this inhibitory effect was blocked by 1 μM MDL 72222, a specific 5-HT$_3$ receptor antagonist. These results suggest that 5-HT is released from serotoninergic neurons, their processes and EC cells. The effect of 5-HT mediated by 5-HT$_3$ receptors involves distinct neuronal and non-neuronal pathways which modulate gastric acid secretion.

Key Words: 5-HT, 5-HT$_3$ receptors, enteric plexus, acid secretion, isolated stomach

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. It is found extensively in the gastrointestinal (GI) tract of animals. About 80%~90% of the human body's total 5-HT is found in the GI tract and the remainder is being divided between platelets, which avidly take up free 5-HT, and the nervous system. Ninety-five percent of 5-HT is found within granules of enterochromaffin (EC) cells (2) and enteric neurons (3, 42) of the GI tract, and it regulates GI functions including gastric acid secretion (6), motility (7, 30), mucosal blood flow (23), mucus formation (31) and fluid secretion (19).

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Received: May 20, 2009; Revised: September 15, 2009; Accepted: September 30, 2009.
 ISSN : 0304-4920. http://www.cps.org.tw

Of the 5-HT receptor subtypes, 5-HT_3 receptors (5-HT_3Rs) are present in enteric neurons (16) and mucosal terminals of extrinsic afferent neurons (20) in the intestines. 5-HT_3 antagonists inhibit small intestinal (7) and pancreatic secretions (27) and delay large bowel transit (33). The mucosal terminals of extrinsic vagal afferents are also excited by activation of 5-HT_3Rs (25). Literature on functional and localization studies of 5-HT_3Rs in the intestinal tract is extensive (16) but localization of 5-HT_3Rs responsible for modulating gastric secretion in the stomach is still obscure. Although endogenous 5-HT inhibits gastric secretory function (35) and pepsin output (13) through 5-HT_1 receptors (24), whether this inhibition occurs through 5-HT_3Rs in the stomach of mammals remains unknown.

To elucidate the relation between the distribution and function of 5-HT_3Rs in the stomach, we attempted to localize 5-HT_3Rs involved in gastric acid secretion of the rat and to study their function. The aims of the study are (A) to quantify 5-HT contents in the stomach, (B) to show whether 5-HT_3Rs are present in the stomach, (C) to determine if 5-HT can regulate oxotremorine-, histamine- and pentagastrin-induced acid secretion and pepsin output, and (D) to further determine whether 5-HT_3Rs are involved in modulating gastric acid secretion.

Materials and Methods

Chemicals and Reagents

5-Hydroxytryptamine creatinine sulfate (5-HT), 2-methyl-5-hydroxytryptamine maleate salt (2-methyl-5-HT; 2-Me-5-HT), oxotremorine, histamine dihydrochloride, pentagastrin and pepsin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in distilled water before being added to a physiological solution. MDL 72222 (tropanyl 3,5-dichlorobenzoate) (Tocris, Bristol, UK) was initially dissolved in dimethyl sulfoxide before being added to the physiological solution. All other chemicals were of analytical grade and were purchased from Sigma-Aldrich.

Animals

We used male Sprague-Dawley rats (National Laboratory Animal Center, Taipei, Taiwan, ROC) weighing 180~250 g at the beginning of the experiment, and they were housed in group cages under controlled illumination (light cycle, 08:00~20:00), a relative humidity of 30-70%, and temperature (23 ± 1°C) with free access to a laboratory diet (LabDiet, Brentwood, MO, USA) and tap water. The Animal Care and Use Committee of Taipei Medical University approved all procedures for use of the animals in this study.

Determination of 5-HT Concentrations

We determined 5-HT_3 concentrations in the stomach with high-performance liquid chromatography with electrochemical detection (HPLC-ECD) according to a previously described modified method (21, 29). We separated the stomach into three regions (the fundus, corpus and antrum), and scraped the mucosa of the corpus with a spatula to remove the mucosal layer. To determine the 5-HT contents in this layer and in the remaining tissues (muscularis mucosa, submucosa, muscle and intramural plexus), we homogenized the tissue in ~10 volumes of ice-cold 0.1 M perchloric acid buffer with 0.05 M EDTA. A 10-fold volume of 0.1 M perchloric acid buffer with a 0.05 M EDTA solution was added to weighed tissue and then centrifuged (5,000 g, 15 min), and an aliquot of 1 ml of the mixture was transferred to another tube. We added 20 µl of N-methyl serotonin (0.1 mM) as the internal standard, and added a proper amount of Tris buffer to adjust the pH to 7.9. The mixture was transferred to an alumina column and eluted with 90 µl of perchloric acid. The eluent was passed through a 0.22 µm Millipore filter and was injected into the HPLC system consisting of a Shimadzu LC-9A pump (Kyoto, Japan), a Nucleosil C18, 150 × 4.6 mm, 5-µm particle size (Alltech, Deerfield, IL, USA), a BAS LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), a Shimadzu SIL-9A autoinjector, and an integrative software of Chem-Lab (Shiunn-Hwa, Taipei, Taiwan, ROC). The mobile phase was 2% acetic acid and the flow rate was set to 1 ml/min. We chose a potential value of +0.8 V versus the Ag/AgCl reference electrode for the analysis. The detection limit of the assay, based on a signal-to-noise ratio of ≥ 3, was set at 0.1 ng/ml 5-HT. We created a calibration curve of 5-HT between the concentrations of 50 nM and 4.5 µM. We also studied the recovery of 5-HT from solid phase extraction with alumina column at concentrations of 0.1, 0.2, 1 and 4.5 µM. The mean recovery was 97.81% and standard errors were all below 8.5%.

Immunohistochemical Localization

The immunohistochemical procedure used in this study was similar to that described elsewhere (40). Briefly, male Sprague-Dawley rats were anesthetized followed by perfusion with 1 l of saline at 37°C and subsequent fixation with 4% paraformaldehyde in phosphate-buffered saline (PBS) (50 mM potassium phosphate buffer (pH 7.4) containing 0.9% NaCl) at 4°C. After fixation, and immediately frozen,

embedded in cryomatrix™ (Thermo Electron Corporation, Pittsburgh, PA, USA), it was sectioned at 20~30 µm thick and mounted on a gelatinized-slide. We used the antrum and corpus of the stomach for immunohistochemical studies with the peroxidase-antiperoxidase (PAP) technique. Tissue sections were (A) incubated with 5-HT (1: 2500) (Sigma-Aldrich) and the 5-HT_3 receptor antibody (1: 20) (Calbiochem, San Diego, CA, USA) for 16 h at 4°C; (B) rinsed with 0.1 M PBS twice; (C) incubated in PAP solution (1:50 dilution) in 50 mM Tris-HCl (pH 7.6) for 2 h at room temperature (RT); (D) rinsed with 50 mM Tris-HCl (pH 7.6) twice; (E) incubated in a solution containing 0.05% diaminobenzidine and 0.01% H_2O_2 in 50 mM Tris-Cl (pH 7.6) for 8~10 min at RT; (F) dehydrated and mounted on slides with Permount (Fisher Scientific, Fair Lawn, NJ, USA); and (G) covered with coverslips for the light microscopic examination. For control experiments, sections were treated exactly the same as those described above for the experimental group except that anti-5-HT or anti-5-HT_3R was replaced with antibodies which had been preabsorbed with an excess of the respective antigens.

Experiments on the Everted Whole Stomach

We performed experiments on the everted whole stomach as described elsewhere (40). Briefly, male Sprague-Dawley rats were deprived of food overnight but were allowed free access to water to ensure that the stomach was free of solid contents. Rats were decapitated and the stomach was immediately removed for use. The entire everted organ was then placed in a 20-ml organ bath containing a mucosal saline solution (in mM: NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; and glucose, 5.6; at pH 5.2) at 30 ± 1°C and continuously bubbled with 100% O_2. The serosal side was perfused with a serosal saline solution (in mM: NaCl, 119; HCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.03; and glucose, 5.6; at pH 7.4) at a rate of 1 ml/min under the same conditions as described above except that 100% O_2 was replaced by a mixture of 95% O_2 and 5% CO_2. One hour after stabilization of the organ, we began the experiment and replaced the mucosal saline solution every 15 min. Spontaneous acid secretion was allowed for 1 h and, thereafter, the effects of applied drugs were followed for another 2 h. We exposed only the serosal side of the preparation to the test drugs. Two experimental protocols were used:

i. basal acid secretion was collected for 180 min; and
ii. basal secretion was collected for 30 min, followed by the addition of an agonist.

After 1 h, the tested drug was added to the serosal solution and the acid secreted was continuously collected for 1 h. The acid solutions collected from the mucosal side were titrated to pH 5.2 and then to pH 7.0 with 0.1 mM NaOH using a pH stat (Autotitrator VIT90, Radiometer, Copenhagen, Denmark). The amount of NaOH consumed was used as an index of acid secretion. Responses to drug treatments in the stomach were expressed as the secretory ratio (R) which was defined as

R = acid secretion evoked by drugs/average spontaneous acid secretion.

Average of the spontaneous acid secretions was calculated from the samples for the four periods immediately before exposure to the test drugs. The concentration-response curves were assessed by measuring the secretory ratio at the peak response.

Assay of Pepsin Output

To measure the pepsin output, we used a method modified from Bunce *et al*. (5). The perfusate was collected at 15-min intervals, and duplicate 0.625-ml aliquots were mixed with an equal volume of 0.1 M glycine buffer at pH 1.9. We then preincubated the solutions at 37°C for 15 min. We initiated the reaction by adding 1.75 ml of a 2% (w/v) hemoglobin solution taken to pH 1.9 by adding 2 N HCl. We incubated the reaction mixtures for 1 h at 37°C in a shaking water bath and stopped the reaction by adding 4 ml of 5% (w/v) trichloroacetic acid. The solutions were filtered through Whatman no. 50 filter papers and the absorbance of the filtrate was measured at 280 nm (Spectrophotometer 7800, Jasco, Tokyo, Japan). The concentration of pepsin in each sample was then calculated by referring to a standard graph which ranged 0.5~10.0 pepsin units/ml.

Statistical Analyses

Quantitative data are presented as the means ± SEM. Results were analyzed for statistically significant differences using analysis of variance (ANOVA) followed by Student's *t*-test or Bonferroni's *t*-test. A P value of ≤ 0.05 was considered statistically significant.

Results

Endogenous 5-HT Content in the Stomach

Table 1 shows that the mean concentrations of 5-HT in the fundus, mucosal layers of the corpus, remaining layers of the corpus, and antrum. Thus, the corpus had 1992 (498 +1494) nmol/mg protein indi-

Table 1. Regional distribution of serotonin (5-HT) in the stomach of male Sprague-Dawley rats

Region	5-HT (nmole/mg protein)	Sample size
Fundus	152 ± 16	6
Corpus		
mucosal layer	498 ± 46	6
remaining layer	1,494 ± 139	6
Antrum	972 ± 98	6

Values are the means ± SEM of 6 animals. The remaining layer included the muscularis mucosa, submucosa and intramural plexus.

cating that the corpus was the main organ containing 5-HT cells in the stomach.

Immunohistochemical Staining

Numerous 5-HT-containing cells were found to be distributed with 5-HT immunoreactive (IR) cells in the mucosal layer of the antrum (Fig. 1, A and B) more than in the body (date not shown). 5-HT-positive fibers were also found to be distributed throughout the smooth muscle layers of the body. 5-HT-positive fibers were concentrated in the enteric plexus (Fig. 1, C and D). 5-HT-positive fibers were seen to run in muscle layers and EC cells in the deep mucosal layer (Fig. 1).

Similar results were obtained as 5-HT_3R-positive cells could easily be identified in the mucosal (Fig. 2, A and B) and muscle layers (Fig. 2, C and D). 5-HT_3Rs-IR was observed in numerous myenteric and submucosal neurons and was abundant in fibers within the enteric plexus and in the interconnecting strands of the stomach. The fibers in the myenteric plexus were abundant and often formed dense networks. 5-HT_3Rs-IR cells were seen to run in the lamina propria of the mucosal layer (Fig. 2). No specific staining was observed in the controls treated with normal rabbit serum or anti-5-HT/anti-5-HT_3Rs preabsorbed with an excess of the respective antigens (data not shown).

Effects of 5-HT, 2-methyl-5-HT (2-Me-5-HT) and MDL 72222 on Spontaneous Acid Secretion

One hour after stabilization of the organ, the experiment began, and the mucosal saline solution was replaced every 15 min. In general, spontaneous acid secretion reached a steady state of 1.591 ± 0.146 (mol/15 min. Application of 5-HT, 2-Me-5-HT and MDL 72222 was used to study the spontaneous acid secretion. Fig. 3 shows that 5-HT, 2-Me-5-HT and MDL 72222 had no significant effect on the spontaneous acid secretion at 10, 3 or 1 μM, respectively.

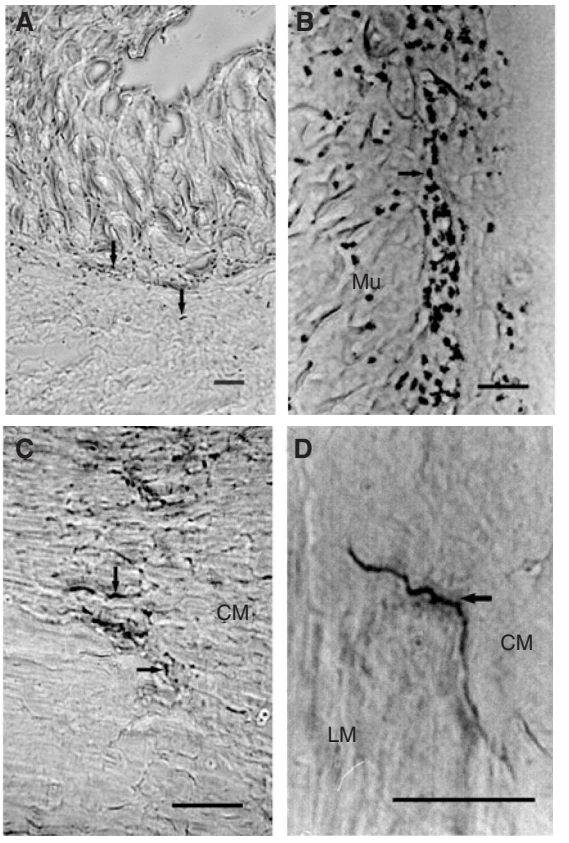

Fig. 1. Immunohistochemical localization of serotonin (5-HT)-containing cells in the rat stomach. A and B: Tissue sections were treated with anti-5-HT. A light micrograph of a cross-section showing 5-HT-positive cells in the mucosal layers of the antrum is shown. C and D: Light micrograph of a transverse section showing 5-HT-positive cells in the muscle layers of the body. Arrows indicate 5-HT-positive processes. Mu, mucosa; CM, circular muscle; LM, longitudinal muscle. Bar, 50 μm.

Effect of 5-HT on the Dose Response of Cholinergic Agent-Induced Acid Secretion and Pepsin Output

Oxotremorine, a muscarinic cholinergic receptor agonist, at 1 μM induced an increase in acid secretion, and the reaction reached a maximal response about 90 min after oxotremorine administration. The secretory ratio at the maximal response obtained was 1.75 ± 0.04 (n = 7). The IC_{50} of inhibition by 5-HT on oxotremorine-induced acid secretion was calculated to be 3.88 ± 0.88 μM. 5-HT was added 1 h after the administration of oxotremorine. 5-HT added to over 100 μM almost completely blocked acid secretion. Figs. 4 A and 4B show that 10 μM 5-HT significantly inhibited oxotremorine-stimulated acid secretion. Fig. 4C reveals that 10 μM 5-HT significantly inhibited oxotremorine-stimulated pepsin output and inhibited

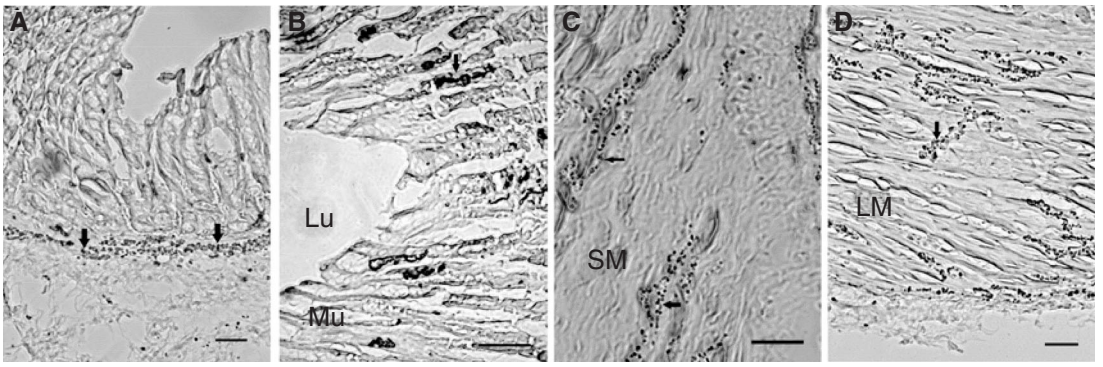

Fig. 2. Immunohistochemical localization of serotonin (5-HT) receptor subunits of 5-HT$_3$ receptors in the rat stomach. A and B: Tissue sections treated with anti-5-HT$_3$ receptors. Light micrograph of a cross-section showing 5-HT$_3$ receptors-IR cells and processes in the mucosal layer of the body (arrows). C and D: Light micrograph of a transverse section showing 5-HT$_3$R-positive cell processes in the muscle layers (arrows). Lu, lumen; Mu, mucosa; SM, smooth layer. Bar, 50 μm.

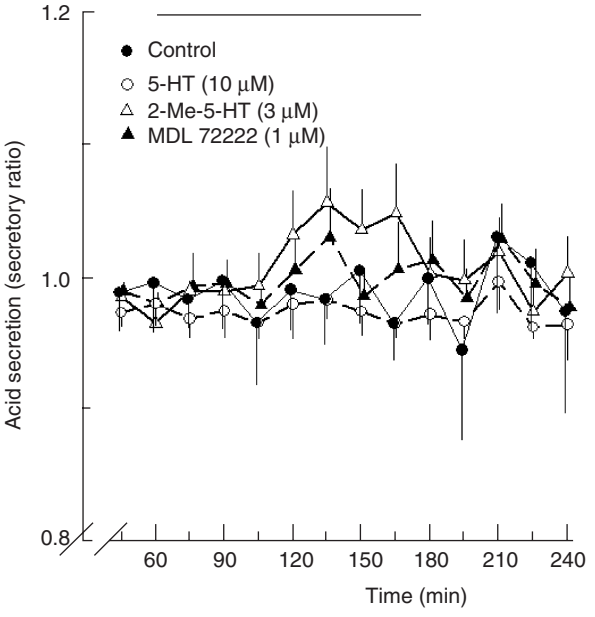

Fig. 3. Effects of serotonin (5-HT), 2-methyl-5-HT and MDL 72222 on spontaneous acid secretion in the isolated rat stomach. Acid secretion expressed as a secretory ratio was plotted as a function of time expressed in minutes. Control, serosal solution alone (●); 5-HT (○, 10 μM); 2-methyl-5-HT (△, 3 μM); or MDL 72222 (▲, 1 μM) was given after 2 h of perfusion. Data are presented as the means ± SEM (n = 6).

> 60% of the oxotremorine-stimulated pepsin output at a duration of 180 min.

Effects of 5-HT on Non-Cholinergic Agent-Induced Acid Secretion and Pepsin Output

Histamine and gastrin, two non-cholinergic agents, were also employed to stimulate acid secretion in the rat stomach. Fig. 5 (A and B) show that both histamine (300 μM) and pentagastrin (1 μM) stimulated acid secretion with secretory ratios of 2.09 and 1.77, respectively, at a duration of 150 min. 5-HT at 10 μM significantly inhibited histamine- and pentagastrin-stimulated acid secretion. Under the same conditions, Fig. 5 (C and D) show that 5-HT at 10 μM inhibited histamine-stimulated pepsin output only slightly but completely inhibited pentagastrin-induced pepsin output at a duration of 180 min or longer.

Effects of 2-Me-5-HT on Oxotremorine-Induced Acid Secretion

Fig. 6 shows that 2-Me-5-HT, a 5-HT$_3$R agonist, significantly inhibited oxotremorine-stimulated acid secretion at 0.1~10 μM. The IC$_{50}$ of 2-Me-5-HT inhibition of oxotremorine-induced acid secretion was 1.456 ± 0.80 μM. 2-Me-5-HT, a 5-HT$_3$ antagonist, at 3 μM inhibited > 55% of the oxotremorine-stimulated acid secretion at a duration of 180 min or longer.

MDL 72222 Reversed the Inhibitory Action of 5-HT or 2-Me-5-HT on Oxotremorine-, Histamine- and Pentagastrin-Stimulated Acid Secretion

Fig. 7 shows that 5-HT at 10 μM inhibited oxotremorine-, histamine-, and pentagastrin-stimulated acid secretion by 78%, 63%, and 68%, respectively, at a duration of 180 min of stimulated acid secretion. Fig. 7 reveals that MDL 72222 at 1 μM partially reversed the inhibition of oxotremorine-, histamine-, and pentagastrin-induced acid secretion by 5-HT. 2-Me-5-HT at 3 μM also greatly inhibited oxotremorine-, histamine-, and pentagastrin-stimulated acid secretion by 69%, 55%, and 65%, respectively, at a duration of 180 min. Fig. 8 reveals that 1 μM MDL 72222 reversed the inhibition of

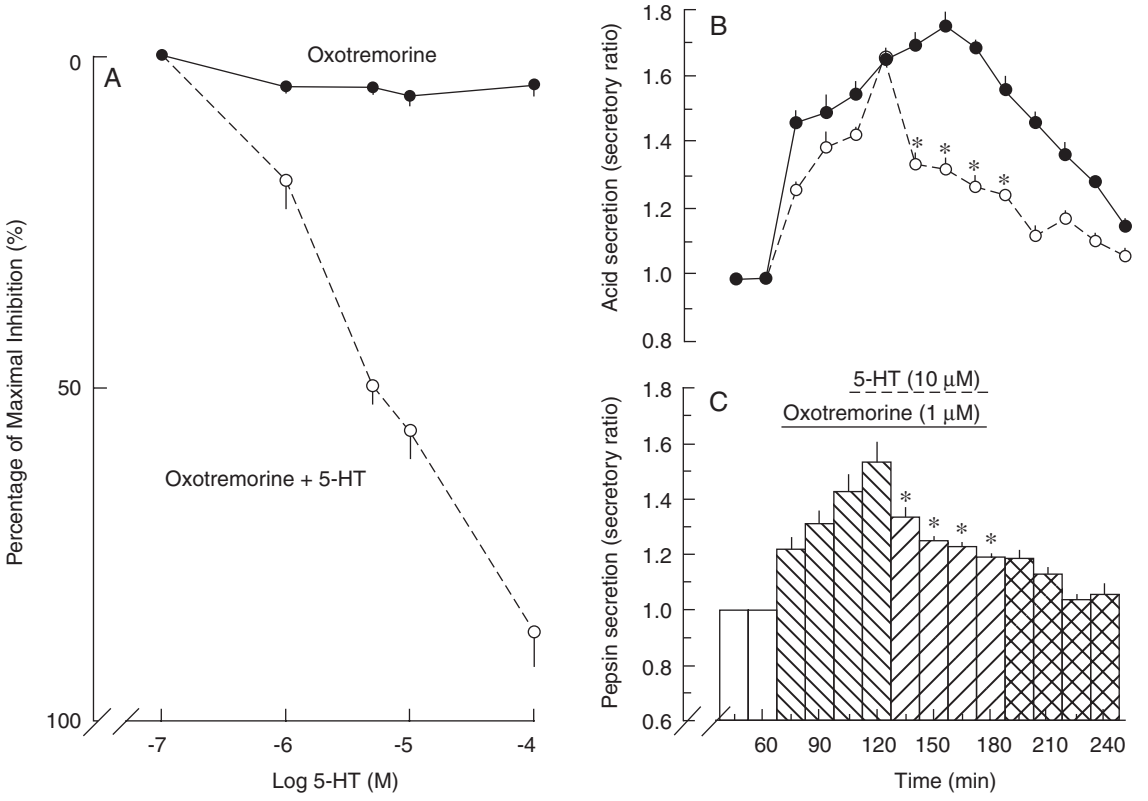

Fig. 4. A: Concentration-response curves for serotonin (5-HT) on oxotremorine-induced acid secretion in isolated rat stomach. Inhibition of oxotremorine-induced acid secretion (%) was plotted against the concentration of 5-HT used (○). Acid secretion induced by oxotremorine (●, 1 μM) alone was determined at each concentration of 5-HT and served as the control. B: Effects of 5-HT on oxotremorine-induced acid secretion. Oxotremorine was given after 1 h of perfusion, and 5-HT 10 μM (○) was added 1 h after administration of 1 μM oxotremorine. C: Effects of 5-HT on oxotremorine-induced pepsin output. Data are presented as the means ± SEM from 3 animals. *$P < 0.05$, significantly different from the oxotremorine alone group at a duration of 120 min.

oxotremorine-, histamine-, and pentagastrin-induced acid secretion by 2-Me-5-HT.

Discussion

In this communication, we further support the notion that 5-HT through 5-HT_3Rs may play an important role in the isolated rat stomach. First of all, 5-HT markedly reduced gastric acid secretion and pepsin output induced by oxotremorine, histamine and pentagastrin. Secondly, 5-HT alone had no significant effect on spontaneous acid secretion in the isolated stomach. Thirdly, MDL 72222 reversed the inhibitory effect of 5-HT or 2-Me-5-HT on oxotremorine-, histamine- and pentagastrin-stimulated acid secretion. Fourthly, the presence of serotoninergic neurons and 5-HT_3Rs in the rat stomach were confirmed, as indicated by the 5-HT content and 5-HT_3Rs, which was also consistent with the above hypothesis.

Our previous investigation first comprehensively described the distributions of 5-HT-containing cells, serotonergic neurons and 5-HT_3Rs in the stomach. The highest concentration of 5-HT was detected in the layers including the muscularis mucosa, and the submucosal and intramural plexus of the corpus (Table 1). In the present study, we examined the distribution and potential roles of 5-HT in the stomach by immunohistochemical methods. We could not detect 5-HT immunopositive nerve elements in the myenteric plexus without pretreatment. 5-HT-containing cells were found in the enteric plexus and EC cells in the deep mucosal layer (Fig. 1) at the same time, as we have proved in this study. Fig. 1 shows that more EC cells were present in the antrum than the body (data not shown). 5-HT is secreted by EC cells that are mucosal projections of primary afferent neurons. These include extrinsic nerves, the mucosal projections of intrinsic primary afferent neurons (IPANs); submucosal IPANs initiate peristaltic and secretory reflexes, while myenteric IPANs initiate giant migrating contractions (28). 5-HT secreted by enteric neurons mediates fast and slow excitatory neurotransmission and is involved in regulating GI motility

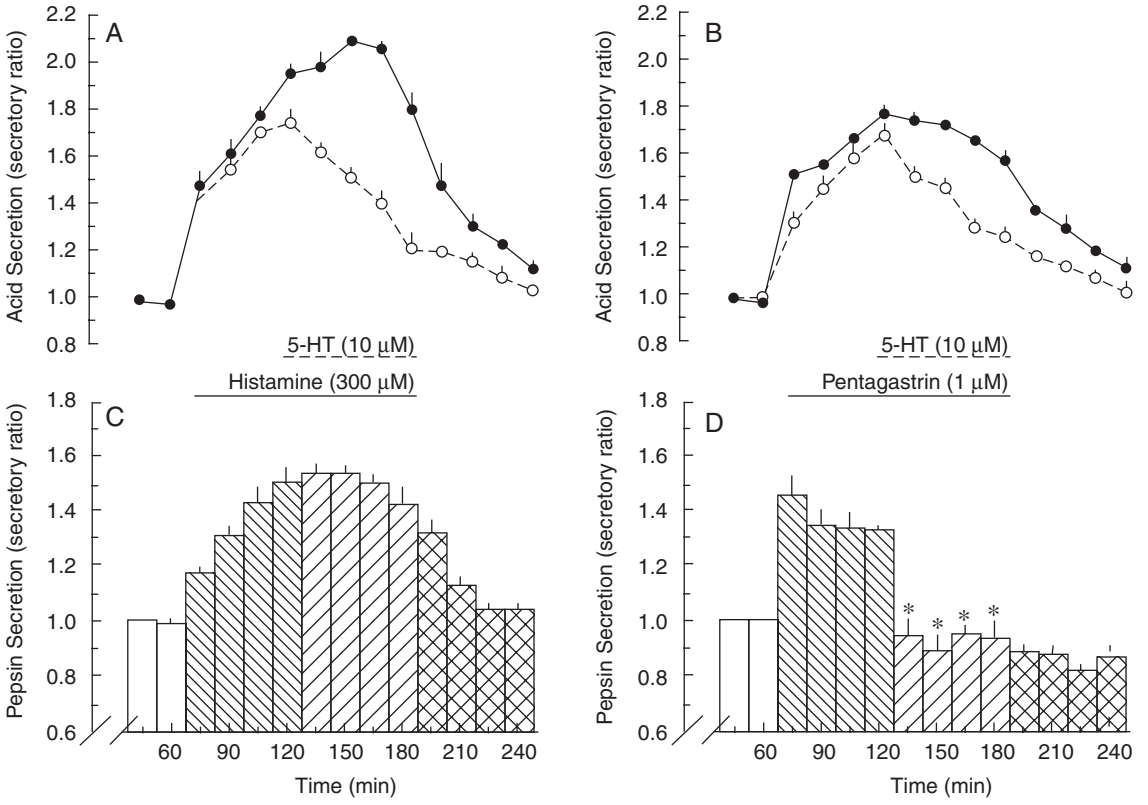

Fig. 5. Effects of serotonin (5-HT) on histamine- and pentagastrin-induced acid secretion (A and B) and pepsin output (C and D) in the rat isolated stomach. Histamine at 300 μM alone (●, n = 3) and1 μM pentagastrin alone (●; n = 3). Histamine or pentagastrin was given after a 1-h perfusion, and 5-HT (○, 10 μM) was added 1 h after administration of histamine or pentagastrin. Data are presented as the means ± SEM. *$P < 0.05$, significantly different from the histamine or pentagastrin alone group.

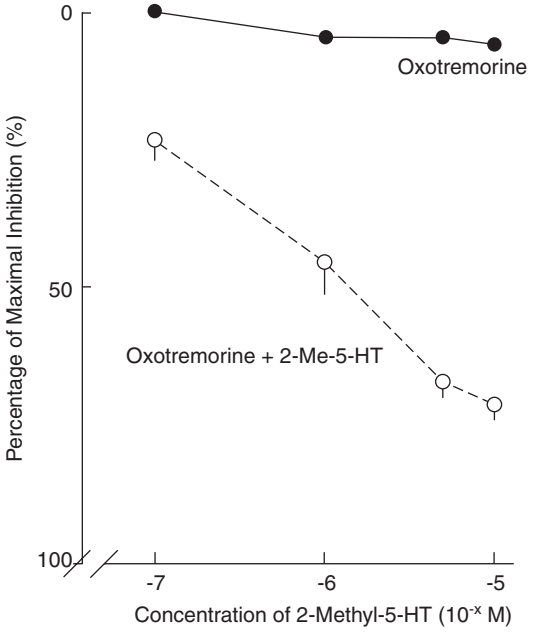

Fig. 6. Concentration-response curves for 2-methyl-5-HT on oxotremorine-induced acid secretion in the isolated rat stomach. Control, oxotremorine alone (●, n = 6). 2-Methyl-5-HT (○, n = 6) was added 1 h after oxotremorine perfusion. Data are presented as the means ± SEM.

(15).

5-HT$_3$Rs were expressed throughout the stomach and were located predominately on the cell surface of neurons and in dense networks of fibers in the myenteric plexus, muscle, mucosa and interestingly in cell bodies and processes of the stomach. 5-HT$_3$Rs were expressed by enteroendocrine cells in the mucosal layer (Fig. 2). These findings support the role of 5-HT$_3$Rs in excitatory and inhibitory secretion and motor function of the stomach and possibly in the pacemaker function of gastric smooth muscle.

5-HT$_3$R immunoreactivity was localized to many nerve fibers in the mucosa along the length of the stomach. 5-HT$_3$Rs mediate a number of neural reflexes involved in the physiological regulation of gastric function including reflexes, pattern generators and motility (15). Strong evidence supports the concept that these responses are mediated by 5-HT$_3$Rs on the peripheral terminals of vagal afferents (1, 25). However, immunoreactive fibers in the mucosa may also be axons and terminals of IPANs; results from the present study certainly suggest that in the stomach, most of the immunoreactive mucosal fibers are not of external origin and could be intrinsic afferents. 5-HT$_3$Rs immunoreactivity was expressed on neurons

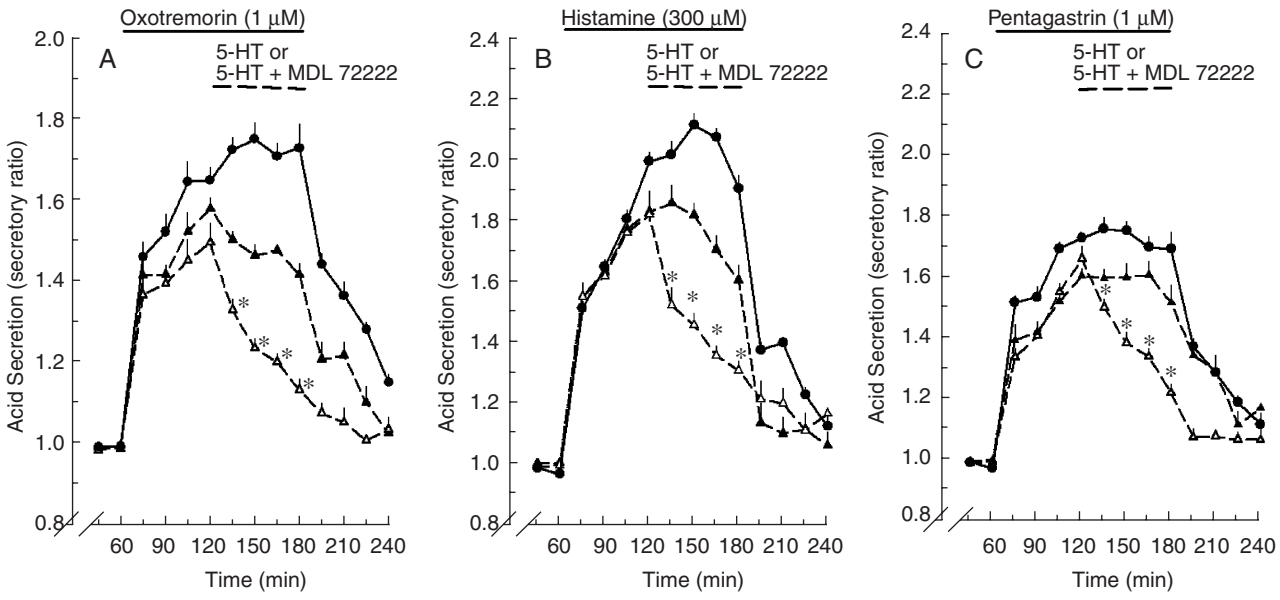

Fig. 7. Effects of serotonin (5-HT) and/or MDL 72222 on oxotremorine- (A), histamine- (B), and pentagastrin (C)-induced acid secretion. Oxotremorine (1 μM), histamine (300 μM) or pentagastrin (1 μM) was given after a 1-h perfusion, and 5-HT (10 μM) or 5-HT plus MDL 72222 (1 μM) was added 1 h after the administration of oxotremorine, histamine or pentagastrin. Oxotremorine, histamine and pentagastrin alone (●); in the presence of 5-HT (△); and in the presence of 5-HT + MDL 72222 (▲). Data are presented as the means ± SEM from 6~8 animals. *$P < 0.05$, significantly different from the oxotremorine-, histamine- and pentagastrin-alone groups.

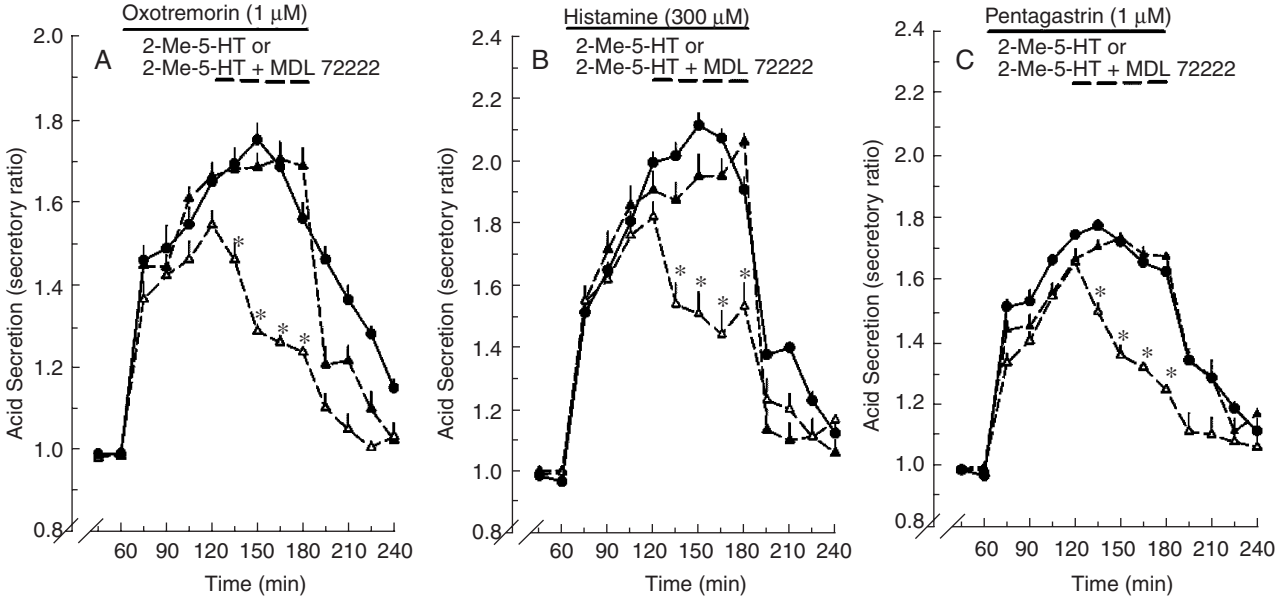

Fig. 8. Effects of 2-methyl-5-HT and/or MDL 72222 on oxotremorine-, histamine- and pentagastrin-induced acid secretion. The conditions were the same as those described in Fig. 7. Oxotremorine, histamine and pentagastrin alone (●); in the presence of 2-methyl-5-HT (△); and in the presence of 2-methyl-5-HT + MDL 72222 (▲). Data are presented as the means ± SEM from 6-8 animals. *$P < 0.05$, significantly different from the oxotremorine-, histamine- and pentagastrin-alone groups.

of the myenteric and submucosal plexuses and fibers in the circular and longitudinal muscle layers, submucosa and mucosa (Fig. 2). This fits well with electrophysiological evidence which has demonstrated that the receptor is localized to the cell body of many enteric neurons where it mediates a rapidly developing and desensitizing depolarization similar to that caused by activation of nicotinic receptors (36). Although

the 5-HT_3R can contribute to fast excitatory postsynaptic potentials in the plexus (45), it is unlikely to be of major functional importance because in the myenteric plexus, 5-HT neurons account for only 1% of the total number of neurons (45). The fast EPSPs in the guinea pig myenteric plexus were likely recorded from descending interneurons which might be involved in mediating descending motor or secretomotor responses (44).

5-HT regulation of acid secretion was investigated using an everted stomach preparation. 5-HT alone had little effect on spontaneous acid secretion. The spontaneous acid secretion from the preparation was 1.591 (mol/15 min, a value similar to the basal acid level *in vivo* (8, 39) and *in vitro* (40). Although 5-HT inhibited acid secretion and pepsin output induced by oxotremorine, histamine, and pentagastrin, under the same conditions, 5-HT inhibited histamine-stimulated pepsin output only slightly, but significantly inhibited oxotremorine- and pentagastrin-induced pepsin output. Pepsin precursors which are called pepsinogens are activated by gastric acid. Different pathways may exist for gastric acid secretion and pepsin output (22). When 2-Me-5-HT, a selective agonist of 5-HT_3Rs (17), was used instead of 5-HT, the inhibition of oxotremorine-induced acid secretion was more potent than with 5-HT, and the IC_{50} value of 2-Me-5-HT (2.0 ± 0.1 μM) was lower than that of 5-HT (7.5 ± 0.3 μM). Similar results of our study were observed in receptor-binding studies and functional studies (26).

It is interesting that 5-HT on the serosal side was taken up by the neural plexus but very little appeared in mucosal EC cells, whereas 5-HT from the mucosal surface penetrated EC cells but not significantly into the nervous tissue (6). We do not know if a similar barrier exists in the rat stomach. However, when 5-HT was placed on the mucosal side, it had no inhibitory action. 5-HT is a potent blocker of gastric acid secretion induced by the cholinergic agonist oxotremorine, or non-cholinergic agents such as histamine or pentagastrin. The action of 5-HT was unlikely to be due to direct blocking of acetylcholine (ACh), histamine or gastrin receptors on parietal cells but more likely to act through second messenger systems, *e.g.* Ca^{2+}, which is involved in acid secretion induced by ACh, histamine or gastrin (12). This is compatible with the notion that the primary action of 5-HT in inhibiting acid secretion occurs through a second messenger system such as Ca^{2+}. Since oxotremorine can increase intracellular [Ca^{2+}] through the metabotropic muscarinic receptor, an increase in intracellular Ca^{2+} concentrations is associated with 5-HT release (34). Intragastric low pH seems to be important in regulating the release of 5-HT because 5-HT itself plays a role in regulating gastric acid secretion (23). Neuronal regulation of 5-HT release is well documented in the stomach where the cholinergic (18, 32), adrenergic (41) and non-adrenergic, non-cholinergic (NANC) neuronal pathways are involved (38, 41). The action of 5-HT is mediated by the 5-HT_3R which induces fast depolarization in the myenteric plexus neurons of the guinea-pig stomach antrum (10). 5-HT_3R activation is associated with increased electrically evoked contractions of guinea pig and mouse stomach corpus or fundus circular smooth muscle (4, 43). The majority of myenteric and submucosal neurons maintained in primary culture responds to 5-HT with a fast inward current that is inhibited by 5-HT_3R antagonists (14).

In isolated guinea pig antrum, 5-HT_3R activation produces a contraction (37). Nitric oxide is the major NANC inhibitory neurotransmitter (9, 11) and ACh is the main excitatory neurotransmitter as well as the preganglionic neurotransmitter of vagal motor innervations in the guinea-pig stomach. Although there is a possibility that 5-HT could be involved in other circuits controlling gastric motility, 5-HT inhibited oxotremorine-, histamine- and pentagastrin-induced acid secretion. The inhibitory effect of MDL 72222 was significantly reversed. Our data also strengthen the idea that potent oxotremorine-, pentagastrin-, and histamine-stimulated acid secretion is involved in regulating the release of 5-HT because 5-HT itself plays a role in regulating gastric acid through 5-HT_3Rs. Expression of 5-HT_3Rs presents on afferent nerve endings in the lamina propria and enteric neurons could be studied. These findings suggest that some 5-HT effects in the stomach, mediated through activation of 5-HT_3Rs, involve distinct neuronal and non-neuronal pathways. 5-HT released by neuronal or endocrine elements might activate 5-HT_3Rs at nerve terminals or at cellular plasma membranes thus influencing gastric functions.

In conclusion, 5-HT or 2-methyl-5-HT has the ability to inhibit oxotremorine-, pentagastrin-, and histamine-stimulated acid secretion but this effect was reversed by MDL 72222, a specific 5-HT_3R antagonist. 5-HT released by neuronal or endocrine elements might activate 5-HT_3R nerve terminals or cellular plasma membranes and influence gastric functions through the release of different transmitters from neurons bearing 5-HT_3Rs. We will continue to explore the evolution of physiological and pathophysiological functions of 5-HT receptors in the stomach in our laboratory.

Acknowledgments

We would like to thank Dr. Shu-Jan Lan (School of Nutrition and Health Sciences, Taipei Medical University, Taiwan, ROC) for her critical reading of

the manuscript. This research was supported by a grant (94CGH-TMU-13) from Cathay General Hospital, Taipei, Taiwan. Present address of Kai-Han Huang: Department of Ophthalmology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, ROC.

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