Cholinergically stimulated gastric acid secretion is mediated by $M_3$ and $M_5$ but not $M_1$ muscarinic acetylcholine receptors in mice

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In addition to $M_3$ receptors, parietal cells also express histamine $H_2$ receptors and gastrin/cholecystokinin (CCK) receptors. It has been thought that ACh indirectly stimulates parietal cells via release of histamine and gastrin from enterochromaffin-like (ECL) cells and antral G cells, respectively (10). It is known that an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) is important in the process leading up to histamine and gastrin release (24, 28). Carbachol, an mAChR agonist, has been reported to increase [Ca$^{2+}$] in ECL and G cells (35). Among the five mAChR subtypes, $M_1$, $M_3$, and $M_5$ receptors positively couple to phospholipase C through $G_q/11$, inducing inositol 1,4,5-trisphosphate and diacylglycerol generation, followed by an increase in [Ca$^{2+}$] (4, 32). Accordingly, it is possible that these mAChR subtypes are involved in vivo gastric acid secretion by regulating hormones and transmitters released from endocrine cells. In particular, in vivo vagal and cholinergic stimulation of gastric acid secretion appears to involve $M_1$ receptors, as evidenced by inhibition of acid secretion with pirenzepine and telenzepine, known as $M_1$-receptor antagonists (11, 14). The present study examined the role of mAChRs involved in gastric acid secretion using mice lacking $M_1$, $M_3$, or $M_5$ receptors.

MATERIALS AND METHODS

Animals. The generation and characterization of each mAChR-KO mouse strain has been previously described [M1 KO: Ohno-Shosaku et al. (22); M3 KO: Matsui et al. (17); M5 KO: Nakamura et al. (21)]. M1/M3 double KO and M1/M5 double KO mice were generated by successive crossing of M1 KO and M3 or M5 KO mice (20, 22). These hybrid lines were back crossed with C57BL/6J mice to yield more than N8 generations, except M1 KO, which was taken to the N3 generation. In the studies with M1 KO mice, littermates generated by intermating heterozygous $M_1$-receptor mutant mice (genetic background: 129SvJ/C57Bl/6J) were used. In other experiments, age-matched C57BL/6J mice were used as wild-type (WT) mice. Animals were housed in plastic cages with hardwood chips in an air-conditioned room (25°C) and were given standard dry pellets, CA-1 (CLEA Japan, Tokyo, Japan), and water ad libitum. Animal maintenance and experimental procedures were approved by the Animal Care and Use Committee of Kyoto Pharmaceutical University.

Drugs. Carbamylcholine chloride (carbachol, Wako Pure Chemical Industries, Osaka, Japan), histamine dihydrochloride (Sigma, St. Louis, MO), human gastrin-17 (gastrin; Sigma), pirenzepine dihydrochloride (pirenzepine; Sigma), atropine sulfate monohydrate (atropine; Wako Pure Chemical Industries), and famotidine (Yamanouchi...
Pharmaceutical, Tokyo, Japan) were all dissolved in saline before administration.

RT-PCR analysis. Whole stomachs, fundic mucosa, antral mucosa, and brains were collected from C57BL/6J mice and immediately frozen in liquid nitrogen and stored at −80°C until use. Total RNA was extracted by the acid-guanidium thiocyanate-phenol-chloroform method using Sepazol RNA-I (Nacarai Tesque). Extracted RNA samples were treated with RNase-free DNase I (Takara Shuzo, Shiga, Japan) at 37°C for 40 min to remove residual genomic DNA. RNA samples were then used as the template for first-strand cDNA synthesis with Maloney murine leukemia virus reverse transcriptase (GIBCO-BRL, Gaithersburg, MD). The reverse transcription step was omitted in control samples to test for the presence of contaminating genomic DNA. The reverse-transcribed products were screened for the presence of muscarinic-receptor cDNA by PCR. The PCR-amplified products were subjected to electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. The sequences of primers used and sizes of the expected RT-PCR products are as follows: M1 receptor: forward, 5'-gcagcagctcagagaggtcacag-3'; reverse, 5'-gcaaac-3' (413 bp); M2 receptor: forward, 5'-gctgagctg-3'; reverse, 5'-gcagcagctcagagaggtcacag-3' (441 bp); M3 receptor: forward, 5'-gcagcagctcagagaggtcacag-3'; reverse, 5'-gcagcagctcagagaggtcacag-3' (511 bp); M4 receptor: forward, 5'-gcaaac-3'; reverse, 5'-gcaaac-3' (345 bp); M5 receptor: forward, 5'-gcagcagctcagagaggtcacag-3'; reverse, 5'-gcagcagctcagagaggtcacag-3' (230 bp).

Measurements of gastric acid secretion. Before experimentation, mice were deprived of food for 18 h and water for 2 h. Intragastric pH was determined by anesthetizing mice with ether. Approximately 30 μl of gastric juice were collected through an incision in the stomach of each mouse. A pH meter (model M-11; Horiba, Kyoto, Japan) with a flat probe (model 6261–10C; Horiba) was used to directly measure the gastric juice pH, which was taken to represent the intragastric pH of the mice.

Secretagogue-stimulated gastric acid secretion and the effects of pirenzepine on carbachol-stimulated gastric acid secretion in WT and M1 KO mice were examined with the pylorus-ligation method. Briefly, after deprivation of food for 18 h and water for 2 h, mice were subjected to epigastric laparotomy and pylorus ligation under light ether anesthesia. Either histamine or gastrin was subcutaneously injected immediately after pylorus ligation. Carbachol was subcutaneously injected immediately and 45 min after pylorus ligation. Pirenzepine was orally administered 1 h before operation. Stomachs were removed, and gastric juice was collected after 1.5 or 3 h. The acidity of the collected gastric juice was determined by titration against 0.01 M NaOH to pH 7.0 using an automatic titrator (Hiranuma; Comitite 550, Tokyo, Japan). Gastric acid output (volume of the collected gastric fluid and expressed as micromoles H+ per hour).

Determination of gastric acid secretion and histamine secretion in mice with a gastric fistula. To determine time-course changes in gastric acid secretion stimulated with carbachol, mice with a gastric fistula were used (7). In brief, each mouse was anesthetized with an intraperitoneal injection of urethane at a dose of 1.25 g/kg. The abdomen was incised, the stomach and duodenum were exposed, and a catheter made from a polyethylene tube was inserted into the stomach via a small incision made in the duodenum and secured with a suture around the pylorus. At the beginning of each experiment, each stomach was rinsed several times with physiological saline and filled with 400 μl of saline through the catheter. Physiological saline (400 μl) was injected through the fistula and collected every 20 min. Carbachol (30 μg/kg) was subcutaneously injected. Atropine (100 μg/kg) or famotidine (1 mg/kg) was subcutaneously injected 20 min before carbachol injection. Control animals received vehicle alone. Gastric acid output was determined by measuring the acidity and volume of the collected gastric fluid and expressed as micromoles H+ per 20 min. Histamine concentration in the collected gastric fluid was determined with the histamine ELISA kit (Medical & Biological Laboratories, Nagoya, Japan).

Measurements of serum gastrin and gastric mucosal histamine levels. Blood samples collected from each mouse were centrifuged at 6,000 g for 15 min to obtain serum, which was then frozen at −20°C until gastrin levels were measured. Gastrin concentrations were determined by means of a radioimmunoassay (Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan). To measure histidine gastrin levels, a sample was collected from the stomach according to the procedure described by Kobayashi et al. (13). Quantitative determination of the histamine level for each sample was performed with an ELISA kit.

Histological analysis. Each stomach was removed from the age-matched WT and KO mice and opened along the greater curvature. The stomachs were fixed with formalin, embedded in paraffin wax, and then sectioned at a thickness of 4 μm. The sections were stained with hematoxylin and eosin and examined under a light microscope.

Data analysis. Data are presented as means ± SE. Differences between two groups were evaluated with the Student’s t-test. Differences between multiple groups were evaluated with analysis of variance followed, when necessary, by a Dunnett’s multiple comparison test or Bonferroni multiple-comparison test. A P value <0.05 was considered statistically significant.

RESULTS

Muscarinic ACh receptor mRNA expression in mice stomachs. To examine the expression of mAChR-subtype mRNA in mice stomachs, total RNA prepared from whole stomachs, oxyntic mucosa, or antral mucosa of C57BL/6J mice was subjected to RT-PCR using mouse M1-M5 muscarinic receptor-specific primers. In the stomach, as well as in the brain, which is known to express all mAChR subtypes, all five muscarinic receptors were found to be expressed (Fig. 1A).

Unexpectedly, the intragastric pH of M1 KO mice was not significantly different from that of WT mice (Fig. 2A). Carbachol-induced gastric acid secretion in M3 KO mice in further experiments was confirmed with another set of primers, MF47 and M545 (21).

Impaired gastric acid secretion in response to carbachol in M3 KO gastric-fistula mice. In a previous study, we reported that deficiency of the M3-receptor gene resulted in impaired gastric acid secretion in mice (1). To investigate carbachol-stimulated gastric acid secretion in M3 KO mice in further detail, the present study used a gastric-fistula mouse model. As shown in Fig. 2A, carbachol induced a remarkable increase in gastric acid secretion in WT mice. M3 KO mice exhibited increased gastric acid secretion in response to carbachol, whereas cumulative analysis of gastric acid output revealed a significant decrease in the KO mice compared with WT mice (2.2 ± 0.8 vs. 12.8 ± 1.1 μmol H+·2 h; P < 0.01; Fig. 2A and B). Carbachol-stimulated gastric acid secretion was completely inhibited by atropine in both WT and M3 KO mice (0.1 ± 0.04 and 0.05 ± 0.02 μmol H+·2 h, respectively). It should be noted that famotidine also completely inhibited carbachol-induced gastric acid secretion in both WT and M3 KO mice (0.2 ± 0.1 and 0.1 ± 0.1 μmol H+·2 h, respectively; Fig. 2B).

Intragastric pH, serum gastrin, and gastric histamine levels in M1 KO mice. Because putative M1-receptor antagonists have been reported to inhibit cholinergergically stimulated gastric acid secretion, the gastric function of M1 KO mice was also studied. Unexpectedly, the intragastric pH of M1 KO mice was not
changed compared with WT mice (2.1 ± 0.1 for both; n = 5). To investigate whether M1 receptors are involved in cholinergically stimulated gastric acid secretion, carbachol-stimulated gastric acid secretion was measured in M1 KO mice subjected to gastric fistula production under urethane anesthesia. In M1 KO mice, carbachol stimulated gastric acid secretion to a similar degree as was observed in WT mice (8.6 ± 2.0 vs. 9.2 ± 1.4 mmol H⁺/2 h; Fig. 3).

Stimulated gastric acid secretion in M1 KO mice was examined by the pylorus ligation method, which evokes gastric acid secretion by eliciting vagal excitation (5). As shown in Fig. 4A, gastric acid output after a 3-h pylorus ligation was similar for WT and M1 KO mice. There was no difference in carbachol-, histamine-, or gastrin-stimulated gastric acid secretion for WT and M1 KO mice (Fig. 4B). Serum gastrin and gastric histamine levels in M1 KO mice were similar to those measured in WT mice (serum gastrin: 160 ± 40 vs. 136 ± 4 pg/ml, and gastric histamine: 7.5 ± 1.0 vs. 7.1 ± 0.8 μg/g tissue; n = 5).

Carbachol-stimulated gastric acid secretion in M1/M5 double KO and M5 KO mice with gastric fistulas. To examine whether or not carbachol-stimulated gastric acid secretion involves Gq/11-coupled mAChRs other than the M3 receptor, M1/M5 double KO mice were used. It is of note that the double KO mice with gastric fistula exhibited a remarkable decrease in gastric acid secretion in response to carbachol, with a significant decrease in cumulative acid output compared with WT mice (4.4 ± 1.1 vs. 9.5 ± 1.1 mmol H⁺/2 h; P < 0.01; Fig. 5A). The results indicate that the M4 receptor is involved in gastric acid secretion. As expected, carbachol-stimulated gastric secretion was significantly decreased in M5 KO mice compared with WT mice (5.3 ± 1.3 vs. 10.9 ± 1.2 mmol H⁺/2 h; P < 0.01). Moreover, M5 KO and M1/M5 double KO mice exhibited similar profiles with respect to their response to carbachol (Fig. 5B).

Carbachol-stimulated histamine synthesis and secretion in WT and KO mice. To elucidate whether or not carbachol stimulates histamine synthesis in the stomach, the time-course changes in gastric histamine contents were determined after carbachol injection to WT mice. As shown in Fig. 6A, gastric histamine contents significantly increased 40 min after carbachol injection compared with immediately after injection (13.3 ± 2.2 tissue vs. 6.6 ± 1.6 μg/g tissue; P < 0.05).
response to carbachol was completely abolished by atropine
treatment 20 min before carbachol injection (Fig. 6B).

In WT mice, cumulative histamine secretion for 1 h before
carbachol injection (basal secretion) was 7.9 ± 1.8 pmol.
Carbachol injection caused a dramatic increase in histamine
secretion accumulated for 1 h (34.6 ± 6.5 pmol; \( P < 0.01 \))
immediately following carbachol injection (Fig. 6C). This
response to carbachol was also abolished by atropine (data not
shown). Similarly, in M1 KO mice, carbachol significantly
stimulated histamine secretion compared with basal secretion
(34.2 ± 3.8 vs. 8.8 ± 1.2 pmol/h; \( P < 0.01 \)). In M3 KO mice,
carbachol tended to increase histamine secretion accumulated with basal secretion (22.0 ± 3.1 vs. 14.7 ± 2.4 pmol/h),
however, stimulated secretion was less than that observed for
WT mice. Carbachol also tended to increase histamine secretion
compared with basal secretion in M3 KO mice (18.8 ± 3.9
vs. 7.5 ± 1.2 pmol/h). It is of note that carbachol-stimulated
histamine secretion was significantly decreased in M3 KO mice
compared with stimulated secretion in WT mice (Fig. 6C).

Effects of pirenzepine on carbachol-stimulated gastric acid
secretion and histamine secretion. As shown in Fig. 7A,
pirenzepine significantly inhibited carbachol-stimulated gastric
acid secretion in a dose-dependent manner in WT mice. To
determine whether or not the inhibitory effect of pirenzepine
on gastric acid secretion results from M1-receptor inhibition,
the effects of pirenzepine on carbachol-stimulated gastric acid
secretion were examined in pylorus-ligated M1 KO mice. It
should be noted that pirenzepine inhibited carbachol-stimu-
lated gastric acid secretion in a dose-dependent manner in M1
KO mice as well as in WT mice (Fig. 7B).

In WT mice with gastric fistula, pirenzepine completely
inhibited carbachol-stimulated gastric acid secretion and hista-
mine secretion. Similarly, in M3 KO mice, gastric acid and
histamine secretion in response to carbachol were completely
inhibited by pirenzepine (Fig. 8, A and B).

Normal gastric mucosal histology in mAChR-KO mice.
Some mouse strains deficient in gastric acid-regulating genes
have been shown to exhibit impaired gastric acid secretion
accompanied by histological changes in gastric mucosal cells, such as decreased parietal cell counts or the presence of immature parietal cells (2). All KO mouse strains used in the present study exhibited normal gastric mucosal histology (data not shown).

DISCUSSION

For the present study, we first performed RT-PCR analysis to demonstrate that all mAChR subtypes including M1-M5 are expressed in the stomach. It is of note that M1, M2, M3, and M4 receptors are expressed in the gastric mucosal layer. Such results are consistent with a previous report that demonstrated that M1 receptors were expressed on zymogen cells and surface mucosal cells (9). M3 receptors have been shown to be expressed on parietal cells (12), whereas M2 and M4 receptors were considered to be involved in D cell (25).

Next, we examined gastric acid secretion in M1, M3, M5, and M1/M5 double KO mice. To date, it has been difficult to determine the role of each mAChR subtype for in vivo gastric acid secretion by a pharmacological approach due to a lack of muscarinic agonists and antagonists that exhibit a high degree of mAChR-subtype selectivity. To circumvent such difficulties, mutant mice lacking each of the five mAChR subtypes (M1-M5 KO mice) were established (18, 33). Genetically engineered mouse studies (2, 26) have recently provided new data concerning the roles of specific molecules and pathways involved in the regulation of gastric acid secretion. In fact, it has previously been reported (7, 30) that deletion of the gene for histidine decarboxylase (HDC), the histamine synthesizing enzyme, resulted in impaired gastric acid secretion in response to gastrin and carbachol, suggesting a crucial role of histamine in gastric acid secretion in mice. We have also revealed the critical role of M3 receptors on parietal cells in gastric acid secretion using M3 KO mice (1). Clearly, mutant mice represent very useful tools to evaluate specific in vivo functions in combination with pharmacological analysis.

The mechanism underlying regulation of gastric acid secretion from parietal cells by ACh is thought to involve at least two pathways. The first pathway involves direct activation of M3 receptors on parietal cells, as evidenced by the fact that carbachol enhanced gastric acid secretion in HDC-KO mice, in which histamine release from ECL cells is absent (7). The second pathway involves indirect stimulation of parietal cells via release of hormones or transmitters from endocrine cells (10). In particular, in vivo histamine release from ECL cells has been reported to be enhanced by cholinergic stimulation (3,

\[ \text{Fig. 5. Gastric acid secretion induced by CCh (30} \mu\text{g/kg}) \text{ in WT and M1/M5 double KO (DKO; A) mice and WT and M5 KO mice (B). Gastric acid secretion was measured with the gastric fistula method under urethane anesthesia. CCh was subcutaneously injected, and gastric acid output was measured. Gastric acid output profiles are shown on the left. Cumulative gastric acid outputs after CCh stimulation are shown on the right. Data are presented as means} \pm \text{ SE (n = 7–9).}^* \text{p < 0.05, significantly different from WT mice.} \]
In the present study, in WT mice, carbachol increased gastric histamine synthesis and secretion, which was completely inhibited by atropine, whereas famotidine significantly inhibited carbachol-stimulated acid secretion in WT mice. Such results indicate that histamine secretion from ECL cells is evoked by cholinergic stimulation via mAChRs activation. Histamine secretion thus plays a crucial role in carbachol-stimulated gastric acid secretion in mice, consistent with pre-
vious findings with HDC-KO mice (7). Interestingly, Lindstrom et al. (15) reported both that muscarinic agonists had no effect on highly enriched, cultured ECL cells and that ECL cell histamine mobilization responds to neuropeptides from enteric neurons, such as pituitary adenylate cyclase-activating peptide (PACAP) and VIP. It has been reported that PACAP is involved with in vivo gastric acid secretion in rats (27, 36). Accordingly, these neuropeptides might be involved in carbachol-stimulated histamine secretion from ECL cells in mice. In contrast, H2-receptor antagonists are known to partially block cholinergic stimulation in normal rats and dogs (10). Accordingly, the role of histamine in cholinergically stimulated acid secretion might be different between mice and other animals.

In the present study, carbachol significantly stimulated gastric acid secretion in M3 KO mice, although the increase was impaired when compared with WT mice. In addition, M3 KO mice exhibited increased histamine secretion in response to carbachol. Moreover, carbachol-stimulated gastric acid secretion was completely inhibited by famotidine in M3 KO mice. Such results indicate that direct activation of M3 receptors on parietal cells is essential, but the other mAChR(s) must be involved in the mechanism underlying cholinergically stimulated gastric acid secretion in mice by regulating histamine secretion. On this note, we found that M3 KO mice exhibited impaired histamine secretion in response to carbachol, suggesting that M3 receptors are also involved in ECL activation. Because gastrin release from G cells has been reported to be directly stimulated by ACh via muscarinic receptors (16), M3 receptor-mediated gastrin might be involved in carbachol-stimulated histamine secretion from ECL cells. Another possibility might be that M3 receptors are involved in release of neuropeptides, leading to stimulated histamine secretion from ECL cells.

The pylorus ligation method is useful for measuring gastric acid secretion in conscious animals; the method is known to artificially evoke acid secretion via vagal excitation (5). Consequently, we used this method for evaluating the acid secretory function stimulated by the vagal system as well as by secretagogues. In the pylorus-ligated condition, there was no difference in gastric acid output between M1 KO and WT mice in basal, carbachol-, histamine-, and gastrin-stimulated conditions, indicating a normal acid secretory response even to vagal stimulation in the KO mice. In addition, both intragastric pH and carbachol-stimulated gastric acid secretion in M1 KO mice with the gastric fistula method were similar to WT mice. M1/M3 double KO mice exhibited similar profiles of acid response to carbachol as M3 KO mice (data not shown). Histamine content in M1 KO mice stomachs was not different from WT mice. Along this same line, there was no significant change in histamine secretion induced by carbachol for WT and M1 KO mice, suggesting that the M1 receptor is not

![Fig. 8. A: effects of pirenzepine (Pir) on gastric acid secretion induced by CCh (30 μg/kg) in WT and M3 KO mice. Pir or Veh was subcutaneously injected 20 min before subcutaneous injection of CCh. Cumulative gastric acid outputs 2 h after CCh stimulation are shown as means ± SE (n = 6). * and †P < 0.05, significantly different from CCh-stimulated WT mice or the corresponding control mice, respectively. B: effects of pirenzepine on histamine secretion in response to CCh (30 μg/kg) in WT and M3 KO mice. Data are presented as means ± SE (n = 6). P < 0.05, significantly different from basal levels.

![Fig. 9. Schematic drawing of proposed mechanism underlying cholinergically stimulated gastric acid secretion in mice. ACh released from enteric nerve endings directly stimulate parietal cells by activation of M1 receptors. M5 receptors could be involved in cholinergic stimulation of histamine release from ECL cells, which might be mediated by release of neuropeptides, such as pituitary adenylate cyclase-activating peptide, from postganglionic enteric nerve fibers. M1 receptors might be also involved in cholinergic regulation of ECL cell activation via gastrin release from G cells and/or neuropeptide release.]
involved in regulation of ECL histamine. This is consistent with the report by Helander et al. (9), showing M1-receptor mRNA expression in chief cells, surface mucous cells, and muscle layers but not in parietal or ECL cells in rats. Thus our present results indicate that the M1 receptor is not involved in gastric acid secretion in mice.

The M3 receptor, the last mAChR subtype to be cloned, is expressed at low levels on both neuronal and nonneuronal cells. Pharmacological studies (6) indicate that the M3 receptor shares very similar functional and ligand-binding properties with M1 receptors. Until recently, little was known about the precise physiological functions of M3 receptors. In the present study, M1/M3 double KO mice and M3 KO mice surprisingly exhibited significant reduction in carbachol-stimulated gastric acid secretion compared with WT mice. Moreover, M3 KO mice exhibited impaired histamine secretion in response to carbachol, indicating that M3 receptors are involved in ACh-mediated gastric acid regulation via histamine secretion from ECL cells. In the present study, RT-PCR analysis failed to detect M2-receptor mRNA in either oxyntic or antral mucosa despite obvious gastric expression. Accordingly, M3 receptors are unlikely to be expressed on endocrine cells related to gastric acid secretion. In the submucosal region of the gastrointestinal tract, neural plexi known as the enteric nervous system are widely distributed and loosely innervate mucosal endocrine cells through the release of ACh and possibly additional neuropeptides (8). M3 receptors might be expressed in the enteric nervous system so as to enhance cholinergic stimulation of gastric acid secretion by increasing ACh release from enteric neurons innervating oxyntic mucosa and/or stimulating release of neuropeptides, such as PACAP, which in turn stimulate histamine secretion from ECL cells (Fig. 9). Synergistic effects of ACh and histamine on acid secretion have been observed using isolated parietal cells (19, 29). M3 receptor-mediated histamine release might play an important role in complete acid secretion in response to cholinergic stimulation. Further studies are required to elucidate the mechanism underlying histamine release from gastric ECL cells following M3-receptor activation by carbachol.

Pirenzepine, a putative M1-receptor antagonist, inhibits vagal- and carbachol-stimulated gastric acid secretion (11), suggesting involvement of M1-receptor activation in cholinergically stimulated in vivo gastric acid secretion. Interestingly, the present study demonstrated that pirenzepine exhibited similar inhibitory effects on carbachol-stimulated gastric acid secretion in both WT and M1 KO mice. Such results indicate that inhibition of acid secretion by pirenzepine is unlikely to result from M1-receptor blockade. Previously, Nakamura et al. (20) have demonstrated that specific pirenzepine-binding sites in rat stomachs were predominantly localized on parietal cells, as well as chief cells, elucidated with 3H-labeled pirenzepine. Pirenzepine prefers mAChR subtypes M1–M3; nonetheless, the selectivity is not so high (log affinity constants for M1: 7.8–8.5, M2: 6.7–7.1) (4). It is also intriguing that pirenzepine completely inhibited carbachol-stimulated gastric acid secretion in M2 KO mice. Taken together, the inhibitory effect of pirenzepine on gastric acid secretion probably mainly results from blocking M3 receptors on parietal cells and M5 (and M3) receptors upstream of ECL activation, not due to M1 receptor inhibition.

In summary, the present study with mAChR-KO mice revealed that cholinergically stimulated gastric acid secretion is primarily mediated by M3 receptors on parietal cells and possible activation of M4 receptors in the submucosal plexus, leading to neurotransmitter and/or neuropeptide release, which in turn stimulates histamine release from ECL cells. M1 receptors are not involved in the regulation of in vivo gastric acid secretion in mice.

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