

Associate editor: M. Kimura

Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present, and future

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Abstract

Pharmacological agents, such as histamine H₂ receptor antagonists and acid pump inhibitors, are now the most frequently used treatment for such acid-related diseases as gastroduodenal ulcers and reflux esophagitis. Based on increased understanding of the precise mechanisms of gastric acid secretion at the level of receptors, enzymes, and cytoplasmic signal transduction systems, further possibilities exist for the development of effective antisecretory pharmacotherapy. Gastrin CCK₂ receptor antagonists and locally active agents appear to represent promising therapies for the future. Development of gene targeting techniques has allowed production of genetically engineered transgenic and knockout mice. Such genetic technology has increased the investigative power for pharmacotherapy for not only antisecretory agents, but also treatment of mucosal diseases, such as atrophy, hyperplasia, and cancer. Elucidation of the origin of gastric parietal cells also represents an interesting investigative target that should allow a better understanding of not only acid-related diseases, but also the evolution of the stomach as an acid-secreting organ.

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Keywords: Gastric acid secretion; Ulcer disease; H₂ receptor; M₃ receptor; Gastrin/CCK₂ receptor; Histidine decarboxylase

Abbreviations: ACh, acetylcholine; cAMP, cyclic AMP; ECL, enterochromaffin-like; HDC, histidine decarboxylase; KO, knockout.

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1. Introduction

Since Prout's (1823) discovery of gastric hydrochloric acid and Heidenhain's (1875) and Golgi's (1893) identification of oxyntic gland parietal cells as the gastric acid secretory cells, the mechanisms underlying acid secretion have proven an interesting area of investigation. To date, the topic of acid secretion has been well reviewed (Davenport, 1992; Hirschowitz et al., 1995; Modlin & Sachs, 1998; Sachs et al., 1995). It has been found that gastric acid is secreted at the time of ingestion of food or alcoholic beverages via both a conditioned and an unconditioned reflex. Both reflexes involve stimulation of several parietal cell receptors, which, in turn, results in transfer of the signal to H⁺/K⁺-ATPase molecules. In addition, it has been empirically determined that gastric acid represents the underlying cause of acid-related peptic disease. In an attempt to protect the gastric mucosa from gastric acid, enhance ulcer healing, and prevent ulcer recurrence, pharmacological control of gastric acid secretion has long represented a desirable goal. Through the painstaking efforts of inspired researchers, various pharmaceuticals, such as histamine H₂ receptor (H₂R) antagonists (Black et al., 1972; Brimblecombe et al., 1975) and H⁺/K⁺-ATPase (acid pump) inhibitors (Fellenius et al., 1981, 1982; Larsson et al., 1983; Sachs & Wallmark, 1989), have been developed and utilized for the treatment of acid-related peptic diseases (Huang & Hunt, 2001). Through such pharmacotherapy, the mechanism by which parietal cells secrete hydrochloric acid was elucidated in detail. In brief, neural and hormonal systems stimulate various receptors on the basolateral membrane, as well as enzymes located on the surface of parietal cell secretory canaliculi, to secrete acid. The mediators for the neural and hormonal pathways are acetylcholine (ACh), released from the vagus nerve; gastrin, released from antral G cells; and histamine, released from enterochromaffin-like (ECL) cells.

Recent genetic technology has allowed deletion and overexpression of specific receptors and enzymes that are involved in gastric acid secretion in mice. The following represents a current review both of the physiology of gastric acid secretion in humans and laboratory animals, with emphasis on data elucidated with genetically engineered

knockout (KO) mice, and of the pharmacological control of gastric acid secretion in acid-related peptic disease.

2. Gastric acid secretion

2.1. Postprandial gastric acid secretion

In daily life, gastric acid is secreted upon ingestion of food or alcoholic beverages. Initially, gastric secretion is commenced by the thought, sight, smell, or taste of appetizing food products via the vagus nerve and enteric nerve system; this process represents the conditioned reflex. Neural pathways directly stimulate parietal cells and antral G cells, leading to gastrin release, as well as oxyntic gland ECL cells, leading to histamine release. Such direct and indirect parietal cell stimulation is principally mediated by three receptors, that is, cholinergic muscarinic M₃ receptors (M₃R), H₂R, and CCK-B/gastrin receptors (CCK₂R). Of these three receptors, however, H₂R appears to represent the key receptor, as H₂R antagonists inhibit not only histamine-stimulated acid secretion, but also gastrin- and ACh-stimulated acid secretion. H₂R activation results in an increase in intracellular cyclic AMP (cAMP) levels, which acts as a second messenger to transfer the signal to the final step of acid secretion (i.e., the acid pump). In contrast, stimulation of either M₃R by ACh or CCK₂R by gastrin results in a signal mediated by an intracellular increase in free Ca²⁺ ions. It is of interest that a threshold concentration of cAMP is required for gastrin-induced acid secretion (Geibel et al., 1995). Furthermore, both gastrin and vagal stimulation enhance histamine release from histamine-containing cells, such as ECL cells, via CCK₂R or pituitary adenylate cyclase-activating polypeptide (PACAP) receptors (Zeng et al., 1999; Sundvik et al., 2001). Activation of such receptors eventually stimulates gastric acid pumps in secretory canaliculi of parietal cell. After sufficient acid secretion has occurred, a feedback system terminates gastric acid secretion. A decrease in intragastric pH stimulates somatostatin release from antral D cells so as to inhibit gastrin release from G cells and to inhibit the stimulative effect of gastrin on CCK₂R located on parietal and ECL cells. Furthermore, acidification of the duodenum also causes a release of the hormone secretin, which acts to inhibit gastric acid secretion.

2.2. Gastric acid secretion resulting from alcoholic beverages

In addition to food, alcoholic beverages, such as beer and red wine, are known to represent potent secretagogues in humans and animals (Chari et al., 1993; Lenz et al., 1983; Teyssen et al., 1997). We have confirmed in dogs that oral administration of either beer or wine, both alcoholic and nonalcoholic, markedly stimulates gastric acid secretion for ~30–45 min in denervated gastric pouch dogs. During active gastric acid secretion, serum gastrin levels were observed to increase. Both Singer et al. (Chari et al., 1993; Teyssen et al., 1997) and our group (Matsuno et al., 2000; Sasaki et al., 2000) have provided evidence that the stimulatory effect of beer and wine on acid secretion is due to not only alcohol, but also other unidentified substances. It is of interest that acid secretion stimulated by beer or wine was found to be significantly inhibited by anticholinergic drugs (e.g., atropine and pirenzepine), H₂R antagonists (e.g., cimetidine and famotidine), a CCK₂R antagonist (e.g., S-0509), and acid pump inhibitors (e.g., omeprazole). These results strongly suggest that substances present in alcoholic beverages stimulate gastric acid secretion via M₃R, muscarinic M₁ receptor (M₁R), H₂R, and CCK₂R on parietal or ECL cells. Identification of such substances might enhance the understanding of the physiology underlying gastric acid secretion.

For the pharmacological control of either gastric acid secretion stimulated by food or alcoholic beverages or diseases involving excessive gastric acid secretion, such as Zollinger-Ellison syndrome or reflux diseases, the following medications are currently used to treat acid-related peptic disease.

3. Antisecretory drugs

3.1. Antacids and anticholinergic drugs

Long ago, people with an upset stomach commonly ingested powdered shells to alleviate the discomfort. Accordingly, it was empirically discovered that CaCO₃, a natural antacid, buffered gastric acid. After demonstration of the presence of hydrochloric acid in the stomach, antacid therapy became the popular treatment for peptic acid-related diseases, such as peptic ulcers. Antacids, such as sodium bicarbonate, calcium carbonate, aluminum hydroxide, magnesium hydroxide, or combined preparations, promptly provide effective pain relief via neutralization of intraluminal acid (Feurle, 1975). The effective time for antacids to last in the human stomach, however, is too short to exert a neutralizing effect. Moreover, given that more potent and safe antisecretory drugs, such as H₂R antagonists and acid pump inhibitors, are readily available, antacid therapy is not commonly utilized for current peptic ulcer treatment. To prolong the effect of antacids, anticholinergic drugs, such as propanthe-

line bromide and benactidine methobromide, have been concurrently administered to delay emptying of the agents into the duodenum. Anticholinergics can also inhibit acid secretion by themselves. Similar to antacids, however, the use of anticholinergic drugs is generally limited, as anticholinergics delivered at dosages capable of inhibiting acid secretion almost invariably induce adverse effects, such as dry mouth, blurred vision, tachycardia, and bladder dysfunction. In contrast to anticholinergics, selective M₁R antagonists, such as pirenzepine and tenipipazine, have been clinically utilized, as side effects occur less frequent (Fig. 1A). Such agents are useful in both providing pain relief and enhancing ulcer healing via suppression of gastric acid secretion.

3.2. Histamine H₂ receptor antagonists

Since histamine was considered to represent a final common mediator for acid secretion, several groups fervently sought an antagonist capable of inhibiting histamine-stimulated acid secretion. After painstaking efforts, Black and colleagues (1972) succeeded in developing the first H₂R antagonist, burimamide, followed by metiamide. With minor alterations, Brimblecombe and colleagues (1975) eventually developed cimetidine, which is widely prescribed throughout the world for treatment of acid-related peptic disease (Brimblecombe et al., 1975). By modifying the chemical structure of cimetidine, the potent H₂R antagonists ranitidine, famotidine, and nizatidine were all developed, resulting in remarkable treatment for acid-related disease, including reflux esophagitis (Fig. 1B). These new compounds were later found to inhibit gastric acid secretion stimulated by not only histamine, but also carbachol and gastrin in both humans and animals. These findings suggest that H₂R stimulation might be required for the effect of such secretagogues. Recently, H₂R antagonists have become first-line therapy for acid-related peptic disease, leading to a marked improvement in the quality of life for a large number of patients. Paralleling the development of such pharmacotherapy, there has been a dramatic reduction in the use of surgical intervention for ulcer treatment. Interestingly, in the beginning of clinical application, many physicians expressed concern over potential regurgitation of intestinal bacteria into the stomach, as the antisecretory effect of H₂R antagonists is much more powerful than conventional anticholinergic drugs. Sustained inhibition of gastric acid might predispose to gastric bacterial contamination, resulting in an increase in *N*-nitrosoamine, a metabolite of ingested nitrites and a known carcinogen. Accordingly, the use of H₂R antagonists initially was limited to only 4 weeks. Nonetheless, long-term clinical experience has demonstrated that such a risk was a groundless fear. In fact, certain H₂R antagonists are currently even considered safe enough to be marketed as over-the-counter pharmaceuticals.

It is well known that cimetidine strongly inhibits cytochrome P450 (CYP) enzymes, particularly CYP3A4 (Rendic, 1999). Accordingly, caution is warranted when

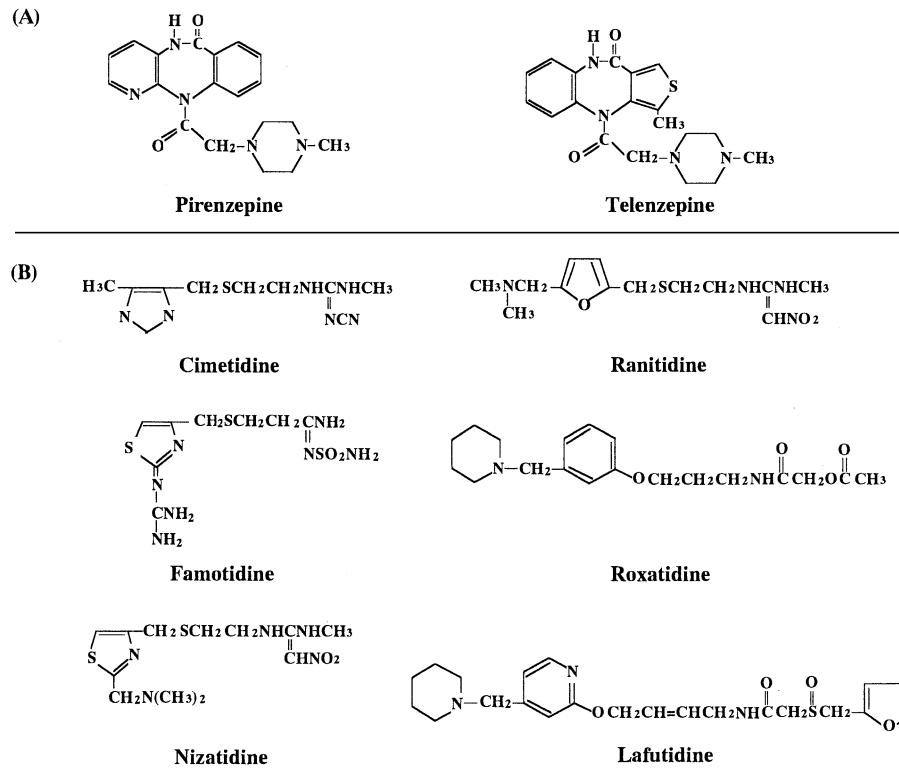


Fig. 1. Chemical structures of muscarinic cholinergic M_1 receptor (A) and histamine H_2 receptor (B) antagonists. Note that roxatidine and lafutidine possess structures completely different from cimetidine, ranitidine, famotidine, and nizatidine.

cimetidine is concurrently prescribed with other pharmacologic agents, such as warfarin and diazepam, that are metabolized by this enzyme. In contrast, it has been reported that CYP inhibition by ranitidine is relatively weak compared to cimetidine.

Black and colleagues (1972) first contended that histamine H_2 R antagonists require an imidazole ring in their chemical structure to antagonize the effect of histamine. Nonetheless, ranitidine and famotidine were found to exert a stronger effect on H_2 R than cimetidine, despite the absence of imidazole rings and the presence of furan and thiazole rings, respectively. Furthermore, new H_2 R antagonists, such as roxatidine and lafutidine, which were developed by random screening in Japan, appear to have no relationship to the chemical structure of either the histamine molecule or the established H_2 R antagonists (Shibata et al., 1993; Shiratsuchi et al., 1988; Tarutani et al., 1985a, 1985b; Umeda et al., 1999). The effects of such newly developed antagonists were much the same as cimetidine, ranitidine, and famotidine in both clinical trials and animal studies. Animal studies demonstrated that cimetidine and other representative H_2 R antagonists had little or no effect on pharmacologic agent-induced necrotizing gastric mucosal damage (Robert et al., 1979). In contrast, the two newly developed antagonists were found to have a cytoprotective effect for gastric mucosa against pharmacologic agent-induced necrotizing damage in animal studies.

Although pharmacotherapy for acid-related peptic diseases has greatly improved since the development of H_2 R antagonists, the agents still possess a number of shortcomings. For instance, it was found that H_2 R antagonists have little effect on daytime acid control, despite marked suppression of nocturnal acid output. It has also been reported that H_2 R antagonists evoke rapid tolerance during therapy. Such tolerance does not depend on overexpression of H_2 R, but rather, appears to be related to up-regulation of other pathways that elevate cAMP levels in parietal cells. In addition, acid rebound following H_2 R antagonist withdrawal represents another disadvantage. Such a phenomenon is considered to represent the result of either up-regulation of H_2 R on parietal cells or increased production of H^+/K^+ -ATPase in parietal cells.

3.3. Acid pump inhibitors

Identification of the acid pump, H^+/K^+ -ATPase, as the final pathway of gastric acid secretion provided a unique opportunity to develop a new class of agents that inhibit acid secretion. Research groups at AB Hassle Company in Sweden serendipitously discovered that benzimidazole derivatives could potentially inhibit gastric acid secretion in rabbit or guinea pig isolated gastric glands stimulated by dibutyryl-cAMP (Fellenius et al., 1981, 1982). This finding resulted in the hypothesis that such compounds might inhibit parietal cell acid pumps. After screening a vast

number of derivatives, the first acid pump inhibitor (i.e., omeprazole) was finally discovered. Omeprazole was promptly utilized for the treatment of acid-related peptic diseases, including upper gastrointestinal ulcers, reflux esophagitis, and Zollinger-Ellison syndrome. Clinical and animal studies demonstrated that the antisecretory effect of omeprazole persisted for a long time (> 24 hr after a single dose) due to covalent binding of omeprazole to H^+/K^+ -ATPase (the precise mechanism is explained in the following section). Similar to the development of H_2R antagonists, lansoprazole, pantoprazole, and rabeprazole were subsequently developed by modifying the chemical structure of omeprazole (Fig. 2A) (Lee et al., 1992; Robinson et al., 1997; Satoh et al., 1989; Williams & Pounder, 1999). In contrast to H_2R antagonists, however, all acid pump inhibitors possessed a common structural element (i.e., a benzimidazole ring).

As expected based on the sight of inhibition, omeprazole and lansoprazole are able to inhibit gastric acid secretion stimulated by ACh, gastrin, and histamine in a dose-related manner (Fig. 3). It was demonstrated that omeprazole and other inhibitors represent prodrugs that are converted into active forms in acidic environments (Nagaya et al., 1989; Satoh et al., 1989). It is of note that acid pump inhibitors were found to accumulate in parietal cell secretory canaliculi, resulting in an antisecretory effect that lasts much longer than that of receptor antagonists. Animal studies have

indicated that as a result of long-term inhibition of gastric acid secretion, circulating gastrin levels increase, resulting in mucosal hyperplasia and carcinoid tumor development in rat gastric mucosa (Carlsson et al., 1990; Hakanson et al., 1986). Nonetheless, there has been no report of development of mucosal hyperplasia in human stomachs, despite presence of hypergastrinemia. It remains most likely that the degree of increase in circulating gastrin in humans is much less than that observed in rodents.

Animal studies have clearly demonstrated the tremendous difference between H_2R antagonists and acid pump inhibitors in terms of efficacy for ulcer treatment. Indeed, ulcer healing following administration of H_2R antagonists in rats is rather inconsistent, despite frequent and adequate dosings. In contrast, ulcer healing following administration of acid pump inhibitors is reproducible, even with only single daily dosing (Satoh et al., 1989; Yamamoto et al., 1984). Such a marked difference might stem from the differing effective time for acid inhibition.

As described above, increased serum gastrin levels, due to suppressed acid secretion, are observed during omeprazole administration. Consequently, it was suggested that the ulcer healing effect of omeprazole might result from increased gastrin levels, as gastrin exerts a potent trophic effect on gastric mucosa. Nonetheless, it was demonstrated that the ulcer healing effect of omeprazole was not affected by concomitant administration of a somatostatin derivative

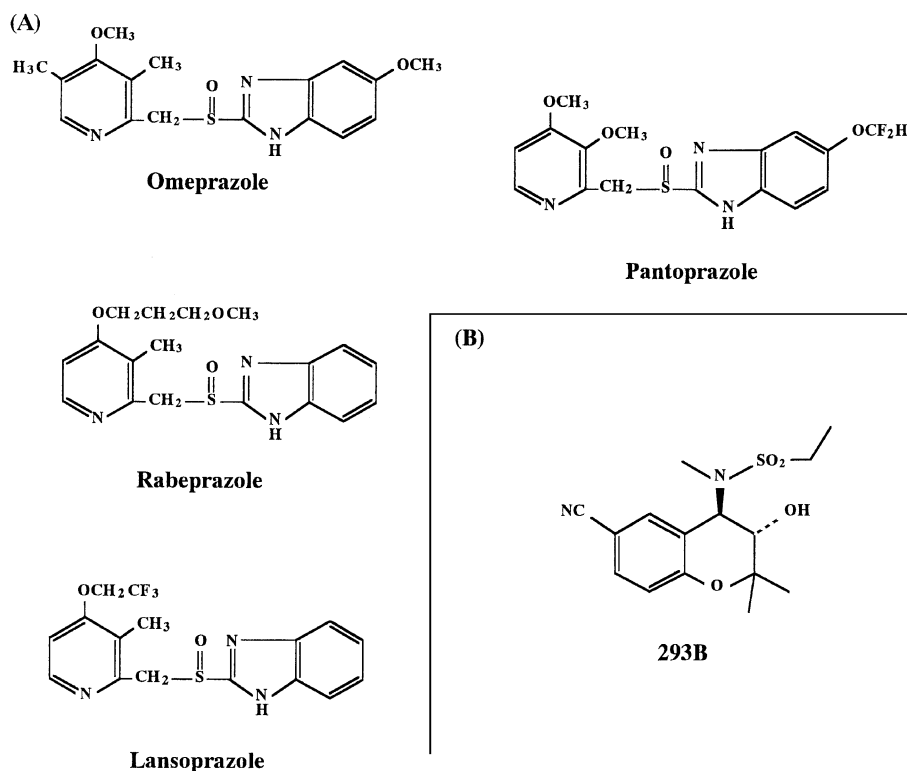


Fig. 2. Chemical structures of acid pump inhibitors with benzimidazole rings (A) and the K^+ channel blocker 293B (B).

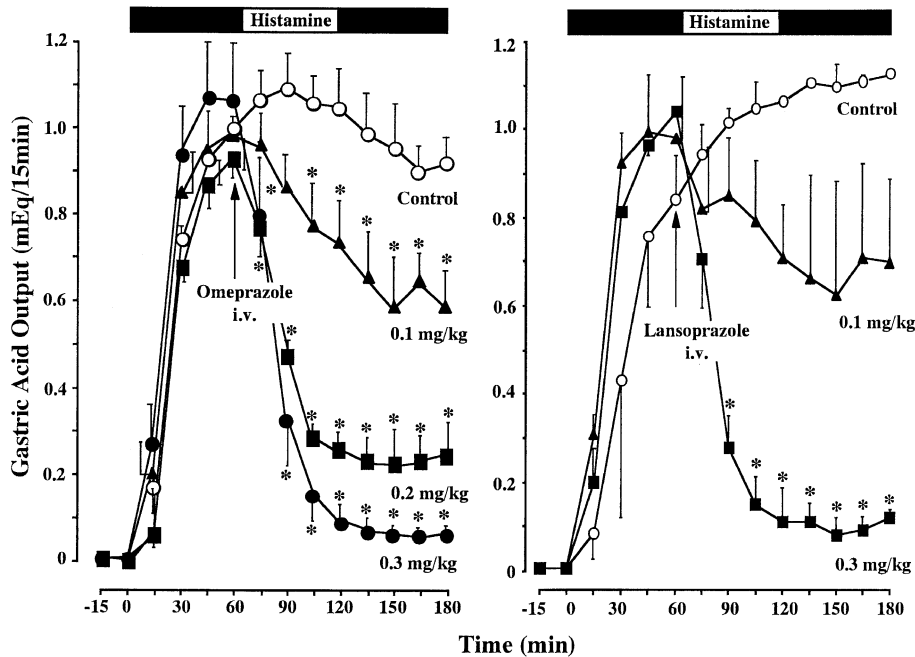


Fig. 3. Antisecretory effects of omeprazole and lansoprazole on histamine-stimulated gastric acid secretion in the denervated (Heidenhain) pouch in dogs. Data are presented as means ± SEM (n = 3–4). * Significantly different from control values, P < 0.05.

and the CCK₂R antagonist YM022, indicating that increased gastrin failed to exert an influence on ulcer healing (Hirschowitz et al., 1995; Okabe et al., 1997; Okabe & Tsukimi, 1996). Accordingly, omeprazole most likely enhances ulcer healing due to its potent and persistent antisecretory effect (Fig. 4).

3.4. Eradication of *Helicobacter pylori* with acid pump inhibitors

Since Marshall and Warren (1984) discovered the bacteria *Helicobacter pylori* in stomachs, *H. pylori* has been known to represent a risk factor for not only development of

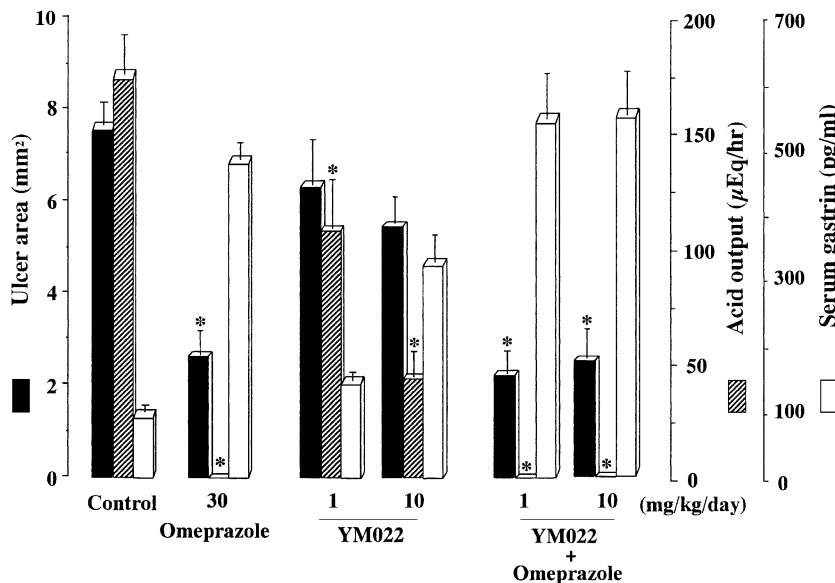


Fig. 4. Effects of omeprazole, YM022 (a selective CCK₂R antagonist), and their combination on ulcer healing, acid secretion, and serum gastrin levels in rats. Acetic acid ulcers were used as the experimental ulcer model. Note that the ulcer healing effect of omeprazole was not affected by combined treatment, suggesting that increased gastrin does not contribute to ulcer healing. Data are presented as means ± SEM (n = 10–17). * Significantly different from control values, P < 0.05.

gastritis and ulcers, but also relapse of healed ulcers (Osato & Graham, 1999). In addition, epidemiological investigations have demonstrated that *H. pylori* infection directly and indirectly induces neoplasms, such as gastric carcinoma and MALT lymphoma (Danesh, 1999; Huang et al., 1998; Konturek et al., 1999; Uemura et al., 2001). Various studies with animals, including primates, have demonstrated that long-term infection with *H. pylori* induces atrophic gastritis, ulcers, and intestinal metaplasia at a high incidence. Our group found that *H. pylori* infection invariably causes acute gastritis, ulcers, and a relapse of healed gastric ulcers in Mongolian gerbils (Keto et al., 1999, 2001). In addition, Watanabe et al. (1998) demonstrated that *H. pylori* infection induced intestinal-type gastric adenocarcinoma 62 weeks following inoculation in Mongolian gerbils. Our group (Keto et al., 2001) has also found that both atrophic gastritis with intestinal metaplasia and intestinal-type adenocarcinoma develop 18–24 months after *H. pylori* inoculation in Mongolian gerbils. A recent study by Higashi and colleagues (2002) demonstrated that cytotoxin associated gene (CagA) protein highjacks a signaling pathway in gastric cells, prompting a morphological change that begins an oncogenic cascade. Given such findings, it is reasonable to consider that *H. pylori* represents a carcinogenic agent for the stomach that probably acts as an initiator and/or a promoter. Accordingly, eradication of *H. pylori* in humans with or without current gastric disease doubtless represents the most important initial step for the prevention of benign and malignant gastric disease.

Crabtree and Figura (1999) penned the following statement, “The key current questions are whether the long-term consequences of mucosal damage, such as atrophy and intestinal metaplasia, can be reversed after elimination of infection and whether, with the development of a simple 1-week therapeutic regimen for eradicating *H. pylori*, large-scale treatment of infected patients will have a beneficial effect on the incidence of gastric cancer.”

In response to the above proposal, we examined the timing of eradication following *H. pylori* (VacA- and CagA-positive strains) inoculation in Mongolian gerbils (Keto et al., 2001). Eradication was achieved 4 or 8 months post-inoculation with a combined treatment of omeprazole (4 weeks) plus clarithromycin (2 weeks). Upon autopsy, 18–24 months postinoculation, it was demonstrated that both visible and histological changes were clearly prevented by *H. pylori* eradication 4 months postinfection. In contrast, atrophy and intestinal metaplasia persisted in animals that underwent *H. pylori* eradication 8 months postinoculation. Accordingly, it was concluded that although early eradication of *H. pylori* clearly prevented development of visible and histologic changes in Mongolian gerbils stomachs 18 and 24 months postinoculation, late eradication failed to prevent development of atrophic gastritis with intestinal metaplasia. These results strongly suggest that the timing of eradication plays a critical role in the prevention of *H. pylori*-associated mucosal changes. Namely, eradication

should be undertaken as early as possible to prevent the occurrence of irreversible gastric damage.

3.5. The potential for K^+ channel therapy

During gastric acid secretion, H^+/K^+ -ATPase couples H^+ exit to K^+ uptake. Consequently, luminal K^+ recycling and Cl^- exit are essential for HCl secretion. Nonetheless, the molecular characterization of luminal K^+ channels that enable K^+ recycling in parietal cells remains unknown. Recently, several luminal K^+ channels were reported and their potential to inhibit gastric acid secretion was demonstrated with in vitro cell cultures and in vivo animal models. KCNQ1, which is mutated in the hereditary long QT syndrome type 1, is present in abundance in a variety of epithelia, including kidney, pancreas, small and large intestines, and stomach epithelia (Bleich & Warth, 2000; Chouabe et al., 1997; Yang et al., 1997). KCNQ1 coassembles with several different subunits, such as KCNE1, KCNE2, and KCNE3, to form native K^+ channels. As KCNE2 and KCNE3 are expressed to a high degree in the stomach, it has been postulated that KCNQ1 coassembled with such subunits may constitute the gastric K^+ channels that contribute to luminal K^+ recycling in parietal cells (Grahammer et al., 2001). 293B is a kind of chromanol compound known as KCNQ1 inhibitor (Bleich et al., 1997; Lohrmann et al., 1995; Yang et al., 1997). Interestingly, 293B (Fig. 2B) reversibly and completely inhibited gastric acid secretion stimulated by histamine, carbachol, and gastrin (Grahammer et al., 2001). Consequently, 293B might represent a potential candidate for inhibiting gastric acid secretion via a new mechanism.

Another K^+ channel designated as inwardly rectifying K^+ channel 4.1 (Kir4.1) has also been reported to localize to the apical membrane of gastric parietal cells (Fujita et al., 2002; Hagen & Ouellette, 2001). Blockade of Kir4.1 led to inhibition of acid secretion stimulated by both histamine and carbachol in isolated parietal cells. Kir4.1 channel activity is sensitive to intracellular H^+ , but resistant to extracellular H^+ , which might reflect the channel's involvement in the K^+ recycling pathway for H^+/K^+ -ATPase at the apical membrane of parietal cells. Accordingly, Kir4.1 might also represent a potential therapeutic target for acid-related diseases. ROMK channels are one of the other inwardly rectifying ATP-sensitive K^+ channels (Sritharan et al., 2001; Vucic et al., 2002). Gastric glands isolated from ROMK channel gene-KO mice exhibited no H^+/K^+ -ATPase activity, indicating that ROMK channel is also associated with gastric acid secretion. ROMK channels thus might also represent an interesting target for acid inhibition.

As many kinds of K^+ channels have been identified (Shieh et al., 2000; Yamada et al., 1998), specific and selective pharmacotherapy that inhibits distinct K^+ channel activities might prove to be ideal for treating acid-related diseases.

3.6. Cholecystokinin-2 receptor antagonists

Similar to ACh and histamine, both endogenous and exogenous gastrin stimulate gastric acid secretion via CCK₂R on parietal and ECL cells upon histamine release. Accordingly, many groups have attempted to develop nonpeptide anti-CCK₂R antagonists as antisecretory drugs. Proglumide was first developed as an CCK₂R antagonist, but was subsequently found to represent a nonselective antagonist (Galeone et al., 1978; Hirst et al., 1991). Thereafter, various selective antagonists, such as L365260, YM022, YF476, S-0509, Z-360, and PD136450, were developed and subjected to preclinical and clinical trials as antisecretory and anti-ulcer drugs (Fig. 5) (Amagase et al., 1999; Hagishita et al., 1997; Huang et al., 1989; Nishida et al., 1994; Powell & Barrett, 1991; Semple et al., 1997). Animal studies indicated that such antagonists possess a more selective effect on gastric acid secretion than proglumide. We have also confirmed that although S-0509 results in dose-dependent inhibition of gastrin-stimulated acid secretion in dogs (Fig. 6), it has little or no effect on histamine- and carbachol-stimulated acid secretion. Z-360 is now under phase 1 study in clinical trial.

Despite the clear-cut efficacy of the above CCK₂R antagonists in animal studies, however, clinical testing

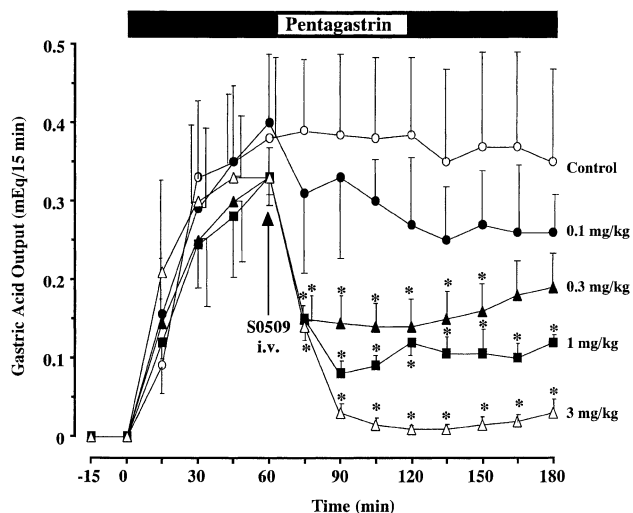


Fig. 6. Effects of S-0509 (a selective CCK₂R antagonist) on pentagastrin (8 μ g/kg/hr)-stimulated gastric acid secretion in denervated (Heidenhain) gastric pouch dogs. Intravenously administered S-0509 inhibited gastric acid secretion in a dose-related manner. Data are presented as means \pm SEM ($n = 5$). * Significantly different from control values, $P < 0.05$.

indicated inconsistent antisecretory and anti-ulcer effects compared with other receptor antagonists and pump inhibitors. Accordingly, no CCK₂R antagonists have been approved for the treatment of acid-related disease to date.

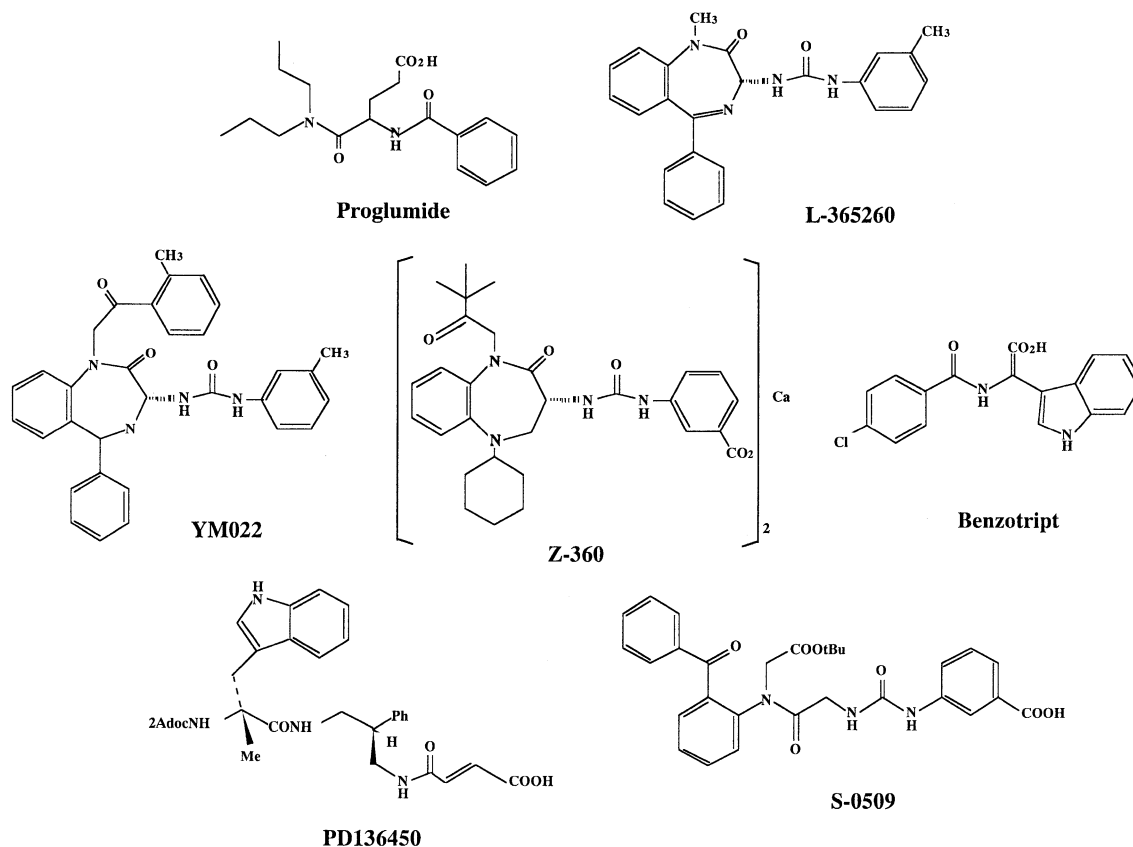


Fig. 5. Chemical structures of CCK₂R antagonists.

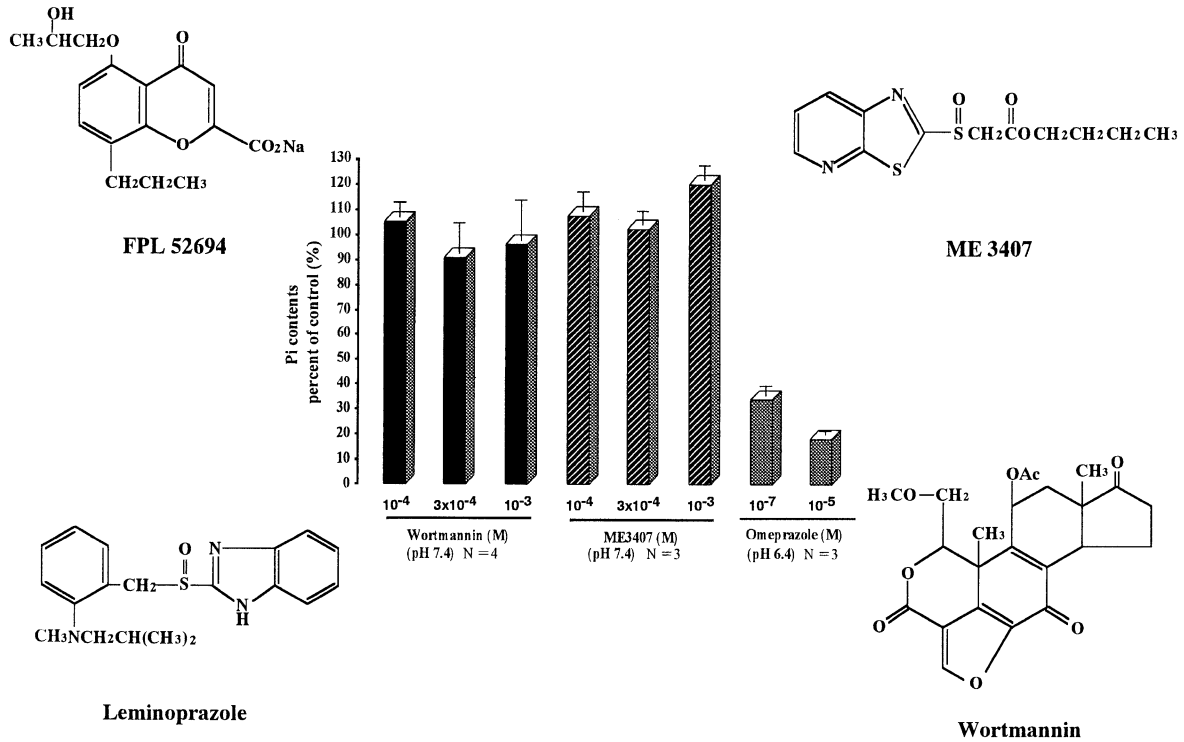


Fig. 7. Chemical structures of locally active antsecretory drugs and effects of H⁺/K⁺-ATPase.

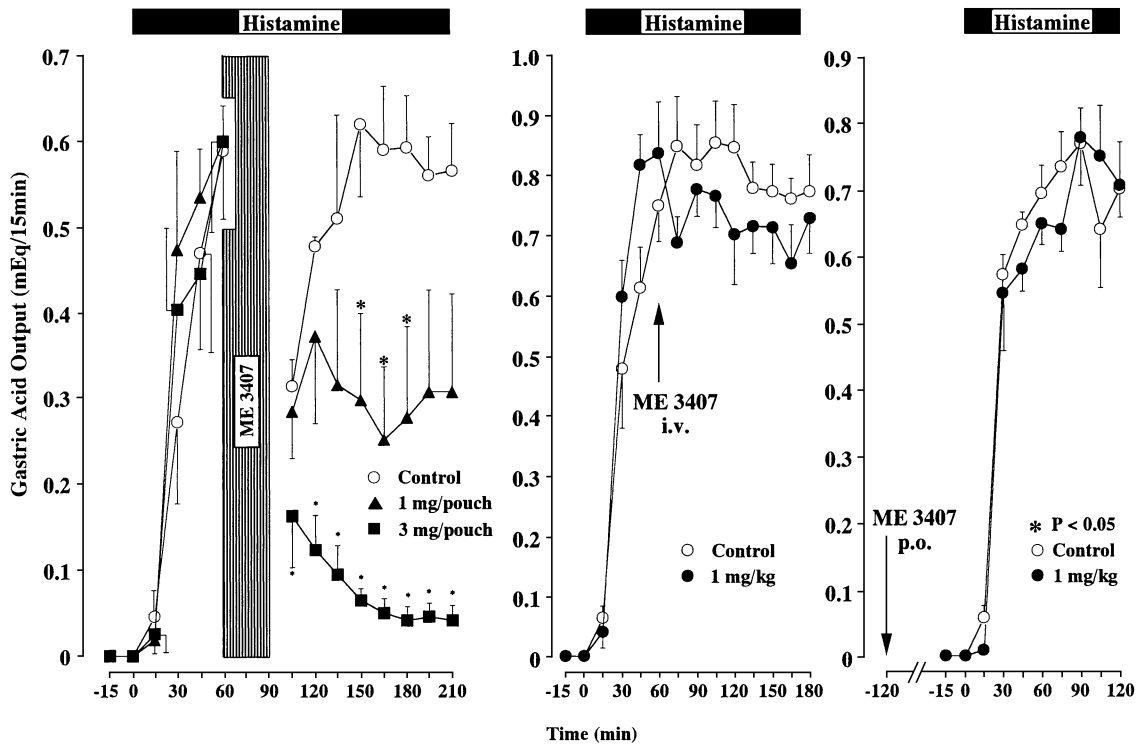


Fig. 8. Local and systemic effects of ME3407 on histamine-stimulated gastric acid secretion (80 μ g/kg/hr) in denervated gastric pouch dogs. ME3407 was administered into the pouch for 30 min, intravenously or orally either before or after histamine infusion. Note that while ME3407 significantly inhibited acid secretion, it had no effect with intravenous or oral administration. Data are presented as means \pm SEM ($n=4$). * Significantly different from control values, $P<0.05$. Data from Okabe et al. (2002a, 2002b).

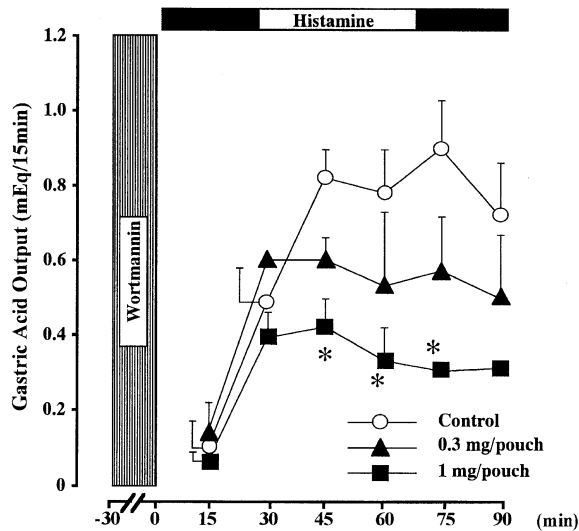


Fig. 9. Local effects of Wortmannin on histamine-stimulated gastric acid secretion (80 $\mu\text{g}/\text{kg}/\text{hr}$) in denervated gastric pouch dogs. Wortmannin was locally applied to the pouch in a volume of 15 mL for either 15 or 30 min. Data are presented as means \pm SEM ($n=5$). * Significantly different from control values, $P<0.05$.

3.7. Locally acting antisecretory drugs

Several drugs have been reported to exert an antisecretory effect when locally applied to the stomach (Bond & Hunt, 1956; Canfield & Curwain, 1983; Curwain & Canfield, 1983; Konturek et al., 1981, 1984; Nicol et al., 1981; Reed & Smy, 1980; Urushidani et al., 1997). Nonetheless, attention has not been directed toward such drugs as either antisecretory drugs or investigational tools useful for the elucidation of parietal cell function. We examined whether or not certain agents could inhibit secretagogue-stimulated

gastric acid secretion when applied to gastric pouches in dogs. Our studies demonstrated that omeprazole, lincloprazole (an acid pump inhibitor), FPL52694 (a mast cell stabilizer), ME3407 (a myosin light chain kinase inhibitor and functional analogue of Wortmannin) (Fig. 7) (Okabe et al., 1995, 2002), and 16,16-dimethyl prostaglandin E_2 significantly inhibited gastric acid secretion stimulated by histamine, gastrin, or carbachol by local application. The duration of the antisecretory activity following local application for 30 min was ~ 5 –6 hr for FPL52694 and ME3407; that is, inhibition was reversible. For omeprazole, lincloprazole, and the prostaglandin analogue, it is possible that the compounds will have inhibited acid pumps following either absorption from the gastric mucosa or interaction with prostaglandin receptors on parietal cells. In contrast, neither FPL52694 nor ME3407 exerted an inhibitory effect on acid pumps or an antisecretory effect following oral or intravenous delivery (Fig. 8). Since ME3407 resulted in marked inhibition of gastric acid secretion, we further examined whether or not another inhibitor of myosin light chain kinase, Wortmannin, achieved a similar local effect on acid secretion. Our studies demonstrated that locally applied Wortmannin, administered at 1 mg per pouch either before or after histamine infusion, resulted in a strong antisecretory effect on histamine-stimulated acid secretion (Figs. 9 and 10). It is of interest that a 15-min application of the agent to the pouch significantly inhibited acid secretion. The antisecretory effect of Wortmannin persisted for more than 10 hr after a single application, suggesting that the duration of the effect is nearly the same as that of acid pump inhibitors systemically delivered (Fig. 10). Accordingly, it remains most likely that FPL52694, ME3407, and Wortmannin act on a pharmacologically sensitive portion of the apical membrane of parietal cells. Other MLCK inhibitors, such

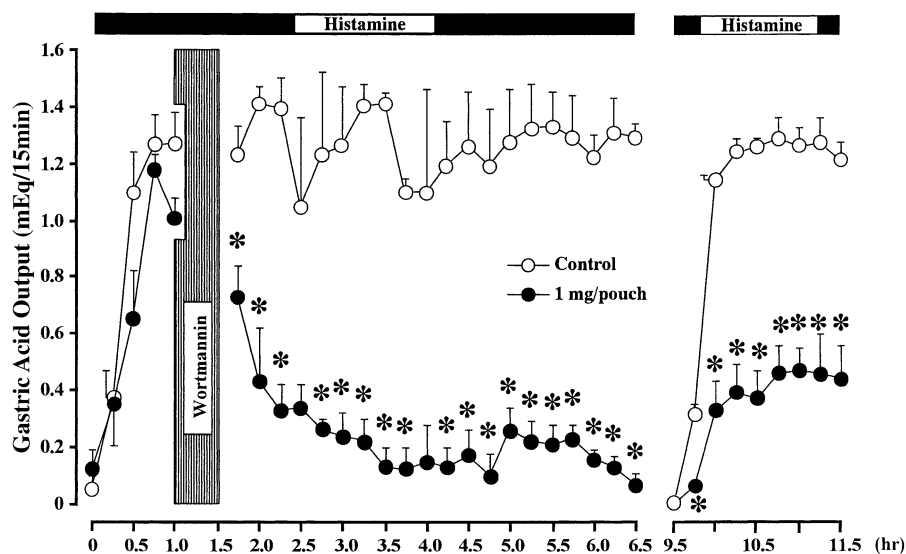


Fig. 10. Duration of antisecretory effects of locally applied Wortmannin. Wortmannin was administered for 30 min to dog denervated gastric pouches 1 hr after histamine infusion (80 $\mu\text{g}/\text{kg}/\text{hr}$). It is of note that the antisecretory effects significantly persisted >10 hr after a single application. Data are presented as means \pm SEM ($n=5$). * Significantly different from control values, $P<0.05$.

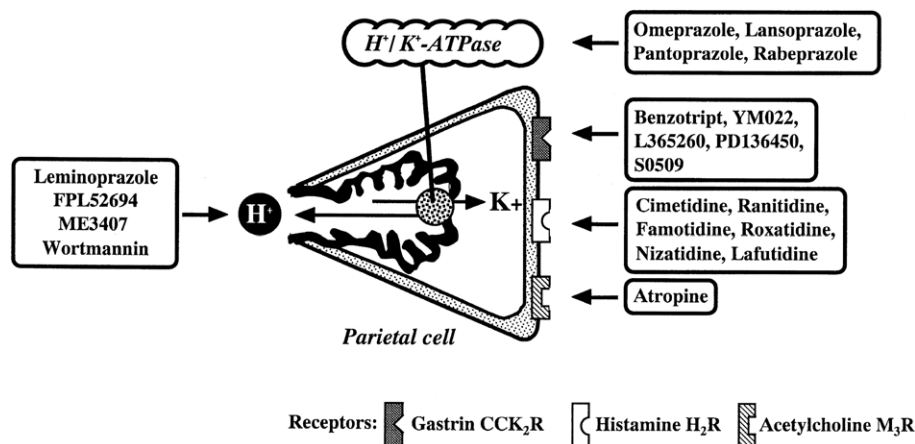


Fig. 11. Schematic drawing of a parietal cell and the various targets for antisecretory drugs.

as HA-100 and HA-1077, failed to exert an effect on acid secretion, despite administration of a dose of 6 mg per pouch. The lipid-soluble substances ME3407 and Wortmannin appeared to penetrate the apical membrane and to proceed into the cytoplasm, while the water-soluble substances HA-100 and HA1077 did not penetrate the membrane, which probably explains their inability to achieve an effect. Accordingly, it is most likely that both ME3407 and Wortmannin act in the cytoplasm to inhibit acid secretion by first inhibiting MLCK, which represents a key point in the acid-secreting process. The exact mechanism by which MLCK effects the normal functioning of gastric acid secretion remains unknown. One consideration is that the substances might prevent the fusion of tubulovesicles to secretory canaliculi. Incidentally, both ME3407 and Wortmannin failed to exert a direct effect on H^+/K^+ -ATPase isolated from rabbit oxyntic mucosa (Fig. 7). In any event, the apical membrane represents not only an intriguing target

for new antisecretory drugs, but also a new medium to further elucidate parietal cell functioning. Finally, the site of action of various antisecretory drugs is illustrated as shown (Fig. 11).

4. Studies with genetically engineered mice

The understanding of the regulatory mechanisms of gastric acid secretion has been greatly deepened by the use of various animal models, including dogs, cats, rabbits, guinea pigs, rats, and mice, as well as clinical studies in both ailing and healthy humans. In the vast majority of cases, the underlying mechanism for acid secretion was elucidated by means of the mechanism of action of the pharmacologic agents, such as receptor antagonists or acid pump inhibitors. Nonetheless, it proved quite difficult to precisely understand the mechanisms underlying gastric acid secretion via phar-

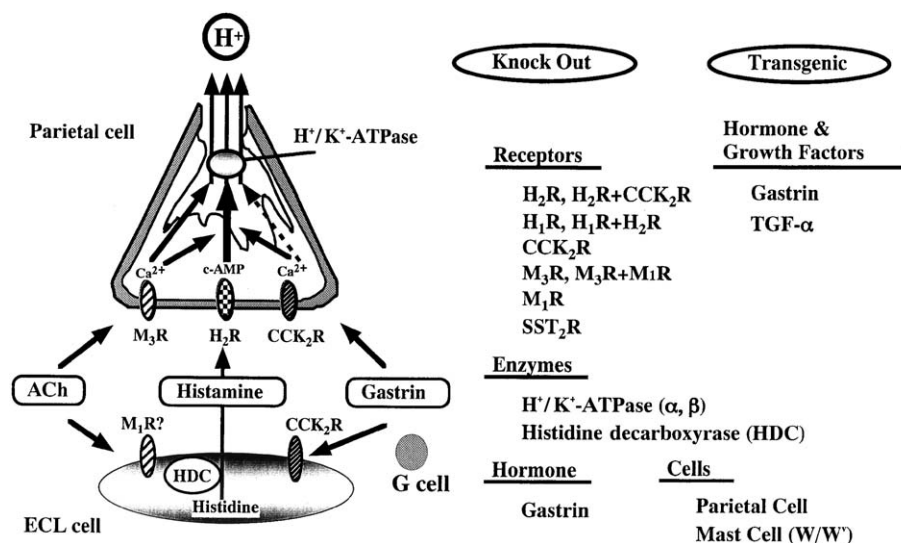


Fig. 12. Genetically engineered KO and transgenic mice. KO mice stand to offer many important findings that selective antagonists cannot reveal.

macotherapy, as most of agents were not selective enough to inhibit each and every receptor for a sufficient length of time due to rapid metabolism. Recently, most of the genes that express the above-mentioned receptors and related enzymes involved in acid secretion were cloned, allowing production of genetic modification (i.e., KO or transgenic) animals (Fig. 13). H^+/K^+ -ATPase (α , β subunit)-KO mice (Francic et al., 2001; Scarff et al., 1999; Spicer et al., 2000), histamine H_2 R-KO mice (Kobayashi et al., 2000; Ogawa et al., 2003), histidine decarboxylase (HDC)-KO mice (Tanaka et al., 2002), muscarinic M_3 R-KO mice (Aihara et al., 2002; Matsui et al., 2000; Yamada et al., 2001), gastrin-KO mice (Francic et al., 2001; Friis-Hansen et al., 1998; Hinkle & Samuelson, 1999; Hinkle et al., 2003; Koh et al., 1997; Zavros et al., 2002a, 2002b), gastrin transgenic mice (Wang & Dockray, 1999; Wang et al., 2000), CCK_2 R-KO mice (Chen et al., 2002; Langhans et al., 1997; Nagata et al., 1996), and somatostatin SST_2 R-KO mice (Martinez et al., 1998) have been generated to date (Fig. 12).

4.1. Parietal cell-knockout mice

It is of note that even parietal cell-deficient mice were generated without manifestation of any apparent aberrancy (Canfield et al., 1996; Li et al., 1996), suggesting that parietal cells are not requisite for the stomach. Interestingly, the stomachs of mice lacking parietal cells exhibited extensive hyperplasia within the oxyntic mucosa. These findings strongly suggest that parietal cells greatly contribute to not only gastric acid secretion, but also to maintenance of gastric mucosal integrity.

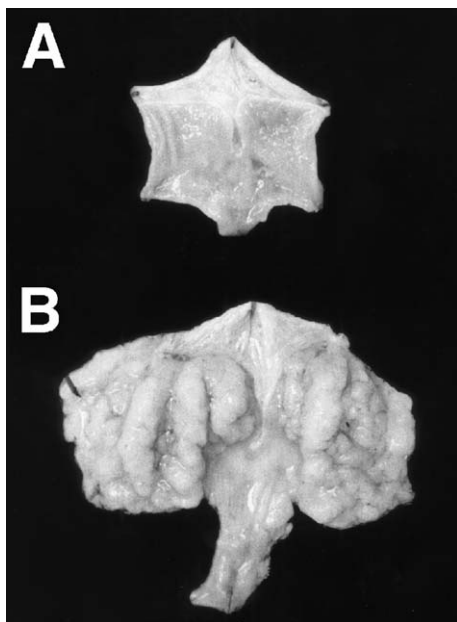


Fig. 13. The stomach of a 17-month-old wild-type mouse (A) and a histamine H_2 R knockout mouse (B). Note that the stomach of the KO mouse is enlarged with a mucosal surface exhibiting large folds reminiscent of Menetrier's disease.

4.2. H^+/K^+ -ATPase (α , β subunit)-knockout mice

Gastric H^+/K^+ -ATPase, present in tubulovesicular and secretory canaliculi of gastric parietal cells, exchanges luminal K^+ for cytoplasmic H^+ and is thus primarily responsible for gastric acid secretion. This enzyme is composed of two noncovalently linked subunits, α and β . The α subunit contains catalytic sites of the enzyme, while the β subunit is required for transport of an active complex from the endoplasmic reticulum to the apical membrane. Recently, mice lacking the genes encoding for both of these subunits were reported (Francic et al., 2001; Scarff et al., 1999; Spicer et al., 2000). It is of note that both mutant mice exhibit achlorhydria and hypergastrinemia (Francic et al., 2001; Scarff et al., 1999; Spicer et al., 2000) compared with wild-type mice. Gastrin, released from G cells, is the major hormone to stimulate gastric acid secretion in response to elevated gastric luminal pH. For instance, treatment with anti-ulcer drugs, such as H_2 R antagonists and acid pump inhibitors, induce hypergastrinemia and gastric mucosal hypertrophy (Larsson et al., 1986). Accordingly, increased serum gastrin in such KO mice may also be due to a positive feedback response by G cells in response to achlorhydria. In adult (10–12 weeks old) H^+/K^+ -ATPase α subunit-KO mice, severe histopathological alterations were observed, including metaplasia and serious disruption of gastric gland architecture (Spicer et al., 2000). The number of parietal and chief cells appeared to be equivalent for wild-type and H^+/K^+ -ATPase α subunit-KO mice; however, pepsinogen expression in chief cells was significantly reduced and parietal cell secretory membranes were severely perturbed. The overall thickness of the mucosa in H^+/K^+ -ATPase β subunit-KO mice (15 days old) was comparable with that of wild-type mice; however, the pit and neck/isthmus regions of the gastric units appeared enlarged, while the bases were reduced in size. In 35-day-old H^+/K^+ -ATPase β subunit-KO mice, most parietal cells did not contain typical tubulovesicular membranes or canaliculi; the number of zymogenic cells was also greatly reduced (Francic et al., 2001; Scarff et al., 1999). Nonetheless, the total number of parietal cells in each gastric unit did not greatly differ between KO mice and wild-type mice. These results indicate that immature parietal cells resulting from H^+/K^+ -ATPase subunit KO-induced abnormal differentiation of gastric mucosal cells, suggesting that unknown factors might be supplied from parietal cells to maintain gastric mucosal integrity.

4.3. Histamine H_2 receptor-knockout mice

We recently reported on gastric phenotypes observed in H_2 R-KO mice (Kobayashi et al., 2000; Ogawa et al., 2003; Okabe et al., 2001, 2002a). H_2 R-KO mice were viable and developed without obvious abnormalities noted on gross inspection. Unexpectedly, contrary to long-term effects on inhibition of gastric acid secretion of H_2 R blocking with antagonists, H_2 R-KO mice (12–16 weeks old) exhibited no

change in basal gastric pH and basal gastric acid output compared with wild-type mice. Although both histamine- and gastrin-stimulated gastric acid secretion were completely abolished in H₂R-KO mice, carbachol-stimulated gastric acid secretion in these KO mice was similar to that of wild-type mice. These results suggest both that gastrin stimulates gastric acid secretion mainly via histamine released from ECL cells and that carbachol stimulates gastric acid secretion independently of histamine secretion. In addition, it would appear that basal gastric acid secretion in H₂R-KO mice is retained by cholinergic regulation. Interestingly, H₂R-KO mice exhibited hypergastrinemia (~4-fold higher than wild-type mice) without elevation of basal gastric pH. Hypergastrinemia is generally considered to result from a simple positive feedback response of G cells to achlorhydria; however, our study suggests that, at least in H₂R-KO mice, other factors unrelated to decreased acid secretion can also induce hypergastrinemia.

In H₂R-KO mice, gastric wet weight and DNA levels were significantly higher than those measured for wild-type mice, suggesting that H₂R-KO mice develop gastric hypertrophy resulting from an increased number of mucosal cells. Histologic studies in H₂R-KO mice demonstrated a remarkable increase in gastric mucosal thickness. Furthermore, such hypertrophy resulted in formation of enlarged gastric folds in the glandular region. The number of parietal and endocrine cells in H₂R-KO mice gastric mucosa was increased compared with wild-type mice. It should be noted that the size of parietal cells in H₂R-KO mice was remarkably smaller. Moreover, electron microscopy of these parietal cells revealed an abnormal structure with enlarged secretory canaliculi, a lower density of microvilli, and few typical tubulovesicles in the narrow cytoplasm. These findings suggest that H₂R-mediated histamine signaling controls the cellular size of parietal cells and preserves the normal structure of secretory membranes in parietal cells. Following a study by our group that demonstrated that H₂R-KO mice develop Menetrier's disease-like gastric mucosa 6–17 months after birth (Fig. 13), it was proposed that increased gastrin and/or TGF- α are involved in such a hypertrophic mechanism (Ogawa et al., 2003). Accordingly, H₂R might play an important role in not only gastric acid secretion, but also gastric mucosal integrity. H₂R-KO mice have afforded new information that had not been discovered with previous pharmacological studies. Further studies with H₂R-KO mice will increasingly elucidate the relationship between H₂R and gastric functioning.

4.4. Histidine decarboxylase-knockout mice

Histamine is synthesized from histidine by the enzyme HDC and is stored in mast cells, ECL cells, and enteric nerve fibers in the stomach. Our group reported that HDC-KO mice express trace levels of histamine secretion and exhibit very little *de novo* histamine synthesis in gastric mucosa (Tanaka et al., 2002). In addition, in HDC-KO mice, low

basal acid secretion, increased sensitivity of acid secretion to exogenous histamine, and hypergastrinemia were observed. Interestingly, in HDC-KO mice, both carbachol and gastrin alone exerted little to no effect on acid secretion; however, the presence of a small amount of exogenous histamine resulted in significant stimulation of gastric acid secretion. In addition, both carbachol and gastrin significantly increased gastric mucosal histamine levels in wild-type mice. These results both indicate that the presence of histamine is essential for carbachol and gastrin to stimulate acid secretion *in vivo* and suggest that both carbachol and gastrin indirectly stimulate parietal cells to secrete acid via release of histamine from ECL cells. It follows that HDC-KO mice represent a convenient model to observe the direct effect of acid secretagogues on parietal cells.

No significant changes were observed in the general histological appearance of gastric mucosal cells in young HDC-KO mice (8–12 weeks old). Nonetheless, in HDC-KO mice older than 3 months, the stomach weight was significantly greater than wild-type mice. Histological analysis revealed that the gastric mucosa in HDC-KO mice was hypertrophic at the glandular base region, with increased parietal and ECL cells. In addition, the morphology of HDC-KO mice appeared to differ from that of H₂R-KO mice. Further studies are needed to compare HDC-KO and H₂R-KO mice.

4.5. Muscarinic M₃R-knockout mice

It has been recognized that ACh activates M₃R on parietal cells, resulting in acid secretion. In addition, *in vivo* cholinergic stimulation of acid secretion appears to involve M₁R, as evidenced by a high sensitivity of acid secretion to a selective M₁R antagonist, pirenzepine. Nonetheless, due to a lack of ligands with sufficient subtype selectivity, it has been difficult to study the specific role of each subtype (Caulfield & Birdsall, 1998). Consequently, the precise *in vivo* role of each muscarinic receptor subtype with respect to gastric acid secretion remained undetermined. Recently, availability of M₃R-KO mice (Matsui et al., 2000; Yamada et al., 2001) enabled us to study the physiological significance of M₃R in the regulation of gastric acid secretion (Aihara et al., 2002). Twelve-week-old M₃R-KO mice exhibited decreased gastric acid secretion, as evidenced by a significantly higher gastric pH and a significantly lower gastric acid output. At this age, histologic observation revealed no difference in the number of parietal and endocrine cells in the gastric mucosa between M₃R-KO and wild-type mice. Nonetheless, electron microscopy demonstrated that the number of active (secreting) parietal cells was significantly reduced in M₃R-KO mice. Carbachol-stimulated gastric acid secretion in M₃R-KO mice was ~30% of that measured in wild-type mice. Histamine and gastrin were still able to stimulate acid secretion in M₃R-KO mice, but the acid output was ~50% of the respective values in wild-type mice. These results suggest that M₃R

stimulation via ACh signaling is required for not only basal acid secretion, but also stimulated gastric acid secretion. In addition, vagal stimulation with 2-deoxy-D-glucose increased the acid output in wild-type mice by 6.5-fold, but had little impact in M₃R-KO mice, strongly suggesting the physiological importance of M₃R in gastric acid secretion. It is of great interest that carbachol is still able to stimulate gastric acid secretion in M₃R-KO mice, albeit to a degree much less than that observed in wild-type mice. Carbachol-stimulated gastric acid secretion was clearly inhibited by either pirenzepine or famotidine in M₃R-KO mice, suggesting that histamine and activation of M₁R appear to be involved in the mechanism underlying carbachol stimulation. It is of note, however, that it has been reported that ECL cells lack functional muscarinic receptors (Helander et al., 1996; Lindstrom et al., 1997). In the future, further investigations to elucidate the detailed mechanisms underlying carbachol-induced gastric acid secretion in M₃R-KO mice are requisite. In addition, improved understanding of the particular *in vivo* participation of each muscarinic receptor subtype in gastric acid secretion will be achieved with phenotype studies, such as the investigation we are currently conducting with M₁R-KO mice.

4.6. Gastrin-knockout mice

Gastrin-KO mice are achlorhydric and fail to respond to exogenously administered histamine, carbachol, and gastrin (Friis-Hansen et al., 1998; Hinkle & Samuelson, 1999; Zavros et al., 2002a, 2002b). Histological analysis revealed a reduction in the gastric mucosal thickness and marked changes in the number of parietal and ECL cells in the oxyntic mucosa of gastrin-KO mice (Francic et al., 2001; Friis-Hansen et al., 1998; Hinkle & Samuelson, 1999; Koh et al., 1997; Zavros et al., 2002a, 2002b). The number of parietal cells was remarkably decreased with a distinct accumulation of immature cells lacking H⁺/K⁺-ATPase. In comparison with wild-type mice, gastrin-KO mice exhibited a marked reduction in HDC-positive cells, with very weak staining in the gastric glands of gastrin-KO mice; the number of chromogranin A-positive cells, however, was unchanged. These findings suggest that gastrin is critical for functional maturation of the acid-secretory system (Friis-Hansen et al., 1998; Hinkle & Samuelson, 1999; Koh et al., 1997). These findings are consistent with results obtained with H⁺/K⁺-ATPase β subunit and gastrin-double KO mice. Namely, hypergastrinemia in H⁺/K⁺-ATPase β subunit-deficient mice is responsible for mucosal cell hypertrophy, but not for depletion of zymogenic cells (Francic et al., 2001).

4.7. Gastrin transgenic mice

Insulin-gastrin (INS-GAS) transgenic mice that overexpress amidated gastrin in pancreatic islets exhibit early (1–4 months) mild hypergastrinemia, that is, a near 2-fold

increase in plasma gastrin levels compared with wild-type mice (Wang & Dockray, 1999; Wang et al., 2000). These INS-GAS mice demonstrate an initial increase in the maximal gastric acid output and the number of both parietal and ECL cells; however, the mice later progress to hypochlorhydria, with decreased numbers of parietal and ECL cells. Hypergastrinemia leads to marked thickening of the fundic mucosa and multifocal hyperplasia in INS-GAS mice after 1 year. At 20 months, INS-GAS mice exhibit gastric metaplasia, dysplasia, and invasive gastric carcinoma. Interestingly, *H. felis* infection accelerates development of intramucosal carcinoma with submucosal and intravascular invasion in INS-GAS mice (Wang et al., 2000).

4.8. Cholecystokinin-2 receptor knockout mice

Several groups have reported on the functional and morphological changes observed in the gastric mucosa of CCK₂R-KO mice (Chen et al., 2002; Langhans et al., 1997; Nagata et al., 1996). We also confirmed that CCK₂R-KO mice exhibit achlorhydria and hypergastrinemia. In comparison with wild-type mice, the basal gastric acid output was lower and intragastric pH was higher in CCK₂R-KO mice (Langhans et al., 1997; Nagata et al., 1996). Histamine and carbachol increased gastric acid secretion in CCK₂R-KO mice; however, the degree of increase in gastric acid output in CCK₂R-KO mice was less than that observed for wild-type mice. Gastrin did not increase gastric acid secretion in CCK₂R-KO mice. In spite of hypergastrinemia, CCK₂R-KO mice had a decreased stomach wet weight with severe atrophy of the gastric oxyntic mucosa accompanied by a reduced number of parietal and ECL cells. These findings are consistent with northern blot analysis that revealed decreased levels of HDC and H⁺/K⁺-ATPase mRNA. In addition, electron microscopy demonstrated a decreased number of active parietal cells and an increased number of immature ECL cells in CCK₂R-KO mice compared with wild-type mice. These results suggest CCK₂R directly or indirectly regulate differentiation of gastric mucosal cells, including parietal and ECL cells.

4.9. Somatostatin receptor subtype 2 SST₂R-knockout mice

Somatostatin is a physiological inhibitor of gastric acid secretion that is released from D cells in gastric fundic and antral mucosa (Patel et al., 1996). Somatostatin is known for its potent inhibition of histamine release by ECL cells, gastrin production by G cells, and acid secretion by parietal cells. Somatostatin has five different receptor subtypes (i.e., SST₁R to SST₅R) (Makhlouf & Schubert, 1990). It has been previously reported that inhibition of gastric acid secretion is mediated by SST₂R in ECL and parietal cells. SST₂R has two different isoforms with long (SST_{2A}R, in ECL cells) and short (SST_{2B}R, in parietal cells) C-terminal variants generated by alternative mRNA splicing (Vanetti et al.,

1993). Martinez et al. (1998) reported that SST₂R-KO mice exhibited high basal gastric acid secretion without a change in serum gastrin compared with wild-type mice. Somatostatin antibodies were found to increase basal secretion by 4-fold in wild-type mice, but failed to exert an effect on SST₂R-KO mice. In addition, somatostatin analogues inhibited gastrin-stimulated acid secretion in wild-type mice, but did not affect basal secretion in SST₂R-KO mice. These results indicate that somatostatin suppresses gastrin-stimulated gastric acid secretion via SST₂R. Consequently, the role of somatostatin in hypergastrinemia should prove an interesting area of investigation for a variety of KO mice.

Based upon physiological and pharmacological analysis with the above KO mice, regulatory mechanisms for gastric acid secretion are rapidly being developed. Double or triple receptor KO mice, such as H₂R+M₃R, H₂R+CCK₂R, M₃R+HDC, H₂R+HDC, and H₂R+M₃R+CCK₂R, as well as the previously generated M₃R+M₁R, will afford new insight into the mechanisms underlying gastric acid secretion. The above results indicate that KO mice stand to offer many important findings that selective antagonists cannot reveal. In addition, these results suggest that abnormalities, particularly regulatory factors for gastric acid secretion, can induce gastric mucosal cancer-like diseases similar to those observed in clinical situations. It follows that these mice models may prove useful for clarifying the unknown mechanisms underlying such diseases, allowing development of novel treatment strategies.

5. Origin of parietal cells

One of the unique and unusual properties of the mammalian stomach is its ability to secrete a highly concentrated inorganic acid (i.e., hydrochloric acid [>0.15 N]) from parietal cells in the oxyntic mucosa. The rationality underlying stomach acid secretion remains a much debated topic. Nevertheless, it has been postulated that acidification of the gastric contents both assists in digestion of ingested food and sterilization of contaminant virulent and/or regurgitated intestinal bacteria. It is interesting, however, that the origin of parietal cells remains unstudied. The most intriguing issue remains the evolution of parietal cells, which are characterized by a p-type H⁺/K⁺-ATPase from stem cells. Namely, the evolution of the H⁺/K⁺-ATPase gene from stem cells located in the fundic mucosa represents the most puzzling topic of discussion. One of the present authors (S.O.) hypothesized that parietal cells might have evolved via transfer of the H⁺/K⁺-ATPase gene from a microbe that possessed a p-type H⁺-ATPase or H⁺/K⁺-ATPase during the early Cambrian era (Okabe, 1997, 1999). The hypothesis is grounded upon the following observations. Microorganisms, including aerobic and anaerobic bacteria, are able to pump H⁺ into the extracellular environment by means of an H⁺-ATPase that is coupled to nutrient influx. It is of interest that 19% of the human H⁺/K⁺-ATPase (α subunit) is

comprised of amino acid residues identical to those of an H⁺-ATPase found in *Neurospora crassa*. In addition, the amino acid sequence for the ATP binding sites of animal Na⁺/K⁺-ATPase, which resembles H⁺/K⁺-ATPase in terms of amino acid residues (Maeda, 1994), and the phosphorylated intermediates of yeast H⁺-ATPase is highly conserved (Serrano et al., 1986). Such data appear to indicate that parietal cells might have originated from a parabiogenic microorganism that was subsequently incorporated into a stem cell. Thereafter, the gene encoding for H⁺/K⁺-ATPase and/or GATA DNA-binding proteins (transcriptional regulators of the gastric H⁺/K⁺-ATPase gene) could have been translocated into the nucleus, most likely with the aid of a virus and/or transposon. Such a gene translocation most likely occurred during the Cambrian era when Prochordata and Chordata, which have no parietal cells, were abundant. Accordingly, during evolution, stem cells for Chordata digestive organs might have differentiated into two cell types (i.e., surface epithelial cells and parietal cells) prior to the appearance of fish, which possess parietal cells with H⁺/K⁺-ATPase.

6. Conclusion

Although the origin of parietal cells has yet to be elucidated, it appears that parietal cells function to secrete highly concentrated gastric acid following ingestion of food or alcoholic beverages to permit digestion of food and/or prevention of pathogenic disease. It is well known that excessive, or even normal, acid secretion is problematic for maintenance of the normal integrity of the upper gastrointestinal tract, resulting in acid-related peptic disease. Accordingly, rigid control of acid secretion via a therapeutic modality is necessary to enhance healing of gastroduodenal ulcers and reflux esophagitis. To that end, currently available H₂R antagonists and acid pump inhibitors represent powerful and reliable pharmacotherapy for the treatment of acid-related peptic disease with few side effects. Since a prototype for locally acting drugs was discovered, new classes of antisecretory drugs, as well as CCK₂R antagonists, represent the next target for drug development. Utilizing genetically engineered KO and transgenic mice, the mechanisms by which parietal cells secrete acid will be further characterized. Finally, early eradication of *H. pylori* infection with acid pump inhibitors and antibiotics will most likely reduce the potential for development of atrophic gastritis, intestinal metaplasia, and gastric carcinoma.

Acknowledgements

The authors wish to thank C. J. Hurt (John Hopkins University School of Medicine, USA) and Dr. Y. Tsukimi (Bayer Yakuin, Research Center Kyoto) for a critical reading of the manuscript. Various gene KO mice were

provided by the following colleagues: H₂R-KO mice, Drs. T. Kobayashi and T. Watanabe (Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University); M₃R-KO mice, Drs. M. Matsui (Division of Neuronal Network, Department of Basic Medical Sciences, The Institute of Medical Science, The University of Tokyo) and M. M. Taketo (Department of Pharmacology, Graduate School of Medicine, Kyoto University); CCK₂R-KO mice, Dr. M. Matsui (Department of Medicine, Kobe University School of Medicine); HDC-KO mice, Drs. A. Ichikawa and S. Tanaka (Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University) and H. Ohtsu (Department of Cellular Pharmacology, Tohoku University School of Medicine).

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