

S. OKABE, K. SHIMOSAKO, K. AMAGASE

PHARMACOLOGICAL REGULATION OF GASTRIC ACID SECRETION IN THE APICAL MEMBRANE OF PARIETAL CELLS; A NEW TARGET FOR ANTISECRETORY DRUGS

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Kyoto, Japan

We examined the local effect of several drugs against secretagogue-stimulated acid secretion in dogs. Test drugs were applied to denervated gastric pouches in conscious dogs either for 5 to 30 min beginning 1 hr after or for 30 min before intravenous infusion of gastric secretagogues (histamine, pentagastrin, or carbachol). The antisecretory effect of test drugs delivered by an intravenous or oral route was also examined. Local application of acid pump inhibitors (omeprazole, leminoprazole) for 30 min beginning 1 hr after histamine infusion significantly inhibited gastric acid secretion. The effect of leminoprazole persisted for more than 8 hr after a 30 min application. A mast cell stabilizer (FPL 52694) applied to pouches for 15 to 30 min also potently inhibited histamine-stimulated gastric acid secretion in a time-dependent manner. The duration of the antisecretory effect of such drugs after a 30 min application was greater than 4 hr. Locally applied leminoprazole and FPL 52694 for 30 min also significantly inhibited pentagastrin- and carbachol-stimulated gastric acid secretion. Although intravenous omeprazole and leminoprazole exerted a potent antisecretory effect on histamine-induced acid secretion FPL 52694 had little or no antisecretory effect following intravenous or oral administration. 16, 16-dimethyl prostaglandin E2 also locally inhibited histamine-stimulated acid secretion. Acid stable local anesthetics (tetracaine, ethyl-4-aminobenzoate), histamine H₂-receptor blockers (cimetidine, ranitidine, and famotidine), and a muscarinic M₁-receptor antagonist (pirenzepine) did not exhibit local antisecretory effects. Such results strongly suggest that the apical membrane of parietal cells possesses a pharmacologically sensitive portion similar to the basolateral membrane, which usually mediates gastric acid secretion. The apical membrane represents an intriguing target for new antisecretory drugs, as well as a new medium for further elucidating the functional features of parietal cells.

Key words: *gastric acid secretion, Heidenhain pouch dogs, omeprazole, leminoprazole, FPL 52694*

INTRODUCTION

More than 100 years has passed since Prout (1823) discovered the presence of hydrochloric acid in the human stomach and Golgi (1893) identified parietal cells as the acid secretory cell in oxyntic glands. During this past century, a number of papers have been published concerning the significance of the

presence of strong acid in the stomach (1, 2). In addition, the mechanism by which parietal cells secrete gastric acid through a combination of various receptors on the basolateral membrane and an enzyme in the apical secretory canaliculi has been elucidated (3—5). The development of several selective drugs, including histamine H₂-receptor blockers (H₂R-blockers) and proton pump inhibitors (PPI), has greatly contributed to the understanding of the mechanism underlying acid secretion (6—9). Utilizing such drugs, it is now understood that gastric acid secretion is mainly regulated by stimulation of corresponding receptors on the cell membrane via acetylcholine released from the vagus nerve, gastrin released from antral G cells, and histamine released from ECL cells. Gastrin-stimulated acid secretion is controlled by the release of inhibitory mediators, such as somatostatin released from D cells. Needless to say, H₂R-blockers, such as cimetidine, ranitidine, and famotidine, and PPI, such as omeprazole and lansoprazole, have been successful in the treatment of acid-related diseases. Accordingly, it appears that further development of antisecretory drugs for ulcer therapy is no longer necessitated.

Several substances or drugs have been reported to have an antisecretory effect when locally applied to the stomach (10—17). Nonetheless, attention has not been directed towards such drugs as either clinically useful antisecretory drugs or investigational tools useful for the elucidation of parietal cell function. We examined whether or not certain drugs could inhibit histamine-stimulated gastric acid secretion when applied into denervated (Heidenhain) gastric pouches in dogs. It was found that omeprazole, leminoprazole (acid pump

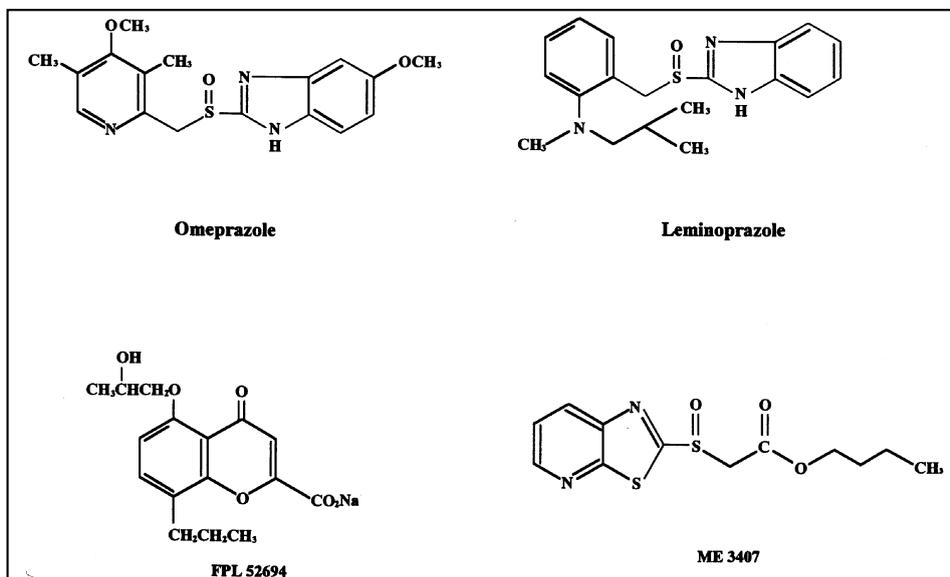


Fig. 1. Chemical structures of the main compounds that were used in this study.

inhibitors) (18, 19), FPL 52694 (a mast cell stabilizer), ME 3407 (a myosin light chain kinase inhibitor and a functional analogue of wortmannin) (*Fig. 1*) and 16, 16-dimethyl prostaglandin E2 significantly inhibited histamine-stimulated acid secretion. This report describes both the effect of locally active antisecretory drugs and the underlying mechanisms, emphasizing the important role of the parietal cell apical membrane for acid secretion.

MATERIALS AND METHODS

Experimental protocol

Ten male and female beagles (10–13 kg), each with a cannulated, denervated (Heidenhain) pouch, were used in the study no earlier than 2 months after their operation. The interval between the experiments was no less than 7 days. Food was withheld for 18 hr before each experiment, but water was freely provided. The pouch of each animal was washed with 15 ml of warm saline several times until the washings became clear. Gastric juice samples were collected throughout the experiments every 15 min by gravity drainage (*Fig. 2*). Following collection of the basal secretion for the initial 30 min, acid secretion was stimulated by continuous intravenous infusion of histamine 2HCl (Nacalai Tesque, Kyoto, Japan, 160 $\mu\text{g}/\text{kg}/\text{hr}$), pentagastrin (Sigma Chemical Co., St. Louis, 8 $\mu\text{g}/\text{kg}/\text{hr}$) and carbachol (Sigma Chemical, 8 $\mu\text{g}/\text{kg}/\text{hr}$) at a rate of 10 mL/hr via a catheter inserted into a leg vein. Test drugs were generally applied to the pouches 1 hr

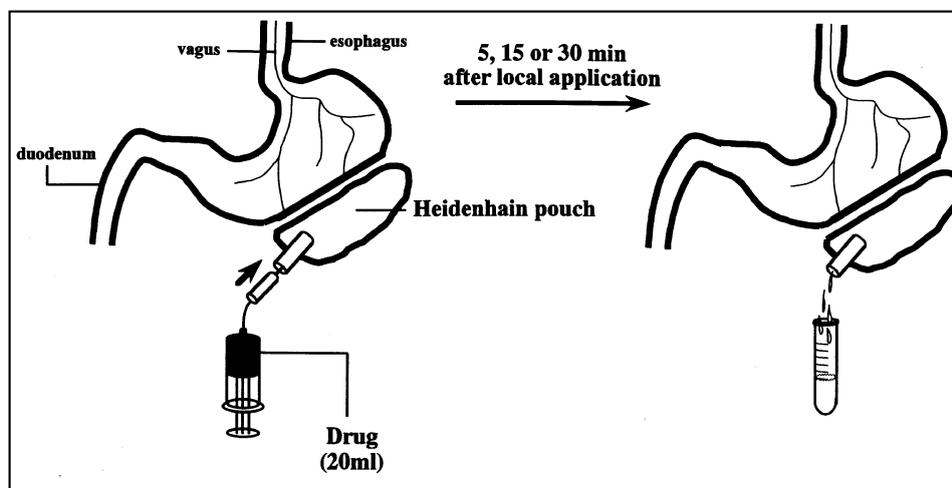


Fig. 2. General design for the experimental procedure in Heidenhain pouch dogs. Test drugs were applied to the pouch for 5 to 30 min and then withdrawn. Gastric acid stimulated by intravenous infusion of histamine, pentagastrin, or carbachol was collected every 15 min before and after drug application.

after commencement of histamine infusion, yet in certain cases drugs were either intravenously or orally administered. During the application of the drugs, histamine infusion was maintained at the same rate. After local application, test drugs were removed from the pouches and each pouch was washed out with saline three times. Thereafter, gastric juice samples were continuously collected every 15 min for 1.5 to 5 hr for analysis of volume and acidity. Total acidity was determined by titration of the gastric juice against 0.1N NaOH to pH 7.0, using an automatic titrator (Radiometer; Copenhagen, Denmark); acid output was expressed as mEq/15 min. The protocol of this study was approved by Ethic Committee for Animal Research of Kyoto Pharmaceutical University.

Drugs

Omeprazole (Astra-Japan, Osaka, Japan), leminoprazole (Nippon Chemiphar, Tokyo, Japan), FPL-52694 and sodium chromoglycate (Fison Pharmaceutical Co., Osaka, Japan) were suspended in 0.5% carboxymethylcellulose (CMC, Nacalai Tesque, Osaka, Japan). Histamine H₂R-blockers, such as cimetidine (Sigma Chemical Co., St. Louis, U.S.A.), ranitidine (Sigma), and famotidine (Yamanouchi Pharmaceutical Co., Tokyo, Japan), were also suspended in 0.5% CMC. Local anesthetics, such as tetracaine (Kyorin Pharmaceutical Co., Tokyo, Japan) and ethyl-5-aminobenzoate (Wako, Osaka, Japan), were also suspended in 0.5% CMC. 16, 16-dimethylprostaglandin E₂ (Ono, Osaka, Japan) was dissolved in a trace of ethanol and then diluted with physiological saline. All drugs were prepared prior to administration.

Statistical analysis

Data are presented as means \pm S.E.M. Statistical differences were evaluated using the Student's t-test or the Dunnett's multiple comparison test, with a P value of < 0.05 regarded as significant.

RESULTS

Stimulation of gastric acid secretion in dogs

Continuous infusion of histamine 2HCl (160 μ g/kg/hr) inevitably induced maximal stimulation of gastric acid secretion 1 hr later; the plateau level was maintained for more than 3 hr. During histamine infusion, the local application of 0.5% CMC alone for 30 min essentially exerted no effect on gastric acid secretion. Acid output was transiently reduced for 15 min after removal of the

solution, but returned to the stimulated level thereafter. Nearly the similar results were observed with pentagastrin and carbachol infusion.

Effects of omeprazole and leminoprazole on gastric acid secretion

Omeprazole, administered at a dose of 240 mg/pouch for 30 min, significantly inhibited gastric acid secretion for approximately 1.5 hr (Fig. 3). As expected, intravenous administration of omeprazole at a dose of 0.3 mg/kg markedly inhibited gastric acid secretion. Local administration of leminoprazole at a dose of 240 mg/pouch for 30 min resulted in significant inhibition of gastric acid output stimulated by histamine, pentagastrin, and carbachol (Figs 4, 5). The antisecretory effect for histamine-stimulated secretion persisted for more than 8 hr following local application (Fig. 6). Local application of the drug 30 min before histamine infusion also resulted in significant inhibition of acid output, although the degree of inhibition was slightly less than the inhibition observed 1 hr after histamine infusion. Intravenous administration of leminoprazole at dosages of 3, 6, and 10 mg/kg significantly inhibited gastric acid secretion in a

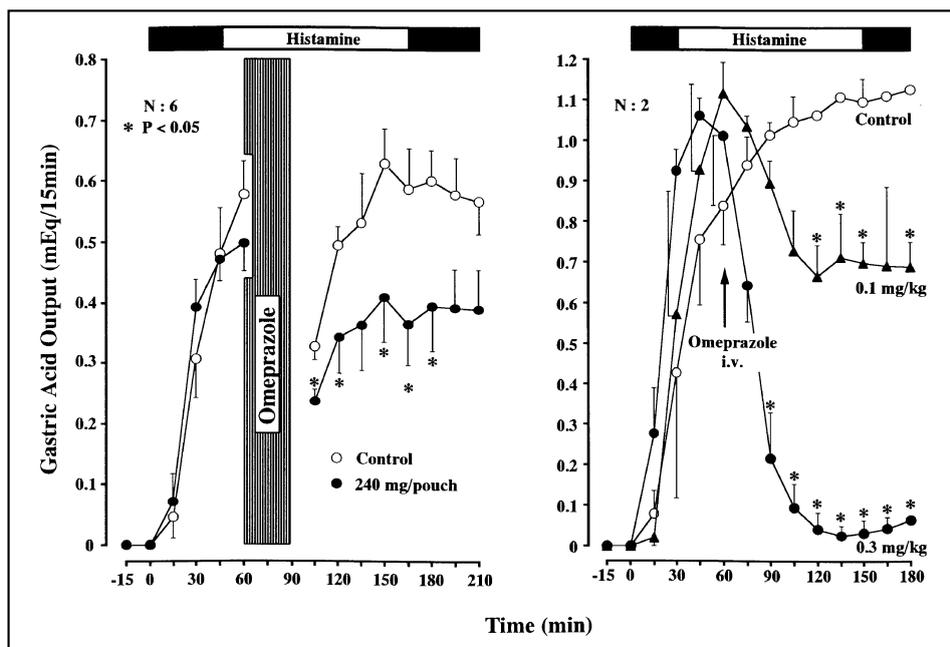


Fig. 3. Effects of omeprazole on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. The drug was administered either directly into the pouch for 30 min or intravenously 1 hr after intravenous histamine infusion. Gastric acid secretion was inhibited following both local and intravenous administration. Data is expressed as means \pm S.E. for 2–6 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

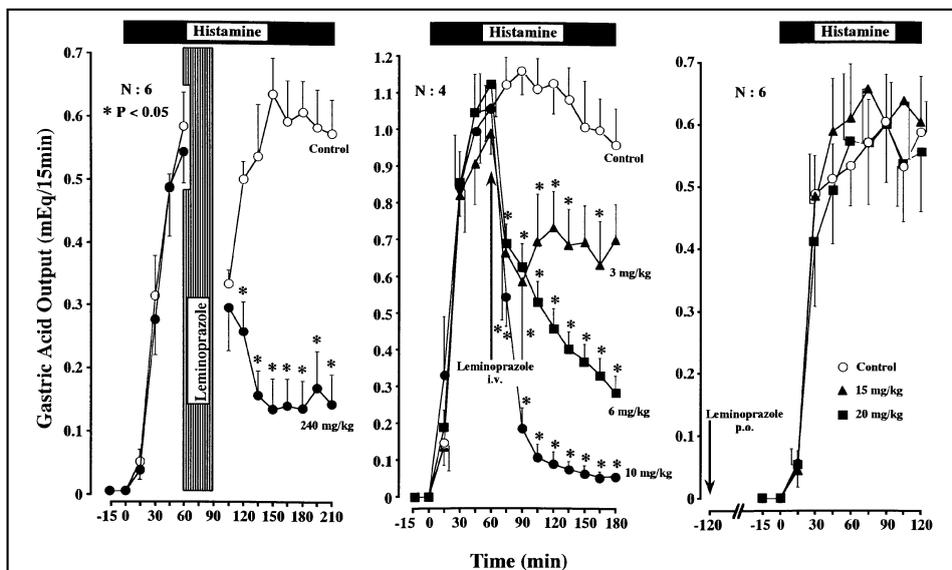


Fig. 4. Effects of leminoprazole on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. The drug was administered locally into the pouch for 30 min, intravenously 1 hr after intravenous histamine infusion, or orally 2 hr before histamine infusion. Note that leminoprazole significantly suppressed gastric acid secretion when either directly applied into the pouch or intravenously delivered. Data is expressed as means \pm S.E. for 4–6 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

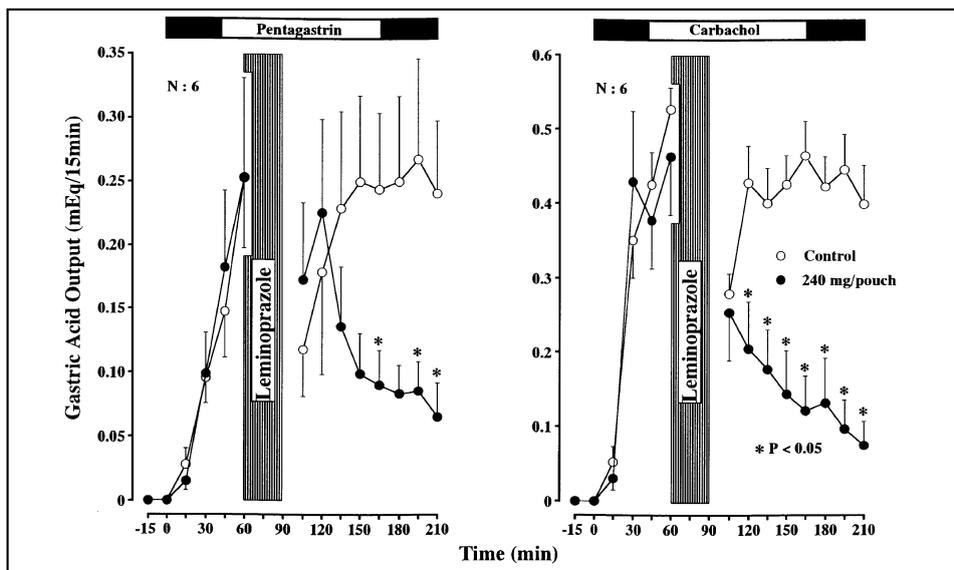


Fig. 5. Effects of leminoprazole on pentagastrin (8 $\mu\text{g}/\text{kg}/\text{hr}$) and carbachol (8 $\mu\text{g}/\text{kg}/\text{hr}$)-stimulated gastric acid secretion in Heidenhain pouch dogs. A 30 min administration of leminoprazole into the pouch was performed 1 hr after intravenous infusion of each stimulant. Note that leminoprazole significantly suppressed gastric acid secretion when directly administered into the pouch. Data is expressed as means \pm S.E. for 6 dogs.

* indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

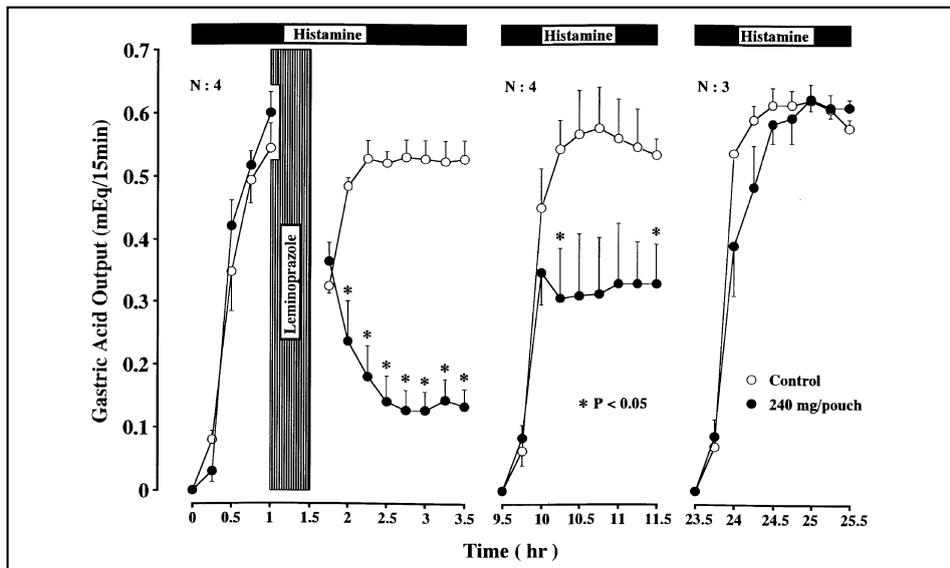


Fig. 6. Duration of antisecretory effects of leminoprazole, locally applied for 30 min, on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Note that significant inhibition persisted for more than 8 hr. Data is expressed as means \pm S.E. for 4 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

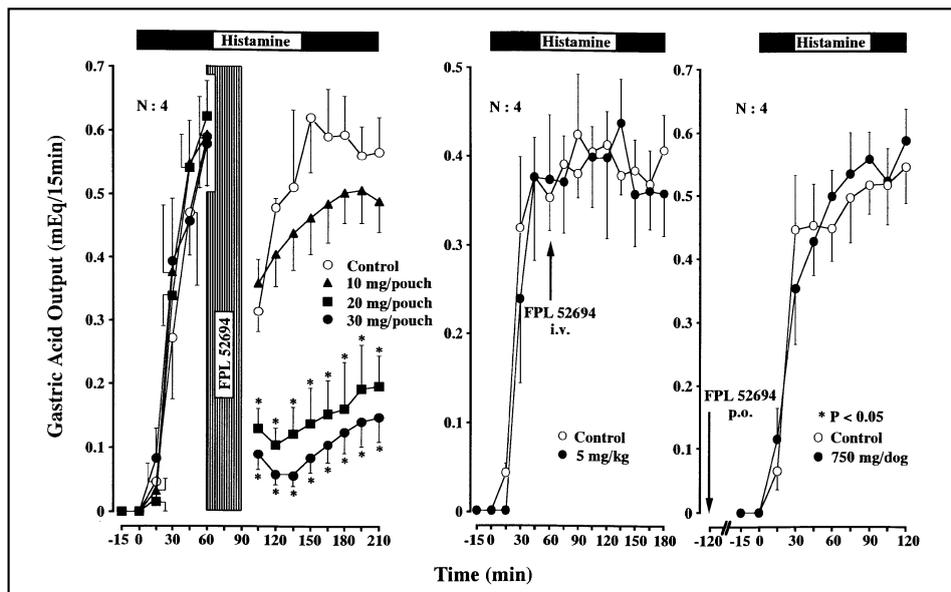


Fig. 7. Effects of FPL 52694 on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Although locally applied FPL 52694 significantly inhibited gastric acid secretion, the drug had no effect on acid secretion following intravenous or oral delivery. Data is expressed as means \pm S.E. for 4 dogs.

* indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

dose-related manner. Nonetheless, oral administration of the drug at a dosage of 15 or 20 mg/kg 2 hr before histamine infusion exerted no effect on gastric acid secretion. Such a protocol also confirmed that the leminoprazole serum concentration following local application was negligible (data not shown). In contrast, 15 min after intravenous injection of 1 mg/kg of leminoprazole, the serum concentration was >500 ng/mL. In a subsequent study, at the time of autopsy 18.6 µg of leminoprazole/g tissue was found in the gastric mucosa following a 30 min local application of the drug at a dose of 240 mg/pouch.

Effects of FPL 52694 on gastric acid secretion

FPL 52694 delivered at doses of 10, 20, and 30 mg/pouch also inhibited histamine-stimulated gastric acid secretion in a dose-related manner; the inhibition was significant at dosages of 20 and 30 mg/pouch (*Fig. 7*). In addition, drug application at a dosage of 30 mg/pouch clearly inhibited pentagastrin- and carbachol-stimulated acid secretion for 30 min (*Fig. 8*). In contrast, intravenous administration of the drug at a dose of 5 mg/kg exerted no effect on gastric acid secretion. In addition, oral administration of the drug at a dose of 750 mg/dog 2 hr before histamine infusion failed to affect gastric acid secretion. Following a 15 min application, the antisecretory effect of the drug at a dose of 30 mg/pouch approximated the effect observed following a 30 min application (*Fig. 9*). Even application of the drug for only 5 min resulted in significant inhibition of gastric acid secretion. The duration of the antisecretory effect exerted by FPL-52694 administered at a dose of 30 mg/pouch was approximately 4 hr (*Fig. 10*).

Effects of miscellaneous drugs on gastric acid secretion

Both tetracaine (160, 320 and 400 mg/pouch) and ethyl-4-aminobenzoate (300 mg/pouch) had little or no effect on gastric acid secretion (*Fig. 11*). In addition, topical application for 30 min of other local anesthetic drugs, such as sulcain (200 mg/pouch) and oxethazaine (15 mg/pouch), exerted no effect on histamine-stimulated gastric acid secretion. The dosages of the drugs were based on the oral antisecretory dose used for patients suffering from gastritis and ulcers. Both sodium chromoglycate (a mast cell stabilizer), delivered at a dose of 40 mg/pouch, and ketochifen (histamine H1-receptor antagonist), delivered at a dose of 1 mg/pouch, resulted in no effect on acid secretion (data not shown). 16, 16-dimethyl prostaglandin E2 significantly inhibited histamine-stimulated gastric acid secretion when intravenously administered at a dose of 1.0 µg/kg. In addition, the drug also resulted in significant inhibition for 30 min after local application to the pouch at a dose of 30 µg/kg. Local

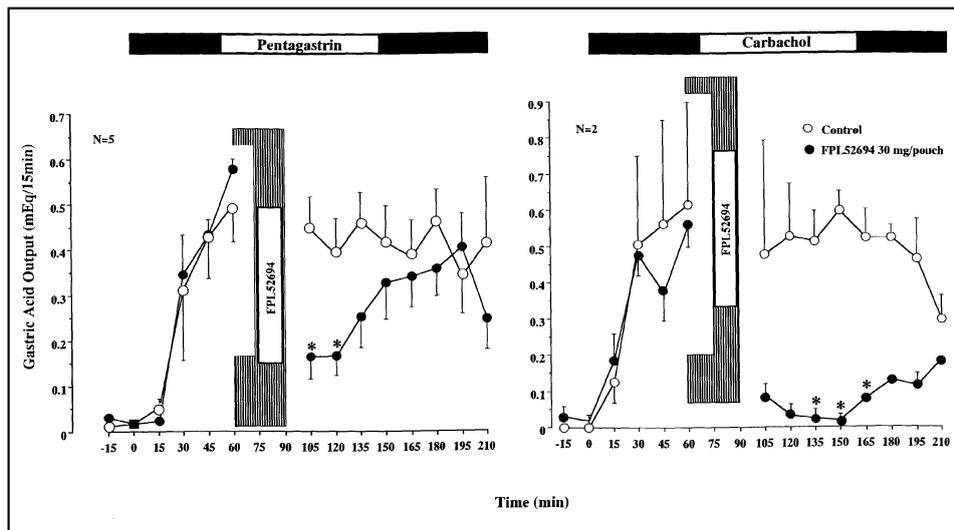


Fig. 8. Effects of FPL 52694 on pentagastrin (8 $\mu\text{g}/\text{kg}/\text{hr}$)- and carbachol (8 $\mu\text{g}/\text{kg}/\text{hr}$)- stimulated gastric acid secretion in Heidenhain pouch dogs. Note that locally applied FPL 52694 for 30 min significantly inhibited acid secretion stimulated by each stimulant. Data is expressed as means \pm S.E. for 2–5 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

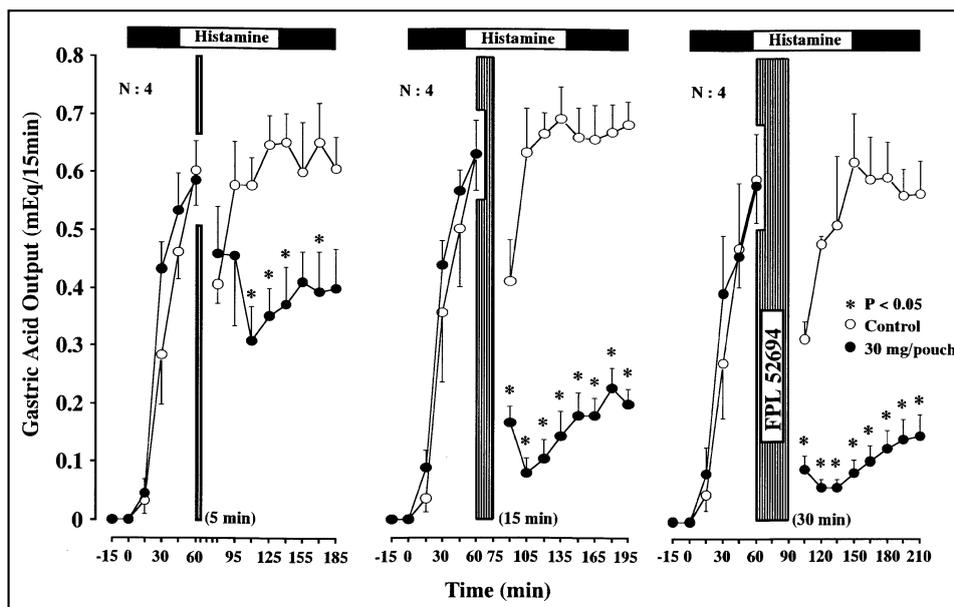


Fig. 9. Effects of locally applied FPL 52694 on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs for 5, 15, or 30 min. Note that the significant antisecretory effect was observed even after the 5 min application. Data is expressed as means \pm S.E. for 4 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

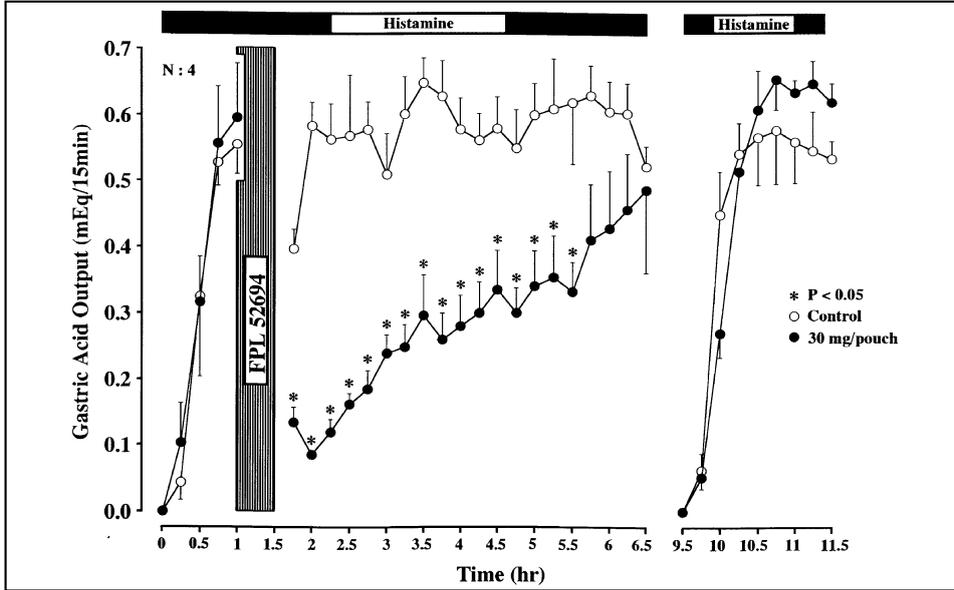


Fig. 10. Duration of the antisecretory effect of FPL 52694, locally applied for 30 min, on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Note that significant inhibition persisted for more than 4 hr. Data is expressed as means \pm S.E. for 4 dogs. * indicates a statistically significant difference from the corresponding control, with $P < 0.05$.

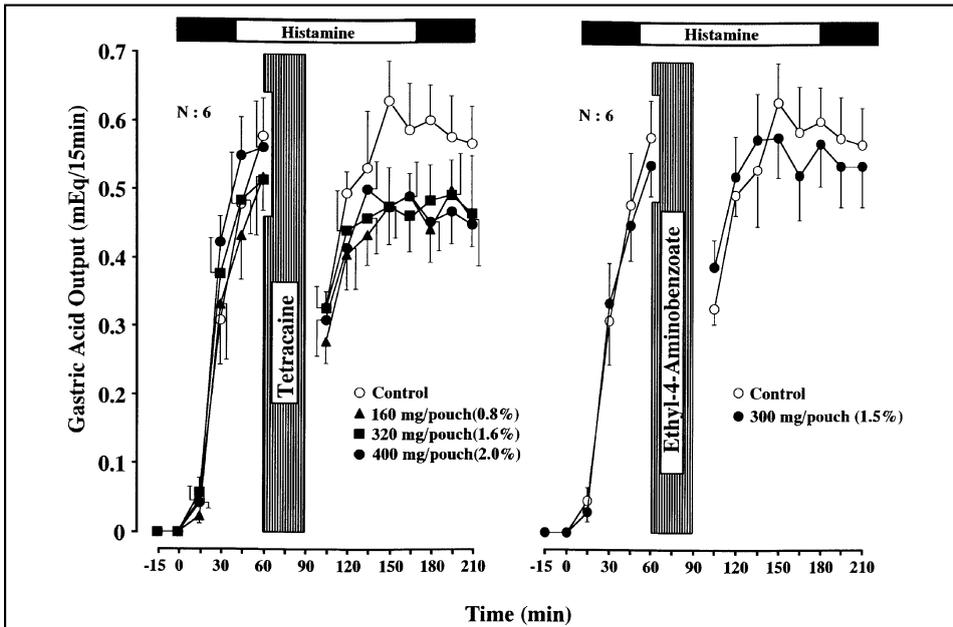


Fig. 11. Effects of 30 min local application of local anesthetics in Heidenhain pouch dogs. Note that the drugs had little or no antisecretory effect. Data is expressed as means \pm S.E. for 6 dogs.

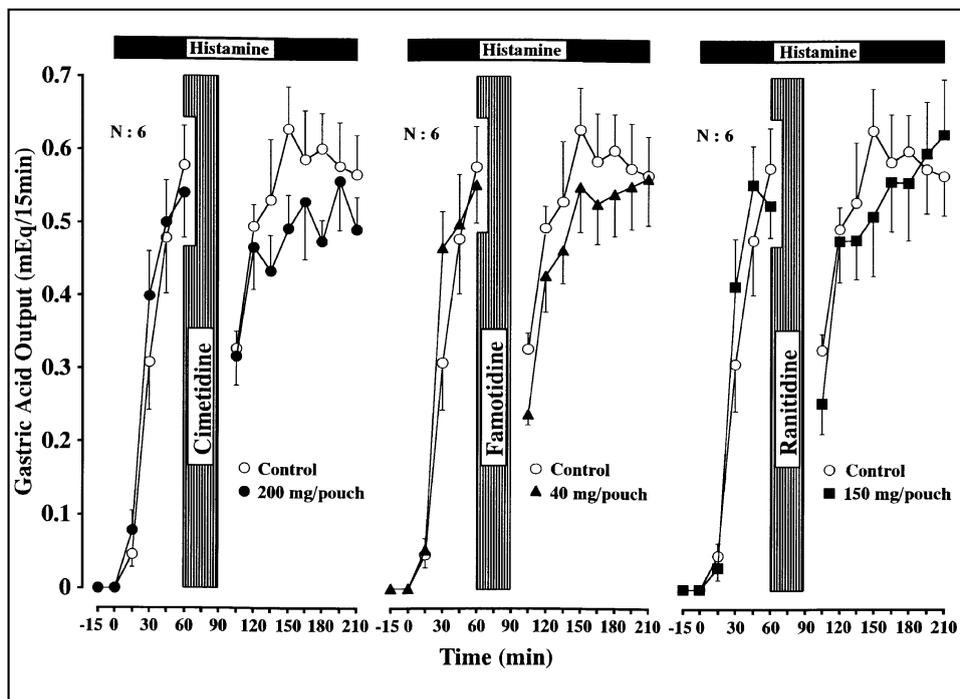


Fig. 12. Effects of H₂R-blockers, locally applied for 30 min, on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Note that the drugs had no antisecretory effect. Data is expressed as means \pm S.E for 6 dogs.

application of histamine H₂-receptor antagonists, such as cimetidine, (200 mg/pouch), ranitidine (150 mg/pouch), and famotidine (40 mg/pouch), for 30 min had no effect on histamine-stimulated gastric acid secretion (Fig. 12). Similar results were obtained with local application of pirenzepine (25 mg/pouch) for 30 min. The dosage of the drugs utilized was based on the oral antisecretory dose determined for patients suffering from ulcers.

DISCUSSION

The above results undisputedly confirmed the notion that drugs that are able to directly inhibit gastric acid secretion from the luminal side exist (Fig. 13).

First, Konturek *et al.* (16) reported that although continuous infusion of omeprazole into denervated gastric pouches of dogs significantly inhibited pentagastrin- and histamine-stimulated acid secretion, it did not affect acid secretion from the gastric fistula in response to such stimulants. Since serum omeprazole level was only slightly increased at a dose that significantly inhibited gastric acid secretion from the denervated pouch, the authors suggested that

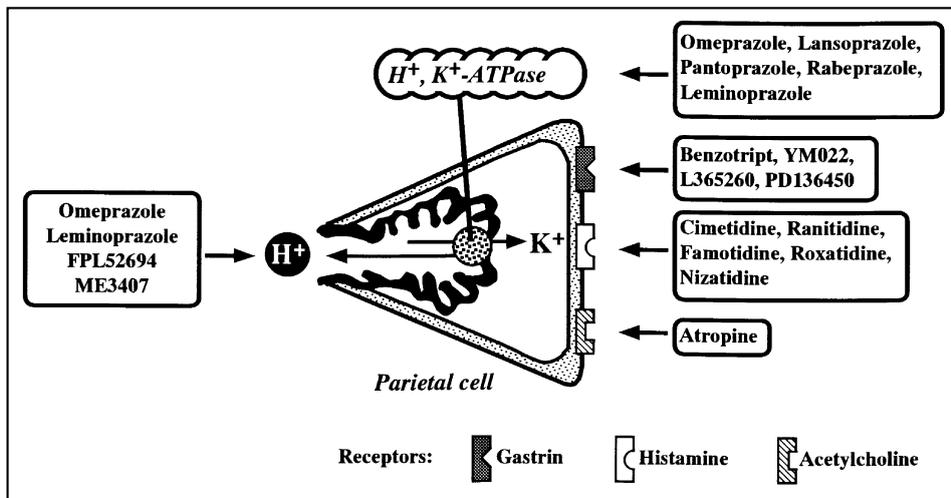


Fig. 13. Schematic drawing for the site of action of the drugs that inhibit the acid secretory function of parietal cells via basolateral membrane (receptors), the acid pump, and the apical membrane (receptors?).

omeprazole exerted a local antisecretory effect in addition to a systemic effect. In our previous report (17), we demonstrated that locally administered omeprazole (160 mg/pouch) with NaHCO_3 into denervated pouches resulted in no antisecretory effect. Nonetheless, we recently found that an increased dose of 240 mg/pouch of omeprazole, even without NaHCO_3 , significantly inhibited histamine-stimulated acid secretion. Although we did not determine the omeprazole blood concentration following local application, it appears that omeprazole also inhibits parietal cells from the mucosal surface, as previously described by Kontureck *et al.* (16).

Similar to omeprazole, locally applied leminoprazole also suppressed acid secretion for more than 9 hr by inhibiting parietal cells from the mucosal side. Such a duration of the local antisecretory effect was clearly longer than that observed following a single intravenous injection of ranitidine and famotidine. The effect, however, was found to be reversible, as it was found to have disappeared after 20 hr. Indeed, as we have previously reported (17), leminoprazole, delivered at a dose of 160 mg/pouch, was not detected in the blood of dogs after local application at a dose that inhibited acid secretion. When parietal cells are stimulated by gastric secretagogues, the activated acid pump is expressed on the apical secretory canaliculi (20–22). It is reasonable that both omeprazole and leminoprazole inhibited the activated acid pump following histamine infusion. Certainly, there is a possibility that the drugs penetrated into the oxyntic gland area and exerted an effect on the acid pumps by entering into the parietal cells from the basolateral membrane, similar to the effect derived from delivery by oral or intravenous routes.

It must also be noted that omeprazole has not been found to stimulate gastric and duodenal bicarbonate secretion in rats and dogs (16, 23). We have also demonstrated that leminoprazole and omeprazole exert no effect on bicarbonate secretion upon local application to the gastric mucosal surface of rats (24). Indeed, we have confirmed that upon local application of leminoprazole, the drug failed to stimulate gastric bicarbonate secretion in dogs. Accordingly, it becomes easy to eliminate the hypothesis that reduced gastric acid secretion observed following local application of acid pump inhibitors is due to neutralization of secreted acid by stimulated bicarbonate secretion. Furthermore, Larson *et al.* (8) and Konturek *et al.* (16) have both found that omeprazole failed to exert an effect on the circulation of the gastric mucosa of dogs.

We have also confirmed findings revealed by Nicol *et al.* (13) and Curwain *et al.* (14, 15) that locally applied FPL 52694 significantly inhibited gastric acid secretion stimulated by histamine, pentagastrin, and carbachol in dogs. In contrast to the results following application of omeprazole and leminoprazole, we found that FPL 52694 failed to exert an inhibitory effect on acid pumps (unpublished data). Indeed, as demonstrated in the present study, FPL 52694 exerted no effect on acid secretion following either intravenous or oral administration. Such results strongly suggest that the antisecretory effect of the drug is solely explained by a local effect, rather than a systemic effect. In addition, we demonstrated that the local antisecretory effect persisted for more than 4 hr after application, yet disappeared following 9 hr. Such a result suggests that the local effect on parietal cells is reversible.

As previously reported (25), we discovered that ME 3407 possesses a strong inhibitory effect on acid secretion, even at doses of only 1 to 3 mg/pouch and even after only a 15 min application to the pouches. It is even more interesting that the antisecretory effect persisted for more than 5 hr. Nonetheless, the underlying mechanism remains unknown. Similar to FPL 52694, ME 3407 failed to exert an effect when orally or intravenously administered, thus suggesting that the drug exerts its effect on parietal cells from the mucosal side of the parietal cells.

Urushidani *et al.* (26) reported that ME 3407 significantly enhanced the healing of acetic acid-induced gastric ulcers in rats, by probably exacting a potent antisecretory effect that did not inhibit the acid pump. Urushidani further demonstrated that the inhibitory effect of ME 3407 on gastric acid secretion was more potent when orally administered than when subcutaneously or intraperitoneally administered. Citing our report (25), Urushidani *et al.* (26) also concluded that ME 3407 directly acts on the mucosa to inhibit acid secretion. In addition, using isolated rabbit gastric glands, Urushidani *et al.* demonstrated that ME 3407 inhibited acid secretion not only by preventing stimulation-associated redistribution of H^+ , K^+ -ATPase from microsomes into the apical plasma membranes, but also by delocalizing ezrin from the apical membrane. Should their findings be applicable to our study, the conclusion

could be drawn that ME 3407 exerts its local antisecretory effect through both inhibition of redistribution of the acid pump and delocalization of ezrin following penetration into the parietal cells from the mucosal side. Since ME 3407 resulted in no inhibitory effect after intravenous administration, penetration of the drug into parietal cell cytoplasm to exert its effect must occur from the apical membrane side, quite possibly from the apical secretory canaliculi. Certainly, there also exists the possibility that ME 3407 directly acts on apical secretory canaliculi without penetrating into parietal cell cytoplasm.

Agnew *et al.* (22), using cultured rabbit parietal cells, also provided evidence that ME 3407 is able to prevent histamine (+isomethylxanthine)-stimulated acid secretion. Agnew contends that the underlying mechanism follows from prevention of translocation of H⁺,K⁺-ATPase-rich tubulovesicles to apical membrane vacuoles and dissociation of ezrin from the putative cytoskeletal-membrane functional site. Similar to Urushidani *et al.* (26), however, Agnew *et al.* did not describe whether or not ME 3407 penetrated into the parietal cells to exert its effect or directly acted on the apical secretory canaliculi render prevention of the secretion-activation cascade. Clarification of the action of ME 3407 on apical secretory canaliculi represents an important step towards elucidation of the mechanism for acid secretion from the apical membrane.

In regards to gastric integrity following local application of drugs, we have confirmed that acid loss from the gastric lumen following local application of leminoprazole and omeprazole does not differ from controls. Should leminoprazole injure the gastric mucosa to result in a reduced acid output, then the drug should also inhibit acid secretion when administered prior to histamine infusion. Accordingly, the possibility that gastric acid secreted into the pouch might diffuse back into the mucosa through the damaged mucosal barrier in response to local application of the drugs is eliminated. As was previously described, leminoprazole failed to exhibit an antisecretory effect on local application prior to histamine infusion. Furthermore, histological studies have demonstrated that leminoprazole does not result in injurious effects on the gastric mucosa. In consideration of the above, it would appear that leminoprazole locally penetrates into the oxyntic mucosa to inhibit gastric acid secretion without entering the systemic circulation and without affecting bicarbonate secretion, mucosal blood flow, or mucosal integrity.

We have also previously demonstrated that 2 kinds of acid-stable local anesthetics, namely tetracaine and ethyl-4-aminobenzoate, exerted no inhibitory activity following local applications at concentrations similar to those used in clinical settings. Consequently, the hypothesis that FPL 52694 and ME 3407 inhibit parietal cells by a local anesthetic effect is easily eliminated.

As was expected, we confirmed in denervated dogs that three H₂R-blockers, i.e. cimetidine, ranitidine, and famotidine, either orally or intravenously

administered, markedly suppressed gastric acid secretion irrespective of the stimulant used. Nonetheless, we were unable to confirm the local antisecretory effect of H₂-blockers, to include ranitidine, following 30 min administration into the pouches 1 hr after starting intravenous histamine infusion. Such results suggest that H₂-blockers not only cannot reach the receptors on the basolateral membrane of parietal cells from the mucosal surface, but also cannot be absorbed from the gastric mucosa into the circulation.

It is of interest that Konturek *et al.* (12) reported that locally applied histamine in denervated pouches of dogs resulted in a dose-dependent increase in acid secretion, without affecting acid secretion in the main stomach. It remains uncertain whether or not locally administered histamine stimulated the parietal cells through the apical membrane. It remains likely that trace histamine absorption from the gastric mucosa might have stimulated H₂-receptors on the basolateral membrane, even if the serum histamine concentration was not elevated to detectable levels. In contrast to our results, Konturek *et al.* (12) found that locally applied ranitidine significantly inhibited gastric acid secretion stimulated by both locally and intravenously administered histamine, without increasing the serum ranitidine level. Accordingly, Konturek suggested that, in addition to the established systemic effect, ranitidine might also inhibit gastric acid secretion via interaction with the mucosal membrane.

We remain unable to convincingly explain the disparity between our results and those obtained by Konturek, despite the fact that the ranitidine dose (5 to 10 mg/ml, i.e., approximately 150 to 300 mg/pouch) was equal to or double our dose. The difference might be due to the variation in either the histamine dosage (80 µg/kg/hr in Konturek's study vs. 160 µg/kg/hr in our study) and/or the method of local application (infusion in Konturek's study vs. single application in our study).

Similar results were observed in the case of pirenzepine, an M₁-receptor antagonist; i.e. the drug exerted no effect on gastric acid secretion when administered into the pouches for 30 min. Such a finding also suggests that pirenzepine, localized to the mucosal surface, was unable to interact with M₁-receptors, which are believed to exist on ECL cells.

In summary, such findings suggest that omeprazole, leminoprazole, FPL 52694, and ME 3407 directly suppress the function of parietal cells on the luminal surface, resulting in a reduction of acid secretion. Regardless of the precise mechanism for a local antisecretory effect, such drugs represent a new armamentarium that targets the apical membrane of parietal cells to control gastric acid secretion in the treatment of acid-related diseases. Moreover, such drugs will no doubt prove useful in the study of the function of parietal cells, particularly in elucidating the role of the apical membrane.

In recent years, most of the genes for the expression of the above receptors or related enzymes involved in acid secretion have been cloned, offering the

opportunity to generate gene deficient, i.e. knockout (KO), animals. To date, histamine H₂R-KO mice (27, 28), muscarinic M₃R-KO mice (29, 30), CCK₂R-KO mice (31, 32), histidine decarboxylase KO mice (33), gastrin KO mice and gastrin transgenic mice (34, 35), and H⁺, K⁺-ATPase (α , β) KO mice (36, 37) have all been generated. It is also of note that even parietal cell deficient mice have been generated, which appear to be able to live normal lifespans without excessive problems (38, 39, 40), suggesting that parietal cells are not requisite cells for stomach development or function. Based upon physiological and pharmacological analyses of such KO mice, understanding of the regulatory mechanisms of gastric acid secretion is rapidly progressing. Elucidation of the antisecretory effect of locally active drugs, such as acid pump inhibitors, FPL 52694, and ME 3407, on H⁺, K⁺-ATPase KO mice and receptor KO mice should afford new and important insights requisite for understanding the functional mechanisms underlying parietal cells.

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Author's address: Susumu Okabe, Ph. D. Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan Tel. +81-75-595-4651, Fax. +81-75-595-4757.

E-mail: okabe@mb.kyoto-phu.ac.jp