Regulation of parietal cell calcium signaling in gastric glands

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ATHMANN, Christoph, Ningxin Zeng, David R. Scott, and George Sachs. Regulation of parietal cell calcium signaling in gastric glands. Am J Physiol Gastrointest Liver Physiol 279: G1048–G1058, 2000.—The ligands interacting with enterochromaffin-like (ECL) and parietal cells and the signaling interactions between these cells were investigated in rabbit gastric glands using confocal microscopy. Intracellular calcium concentration ([Ca\(^{2+}\)]_i) changes were used to monitor cellular responses. Histamine and carbachol increased [Ca\(^{2+}\)]_i in parietal cells. Gastrin (1 nM) increased [Ca\(^{2+}\)]_i in ECL cells and adjacent parietal cells. Only the increase of [Ca\(^{2+}\)]_i in parietal cells was inhibited by H\(_2\) receptor antagonists (H\(_2\)RAs). Gastrin (10 nM) evoked an H\(_2\)RA-insensitive [Ca\(^{2+}\)]_i increase in parietal cells. Carbachol produced large H\(_2\)RA- and somatostatin-insensitive signals in parietal cells. Pituitary adenylate cyclase-activating peptide (PACAP, 100 nM) elevated [Ca\(^{2+}\)]_i in ECL cells and adjacent parietal cells. H\(_2\)RAs abolished the PACAP-stimulated [Ca\(^{2+}\)]_i increase in adjacent parietal cells. Somatostatin did not inhibit the increase of [Ca\(^{2+}\)]_i in parietal cells stimulated with histamine, high gastrin concentrations, or carbachol but abolished ECL cell calcium responses to gastrin or PACAP. Hence, rabbit parietal cells express histaminergic, muscarinic, and CCK-B receptors coupled to calcium signaling but insensitive to somatostatin, whereas rabbit and rat ECL cells express PACAP and CCK-B calcium coupled receptors sensitive to somatostatin.

gastrin; histamine; pituitary adenylate cyclase-activating peptide; somatostatin; enterochromaffin-like cell

Over the years, three major stimulants of acid secretion have emerged: ACh released from vagal nerve fibers within the enteric nervous system or in the vicinity of gastric epithelial cells (42), gastrin released from the G cells of the antral glands (5), and histamine released from enterochromaffin-like (ECL) cells in the fundic gland (17, 31). Quite recently, pituitary adenylate cyclase-activating peptide (PACAP) has also been considered to be important in gastric acid stimulation (46).

The neurotransmitter ACh exhibits a number of different actions when it is released from postganglionic nerve fibers in the fundic mucosa. Regional release of ACh activates the parietal cell directly by binding to an M\(_3\) muscarinic receptor subtype, which mainly leads to calcium release and calcium entry, presumably coupled to a G\(_q\) trimeric protein (30, 41).

There is little sensitivity to carbachol (CCh) stimulation in vivo or in vitro to H\(_2\) receptor antagonists (4). In accordance with these findings, studies with highly purified, isolated ECL cells show that CCh stimulates only a few ECL cells and therefore does not release histamine in these preparations (24, 47).

The major stimulatory endocrine peptide for histamine release from ECL cells is gastrin (33). It is released from antral G cells in response to aromatic amino acids and amines in the gastric lumen or in response to gastrin-releasing peptide from postganglionic fibers of the vagus (40). Gastrin is the major endocrine regulator of gastric acid secretion because changes in circulating gastrin can account for most of the gastric secretory response to feeding (40).

Gastrin binds to CCK-B receptors present on ECL cells and parietal cells, releasing histamine from ECL cells via a calcium-signaling cascade (33). Binding to the CCK-B receptor on the parietal cell also enables a calcium cascade, but it is thought that this signaling pathway in the parietal cell and its consequences also depend on concomitant elevation of cAMP (6, 16). In previous studies on gastric glands and in vivo, the presence of H\(_2\) receptor antagonists abolished the gastrin stimulatory effect on the parietal cell, suggesting...
that the gastrin stimulation of acid secretion is mediated mainly by histamine release from the ECL cell (2). It was thought that the histamine receptor was coupled only to adenylate cyclase and that elevation of CAMP was in itself sufficient for stimulation of acid secretion in vitro models (10). More recently, histamine was also shown to elevate intracellular calcium in parietal cells, suggesting that this receptor has at least a dual coupling system in this cell type (9).

The gastric source of histamine was shown to be the ECL cell, first by histological observation and later by studies using inhibitors of histidine decarboxylase or by detailed analysis of the properties of isolated, purified ECL cells in short-term culture (1, 31, 33, 47). In the latter case, gastrin, but not ACh, was shown to be an effective stimulant of histamine release from ECL cells. This accounts not only for the acid secretory stimulation of gastrin but also for the inhibitory effects of H2 receptor antagonists on gastrin stimulation.

PACAP has been shown to be a stimulant of ECL cell calcium signaling and histamine release as well as ECL cell growth (24, 46). This neuropeptide is present in nerve fibers of the gastric mucosa (37). The finding that PACAP injection inhibits gastrin stimulation of acid secretion in vivo obscured its candidacy as a stimulant of acid secretion (27). However, somatostatin antibody prevented the inhibition due to PACAP injection, showing that the inhibition in vivo was due to stimulation of vasoactive intestinal polypeptide (VPAC) receptors on D cells (46). This paradoxical finding that a stimulant of histamine release from ECL cells in vitro inhibits acid secretion in vivo and the apparently direct effect of gastrin in vivo and its indirect effect in vitro illustrate the complexity of interactions that can occur in the regulatory pathways of acid secretion when studied in intact animals and even in isolated cells not in an integrated environment.

The isolated rabbit gastric fundic gland is probably the simplest integrated secretory component that has been studied to elucidate mechanisms of stimulation of acid secretion as well as mechanisms of acid secretion per se (3). The advent of confocal microscopy now enables direct studies of signaling pathways in the ECL and parietal cells in this multicellular structure. The present study describes some of the novel characteristics elucidated by confocal observation of calcium signaling in response to ligands in the ECL and parietal cells of superfused rabbit gastric glands.

MATERIALS AND METHODS

Gland preparation. Pathogen-free New Zealand White rabbits (1–1.5 kg) were used in this study. The animals were anesthetized with 50 mg/kg body wt ketamine and 6 mg/kg body wt xylazine by intramuscular injection followed by an intravenous injection of pentobarbital (50 mg/kg). Gastric glands were isolated by a modification of the method described previously (3). Briefly, a high-pressure perfusion with PBS was used to separate the gastric epithelium from the submucosa. The oxyntic mucosa was stripped off and cut into small pieces. Glands were isolated by collagenase digestion at a concentration of 8 mg/25 ml. Ringer buffer (see Media and ligands used for gland preparation and confocal microscopy) containing 100 μM ranitidine was used with shaking at 37°C in an atmosphere of 95% O2-5% CO2. After 30 min, aliquots were taken to monitor the process of digestion. After several washes, intact glands were obtained. They were put on round coverslips (no. 1, Fisher) that were coated with Cell-Tak (Collaborative Biomedical Products) and transferred into a six-well plate containing Ringer buffer.

Confocal microscopy. All confocal experiments were conducted on a Zeiss LSM 410 confocal microscope. An Attofluor perfusion chamber allowing temperature control and rapid medium exchange was used in these experiments as detailed below.

For intact gland visualization, a ×63 objective was used. In some experiments, a ×100 objective was used to observe the origin of the intracellular calcium concentration ([Ca2+]i), signal in individual parietal cells. For calcium signals, the glands were loaded with fluo 4 and excited at 488 nm, and fluorescence reflecting changes of [Ca2+]i was monitored at 510–525 nm. Subsequent images were monitored over the same area in 2-s intervals and stored on a hard disk. In each experiment, a region of interest (ROI) was defined on the system by highlighting a particular cell type or region of the cell, and the signal intensity was followed as a function of time after addition of ligand. In Figs. 2–10, the image displayed corresponds to the number noted in the signal trace below the image sequence. The color of the trace corresponds to the color of the ROI box illustrated in the images. Each experiment is representative of at least four such experiments. For measurement of pH changes in the secretory canaliculi, LysoSensor yellow/blue (Molecular Probes) was used and the ultraviolet (UV) laser was used to excite the dye at 364 nm.

Cell identification. For each experiment, a single intact gland was chosen. Because the morphology of the parietal cell is unique, its conical shape, with a small apical and large basolateral membrane, was quite apparent in the preparation. Additionally, parietal cells represent the largest cell type in the gastric gland and are peripheral, allowing unequivocal identification. ECL cells are small, 8- to 10-μm diameter cells that are found close to the parietal cell and are subepithelial with no direct contact with the lumen of the gland. They have a large, eccentrically located nucleus surrounded by numerous electron-translucent cytoplasmic vesicles that store histamine. The vesicles generate an internal acidity and accumulate histamine driven by the pH gradient (24, 47). Acidine orange accumulates and stacks in acidic compartments with a metachromatic shift. Consequently, the emission wavelength at neutral pH shifts from a maximum of 510 nm to a higher wavelength in acidic compartments (>590 nm). After incubation with 1 μM acidine orange, there was an accumulation in the acidic vesicles of the ECL cells, resulting in a bright red fluorescence. This gives a unique pattern, making it easy to identify these cells within the gland. Because the wavelength of >590 nm was not affected by the emission of fluo 4, it was possible to identify the ECL cells before each experiment was started. The presence of acidine orange in the histamine-containing vacuoles did not inhibit the ECL calcium signals or result in any obvious damage to those cells.

Media and ligands used for gland preparation and confocal microscopy. In the experiments reported, the Ringer buffer (pH 7.4) used for superfusion contained (in mM) 10 HEPES, 140 NaCl, 5 KCl, 1.3 CaCl2, 1.2 MgSO4, 40 KOH, and 11 glucose with 0.1% bovine albumin. For calcium-free media the CaCl2 was omitted, and for experiments with lanthanum 25 μM LaCl3 was added. For each experiment, ligands (histamine, gastrin, carbachol, PACAP, and soma-
histamine) were added to the media at the indicated concentrations.

**Verification of acid secretion by parietal cells.** The glands were incubated with the dye LysoSensor yellow/blue at a concentration of 1 μM at 37°C for 30 min before the superfusion containing histamine at 10 μM was started. Visualization of the dye was by excitation at 364 nm using the UV laser of the microscope, and the emission was monitored with a 515 nm long-pass filter. This dye has a pH of ~4.1 and is the best suited for confocal visualization of acid secretion.

**Measurement of [Ca2+]i levels.** For each experiment, a coverslip with attached gastric glands was placed into an Attofluor chamber (Molecular Probes) and incubated with 5 μM fluo 4-AM (Molecular Probes) for 30 min at 37°C. Then the chamber was placed on a heated stage (Medical Systems, Greenvale, NY) maintained at 37°C and attached to a peristaltic pump superfusing the coverslip in the chamber with Ringer solution at a rate of 5 ml/min, which provided a 90% solution exchange within 30 s. The coverslip was superfused for 5 min with Ringer buffer to remove any extracellular dye.

We chose fluo 4 because it results in increased fluorescence in response to calcium binding when excited at 488 nm. However, because this is a single-wavelength excitation, single-emission dye, the data are expressed in arbitrary units of fluorescence intensity. Therefore, variations in dye loading that may account for some variations in basal fluorescence cannot be corrected for in the same way that is used for dual-wavelength ratiometric fluorescent dyes. However, a simple means of correction for variations in dye loading is to ratio the signal generated by ligand with the baseline fluorescence in a given cell, particularly when conducting high-frequency scans. Because only the relative changes in fluorescence are therefore being monitored in a single section of 1 μm, changes in focal plane, if they were to occur on a floating table, would be compensated with equivalent change in baseline and signal.

In Figs. 2–10, representative images during an experiment before and after ligand addition are shown at the top and the number on the image corresponds to the time point defined by a line and a boxed number on the continuous recording below. The time of addition of ligands is indicated above the graph. The scanned regions of interest are color coded to correspond to the colors in the graphic representation.

**Materials.** Dyes were obtained from Molecular Probes. All other reagents were of analytical grade and were purchased from the indicated sources: collagenase A (Boehringer Mannheim), gastrin G-17 (Peninsula Laboratories), and carbachol, histamine, ranitidine, and acridine orange (Sigma).

**RESULTS**

**Confocal verification of acid secretion in gastric glands.** Previous experiments defining the site of acid secretion in this preparation used the metachromatic shift of acridine orange (14). However, the laser used in confocal microscopy rapidly bleaches the acridine orange stacked in the secretory canaliculus, resulting in a rapid fade of the signal. Therefore, to illustrate acid secretion in this model, the dye LysoSensor yellow/blue was used. LysoSensor yellow/blue shifts its fluorescence from blue to yellow in acidic compartments as shown in Fig. 1. In the absence of added stimulant, some cells were generating acid in the canaliculus (Fig. 1A). With the addition of histamine, virtually every cell was activated as shown by the increase in fluorescence (Fig. 1B). This technique provided a qualitative index of the responsiveness of a gland preparation in this series of experiments because the experiments were not designed to measure acid secretion directly. The dye showed that the majority of parietal cells in a given gland preparation responded to histamine by an increase in acid secretion, although some cells had a basal level of acid secretion before stimulation, as is typical of a rabbit gastric gland preparation when monitored by the uptake of the weak base aminopyrine (2). The identification of the ECL cells by the metachromatic shift of acridine orange fluorescence is shown in Fig. 1C. These images are representative of at least four experiments with the same number of gastric glands in each experiment.

**Effect of histamine.** The addition of 10 μM histamine to the perfusate resulted in a robust increase in [Ca2+]i. Histamine had no effect on any ECL cell in terms of changes in cell calcium (Fig. 2). The majority of parietal cells observed in a series of experiments responded with a change in [Ca2+]i, in general similar to the number responding in the LysoSensor dye experiments that visualized acid secretion in the parietal cells of the gastric gland after histamine addition. Although the presence of a histamine-induced calcium signal has been described previously (9), the relative response to histamine in the intact gland was greater.
than expected. Although an inhibitory H₃ receptor has been postulated as being able to downregulate histamine release from this preparation (31), no effect even of high histamine concentrations was observed on calcium signals in the ECL cell induced by gastrin. The effect of histamine on the parietal cell was abolished by a 10-fold higher concentration of the H₂ receptor antagonists cimetidine and ranitidine, showing that the change of [Ca²⁺]ᵢ was caused by activation of the H₂ receptor on the parietal cell (data not shown). The elevation of intracellular calcium in the parietal cells of gastric glands allowed a study of the orientation of this signal in this cell type while still in a polarized state. Again, these results are typical of at least four separate experiments with the same number of gastric glands in each experiment.

Spatial characteristics of calcium signaling in the parietal cell. The parietal cell possesses a variety of receptors for both stimulating and inhibiting ligands. With single-cell video imaging, the [Ca²⁺]ᵢ responses in individual parietal cells typically are found to be biphasic, consisting of an initial peak elevation that rapidly declines to a steady-state elevation (6). This pattern is seen in many cell types and is interpreted as being caused by an initial release of calcium from intracellular stores followed by a sustained influx of extracellular calcium. The parietal cell in an intact gastric gland retains a distinction between apical and basolateral surfaces. Activation of acid secretion at the cellular level involves binding to receptors on the basolateral surface, cytoplasmic events consequent to ligand interaction with these receptors, and finally the regulation of the activity of the gastric H-K-ATPase itself. After stimulation, the secretory canalculus is formed.

The LSM 410 confocal microscope is capable of increasing the frequency of scans by reducing the ROI. In this set of experiments, ROIs overlaying different areas of a single parietal cell (×100 magnification) were scanned at 7.7 frames/s, which equals a single scan time of 130 ms. [Ca²⁺]ᵢ was followed after superfusion with 100 μM histamine. Three ROIs of equal size were placed from the basolateral area toward the apical surface. The graph represents changes in fluorescence over a parietal cell and an ECL cell and is representative of several such experiments.
surface monitoring the fluorescence intensity. As shown in Fig. 3, the calcium signal was first evident at the basolateral region of the cell, followed by spread of the signal to the apical pole along with a secondary larger increase over the basal region. The initial signal at the basal region was also larger than the signal at the apical pole. The lower fluorescence intensity seen here is a function of the rapid scan time and does not imply a lesser signal than in Fig. 2. The secondary increase over the basal region of the cell was absent in calcium-free medium and prevented in the presence of 25 μM lanthanum previously shown to prevent calcium entry into parietal cells (41). These results represent the images observed in at least four experiments.

**Effect of gastrin.** The ECL cell responds to gastrin binding to a CCK-B receptor, as does the parietal cell (21, 33). In this study, different concentrations of gastrin were used. Figure 4 shows the effect of addition of 1 nM gastrin to the gastric gland. This resulted in an increase of \([Ca^{2+}]_i\) initially in the ECL cells. After a time delay of 1–2 s, an increase in \([Ca^{2+}]_i\) occurred only in adjacent parietal cells. Other parietal cells did not respond even after 200 s of perfusion. The ECL cell releases histamine from cytoplasmic vesicles, which then acts as a paracrine agent to stimulate the parietal cells. To see whether the gastrin-induced \([Ca^{2+}]_i\) signal in the parietal cells was caused only by histamine release from the ECL cells, the same experiments as described above were conducted in the presence of 100 μM ranitidine to block the H2 receptor on the parietal cell. As shown in Fig. 5, with the addition of 1 nM gastrin, the presence of ranitidine totally abolished the rise of \([Ca^{2+}]_i\) in the adjacent parietal cells in every experiment performed with this protocol, whereas the \([Ca^{2+}]_i\) signal in the ECL cells remained unaffected.

However, superfusion with 10 nM gastrin in the presence of 100 μM ranitidine still resulted in a \([Ca^{2+}]_i\) signal in most of the parietal cells, as shown in Fig. 6. The general effect of 10 nM gastrin on parietal cells contrasts with the local effect of 1 nM gastrin. Hence, there are actions of gastrin on parietal cells independent of effects due to ECL cell histamine release. At
At least four experiments were carried out with the same results as those illustrated.

**Effect of carbachol.** Regional release of ACh activates the parietal cell directly by binding to an M₃ muscarinic receptor subtype (30, 41). At best, 10% of purified ECL cells can be stimulated, perhaps via an M₁ receptor (36), to give histamine release, although neither a calcium signal nor release of histamine is observed in our preparations or in rabbit gastric glands (24, 47). In vitro, cholinergic stimulation of acid secretion in rabbit gastric glands is transient (3). The stimulation is blocked by atropine, indicating a direct action of ACh on the parietal cell (2). Superfusion with 100 μM carbachol showed a large [Ca²⁺]ᵢ signal in virtually all parietal cells that was unaffected by the presence of 100 μM ranitidine, as shown in Fig. 7. For example, in Fig. 7, all the parietal cells in the field show an increase in calcium after carbachol addition. No signal was ever seen in any of the ECL cells observed under these conditions, and the direct parietal cell signal is larger in response to carbachol than that found with histamine or gastrin when the relative intensities are compared (cf. Figs 2, 6, and 7).

**Effect of PACAP.** When the gastric glands were superfused with 10–100 nM PACAP-containing media, a calcium signal was seen first in the ECL cell followed by a signal in adjacent parietal cells, as previously described (46) (Fig. 8). In the presence of ranitidine, although the calcium signal was retained in the ECL cell, the calcium signal was abolished in the parietal cell, indicating that the effect of PACAP on parietal cell calcium signaling was secondary to histamine release from the ECL cell (Fig. 9). This result suggests that PACAP, rather than ACh, is the neural mediator for ECL cell activation and that PACAP links neural-to-paracrine stimulation of acid secretion. These results reflect four individual experiments with at least twice that number of ECL and parietal cells observed in each experiment.

**Effect of somatostatin.** Somatostatin inhibits gastrin-mediated stimulation of histamine release from ECL cells (32). In vivo and in vitro, this short-lived peptide

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**Fig. 6.** A high concentration of gastrin restores the gastrin-stimulated increase of intracellular calcium in the parietal cell in the presence of ranitidine. Gastrin at 10 nM was added in the presence of 100 μM ranitidine. Gastrin (10 nM) elicited a calcium signal in both the ECL cell and virtually every parietal cell. **Top:** a series of confocal images. **Bottom:** fluorescence intensity representing intracellular calcium.

**Fig. 7.** The effect of the addition of 100 μM carbachol in the presence of 100 μM ranitidine on calcium levels in ECL and parietal cells. In the confocal images (top), as indicated in the graph of fluorescence intensity (bottom), carbachol (100 μM) elicits a transient elevation in intracellular calcium in the parietal cells but has no effect on ECL cell calcium levels.
also inhibits acid secretion in part because of blockade of gastrin release from G cells (23). It also acts directly on parietal cells to inhibit acid secretion caused by histamine through inhibition of adenylate cyclase (20, 29). In our experiments, 100 nM somatostatin inhibited the \([\text{Ca}^{2+}]_i\) signal in both ECL and parietal cells superfused with 1 nM gastrin (data not shown). Previously, it was shown that gastrin-induced calcium signals and histamine release in isolated ECL cells were inhibited by somatostatin acting at a type 2 receptor (32). However, as shown in Fig. 10A, after effective stimulation of an ECL cell with 1 nM gastrin, the presence of 100 nM somatostatin inhibited the fluorescence signal in the ECL cell due to 10 nM gastrin but not the signal in parietal cells. This again emphasizes the difference between the effect of gastrin at low and high concentrations. The calcium response of the parietal cell to the addition of histamine (Fig. 10B) or carbachol (data not shown) was also unaffected by the presence or addition of somatostatin. The effects of somatostatin illustrated here reflect observations in at least four experiments with ECL and parietal cells.

DISCUSSION

The intact rabbit gastric gland provided the first reliable in vitro model of mammalian gastric acid secretion in terms of ligand responses (3). This preparation responds to histamine and gastrin with a steady-state elevation of the accumulation of the weak base aminopyrine and displays a transient response to stimulation by carbachol. Histamine elevates cAMP in the gland (10), but study of the cellular localization of calcium signaling in response to a variety of ligands required the advent of confocal microscopy, given the 50-μm thickness of the preparation. Histamine stimulation of acid secretion was blocked by \(\text{H}_2\) receptor antagonists, as was the gastrin response (2). The latter was also inhibited by diamine oxidase in the medium (20). Isolated parietal cells respond to histamine with an increase in both cAMP and calcium (8). They also respond to carbachol via a calcium signaling pathway because of the presence of an \(M_3\) receptor subtype on the parietal cell (30, 35, 41).
ECL cells, the neuroendocrine cells responsible for release of histamine as a paracrine stimulant of gastric acid secretion, respond to gastrin and PACAP with a release of histamine and are inhibited by somatostatin binding to a SST-2 receptor subtype when studied in vitro but do not respond to carbachol (24, 32, 47). The gastrin receptor was cloned from isolated parietal cells, and gastrin was able to stimulate elevation of parietal cell calcium, apparently provided that cAMP was also elevated (6, 16, 21). Elevation of parietal cell calcium is not able to stimulate acid secretion (41); therefore, other, unknown messenger pathways must also be involved.

In the present study, gastrin at close to physiological concentrations produced a calcium response in adjacent parietal cells that was blocked by ranitidine. This is consistent with in vivo data showing that pentagastrin stimulation is sensitive to H₂ blockade (4). Previous experiments showed that stimulation of acid secretion in glands and isolated parietal cells appears to require elevation of cAMP, presumably that generated by histamine action on the parietal cell (6, 16). In the experiments described here, at supraphysiological concentrations, gastrin produced a calcium signal insensitive to ranitidine. Perhaps this change of intracellular calcium at 10-fold physiological levels results in effects other than acid secretion, such as stimulation of growth of the parietal cell mass in the gastric mucosa, as has been shown to occur with treatment with proton pump inhibitors (13). Lower concentrations of gastrin appear to stimulate growth of ECL cells than those required to stimulate calcium and histamine release (33). It seems that the CCK-B receptor stimulates events in the gastric mucosa other than secretion but with different affinities.

The lack of effects of carbachol on isolated, purified ECL cells has been described previously (24, 47), although it was not possible to exclude selective digestion of the muscarinic receptor by the pronase necessary for cell isolation. In the present study, in intact
gastric glands, the $M_3$ receptor is clearly retained during collagenase digestion on the parietal cell, and this makes it extremely unlikely that there is selective removal of a muscarinic receptor on the ECL cell. Hence it can be firmly concluded that ECL cells in the rabbit do not express muscarinic receptors, at least not the $M_1$, $M_3$, and $M_5$ subtypes that are coupled to elevation of intracellular calcium. There is, however, a report of $M_1$ receptor expression on the ECL cells of the rat (37). $M_3$ receptors are also expressed on the G cell (15, 47). Because the $M_1$ antagonist pirenzepine is an effective inhibitor of acid secretion, this inhibition must occur before either the parietal cell or the G cell. Presumably, the $M_1$ receptor is expressed on the ganglionic cells of the myenteric plexus and pirenzepine inhibits transmission of neural signaling in the myenteric plexus to account for its inhibitory action on gastric acid secretion (15).

The $H_2$ receptor in the parietal cell is coupled to both $G_s$ and $G_q$ signaling pathways. Because cAMP stimulates acid secretion in the parietal cell and phosphodiesterase inhibition also stimulates acid secretion, cAMP elevation is sufficient for parietal cell stimulation (3). The robust calcium signal observed here may reflect promiscuity of the $H_2$ receptor at high concentrations of ligand, although the actual histamine concentration at the parietal cell is unknown. On the other hand, because this elevation of calcium is found concurrently with elevation of cAMP at the concentrations used here, a physiological role for the coupling of the $H_2$ receptor to $G_q$ proteins may be present, although it is as yet undefined.

The time course of elevation of calcium at different locations in the parietal cell follows from the basolateral location of the $H_2$ receptor and is the converse of what is found in the pancreatic acinar cell. In pancreatic acinar cells, $Ca^{2+}$ is released in the luminal cell pole followed by spreading of the $[Ca^{2+}]_i$ signal toward the basolateral side of the cell. It is thought that the CCK-A receptor may be present at the apical pole of the acinar cell, that the various inositol 1,4,5-triphosphate receptors have a different localization placing the most sensitive at the apical pole of the acinar cell, or that $G$ proteins are associated with the zymogen granules (28, 34, 39, 45).

Another striking result was the elevation of calcium at the basolateral pole of the cell after the signal had decayed elsewhere in the cell. This elevation was absent in calcium-free media or with the addition of 25 $\mu$M lanthanum, indicating that calcium entry was responsible for this localized elevation. Because the elevation of calcium followed the initial activating signal, it is likely to be caused by reloading of calcium stores. Electron microscopic analysis of the basolateral surface region of the parietal cell reveals deep infolding of the plasma membrane but a relative paucity of endoplasmic reticulum (18). The elevation of calcium seems to be related morphologically to these infoldings that provide an entry pathway for calcium. Recent data implicating mitochondria as transient stores for calcium and the high content of parietal cell mitochondria may explain this phenomenon (25).

PACAP may be the transmitter for neural stimulation of ECL cell calcium and histamine release rather than ACh (46). This explains the relative insensitivity of in vivo acid secretion stimulated by carbachol to histamine antagonism, because carbachol stimulates the parietal cell directly (4). On the other hand, $\sim 60\%$ of centrally stimulated acid secretion in the rat is blocked by $H_2$ antagonism (44), suggesting that neural mediation of ECL cell histamine release is present to explain this effect of $H_2$ antagonists. The effect of PACAP on calcium signaling in ECL cells and the $H_2$-sensitive activation of calcium signals in only parietal cells close to ECL cells would explain the effect of antagonists on central stimulation of acid secretion. Pituitary adenylate cyclase (PAC 1) receptors in ECL cells are coupled to both $G_s$ and $G_q$ signaling pathways, and PACAP also stimulates growth of ECL cells (22, 46). PACAP therefore seems to be the neural analog of gastrin with respect to ECL cells.

Somatostatin is known to inhibit gastric acid secretion stimulated by a variety of ligands including gastrin and a specific $H_2$ receptor ligand, dimaprit (7, 11, 43). The former action is caused by blockade of gastrin-induced histamine release from the ECL cell and the

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**Fig. 11.** A model representing the data illustrated in the present study and elsewhere. The parietal cell is stimulated in terms of calcium signaling directly by histamine at an $H_2$ receptor, by gastrin at a CCK-B receptor, and by acetylcholine at an $M_3$ receptor. The $H_2$ receptor is coupled to adenylate cyclase that is inhibited by the somatostatin receptor on the parietal cell, but this latter receptor is not coupled to inhibition of the calcium signaling cascade in this cell. The ECL cell responds to both gastrin and PACAP by elevation of cell calcium and release of histamine, and this response is blocked by somatostatin.
latter by inhibition of adenylyl cyclase in the parietal cell. Rat parietal cells express the SST-2 subtype of somatostatin receptor, as do the ECL cells (43). Although inhibition of the calcium signal in ECL cells would account for inhibition of gastrin stimulation of acid secretion, it would not account for the inhibition of histamine-stimulated acid secretion because here the calcium signal due to histamine was not inhibited by somatostatin. Because the calcium signal due to high gastrin concentration was not inhibited by somatostatin, and yet acid secretion due to gastrin is inhibited, these data emphasize that this calcium signal in the parietal cell, either histamine or high gastrin induced, is not sufficient for stimulation of acid secretion. The SST-2 receptor is coupled to G<sub>i</sub> trimeric proteins that inhibit G<sub>i</sub>-coupled pathways and in some cells, such as ECL cells, may also inhibit G<sub>s</sub>-coupled pathways. Inhibition of the ECL cell signaling pathways that depend on G<sub>q</sub> explains the effect of the SST-2 receptor in inhibition of the ECL cell signaling pathways that de-
pended via a G<sub>s</sub> trimeric set of proteins (43). Evidently, methylxanthine (a phosphodiesterase inhibitor)-stim-
blocade of either PACAP or gastrin stimulation of

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From the data presented here, the pathways in-
volved in direct and indirect stimulation of calcium signaling in the parietal cell have been defined for the first time in an integrated in vitro heterocellular model. As illustrated in Fig. 11, PACAP acts exclu-
sively on the ECL cell, whereas ACh and histamine act exclu-
sively on the parietal cell. Gastrin acts only on the ECL cell at the 1 nM level but at higher concentrations also acts directly on the parietal cell, although the physiological significance of this latter observation is obscure. H<sub>2</sub> receptor antagonist action is confined to the parietal cell, and M<sub>1</sub> receptor antagonists can be deduced to act at the myenteric plexus and not directly on either parietal or ECL cells. Somatostatin inhibits ECL cell function and adenylyl cyclase in the parietal cell but not the histamine or gastrin calcium signals in the parietal cell. These observations deserve further investigation. The relative magnitudes of the calcium signals observed in ECL cells were similar for gastrin or PACAP, but in parietal cells the magnitudes were carbachol > histamine > gastrin.

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