Simultaneous detection of gastric acid and histamine release to unravel the regulation of acid secretion from the guinea pig stomach

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Bitziou E, Patel BA. Simultaneous detection of gastric acid and histamine release to unravel the regulation of acid secretion from the guinea pig stomach. Am J Physiol Gastrointest Liver Physiol 303: G396–G403, 2012. First published May 16, 2012; doi:10.1152/ajpgi.00548.2011.—Gastric acid secretion is regulated by three primary components that activate the parietal cell: histamine, gastrin, and acetylcholine (ACh). Although much is known about these regulatory components individually, little is known on the interplay of these multiple activators and the degree of regulation they pose on the gastric acid secretion mechanism. We utilized a novel dual-sensing approach, where an iridium oxide sensor was used to monitor pH and a boron-doped diamond electrode was used for the detection of histamine from in vitro guinea pig stomach mucosal sections. Under basal conditions, gastrin was shown to be the main regulatory component of the total acid secretion and directly activated the parietal cell rather than by mediating gastric acid secretion through the release of histamine from the enterochromaffin-like cell, although both pathways were active. Under stimulated conditions with ACh, the gastrin and histamine components of the total acid secretion were not altered compared with levels observed under basal conditions, suggestive that ACh had no direct effect on the enterochromaffin-like cell and G cell. These data identify a new unique approach to investigate the regulation pathways active during acid secretion and the degree that they are utilized to drive total gastric acid secretion. The findings of this study will enhance our understanding on how these signaling mechanisms vary under pathophysiology or therapeutic management.

parietal cell; enterochromaffin-like cell

The regulation of gastric acid secretion and the parietal cell have been widely investigated in a host of different organisms. It is well established that there are three core stimulatory regulatory components of the parietal cell: histamine (paracrine signaling molecule), gastrin (secreted hormone), and ACh (neural, (36, 37)). The regulation of the parietal cell is a dynamic process and is also partly based on sensory inputs (5). Improved understanding of gastric acid regulation has led to the development of acid-suppressive therapies that have enhanced our management of acid-related disorders, such as gastroesophageal reflux disease and peptic ulcer disease (36).

Studies have shown that the acid secretion is mainly regulated by histamine and that the enterochromaffin-like (ECL) cell plays the central role in this process (1, 19, 29, 35, 39). Gastrin is released from G cells and elevates acid secretion by activation of cholecystokinin-2 (CCK2) receptors on ECL cells. Studies utilizing isolated ECL cells and in vitro assays have shown that gastrin stimulation induces histidine decarboxylase to synthesize histamine (11, 15, 30). ACh is released from postganglionic neurons and stimulates the parietal cells directly via M3 receptors and indirectly via M2 and M4 inhibition of somatostatin secretion (34, 35). Many studies have shown that ACh stimulates the ECL cells to drive acid secretion (6, 29, 31). Many other important regulators and modulators are also involved in the acid secretion mechanism (17, 18, 45). Although there is no doubt that these three signaling molecules play a key role in gastric acid secretion, there is still much debate about the interplay between these regulators under normal conditions and the contributions they play on regulating the ECL and parietal cell.

The difficulty in understanding the regulation of the gastric acid secretion process is mainly due to limitations on the analytical approaches utilized. The majority of studies have focused on either direct sole measurements of either gastric acid or histamine. Gastric acid has been monitored using simple approaches such as titrations from acid perfusates or using pH sensors (4, 10, 20, 33). Histamine has been widely monitored using radioimmunoassays (9, 23, 32, 41) and chromatographic approaches (22, 38) and via mass spectrometry (14, 40). These studies have provided insight into the regulation of the ECL and parietal cell, but not the whole mechanism (19, 20, 39). On the other hand, simultaneous monitoring of multiple markers can provide greater information on contributions played by chemicals on the regulation of gastric acid secretion. Electroanalytical approaches offer the ability to conduct such measurements, as they allow for simultaneous monitoring of multiple signaling molecules and have been successfully utilized to monitoring various neurochemicals in the gastrointestinal tract (26).

Here we demonstrate a novel approach to monitor the contributions of regulatory components on the ECL and parietal cell to drive gastric acid secretion by simultaneous monitoring of pH and histamine from the guinea pig stomach. We have used an iridium oxide pH microelectrode and a boron-doped diamond (BDD) electrode for monitoring pH and histamine, respectively. We investigated the alterations in the regulation process under basal conditions and when stimulated with ACh.

Materials and Methods

Ethical Approval. Animal use was carried out in compliance with the relevant laws and institution guidelines. Male guinea pigs weighing 300–400 g were euthanized using CO2 gas.

Biological Preparation. The stomach was isolated and placed in oxygenated (95% O2–5% CO2) DMEM high glucose buffer solution, pH 7.4 (PAA Laboratories) prior to sample preparation. A segment of...
stomach tissue covering the fudus and body region was then cut along the mesenteric border, lightly stretched and pinned flat in the recording bath using stainless steel pins (Ø, 50 µm). The mucosal surface was face-up at the bottom of the chamber. This tissue was constantly perfused with warm (37°C) oxygenated (95% O2-5% CO2) DMEM high glucose buffer solution, pH 7.4. The tissue was exposed to the flowing solution for ~30 min prior to measurements.

**pH sensor fabrication and characterization.** Needle-type electrodes were fabricated in-house according to previously described method (4). Briefly, a 75-µm gold wire insulated in Teflon (A-M Systems) was threaded through a 27-gauge hypodermic needle and Robnor epoxy resin (CY1301 and HY1300) was used to fill the internal volume of the needle to secure the wire in place. Once the epoxy cured, the sharp end of the needle was cut in a 45-degree angle using a diamond saw (Buehler) to expose the gold microelectrode. Alumina slurries (1, 0.3, and 0.05 µm) were used sequentially to polish the beveled end to ensure a flat electrode surface.

Anodic electrodeposition of a thin iridium oxide film onto the gold microelectrode was performed using an iridium oxalate solution described elsewhere (42, 43). Amperometric deposition using a constant-potential of 0.6 V vs. no-leak Ag/AgCl (model E9009; Cypress Systems) was applied for 2 min. The coated microelectrodes were placed in deionized water for at least 2 days prior to use to hydrate the film and stabilize the potential reading.

The pH sensor was calibrated in DMEM buffer at different pH values (pH 6.0–8.0) before and after the in vitro application to access the effect of extensive use of the probe over the tissue.

**BDD microelectrode fabrication and characterization.** Polycrystalline BDD thin film was deposited on a 40-µm diameter Pt wire (99.99%; Aldrich Chemical) by microwave-assisted chemical vapor deposition (1.5 kW, 2.54 GHz; ASTeX, Woburn, MA), as detailed previously (7, 24, 27). The diamond-coated Pt wire was insulated with polypropylene using a pipette tip so that a length of 100–200 µm diameter Pt wire exposed BDD was protruding at the end (7, 24). The resulting microelectrode was conical cylindrical with a maximum diameter of ~40 µm. The electrode can be reproducibly insulated with a thin and continuous polymer film using this method, but precise control of the exposed electrode length is difficult to achieve.

Differential pulse voltammetry in 50 µM histamine was carried out to identify the BDD electrode where histamine was oxidized and compared with tissue responses. Measurements were obtained in the potential range from 0 to 1.4 V vs. Ag/AgCl, using a pulse height of 50 mV and a pulse step of 50 s. Calibrations for histamine using the BDD microelectrode were carried out as previously described (4) using flow injection analysis. Each electrode was subjected to a calibration response prior to in vitro recordings. The response of the BDD electrode to 4 µM histamine before and after in vitro application was carried out to access the effect of extensive use of the probe over the tissue.

**Electrochemical recordings of pH and histamine from in vitro tissue sections.** For the potentiometric recording of pH, a two-electrode system was used, where a no-leak Ag/AgCl reference was placed into the flow bath. For the continuous amperometric detection of histamine, a three-electrode system was used consisting of the BDD working electrode, a Pt wire counter, and a commercial no-leak Ag/AgCl reference electrode. The BDD was held at a potential of +1.2 V vs. Ag/AgCl for amperometric detection of histamine overflow. Both the amperometric and potentiometric modes were controlled using a Biostat potentiostat (ESA Biosciences). The flow bath was made from Teflon (6.5 cm wide, 6.5 cm long, and 0.4 cm deep) and was lined with a silicone elastomer (model Sygard184; Dow Corning). The bath was mounted on the stage of an inverted microscope (model 1130A; VWR Scientific), and the solution flow was controlled with a peristaltic pump (Masterflex, Cole Parmer).

The BDD microelectrode was mounted on one micromanipulator (model 25033; Fine Scientific Tools), and the pH sensor affixed to another for reproducible positioning near the fudus mucosa as described previously (4). For in vitro measurements, the electrodes were initially placed at least 1 cm away from the tissue to establish a stable baseline response. The beveled-shaped pH sensor was therefore positioned parallel to the sample. During recordings, the BDD and pH electrodes were carefully moved to 0.5 mm over the tissue for 40 s and then removed back into the bulk of the media. This measurement was made over 4 or 5 regions of each tissue section to gain an understanding of variation within a tissue section. The differences in the current (ΔI(t)) and pH (ΔpH) between the perfusing media and immediate vicinity of the tissue were recorded and assessed.

Measurements were then conducted on the same tissue sample that was perfused continuously, either individually or a with combination of the following treatments: 1 µM ranitidine (RAN; H2-receptor antagonist), 1 µM proglumide (PRO; CCK2-receptor antagonist), and 1 µM scopolamine (SCO; M3-receptor antagonist), all Sigma-Aldrich. ACh (1 µM; Sigma-Aldrich) was then used to stimulate acid secretion and understand the alterations in the regulation of the parietal cell in the presence of the neural activator. Recordings were commenced after the tissue was perfused with the specific treatment for 30 min.

**Data analysis and statistics.** The mean change in pH from baseline to over-tissue measurements were measured and reported as ΔpH, while the mean change in current from baseline to over-tissue measurements were measured and converted to concentration using calibration curve responses for histamine. Data are presented as means ± SE and were compared using one-way ANOVA followed by post hoc pairwise comparisons with Tukey’s test, where P < 0.05 was accepted as a level of statistically significant difference.

**RESULTS**

**Dynamic and stable recordings of pH and histamine in vitro.** Histamine release was observed when the BDD microelectrode was positioned over the mucosal surface during differential pulse voltammetry measurements. Histamine was shown to be oxidized at 1.2 V vs. Ag/AgCl (Fig. 1C). No other peaks were observed, suggesting the current response is solely from histamine.

Calibrations of the pH sensor before and after tissue recordings showed no significant difference in the sensitivity (Fig. 2A). No significant differences were observed in the current response to a fixed concentration of histamine before and after tissue measurements (Fig. 2B).

**Regulation of gastric acid release under basal conditions.** Dose-response curves of ΔpH under SCO, PRO, and RAN were carried out to find the suitable concentration for maximum activity of the various pharmacological agents (Fig. 3A). All treatments significantly decreased ΔpH (n = 5, P < 0.001); however, maximum steady-state effects were observed at 1 µM for SCO, PRO, and RAN, and thus this concentration was utilized to study the regulation of gastric acid secretion.

When the pH electrode was positioned over the mucosal tissue surface there was a significant decrease in the pH (Fig. 4A). When recordings were carried out without the presence of the mucosal layer or in the absence of the tissue, no responses was observed (not shown herein).

Under control basal conditions ΔpH was −0.289 ± 0.005 (n = 5, Fig. 4B). This was significantly reduced in the presence of H2 antagonist RAN (−0.174 ± 0.005, n = 5, P < 0.001 vs. control). In the presence of the CCK2 inhibitor PRO, there was a significant reduction in ΔpH (−0.112 ± 0.006, n = 5, P <
When combining RAN+PRO (\(0.048 \pm 0.002, n = 5, P < 0.001\) vs. control) and PRO+RAN with the M3 inhibitor SCO (\(-0.01 \pm 0.0003, n = 5, P < 0.001\) vs. control) levels were further reduced. When SCO was utilized alone, for the degree of component observed did not vary to that shown in Fig. 4C. The difference in control and RAN response accounts for the contribution of histamine to the total acid secretion, while the difference in control and PRO provides the contribution of gastrin. The difference between RAN+PRO+SCO treatment and RAN+PRO treatment provides information on the cholinergic contribution. Gastrin was responsible for 53.8 ± 2.0\% of the total acid secretion and was significantly greater than histamine (35.0 ± 1.6\%, \(P < 0.001\) vs. gastrin and ACh (11.2 ± 0.7\%, \(P < 0.001\) vs. gastrin and histamine).

**Investigation of the ECL cell regulation under basal conditions.** Dose-response curves were utilized to study the suitable concentration of PRO used to reduce histamine levels. Fig. 3B shows that at concentrations greater than and equal to 1 \(\mu M\) PRO, there was no significant variations in the histamine levels observed. As experiments were carried out at basal conditions, reductions in the histamine response to SCO were observed at 5 \(\mu M\), and no alterations in the histamine levels were observed in the presence of RAN at all the concentrations utilized.

Fig. 1. Simultaneous monitoring of pH and histamine release. A: schematic diagram of the recording sensors used for this study. The boron-doped diamond (BDD) [used for the detection of histamine (His)] and iridium oxide pH sensors [used to monitor gastric acid secretion (GA)] were placed 0.5 mm above the mucosal surface of the stomach wall. B: photographic image of the sensors located above the mucosal surface of the guinea pig stomach. C: differential pulse voltammetry response obtained from the tissue of 1 \(\mu M\) histamine and the background perfusion media. Histamine was shown to be oxidized at \(-1.2\) V vs. Ag/AgCl and a similar response is obtained from tissue recordings, confirming the detection of histamine. No other peaks were observed. D: recording protocol, showing the traces observed for both histamine and pH monitoring. The sensor is located 5 mm above the tissue surface and for a duration of 40 s brought to a distance of 0.5 mm above the tissue surface (shown by the shaded bar). Following this period, the sensor is retracted back to the bulk media (distance of 5 mm away from the tissue surface). The difference in the background response to the over-tissue response was recorded for both histamine (\(\Delta{His}\)) and pH (\(\Delta{pH}\)).

Fig. 2. Stability of the sensor devices for in vitro recordings. A: pH electrode sensitivity measurements from a calibration of varying pHs before and after in vitro recordings. No significant difference was observed in the pH sensor response (data are means ± SD, \(n = 5\)). B: current recordings of 4 \(\mu M\) histamine in DMEM solution before and after in vitro tissue measurements. No significant differences were observed in the performance of the BDD electrode (\(n = 5\)). For both measurements, in vitro tissue recordings were carried out for 3 h.
When the BDD microelectrode was positioned within 500 µm of the mucosal surface, the oxidation current increased until plateau. Following retraction of the sensor from the tissue surface the response decayed back to baseline (Fig. 5A). When recordings were carried out either in the absence of the tissue section or in a tissue segment where the mucosa was removed, no current responses were observed. Under basal conditions, 2.95 ± 0.07 µM histamine was released from the mucosal surface (n = 5, Fig. 5B). The amount of histamine observed was not altered in the presence of RAN; however, the concentration of histamine significantly reduced to 2.67 ± 0.05 µM in the presence of PRO (n = 5, P < 0.05). Similar reductions in histamine levels when combinations of RAN+PRO and RAN+PRO+SCO were used (n = 5, P < 0.01). Gastrin-mediated histamine release via the CCK2 receptor on the ECL cell accounted for 9.5 ± 1.0% of the total released histamine.

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Fig. 3. Dose-response curves of pharmacological agents known to influence release of gastric acid. A: all treatments significantly decrease ∆pH (n = 5; ***P < 0.001). For matched concentrations, proglumide (PRO) caused the greatest decrease in ∆pH [n = 5; ***P < 0.001 vs. ranitidine (RAN) and scopolamine (SCO)]. B: only PRO decreased histamine levels under basal conditions (n = 5; ***P < 0.001 vs. RAN, *P < 0.05 vs. RAN and SCO; #P < 0.05 vs. SCO). As anticipated, no alterations in the level of histamine were observed in the presence of RAN and SCO. On the basis of these responses, the concentration of 1 µM RAN, SCO, and PRO were chosen to effectively inhibit acid secretion and histamine release. Data are means ± SD.

Fig. 4. Regulation of gastric acid secretion under basal conditions. A: original recordings of gastric acid secretion showing decrease in the pH when the sensor is located over the tissue (grey bar) in the presence of individual and combinations of RAN, PRO, and SCO. B: ∆pH responses in the presence of various combinations of RAN, PRO, and SCO. There is a significant decrease in ∆pH under each treatment; however, the total response is diminished in the presence of all pharmacological agents (data are means ± SE, n = 6, ***P < 0.001), suggesting that regulation is via histamine, gastrin, and acetylcholine (ACh) solely. C: contributions of histamine, gastrin, and ACh regulating the total ∆pH based on data shown in B. The histamine component is obtained from the difference between the control response and that in the presence of RAN. The gastrin component is obtained from showing the difference between the control and PRO response. The ACh component is analyzed by obtaining the difference between response of SCO+PRO+RAN and that of PRO+RAN. From the 3 signaling molecules, gastrin was responsible for the greatest contribution to the total observed gastric acid secretion under basic conditions (data are means ± SE, ***P < 0.001 vs. control, ###P < 0.01 vs. histamine, and †††P < 0.001 vs. gastrin).
Alteration in the gastric acid secretion mechanism in the presence of cholinergic stimulation. Responses of pH were not significantly different between 1/1000 M ACh and 1/1000 M carbachol (data not shown). There was a significant increase of pH in the presence of ACh (ANOVA P < 0.001). Fig. 6A shows that ACh increased the pH from 0.289 ± 0.005 to 0.452 ± 0.020 (n = 6, *P < 0.001, **P < 0.01 vs. control, and ***P < 0.001 vs. control). Only 10% of the histamine released is via CCK2 activation, suggesting that the ECL cell is only partly regulated by gastrin.

Fig. 5. Histamine release from the enterochromaffin-like (ECL) cell is not regulated by gastrin. A: original recordings of histamine release showing no differences in the presence of RAN but small decreases in the presence of PRO. The grey bar indicates when the sensor is located over the tissue. B: histamine concentrations in the presence of various combinations of RAN, PRO, and SCO (n = 6). There is a significant decrease in the concentration of histamine in the presence of PRO (n = 6, *P < 0.001, **P < 0.01 vs. control, and ***P < 0.001 vs. control). Only 10% of the histamine released is via CCK2 activation, suggesting that the ECL cell is only partly regulated by gastrin.

Fig. 6. Influence of ACh on regulation of gastric acid secretion compared with basal conditions. A: ΔpH levels significantly increased in the presence of ACh (n = 6, ***P < 0.001). All the treatments showed to significantly decrease acid secretion levels (n = 6, ***P < 0.001). B: contributions of ΔpH significantly varied between basal conditions (from Fig. 4C) and those under ACh stimulation (derived from Fig. 6A). No increases in the histamine and gastrin components were observed in the presence of ACh. Significant increases in ΔpH were observed under control experiments and when studying the ACh component of acid secretion (n = 6,***P < 0.001). C: ACh stimulation increased histamine levels; however, there was no difference in the presence of RAN. This suggests that ACh did not directly stimulate increased histamine release (n = 6, #P < 0.05 and ***P < 0.001 basal vs. ACh). PRO significantly reduced levels of histamine; however, decreases in the presence of ACh were similar to that under basal conditions (n = 6, ***P < 0.001 vs. control). ACh doesn’t stimulate increases in gastrin either directly on the G cell or via the ECL cell.
Most importantly, as shown in Fig. 4C, the responses obtained in Fig. 6A can be converted into components that regulate the acid secretion mechanism. Fig. 6B shows the contributions of histamine, gastrin, and ACh to the total observed ΔpH in the absence (basal state) and presence (stimulated state) of ACh. No significant differences were observed in the contributions of histamine and gastrin in the presence of ACh. The contribution of ACh on total observed ΔpH significantly increased from 11.2 ± 0.7% under basal conditions to 27.9 ± 0.8% (n = 5, P < 0.001, Fig. 6C).

A significant increase in the amount of histamine was observed in the presence of ACh (n = 5, P < 0.001, Fig. 6C) compared with the control levels. Similar to basal results, the concentration of histamine decreased from 3.35 ± 0.10 μM to 2.88 ± 0.06 μM in the presence of PRO under ACh stimulation (n = 5, P < 0.001). A similar trend was observed in the presence of PRO+RAN and PRO+RAN+SCO compared with the control response under ACh (n = 5, P < 0.001, Fig. 6C).

DISCUSSION

Various analytical approaches have been utilized for monitoring gastric acid secretion and the regulation mechanism. These have relied on the monitoring of pH and histamine. pH sensors and devices offer excellent dynamic information and have shown excellent clinical use (2, 10, 12); however, histamine monitoring is far more difficult. Histamine levels have been monitored at present using microdialysis sampling with either chromatographic or radioimmunoassay analysis (8, 23, 32, 41). These approaches, although they offer excellent sensitivity, are limited by the time scales where data points can be collected. It has been well noted that acid secretion is a dynamic and intrinsic process, and thus a real-time monitoring approach can provide more insight during pharmaceutical manipulation. We have shown previously that by using BDD microelectrodes we can record histamine release with excellent spatial resolution (4). Within this study we have shown the ability to conduct real-time recordings of histamine release and pH recordings simultaneously. The major advantage of using the BDD microelectrode is that it is not prone to influence by pH fluctuations, which are well known to shift oxidation potentials of the measured analyte and thus provide inaccurate responses (24).

Histamine release was observed from the gastric mucosa; however, no other responses influenced this signal. This is slightly surprising as serotonin is electroactive and known to be present within mast cells located within the stomach (28). Yet we believe the release of serotonin may be under the limit of detection for the electrode. All other known key released signaling molecules are either not electroactive or not oxidized within our potential window and thus were not observed or posed an issue for recordings. The electrodes were also shown to be highly stable, which is a major issue for in vitro and in vivo recordings. Electrodes are well known to be prone to fouling from matrix components and thus can limit the lifespan of the electrode. Our data shows that the sensor responses were not affected by conducting 3 h in vitro recordings. This is partly due to the sensor materials utilized, as the BDD electrode has been shown to be less prone to electrode fouling (24, 25, 27). Measurements were carried out when the sensors were not in any contact with the mucosal surface, thus preventing fouling from mucus and other matrix components.

Our study showed that hydrogen ion concentration can be monitored from above the mucus layer of the guinea pig gastric mucosa. The iridium oxide pH sensor showed good stability during the course of long-term mucosal recordings. The electrode is well suited for such measurements as it offers better spatial resolution and provides limited drift compared with conventional glass pH electrodes. These results are supported by a study using pH-sensitive micro-electrodes in rat gastric mucosa, where similar levels were observed (33).

Many studies have shown that gastric acid secretion is primarily regulated by three main components: gastrin, ACh, and histamine. Our results indicate that the majority of the total gastric acid output observed on the pH electrode was diminished when these three components were inhibited. A small fraction of acid secretion remained under basal conditions, but ~5% of the acid secretion under ACh stimulation was unaccounted for. This could be due to other key regulators or modulators known to influence acid secretion, such as vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide and galanin (17, 18, 45). One other study has investigated the regulation of total acid production in rats induced using peptone, where ~30% of the output was due to other unidentified mechanisms, such as secretagogues, direct effect of amino acids, or novel peptides (20). Although limited studies exist for studying the contributions of the total acid secretion, the receptors for gastrin, ACh, and histamine have been identified on parietal cells from various species (3, 44).

Under basal conditions, we observed that gastrin had the greatest contribution of the total gastric acid output in the guinea pig. Direct or indirect gastrin-induced acid secretion accounts for ~55% of the total output, which was greater than histamine at ~35% and ACh at ~10%. There are limited studies that have investigated basal acid secretion regulation in any species, but studies have focused on investigating the components of acid secretion during stimulation with various agents such as peptone, methacholine, and gastrin (13, 20, 21). Our studies in a different species have demonstrated that gastrin predominately stimulates gastric acid secretion via direct activation of CCK2 receptors on the parietal cell rather than through the histaminergic pathway. Histamine production is diminished by ~10% in the presence of the CCK2 blocker proglumide, suggesting that gastrin is active along this pathway. This finding is different than other studies, where in various species histamine is believed to be predominately mediated by gastrin to elevate acid secretion (11, 15, 19, 20). Although we show that this pathway is active in the guinea pig, no other studies have monitored both the direct and indirect gastrin pathways leading to the activation of the parietal cell in one experiment on any species. However, other studies have shown that when acid secretion is monitored, inhibitors of the gastrin pathway diminish acid secretion (16, 20) or when histamine is monitored, gastrin elevates levels of histamine (15, 19, 23, 29). Our data are supportive of both these studies, but provide further information in the degree gastrin plays in both pathways.
We have stimulated acid secretion using ACh to enhance acid output, but importantly, to activate the release of somatostatin, which is known to inhibit acid secretion and histamine release from the ECL cell (34, 35) under basal conditions. The activation of the ACh acts as a mimic to the meal ingestion state and thus can provide useful information on the alterations in acid secretion during this phase. Other studies have mimicked this process by using either somatostatin monoclonal antibodies or other cholinergic stimulants, such as methacholine (20, 21). ACh was utilized instead of any other nonmetabolized carbachol as the tissue was constantly perfused with ACh, thus limiting the influence of enzymatic hydrolysis. Additionally gastric acid responses under our conditions to ACh were identical to that stimulated by carbachol. ACh also did not interfere with the electrochemical monitoring of pH and histamine.

We observed a significant increase in the pH during ACh stimulation, which other studies have shown using various stimulatory agents (13, 20, 21). However, interestingly, we noticed that the components of acid secretion under ACh stimulation that are attributed to histamine and gastrin are not altered compared with basal conditions. There is a significant increase in the cholinergic component as anticipated during ACh stimulation. This is similar to studies in various species, which have shown that either the histamine contribution of acid secretion is only marginally increased during vagus excitation (21) or that elevated acid secretion levels were attributed to other chemical secatragoogues (20).

Importantly this data suggests that ACh has limited mediation on gastrin and no mediation on histamine levels. The latter statement is supported by the fact that histamine levels are not altered in the presence of scopolamine between basal and ACh-stimulated conditions. Therefore, the increased levels of histamine observed between basal and ACh-stimulated conditions are most likely due to the removal of the inhibitory block of somatostatin on the ECL cell. Another important feature is that the degree of indirect or direct mediation of histamine by gastrin is not altered during ACh stimulation, suggesting that under our experimental conditions ACh may have no effect on the G cell. This is different from other studies on various species where gastrin levels have been shown to be elevated or inhibited by cholinergic agents (13, 34).

Our study illustrates that multiple monitoring can provide insights into the contributions and mechanism that influence the regulation of gastric acid secretion. Importantly, this approach can provide insight into the contribution one signaling molecule can have on activating receptors on multiple cells. Fig. 7 shows a schematic representation of the gastric acid secretion mechanism in the guinea pig stomach under basal and stimulated conditions. Although many of the key mechanisms are active, the degree of activation significantly varies between direct and indirect pathways. Specifically we propose that in the guinea pig stomach, under basal conditions, gastrin directly mediates acid secretion and has the greatest contribution; and under stimulated conditions, ACh directly stimulates the parietal cell rather than directly elevating levels of gastrin. ACh does, however, increase histamine release by preventing release of somatostatin.

In conclusion, this new approach will enhance our knowledge of the gastric acid secretion mechanism and the role of the ECL cell as well as provide a means of monitoring the varying contributions of active pathways utilized to regulate acid secretion and to understand the interplay between different stimulatory agents involved in regulating acid secretion. Such studies will have an impact on our understanding of the signaling mechanisms in various physiological areas and will enhance our understanding of how such pathways are varied during disease, which may advance our therapeutic management of such conditions.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: E.B. and B.A.P. performed experiments; E.B. and B.A.P. analyzed data; E.B. and B.A.P. edited and revised manuscript; B.A.P. conception and design of research; B.A.P. interpreted results of experiments; B.A.P. prepared figures; B.A.P. drafted manuscript; B.A.P. approved final version of manuscript.
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