Coordinated regulation of gastric chloride secretion with both acid and alkali secretion

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Received 14 May 2002; accepted in final form 8 July 2002


It was first demonstrated almost 200 years ago that the stomach secreted hydrochloric acid (23). In the first half of the 20th century, many investigators explored chloride secretion as a means to understand mechanisms of hydrochloric acid formation (3, 8, 16, 23, 24, 33). First, proton pump, fluorescence, rat, stomach gastric luminal pH

IT WAS FIRST DEMONSTRATED almost 200 years ago that the stomach secreted hydrochloric acid (23). In the first half of the 20th century, many investigators explored chloride secretion as a means to understand mechanisms of hydrochloric acid formation (3, 8, 16, 23, 24, 33). These early experiments identified three separable routes for chloride secretion: an active transport component, an ion-exchange component, and a passive ionic diffusion component (10, 13, 22, 33). Active transport of chloride requires cellular metabolism and is correlated with acid secretory rates (12, 22, 33). There is also a Cl−-dependent exchange reaction, defined as the chloride secretory component stimulated by the presence of luminal Cl− (13, 17, 21). Finally, passive electrodiffusion of Cl− is driven by the transepithelial potential according to the flux ratio equation (13). In some cases, Cl− transport was more simply separated into “acidic chloride transport” (coupled to gastric acid secretion) and “nonacidic chloride transport” (19, 32, 35). These early investigations defined most of our knowledge about gastric chloride secretion but are derived solely from in vivo installation (steady state) experiments (14, 15, 25, 27, 38) or in vitro preparations of isolated amphibian mucosa (9–11, 13, 17–19, 22, 29, 32, 35). More recent information from mammalian systems has identified basolateral Na+/K+/Cl− cotransporter NKCC1 and Cl−/HCO3− exchanger AE2 as potential routes for chloride uptake by gastric epithelial cells (30, 34) and has identified a CLC-2 as a pH-sensitive Cl− channel that may represent an apical efflux route for Cl− secretion in parietal cells (6, 28, 31, 36). Even with these more recent advances, we remain limited in our knowledge about mechanisms and regulation of mammalian gastric chloride secretion. In particular, we are aware of no studies that explore whether gastric chloride secretion is coordinated with gastric bicarbonate secretion. Such questions are best answered by evaluating the dynamic regulation of mammalian chloride secretion.

Here, we report on-line quantitative measurements of chloride secretion from the rat stomach in vivo. Results build on our prior development of a method for simultaneous measurement of gastric effluents via pH electrode and fluorescent indicators. We show that gastric chloride secretion is increased in synchrony with both acid and alkali secretion. Results suggest that understanding the regulation and transport mechanisms of chloride secretion may provide a new window for understanding gastric physiology.

METHODS

Animals. Male Sprague-Dawley rats (Harlan) were housed individually in cages with raised mesh floors to prevent coprophagia. Animals were deprived of food for 18–20 h

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before experimental use but had free access to water at all times. All experimental procedures were approved by the Animal Care and Use Committee of Indiana University.

Surgery. Animals were prepared for experiments as described previously (5). Briefly, 250–300 g rats were anesthetized with Inactin (100 mg/kg ip), and the right jugular vein was cannulated for intravenous administration of saline or drugs. The stomach was cannulated to permit continuous perfusion of the gastric lumen. The inlet tubing was routed through the esophagus and ligated at the cervical level. A CO2-impermeable tubing (Saran, Chicago; OD 3.28 × ID 1.79 mm) was ligated at the terminal pylorus and used to collect gastric effluent. Surgically prepared animals were allowed to stabilize 2–3 h before experimental use.

Gastric perfusion. The stomach lumen was perfused at 0.7 ml/min using a syringe pump (KDS 260, KD Scientific). In all experiments, stomach distension was avoided and results were not accepted if the stomach became distended during the experiment (e.g., due to perfusion blockage). Perfusates contained 150 mM NaNO3, 4 mM Homopipes, and 5 μM N-ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE; a chloride-sensitive fluorescent dye). In some experiments, NaCl replaced NaNO3. Before use, perfusates were titrated to either pH 5.0 or 3.0 with nitric acid (HNO3) or 10 N NaOH in chloride-free experiments and with 10 N HCl or 10 N NaOH in 150 mM NaCl experiments to approximate the physiological luminal pH measured in fed or fasted rat stomach, respectively (4). Gastric perfusion was run continuously except for defined periods in which perfusion was stopped transiently for 10 min. This "stop-flow" interval allowed intraluminal accumulation of gastric secretions (5) and thereby amplified observed changes. These enhanced changes of pH and chloride in perfusate were detected when perfusion was restarted and gastric contents left the stomach to flow past the downstream pH and chloride sensors. All measurements reported in the text were made at times when perfusion was running at 0.7 ml/min.

Gastric acid/base and chloride secretion. Gastric perfusion effluent was sequentially routed past an in-line reference and pH electrode (Microelectrodes, Bedford, NH) and then into a pH meter. The pH electrode was calibrated by conventional pH standards followed by Dunnett’s multiple-comparison test. P < 0.05 was considered significant.

RESULTS

We perfused the stomachs of anesthetized rats with either pH 5 or 3 solution and measured pH of the gastric effluent to detect net gastric acid/base secretion. In a previous study, we demonstrated that luminal pH regulated the conversion from net acid (luminal pH 5) to net alkaline secretion (luminal pH 3) in the whole stomach (5). Perfusion solutions were weakly pH buffered (4 mM Homopipes, pHs = 4.32) and contained 5 μM of the chloride-sensitive fluorescent dye MQAE. We used lightly buffered solution to control pH excursions during perfusion.

MQAE is a chloride-sensitive fluorescent dye, shown in a previous study (39) to be insensitive to changes in HCO3−, SO42−, and PO43−, cations, or pH. However, only the pH range of 4–8 had been tested previously, which was not adequate for measurements in gastric effluents in which more acidic pH values can be expected. Therefore, we tested the effect of a wider range of pH on MQAE fluorescence. As shown in Fig. 1, the MQAE fluorescence emission spectrum was identical over a pH range of 1–7. Examination of MQAE emission at 0, 5, and 150 mM chloride confirmed that there was no change in the chloride concentration dependency or wavelength dependence of MQAE emission over this pH range. We noted at pH 10 that MQAE demonstrated 10, 33, and 108% increases in fluorescence intensity at 0, 5, and 150 mM chloride concentrations, respectively, and a shift from peak emission at 454 to 444 nm (data not shown). Results suggested MQAE was a suitable indicator for measuring chloride concentrations at the pH values found in gastric effluents.

We first compared the effect of chloride removal from the gastric lumen on gastric acid and alkali secretion. We examined two conditions where either acid secretion predominated (luminal pH 5) or bicarbonate secretion predominated (luminal pH 3) (5). As seen in Fig. 2, substitution of nitrate for chloride did not have any effect on the net basal acid/alkali secretion detected during steady-state perfusion at either luminal pH 5

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and 3. There was also no difference after a stop-flow period had amplified the amount of secreted acid or base in the perfusate. Results suggested that removal of luminal chloride does not alter the balance between basal gastric acid and bicarbonate secretion in rat. Therefore, subsequent experiments measured gastric chloride secretion using a luminal environment without added chloride in the presence of NO3.

By flowing gastric effluent containing MQAE past a pH electrode and then into a fluorometer cuvette, both pH and chloride concentration of the effluent could be measured on-line. After calibration of both signals to milliequivalents per liter of gastric effluent, it was possible to directly compare the chloride ions and protons added by gastric secretions. Figure 3 shows a representative experiment with luminal pH 5 perfusion. During continuous perfusion, pH was below that of the fresh perfusate, demonstrating net acid secretion (5). A net secretion of chloride was also observed into the chloride-free perfusate. After the addition of pentagastrin (16 μg·kg⁻¹·h⁻¹ iv infusion), similar increases in proton and chloride concentration were observed, although chloride concentration was always higher than proton concentration (n = 7 experiments; data not shown). Blocking H⁺,K⁺-ATPase activity (omeprazole, 60 mg/kg ip) diminished proton and chlo-
ride concentrations in parallel. However, although proton secretion was completely abolished, a significant amount of chloride continued to be reported in the lumen. Results qualitatively confirm the presence of at least two components of chloride secretion: one component coupled to proton secretion and a second that is independent of proton secretion. These points are confirmed in a more quantitative analysis in the following figures.

**Luminal pH 5.** Experiments were performed to quantify pH and chloride secretion simultaneously during stimulated acid secretion. Results from the pH measurement are shown in Fig. 4A. During continuous superfusion, steady-state pH in gastric effluents was $4.74 \pm 0.04$ ($P < 0.001$ vs. fresh perfusion solution pH $5.02 \pm 0.01, n = 7$), and the peak pH after stop-flow was $3.63 \pm 0.30$ ($P = 0.007$ vs. basal pH). Both results confirm the existence of net acid secretion in the basal condition. As in Fig. 3, pentagastrin was then used to stimulate gastric acid secretion at luminal pH 5. After $16 \mu g\cdot kg^{-1}\cdot h^{-1}$ iv pentagastrin, effluent pH reached a new steady-state level after 20 min ($3.76 \pm 0.20; P = 0.0011$ vs. basal steady-state pH). The peak pH after stop-flow was $1.84 \pm 0.11$ ($P < 0.001$ vs. pentagastrin steady-state pH).

In the same experiments, chloride concentration in the gastric effluents was independently determined using both MQAE and a chemical determination of chloride with chloride titrator. MQAE fluorescence was recorded every 5 s within the flow-through cuvette, and gastric effluent was then accumulated in a test tube for 1 min to get a sample of adequate volume for chemical determination of chloride concentration. In an attempt to partially address the different methods of sampling, nonoverlapping sets of 12 sequential MQAE measurements were averaged to yield the mean MQAE value observed over 1 min. In this scheme, MQAE values will likely miss the true maxima of any transient change in chloride concentration but can be at least roughly compared with titrator values. The measured amount of chloride in the fresh perfusate, containing no added chloride, was $0$ mM via MQAE and $0$ mM via chemical determination (data not shown).

![Fig. 4. Effect of pentagastrin stimulation on gastric pH and chloride secretion determined by MQAE fluorescence and chloridometer. Stomach lumen was perfused with luminal pH 5 solution, and gastric effluent was continuously measured by pH electrode (A) and MQAE fluorescence (B). Effluent was collected at 1-min intervals 5 min before and 10 min after stop-flow (hatched box) and used to chemically determine chloride concentration by chloridometer (C). Two stop-flow periods were used: the first under basal conditions and the second 1 h after pentagastrin treatment. Results in each panel are means ± SE of $n = 5–7$ preparations. [Cl$^-$], Cl$^-$ concentration.](http://ajpgi.physiology.org/)

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In gastric effluents, the basal steady-state chloride secretion measured with MQAE (Fig. 4B) was 1.8 ± 0.4 mM (n = 5) during continuous perfusion. After stop-flow, the peak chloride concentration was 6.0 ± 1.2 mM (P = 0.008 vs. basal chloride concentration). Both measurements confirm continuous secretion of chloride in the basal condition. After pentagastrin treatment, steady-state chloride secretion was 4.4 ± 1.1 mM (P = 0.03 vs. basal steady-state chloride secretion) and the peak concentration after stop-flow period was 20.8 ± 5.7 mM (P = 0.02 vs. omeprazole steady-state chloride secretion). The same tendency was observed with chemical determination of chloride (Fig. 4C). Basal chloride concentration determined by chloridometer was 1.1 ± 0.2 mM (n = 6). The peak chloride concentration after stop-flow was 4.8 ± 0.5 mM (P = 0.0002 vs. basal chloride concentration). After pentagastrin treatment, chloride concentration was 2.72 ± 0.40 mM (P = 0.02 vs. basal chloride concentration). The peak value after stop-flow was 12.7 ± 2.3 mM (P = 0.02 vs. basal chloride concentration). On the basis of the mixing that occurs during progression through the flow system, it is predicted that chemical determinations should underestimate peak values compared with the MQAE determinations performed earlier in the flow path. Results confirm that chloride secretion can be stimulated by increased acid secretion.

To ask the complementary question of how chloride secretion was affected when acid secretion was diminished, we used omeprazole to block activity of the H⁺,K⁺-ATPase. In this series of experiments (Fig. 5A), the steady-state gastric effluent pH of untreated tissue was 4.79 ± 0.03 (n = 12) during continuous perfusion, and after the stop-flow period, the peak pH was 3.99 ± 0.23 (P = 0.003 vs. steady-state pH). One hour after omeprazole treatment, steady-state pH alkalinized (4.95 ± 0.02; P < 0.001 vs. basal steady-state pH, n = 5), and after stop-flow, the peak pH was 5.21 ± 0.12 (P = 0.13 vs. omeprazole steady-state pH). These pH measurements confirmed effectiveness of omeprazole.

In gastric effluents, steady-state chloride secretion measured with MQAE (Fig. 5B) was 1.6 ± 0.2 mM (n =

![Fig. 5. Effect of omeprazole treatment on gastric chloride secretion. Stomach lumen was perfused with luminal pH 5 solution, and gastric effluent was continuously measured by pH electrode (A) and MQAE fluorescence (B). Effluent was collected in 1-min intervals 5 min before and 10 min after stop-flow (hatched box) for chemical determination of chloride concentration by chloridometer (C). After basal pH and chloride concentration were measured, omeprazole was given intraperitoneally to block gastric acid secretion. Two stop-flow periods were used: the first under basal conditions, and the second 1 h after omeprazole treatment. Results in each panel are means ± SE of n = 5–12 preparations.](http://ajpgi.physiology.org/)

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11) during continuous perfusion of untreated tissue. After stop-flow, the transient peak chloride concentration was 5.6 ± 0.7 mM ($P < 0.001$ vs. steady-state chloride concentration). After omeprazole treatment, steady-state chloride secretion was 2.1 ± 0.3 mM ($P = 0.24$ vs. basal steady-state chloride secretion, $n = 5$) and the peak concentration after stop-flow period was 6.9 ± 1.1 mM ($P = 0.01$ vs. omeprazole steady-state chloride secretion). Results were qualitatively similar from the chemical determination of chloride in the gastric effluents (Fig. 5C). Steady-state chloride concentration determined by chloridometer was 1.0 ± 0.1 mM ($n = 12$) during continuous perfusion of untreated tissue. The peak chloride concentration after stop-flow was 3.1 ± 0.4 mM ($P < 0.001$ vs. basal chloride concentration). After omeprazole treatment, steady-state chloride concentration was 1.4 ± 0.1 mM ($P = 0.08$ vs. basal chloride concentration, $n = 8$). The peak value after stop-flow was 4.5 ± 0.4 mM ($P < 0.001$ vs. basal chloride concentration). In contrast to the linkage between stimulated acid secretion and stimulated chloride secretion, these results show that blocking acid secretion does not diminish chloride secretion.

**Luminal pH 3.** Results at luminal pH 5 showed the presence of chloride secretion even when gastric acid secretion was blocked by omeprazole. We therefore used an alternative way to limit acid secretion by switching to luminal pH 3. Previously, Coskun et al. (5) showed that net alkaline secretion dominated over acid secretion at this luminal pH.

At luminal pH 3 (Fig. 6A), basal steady-state pH was 2.93 ± 0.02 ($P = 0.03$ compared with fresh perfusion solution pH 2.99 ± 0.01, $n = 6$). In contrast to this incorrectly low pH [as observed earlier (5)], net alkali accumulation was clearly observed after stop-flow, with the peak pH 3.24 ± 0.11 ($P = 0.04$ vs. steady-state pH). Subsequent omeprazole treatment blocked residual acid secretion, and steady-state pH was 3.02 ± 0.02 ($P = 0.0003$ vs. basal steady-state pH). After stop-flow, the gastric effluent accumulated substantial alkali, with the peak pH 3.66 ± 0.13 ($P = 0.006$ vs. omeprazole steady-state pH).

**Fig. 6.** At luminal pH 3, gastric alkaline secretion predominates, and chloride secretion is enhanced. Gastric effluent pH was measured by flow-through pH electrode (A), and chloride concentration was measured by MQAE fluorescence (B) and chloridometer (C). Data collection before and after omeprazole treatment was identical to methods described in Fig. 5. Results in each panel are means ± SE of $n = 4$–6 preparations.
As shown in Fig. 6, B and C, basal chloride secretion at luminal pH 3 was higher than secretion at luminal pH 5, reported as 2.4 ± 0.3 mM with MQAE and 1.7 ± 0.2 mM with chloridometer (both $P = 0.03$ vs. basal chloride secretion at luminal pH 5, $n = 4$). The peak values after stop-flow were 8.6 ± 1.2 mM with MQAE ($P = 0.02$ vs. basal chloride secretion) and 5.7 ± 0.2 mM with chloridometer ($P = 0.002$ vs. basal values).

Figure 6 also shows that omeprazole treatment significantly increased chloride secretion. During steady-state luminal perfusion, values were 3.9 ± 0.32 mM with MQAE ($P = 0.02$ vs. basal steady-state value) and 2.7 ± 0.3 mM with chloridometer ($P = 0.047$ vs. basal steady-state value). After stop-flow, the peak values were 12.4 ± 2.6 mM with MQAE ($P = 0.03$ vs. basal value) and 9.7 ± 2.1 mM with chloridometer ($P = 0.03$ vs. basal value).

Because the “nonacidic chloride secretion” was observed in tissues mediating net alkali secretion, we questioned whether chloride secretion was affected by a regulator of bicarbonate secretion. In rats treated with omeprazole at luminal pH 3.0, bicarbonate secretion was stimulated with PGE$_2$. As shown in Fig. 7A, PGE$_2$ stimulated bicarbonate secretion that could be detected during continuous perfusion. The maximal pH (3.58 ± 0.08, $P = 0.0004$ vs. basal steady state pH) was attained in 5 min, and stimulated secretion lasted >30 min. Simultaneously measured chloride secretion increased in synchrony with bicarbonate secretion (maximal value 7.34 ± 0.49 mM Cl$^-$ concentration, $P = 0.001$ vs. basal chloride secretion) as seen in Fig. 7B.

DISCUSSION

Chloride ion is an important component of gastric secretion. Our goal was to introduce a method for measurement of gastric chloride secretion that complemented those applied previously. Earlier measurements of chloride isotopic flux or steady-state luminal accumulation had limited time resolution. Nonetheless, use of isotopic fluxes gave valuable information on some dynamic changes in chloride secretion and yielded high-fidelity measurements of transepithelial unidirectional fluxes (13, 17, 21). However, isotopic fluxes were difficult to apply in vivo, or even in vitro, to studies of mammalian stomach. Here, we introduce in vivo methods for the continuous and simultaneous measurement of chloride secretion and gastric pH in rats. During continuous perfusion in the baseline conditions at either pH 3 or 5, there was no significant difference between the steady-state chloride values reported by MQAE fluorescence vs. chloride titrator ($P = 0.13$ or 0.10, respectively), although the titrator values tended to be lower. A quantitative comparison of chloride transients between the fluorescence and chemical methods was not possible, because physically different samples were analyzed: fluorometric measurements are made every 5 s within the flow stream of the gastric effluent, whereas gastric effluent must be collected in a test tube for 1 min to provide adequate sample for subsequent titrator measurements. The optical method has excellent time resolution, and our experiments confirm that MQAE fluorescence, at least qualitatively, mirrors results from more conventional chemical determination of chloride.

A notable limitation of the new method is that it requires use of a chloride-free luminal perfusate. In the classic nomenclature of Forte (13), this experimental condition eliminates the Cl$^-$-exchange diffusion or the component of Cl$^-$ secretion that requires luminal Cl$^-$. This may not be a crucial limitation, because there is no known physiological significance of the Cl$^-$ exchange diffusion. Furthermore, several of our results confirm previous reports from amphibian preparations that numerous physiologically important functions remain intact in the absence of the Cl$^-$-exchange diffusion. As reported in bullfrog stomach (10, 13), changing the luminal chloride compositions did not affect net acid secretion from rat stomach. In the absence of luminal Cl$^-$, the bullfrog stomach still manifests at least two distinct routes for Cl$^-$ secretion: the active component (sensitive to anoxia, stimulated by acid secretion) and the ionic diffusion (driven by transepithelial voltage). In rat, we observed that one component of Cl$^-$ secretion was stimulated by the activation of acid secretion (pentagastrin), in contrast to another that could actually be stimulated in the absence of acid secretion. In both rat and frog, addition of omeprazole blocks acid secretion but not Cl$^-$ secretion (35). We do not know whether the “nonacidic” Cl$^-$ secretion is due to ionic diffusion, because we can neither measure nor reliably manipulate transepithelial potential in the in vivo preparation. However, our experiments can reli-

![Fig. 7. PGE$_2$ stimulates chloride secretion at luminal pH 3.0. The residual acid secretion existing at luminal pH 3 is blocked by omeprazole (60 mg/kg ip), and then bicarbonate secretion is stimulated by PGE$_2$ (1 mg/kg iv), as reported by pH electrode (A). Chloride secretion is measured simultaneously by MQAE fluorescence (B). Results in each panel are means ± SE of $n = 6$ preparations.](http://ajpgi.physiology.org/)

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Alternatively, it could be because the nonacidic Cl\(^{-}\) secretion has properties that strongly suggested its magnitude was correlated with the magnitude of bicarbonate secretion. Omeprazole unmasks bicarbonate secretion without affecting accompanying chloride secretion. In the presence of omeprazole, Coskun et al. (5) previously observed that gastric bicarbonate secretion was greater at luminal pH 3 compared with luminal pH 5. In the current report, we observed that Cl\(^{-}\) secretion was also greater at luminal pH 3 compared with luminal pH 5 (compare Figs. 5 and 6). At a minimum, this implies that some component of Cl\(^{-}\) secretion is controlled by luminal pH. This could be a direct effect of extracellular protons on an apical ClC-2 Cl\(^{-}\) channel, known to cause channel opening with a pK\(_{a}\) of ~5 (6, 36). It is also consistent with activation of the CIC-2 channel through the direct effect of omeprazole or changes in membrane potential (7) or recruitment of alternative chloride secretory mechanisms in diverse cell types of the stomach. Alternatively, it could be because the nonacidic Cl\(^{-}\) secretion is a facet of bicarbonate secretion. The ability of PGE\(_{2}\) to elicit synchronous undulations in bicarbonate and Cl\(^{-}\) secretion (Fig. 7) supports this last possibility.

At a minimum, it seems unlikely that an apical Cl\(^{-}\)/HCO\(_{3}\) exchanger mediates bicarbonate secretion in rat stomach. The use of such a transporter would be difficult to reconcile with the insensitivity of bicarbonate secretion to removal of luminal Cl\(^{-}\) (Fig. 2) and the surge in Cl\(^{-}\) secretion that occurs following stimulation of bicarbonate secretion with PGE\(_{2}\) in the acid-suppressed stomach. In analogy to duodenal models, it may be that the activation of an apical anion conductance (CIC-2?) serves as a route for both bicarbonate and chloride exit into the lumen (20, 26).

In summary, we have developed and applied a new in vivo method for measurement of gastric Cl\(^{-}\) secretion. Results reveal a connection between the mechanisms that either mediate or regulate gastric bicarbonate and Cl\(^{-}\) secretion. It is hoped that further research will be able to resolve the basis for the connection and further develop our understanding of the nonacidic Cl\(^{-}\) secretory mechanism.

We acknowledge the pivotal role of S. Chu in the initial development of this technique.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant RO1-DK-54940.

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