Agonist-induced cytoplasmic volume changes in cultured rabbit parietal cells

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Sonntag, Thorsten, Wolf-Kristian Siegel, Oliver Bachmann, Heidi Rossmann, Andreas Mack, Hans-Joachim Wagner, Michael Gregor, and Ursula Seidler. Agonist-induced cytoplasmic volume changes in cultured rabbit parietal cells. Am J Physiol Gastrointest Liver Physiol 279: G40–G48, 2000.—Concomitant Na+/H+ and Cl-/HCO3 exchange activation occurs during stimulation of acid secretion in cultured rabbit parietal cells, possibly related to a necessity for volume regulation during the secretory process. We investigated whether cytoplasmic volume changes occur during secretagogue stimulation of cultured rabbit parietal cells. Cells were loaded with the fluorescent dye calcein, and the calcein concentration within a defined cytoplasmic volume was recorded by confocal microscopy. Forskolin at 10^{-5} M, carbachol at 10^{-5} M, and hyperosmolarity (400 mosmol) resulted in a rapid increase in the cytoplasmic dye concentration by 21 ± 6, 9 ± 4, and 23 ± 5%, respectively, indicative of cell shrinkage, followed by recovery to baseline within several minutes, indicative of regulatory volume increase (RVI). Depolarization by 5 mM barium resulted in a decrease of the cytoplasmic dye concentration by 10 ± 2%, indicative of cell swelling, with recovery within 15 min, and completely prevented forskolin- or carbachol-induced cytoplasmic shrinkage. Na+/H+ exchange inhibitors slightly reduced the initial cell shrinkage and significantly slowed the RVI, whereas 100 µM bumetanide had no significant effect on either parameter. We conclude that acid secretagogues induce a rapid loss of parietal cell cytoplasmic volume, followed by RVI, which is predominantly mediated by Na+/H+ and Cl-/HCO3 exchange.

THE STOMACH SECRETES ~2 liters of gastric juice per day, a fluid mainly consisting of H+, Cl−, K+, and water. Transport pathways for H+, Cl−, and K+ have been identified in the apical membrane of the secreting parietal cell, but not for water. Quite the contrary, isolated apical membrane vesicles from secreting gastric mucosa, so-called “stimulation-associated” vesicles, were found to have a very low water permeability (31). Therefore, it has been assumed that the water permeates through paracellular pathways.

However, we and others observed a strong immunoreactivity of parietal cell basolateral membranes with antibodies against aquaporin-4 (21, 27, 44), and it has recently been shown that the aquaporin-4 knockout mouse has a reduced pentagastrin-stimulated acid secretion (19), suggesting that water uptake into the parietal cell is necessary for acid secretion. Moreover, we had observed that acid secretagogues activate parietal cell Na+/H+ exchange, but that in the presence of CO2/HCO3, this activation is not associated with an increase in intracellular pH (pHi) or basolateral proton efflux, suggesting 1) that a Cl-/HCO3 exchanger is activated concomitantly and 2) that the physiological role for Na+/H+ exchange activation during stimulation of acid secretion is not to increase parietal cell pHi (1). Since the concomitant activation of Na+/H+ and Cl-/HCO3 exchange is one of the known mechanisms that cells use to increase their volume (20), we speculated that the secretagogue-induced Na+/H+ exchange activation may occur in the context of parietal cell volume regulation. Therefore, we investigated 1) whether the cytoplasmic volume of parietal cells in primary culture changes during stimulation of acid secretion, 2) whether parietal cells regulate their cell volume during this process, and 3) whether Na+/H+ exchangers are involved in the volume regulation observed after stimulation of acid formation.

MATERIAL AND METHODS

Materials. The materials used for cell preparation and culture were the same as previously described (1, 33). Dimethylamiloride (DMA), forskolin, and carbachol were from Sigma (Deisenhofen, Germany); the acetoxymethyl ester of 2′,7′-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF/AM) and calcein/AM were purchased from Molecular Probes (Leiden, The Netherlands). 4-Isopropyl-3-methylsulfonfylbenzoyl-
guanidine methanesulfonate (Hoe-642) was a generous gift from Hoechst (Frankfurt, Germany).

**Cell isolation and culture.** Cell isolation and culture were carried out as previously described (1, 33). Parietal cells for volume measurements were used during the first 3 days after plating, when they still retained a relatively round shape.

**Cytoplasmic volume measurements.** During stimulation of acid secretion, a network of parietal cell intracellular membrane structures enlarges to become the so-called "secretory membranes" into which the acid is secreted (13, 16). In isolated parietal cells, gastric glands, or cultured parietal cells, these secretory membranes enlarge into vacuolar structures, whose interior becomes acidic (2, 5, 28). Because of these marked vacuolar volume changes, total cell volume changes are unlikely to parallel cytoplasmic volume changes (which we wanted to measure). Therefore we used fluorescence optical measurements to assess the concentration change of the intracellularly trapped dye calcein within a defined intracellular volume as a reflection of cytoplasmic volume changes. Calcein was used as the fluorescent probe because it is well retained in viable cells (9), is insensitive to pH changes (11), and has been used by others to monitor cell volume changes (24).

Parietal cells cultured on coverslips were incubated with 3 μM calcein-AM for 30 min at 37°C in perfusion buffer (in mM: 118 NaCl, 22 NaHCO3, 14 HEPES, 7 Tris, 3 KH2PO4, 1.2 CaCl2, 1.2 MgSO4, and 20 glucose, pH 7.4, gassed with 5% CO2-95% O2), then washed twice and incubated a further 30 min in buffer without dye. The coverslips were then mounted in a custom-made heated perfusion chamber that allowed extremely fast buffer change. Measurements were performed using a model LSM 410 inverted laser-scanning microscope (Carl Zeiss, Oberkochen, Germany). The light from a single-line argon laser (488 nm) was directed through the objective (Plan Neofluar, 40×/1.3 NA, oil immersion). Emitted fluorescence was directed through a long-pass filter (LP 515, Zeiss), resulting in a 1,000-fold reduction of the light intensity, and a pinhole of 20 (which defines the accuracy of one point in the xy-plane, not relevant for z-scans) before it was detected by a photomultiplier. A time series of z-scans (z-step height of 1 μm) was made through the middle of a parietal cell. The thickness of the cell sectioning in the z-plane was ~500 nm. The duration of each z-scan was ~1.5 s, and the interval between scans was 15 s in the first 5 min of the experiment and 30 s thereafter. The perfusion buffer was changed 90 s after the start of the z-scan series to the experimental solution. The images were analyzed utilizing the integrated software. An area within the cytoplasm was marked, fluorescence intensity was measured, and the voxels in each 1 μm3 within this area in all z-scans of the series were calculated. This procedure was used to maximize the scanning speed and to minimize the light exposure. A series of z-scans were made to assess dye bleaching/leakage over time and to construct a bleaching curve for subtraction (Fig. 1). The percentage of dye bleaching/leakage over the 20 min of the experiment was found to be ~10%. If the initial recordings before the switch to the test solution did not show a horizontal slope, then the experiment was discarded. Background fluorescence was found to be negligible and was not corrected for. If vesicles appeared within or enlarged into the selected intracytoplasmic areas, if the cells detached or moved during the rapid perfusion, or if bleaching/leakage was pronounced, then the measurement was stopped or the experiment was not used after evaluation.

**Statistics.** To assess the effect of inhibitors on volume regulation, we compared the slopes of the individual time courses for calcein concentration change of the control and the inhibitor-treated cells in the linear phase of volume recovery using the Student’s t-test or the Spearman rank test for paired samples. Identical time periods of the scans were compared between control and inhibitor-treated cells; n = number of separate experiments. Results were considered significant if P < 0.05.

**RESULTS**

**Changes in parietal cell morphology during stimulation of acid formation.** Figure 2 shows a z-scan through three parietal cells on the second day of culture, before and 15 min after stimulation with 10−5 M forskolin. Albeit the chosen picture is an extreme example of variability, it is evident that cellular morphology may vary considerably within individual cells. Although one cell has no discernible intracellular vacuoles and does not develop any during stimulation, one has large vacuoles that further enlarge during stimulation, and a third has small vacuoles that enlarge during stimulation. Experiments with a conventional fluorescent microscope and the fluorescent dye 9-aminoacridine has shown that the pH in these vacuoles is acidic, indicative of acid formation ongoing in cultured parietal cells in the resting state, and becomes more acidic when stimulated (38). All cells stain for H+−K+−ATPase and also show other morphological features of parietal cells (1). The variability of individual parietal cells in culture to acid secretagogues has been observed before (4). We usually selected parietal cells with small- or medium-size intracytoplasmic vesicles that enlarged during stimulation for assessment of volume changes.
which vesicles appear or enlarge that progressively acidify. Thus total parietal cell volume may not correlate with cytoplasmic volume during stimulation of acid secretion. The latter, however, is likely the regulated parameter. Therefore, we assessed cytoplasmic volume changes by measuring the concentration change of a fluorescent dye within a defined intracellular volume by confocal microscopy. To validate the method, we investigated whether passive volume changes, induced by changes of the medium osmolarity or depolarization, resulted in the expected cytoplasmic volume changes. Switching the medium osmolarity from 300 to 400 mosmol/l resulted in a transient increase in cytoplasmic calcein concentration by 23 ± 5% at 200 s, indicative of cell shrinkage, followed by recovery to baseline within 13 min, indicative of a regulatory volume increase (RVI) (Fig. 3A). When 500 μM DMA and 100 μM bunetanide were applied together with the hyperosmolar medium, the percentage of volume loss was reduced to 16 ± 4% and the slope of recovery was reduced by 35% (Fig. 3, B and C). The results were not different when only 500 μM DMA was applied (42% reduction of recovery, data not shown). It was not our aim to study parietal cell volume recovery mechanisms after hyperosmolar shrinkage, but, because of the less pronounced contribution of Na+/H+ exchange, they appear different than after secretagogue-associated shrinkage. Since the concomitant application of a secretagogue and hyperosmolarity results in a marked reduction of acid formation in isolated parietal cells (data not shown), it was clear to us that passive and secretagogue-associated shrinkage may result in totally different intracellular ion compositions, and parietal cells shrunken by different methods may use different means of volume recovery.

Barium, 5 mM, which will at this concentration block both Ca2+ and cAMP-dependent basolateral K+ channels and therefore cause depolarization, caused a transient decrease in the cytoplasmic dye concentration, indicative of cell swelling, followed by subsequent recovery to baseline levels (Fig. 3D). Since it is known that depolarization results in cell swelling, our results indicate that we are able to monitor parietal cell volume changes in either direction. Furthermore, the results show that parietal cells are able to regulate cell volume both after cell shrinkage and swelling. They also show that even in the absence of secretagogues, parietal cells lose K+ and Cl−. This is in agreement with previous findings by Coppola et al. (8), who obtained findings supportive of the presence of basolateral Cl− and K+ conductances in resting state bullfrog oxyntic cells (8).

Effect of forskolin and carbachol on parietal cell cytoplasmic volume. The application of 10−5 M forskolin, which at this concentration elicits a rapid stimulation of acid formation in cultured parietal cells, resulted in a rapid increase in cytoplasmic dye concentration with a maximum of 51 ± 6% after 120 s, indicative of cell shrinkage, followed by recovery to baseline within the next 6 min (Fig. 4A). The rapidity of shrinkage, which was much faster than the enlargement of intracellular vesicles, suggested to us that volume loss occurred secondary to opening of basolateral K+ channels in addition to apical Cl− and K+ channels. Barium, 5 mM, resulted in cell swelling and prevented forskolin-induced cell shrinkage (data not shown). Carbachol, 10−4 M, is a weaker agonist of acid formation in rabbit parietal cells, resulting in a rapid increase in cytoplasmic dye concentration with a maximum of 21 ± 6% after 120 s, indicative of cell shrinkage, followed by recovery to baseline within the next 6 min (Fig. 4A). The rapidity of shrinkage, which was much faster than the enlargement of intracellular vesicles, suggested to us that volume loss occurred secondary to opening of basolateral K+ channels in addition to apical Cl− and K+ channels. Barium, 5 mM, resulted in cell swelling and prevented forskolin-induced cell shrinkage (data not shown). Carbachol, 10−4 M, is a weaker agonist of acid formation in rabbit parietal cells, resulting in a rapid increase in cytoplasmic dye concentration with a maximum of 21 ± 6% after 120 s, indicative of cell shrinkage, followed by recovery to baseline within the next 6 min (Fig. 4A). This indicates that the magnitude of cell shrinkage correlates with the acid stimulatory capacity of the secretagogue. A comparison of Figs. 3C, 4C, and 6C demonstrates that the speed of recovery is not directly linked to the magnitude of cell shrinkage, because the recovery was slower after hyperosmolarity- compared with forskolin- or carbachol-associated shrinkage but may be influenced by direct agonist-induced activation of volume regulatory mechanisms. Again, 5 mM barium resulted in cell...
swelling and completely prevented carbachol- or forskolin-induced shrinkage (data not shown).

Effect of Na\textsuperscript{+}/H\textsuperscript{+} exchange and Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{–} cotransport inhibition on secretagogue-induced cytoplasmic volume changes. Na\textsuperscript{+}/H\textsuperscript{+} exchangers have been shown to be involved in RVI in several cell types (15, 20, 30). We have previously demonstrated that forskolin activates Na\textsuperscript{+}/H\textsuperscript{+} exchange in cultured parietal cells and that the involved isoforms are NHE1 and NHE4 (1). Since high DMA concentrations are needed to inhibit NHE4, we tested the effect of 500 µM DMA on forskolin-induced parietal cell volume changes. DMA reduced forskolin-induced cell shrinkage by ~25% (Fig. 4), as had been found with hyperosmolarity, inhibited the speed of volume recovery by 60%, and prolonged the time to complete recovery from ~6 min to ~16 min. A low concentration of DMA (10\textsuperscript{−}5 M) did not result in a significant reduction in the speed of volume recovery, suggesting that the effect of DMA is primarily due to Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition and not due to inhibition of an amiloride-sensitive Na\textsuperscript{+} channel. In many cell types, the bumetanide-sensitive Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{–} cotransporter, which is also expressed in the stomach (14), is involved in RVI. We therefore investigated whether inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{–} cotransport by 100 µM bumetanide had an effect on forskolin-induced parietal cell volume changes. Bumetanide had no significant effect on forskolin-induced shrinkage or recovery (Fig. 5) and, when added together with DMA, did not result in a stronger inhibition of recovery than DMA alone (Fig. 4C). Therefore, the Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{–}
cotransporter does not appear to regulate volume in acid-secreting parietal cells.

In a previous study, carbachol was found to primarily activate NHE1 (1). Therefore, we speculated that if carbachol-induced Na\(^+\)/H\(^+\) exchange activation was related to volume regulation, a selective inhibition of NHE1 would have a significant effect on cytoplasmic volume recovery after stimulation-associated shrinkage. Indeed, 1 μM Hoe-642, a substance that selectively inhibits NHE1 at this low concentration, inhibited the speed of volume recovery by 50% (Fig. 6). This indicates that carbachol-induced NHE1 activation in rabbit parietal cells is likely related to NHE1-mediated volume recovery after shrinkage.

DISCUSSION

In this study, we have shown that 1) isolated rabbit parietal cells in primary culture are able to regulate their cell volume after hyperosmolarity-induced cell shrinkage and depolarization-induced swelling; 2) secretagogue activation causes cytoplasmic shrinkage, from which parietal cells recover even more rapidly than after hyperosmolar shrinkage; and 3) Na\(^+\)/H\(^+\) exchange is one of the mechanisms of rabbit parietal cell RVI after secretagogue activation, whereas Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport appears not to be involved during RVI in this cell type.

Despite considerable scientific interest in the parietal cell pathways for ion and fluid movement, nothing is known about the ability of gastric parietal cells for volume regulation and the involved ionic mechanisms (20). This is probably due to the fact that membrane and cytoskeletal perturbances occur during any form of enzymatic parietal cell or gastric gland isolation and the freshly isolated cells swell. A few years ago, Chew and colleagues (5) developed a method for culture of

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**Fig. 4.** A: 10\(^{-5}\) M forskolin results in rapid cytoplasmic shrinkage, followed by recovery to baseline values. B–D: 500 μM DMA, which inhibits all forskolin-activated Na\(^+\)/H\(^+\) exchanger isoforms in rabbit parietal cells (1), results in a significant slowing of volume recovery after forskolin application, both in the absence and additional presence of 100 μM bumetanide. As observed with hyperosmolarity, 500 μM DMA also reduces the initial shrinkage from 21% to 14% (n = 5–7). *P < 0.05, **P < 0.01. Recovery slopes were measured within the time period of 450–650 s.
parietal cells for a period of several days and reported an increased sensitivity of these cultured cells to secretagogues. It was further shown that these cells develop a polarized distribution of membrane proteins and that they secrete acid into intracellular secretory vesicles whose membrane contains the apical proteins involved in HCl secretion and which enlarge during stimulation (4, 25, 41). Although this latter feature suggests that they do not fully resemble native parietal cells in situ, probably due to the lack of specialized cellular contacts, they proved useful for the study of signal transduction (4, 6, 7), cellular trafficking events (3), and gene transcription (17, 29). We adapted this technique for the fluorometric study of ion transport processes and found, for example, that the Na\(^+/\)H\(^+\) exchange rate stimulated by a given low pH\(_i\) was markedly higher in the cultured cells compared with freshly isolated cells (33, 36). Moreover, we found that, in contrast to the situation in freshly isolated parietal cells, secretagogues strongly stimulated Na\(^+/\)H\(^+\) exchange in cultured parietal cells (1, 37). Interestingly, a concomitant pH\(_i\) increase and proton efflux was only observed in the absence, not in the presence, of CO\(_2\)/HCO\(_3\)\. Since the latter is the physiological situation, this suggested to us that 1) the physiological significance of secretagogue-induced Na\(^+/\)H\(^+\) exchanger activation does not lie in an increase in pH\(_i\) and that 2) Cl\(^-/\)HCO\(_3\) exchange activation is likely occurring concomitantly with, and not sequentially to and caused by, Na\(^+/\)H\(^+\) exchange activation, as has been previously suggested (28). Since the concomitant activation of Na\(^+/\)H\(^+\) and Cl\(^-/\)HCO\(_3\) exchange is one mechanism for RVI, we speculated that parietal cells may shrink during stimulation of acid secretion and that the observed Na\(^+/\)H\(^+\) exchange activation was related to a parietal cell volume regulatory phenomenon during stimulation of acid secretion.

The present investigation demonstrates that secretagogue stimulation of cultured parietal cells does indeed result in a rapid cytoplasmic shrinkage, followed by volume recovery. It was not the aim of the present study to define the cellular mechanisms of this secretagogue-induced shrinkage, but we believe that the concomitant activation of basolateral K\(^+\) channels and apical K\(^+\) and Cl\(^-\) channels will be the predominant reason for cellular volume loss. Parietal cells possess both Ca\(^{2+}\)- and cAMP-activated basolateral K\(^+\) channel (43), at least one, possibly more, apical K\(^+\) channel (32), and a cAMP-activated (9, 23, 39) and possibly a Ca\(^{2+}\)-activated apical Cl\(^-\) channel (10), and each of our
agonists has likely activated a different set of channels. Interestingly, the shrinkage observed with forskolin and carbachol paralleled their acid stimulatory capacity in cultured gastric parietal cells (1), suggesting a causal relationship. DMA, 500 μM, caused a ~25% reduction of both hyperosmolarity- and forskolin-induced cell shrinkage, probably due to a direct or indirect, via a pH, decrease, effect on K⁺ channels. McLeod and Hamilton (22) have found that in isolated enterocytes, cellular volume decrease after nutrient-induced mild swelling requires Na⁺/H⁺ exchange activation and that the Na⁺/H⁺ exchanger-induced alkalinization was essential for the activation of a K⁺ channel. In cultured parietal cells, an alkalinization does not appear essential for cell shrinkage, since none occurs in the presence of 500 μM DMA, whereas the cells shrink to 75% of the control value.

Secretagogue-induced Na⁺/H⁺ exchange activation occurs concomitantly with the onset of cellular volume loss (1). Rabbit parietal cells express NHE1, NHE2, and NHE4 (35), and we have found that the forskolin-induced Na⁺/H⁺ exchange activation is mediated largely by NHE4 (1). The high DMA concentrations necessary to inhibit NHE4 reduced the speed of recovery by >60%, consistent with an important role of Na⁺/H⁺ exchangers in parietal cell volume regulation during acid secretion. However, this DMA concentration would likely also inhibit an epithelial sodium channel that has been described to mediate a part of volume recovery in hepatocytes (45) and, furthermore, exert secondary effects due to the strong pH, decrease during DMA treatment. Therefore, we also studied carbachol-induced volume changes in cultured rabbit parietal cells. We have previously shown that carbachol predominantly activates the NHE1 isoform in cultured rabbit parietal cells and that 1 μM Hoe-642 is able to inhibit this activation almost completely and very specifically (1). We have further found that in cultured parietal cells, even prolonged preincubation with 1 μM Hoe-642 does not influence basal- and forskolin-stimulated acid formation and that the pH, decrease observed under 1 μM Hoe-642 is rather mild and transient (40). Thus we speculated that if the carbachol-induced NHE1 activation was related to a volume regulatory phenomenon, we may inhibit the RVI after carbachol-induced shrinkage by selectively

Fig. 6. Carbachol predominantly activates NHE1 in rabbit parietal cells (1). Hoe-642, 1 μM, which very specifically inhibits NHE1, markedly slows volume recovery after carbachol-induced cytoplasmic shrinkage (B and C). Unlike 500 μM DMA, 1 μM Hoe-642 does not reduce carbachol-induced shrinkage. This demonstrates that the carbachol-induced NHE1 activation in rabbit parietal cells is indeed related to shrinkage-associated volume recovery (n = 5). *P < 0.05. Recovery slopes were measured within the time period of 250–400 s.
inhibiting NHE1. This was indeed the case. Therefore, the results of this study strongly support the concept that, in rabbit parietal cells, the NHE isoforms activated during stimulation of acid secretion play an important role in the volume regulation necessary during acid secretion. The fact that the activation of Na+/H+ exchange is not accompanied by a pHi increase demonstrates that a Cl-/HCO3- exchange process, likely the AE2 isoform, which is strongly expressed in rabbit parietal cells (34, 42), is activated concomitantly.

However, volume recovery was not completely prevented by Na+/H+ exchange inhibition, indicating that other mechanisms may exist for a RVI in rabbit parietal cells. Inhibition of the bumetanide-sensitive Na+-K+-2Cl- cotransporter NKCC did not result in an additional inhibition of volume recovery, suggesting that in rabbit parietal cells, this transporter is not involved in secretagogue-associated volume regulation. We also observed no effect of bumetanide on forskolin-stimulated acid formation in cultured rabbit parietal cells, and although bumetanide causes a marked reduction in short-circuit current isolated in inflated mouse gastric mucosa in the Ussing chamber, it has no effect on forskolin-stimulated acid secretion (data not shown). This suggests that although NKCC is clearly involved in gastric Cl- transport, it is not a Cl- supply mechanism in acid-secreting parietal cells, as has been speculated (18). This is further supported by the normal gastric pH of NKCC knockout mice (12). Recent immunohistochemical data show that NKCC1 antibodies stain only parietal cells in the base of the glands and that these parietal cells do not stain with AE2 antibodies, suggesting that they may not mediate HCl secretion but may instead mediate NaCl or KCl secretion (26). Since we have chosen parietal cells that responded to acid secretagogues with an increase in the size of their intracellular vesicles, we may have selected parietal cells that secrete acid, as was our aim, and thus not studied those parietal cells that express an NKCC.

Additional putative mechanisms for the DMA-insensitive volume increase are 1) the Na+-HCO3- cotransporter Nbc1, which is expressed in these cells and is activated by a low pHi (33) but whose role in volume regulation is as yet speculative, 2) nonspecific cation channels, 3) the H+-K+-ATPase, which reabsorbs K+ ions from the secretory vesicular space, or 4) the fact that after stimulation and low-pH-associated depolarization, cells gradually re-swell because the equilibrium potential for Cl- is reversed.

In summary, this study demonstrates that cultured parietal cells are a useful model to study parietal cell volume regulatory mechanisms. Agonists of acid secretion cause a rapid cytoplasmic shrinkage, followed by volume recovery, which is predominantly mediated by Na+/H+ and Cl-/HCO3- exchange.

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