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Local and global calcium signals and fluid and electrolyte secretion in mouse submandibular acinar cells

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Hamer, A. R., P. M. Smith, and D. V. Gallacher. Local and global calcium signals and fluid and electrolyte secretion in mouse submandibular acinar cells. Am J Physiol Gastrointest Liver Physiol 288: G118–G124, 2005. First published August 12, 2004; doi: 10.1152/ajpgi.00096.2004. — Polarized Ca2+ signals that originate at and spread from the apical pole have been shown to occur in acinar cells from lacrimal, parotid, and pancreatic glands. However, “local” Ca2+ signals, that are restricted to the apical pole of the cell, have been previously demonstrated only in pancreatic acinar cells in which the primary function of the Ca2+ signal is to regulate exocytosis. We show that submandibular acinar cells, in which the primary function of the Ca2+ signal is to drive fluid and electrolyte secretion, are capable of both Ca2+ waves and local Ca2+ signals. The generally accepted model for fluid and electrolyte secretion requires simultaneous Ca2+-activation of basally located K+ channels and apically located Cl− channels. Whereas a propagated cell-wide Ca2+ signal is clearly consistent with this model, a local Ca2+ signal is not, because there is no increase in intracellular Ca2+ concentration at the basal pole of the cell. Our data provide the first direct demonstration, in submandibular acinar cells, of the apical and basal location of the Cl− channel (14, 18) and an apical Cl− channel (13, 18) and an apical Cl− channel on the grounds that activation of the K+ channel is necessary to maintain the driving force for Cl− efflux from the cell (21, 26). It follows that a Ca2+ signal restricted to the apical pole would not activate the Ca2+-dependent K+ channel, and thus fluid secretion could not occur because of the lack of driving force (6).

Previous work has indicated that, in pancreatic acinar cells, global Ca2+ signals in lacrimal (25, parotid (6, 11), and submandibular (10) acinar cells start from the apical pole of the cell. What is not known is whether Ca2+ signals in fluid secreting acinar cells invariably propagate across the whole cell or whether they may be spatially restricted to the apical pole. A recent study (6) indicates that the former is the case in rat parotid acinar cells. We have used a combination of patch-clamp and microfluorimetry techniques to address this question in mouse submandibular acinar cells. The pulse protocol variant of the patch-clamp whole cell technique allows near simultaneous measurement of both the Ca2+-activated Cl− conductance and the Ca2+-activated K+ conductance. Thus we can simultaneously in [Ca2+], on the Ca2+-dependent ion channels that underlie secretion.

MATERIALS AND METHODS

Adult male CD1 mice were humanely killed by stunning and cervical dislocation [Schedule 1 to the Animals (Scientific Procedure...
Submandibular cells were isolated by collagenase (Worthington Biochemical, Lakewood, NJ) digestion in extracellular medium containing 1 mM Ca²⁺, followed by a short treatment (0.5–2 min) with 0.8 mg/ml trypsin as described previously (20). After dispersal, cells were suspended in serum-free 50:50 DMEM/Ham’s F-12 medium (GIBCO-BRL, Life Technologies, Paisley, UK) and were used within 3 h.

For each experiment, cells were allowed to settle onto a poly-l-lysine-coated coverslip that formed the base of a perfusion chamber and was placed on the stage of an inverted microscope (model TMD 100; Nikon, Kingston Upon Thames, UK) Cells were superfused continuously at 0.5 ml/min from one of several parallel superfusion pipettes. The solution bathing the cell could be changed in 1 to 2 s. All experiments were carried out at 24 ± 2°C.

A monochromater (Applied Imaging, Sunderland, UK) provided excitation light at 340 and 380 nm, and images of light emitted at 510 nm were captured using a charge-coupled device camera (Photonic Science, Robertsbridge, Sussex, UK). Pairs of images were captured every 120 to 500 ms, depending on the degree of time averaging used. Ratioed images were generated on-line and were subsequently saved and analyzed using the QuantiCell 700 M software package (Applied Imaging). [Ca²⁺]ᵢ was calculated from this ratio using the Grynkiewicz equation. Images presented in the figures have been background corrected and median filtered to remove noise. The cells were loaded with fura-2 either by incubation for 5–10 min in the presence of 1 μM fura-2 AM (Molecular Probes, Cambridge Bioscience, Cambridge, UK) or with fura-free acid (Molecular Probes) via the patch pipette.

The patch-clamp whole cell configuration was achieved with single cells using 2 to 4 MΩ patch-clamp pipettes pulled from borosilicate glass capillaries (Clark Electromedical Instruments, Reading, UK). Access resistance through the patch-clamp pipette was 3 times that of the pipette itself. Cells were voltage clamped to −40 mV using an Axopatch 200a patch-clamp amplifier (Axon Instruments, Foster City, CA). K⁺ and Cl⁻ currents were measured separately by pulsing to 0 mV and −80 mV, respectively, for 100 ms twice a second. Currents were digitized using the CED 1401 interface (Cambridge Electronics Design, Cambridge, England) and were stored and analyzed using a personal computer with custom written software (20).

The patch-clamp pipette contained (in mM) 140 KCl, 1.13 MgCl₂, 10 glucose, 0.5 EGTA, and 1 ATP, buffered to pH 7.4 with 10 HEPES. Where indicated in the text and figure legends, the pipette also contained fura-2 AM (100 μM) and/or inositol(1,4,5)trisphosphate [Ins(1,4,5)P₃], 50 μM. Ins(1,4,5)P₃ was a gift from Robin Irvine, Cambridge, UK.

The extracellular bathing solution contained (in mM) 140 NaCl, 4.7 KCl, 1.13 MgCl₂, 1.2 CaCl₂, and 10 glucose, buffered to pH 7 with 10 HEPES.

Unless otherwise indicated, drugs and chemicals were obtained from Sigma (Poole, Dorset, UK) and BDH (Lutterworth, Leicester, UK).

Many of the experiments shown here take advantage of access to the cell interior offered by the patch-clamp whole cell technique to infuse substances into the cell. In all such experiments, these substances were added to the intracellular medium at the start of the experiment and thus entered the cell as soon as the breakthrough to the whole cell condition was achieved.

RESULTS

We have measured changes in [Ca²⁺]ᵢ in acutely isolated submandibular cells in response to ACh stimulation at concentrations between 5 and 5,000 nM. The data in Fig. 1 are typical of the changes in [Ca²⁺]ᵢ and both K⁺ and Cl⁻ currents seen in response to stimulation with an intermediate concentration (20–500 nM) of ACh. Fig. 1A shows a Ca²⁺ image map of two adjacent fura-1 AM-loaded cells. The apical region of the

Fig. 1. Global changes in [Ca²⁺]ᵢ and ion current activation after stimulation by 50 nM ACh. A: image maps of [Ca²⁺]ᵢ at 0.5-s intervals immediately after stimulation. Both cells of a 2-cell cluster were loaded using fura-2 AM. B: bright-field image showing the location of the apical (a) and basal (b) regions of the cells in which [Ca²⁺]ᵢ was sampled in C. C and D: numbered arrows indicate time points at which first and last images in A were sampled. C: average change in [Ca²⁺]ᵢ within the apical (a) and basal (b) regions. D: K⁺ and Cl⁻ currents. Dashed line indicates the zero current point.
acinar cells was determined by observing the location of the secretory vesicles in the bright-field image (Fig. 1B). When measurements were made from two or more adjacent cells, the apical region invariably included the common border between the cells. The ACh-evoked increase in [Ca\(^{2+}\)]\(_i\) may be seen to start at the apical pole of each cell and then to spread rapidly throughout the rest of the cell. This pattern of Ca\(^{2+}\) signal increased the magnitude of both the K\(^+\) and the Cl\(^-\) current (Fig. 1D). Close examination of the trace reveals a pulsatile component in both currents, although this is most clearly evident in the Cl\(^-\) current. In a series of 30 microfluorimetric experiments that includes those in which ion currents were simultaneously measured using patch-clamp (9:30) technique, this pattern of response was elicited 3:10 times using 20 nM ACh, 9:15 times using 50 nM ACh, and 10:17 times using 500 nM ACh. In a concurrent series of patch-clamp experiments, this pattern of response was elicited 2:8 times using 5 or 10 nM ACh, 7:16 times using 20 nM ACh, 15:24 times using 50 nM ACh, and 3:9 times using 500 nM ACh.

These data confirm the now well-established observations that Ca\(^{2+}\) signals in polarized acinar cells originate at the apical pole of the cell. Furthermore, these data show that “global” Ca\(^{2+}\) signals in these cells take the form of a Ca\(^{2+}\) wave propagating from an initiation site at the apical pole to the basolateral pole. Stimulation may produce a single Ca\(^{2+}\) wave or a sequence of Ca\(^{2+}\) waves, usually on top of a global elevation in [Ca\(^{2+}\)]\(_i\). These data also show that global Ca\(^{2+}\) signals give rise to activation of both the Ca\(^{2+}\)-dependent Cl\(^-\) channel and the Ca\(^{2+}\)-dependent K\(^+\) channel. A single Ca\(^{2+}\) wave resulting in a sustained increase of [Ca\(^{2+}\)]\(_i\), with consequent ion channel activation was rarely observed after stimulation by ACh at 50 nM or less (1:25). A sustained response was more common after stimulation using 500 nM ACh (7:17) and inevitable after stimulation with 5 μM ACh (23:23) (22).

A typical response to a low dose of ACh (5–50 nM) is shown in Fig. 2. In this experiment, one of several adjacent cells was loaded with fura-2 through the patch pipette. The train of brief, transient, increases in [Ca\(^{2+}\)]\(_i\), evoked by ACh did not spread from the apical pole of the cell (Fig. 2, A and C). The patch-clamp data (Fig. 2D) show activation of the Cl\(^-\) current only. Comparison of the data in Fig. 2C to that in Fig. 2D shows a clear temporal correspondence between the peaks of the Ca\(^{2+}\) signal and those of the Cl\(^-\) current. There was a small, gradual increase in [Ca\(^{2+}\)]\(_i\) at the basal pole of the cell, which did not increase the K\(^+\) current. In a series of 30 microfluorimetric experiments, which includes those in which ion currents were simultaneously measured using patch-clamp techniques, responses that were largely or completely restricted to the apical pole of the cell were observed 7:10 times using 20 nM ACh, 5:15 times using 50 nM ACh, and 0:17 times using 500 nM ACh. In a concurrent series of patch-clamp experiments, responses that were largely or completely manifest in the Cl\(^-\) current were observed: 6:8 times using 5 or 10 nM ACh.

![Fig. 2. Localized changes in [Ca\(^{2+}\)]\(_i\), and ion current activation after stimulation by 20 nM ACh.](image-url)
ACh, 9:16 times using 20 nM ACh, 8:24 times using 50 nM ACh, and 0:9 times using 500 nM ACh.

Our data clearly show Ca<sup>2+</sup> signals that remain localized to the apical pole of the cell and that are sufficient to Ca<sup>2+</sup>-activate the Cl<sup>-</sup> but not the K<sup>-</sup> channels.

Data similar to that shown in Fig. 2 were obtained by infusion of 50 μM Ins(1,4,5)P<sub>3</sub> into the cell via the patch-clamp pipette and are shown in Fig. 3. In this experiment, one of three adjacent cells was loaded with fura-2 through the patch-clamp pipette. Again, the brief transient increases in [Ca<sup>2+</sup>]<sub>i</sub> were restricted to the apical pole of the cell and caused activation of the Cl<sup>-</sup> and not the K<sup>-</sup> current. The position of the pipette on the cell is visible as a “hot spot” on the Ca<sup>2+</sup> image. It should be noted that the pulsatile Ca<sup>2+</sup> signal in these cells occurred at the apical region of the cell and not around the pipette in which the Ins(1,4,5)P<sub>3</sub> concentration would be greatest. Identical results were obtained in 3:3 experiments in which high-time resolution currents were measured (<i>n</i> = 12). These data are consistent with an apical location of the Cl<sup>-</sup> channels and a predominantly basolateral location of the K<sup>-</sup> channels.

Figure 5A shows a series of transient activations of both the K<sup>-</sup> and Cl<sup>-</sup> currents elicited by 20 nM ACh stimulation and channel. The time lag between activation of the Cl<sup>-</sup> current and activation of the K<sup>-</sup> current (0.5–1 s) corresponds to the time required for the Ca<sup>2+</sup> wave to cross the cell (see Fig. 1).

The time lag between activation of the Cl<sup>-</sup> current and activation of the K<sup>-</sup> current was observed in every experiment in which high-time resolution currents were measured (<i>n</i> = 12).

Figure 4 shows a high-resolution patch-clamp recording of a single transient seen in response to stimulation by 50 nM ACh in which both the K<sup>-</sup> and the Cl<sup>-</sup> currents were activated. It is clear that activation of the Cl<sup>-</sup> channel precedes that of the K<sup>-</sup> channel. The time lag between activation of the Cl<sup>-</sup> current and activation of the K<sup>-</sup> current (0.5–1 s) corresponds to the time required for the Ca<sup>2+</sup> wave to cross the cell (see Fig. 1).

The time lag between activation of the Cl<sup>-</sup> current and activation of the K<sup>-</sup> current was observed in every experiment in which high-time resolution currents were measured (<i>n</i> = 12).

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These data are consistent with an apical location of the Cl<sup>-</sup> channels and a predominantly basolateral location of the K<sup>-</sup> channels.
Local Ca\(^{2+}\) Signals and Salivary Gland Fluid Secretion

**Fig. 5.** Voltage-clamp and current-clamp recordings of global and local transient current activation. **A:** voltage-clamp recording showing a series of transient global responses stimulated by 20 nM ACh in which both the K\(^+\) and Cl\(^-\) current were activated. **B:** current-clamp recording of the same cell as in **A** showing an initial depolarization followed by a rapid hyperpolarization of membrane potential. **C:** voltage-clamp recording showing a series of transient local responses stimulated by 10 nM ACh in which only the Cl\(^-\) current was activated. **D:** current-clamp recording of the same cell as in **C** showing a depolarization of membrane potential. The dashed lines in **A** and **C** indicate the zero current position.

measured under voltage-clamp conditions. Figure 5B shows the next transient measured under current-clamp conditions. The current-clamp recordings of the transient has two components: an initial depolarization, which follows activation of the Cl\(^-\) channels, and then a massive hyperpolarization as the K\(^+\) channels open. The same protocol was used to record the data in Fig. 5, C and D. Figure 5C shows a voltage-clamp recording in response to stimulation by 10 nM ACh in which only the Cl\(^-\) current was activated. The next transient in the sequence, measured under current-clamp conditions shows only transient depolarization of the membrane potential. The depolarization of the membrane potential is clearly due to activation of the Cl\(^-\) channel; however, the repolarization could be due to inactivation of the Cl\(^-\) channels or to activation of K\(^+\) channels. At present, we are unable to distinguish between these alternatives.

**DISCUSSION**

Our data address two aspects of the Ca\(^{2+}\) signaling process in fluid-secreting exocrine acinar cells. First, whether acinar cells that employ Ca\(^{2+}\) signaling to regulate fluid secretion are, like pancreatic acinar cells, capable of localized Ca\(^{2+}\) signals or whether Ca\(^{2+}\) signals in these cells, once initiated, must invariably propagate as a global signal across the cell. Second, if local Ca\(^{2+}\) signals do occur in fluid secreting acinar cells, are they capable of supporting the fluid secretion process?

Our data show first that all agonist or Ins(1,4,5)P\(_3\)-evoked Ca\(^{2+}\) signals originate at the apical pole of mouse submandibular cells. The response to intermediate or high concentration of agonist or Ins(1,4,5)P\(_3\) takes the form of a Ca\(^{2+}\) wave that moves rapidly from the apical to the basal pole. This has been previously observed in all exocrine acinar cells, but what has not been shown previously is the precise correspondence between propagation of the wave across the cell and activation of the K\(^+\) and Cl\(^-\) currents. As shown in Fig. 4, activation of the Cl\(^-\) current occurs simultaneously with initiation of the Ca\(^{2+}\) signal at the apical pole, and activation of the K\(^+\) current occurs only when the Ca\(^{2+}\) wave reaches the basolateral region. These data indicate that the Cl\(^-\) channels are apically located and that the K\(^+\) channels are basolaterally located. This distribution of the channels has been assumed for many years and is fundamental to most models of fluid secretion; however, it has never actually been shown in a fluid-secreting acinar cell type (28).

Further confirmation that the apical membrane of the cell contains only Cl\(^-\) channels is shown by the response of the cells to stimulation by low concentrations of agonist or Ins(1,4,5)P\(_3\). This gentle stimulation produced repeated small increases in [Ca\(^{2+}\)], at the apical pole only and simultaneous activation of the Cl\(^-\) current only. Furthermore, the two were precisely synchronized. Such a precise temporal correspondence can result only from a close spatial congruence between the localized Ca\(^{2+}\) signal and the Ca\(^{2+}\)-activated Cl\(^-\) channels. Localized Ca\(^{2+}\) signals, which do not spread from the apical pole of the cell, have not previously been observed in fluid-secreting acinar cells.

Our first conclusion is, therefore, that mouse submandibular acinar cells, unlike rat parotid acinar cells (6), may be shown to produce localized Ca\(^{2+}\) signals. The implication of this conclusion is that localized Ca\(^{2+}\) signals are not solely the province of pancreatic exocrine acinar cells (24) in which their primary function is to regulate protein secretion (12), but rather that local Ca\(^{2+}\) signals may be associated with both protein and fluid secretion. Obligate global Ca\(^{2+}\) signals are not, therefore, a characteristic of fluid secreting acinar cells.

Having shown that fluid secreting acinar cells are capable of local Ca\(^{2+}\) signals that give rise to activation of the Cl\(^-\) and not the K\(^+\) current, the second question is whether these Ca\(^{2+}\)
signals can serve any useful purpose in the regulation of fluid secretion. The conventional model for fluid secretion (21, 26) is that simultaneous, Ca\(^{2+}\)-dependent activation of the K\(^+\) and Cl\(^-\) channels permits Cl\(^-\) efflux across the apical membrane (which drags Na\(^+\) across the tissue to preserve electroneutrality and then fluid by osmosis), whereas K\(^+\) efflux across the basolateral membrane preserves the driving force for Cl\(^-\) efflux. At first glance, it would appear that activation of the Cl\(^-\) current alone would not be sufficient to support fluid secretion. However, closer examination of the model indicates that this may not be the case.

First, Cl\(^-\) efflux from the cell will occur for as long as the membrane potential is more negative than the Nernst or reversal potential for Cl\(^-\). There is no requirement for increased K\(^+\) conductance in order for this condition to be met. The observation that resting acinar cells have a K\(^+\)-sensitive cell negative membrane potential in the absence of stimulation (4, 19) indicates that these cells have a significant K\(^+\) conductance at rest. Only if this K\(^+\) conductance vanished or was displaced by, for example, a Na\(^+\) or nonselective conductance could the membrane potential fall to the Nernst potential for Cl\(^-\). Our patch-clamp data demonstrate that neither of these phenomena occurs.

The second reason why fluid secretion may occur after Ca\(^{2+}\)-activation of the Cl\(^-\) conductance only is that the K\(^+\) channel in submandibular acinar cells is voltage as well as Ca\(^{2+}\) activated (4). Our patch-clamp experiments are conducted under voltage-clamp conditions that prevent stimulus-evoked changes in membrane potential. These are optimal conditions for showing Ca\(^{2+}\) activation of ion channels and show that the K\(^+\) channel is not Ca\(^{2+}\)-activated by local Ca\(^{2+}\) signals. Under open-circuit conditions, the situation that prevails in vivo, Ca\(^{2+}\) activation of the Cl\(^-\) channels will depolarize the membrane potential, and this depolarization will itself activate the K\(^+\) channels. An agonist-evoked membrane depolarization of 20 mV (see Fig. 5) could increase the K\(^+\) conductance 5- to 10-fold (4). In short, depolarization of the membrane potential in submandibular cells is a self-limiting phenomenon, even in the absence of a global Ca\(^{2+}\) signal, because it stimulates increased K\(^+\) channel activity and membrane repolarization.

Distinctive “signature” patterns in Ca\(^{2+}\) signaling have been and continue to be reported in a wide range of cell types (1–3, 8, 9, 15, 23). There are many factors that determine the spatial and temporal characteristics of a Ca\(^{2+}\) signal, including the agonist and the second messengers that initiate the Ca\(^{2+}\) cascade (2) and cellular geometry, including the state and distribution of organelles, especially the endoplasmic reticulum (7) and mitochondria (8, 9). However, it is as important to determine whether a particular signature Ca\(^{2+}\) signal can support the primary function of a cell as it is to identify the Ca\(^{2+}\) signature in the first place. The most simple argument to justify local, apical, Ca\(^{2+}\) signals in acinar cells is that secretion of both protein and fluid occurs at this pole of the cell. Cell-wide increases in [Ca\(^{2+}\)], may be seen as an unnecessary energetic expense (24). We extend the range of Ca\(^{2+}\) signaling signatures seen to fluid-secreting acinar cells by showing submandibular acinar cells to be capable of highly localized Ca\(^{2+}\) signals. Furthermore, we show how the impact of an apically restricted Ca\(^{2+}\) signal may extend to the whole cell by means of a transient depolarization of membrane potential mediated by activation of apically located Cl\(^-\) channels. Fluid secretion is clearly supportable under these conditions, and therefore a Ca\(^{2+}\) signal that originates from and is restricted to the apical pole of the cell is therefore a useful signature signal in fluid secreting acinar cells.

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GRANTS

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