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Initiation Site of Ca^{2+} Entry Evoked by Endoplasmic Reticulum Ca^{2+} Depletion in Mouse Parotid and Pancreatic Acinar Cells

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Purpose: In non-excitable cells, which include parotid and pancreatic acinar cells, Ca^{2+} entry is triggered via a mechanism known as capacitative Ca^{2+} entry, or store-operated Ca^{2+} entry. This process is initiated by the perception of the filling state of endoplasmic reticulum (ER) and the depletion of internal Ca^{2+} stores, which acts as an important factor triggering Ca^{2+} entry. However, both the mechanism of store-mediated Ca^{2+} entry and the molecular identity of store-operated Ca^{2+} channel (SOCC) remain uncertain. **Materials and Methods:** In the present study we investigated the Ca^{2+} entry initiation site evoked by depletion of ER to identify the localization of SOCC in mouse parotid and pancreatic acinar cells with microfluorometeric imaging system. **Results:** Treatment with thapsigargin (Tg), an inhibitor of sarco/ endoplasmic reticulum Ca^{2+}-ATPase, in an extracellular Ca^{2+} free state, and subsequent exposure to a high external calcium state evoked Ca^{2+} entry, while treatment with lanthanum, a non-specific blocker of plasma Ca^{2+} channel, completely blocked Tg-induced Ca^{2+} entry. Microfluorometric imaging showed that Tg-induced Ca^{2+} entry started at a basal membrane, not a apical membrane. **Conclusion:** These results suggest that Ca^{2+} entry by depletion of the ER initiates at the basal pole in polarized exocrine cells and may help to characterize the nature of SOCC.

Key Words: Parotid, Ca^{2+} signaling, store-operated calcium channel

INTRODUCTION

The process of cellular Ca^{2+} signaling involves regulated changes in the concentration of Ca^{2+} in the cytoplasm ($[Ca^{2+}]_i$) as well as other cellular compartments. A multitude of cellular processes are controlled through Ca^{2+} signaling and, in turn, a multitude of external cellular signals induce or regulate Ca^{2+} signaling. Because so many systems respond to or regulate Ca^{2+} signaling, it is not surprising that dysfunctions of various aspects of Ca^{2+} signaling pathways underlie several important diseases.[1] When Ca^{2+} signaling is stimulated in a cell, Ca^{2+} enters the cytoplasm from one of two general sources: it is either released from intracellular Ca^{2+} stores or enters the cell across the plasma membrane. Both processes often occur simultaneously or sequentially. In all non-excitable cells, and some excitable cells, an important initiating step is the intracellular release of Ca^{2+} from internal stores by binding of a second messenger to its receptor in the endoplasmic reticulum (ER). Commonly, this messenger is inositol 1,4,5-trisphosphate (IP_3),[2] but a number of other potential messengers have been discovered in recent years.[3,4] Ca^{2+} entry can be signaled by a variety of processes, including direct activation by surface receptors and activation by a variety of second messengers;[5] however, the most commonly observed mechanism of regulated Ca^{2+} entry in non-excitable cells is a process knows as capacitative Ca^{2+} entry or store-operated Ca^{2+} entry (SOCE).[6,7] In many non-excitable cells, depletion of intracellular Ca^{2+} stores by IP_3 is the primary mechanism by which cell surface receptors activate Ca^{2+} influx. This phenomenon, which is termed capacitative Ca^{2+} entry,[8] has been identified in the control of Ca^{2+} oscillations,[9] secretion,[10] and enzymatic regulation.[11] Despite

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the wide range of processes in which SOCE is involved, the signal mechanism that couples store depletion to Ca^{2+} entry has not yet been fully identified.[8] The exocrine acinar cells of the pancreas and the parotid gland are classic examples of non-excitable cells whose key physiological activities are known to be dependent on $[Ca^{2+}]_i$ signals that involve a component of Ca^{2+} entry.[12,13] To determine the site of initiation of store- operated Ca^{2+} entry in non-excitable cells, we investigated the initiation site of SOCE in parotid and pancreatic acinar cells. Our results suggest that the commencement site of SOCE has significance for the future study of the physiological importance of SOCE in parotid and pancreatic acinar cells.

MATERIALS AND METHODS

Preparation of parotid and pancreatic acinar cells from mice

ICR strain mice (23 to 28 g) were sacrificed by cervical dislocation. Cells were prepared from the parotids and pancreases of ICR mice by limited collagenase digestion as previously described.[14] In order to achieve a pure isolation of acinar cells, density gradient centrifugation was performed with Accudenz (Accurate Chemical and Scientific corp., Westbury, NY, USA), and pure acinar cells were confirmed via light microscope.[15] After isolation, the acinar cells were resuspended in an extracellular physiologic salt solution (PSS: NaCl, 140 mM; KCl, 5 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1 mM; HEPES, 10 mM; and glucose, 10 mM, titrated to pH 7.4 with NaOH). The osmolality of the extracellular solution (measured with a FISKE 110 osmometer) was 310 mOsm.

$[Ca^{2+}]_i$ measurement

Cells were incubated for 40 min in PSS containing 5 μM fura 2-acetoxymethyl ester (Teflabs Inc., Austin, TX, USA) with Pluronic F-127 to enhance dye loading. Changes in $[Ca^{2+}]_i$ were measured by means of fura 2 fluorescence, with excitation wavelengths of 340 nm and 380 nm, and an emission wavelength of 510 nm at room temperature. Background fluorescence was subtracted from the raw signals at each excitation wavelength prior to calculating the fluorescence ratio, which was as follows: Ratio = F340/F380. The emitted fluorescence was monitored with a CCD camera (Photon Technology International Inc., Lawrenceville, NJ, USA) attached to an inverted microscope. Fluorescence images were obtained at 0.14s intervals.[16] The endoplasmic reticulum of the each cell was emptied with 1 μM of thapsigargin in a Ca^{2+} free state then stimulated by 7.5 mM external calcium.

RESULTS

In order to find the initiation sites of store-operated Ca^{2+} entry (SOCE) in mouse parotid and pancreatic acinar cells, we measured $[Ca^{2+}]_i$ increase as follows. The initial step to trigger the SOCE was conducted by depletion of intracellular Ca^{2+} stores. For this purpose, the endoplasmic reticulium (ER) in parotid and pancreatic acinar cells were depleted by 1 μM thapsigargin (Tg), an inhibitor specific to sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), in the absence of Ca^{2+}. To confirm whether ER was definitely emptied by Tg, cells were stimulated with 1 mM carbachol in a Ca^{2+} free state after depletion of ER. When a cellular response to carbachol was no longer observed, the cells were incubated with 7.5 mM external calcium for 200 to 400 s so as to trigger SOCE. As a result, we confirmed that vacancy of ER is a kind of signal occurring Ca^{2+} entry (Fig. 1Aa and Ba. n = 5). However, this finding required us to determine whether the late $[Ca^{2+}]_i$ increases were accomplished across the plasma membrane. Thus, lanthanum (La^{3+}), a non-specific blocker of plasma Ca^{2+} channel, was used to elucidate whether Tg-induced Ca^{2+} increases were indeed from extracellular sources. Both cell types were treated by 1 μM Tg to deplete internal Ca^{2+} stores, and the cells were then treated with 1 mM La^{3+} for 3 min prior to exposure 7.5 mM external Ca^{2+}. We found that the Ca^{2+} input from external Ca^{2+} had nearly disappeared in both mouse parotid and pancreatic acinar cells (Fig. 1Ab and Bb).

Next, to identify the initiation sites of SOCE, the initiation site of Tg-induced Ca^{2+} entry was imaged with $fura^2$ fluorescence. The Ca^{2+} response

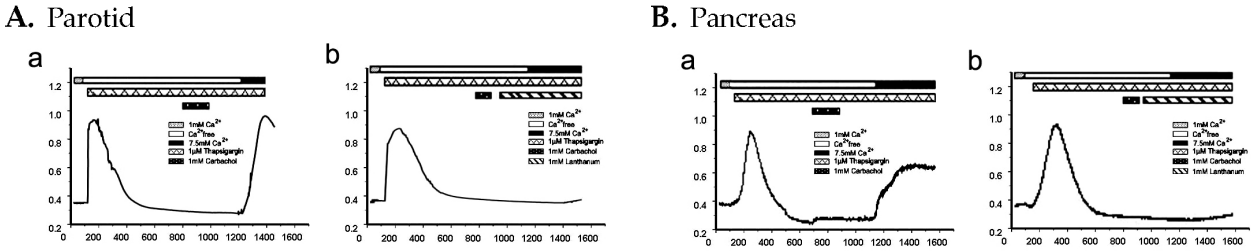

Fig. 1. Measurement of thapsigargin-induced $[Ca^{2+}]_i$ increases. A (a) and B (a): parotid and pancreatic acinar mouse cell responses, respectively, after depletion of internal Ca^{2+} stores with 1 μM thapsigargin (Tg) in nominally Ca^{2+}-free media. Cells were stimulated with 1mM carbachol, and then thapsigargin-induced $[Ca^{2+}]_i$ increases were measured by exposure to external 7.5 mM Ca^{2+}. A (b) and B (b): parotid and pancreatic acinar mouse cell responses, respectively, after the cells were treated with 1 mM lanthanum before exposure to external high Ca^{2+}. Each result was the Trepresentative of 5 independent experiments.

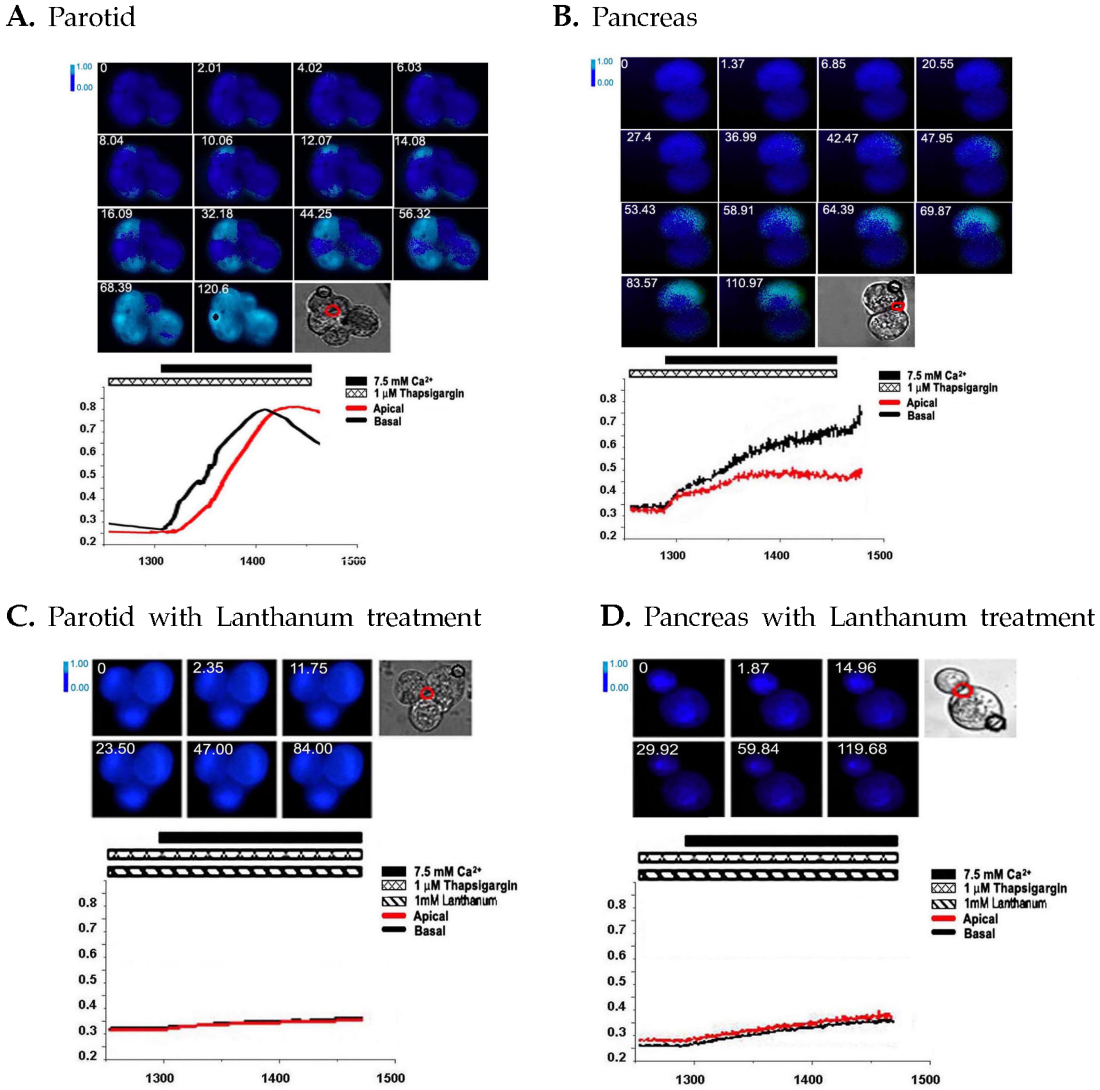

Fig. 2. Measurement of the initiation site of Tg-induced $[Ca^{2+}]_i$ increases. (A) and (B) parotid and pancreatic acinar mouse cell responses, respectively. Initiation site of Tg- induced $[Ca^{2+}]_i$ increases were imaged with fura2 fluorescence. (C) and (D) The cells were treated with 1 mM lanthanum before exposure to external high Ca^{2+}. In each transmission image, red and black circles shows apical and basal pole, respectively. Each result was the representative of 5 independent experiments.

generated by the depletion of internal Ca^{2+} store was not initiated at apical membrane (AM), but rather basal membrane (BM) in parotid and pancreatic acinar cells (Fig. 2A and B, n = 5). In addition, the propagation direction of the initiated Ca^{2+} entry was from the BM to the AM. There were no differences in Ca^{2+} entry patterns between parotid acinar cells and pancreatic acinar cells: however, the time scores for Ca^{2+} entry were different between the two cell types. The half time value of Ca^{2+} entry (T1/2) in parotid acinar cells was 55.38 ± 6.63 sec following exposure to a 7.5 mM Ca^{2+} solution, while the T1/2 in pancreatic acinar cells was 74.28 ± 6.80 sec., suggesting that the Ca^{2+} entry in parotid acinar cells was faster than that in pancreatic acinar cells.

DISCUSSION

In the present work, we investigated the location of the initiation site of SOCE in mouse parotid and pancreatic acinar cells, as identification of this site may be helpful to identify the nature of SOCC. We showed that the store-regulated Ca^{2+} response initiated at BM of mouse parotid and pancreatic acinar cells after ER depletion by Tg and stimulation by external Ca^{2+}. The mechanism by which Tg effectively empties intracellular Ca^{2+} stores in exocrine gland acinar cells is through its ability to completely suppress the SERCA.[7] Moreover, the Ca^{2+} response from an external source was diminished when cells were treated with La^{3+} prior to exposure to external Ca^{2+}, suggesting that Tg-induced Ca^{2+} entry is mediated by SOCE. Therefore, it is a notable discovery that SOCE initiation begins at the BM in the cells, since there are many Ca^{2+}-signaling related proteins in the apical region in exocrine acinar cells.[17]

Exocrine acinar cells such as salivary gland acinar cells are structurally and functionally polarized between the basal region and apical region.[18,19] The receptors for secretagogues are localized in the basal region, whereas the apical region has a high density of secretory granules that are ultimately released by exocytosis at the apical plasma membrane.[20] Many studies have shown that the increase in cytoplasmic free Ca^{2+} is initiated at the apical region and propagated to the basal region through a Ca^{2+} wave.[21-23] Therefore, it is likely that in polarized exocrine cells, other Ca^{2+} signaling proteins together with SOCC exist in the basal region of the cells. Recently, a strong candidate and regulator of SOCC were reported as Orai1 and stromal- interaction molecule 1 (STIM1), respectively.[24,25] Therefore, it will be necessary to investigate the localization of Orai1 and STIM1 in exocrine gland acinar cells.

Our findings are important, as SOCCs play a fundamental role in both the immediate and long-term regulation of cells. It follows that unexpected perturbations in this process may have the potential for pathological outcomes, while planned pharmacological manipulations may find clinical utility in certain disease states. We look forward to increasing the body of information on the role of SOCCs in disease as well as in the therapy of disease.

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