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Characterization of store-operated Ca^{2+} channels in pancreatic duct epithelia

Mean-Hwan Kim [a,1,2], Jong Bae Seo [b,1], Lindsey A. Burnett [b], Bertil Hille [b], Duk-Su Koh [a,b,*]

[a] Department of Physics, Pohang University of Science and Technology, Pohang, Republic of Korea
[b] Department of Physiology & Biophysics, University of Washington, Seattle, WA, USA

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ABSTRACT

Store-operated Ca^{2+} channels (SOCs) are activated by depletion of intracellular Ca^{2+} stores following agonist-mediated Ca^{2+} release. Previously we demonstrated that Ca^{2+} influx through SOCs elicits exocytosis efficiently in pancreatic duct epithelial cells (PDEC). Here we describe the biophysical, pharmacological, and molecular properties of the duct epithelial SOCs using Ca^{2+} imaging, whole-cell patch-clamp, and molecular biology. In PDEC, agonists of purinergic, muscarinic, and adrenergic receptors coupled to phospholipase C activated SOC-mediated Ca^{2+} influx as Ca^{2+} was released from intracellular stores. Direct measurement of $[Ca^{2+}]$ in the ER showed that SOCs greatly slowed depletion of the ER. Using IP_3 or thapsigargin in the patch pipette elicited inwardly rectifying SOC currents. The currents increased ~8-fold after removal of extracellular divalent cations, suggesting competitive permeation between mono- and divalent cations. The current was completely blocked by high doses of La^{3+} and 2-aminoethoxydiphenyl borate (2-APB) but only partially depressed by SKF-96365. In polarized PDEC, SOCs were localized specifically to the basolateral membrane. RT-PCR screening revealed the expression of both STIM and Orai proteins for the formation of SOCs in PDEC. By expression of fluorescent STIM1 and Orai1 proteins in PDEC, we confirmed that colocalization of the two proteins increases after store depletion. In conclusion, basolateral Ca^{2+} entry through SOCs fills internal Ca^{2+} stores depleted by external stimuli and will facilitate cellular processes dependent on cytoplasmic Ca^{2+} such as salt and mucin secretion from the exocrine pancreatic ducts.

1. Introduction

Ca^{2+} ions have versatile functions in cellular physiology, such as muscle contraction, neurotransmitter release, hormone secretion, gene expression, and cell proliferation [1]. Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is kept very low at rest but can be dynamically raised by the opening of Ca^{2+}-permeable ion channels such as IP_3 or ryanodine receptors on the intracellular Ca^{2+} stores and voltage-gated or store-operated Ca^{2+} channels (SOCs) on the plasma membrane [2]. SOCs are one of the major sources for Ca^{2+} influx in many non-excitable and some excitable cells. SOCs are activated by sensing reduced Ca^{2+} levels in the intracellular Ca^{2+} stores following activation of certain G-protein coupled receptors (GPCR).

SOCs seem to be heterogeneous. Their detailed activation profiles and single-channel properties depend upon the cell type [3–6]. This variability suggests diverse molecular compositions, activation mechanisms, and physiological functions. Early studies suggested transient receptor potential (TRP) channels might be the molecular correlate of SOCs [7,8]. Later, two key molecules, the STIM and Orai proteins, were found to better fit the criteria of activation and permeation mechanisms for SOCs [9,10]. They were identified with the Ca^{2+}-release-activated Ca^{2+} current (I_{CRAC}) in mast cells and T lymphocytes, a well characterized class of SOC current. It is proposed that some SOCs distinct from I_{CRAC} may still require TRP channel subunits [11].

Compared to immune cells, the roles and identity of SOCs in epithelial cells are less understood. In epithelia, CaT1 (TRPV6) from the intestine [12] and ECaC (TRPV5) from the kidney [13] were identified as apical Ca^{2+} entry channels. CaT1 has some of the properties of the CRAC channels of the human Jurkat T-lymphocytes [14] and LNCaP prostate cancer cells [15]. Functionally, CaT1 expression is

Abbreviations: IP_3, inositol 1,4,5-trisphosphate; ATP, adenosine-5′-triphosphate; UTP, uridine-5′-triphosphate; SOCs, store-operated Ca^{2+} channels; PDEC, pancreatic duct epithelial cells.

* Corresponding author at: Department of Physiology and Biophysics, University of Washington, Health Sciences Building, Room G-424, Seattle, WA 98195-7290, USA. Tel.: +1 206 543 6661; fax: +1 206 685 0619.
E-mail address: koh@uw.edu (D.-S. Koh).
[1] These authors contributed equally to this work.
[2] Present address: The Vollum Institute, Oregon Health & Science University, Portland, OR, USA.

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upregulated in diverse pathological conditions, including apoptosis and abnormal cell proliferation [1].

Pancreatic duct epithelial cells (PDEC) have diverse GPCRs coupled to phospholipase C, PIP_2 hydrolysis, and IP_3 generation [16–18]. For example, when protease-activated receptor-2 (PAR-2) is activated by trypsin, initial Ca^{2+} release from stores and delayed Ca^{2+} influx through SOC are observed, and both mechanisms evoke exocytosis and mucin secretion [18]. SOC-mediated Ca^{2+} elevations also decrease the mobility of intracellular organelles including secretory granules and mitochondria [19,20]. Since SOCs are the major and the only known Ca^{2+} entry channels in the non-excitable exocrine epithelia, we here investigated SOC in dog PDEC in detail. Our results indicate that SOC in PDEC is probably composed of STIM/Orai channels that are functionally expressed on the basolateral membrane and tightly regulated by the level of ER Ca^{2+} depletion ($[Ca^{2+}]_{ER}$).

2. Materials and methods

2.1. Cell cultures

The epithelial cells originated from the accessory pancreatic duct of a normal dog [21]. Cells grow on Vitrogen-coated Transwell insert (0.7 ml of equal volume mixture of Vitrogen and culture medium), and the inserts were suspended above a confluent feeder layer of cultured human gall bladder myofibroblasts. Cells were maintained at 37 °C in 5% CO_2/95% air and fed twice weekly with Eagle's minimum essential medium (EMEM) containing 10% fetal bovine serum, 2 mM L-glutamine, 20 mM HEPES, 2% penicillin/streptomycin solution, 1% insulin-transferrin-sodium selenite medium supplement from Sigma (St. Louis, MO). When confluent, the cells form a tight monolayer (>1 kOhm/cm^2) and show polarized expression of GPCRs and ion channels [21–23]. For subculture, the cells in a monolayer were treated with 0.05% trypsin/EDTA at 37 °C for 45 min and passaged to newly coated inserts with fresh feeder layers. Cells of passage number 10–30 were used for studies. For single-cell experiments, cells were plated on 5 mm round coverglasses coated with a thick layer of Vitrogen and used for measurement after 2–4 days. These subconfluent epithelial cells were presumably not completely differentiated or polarized.

2.2. Solutions and chemicals

Normal Ringer's solution, used in most experiments, contained (in mM): 137.5 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose and 10 HEPES, pH 7.3. In Ca^{2+}-free Ringer's solution, $CaCl_2$ was omitted and 100 μM EGTA was added to remove contaminating free Ca^{2+}. Dimethyl sulfoxide was used to prepare stock solution of 5 mM thapsigargin. Fura-2 AM was from Molecular Probes (Eugene, OR). Other chemicals and culture reagents were from Sigma (St. Louis, MO). Solution exchange was achieved with a local perfusion system that allowed complete exchange within 0.5 s. All experiments were performed at room temperature (22–24 °C).

2.3. Fluorescence measurements of Ca^{2+}

To measure $[Ca^{2+}]_i$ in single cells, PDEC were loaded with 2 μM of the membrane-permeant Ca^{2+}-sensitive dye Fura-2 AM for 30 min. Single cells were excited at 340 nm and 380 nm at 1 or 2 s intervals and the emitted fluorescence at 510 nm detected using CCD camera (Pixelfly; PCO-TECH, Inc., Romulus, MI). A cell-free region was used for background fluorescence correction. $[Ca^{2+}]_i$ was calculated as $K^* \times (R - R_{min})/(R_{max} - R)$, where R is the ratio of fluorescence at 340 nm/fluorescence at 380 nm, K^* is the effective dissociation constant, and R_{min} and R_{max} are the ratios at minimal and maximal $[Ca^{2+}]_i$. R_{min}, R_{max}, and K^*, determined with cells perfused with K^+-rich external solutions containing 20 μM ionomycin and 5 μM thapsigargin plus 20 mM EGTA or 15 mM Ca^{2+}, or 20 mM EGTA and 15 mM Ca^{2+}, were 0.334, 3.733, and 2874 nM, respectively (n = 10–14 cells for each measurement). For $[Ca^{2+}]_i$ with polarized PDEC, monolayers grown in Snapwell inserts (Cat. No. 3407; Corning Costar, Tewksbury, MA) were loaded with 4 μM Fura-2 AM for 30 min and then mounted on top of a customized chamber made with a glass slide. In this chamber the apical and basolateral sides are separately perfused. The result is presented as fluorescence ratio (F340/F380 nm) since no cell-free region was present for background subtraction.

In order to monitor the Ca^{2+} level within the ER Ca^{2+} stores ($[Ca^{2+}]_{ER}$), we used a genetically encoded, ER-targeted Ca^{2+} indicator, D1-ER cameleon (kind gift from Dr. R.Y. Tsien, University of California) [24]. cDNA of D1-ER cameleon (1 μg/30 mm dish) was transfected into PDEC for 6 h with X-tremeGENE 9 DNA Transfection Reagent (Roche Applied Science, Indianapolis, IN). Cells expressing the fluorescent probes were measured with a Zeiss 710 laser-scanning confocal microscope 1–2 days after transfection. For this fluorescence resonance energy transfer (FRET)-based probe, cyan fluorescence protein (CFP) was excited at 405 nm and emission was detected at 420–481 nm. Yellow fluorescence protein (YFP) was excited by the CFP emission and its fluorescence was detected at 560–616 nm. The uncalibrated result is given as the FRET ratio, YFP emission divided by CFP emission.

2.4. Fluorescence microscopy of Orai1 and STIM1

Cells were transfected with cDNAs as pairs of Orai3-GFP/STIM1-YFP or Orai1-orange/STIM1-GFP (1 μg each/30 mm dish for single cells and 3 μg each/100 mm dish for PDEC monolayers. All constructs based on human sequences were kind gifts from Dr. M. Cahalan, University of California, Irvine and Dr. L. Chen, Peking University) and monitored with a Zeiss 710 confocal microscope (63× 1.40-NA objective). Orai3-GFP was excited at 488 nm and the emission was detected at 492–516 nm, while STIM1-YFP was excited at 514 nm and the emission was recorded at 518–621 nm. For the Orai1/STIM1 pair, we used respectively orange (ex. 514 nm, em. 550–681 nm) and GFP (ex. 488, em. 492–543 nm) channels. These protocols minimize the cross-over of fluorescence signals between channels. Colocalization of the two probes was estimated using Pearson's analysis implemented in Nikon Elements software (Nikon Instruments Inc., Melville, NY). Pearson's linear correlation coefficient (r_P) measures the mean overlap of pixels with two different colors:

$$r_P = \frac{\sum (A_i - A_{avg})(B_i - B_{avg})}{\sqrt{\sum (A_i - A_{avg})^2 \sum (B_i - B_{avg})^2}},$$

where A_{avg} and B_{avg} are the averages of A and B colors, respectively, and i is the ith pixel of the image. r_P ranges from −1 to +1. Values of +1 and −1 suggest perfect positive and negative correlation between two variables, respectively. Considering the values obtained in single-cell and monolayer experiments, we set the cut-off criterion for significant colocalization of two colors at 0.25. It is known that STIM1 in the ER translocates toward Orai1 in the plasma membrane after store depletion, and the membrane of PDEC is enriched at the cell periphery in confocal images. Therefore regions of interest (ROIs) for Pearson's analysis were allocated mainly at the cell boundary.