MINI-REVIEW

Store operated calcium entry in NIH-3T3 cells

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Intracellular Ca^{2+} plays numerous physiological roles in regulating cell functions such as chemotaxis, motility or secretion. Stimulation by neurotransmitters or hormones activates intracellular production of inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor on the Ca^{2+} store (endoplasmic reticulum ; ER) membrane and releases the sequestered Ca^{2+} to the intracellular space. Increased intracellular Ca^{2+} functions as a second messenger for activation of many important downstream effectors. DAG activates protein kinase C (PKC), a key enzyme for diverse physiological functions. Capacitative or store operated calcium entry (SOCE) is a process by which depletion of intracellular Ca^{2+} stores activates a Ca^{2+} influx pathway (1). It is an essential pathway for refilling ER Ca^{2+} stores after releasing store Ca^{2+} and also serves as a direct activator of downstream effectors.

SOCE has been investigated by use of inhibitors of sarcoplasmic-endoplasmic Ca^{2+}-ATPase (SERCA), the most common of which is the irreversible inhibitor, thapsigargin. Application of thapsigargin to cells induces sustained SOCE, generally assessed by use of fluorescent Ca^{2+} indicators. Also thapsigargin can be used to study the small, Ca^{2+} selective Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) by the patch clamp technique. Therefore, thapsigargin-induced SOCE is a useful approach to investigate mechanisms of SOCE.

Immortal NIH3T3 cells have been established from the embryonic NIH Swiss mice fibroblasts. The NIH-3T3 cell is a useful cell line for investigation of transformation, stress fiber formation, or feeder cells of keratinocytes. NIH-3T3 cells have epidermal growth factor (EGF) receptors (2), fibroblast growth factor (FGF) receptors (3), platelet derived growth factor (PDGF) receptors (3), bombesin receptors (3) bradykinin receptors (4) and ATP receptors (5), all of those receptors require calcium influx. Stimulation of those receptors mobilizes stored Ca^{2+} through production of IP_3. However, in a series of papers, it was reported that thapsigargin-induced SOCE in NIH-3T3 cells was not sensitive to several kinds of inhibitors (Table 1) (6-9), indicating that in NIH-3T3 cells, SOCE appears to be independent of PKC, calmodulin, microtubules or actin cytoskeleton.

Table 1 Inhibitors which is insensitive on TG-induced SOCE in NIH-3T3 cells

target	reagent	effect of reagent	reference
protein kinase	staurosporin	inhibition of PKC	8
	PMA	activation of PKC	8
phosphatase	ocadaic acid	inhibition of phosphatase	8
calmodulin	KN62	CAMKII inhibitor	9
	cyclosporin A	calmodulin antagonist	9
cytosleleton	nocodazole	microtubule inhibitor	6
	cytochalasin D	actin inhibitor	6

Recent discoveries have revealed the major molecular component proteins in SOCE. STromal Interaction Molecule (STIM) 1 and 2 are Ca^{2+} sensing proteins which initiate signaling to open the CRAC

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channel (10). STIMs have a Ca^{2+} binding site (EF-hand) localized in the N-terminus facing the interior of the Ca^{2+} store. Following depletion of the Ca^{2+} store, the Ca^{2+} dissociates from the EF-hand and STIMs are activated. Activated STIMs then interact with the CRAC channel and to open the channel pore. After the discovery of STIMs, Orai proteins were found to be essential partners of STIM proteins (10). Orai1, 2 and 3 are believed to function as pore-forming subunits of the store-operated channels. The Orai1 channel is blocked by low concentrations of Gd^{3+} (~1 μM) or by of 2-aminoethoxydiphenyl borate (2-APB) (11). In the future, molecular manipulations of Orai and STIM expression, as well as use of Gd^{3+} or 2-APB will be useful tools to investigate the mechanism and regulation of SOCE in NIH-3T3 cells.

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