Discovering the mechanism of capacitative calcium entry

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This essay examines the historical significance of an APS classic paper that is freely available online:


A NUMBER OF HORMONES AND NEUROTRANSMITTERS activate cellular functions by mobilizing intracellular Ca2+. In general, Ca2+ mobilization consist of release of Ca2+ from intracellular stores, as well as increased entry of Ca2+ from the extracellular medium through Ca2+-permeable channels. Capacitative Ca2+ entry, also termed store-operated or store-mediated Ca2+ entry, is a major mechanism for Ca2+ influx in nonexcitable cells. This process, which has also been described in several excitable cells, is controlled by the filling state of the intracellular Ca2+ stores. Capacitative Ca2+ entry plays a number of important roles in cell physiology. First, this mechanism refills cellular free Ca2+ stores. Capacitative Ca2+ entry provides a sustained elevation in intracellular Ca2+ concentration required for a number of cellular functions. Third, this process has been shown to be involved in the maintenance of the amplitude of Ca2+ oscillations (9). Capacitative Ca2+ entry was first reported two decades ago by J. W. Putney, Jr. (8) (Fig. 1), as a mechanism for Ca2+ influx controlled by inositol 1,4,5-trisphosphate (IP3) that allows refilling of the intracellular Ca2+ pool once agonist stimulation has finished.

Despite the complexity of the cellular processes involved in capacitative Ca2+ entry, significant advances have been made since its first description in order to link the filling state of the Ca2+ stores with the regulation of Ca2+-permeable channels in the plasma membrane. The topic of this short essay is a relevant and elegant paper published by Kwan, Takemura, Obie, Thastrup, and Putney in 1990. The experiments were mostly performed by Chiu-Yin Kwan, who came from the laboratory of Ed Daniel at McMaster University in Hamilton, Ontario, and was spending a sabbatical year in the laboratory of J. W. Putney, a pioneer in capacitative Ca2+ entry, was responsible for the design and discussion of the manuscript. In this paper and those leading up to it, Putney and coworkers investigated the effect of a muscarinic receptor agonist, methacholine, and the sarcoendoplasmic reticulum Ca2+-ATPase (SERCA) inhibitor, thapsigargin, on capacitative Ca2+ entry in lacrimal acinar cells (6). The authors followed up on earlier studies investigating capacitative Ca2+ entry induced by methacholine and that induced by thapsigargin are mediated by the same mechanism activated by depletion of the intracellular Ca2+ stores, so that the Ca2+ content of the agonist-sensitive stores determines the rate of Ca2+ entry in these cells. Since thapsigargin is able to mobilize Ca2+ in lacrimal cells without increasing the cellular levels of inositol 1,4,5-trisphosphate or inositol 1,3,4,5-tetrasphosphate (14), these findings indicated that capacitative Ca2+ entry does not require rises in the cellular concentrations of these inositol polyphosphates.

The paper by Kwan et al. (6) also raised key conceptual issues concerning the mechanism of Ca2+ influx into agonist-activated cells. Previous observations had suggested that the entry of extracellular Ca2+ involved a process of direct move-
ment of Ca\(^{2+}\) into the intracellular stores, perhaps by a structure analogous to a gap junction and activated when the concentration of free Ca\(^{2+}\) in the stores was reduced (7, 8). This hypothesis limited the role of Ca\(^{2+}\) entry to the refilling of the intracellular Ca\(^{2+}\) pools after or during agonist stimulation. The seminal paper by Kwan et al. provided new information on the pathways for filling agonist-sensitive Ca\(^{2+}\) stores after Ca\(^{2+}\) mobilization in lacrimal cells based on the use of the trivalent cation La\(^{3+}\). The authors were familiar with the studies of C. van Breemen concerning the inhibitory effect of high concentrations of La\(^{3+}\) on the activity of the plasma membrane Ca\(^{2+}\)-ATPase (see Ref. 3), a major mechanism for Ca\(^{2+}\) extrusion. In lacrimal acinar cells, La\(^{3+}\) blocked both Ca\(^{2+}\) extrusion and entry. Therefore, they reasoned that by preventing Ca\(^{2+}\) entry and extrusion they could determine whether Ca\(^{2+}\) released into the cytoplasm could replenish the stores, which was the case, providing evidence to the idea that the refilling process did not involve a direct route into the endoplasmic reticulum, but rather resulted from a sequential Ca\(^{2+}\) entry into the cytoplasm and subsequent accumulation in the Ca\(^{2+}\) stores by SERCA pumps (6).

The key conceptual issues reported by Kwan et al. (6) have been extensively cited (according to the Thomson ISI Web of Knowledge index, this article has been referenced ~180 times, 17 citations during the first year after publication, which indicates that the study received immediate support from the scientific community) and are the basis for subsequent scientific studies by researchers from around the world on the mechanisms involved in the activation of capacitative Ca\(^{2+}\) entry.

The mechanism by which the filling state of the intracellular Ca\(^{2+}\) stores regulates Ca\(^{2+}\)-permeable channels in the plasma membrane has been a topic of much discussion and debate. Current hypotheses fall into four main categories: indirect coupling, classic conformational coupling, de novo conformational coupling, and secretion-like coupling. The indirect coupling assumes the generation of diffusible molecules that gate capacitative Ca\(^{2+}\) channels. The diffusible messengers include a still uncharacterized calcium-influx factor, cGMP, tyrosine kinases, small GTP-binding proteins, a product of cytochrome P-450, and a Ca\(^{2+}\)-calmodulin-dependent step.

The direct (conformational) coupling proposes a physical interaction between capacitative Ca\(^{2+}\) channels in the plasma membrane and IP\(_3\) receptors in the membrane of the intracellular Ca\(^{2+}\) stores. Two possibilities have been described for the conformational coupling model. The classic conformational coupling hypothesis suggests that the Ca\(^{2+}\) store must be close enough to the plasma membrane to allow a constitutive protein-protein interaction between capacitative Ca\(^{2+}\) channels and IP\(_3\) receptors (1). This hypothesis has been supported by the demonstration that expressed exogenous canonical transient receptor potential (TRPC) channels interact with IP\(_3\) receptors under resting conditions and the finding that IP\(_3\) receptor sequences can modulate capacitative Ca\(^{2+}\) entry (5).

A modification of the classic conformational coupling, the so-called de novo conformational coupling model, proposes that portions of the Ca\(^{2+}\) stores, containing IP\(_3\) receptors, initially distant from the plasma membrane, might be transported to the plasma membrane to facilitate de novo protein coupling. Consistent with this hypothesis, Ca\(^{2+}\) store depletion leads to trafficking of portions of the Ca\(^{2+}\) stores toward the plasma membrane to allow a reversible interaction between IP\(_3\) receptors and capacitative Ca\(^{2+}\) channels (10). Supporting this hypothesis, we found coimmunoprecipitation of naturally expressed TRPC1 and the type II IP\(_3\) receptor (IP\(_3\)R1) upon Ca\(^{2+}\) store depletion but not at resting conditions in human platelets (12). The coupling process is regulated by the actin cytoskeleton, which plays a dual role, acting as a negative cortical clamp that prevents constitutive coupling but also providing support for the coupling between IP\(_3\) receptors and capacitative Ca\(^{2+}\) channels (10).

The fourth model for the activation of capacitative Ca\(^{2+}\) entry is the secretion-like coupling hypothesis, based on the translocation and insertion of preformed channels into the plasma membrane by vesicle fusion. The idea of vesicle fusion to explain the activation of capacitative Ca\(^{2+}\) entry has received support from studies reporting that cell stimulation with physiological agonists or treatment with thapsigargin increase the expression of stored TRPC channels in the plasma membrane (2, 4).

Recent studies have shown that some of these models for capacitative Ca\(^{2+}\) entry might coexist in a single cell type, such as human platelets (11) or LNCaP human prostate cancer epithelial cells (15), which further support the complexity of the mechanisms involved in the activation capacitative Ca\(^{2+}\) entry in different cellular models.

Alterations in capacitative Ca\(^{2+}\) entry has been shown to be at least partially responsible for a number of pathologies, including acute pancreatitis, type 2 diabetes mellitus, primary immunodeficiency, Alzheimer disease, hypertension, and some types of cancer. The seminal paper of Kwan and coworkers (6) revealed characteristics of capacitative Ca\(^{2+}\) entry of great significance and provided the groundwork on which subsequent studies are based. The understanding of the mechanisms of capacitative Ca\(^{2+}\) entry is a remarkable finding regarding cellular physiology and pathology.

REFERENCES


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