

Origins of the concept of store-operated calcium entry

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TABLE OF CONTENTS

1. Abstract
2. Origins of the concept of store-operated calcium entry
3. Acknowledgements
4. References

1. ABSTRACT

The concept of capacitative or store-operated calcium entry, a process by which the release of stored calcium signals the opening of plasma membrane calcium channels, has its roots in the late 1970's, and was formalized in 1986. This short introduction to the current volume of Frontiers in Bioscience briefly summarizes the early experimental work that led to the idea of store-operated calcium entry, and provided the initial proofs for it.

2. ORIGINS OF THE CONCEPT OF STORE-OPERATED CALCIUM ENTRY

Store-operated calcium entry, sometimes referred to as capacitative calcium entry, refers to a process whereby the discharge of calcium stores within a cell secondarily activates an influx of calcium into the cell across the plasma membrane (1). The appreciation of the role of calcium as a cell signal dates back to the late nineteenth century (2), but it was not until the second half of the twentieth century that an appreciation of the sources of cellular calcium developed. Although difficult to attribute specifically to a single publication, it is likely that smooth muscle physiologists and pharmacologists first appreciated the potential dual nature of calcium signaling; that is, signaling calcium could enter the cytoplasm either from outside of the cell, or from storage sites within the cell (3). Note that prior to the emergence of Roger Tsien's BAPTA-based calcium indicators in the 1980's, it was not routinely practical to measure cytoplasmic calcium in living cells in real time. Changes in calcium were inferred from responses of tissues that were known or suspected to

be calcium-mediated. For smooth muscle, the presumed calcium-mediated response was muscle contraction.

Owing to the pioneering work of Douglas, perhaps the next major category of cellular phenomena to embrace calcium signaling was cell secretion (4). Exocrine glands present an interesting physiological model for secretion, as they need to secrete various macromolecules by classical exocytosis, while also producing the aqueous medium into which they are secreted. Thus, salivary glands secrete digestive enzymes (for example, α -amylase) and mucins into a relatively dilute solution of electrolytes and water. The flow of fluid in salivary glands, and in other exocrine glands, results from complex generation and regulation of ion gradients to draw water flow osmotically (5). Schramm and Selinger established that activation of salivary gland cells by secretagogue neurotransmitters, such as acetylcholine or norepinephrine, resulted in a major increase in cellular permeability to K^+ ; importantly, they also established that this response was mediated by calcium (6; 7).

In my own laboratory, we investigated the kinetics of the K^+ permeability response in parotid gland by using a unidirectional isotope flux technique. With this technique, we demonstrated that acetylcholine increased K^+ permeability in two phases; an initial transient phase followed by a lesser, but sustained phase. Importantly, the absence of extracellular calcium prevented the sustained phase with little or no effect on the transient phase (8). In a subsequent study, we demonstrated that the initial transient

Origins of store-operated Ca^{2+} Entry

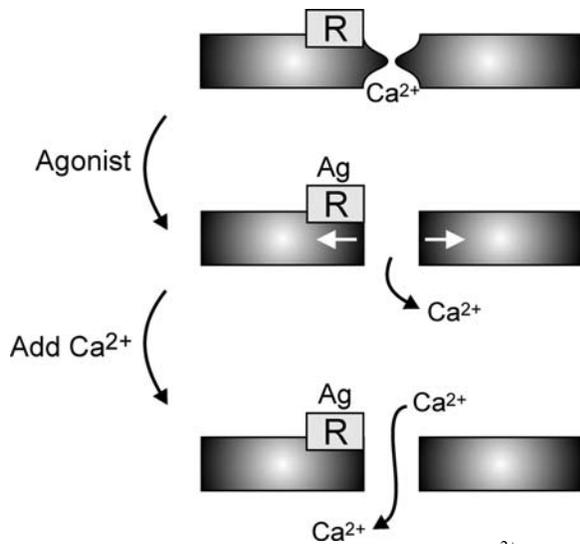


Figure 1. Primitive version of store-operated Ca^{2+} entry: The Ca^{2+} store and channel were seen as a common entity within the plasma membrane. Receptor activation would cause a conformational change, resulting in the release of bound Ca^{2+} , permitting influx of Ca^{2+} through the channels. Based on a model originally published in (9).

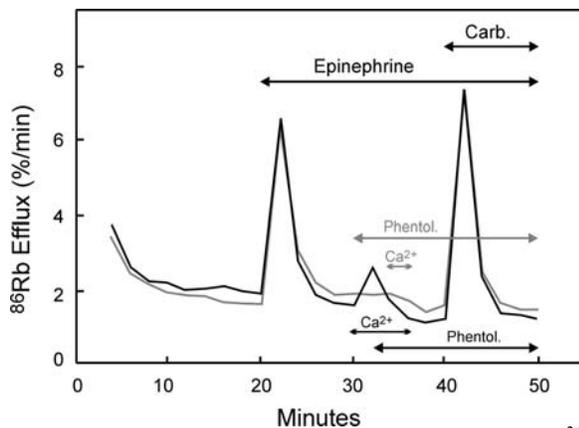


Figure 2. First experimental evidence that refilling Ca^{2+} stores does not require receptor activation: Parotid acinar cells (slices) were pre-incubated with $^{86}\text{Rb}^{+}$ and unidirectional efflux of the nuclide monitored as an index of activity of Ca^{2+} -activated K^{+} channels. The black trace shows the result adding epinephrine (to activate α_1 -adrenergic receptors), in the absence of Ca^{2+} , which releases stored Ca^{2+} causing a transient increase in $^{86}\text{Rb}^{+}$ efflux. This is followed by the addition of Ca^{2+} , causing Ca^{2+} influx, again increasing $^{86}\text{Rb}^{+}$ efflux. This is reversed by the addition of the α -receptor blocking drug, phentolamine, followed by Ca^{2+} removal. The Ca^{2+} stores have refilled as shown by the large transient increase in efflux upon addition of the muscarinic receptor agonist, carbachol. In the experiment shown in gray, the additions are similar, except that the receptors are first blocked by phentolamine before addition of Ca^{2+} . Nonetheless, the response to carbachol is the same in both cases, indicating that the stores refill from the outside with similar efficiency, whether or not the surface receptor is activated. Based on data originally published in (10).

response was indeed dependent on calcium, but utilized calcium from somewhere inside the cell (9). In this same report an important observation was made: in the absence of external calcium, the internal calcium store was lost, and could only be restored by calcium entering the cell through the same channels in the plasma membrane responsible for activation of the sustained response. The locus of the internal store was not known, and at the time, no second messenger (i.e., IP_3) was known. So we assumed the calcium was stored somewhere on the plasma membrane, perhaps associated with the channels themselves. Release of stored calcium and influx of calcium was seen as a coordinated, series event (Figure 1). Although more attention is given to the 1986 paper (discussed below), I have always felt the 1977 study (9) provided the first basic association between release and influx as coordinated processes.

In the following year, a graduate student in the lab, Ralph Parod, carried out a critical experiment on reloading Ca^{2+} stores in lacrimal acinar cells (10). The result is depicted in Figure 2. Exocrine glands express Ca^{2+} -activated K^{+} channels, and thus efflux of K^{+} , or as in this experiment its surrogate, $^{86}\text{Rb}^{+}$, can be used as an indicator of cytoplasmic Ca^{2+} changes. Activation of either muscarinic cholinergic or α_1 -adrenergic receptors present on the acinar cells of the rat lacrimal gland results in a transient increase in $^{86}\text{Rb}^{+}$ efflux in the absence of extracellular Ca^{2+} , indicative of the release of intracellular Ca^{2+} stores through these G-protein, PLC-coupled receptors. Previous experiments had shown that a subsequent stimulation of either of these two receptor types could not induce a second transient response, demonstrating that the intracellular Ca^{2+} store was completely discharged by the first stimulation, that the store was common to the two receptor types, and that it could not be refilled in the absence of extracellular Ca^{2+} . However, restoration of extracellular Ca^{2+} will refill the stores, and in Figure 2, two different protocols are shown. In the black trace, Ca^{2+} is added to the extracellular medium causing an increase in $^{86}\text{Rb}^{+}$ efflux due to Ca^{2+} entry across the plasma membrane. The α_1 -adrenergic receptor activation was then terminated by addition of the α_1 -receptor blocking drug, phentolamine, and then Ca^{2+} is again removed. Addition of carbachol to activate muscarinic receptors causes a second transient $^{86}\text{Rb}^{+}$ efflux response demonstrating that Ca^{2+} entering the cell has refilled the intracellular Ca^{2+} stores. In the experiment shown by the gray trace, the order of the addition of Ca^{2+} and phentolamine is reversed. Thus, phentolamine is added first such that when Ca^{2+} is later added, the α_1 -receptor signaling is presumably already shut down. No visible increase in $^{86}\text{Rb}^{+}$ efflux occurs. Yet, following removal of Ca^{2+} , addition of carbachol causes a transient response indistinguishable from the one when stores were loaded in activated cells. A full ten years ensued before the simple meaning of this experiment was realized: the influx of Ca^{2+} responsible for refilling the stores was not a direct consequence of receptor activation or its various signals, but rather was simply a consequence of the stores being empty. In 1981 Casteels and Droogmans made a similar observation in experiments with vascular smooth muscle and suggested a "protected" route whereby

Origins of store-operated Ca^{2+} Entry

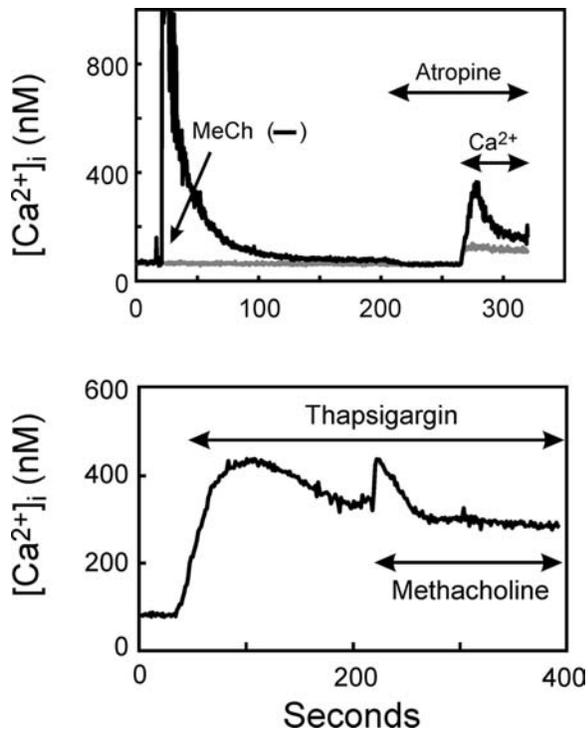


Figure 3. Two key experiments validating the concept of store-operated Ca^{2+} entry: Top: In the experiment shown by the black trace, the muscarinic cholinergic receptor activating drug, methacholine, was applied to Fura-2-loaded parotid acinar cells in the absence of extracellular Ca^{2+} . Atropine was added to block the muscarinic receptors where indicated, and subsequently extracellular Ca^{2+} (10 mM) was added. This resulted in a transient increase in $[\text{Ca}^{2+}]_i$, indicative of Ca^{2+} influx occurring to refill the empty intracellular stores. In the control experiment, shown in gray, no carbachol was added such that stores remained full. Based on data originally published in (13). Bottom: Thapsigargin (2 μM) was applied to parotid acinar cells in the presence of extracellular Ca^{2+} resulting in a sustained elevation in $[\text{Ca}^{2+}]_i$. Subsequent addition of methacholine caused a small transient elevation in $[\text{Ca}^{2+}]_i$, probably because stores were not yet fully empty, but did not further elevate the sustained Ca^{2+} signal. This indicated that full activation of Ca^{2+} entry could be accomplished simply by depleting the intracellular Ca^{2+} stores. Based on data originally published in (15).

Ca^{2+} could move from the extracellular space into intracellular stores without traversing the cytoplasm (11), and idea later demonstrated to be incorrect (12). In 1986, shortly following the demonstration of IP_3 as the second messenger for intracellular Ca^{2+} release I proposed what came to be known as the “capacitance” model for Ca^{2+} entry whereby depletion of intracellular Ca^{2+} store somehow signaled to plasma membrane Ca^{2+} channels opening them thus permitting refilling of the stores (1).

However, capacitance Ca^{2+} entry, or store-operated Ca^{2+} entry as it is now more commonly called was

only a theory. In the following few years, three reports provided the proofs that such a pathway did indeed exist.

The first was essentially a follow-up of the experiment carried out by Ralph Parod, but employing direct monitoring of cytoplasmic Ca^{2+} with Fura-2 (13). Rat parotid acinar cells were activated with a muscarinic-cholinergic agonist, in the absence of extracellular Ca^{2+} , to discharge intracellular Ca^{2+} stores. Subsequently, the receptors were blocked with the potent receptor blocking drug, atropine. After a period of time sufficient for complete degradation of IP_3 , Ca^{2+} was restored extracellularly. A transient rise in cytoplasmic Ca^{2+} was observed that was not seen in cells treated similarly, but with intracellular stores intact. The length of time following atropine addition did not influence the magnitude of this transient Ca^{2+} rise. The experiment demonstrated that there was an influx of Ca^{2+} that occurred independently of receptor activation that apparently depended on the intracellular Ca^{2+} stores having been depleted. As this influx refilled the stores, the influx was terminated, consistent with the transient nature of the response (Figure 3).

The above result provided strong evidence for a store-operated Ca^{2+} influx mechanism, but could not provide any quantitative relationship between this influx and that which occurs during receptor activation. Shortly thereafter, a new pharmacological tool emerged on the scene, thapsigargin, which has been invaluable in the study of store-operated Ca^{2+} entry. My first encounter with thapsigargin came when I attended a Royal Society meeting in London. Mike Hanley presented results with this new plant alkaloid that apparently could release the same pool of Ca^{2+} as IP_3 , but in an IP_3 -independent manner. The mechanism for this release was not known at the time; the action of thapsigargin as an inhibitor of SERCA pumps came somewhat later (14). In listening to Mike Hanley’s presentation, I was dismayed to learn that despite its ability to discharge intracellular Ca^{2+} stores in NG115-401L neuronal cells, it did not activate Ca^{2+} entry. Nonetheless, my laboratory subsequently entered into a collaboration with Ole Thastrup who was at the time the world supplier of thapsigargin, and set out to test its activity in our favorite model, the rat parotid acinar cell (15). In contrast to the earlier result in the neuronal cell line (16), in parotid acinar cells, thapsigargin induced a sustained increase in cytoplasmic Ca^{2+} (Figure 3). (It is now known that the NG115-401L cells do not have store-operated Ca^{2+} entry due to a lack of the Ca^{2+} sensor, STIM1 (17).) Importantly, subsequent activation of the phospholipase C-linked muscarinic receptor did not increase the sustained Ca^{2+} level any further. This provided the first evidence that depletion of Ca^{2+} stores activated Ca^{2+} , and that this accounted for all of the agonist-induced Ca^{2+} entry, at least in this cell type.

The third significant finding that solidified the concept of store-operated Ca^{2+} entry was the demonstration of an electrophysiological current activated by Ca^{2+} store depletion (18). In whole-cell patch-clamp experiments with both mast cells, and the mast cell line, RBL, Markus Hoth

Origins of store-operated Ca²⁺ Entry

and Reinhold Penner demonstrated that depletion of Ca²⁺ stores by IP₃, or passively with an intracellular Ca²⁺ chelator, activated a small inwardly rectifying current that was highly Ca²⁺ selective. They called the current *I*_{crac} for calcium-release-activated-calcium current.

The discovery of a bona fide plasma membrane Ca²⁺ current, together with the Ca²⁺ imaging studies described above, set the stage for the pursuit of two essential ingredients for store-operated Ca²⁺ entry: the message conveying information on the Ca²⁺ content of the stores to the plasma membrane, and the identity of the Ca²⁺ channels. In the ensuing 15-20 years after the phenomenon of store-operated Ca²⁺ was clearly established, a number of laboratories strived to solve these two problems, but with little success. One particular focus for certain groups was the possible role of TRPC channels in store-operated Ca²⁺ entry (19). These channels are clearly activated downstream of phospholipase C and there is considerable evidence the signaling to these channels involves in some way alterations in their lipid environment (20). As to whether they can be activated or regulated by Ca²⁺ store depletion has been a controversial issue (21; 22). TRPC channels are typically relatively non-selective cation channels, making it unlikely that they are involved in the well-characterized *I*_{crac}. In fact, the specific and even somewhat unusual electrophysiological properties of *I*_{crac} proved useful when RNAi screens eventually identified the two molecular players, STIM which serves as the Ca²⁺ sensor in the endoplasmic reticulum (23; 24), and Orai which are the pore-forming subunits of the store-operated CRAC channel (25; 26; 27). Currently, research on store-operated Ca²⁺ entry is focused almost exclusively on understanding the regulation and functions of these two key proteins. This includes studies on how STIM and ORAI proteins interact and how their structures relate to their basic functions as Ca²⁺ sensor and Ca²⁺ channel. Many of the following reviews will discuss recent findings on these fundamental issues. STIM and Orai have also emerged as the molecular correlates for the store-independent Arachidonate-activated Ca²⁺ channels (ARC) (28). One review will discuss the evidence for STIM/Orai involvement in the function of ARC channels. In addition, knowledge of the genes underlying store-operated Ca²⁺ entry affords the opportunity to manipulate the expression of these genes, either in cells or organisms, in order to better understand their function in specific organ systems. This may lead to a better understanding of the molecular basis for a number of important diseases, as well as the possibility of developing novel therapies. Some of the following reviews will highlight recent advances in this fast-moving area that marries basic to translational research.

3. ACKNOWLEDGEMENTS

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