Published in final edited form as:

Crit Rev Immunol. 2009 ; 29(2): 155–186.

Regulation of Ca^{2+} Signaling with Particular Focus on Mast Cells

Hong-Tao Ma and Michael A. Beaven*

Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

Abstract

Calcium signals mediate diverse cellular functions in immunological cells. Early studies with mast cells, then a preeminent model for studying Ca^{2+} -dependent exocytosis, revealed several basic features of calcium signaling in non-electrically excitable cells. Subsequent studies in these and other cells further defined the basic processes such as inositol 1,4,5-trisphosphate-mediated release of Ca^{2+} from Ca^{2+} stores in the endoplasmic reticulum (ER); coupling of ER store depletion to influx of external Ca^{2+} through a calcium-release activated calcium (CRAC) channel now attributed to the interaction of the ER Ca^{2+} sensor, stromal interacting molecule-1 (STIM1), with a unique Ca^{2+} -channel protein, Orai1/CRACM1, and subsequent uptake of excess Ca^{2+} into ER and mitochondria through ATP-dependent Ca^{2+} pumps. In addition, transient receptor potential channels and ion exchangers also contribute to the generation of calcium signals that may be global or have dynamic (e.g., waves and oscillations) and spatial resolution for specific functional readouts. This review discusses past and recent developments in this field of research, the pharmacologic agents that have assisted in these endeavors, and the mast cell as an exemplar for sorting out how calcium signals may regulate multiple outputs in a single cell.

Keywords

store-operated calcium entry (SOCE); calcium channels; TRPC; Orai1; mitochondria; pharmacologic probes; review

I. INTRODUCTION

A. Early Studies of Ca^{2+} Signaling in Mast Cells

Ringer's formulation of his eponymous solution¹ made possible the discoveries that extracellular Ca^{2+} was required for many cellular activities. These included anaphylactic release of histamine from chopped guinea pig lung,² human leukocytes,³ rabbit basophils,⁴ and rat peritoneal mast cells.⁵ An often forgotten observation from these early studies was that Sr^{2+} or Ba^{2+} could replace Ca^{2+} in supporting anaphylactic histamine release from rat peritoneal mast cells.^{5,6} Subsequent studies with $^{45}\text{Ca}^{2+}$ and $^{89}\text{Sr}^{2+}$ showed that uptake of these ions by stimulated peritoneal mast cells correlated with the extent of histamine release.^{6,7} The uptake of $^{45}\text{Ca}^{2+}$ was substantial and rose from ~ 5 to ~ 230 amoles/cell/min after stimulation (recalculated from published data⁷). The same was true for uptake of $^{89}\text{Sr}^{2+}$.⁶ The concept drawn from these studies was that mast cell degranulation was dependent on influx of Ca^{2+} through " Ca^{2+} channels" that can convey Sr^{2+} .⁶ The release of histamine from rat peritoneal

mast cells, whether supported by Ca^{2+} or Sr^{2+} , was effectively blocked by low concentrations of La^{3+} .^{8,9}

Our understanding of Ca^{2+} homeostasis in mast cells was further advanced by the introduction of Ca^{2+} -specific fluorescent probes by Tsien et al.¹⁰ The initial studies with Fura-2 in the RBL-2H3 mast cell line indicated that antigen elicited an increase in concentration of cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) from ~ 0.1 to ~ 1 μM , which was maintained by a La^{3+} -inhibitable influx of Ca^{2+} , to generate an essential signal for degranulation.¹¹ This increase correlated with the production of inositol phosphates¹² of which the generation of inositol 1,4,5-trisphosphate (IP_3)¹³ by phospholipase (PL) $\text{C}\gamma 14$ preceded the increase in $[\text{Ca}^{2+}]_i$. The calculated influx of Ca^{2+} was of similar magnitude to that calculated in the earlier studies with $^{45}\text{Ca}^{2+}$.¹¹ These data pointed to an influx mechanism(s) of relatively high capacity.

Later studies with calcium fluorescent probes and $^{45}\text{Ca}^{2+}$ -equilibrated RBL-2H3 cells demonstrated that Ca^{2+} was released from intracellular stores^{15–18} and that the subsequent influx of Ca^{2+} was associated with replenishment of an antigen/ IP_3 -sensitive pool,¹⁹ accumulation of Ca^{2+} in mitochondria,^{20,19} and depolarization of the plasma membrane.²¹ Repolarization of the plasma membrane was necessary for continued influx of Ca^{2+} and degranulation.^{15,22} The fluxes of Ca^{2+} during this process were substantial. Intracellular $^{45}\text{Ca}^{2+}$ increased from approximately 0.5 mM (about 500 amoles/cell) to about 2.5 mM and then decreased as the levels of cytosolic Ca^{2+} subsided.¹⁵ Other studies reaffirmed that the influx pathway was permeable to Sr^{2+} and other divalent metal ions.²³ The mitochondrial pool was of high capacity and was insensitive to IP_3 and thapsigargin, but was inhibited by oligomycin and antimycin A.^{19,20} The antigen-responsive pool, in contrast, could be depleted by IP_3 and thapsigargin.¹⁹ Both pools, however, could be depleted by the Ca^{2+} ionophore, ionomycin. At low Ca^{2+} concentrations (0.1 μM), uptake of Ca^{2+} was largely confined to the IP_3 -sensitive store, but at higher concentrations (>1 μM), uptake into the mitochondrial pool predominated.¹⁹ Together, these studies suggested that antigen stimulation resulted in release of Ca^{2+} from an IP_3 - and thapsigargin-sensitive store, which was replenished by influx of extracellular Ca^{2+} . This influx was dependent on maintenance of membrane polarity, presumably through efflux of K^+ . During this process, $[\text{Ca}^{2+}]_i$ was buffered at concentrations of around 1 μM by uptake and release of Ca^{2+} in mitochondria. As depicted in Figure 1, these processes were counterbalanced by extrusion of Ca^{2+} from the cell by an ATP-dependent mechanism.¹⁵

B. Studies in Other Types of Cells Further Define the Basic Features of IP_3 -Mediated Calcium Signaling

Studies in other types of cells had identified the source of IP_3 -releasable Ca^{2+} ²⁴ as the endoplasmic reticulum (ER), the receptor for IP_3 (IP_3R)²⁵ as the Ca^{2+} -efflux channel in ER,²⁶ and the mechanism of reuptake of Ca^{2+} into ER as an ATP-dependent process.^{24,27–30} Thapsigargin was shown to block reuptake into ER by inhibiting specifically the sarcoendoplasmic Ca^{2+} ATPase pump (SERCA)^{31,32} and by doing so unmasking a spontaneous loss or “leak” of Ca^{2+} from the IP_3 -sensitive pool without stimulating phosphoinositide hydrolysis.^{33,34} Depletion of the ER pool by thapsigargin or IP_3 inevitably results in entry of Ca^{2+} into the cell. This feature led to the concept, best articulated by Putney in 1986, of “capacitative calcium entry” or “store-operated calcium entry” (SOCE),^{35–37} a process that appeared to be relevant to mast cells.^{19,38} The description of a unique Ca^{2+} -selective calcium-release activated calcium current (I_{CRAC}) in RBL mast cells by Hoth and Penner in 1992 and 1993^{39,40} initiated the search for an elusive I_{CRAC} (CRAC) channel and set the tone for subsequent research on SOCE. However, the mechanism of communication between depleted ER stores and the putative CRAC channel in the plasma membrane remained unclear until recently. The properties and molecular identity of the CRAC channel and other components

that are involved in the mobilization of Ca^{2+} are discussed in subsequent sections. Although our focus is the mast cell, our review is set in the context of IP₃-mediated calcium signaling in other electrically nonexcitable cells because of the generic similarities in signaling mechanisms.

II. IP₃-SENSITIVE AND -INSENSITIVE Ca^{2+} STORES

A. Intracellular IP₃/Thapsigargin-Sensitive Stores

These include Ca^{2+} stores within the ER and, at least in some cells, nucleoplasmic reticulum (NR) to form a contiguous Ca^{2+} store of ~ 0.5 mM.⁴¹ The Golgi also contains high concentrations of Ca^{2+} (~ 0.3 mM), which is released into the adjacent cytosol.⁴² Both Golgi and ER contribute to the increase in $[\text{Ca}^{2+}]_i$ on cell stimulation although at different rates and duration.⁴³ In common with ER and NR, Golgi membranes contain the thapsigargin-sensitive SERCA, which enables reuptake of cytosolic Ca^{2+} against a high concentration gradient (see Section VIII.A). The Golgi membranes in addition contain the thapsigargin-insensitive secretory pathway calcium ATPase (SPCA). An inactivating mutation of the SPCA1 gene in the Hailey-Hailey skin disease indicates a prominent role for Golgi SPCA1 in regulating Ca^{2+} homeostasis at least in certain tissues.⁴⁴ The relative contributions of Golgi and NR to the calcium signal in mast cells, or indeed any immunologic cell, has not been determined.

B. Mitochondria

As noted earlier, the global calcium signal in mast cells is substantially attenuated and prolonged by Ca^{2+} uptake and release from mitochondria.²⁰ It is thought also that uptake into mitochondria influences the spatial as well as temporal profile of the calcium signal to permit more discrete regulation of cellular events.^{45,46} The consequences are not only stimulation of mitochondrial energy production but also localization of the calcium signal to specific subcellular domains. Uptake is believed to occur through the mitochondrial Ca^{2+} uniporter (MCU) in the inner mitochondrial membrane.⁴⁷ This uptake is driven by a negative membrane potential ($\Delta\psi$) maintained by the respiratory chain and is accordingly impaired by the mitochondrial respiratory chain inhibitor, antimycin A, in conjunction with the ATP synthase inhibitor, oligomycin.²⁰ MCU is also inhibited by ruthenium red analogs, Ru360 being a particularly potent inhibitor.⁴⁸ MCU is activated via calmodulin when $[\text{Ca}^{2+}]_i$ is elevated.⁴⁹ Activating levels of $[\text{Ca}^{2+}]_i$ are most likely to occur in the proximity of Ca^{2+} channels in ER, NR, Golgi, and plasma membranes and there is evidence that mitochondria interact with these channels to facilitate mitochondrial Ca^{2+} uptake and thereby restrict increases in Ca^{2+} to specific cellular domains.^{44,46} Mitochondria in the vicinity of Golgi, for example, may limit activation of Ca^{2+} -dependent signaling molecules to those on or located around Golgi.⁴⁴ Mitochondrial activity may also promote Ca^{2+} influx through CRAC channels by scavenging Ca^{2+} released from ER stores and ensuring efficient depletion of these stores.⁵⁰ Impairment of mitochondrial respiration by depolarization suppresses I_{CRAC} activity and Ca^{2+} entry whether stores are depleted by IP₃, thapsigargin, or Adenophostin A.⁵⁰ The actions of thapsigargin and Adenophostin A are described in more detail in Section IX.

It was noted 45 years ago that accumulation of Ca^{2+} in mitochondria requires the simultaneous but independent uptake of phosphate⁵¹ resulting in the formation of a freely dissociable calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) complex within the mitochondrial matrix.⁵² As long as phosphate ions remain in excess, mitochondrial Ca^{2+} can reach millimolar concentrations. Nevertheless, free Ca^{2+} concentrations in mitochondria $[\text{Ca}^{2+}]_m$ are held within the narrow range of 0.5–2 μM regardless of the total Ca^{2+} load within mitochondria. The uncertainties about the nature and kinetic properties of the calcium phosphate complex are discussed in a concise review on calcium signaling and mitochondria.⁵² Egress of Ca^{2+} is mediated by a $\text{Na}^+/\text{Ca}^{2+}$ exchanger,^{53,54} now identified as NCX, which is thought to allow continuous recycling of Ca^{2+} across

the mitochondrial membrane resulting in reciprocal changes in Ca^{2+} concentrations in mitochondria and cytosol.⁵² Ca^{2+} influx and efflux across the membrane of isolated mitochondria reaches rapid equilibrium over a wide range of loading conditions to buffer extramitochondrial $[\text{Ca}^{2+}]$ at 0.5–1.0 μM , the so-called mitochondrial “set point” for $[\text{Ca}^{2+}]_i$.⁵⁵ The importance of NCX to mitochondrial Ca^{2+} homeostasis is evident in disorders such as hamster hereditary cardiomyopathy and human mitochondrial type 1 deficiency when enhanced NCX activity results in reduced mitochondrial Ca^{2+} levels and pathological defects.^{56,57} The Ca^{2+} antagonists, verapamil and diltiazem, inhibit NCX activity and have been tested with positive effects in these disorders.

III. IP3 RECEPTORS (IP3Rs)

IP3Rs are of three subtypes that are closely related in terms of function but show differential expression among tissues and within cells (see previous reviews^{45,58–61}). IP3R subtypes are usually restricted to particular subcellular locations. RBL-2H3 mast cells express all three subtypes; IP3R1 and IP3R2 are located in ER and IP3R3 elsewhere.⁶² In liver HepG2 cells, ER contains IP3R2 and IP3R3, whereas NR contains only IP3R2.⁶³ However, redistribution can occur during cell stimulation or polarization. Stimulation of RBL-2H3 cells with antigen, for example, results in rapid redistribution of IP3R2 in ER and NR from a diffuse to a clustered pattern.⁶⁴ In endothelial and epithelial cell lines, IP3Rs cluster around tight junctions once cells become confluent.^{65,66} These patterns of redistributions are dependent on extracellular Ca^{2+} . Other examples of the heterogeneous distribution and redistribution of IP3Rs are described in detail elsewhere,⁵⁸ but it is apparent that there is no common pattern of distribution, or redistribution, of IP3Rs among different types of cells, and changes may be specific for particular functional responses.

The IP3Rs are expressed predominantly in the ER and to a lesser extent in NR and Golgi.⁴⁵ In addition, a few (~ 2) IP3Rs localize in the plasma membrane of activated B cells, and because of their greater conductance (> 200 pS) than I_{CRAC} (< 1.0 pS), they could conceivably contribute to the influx of Ca^{2+} during cell stimulation.⁶⁷ Activation of these plasma membrane IP3Rs by IP3 requires several minutes, a delay that has been attributed to IP3R translocation to the plasma membrane.⁶⁷

The IP3Rs normally exist as homo- or hetero-tetrameric structures to form functional gated Ca^{2+} channels that bear substantial sequence homology with the corresponding channel region of the sarcoplasmic ryanodine receptor and some structural resemblance to voltage-gated Ca^{2+} and K^+ channels.^{61,68} Unlike I_{CRAC} , IP3Rs conduct other divalent ions and Na^+ but are insensitive to Gd^{3+} or La^{3+} . Each IP3R subunit contains a cytosolic N-terminal ligand-binding domain, an intermediate modulatory domain, and six transmembrane-spanning helices at the C-terminus that constitute part of the tetrameric channel pore. The ligand-binding domain contains a binding pocket for IP3 as revealed by mutagenesis and crystal structure.⁶⁹ Several lines of evidence indicate that the three-dimensional conformation of IP3R places the IP3-binding pocket in close proximity to the channel pore and that only subtle changes in conformation are required for channel gating (see Refs. 61 and 68 for further details). The intermediate modulatory domain is the least homologous region among the IP3Rs but contains binding sites for Ca^{2+} , calmodulin, and other modulatory factors as outlined below.

Apart from IP3, IP3R interacts with a host of regulatory factors including Ca^{2+} , calmodulin, ATP, other nucleotides, kinases, phosphatases, and non-enzymatic proteins that enable fine-tuning of IP3R function.^{58,59,61} Indeed, the recognition sites for these molecules constitute the major portion of the IP3R molecule. Ca^{2+} is the most important regulatory factor exhibiting stimulatory activity at low concentrations and inhibitory activity at high concentrations. Although initially a matter of controversy, it now appears that all three IP3Rs exhibit biphasic

responses to Ca^{2+} but with different affinities in the order $\text{IP3R3} > \text{IP3R2} > \text{IP3R1}$.⁷⁰ Multiple Ca^{2+} -binding sites have been identified on IP3R, some of which may act cooperatively. However, the interactions that underlie the stimulatory and inhibitory effects of Ca^{2+} are still obscure although one enticing model has been proposed to account for both effects.⁷¹ The model invokes an N-terminal suppressor domain that can interact with either the IP3-binding domain or the Ca^{2+} -gatekeeper region in a calmodulin-dependent manner according to the concentrations of Ca^{2+} and IP3. This model is based on reports that Ca^{2+} is an essential coactivator of channel opening,^{72,73} that a Ca^{2+} -binding region within the IP3-binding domain acts in a coordinate manner,⁷⁴ and that inhibition may involve a Ca^{2+} -binding partner such as calmodulin.^{75–77} Other Ca^{2+} -sensor proteins have been implicated as negative or positive regulators of IP3R activity but with conflicting results. In general, the precise roles of the Ca^{2+} sensors, including calmodulin, are unclear.

Many other proteins and small molecules are reported to interact with and regulate function of IP3Rs and it is thought that this multiplicity of interactions regulates the location of IP3R and adds precision to the way IP3Rs respond to IP3-dependent stimulants. These molecules include scaffolding proteins such as ankyrin, myosin, and Homer proteins that allow anchoring of IP3R at appropriate sites within the cell, TRPC family members (see Section V), receptors for activated C-kinase-1 (RACK1), and IP3R-binding proteins released by IP3 (IRBIT), among others.^{61–59} IP3Rs thus have the inherent ability to form macroprotein complexes or “signalosomes” that permit fine-tuning of Ca^{2+} release in a cell- and stimulus-dependent manner. However, many details of the molecular interactions require clarification and the reader is referred to recent reviews for more comprehensive accounts of IP3R.^{58–61,68,71}

IP3Rs are also phosphorylated by multiple serine/threonine protein kinases (PKs) including PKA, PKG, PKC, and calcium/calmodulin-dependent PK. In general, these phosphorylations create positive feedback loops to regulate Ca^{2+} release possibly in spatially restricted areas.⁶¹ IP3R is also phosphorylated by the nonreceptor tyrosine kinases Fyn⁷⁸ and Lyn,⁷⁹ in T and B cells, respectively, thus facilitating release of intracellular Ca^{2+} . Both kinases are activated on ligation of FcεRI in mast cells⁸⁰ and, although not examined specifically, such a mechanism could conceivably contribute to the diminution of the calcium signal in Lyn-deficient mast cells.⁸⁰

IV. I_{CRAC} AND OTHER SOCE CHANNELS IDENTIFIED BY PATCH CLAMP TECHNIQUES

The highly Ca^{2+} -selective current, I_{CRAC} , that was initially characterized by Hoth and Penner in RBL mast cells, was found to be activated by depletion of intracellular Ca^{2+} stores by IP3, ionomycin, and chelation of cytosolic Ca^{2+} .^{39,40} This current was further characterized in T lymphocytes by Zweifach and Lewis.^{81–83} Although I_{CRAC} exhibited some similarities to Ca^{2+} -selective voltage-activated currents, it differed in several respects. It was of low conductance,⁸¹ inhibited by elevated $[\text{Ca}^{2+}]_i$,^{40,82} and potentiated by extracellular Ca^{2+} .⁸³ Of particular note is that replacement of external Ca^{2+} with Sr^{2+} or Ba^{2+} resulted in a decline in I_{CRAC} activity.^{83,84} The apparent abundance of I_{CRAC} channels in T cells (possibly $> 10,000/\text{cell}$)⁸¹ may compensate for the slow conductance of this channel.

Nonetheless, the early patch clamp studies revealed activation of additional cation channels in mast cells and T cells. I_{CRAC} ⁸⁵ and a 50 pS $\text{Ca}^{2+}/\text{Mn}^{2+}$ conducting channel activity⁸⁶ were detected in activated rat peritoneal mast cells with both channels contributing to the increase in $[\text{Ca}^{2+}]_i$. An additional current with characteristics of an amplified I_{CRAC} was noted in Jurkat T cells⁸⁷ and the RBL-2H3 mast cell line⁸⁸ that was preactivated by the absence of external divalent metal ions and by store depletion.⁸⁸ This amplified current appeared to be conducted through relatively few channels (several hundred per cell) and, like I_{CRAC} , it was highly

sensitive to inhibition by physiologic concentrations of intracellular Ca^{2+} .⁸⁸ Whether the amplified current was due to loss of I_{CRAC} regulatory factors or to association with another protein(s) that modified I_{CRAC} activity was unclear and the singular focus has remained on store-operated I_{CRAC} and its putative Ca^{2+} -specific channel, CRAC. However, CRAC defied description until recently with the discoveries of a Ca^{2+} -specific channel protein called Orai1 and the ER Ca^{2+} sensor, STIM1, as described in Sections VI and VII.

V. TRANSIENT RECEPTOR POTENTIAL CHANNELS

At present, the exact physiological role of transient receptor potential (TRP) channels (TRPCs) in calcium signaling is still an issue of debate. Shortly after the initial description of I_{CRAC} , Hardie and Minke⁸⁹ noted that the PLC-dependent *Drosophila* photoreceptor Ca^{2+} channel, TRP, had the attributes of the putative store-operated Ca^{2+} channel for SOCE in vertebrate cells. This possibility was examined by many investigators after the cloning of the mammalian homologs of the TRP gene,^{90,91} which were later classified as canonical TRPs or TRPCs. Although it was clear that knockdown of some TRPCs diminished SOCE and when overexpressed they were activated by store depletion, the verdict was mixed. In particular, none of the TRPCs exhibited the exact characteristics of I_{CRAC} (reviewed in Refs. 91 and 92). In contrast to I_{CRAC} , TRP channels can conduct Sr^{2+} and Ba^{2+} and have single-channel conductances several orders of magnitude greater than I_{CRAC} . We argue later that these properties are still relevant to the characteristics of SOCE in mast cells.

The TRPCs, of which there are seven members, are now recognized as a subset of a superfamily of TRPs that include TRPC, TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin).⁹³ All TRPC channels and a few members of other TRP subfamilies have been considered as SOCE channels. The TRP channel subunits consist of six transmembrane segments (S1–S6) with the N- and C-terminal regions located within the cell. A pore-forming loop exists between S5 and S6 and formation of the complete cation channel requires assembly of homo- or heteromeric complexes of four TRP subunits. TRPCs, in addition, contain a common motif, the TRP box, in the carboxy-terminal tail and several ankyrin repeats in the N-terminal region.

All TRPCs are activated as a consequence of activation of PLC.⁹³ Some but not all TRPCs can be activated by store depletion, although which ones actually operate as store-operated channels is still controversial.⁹⁴ Some are activated by diacylglycerol (i.e., TRPC2, -3, -6, and -7), whereas others are not (i.e., TRPC1, -4, and -5). Like other TRPs, TRPCs can form heteromers among themselves as, for example TRPC1 with TRPC3, TRPC4, TRPC5, or TRPC7 and TRPC3 with TRPC6 or TRPC7.⁹⁵ As a consequence, current properties are significantly altered. Single-channel conductances range from ~ 15 to 75 pS and exhibit a varying selectivity toward Ca^{2+} over Na^{+} from 1.1 to 9.⁹³

It has been suggested that TRPCs can be functionally organized into segregated signaling complexes or “channelsomes” according to composition of the tetrameric TRPC complex and the associated accessory proteins.⁹⁵ As with the IP3Rs, the accessory proteins help regulate the cellular location and function of the TRPCs in a cell- and stimulus-specific manner. These proteins include those involved in cytoskeletal interactions, vesicular trafficking, scaffolding, and calcium signaling and include PLC β , calmodulin, IP3R, immuno-phillins, STIM1, plasma membrane Ca^{2+} ATPase (PMCA), and the sarco/endoplasmic Ca^{2+} ATPase, SERCA.^{96,95} We shall discuss the latter proteins in subsequent sections. The ankyrin regions of TRPCs appear to account for many of the interactions of TRPCs with their associated proteins. The composition of the signaling complex is thought to determine not only the channel properties but also the cellular location and downstream functions of TRPCs. The concept is that in addition to contributing to SOCE, TRPCs like IP3Rs may regulate $[\text{Ca}^{2+}]_i$ in discrete locations

or modify the amplitude of the calcium signal in localized regions for specialized cellular functions.⁹⁵ In other words, the variable subunit composition of TRPC channels and ability to interact with regulatory subunits and other channel proteins provides flexibility of the cell to use TRPCs in diverse ways.⁹⁷

The TRPCs contain an IP3R consensus binding domain but the regulatory consequences have been defined only for TRPC1, TRPC2, and TRPC3. In general, IP3R positively regulates TRPC function. TRPC1 in the plasma membrane is reported to couple to IP3R in the ER,^{98–100} an interaction facilitated by RhoA⁹⁹ or Homer,¹⁰⁰ and thus regulate SOCE in conjunction with STIM1.⁹⁸ As discussed in the next section, STIM1 acts as a Ca²⁺ sensor in the ER and can interact with and activate some TRPCs. The possible functional combinations of TRPC1 and other TRPCs with IP3R and STIM1 are topics of a recent review.⁹⁵

The uncertainty about the exact role of TRPCs stems in part from the modest selectivity toward Ca²⁺, the variability in channel properties of different TRPC complexes, and the lack of correspondence to *I*_{CRAC}. Many of the early studies relied on overexpression of TRPCs and the potential pitfalls of this approach are now well recognized. These include promiscuous interactions with endogenous TRPCs and other proteins or aberrant localization within the cell. As a result, the levels of expression may result in altered properties as exemplified in studies with TRPC3 where TRPC3 exhibited either store-dependent or store-independent activation when expressed at low or high levels, respectively.¹⁰¹ The introduction of siRNA technology and use of TRPC knockout mice circumvent these pitfalls, but even these approaches can introduce new ones such as compensatory expression of other endogenous TRPCs.¹⁰²

VI. THE CRAC CHANNEL PROTEIN, ORAI, AND ITS INTERACTIONS WITH STIM AND TRPC PROTEINS

The field of calcium signaling was galvanized recently by the identification of the ER Ca²⁺ sensor, STIM1, and the Ca²⁺-channel protein, Orai1 (also called CRACM1), as the long-sought-for components underlying *I*_{CRAC}.^{103–107} Over-expression of both, but not individually, in a variety of cells results in large CRAC currents.^{108–110} Also, a mutated form of Orai1 was found to be responsible for severe combined immune deficiency with defective Ca²⁺ influx in T cells that could be rectified by expression of wild-type Orai1.¹⁰⁵ Since STIM1 and Orai1 are featured topics in many contemporary reviews,^{92,111–114} some focused exclusively on immunological cells,^{115–118} we shall summarize the major findings in this and the subsequent section.

Orai1 is an atypical cation channel with four transmembrane domains.^{105–108,110} At present, three mammalian *Orai* gene products have been identified in a wide variety of cells. All three contain a proline/arginine-rich region in the N-terminus and a putative C-terminus coiled-coiled domain, with predicted probabilities that are severalfold higher for Orai2 and Orai3 than Orai1.¹¹⁹ An extracellular loop between the third and fourth transmembrane domains of Orai1 contains an N-glycosylation site but this does not appear to be functionally critical.¹²⁰ The selectivity filter of Orai1 is linked to acidic residues in the first and third transmembrane domains and the first loop segment. However, a CRAC channel is created only after oligomerization of Orai1^{121,122} to form a fully functional channel of four Orai1 subunits.^{123,124} This was demonstrated by coexpression of preassembled Orai1 multimers of varying numbers along with STIM1. In heterologous expression systems, Orai1 can also oligomerize with Orai2 and Orai3 to create CRAC channels in the presence of STIM1, each with slightly different ion-selectivity profiles, feedback inhibition by cytosolic Ca²⁺, and responses to 2-aminoethoxydiphenyl borate (see Section IX.C).¹²⁵ The physiological relevance of these various combinatorial arrangements are unclear but it could add flexibility to the “tool kit” that is available for regulation of calcium signaling in a given cell. All three Orai proteins can be

activated by STIM2 as well as by STIM1.¹²⁶ The historical sequence of key observations in these and earlier studies of calcium signaling are illustrated in Figure 2.

While it is now apparent that STIM1 and Orai1 are sufficient to reconstitute CRAC channels, Orai1 also forms complexes with some TRPCs and in conjunction with STIM1 creates SOCE channels with properties distinct from I_{CRAC} .^{127–130} These interactions include TRPC1 or TRPC5 with Orai1^{127,129–131} and TRPC3 or TRPC6 with Orai1, Orai2, or Orai3.¹²⁸ Although many of these studies were based on heterologous expression systems, the interactions were also apparent by coimmunoprecipitation of endogenous proteins,^{127,131} by knockdown of individual endogenous components of the presumed STIM1/channel complex,¹²⁹ or by introduction of neutralizing antibodies.¹³⁰

There are two views as to whether or not TRPCs are directly involved in SOCE. One view is that STIM1 and Orai1 create functional CRAC channels and are the exclusive SOCE channels. Another is that STIM1, Orai1, and TRPCs can also form SOCE channels with varying degrees of Ca^{2+} selectivity.^{131,132} In support of the latter view are the interactions just noted, that not all cells bearing Orai1 generate I_{CRAC} ^{133–135} (see also Section IV), and that coexpressed TRPC1 and STIM1 are able to activate a nonselective current (I_{SOC}) following store depletion.¹³¹ I_{SOC} is blocked by low concentrations ($\sim 1 \mu M$) of La^{3+} and 2-aminoethoxydiphenyl borate and is attenuated by Orai1 siRNA or expression of inactive mutants of Orai1 to indicate a dependence on Orai1.¹³¹ One proposal is that Orai proteins (i.e., 1, 2, or 3) act as a regulatory subunit of TRPCs (i.e., 3 or 6) to confer sensitivity to STIM1 following store depletion.¹²⁸ The ability of Orai proteins to interact not only among themselves but also with TRPCs would add further flexibility in the assembly of calcium channels from the calcium tool kit within cells. At the moment, the field lacks physiologic examples of how these various combinatorial arrangements are used by cells for specific purposes.

VII. THE Ca^{2+} SENSORS, STIM1 AND STIM2

STIM1 and STIM2 were initially identified as potential tumor suppressors^{136,137} and only some time later as regulators of SOCE.^{103,104,107} Both proteins are ubiquitously distributed in cells and can cooligomerize to indicate possible functional interactions.¹³⁷ Both are highly homologous with single transmembrane domains, an N-terminal helix-loop-helix (EF hand) motif and a sterile a motif (SAM) within the ER lumenal segment, and protein-interacting domains in their lumenal and cytoplasmic portions.^{103,104,138} The STIM proteins can sense the state of depletion of ER Ca^{2+} stores by virtue of the EF-SAM domains that have an affinity for Ca^{2+} that is appropriate for the high concentration of Ca^{2+} within the ER lumen.^{139,140} However, the STIM isoforms differ markedly in their rates of oligomerization and dissociation, which may portend differences in their regulatory roles in calcium signaling.¹⁴¹

STIM1 was the first isoform to be recognized as a regulator of SOCE through RNA interference screens in thapsigargin-stimulated *Drosophila*^{104,107} and HeLa cells.¹⁰³ When ER Ca^{2+} stores are full, STIM1 is localized throughout the ER network in structures organized by the microtubule network¹⁴² but when Ca^{2+} stores are depleted, STIM1 oligomerizes and migrates into punctaelike structures at the cell periphery.^{103,143} Viewpoints differ as to whether STIM1 actually transfers to the plasma membrane following store depletion¹⁴³ or merely redistributes into punctae beneath the plasma membrane^{144,109} to enable activation of I_{CRAC} . In addition to acting as an ER sensor, STIM1 is reported to also regulate I_{CRAC} from within the plasma membrane.¹⁴⁵ Mutating the EF-hand results in constitutive localization of STIM1 in punctae and activation of I_{CRAC} independently of store depletion.^{143,144} A coiled-coil domain in the C-terminus is crucial for its oligomerization as is a serine-proline-rich region for correct targeting of the STIM1 complex at the cell periphery.^{146,147} The process is reversed on store

repletion and, paradoxically, by the myosin light chain kinase inhibitor ML-9, which acts independently of the light chain kinase.¹⁴⁸

When located in peripheral punctae, STIM1 is placed in apposition to Orai1, resulting in Ca^{2+} entry.^{108–110} Although the mechanism of Orai activation by STIM1 is not entirely clear, functional interaction of both proteins is dependent on the coiled-coil domain in the cytoplasmic C-terminal tail of Orai1¹⁴⁹ and two distinct C-terminal domains of STIM1¹⁵⁰ as indicated by genetic manipulations that impact on these domains. The interaction may also occur in the context of macromolecular complexes that contain an unidentified component(s) in addition to STIM1.^{148,151}

As noted earlier (Section VI), STIM1 also interacts with various TRPC channels, either individually or as heteromeric combinations with Orai,^{132,152–153} and appears to do so through electrostatic attraction between positively charged residues in the polybasic domain of STIM1 and matching negatively charged residues in conserved domains of TRPC1 or TRPC3.¹⁵³ Since these domains are not required for functional coupling of STIM1 to Orai1, the coupling mechanisms for Orai and TRPC appear to be different. STIM1 has also been linked to activation of SERCA3 in the refilling of acidic Ca^{2+} stores in platelets.¹⁵⁴

STIM2 has been less well studied. STIM2, when expressed by itself, inhibits SOCE,¹⁵⁵ but when expressed with Orai1, it stimulates Ca^{2+} influx without store depletion.¹⁰⁸ Expressed STIM2 localizes in ER but only migrates into punctae following store depletion if it is coexpressed with STIM1.¹⁵⁵ An RNA interference screen has identified STIM2 as a particularly strong regulator of basal $[\text{Ca}^{2+}]_i$ possibly acting as a feedback modulator that keeps ER and cytosolic Ca^{2+} concentrations “within tight limits.”¹³⁸ Furthermore, STIM2 migrates to peripheral punctae in response to relatively small decreases in concentrations of ER Ca^{2+} and thus stimulates Ca^{2+} influx via Orai1.¹³⁸ In contrast, STIM1 comes to the fore with more profound store depletion. This was indicated in studies of T cells and fibroblasts from mice with conditionally targeted alleles of *STIM1* and *STIM2*.¹⁵⁶ Deficiency of STIM1 severely impaired thapsigargin-induced SOCE in these cells, whereas deficiency in STIM2 had much less but still significant effect. Other studies based on overexpression of STIM proteins and whole-cell dialysis suggest that store-dependent and store-independent mechanisms exist for regulating coupling of STIM2 with Orai1 and the associated activation of I_{CRAC} .¹²⁶ The store-independent mechanism was unmasked by cell dialysis and was attributed to washout of a cytosolic inhibitory factor, tentatively identified as calmodulin. In this model, STIM2 is viewed as constitutively active under basal conditions and negatively regulated by elevation of $[\text{Ca}^{2+}]_i$ through calmodulin. In this respect, the model is a variation of the feedback model noted above.

VIII. OTHER MECHANISMS FOR Ca^{2+} FLUX ACROSS CELL MEMBRANES

A. Ca^{2+} -ATPase Pumps: PMCAs and SERCAs

PMCA removes Ca^{2+} from the cytosol to the cell exterior and SERCA transfers cytosolic Ca^{2+} to ER, and both operate against a concentration gradient. They share many of the same structural and functional features and belong to a large family of the so-called P-type ATPases because the reaction cycle involves formation of a phosphorylated intermediate.^{157,158} The recent determination of the crystal structure of SERCA¹⁵⁹ has confirmed that counterion (i.e., H^+) transport is an obligatory part of the reaction mechanism.¹⁵⁸ In addition to PMCA, $\text{Na}^+/\text{Ca}^{2+}$ exchangers also participate in the removal of excess Ca^{2+} from the cell. It is thought that they enable rapid extrusion of Ca^{2+} because of their high capacity. The more metabolically demanding PMCAs may then act to fine-tune the calcium signal and ensure a return of $[\text{Ca}^{2+}]_i$ to the final basal levels.¹⁶⁰

As noted in Section I, both uptake of Ca^{2+} into the endoplasmic reticulum and efflux of Ca^{2+} from mast cells are ATP-dependent processes. Although it is assumed by analogy with other types of cells that Ca^{2+} -ATPase pumps participate in these processes in mast cells,¹⁶¹ the particular isoforms of PMCA and SERCA involved have not been determined. SERCA is probably responsible for uptake of Ca^{2+} into ER stores because thapsigargin effectively blocks this uptake and localizes within ER of RBL-2H3 cells.¹⁶¹ However, PMCA appears to share an equal role with $\text{Na}^+/\text{Ca}^{2+}$ exchangers in the extrusion of Ca^{2+} from mast cells (see Section 2 below).

1. PMCA—A plasma membrane Ca^{2+} ATP-dependent transporter was first described in erythrocyte ghosts in 1966¹⁶² and subsequently purified from several tissue sources. The purified plasma membrane transporter was found to have the same basic features whatever the source and was classified as a P-type ATPase for reasons noted above.¹⁵⁷ Other identifying features include stimulation by direct interaction with calmodulin and inhibition by vanadate. The complete sequences of the mammalian pumps were first reported in 1988^{163,164} and they are now generally referred to as PMCA. To date four PMCA isoforms, 1–4, and many of their spliced variants have been cloned.^{160,165} They contain 10 transmembrane regions. The second cytosolic loop between the fourth and fifth transmembrane segments contains a critical aspartate residue and an ATP-binding domain. The aspartate residue is phosphorylated during each cycle of Ca^{2+} transport. The C-terminal domain possesses a Ca^{2+} /calmodulin-binding region although alternative splicing results in considerable variation in the affinity of different spliced variants for calmodulin. At low concentrations of $[\text{Ca}^{2+}]_i$ (50 to 100 nM), the C-terminal domain interacts with the first and second cytosolic loops thus masking the ATP-binding and aspartate sites making the PMCA inactive. This inhibitory state is reversed at higher concentrations of $[\text{Ca}^{2+}]_i$ by the interaction of Ca^{2+} /calmodulin with the C-terminal domain. The multiplicity of PMCA variants is thought to accommodate the specific needs of different types of cells for Ca^{2+} .¹⁶⁶ This is likely reflected by the differences in Ca^{2+} extrusion properties and tissue distribution of the various PMCA isoforms and their variants. In addition to activation via calmodulin, PMCA activity may be further modulated by other factors. These include protein kinases and acidic phospholipids, particularly the inositol phospholipids, sphingosine, and phosphatidyl serine. PMCA also interact with other proteins, sometimes in an isoform/variant-specific manner, to allow assembly of multiprotein complexes within specialized membrane domains as described elsewhere.^{160,165}

In addition to restoring $[\text{Ca}^{2+}]_i$ to basal levels, PMCA are thought to influence the dynamics of Ca^{2+} waves, spikes, and oscillations (see Section X.D). There is compelling evidence that PMCA not only help shape transient increases in $[\text{Ca}^{2+}]_i$ during brief periods of SOCE, but also help stabilize the calcium signal during sustained SOCE in part because of an ability to adapt rapidly to CRAC channel activity¹⁶⁷ and act in concert with other components of the calcium signaling machinery.¹⁶⁰ In cells with specialized functions, PMCA are concentrated in locations specific for those functions, as for example, in the apical membranes of pancreatic and salivary gland cells.^{168,169} The presumption is that these cells possess machinery, as yet undefined, for recruitment of PMCA within membrane microdomains,¹⁶⁵ as might well be the case for other proteins involved in calcium signaling.

The functions of individual PMCA isoforms have not been well defined but gene deletion in mice indicates little redundancy among their functions. PMCA1 knockout mice are embryonic lethal, whereas knockout of PMCA2 and PMCA4 genes results in more specific mouse phenotypes.¹⁷⁰ The lethality of PMCA1 deficiency and the ubiquitous distribution of this isoform suggest that it has a generic function in the regulation of cellular calcium. Other isoforms, or combination of isoforms, may have more specialized tissue-specific functions. As far as we are aware, there are no reports of the effects of these genetic deletions on mast cell function.

2. SERCAs—Three genes encode SERCA1, SERCA2, SERCA3, and their splice variants.¹⁶⁰ The sensitivity of these proteins to thapsigargin and another inhibitor, cyclopiazonic acid, is discussed in Section IX.A. Expression of SERCA1 isoforms is restricted almost exclusively to fast-twitch skeletal muscle fibers. SERCA2a is expressed predominantly in cardiac muscle and SERCA2b is found in almost all tissues including smooth muscle. SERCA3 isoforms are found, often in conjunction with SERCA 2b, in non-muscle cells. Although SERCAs and PMCAs have common molecular and functional features, SERCAs lack an extended C-terminal tail and transport two Ca^{2+} ions for each ATP molecule consumed as opposed to one Ca^{2+} ion for PMCAs. Among the Ca^{2+} transporters, SERCAs have the highest affinity for removal of Ca^{2+} from cytosol, which probably accounts for the efficient recapture of released Ca^{2+} by ER from the cytosol such that several Ca^{2+} oscillations can still occur in the absence of external Ca^{2+} . It has also been postulated that in circumstances where ER is in close proximity to SOCE channels, SERCA may ensure capture of Ca^{2+} to allow refilling of ER with minimal perturbation of $[\text{Ca}^{2+}]_i$.¹⁷¹

SERCAs appear to be regulated directly by the concentrations of free Ca^{2+} in ER, which is typically held at $\sim 500 \mu\text{M}$.¹⁷² The Ca^{2+} -sensing mechanism for SERCA is not as well defined as that for PMCA. Suggested mechanisms include interactions of SERCA2b with calreticulin and calnexin,¹⁷³ SERCA3 with STIM1,¹⁷⁴ SERCA2b with presenilins,¹⁷⁵ and *Xenopus* SERCA with the second messenger cyclic ADP-ribose,¹⁷⁶ among others. Calreticulin and calnexin are ER chaperone proteins primarily recognized for their role in protein folding. Calreticulin resides within the ER lumen and contains a high-capacity Ca^{2+} -binding domain of low affinity. Calnexin is a related transmembrane ER protein whose luminal portion shares homology with calreticulin but lacks the high-capacity Ca^{2+} -binding domain. Coexpression of either protein with SERCA2b, but not SERCA2a, inhibits Ca^{2+} oscillations in *Xenopus* oocytes, and certain domains of calnexin and calreticulin appeared to be critical for this inhibition.¹⁷³ Overexpression of wild-type presenilins accelerates ER Ca^{2+} uptake and presenilin-deficient cells have a phenotype similar to SERCA knockdown cells.¹⁷⁵ Clearly, additional studies are required to verify the physiologic significance of these interactions to gain a broader view of the situation.

B. Ion Exchangers

In addition to the established roles of SOCE and ATP-dependent Ca^{2+} channels in calcium signaling, $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCXs) should also share this limelight. These exchangers are widely expressed¹⁷⁷ and have been categorized as K^+ independent (designated as NCX) or K^+ dependent (designated as NCKX). Those of the former category are encoded by a family of three genes (*NCX1*, *NCX2*, and *NCX3*) and the latter by a family of five genes (*NCKX1*, *NCKX2*, *NCKX3*, *NCKX4*, and *NCKX5*). While both families of exchangers are bidirectional and are driven by the electrochemical Na^+ and Ca^{2+} gradients across cell membranes, the NCKX family members are also dependent on the K^+ gradient.¹⁷⁷ Although the normal function of the $\text{Na}^+/\text{Ca}^{2+}$ exchangers is the extrusion of Ca^{2+} in exchange for Na^+ when $[\text{Ca}^{2+}]_i$ is elevated, ion exchange can be reversed by, for example, removal of external Na^+ to cause influx of Ca^{2+} .

$\text{Na}^+/\text{Ca}^{2+}$ exchangers are known to operate in conjunction with voltage-gated channels to regulate localized changes in $[\text{Ca}^{2+}]_i$ ¹⁷⁷ but they could also operate in conjunction with SERCA to limit increases in $[\text{Ca}^{2+}]_i$ following activation of SOCE channels.¹⁷⁸ Hints that this might be the case comes from studies of the effects of expressed $\text{Na}^+/\text{Ca}^{2+}$ exchangers. When activated by store depletion, the exogenous exchangers modulate localized and global changes in cellular Ca^{2+} levels.^{179,180} This also appears to be true for endogenous $\text{Na}^+/\text{Ca}^{2+}$ exchangers in mast cells where significant coupling of Na^+ and Ca^{2+} plasma gradients has been observed.^{181,182} The effects of varying the concentrations of external Na^+ on the influx of Ca^{2+} or

Sr^{2+} , changes in $[\text{Ca}^{2+}]_i$, and mast cell degranulation suggest that these events are tightly coupled.¹⁸² Whole-cell patch clamp recordings indicate that mast cells express both K^+ -dependent and K^+ -independent $\text{Na}^+/\text{Ca}^{2+}$ exchangers (identified as NCKX3, NCKX1, and NCX3), which may account for as much as 50% of the Ca^{2+} extruded from cells once $[\text{Ca}^{2+}]_i$ reaches 200 nM.¹⁷⁸ A further indication of the prominent role played by $\text{Na}^+/\text{Ca}^{2+}$ exchange in mast cells is that its reversal by omission of external Na^+ significantly diminishes SOCE and increases in $[\text{Ca}^{2+}]_i$. In similar experiments in Jurkat T cells, however, Ca^{2+} was extruded predominantly through PMCA.¹⁶⁷ Therefore, the relative contributions of $\text{Na}^+/\text{Ca}^{2+}$ exchange and PMCA may vary from one cell type or experimental condition to another.

As with other channel proteins, an expanding list of interacting partners have been described as regulators of NCX activity.¹⁷⁷ These include lipids, especially phosphatidylinositol 4,5-bisphosphate, as well as cytoskeletal, scaffolding, and signaling proteins. However, the molecular context in which the ion exchangers operate and their proximity to other Ca^{2+} channels is still obscure and many questions remain.

C. Presenilins (PS) and Calcium Homeostasis Modulator 1 (CALM1): The Connections to Alzheimer's Disease

There are two sides to the coin in the debate on the etiology of Alzheimer's disease. On the one hand, there is the well-documented formation of β -amyloid plaques; and on the other, the presumed, but not yet established, dysregulation of calcium homeostasis leading to neuronal "calcium overload" and cell death.^{183,184} The relationship between these two phenomena remains a matter of investigation. Nevertheless, research on either the inherited familial Alzheimer's disease (FAD) or the more prevalent sporadic late-onset form of the disease has brought forth two proteins of relevance to calcium signaling. One is PS and the other is the newly discovered protein, CALM1.

1. PS, the Elusive Ca^{2+} -Leak Protein?—PS is expressed as two isoforms, PS1 and PS2, and resides mainly in ER and Golgi. Both isoforms contain multiple transmembrane regions¹⁸⁵ and undergo endoproteolytic cleavage to form N-terminal and C-terminal fragments that reassociate within a multimeric protein complex.¹⁸⁶ In this form, the PS proteins constitute the core γ -secretase activity that cleaves, among other proteins, membrane β -amyloid precursor protein to release β -amyloid. About 40% of FAD patients express mutated versions of PS that promote formation of the highly fibrillogenic β -amyloid42 protein, which is characteristic of this disease.¹⁸⁷ In addition, many of these PS mutations are also associated with perturbed calcium signaling, which is thought also to contribute to the pathology of Alzheimer's disease.^{186,188}

It was initially shown that several FAD-related PS mutations profoundly affect Ca^{2+} release from Ca^{2+} stores and in turn SOCE,^{189,190} but the mechanisms are still debated.^{183,184} Recently, the PS proteins have been proposed as candidates for the elusive Ca^{2+} -leak protein in ER based on observations that PS1 and PS2 form Ca^{2+} -permeable ion channels in bilayer vesicles.^{191,192} However, other workers detected no such ability but concluded instead that PS1 and PS2 interact with IP3R to enhance IP3-mediated Ca^{2+} release.¹⁹³ PS proteins have also been implicated as interacting active partners with SERCA¹⁷⁵ and the ryanodine receptor.¹⁹⁴ The FAD-related PS1 and PS2 mutants exhibit gain of function in the case of the IP3 and ryanodine receptors^{193,194} and loss of function in the case of the Ca^{2+} -leak protein,^{183,184} all of which theoretically would lead to increased $[\text{Ca}^{2+}]_i$ or "calcium overload." These and earlier studies clearly establish a role for PS proteins in regulating Ca^{2+} homeostasis in ER although it is premature to declare which of the above mechanisms operate under normal or pathological conditions. We also note that PS is widely expressed in tissues including mast cells (our

unpublished results) but the significance of PS, and inherited mutations thereof, in calcium signaling in mast cells still requires investigation.

2. CALHM1—*CALHM1* was recently identified as a risk gene for late-onset Alzheimer's disease,¹⁹⁵ although this is disputed,¹⁹⁶ and was thought to encode a previously uncharacterized membrane Ca^{2+} channel protein.¹⁹⁵ CALHM1 has three transmembrane domains but assumes a multimeric structure when expressed in cells and has sequence similarities with the selectivity filter of the NMDA receptor. Expressed CALHM1 generated a large Ca^{2+} -selective conductance in the plasma membrane and enhanced restoration of basal $[\text{Ca}^{2+}]_i$ on provision of external Ca^{2+} to Ca^{2+} -deprived cells.¹⁹⁵ The pharmacological and channel properties of CALHM1 were quite distinct from those of SOCE, voltage-gated Ca^{2+} channels, and ryanodine receptors as indicated by use of various pharmacologic inhibitors. In addition, the effects CALHM1 on Ca^{2+} flux (and the processing of β -amyloid) were impaired by a single nucleotide polymorphism in *CALM1* that was found to be particularly prevalent in Alzheimer's disease. Inevitably, novel findings such as these raise questions that need to be addressed before CALM1 can be considered as a bone fide Ca^{2+} channel. One question is whether or not CALHM1 forms similar Ca^{2+} channels in ER, the main location of expressed CALHM1,¹⁹⁵ and if so in what orientation. Another is whether expressed CALHM1 recapitulates the exact function of the endogenous protein and if so how are these channels regulated.

IX. PHARMACOLOGICAL TOOLS FOR INVESTIGATING THE CALCIUM SIGNAL

A. SERCA Inhibitors: Thapsigargin and Cyclopiazonic Acid

Thapsigargin, a skin irritant isolated from *Thapsia garganica*,¹⁹⁷ is perhaps the most widely used pharmacological probe for studies of IP₃-dependent calcium mobilization because of its unique ability to block replenishment of IP₃-sensitive stores through SERCA. It was found to be a potent stimulant of mast cell degranulation^{198–200} and, in 1985, to elevate platelet $[\text{Ca}^{2+}]_i$ by an undefined mechanism.²⁰⁰ Later studies in other types of cells showed that thapsigargin caused loss of Ca^{2+} from intracellular stores²⁰¹ without stimulating inositol phosphate formation,^{33,34} and did so by blocking Ca^{2+} uptake via SERCA³¹ into an IP₃-sensitive pool³³ resulting in spontaneous “discharge” of Ca^{2+} from that pool.³² Moreover, thapsigargin and receptor-mediated generation of IP₃ release Ca^{2+} from the same pool.³³ Thapsigargin acted in the same manner in RBL-2H3 cells where it was apparent that Ca^{2+} was released from an IP₃-sensitive pool and that both antigen and thapsigargin stimulated Ca^{2+} influx by ostensibly similar mechanisms in which Sr^{2+} and other divalent metal ions competed for Ca^{2+} entry.¹⁹ The studies with thapsigargin provided the definitive evidence that entry of extracellular Ca^{2+} was directly linked to depletion of the IP₃-sensitive intracellular pool.²⁰² Subsequently, thapsigargin was to prove equally valuable in the identification of the molecular components involved in SOCE.

By 1991, thapsigargin was found to inhibit all isoforms of SERCA at nanomolar concentrations in a stoichiometric manner without affecting PMCA or Na^+/K^+ -ATPases.³¹ Its action could be attributed to binding of one thapsigargin molecule to each molecule of SERCA, presumably at a site that is not shared by PMCA.²⁰³ This site was later identified as a cavity circumscribed by the third, fifth, and seventh transmembrane domains within the cytosolic side of the membrane.²⁰⁴ In this location, thapsigargin interferes with the necessary conformational changes that accompany the ATPase cycle.²⁰⁵ Thapsigargin has very high but differing affinities for all three SERCA isoforms²⁰⁶ and a slow off rate so that its action is virtually irreversible.^{31,203} Thapsigargin rapidly penetrates cells, because its sesquiterpenelike structure

confers high lipid solubility, and its effects on Ca^{2+} are apparent in RBL-2H3 cells at concentrations as low as 1 nM.¹⁹

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, was originally described as a reversible and highly selective inhibitor of the Ca^{2+} -ATPase in skeletal^{207,208} and smooth²⁰⁹ muscle sarcoplasmic reticulum. Comparative analysis of the effects of thapsigargin and cyclopiazonic acid on expressed mutated forms of SERCA1a suggest considerable overlap in their binding domains such that both interfere with the coupling of “ATP utilization in the ATPase cytosolic region and Ca^{2+} binding in the membrane-bound region” of SERCA,²¹⁰ although other data suggest differences in the binding domains.²⁰⁶ This issue is partially resolved by recent X-Ray crystallographic data with SERCA in its ADP/ Ca^{2+} free state.²¹¹ Both inhibitors block Ca^{2+} -ATPase activity by immobilizing the SERCA transmembrane helices albeit on different subsets of transmembrane helices (first, second, and fourth in the case of cyclopiazonic acid). Thus, unlike thapsigargin, cyclopiazonic acid blocks access of Ca^{2+} to the channel.

As a practical matter, cyclopiazonic acid has much lower affinity for all SERCA isoforms than thapsigargin²⁰⁶ and its action is reversible. Data on the effects of cyclopiazonic acid in mast cells are quite limited. It was shown to cause store depletion and activate a Ca^{2+} -influx pathway that was permeable to both Mn^{2+} and Ca^{2+} in RBL-2H3 cells³⁸ and, in this respect, was reminiscent of the nonselective 50 pS influx pathway detected by patch clamp techniques (see Section VI).⁸⁶ Ca^{2+} stores in RBL-2H3 cells and BMMC can be depleted by cyclopiazonic acid as well as by thapsigargin and antigen.²¹² The subsequent Ca^{2+} influx, but not store depletion, is impaired by microtubular disrupting agents and attributed to altered cytoplasmic distribution of ER and loss of communication with putative SOCE channels in the plasma membrane.

B. Adenophostins

The Adenophostins (A and B), products of *Penicillium brevicompactum*,²¹³ and their synthetic derivatives²¹⁴ are the most potent IP3R agonists described to date. Adenophostin A has almost 100-fold greater affinity for IP3R than IP3²¹⁵ and a mechanism has been proposed to account for this greater affinity.^{216,217} Otherwise the interaction of Adenophostins with IP3R is indistinguishable from that of IP3 at the intracellular and single-channel levels. Adenophostin A elicits a quantal pattern of release of Ca^{2+} ,²¹⁸ intracellular Ca^{2+} oscillations,²¹⁹ and changes in electrophysiological parameters that are reminiscent of IP3.²²⁰ However, Adenophostin A diffuses through the cytosol and detaches from IP3R more slowly than IP3. Consequently, with low concentrations of Adenophostin A Ca^{2+} release, the associated Ca^{2+} oscillations and Ca^{2+} entry are spatially restricted to those regions that are presumed to contain sufficient Adenophostin A to activate IP3R.²²¹ Such studies suggest that Ca^{2+} oscillations and entry do not extend into regions beyond the range of activated IP3Rs.

Because the Adenophostins are cell impermeant, their effects on calcium signaling have been studied by conventional patch clamp techniques with single cells, particularly the *Xenopus laevis* oocyte and the RBL1 mast cell line. In RBL1 cells, Adenophostin A can induce Ca^{2+} oscillations when Ca^{2+} entry into the cell is blocked by voltage clamp to indicate that the oscillations are regenerative; that is, reuptake of Ca^{2+} into ER replenishes stores sufficiently for another cycle of release and reuptake.²¹⁹ Unlike IP3, Adenophostin activates I_{CRAC} at low buffered concentrations of intracellular Ca^{2+} .^{219,222,223} This has been attributed to reduced sensitivity to the normal Ca^{2+} -dependent inactivation of either IP3R²²² or the CRAC channel²¹⁹ or alternatively to another unique action of Adenophostin.²²³ As noted earlier, the activation of CRAC channels by Adenophostin A, thapsigargin, or IP3 is substantially diminished by mitochondrial depolarization.⁵⁰

C. Xestospongins and 2-Aminoethoxydiphenyl Borate (2-APB)

Pharmacological antagonists of IP3R-mediated Ca^{2+} release are few and all have limitations.²²⁴ Heparin has been used for such purpose but it is cell impermeable and it has multiple actions including activation of ryanodine receptors. The membrane-permeant xestospongins²²⁵ and 2-APB²²⁶ were initially identified as IP3R antagonists but their mechanisms of action remain undefined and it is now apparent that 2-APB targets other entities that regulate calcium signaling.

The xestospongins (B, C, and D) are macrocyclic bis-1-oxaquinolizidines present in the sponge *Xestospongia* sp and are thought to be allosteric inhibitors of IP3R. Since the initial report that xestospongin C inhibited IP3-elicited Ca^{2+} release without affecting IP3 binding,²²⁵ xestospongin C has been used in various types of cells and tissues for this purpose. In RBL-2H3 cells, for example, xestospongin C (3–10 μM) inhibited the transient antigen-induced increase in $[\text{Ca}^{2+}]_i$ in the absence of external Ca^{2+} , influx of Ca^{2+} in the presence of external Ca^{2+} , and degranulation.²²⁷ IP3-induced depletion of Ca^{2+} stores was also inhibited. However, depletion of stores by thapsigargin and the subsequent influx of external Ca^{2+} were unaffected. Other reports have implied that xestospongin also inhibits SERCA in several^{228–230} but not all tissues.^{231,232} This and other unresolved issues about the specificity and mechanism of action of the xestospongins limit interpretation of published experimental data. Other drawbacks include their slow action and limited availability.

2-APB was initially reported to inhibit IP3-induced Ca^{2+} release from stores without affecting IP3 production or binding.²²⁵ Nor did it appear to activate ryanodine receptors or voltage-operated Ca^{2+} entry. Subsequent studies with cell membranes, permeabilized cells and intact cells pointed to inconsistent actions of 2-APB on IP3R in different types of cells and various explanations were proffered for this inconsistency. These include differences in sensitivity of the different isoforms of IP3R to 2-APB and a dependency on ambient IP3 concentrations that may outcompete 2-APB as discussed in a recent review.²²⁴

Interest in 2-APB was enhanced by the report that 2-APB blocked IP3R-mediated SOCE.²³³ Studies since then have shown that high concentrations of 2-APB can reliably block SOCE, independently of its effects on IP3R,^{234–237} by interacting with components on the exterior surface of the cells.^{88,235,238,239} Moreover, 2-APB blocks SOCE whether mediated by I_{CRAC} ^{235,236} or TRPC channels.^{234,237,239–242} The latter include heterologously expressed TRPC1,²⁴⁰ TRPC3,^{234,241} TRPC5,^{239,242} TRPC6, and several TRPMs.^{239,243,244} However, the action of 2-APB is biphasic, enhancing I_{CRAC} at low concentrations (1–5 μM) and suppressing I_{CRAC} at high concentrations (10 μM or greater) in IP3R-defective DT40 B cells, Jurkat T B cells, and RBL mast cells.²³⁵ This biphasic action has recently been examined in heterologous expression systems, and differences in 2-APB sensitivity were noted among the Orai proteins.^{245–248} In one report, Orai1 was weakly activated, Orai2 was unresponsive, and Orai3 was strongly activated by 2-APB, but when coexpressed with STIM1, Orai1 and Orai2 were activated at low concentrations and inhibited at high concentrations of 2-APB.²⁴⁶ These stimulatory and inhibitory actions of 2-APB were attributed to a variety of actions including alteration of pore architecture, channel blockade, reversal of store-dependent polymerization of STIM1,²⁴⁶ or reduced accumulation of STIM in punctae near the plasma membrane.²⁴⁷ In all studies, 2-APB activated Orai3 independently of STIM1 and store depletion in a manner that resulted in a nonselective cation current possibly due to enlargement of the pore size.²⁴⁸ In contrast to the Orai proteins, only inhibitory effects have been noted with TRPCs. 2-APB inhibits TRPC5 and TRPC6 activities in a monophasic manner to suggest that each TRPC channel is blocked by a single molecule of 2-APB.²³⁹

Other effects of 2-APB include reversible block of SERCA and “leakage” of Ca^{2+} from ER pools,²⁴⁹ release of sequestered Ca^{2+} from mitochondria possibly by acting on the $\text{Na}^+/\text{Ca}^{2+}$

exchanger,²³⁵ and blockade of TRPMs,^{239,243,244} all of which may be relevant to calcium signaling in mast cells. Despite these and other diverse actions of 2-APB,^{224,250,251} the compound can be a useful reagent when used cautiously.

X. ADDITIONAL DETAILS OF CALCIUM SIGNALING IN MAST CELLS

A. Biochemical Signals Regulating Calcium Signaling: IP3 and Sphingolipids

The aggregation of high-affinity receptors for IgE (FcεRI) by antigen results in transphosphorylation of adjacent FcεRI subunits and the initiation of phosphorylation cascades that involve Src kinases and the tyrosine kinase Syk. One consequence is the tyrosine phosphorylation of PLCγ, production of IP3, and a store-dependent calcium signal.²⁵² In contrast, stimulation of mast cells through G-protein-coupled receptors is linked to activation of PLCβ, although some of these receptors undergo rapid desensitization and the associated IP3 production and calcium signal are transient and insufficient to promote functional responses.²⁵³

Less clear is the role of the sphingosine kinases (SKs) in calcium signaling. The SK product, sphingosine 1-phosphate (S1P), is proposed to be equally essential for Ca²⁺ mobilization, degranulation,^{254,255} and the production of eicosanoids and cytokines²⁵⁶ in mast cells. It was originally postulated that IP3 and S1P are both necessary for release of Ca²⁺ from intracellular stores²⁵⁴ with S1P promoting transient release and IP3 a more sustained release coupled to Ca²⁺ entry.²⁵⁵ However, recent evidence suggests that Ca²⁺ entry, and not release, is the process primarily regulated by S1P.²⁵⁶ Potential upstream regulators of the SK/Ca²⁺ pathway include the Src kinases Lyn and Fyn²⁵⁷ as well as PLD1.^{255,258} In addition, activation of the SK/Ca²⁺ pathway, but not the PLC/Ca²⁺ pathway, appears to be dependent on clathrin, which is thought to facilitate transfer of the lipophilic S1P from the plasma membrane to ER.²⁵⁹

There are, nevertheless, several unresolved issues.^{260,261} Of the two isoforms of SK, SK1 was claimed to be the predominant regulator of calcium signaling and downstream events in one study²⁵⁵ and SK2 in another.²⁵⁶ In our view, the latter study is the most carefully crafted investigation and is consistent with our own unpublished results. Another issue is whether or not PLD tightly regulates one or both SKs.²⁶⁰ In our findings, the effects of pharmacologic inhibition or knockdown of the PLDs on calcium signaling are relatively modest (Peng, Ma, and Beaven, unpublished data). It should be noted also that S1P acts both intracellularly and extracellularly—after export from mast cells—to activate G-protein-coupled S1P receptors and cell migration.²⁶² This could be a confounding factor in studies with mast cells because extracellular S1P can mediate increases in [Ca²⁺]_i via PLCβ-coupled S1P receptors in other types of cells.^{263,264} Regardless of these issues, there is consistent evidence that S1P and related sphingolipids are critical for mast cell activation and allergic reactions.^{265–267}

S1P has also been implicated in Ca²⁺ mobilization following stimulation through Fcγ and G-protein-coupled receptors in other types of cells independently of the classical PLC/IP3/IP3R-pathway.^{258,268–271} It is reported that S1P is generated in, and releases Ca²⁺ from, ER-containing vesicles.²⁷² Also, intracellular application of S1P causes release of stored Ca²⁺²⁷¹ and does so independently of S1P receptors on the cell surface.²⁷³

Although the accumulated information is intriguing, the determination of the role of S1P and other sphingolipids in calcium signaling is beset with difficulties.²⁶¹ Early studies, of necessity, relied on the use of the SK inhibitors *DL-threo*-dihydrosphingosine and *N,N*-dimethylsphingosine; which may not have the specificity intended. A particular problem with mast cells is the phenotypic flexibility of these cells in vivo and in culture.²⁷⁴ Deletion of molecules, such as SK and PLD, that perform essential housekeeping functions may well result in altered phenotype or compensatory expression of alternate isoforms of these molecules. In

addition, several questions need to be resolved and some revisited to make a convincing case that production S1P has an integral, rather than indirect, role in FcεRI-mediated calcium signaling in mast cells. Despite numerous studies, the channel(s) targeted by S1P and by another possible candidate, sphingosylphosphorylcholine, has not been identified.²⁶¹ The sites of production and mechanisms of delivery of these phospholipids to targets need to be clearly defined. Unlike IP3 they are not freely diffusible in the cytosol.²⁶⁰

B. Ca^{2+} Stores and Channels in Mast Cells

The generic scheme for IP3-dependent calcium mobilization in RBL-2H3 cells is shown in Figure 3. It is unknown whether certain details of this scheme vary from one mast cell population to another because FcεRI-mediated calcium signaling has been studied almost exclusively with RBL-2H3 cells. The composition of the SOCE channel is probably a variable feature among different cell types, but we propose that TRPC5 addition to Orai1 and STIM1 participates in SOCE channel activity in RBL-2H3 cells.

As noted earlier, RBL-2H3 cells produce IP3 via $\text{PLC}\gamma^{14}$ to induce release of Ca^{2+} from intracellular stores.¹²⁹ This results in sustained influx of Ca^{2+} to maintain an elevated $[\text{Ca}^{2+}]_i$ ¹¹ and replenish IP3-sensitive intracellular stores¹⁹ in an ATP-dependent manner¹⁵ through a thapsigargin-sensitive pump.¹⁹ RBL-2H3 cells express all three forms of IP3R and thapsigargin-sensitive SERCA2b and SERCA3 pumps.⁶² The relative importance of PMCA and $\text{Na}^+/\text{Ca}^{2+}$ exchangers in the extrusion of Ca^{2+} from mast cells was described in Section VIII.B.

Influx is also associated with substantial uptake of Ca^{2+} into mitochondria, which then releases Ca^{2+} once $[\text{Ca}^{2+}]_i$ declines to below the mitochondrial set point for Ca^{2+} .^{19,20} In fact, most mitochondria in RBL-2H3 cells lie in very close proximity to ER to form ER/mitochondrial junctions²⁷⁵ with access to ER SERCA pumps.²⁷⁶ This situation suggests that ER/mitochondrial junctions may be equipped to deliver Ca^{2+} effectively to and from the mitochondria, enabling regulation of mitochondrial dehydrogenases.²⁷⁶ Indeed, IP3 induces incremental Ca^{2+} release from ER and Ca^{2+} uptake into mitochondria in permeabilized RBL-2H3 cells.²⁷⁶ In intact RBL-2H3 cells, transfer of Ca^{2+} to mitochondria is most efficient during Ca^{2+} oscillations where the peak $[\text{Ca}^{2+}]_i$ appears to activate the MCU Ca^{2+} transporter, possibly through calmodulin.²⁷⁷

All indications point to STIM1 and Orai1 as being essential for CRAC and SOCE channel activity in mast cells. Both proteins are absolutely required for FcεRI-mediated mast cell activation in vitro and in vivo. Orai1/CRACM²⁷⁸ or STIM1²⁷⁹ deficient mast cells exhibit defective calcium signaling, degranulation, and cytokine release. Mice, from which these cells were derived, have impaired IgE-dependent allergic responses. Yet, the known features of I_{CRAC} and Orai proteins are not in accord with the permeability of mast cells to divalent metal ions and Ca^{2+} (see Section I) or the non- I_{CRAC} currents in RBL-2H3 cells (as described Section IV). Resolution of this conundrum may come from studies of the less selective TRPCs channels, which appear to act coordinately with Orai1 and STIM1. Knockdown of endogenous TRPC5, STIM1, or Orai1 individually with inhibitory RNAs substantially reduces influx of Ca^{2+} as well as degranulation in RBL-2H3 cells.¹²⁹ Moreover, overexpression of Orai1 with STIM1 promotes constitutive influx of Ca^{2+} but not of Sr^{2+} , whereas overexpression of TRPC5 with STIM1 promotes constitutive influx of both ions. These and other data suggest that Sr^{2+} -permeable TRPC5 acts in conjunction with Orai1 and STIM1 to allow Sr^{2+} and other divalent metal ions to permeate and support degranulation in mast cells¹²⁹ and possibly amplify channel conductance.⁸⁸ Given the phenotypic diversity²⁷⁴ and variable expression of TRPCs among mast cell subpopulations (Ma, Iwaki, Gilfillan, and Beaven, unpublished data), additional studies are necessary to determine whether such subpopulations continue to employ TRPC5 or rely on other TRPCs for SOCE.

The communication between STIM1 and Orai1 has been examined by confocal microscopy and fluorescence resonance energy transfer (FRET) in RBL-2H3 cells that expressed fluorescent tagged versions of these molecules.²⁸⁰ In resting cells, STIM1 was associated with microtubules and was diffusely distributed on ER. Following depletion of ER stores by thapsigargin, STIM1 redistributed along with ER which underwent substantial rearrangement to form peripheral punctae where STIM1 in ER and Orai1 in plasma membrane were placed in close proximity. The extent of this interaction may depend on the extent of depletion of ER stores. Antigen stimulation resulted in less extensive colocalization of STIM1 and Orai1 but was more prominent when refilling of stores was blocked by Gd^{3+} . Without Gd^{3+} , maximal colocalization coincided with the initial spike in $[Ca^{2+}]_i$ following addition of antigen with rapid reversal on decay in $[Ca^{2+}]_i$ and refilling of ER stores.²⁸⁰ These reactions may involve ionic interactions between STIM1 and Orai1 because amphiphilic molecules such as D-sphingosine and *N,N*-dimethylsphingosine inhibit not only IP3-mediated I_{CRAC} ²⁸¹ but also the FRET-monitored interaction of STIM1 with Orai1 and Ca^{2+} influx.²⁸⁰ Similar effects were noted by mutation of acidic residues in the cytoplasmic tail of Orai1.²⁸⁰ The proposed model was that the positively charged sphingosines, which flip to the cytoplasmic surface of the plasma membrane, neutralize the Orai1 acidic residues resulting in Orai1 homooligomerization and preclusion of STIM1. Although these findings may imply a potential regulatory role for sphingolipids on this aspect of SOCE, they exclude a direct role of SKs because D-sphingosine is a substrate and *N,N*-dimethylsphingosine is an inhibitor of SK, and yet both have the same effect.

C. Regulation of Membrane Potential and Its Role in Ca^{2+} Entry

Ca^{2+} influx is driven not only by the high Ca^{2+} concentration gradient, but also by the electrochemical gradient across the plasma membrane²¹ such that depolarization with high external K^+ abolishes Ca^{2+} influx and degranulation.¹⁵ The mechanism(s) responsible for maintenance and restoration of plasma membrane polarity in mast cells is not entirely clear.²⁸² Also, mechanisms may vary from one type of mast cell to another. For example, RBL-2H3 cells express an inwardly rectifying K^+ channel, Kir2.1, which sets the membrane potential at about -80 mV,²⁸³ whereas resting primary human cell lines are electrically silent with a membrane potential of about 0 mV and no demonstrable Kir current.²⁸² Following antigen stimulation, RBL-2H3 cells partially depolarize,²¹ due in part to monovalent cation influx via TRPM4 (described later in this section), and then repolarize by a mechanism attributed to K^+ efflux,²⁸⁴ possibly through the Ca^{2+} -activated $iK_{Ca3.1}$ (also known as iK_{Ca1}) potassium channel.²⁸⁵ In human mast cells, stimulation causes a rapid change in membrane potential from 0 to around -45 mV as a result of activation of $iK_{Ca3.1}$.^{282,286} Blockade of $iK_{Ca3.1}$ impairs degranulation²⁸⁶ and chemotaxis.²⁸⁷ Interestingly, the expression of functional $iK_{Ca3.1}$ is increased when human mast cells are cultured with stem cell factor (SCF), interleukin (IL)-6, and IL-10, which likely accounts for the enhanced reactivity of these cells as compared to their freshly isolated counterparts.²⁸⁶

Chloride channels have also been implicated in regulating membrane polarity and promoting Ca^{2+} -influx and degranulation in mast cells. However, the evidence is indirect and not definitive. A number of chloride channels, including ClC-2, -3, -4, -5, and -7 as well as CFTR, have been identified electrophysiologically or by RT-PCR in rodent and human mast cell lines. The contributions of these channels to mast cell activation are unclear and the reader is referred to a more detailed discussion of this topic in a recent review.²⁸²

Nonselective Ca^{2+} -activated cation channels, of which TRPM4 and TRPM5 are two candidates, are also thought to regulate membrane polarity.¹⁶⁶ One such channel, TRPM4, has been identified in mouse BMMC where it promotes membrane depolarization, limits Ca^{2+} influx, and constrains the extent of mast cell activation.²⁸⁸ TRPM4 deficiency enhances the

activation of mast cells in vitro and in vivo. This is manifested by increased FcεRI-mediated Ca^{2+} entry and release of inflammatory mediators in TRPM4^{-/-} BMMC and increased severity of the acute phase of IgE-mediated cutaneous anaphylactic response in TRPM4^{-/-} mice.²⁸⁸ TRPM4, like TRPM5, conducts Na^+ and other monovalent cations but not divalent cations in mast cells.²⁸⁹ Consistent with the proposed role of TRPM4, it is activated by the immunosuppressive compound YM-58483, and this activation is associated with suppression of Ca^{2+} and cytokine production in lymphocytes.²⁹⁰ Inhibitors include the sulfonylurea, glibenclamide, and hydroxytricyclic compounds such as 9-phenanthrol.²⁹¹ Consideration of TRPM4 as a therapeutic target in allergic disease as suggested would obviously be predicated on the extent of its functional distribution in different types of cells.

D. Ca^{2+} Puffs, Waves, and Oscillations in Mast Cells

Ca^{2+} waves were first described in fertilized *Xenopus* oocytes²⁹² and since then in electrically excitable²⁹³ and nonexcitable cells.^{294,295} Ca^{2+} waves are preceded by localized “ Ca^{2+} sparks”²⁹⁶ or puffs and then spread through the cytosol by Ca^{2+} -induced Ca^{2+} release from ER.^{297,298} This is usually followed by regenerative oscillations once ER stores are replenished with external Ca^{2+} . The dynamics of these oscillations may determine the functional output of the cell.²⁹⁸ In RBL-2H3 cells, Ca^{2+} oscillations^{16,299,300} are temporally correlated to degranulation^{301,302} and are dependent on Ca^{2+} entry to sustain oscillations beyond the initial few oscillations.¹⁶ These oscillations are of variable amplitude and duration and are usually superimposed on a rising then decreasing baseline of elevated $[\text{Ca}^{2+}]_i$.¹⁶ The oscillations are not dependent on transient changes in membrane potential or mitochondrial stores nor are they induced by ionomycin¹⁶ or thapsigargin.¹⁹ Together these results, in the context of studies in other types of cells, suggest that these oscillations result from IP3-induced release of Ca^{2+} from ER.

E. Functional Consequences of Calcium Signals in Mast Cells

Increases in free Ca^{2+} within the cytosol are essential for many cellular activities such as proliferation, gene expression, secretion, migration, and adhesion. A particular activity may require spatial and temporal resolution of the calcium signal, a few examples of which have been described in previous sections. These and other aspects of calcium signaling have been described in numerous reviews,^{44,45,58,60,92,95,111–118,303–305} but the question remains as to how a multifunctional cell, such as the mast cell,^{274,306} directs and regulates its calcium signal to promote a particular response or subset of responses.

The mast cell has remarkably diverse functional outputs. These include degranulation,³⁰⁷ formation of the eicosanoid precursor arachidonic acid,^{308,309} chemotaxis,³¹⁰ and production of cytokines,^{311–313} and all to some degree are dependent on the generation of a calcium signal regardless of stimulant. Some mast cell-activating ligands such as Toll-like receptor ligands can elicit modest production of cytokines in the absence of a calcium signal but this production is markedly enhanced when mast cells are costimulated with antigen that activates the Ca^{2+} /calcineurin/NFAT pathway.³¹⁴ Under certain conditions, mast cells can be stimulated to undergo chemotaxis^{310,315,316} or produce cytokines³¹⁷ without degranulation. Preferential release of either histamine (a secretory granule constituent) or eicosanoids has been noted with different stimulants^{267,318,319} and attributed to differences in the amplitude and duration of the calcium signal.³¹⁹

Examples of coupling of responses to specific components of the calcium signal in mast cells are limited. Early studies indicated that influx of Ca^{2+} from the external medium was absolutely required for degranulation¹¹ and that Ca^{2+} oscillations generated by release of Ca^{2+} from intracellular stores failed to support degranulation in antigen-stimulated RBL-2H3 cells.³⁰¹ Instead, sustained Ca^{2+} oscillations or global increases of $[\text{Ca}^{2+}]_i$ maintained by Ca^{2+} influx

did support degranulation.^{309,320} A similar tight dependency on Ca^{2+} influx was noted for the recruitment of protein kinase C and components of the ERK/PLA₂/5-lipoxygenase pathway in RBL cells activated with thapsigargin³⁰⁹ or receptor agonists.³²⁰ Some mast cell stimulants such as agonists of the adenosine A₃ receptor³²¹ and purinergic P2Y receptor³²⁰ elicit transient production of IP₃ and release of stored Ca^{2+} without activating influx mechanisms, Ca^{2+} -dependent signals, and degranulation. These studies might imply that downstream effectors are most tightly coupled to influx rather than release of Ca^{2+} . A complicating factor in relating the calcium signal to specific responses is that Ca^{2+} entry and activation of I_{CRAC} was found to be an all-or-none reaction in individual cells regardless of concentration of stimulant.³²⁰ Therefore, the typical concentration-response curves for cell populations may reflect an increasing proportion of cells responding in an all-or-none fashion.

The observations to date do not adequately explain how information is transmitted through the calcium signal to direct mast cells to respond in a particular manner to a given stimulant. This is still largely uncharted territory that needs to be examined at a level that, for example, has been described recently for fibroblast migration where “calcium flickers” guide migration down PDGF gradients.³²² Our suspicion is that the details will lie in the spatiotemporal aspects of the calcium signal as outlined in the previous subsection.

XI. CONCLUDING COMMENTS

We have highlighted several issues that will likely guide future research on calcium signaling, especially in regard to the mast cell. These include the temporal and spatial aspects of the calcium signaling and how these relate to specific cellular responses to agonists; how and where channel proteins and their regulatory partners might be recruited to specialized microdomains within membranes; the mechanisms of coordination of these regulatory microdomains that shape the profile and dynamics of the calcium signal; and the potential flexibility of calcium signaling as, for example, the combinatorial arrangements of Orai proteins and TRPCs that might fine-tune influx to fit the particular needs of the cell. Much has been accomplished in recent years in defining the individual components of calcium signaling, but to gain a more global perspective of the signaling process, mathematical modeling of calcium signaling in mast cells has been attempted^{161,323} and may be a useful start in this direction. The mast cell has proved to be a useful experimental model for studies of calcium signaling and much primary information has been gained from studies with this cell in the past 40 years. The diversity of its receptors and responses to different stimuli are an additional asset in examining how calcium signals communicate information for specific responses.

Acknowledgments

This work is supported by the intramural program of the National Heart, Lung, and Blood Institute of the National Institutes of Health.

REFERENCES

1. Ringer S. A further Contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *J Physiol* 1883;4:29–42.
2. Mongar JL, Schild HO. The effect of calcium and pH on the anaphylactic reaction. *J Physiol* 1958;140:272–284. [PubMed: 13514685]
3. Lichtenstein LM, Osler AG. Studies on the mechanism of hypersensitivity phenomena: IX. Histamine release from human leukocytes by ragweed pollen. *J Exp Med* 1964;120:507–530. [PubMed: 14212116]
4. Greaves MW, Mongar JL. The mechanism of anaphylactic histamine release from rabbit leucocytes. *Immunology* 1968;15:743–749. [PubMed: 4177078]

5. Foreman JC, Mongar JL. The role of the alkaline earth ions in anaphylactic histamine secretion. *J Physiol (Lond)* 1972;224:753–769. [PubMed: 4116105]
6. Foreman JC, Hallett MB, Mongar JL. Movement of strontium ions into mast cells and its relationship to the secretory response. *J Physiol (Lond)* 1977;271:233–251. [PubMed: 72148]
7. Foreman JC, Hallet MB, Mongar JL. The relationship between histamine secretion and ⁴⁵Calcium-uptake by mast cells. *J Physiol* 1977;271:193–214. [PubMed: 72146]
8. Foreman JC, Mongar JL. The action of lanthanum and manganese on anaphylactic histamine secretion. *Br J Pharmacol* 1973;48:527–537. [PubMed: 4128659]
9. Pearce FL, White JR. Effect of lanthanide ions on histamine secretion from rat peritoneal mast cells. *Br J Pharmacol* 1981;72:341–347. [PubMed: 6163496]
10. Tsien RY. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochemistry* 1980;19:2396–2404. [PubMed: 6770893]
11. Beaven MA, Rogers J, Moore JP, Hesketh TR, Smith GA, Metcalfe JC. The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. *J Biol Chem* 1984;259:7129–7136. [PubMed: 6202691]
12. Beaven MA, Moore JP, Smith GA, Hesketh TR, Metcalfe JC. The calcium signal and phosphatidylinositol breakdown in 2H3 cells. *J Biol Chem* 1984;259:7137–7142. [PubMed: 6202692]
13. Pribluda VS, Metzger H. Calcium-independent phosphoinositide breakdown in rat basophilic leukemia cells. Evidence for an early rise in inositol 1,4,5-trisphosphate which precedes the rise in other inositol phosphates and in cytoplasmic calcium. *J Biol Chem* 1987;262:11449–11454. [PubMed: 3040702]
14. Park DJ, Min HK, Rhee SG. IgE-induced tyrosine phosphorylation of phospholipase C- α 1 in rat basophilic leukemia cells. *J Biol Chem* 1991;266:24237–24240. [PubMed: 1662204]
15. Mohr FC, Fewtrell C. Depolarization of rat basophilic leukemia cells inhibits calcium uptake and exo-cytosis. *J Cell Biol* 1987;104:783–792. [PubMed: 2950123]
16. Millard PJ, Ryan TA, Webb WW, Fewtrell C. Immunoglobulin E receptor cross-linking induces oscillations in intracellular free ionized calcium in individual tumor mast cells. *J Biol Chem* 1989;264:19730–19739. [PubMed: 2531141]
17. Jones SV, Choi OH, Beaven MA. Carbachol induces secretion in a mast cell line (RBL-2H3) transfected with the m1 muscarinic receptor gene. *FEBS Lett* 1991;289:47–50. [PubMed: 1832647]
18. Marcotte GV, Millard PJ, Fewtrell C. Release of calcium from intracellular stores in rat basophilic leukemia cells monitored with the fluorescent probe chlor-tetracycline. *J Cell Physiol* 1990;142:78–88. [PubMed: 1688862]
19. Ali H, Maeyama K, Sagi-Eisenberg R, Beaven MA. Antigen and thapsigargin promote influx of Ca^{2+} in rat basophilic RBL-2H3 cells by ostensibly similar mechanisms that allow filling of inositol 1,4,5-trisphosphate-sensitive and mitochondrial Ca^{2+} stores. *Biochem J* 1994;304:431–440. [PubMed: 7998977]
20. Mohr FC, Fewtrell C. The effect of mitochondrial inhibitors on calcium homeostasis in tumor mast cells. *Am J Physiol* 1990;258:C217–C226. [PubMed: 2137675]
21. Mohr FC, Fewtrell C. IgE receptor-mediated depolarization of rat basophilic leukemia cells measured with the fluorescent probe bis-oxonol. *J Immunol* 1987;138:1564–1570. [PubMed: 2949017]
22. Mohr FC, Fewtrell C. The relative contributions of extracellular and intracellular calcium to secretion from tumor mast cells. Multiple effects of the proton ionophore carbonyl cyanide m-chlorophenylhydrazone. *J Biol Chem* 1987;262:10638–10643. [PubMed: 2440869]
23. Hide M, Beaven MA. Calcium influx in a rat mast cell (RBL-2H3) line: use of multivalent metal ions to define its characteristics and role in exocytosis. *J Biol Chem* 1991;266:15221–15229. [PubMed: 1869551]
24. Berridge MJ. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J* 1984;220:345–360. [PubMed: 6146314]
25. Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K. Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature* 1989;342:32–38. [PubMed: 2554142]

26. Mignery GA, Newton CL, Archer BT III, Sudhof TC. Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. *J Biol Chem* 1990;265:12679–12685. [PubMed: 2165071]
27. Streb H, Irvine RF, Berridge MJ, Schulz I. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* 1983;306:67–69. [PubMed: 6605482]
28. Burgess GM, Godfrey PP, McKinney JS, Berridge MJ, Irvine RF, Putney JW. The second messenger linking receptor activation to internal Ca^{2+} release in liver. *Nature* 1984;309:63–66. [PubMed: 6325926]
29. Joseph SK, Thomas AP, Williams RJ, Irvine RF, Williamson JR. myo-Inositol 1,4,5-trisphosphate. a second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. *J Biol Chem* 1984;259:3077–3081. [PubMed: 6607924]
30. Prentki M, Biden TJ, Janjic D, Irvine RF, Berridge MJ, Wollheim CB. Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. *Nature* 1984;309:562–564. [PubMed: 6328320]
31. Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca^{2+} -ATPase family of calcium pumps. *J Biol Chem* 1991;266:17067–17071. [PubMed: 1832668]
32. Thastrup O, Cullen PJ, Drbak BK, Hanley MR, Dawson AP. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+} -ATPase. *Proc Natl Acad Sci U S A* 1990;87:2466–2470. [PubMed: 2138778]
33. Takemura H, Hughes AR, Thastrup O, Putney JW Jr. Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool and not an inositol phosphate regulates calcium fluxes at the plasma membrane. *J Biol Chem* 1989;264:12266–12271. [PubMed: 2663854]
34. Jackson TR, Patterson SI, Thastrup O, Hanley MR. A novel tumor promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells. *Biochem J* 1988;253:81–86. [PubMed: 3138987]
35. Putney JW. A model for receptor-regulated calcium entry. *Cell Calcium* 1986;7:1–12. [PubMed: 2420465]
36. Putney JW Jr. Capacitative calcium entry revisited. *Cell Calcium* 1990;11:611–624. [PubMed: 1965707]
37. Putney JW Jr. Recent breakthroughs in the molecular mechanism of capacitative calcium entry (with thoughts on how we got here). *Cell Calcium* 2007;42:103–110. [PubMed: 17349691]
38. Falcone D, Fewtrell C. Ca^{2+} -ATPase inhibitor, cyclopiazonic acid, releases Ca^{2+} from intracellular stores in RBL-2H3 mast cells and activates a Ca^{2+} influx pathway that is permeable to sodium and manganese. *J Cell Physiol* 1995;164:205–213. [PubMed: 7790392]
39. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 1992;355:353–356. [PubMed: 1309940]
40. Hoth M, Penner R. Calcium release-activated calcium current in rat mast cells. *J Physiol (Lond)* 1993;465:359–386. [PubMed: 8229840]
41. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. *Nat Cell Biol* 2003;5:440–446. [PubMed: 12717445]
42. Pinton P, Pozzan T, Rizzuto R. The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum. *EMBO J* 1998;17:5298–5308. [PubMed: 9736609]
43. Vanoevenen J, Raeymaekers L, Dode L, Parys JB, De Smedt H, Callewaert G, Wuytack F, Missiaen L. Cytosolic Ca^{2+} signals depending on the functional state of the Golgi in HeLa cells. *Cell Calcium* 2005;38:489–495. [PubMed: 16122795]
44. Dolman NJ, Tepikin AV. Calcium gradients and the Golgi. *Cell Calcium* 2006;40:505–512. [PubMed: 17023044]
45. Michelangeli F, Ogunbayo OA, Wootton LL. A plethora of interacting organellar Ca^{2+} stores. *Curr Opin Cell Biol* 2005;17:135–140. [PubMed: 15780589]
46. Romagnoli A, Aguiari P, De SD, Leo S, Marchi S, Rimessi A, Zecchini E, Pinton P, Rizzuto R. Endoplasmic reticulum/mitochondria calcium cross-talk. *Novartis Found Symp* 2007;287:122–131. [PubMed: 18074635]

47. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 2004;427:360–364. [PubMed: 14737170]
48. Matlib MA, Zhou Z, Knight S, Ahmed S, Choi KM, Krause-Bauer J, Phillips R, Altschuld R, Katsube Y, Sperelakis N, Bers DM. Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca^{2+} uptake into mitochondria in vitro and in situ in single cardiac myocytes. *J Biol Chem* 1998;273:10223–10231. [PubMed: 9553073]
49. Moreau B, Nelson C, Parekh AB. Biphasic regulation of mitochondrial Ca^{2+} uptake by cytosolic Ca^{2+} concentration. *Curr Biol* 2006;16:1672–1677. [PubMed: 16920631]
50. Glitsch MD, Bakowski D, Parekh AB. Store-operated Ca^{2+} entry depends on mitochondrial Ca^{2+} uptake. *EMBO J* 2002;21:6744–6754. [PubMed: 12485995]
51. Rossi CS, Lehinger AL. Stoichiometry of respiratory stimulation, accumulation of Ca^{2+} and phosphate, and oxidative phosphorylation in rat liver mitochondria. *J Biol Chem* 1964;239:3971–3980. [PubMed: 14257633]
52. Nicholls DG. Mitochondria and calcium signaling. *Cell Calcium* 2005;38:311–317. [PubMed: 16087232]
53. Crompton M, Moser R, Ludi H, Carafoli E. The interrelations between the transport of sodium and calcium in mitochondria of various mammalian tissues. *Eur J Biochem* 1978;82:25–31. [PubMed: 23291]
54. Crompton M, Heid I. The cycling of calcium, sodium, and protons across the inner membrane of cardiac mitochondria. *Eur J Biochem* 1978;91:599–608. [PubMed: 32035]
55. Nicholls DG. The regulation of extramitochondrial free calcium ion concentration by rat liver mitochondria. *Biochem J* 1978;176:463–474. [PubMed: 33670]
56. Visch HJ, Rutter GA, Koopman WJ, Koenderink JB, Verkaart S, de Groot T, Varadi A, Mitchell KJ, van den Heuvel LP, Smeitink JA, Willems PH. Inhibition of mitochondrial Na^{+} - Ca^{2+} exchange restores agonist-induced ATP production and Ca^{2+} handling in human complex I deficiency. *J Biol Chem* 2004;279:40328–40336. [PubMed: 15269216]
57. Kuo TH, Zhu L, Golden K, Marsh JD, Bhattacharya SK, Liu BF. Altered Ca^{2+} homeostasis and impaired mitochondrial function in cardiomyopathy. *Mol Cell Biochem* 2002;238:119–127. [PubMed: 12349899]
58. Vermassen E, Parys JB, Mauger JP. Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. *Biol Cell* 2004;96:3–17. [PubMed: 15093123]
59. Devogelaere B, Verbert L, Parys JB, Missiaen L, De SH. The complex regulatory function of the ligand-binding domain of the inositol 1,4,5-trisphosphate receptor. *Cell Calcium* 2008;43:17–27. [PubMed: 17499849]
60. Choe CU, Ehrlich BE. The inositol 1,4,5-trisphosphate receptor (IP3R) and its regulators: sometimes good and sometimes bad teamwork. *Sci STKE* 2006;2006:re 15.
61. Patterson RL, Boehning D, Snyder SH. Inositol 1,4,5-trisphosphate receptors as signal integrators. *Annu Rev Biochem* 2004;73:437–465. [PubMed: 15189149]
62. Vanlingen S, Parys JB, Missiaen L, De SH, Wuytack F, Casteels R. Distribution of inositol 1,4,5-trisphosphate receptor isoforms, SERCA isoforms and Ca^{2+} binding proteins in RBL-2H3 rat basophilic leukemia cells. *Cell Calcium* 1997;22:475–486. [PubMed: 9502197]
63. Leite MF, Thrower EC, Echevarria W, Koulen P, Hirata K, Bennett AM, Ehrlich BE, Nathanson MH. Nuclear and cytosolic calcium are regulated independently. *Proc Natl Acad Sci U S A* 2003;100:2975–2980. [PubMed: 12606721]
64. Wilson BS, Pfeiffer JR, Smith AJ, Oliver JM, Oberdorf JA, Wojcikiewicz RJ. Calcium-dependent clustering of inositol 1,4,5-trisphosphate receptors. *Mol Biol Cell* 1998;9:1465–1478. [PubMed: 9614187]
65. Fujimoto T, Miyawaki A, Mikoshiba K. Inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae is linked to actin filaments. *J Cell Sci* 1995;108(Pt 1):7–15. [PubMed: 7738118]
66. Colosetti P, Tunwell RE, Cruttwell C, Arsanto JP, Mauger JP, Cassio D. The type 3 inositol 1,4,5-trisphosphate receptor is concentrated at the tight junction level in polarized MDCK cells. *J Cell Sci* 2003;116:2791–2803. [PubMed: 12759372]

67. Dellis O, Dedos SG, Tovey SC, Taufiq UR, Dubel SJ, Taylor CW. Ca^{2+} entry through plasma membrane IP_3 receptors. *Science* 2006;313:229–233. [PubMed: 16840702]
68. Mikoshiba K. The IP_3 receptor/ Ca^{2+} channel and its cellular function. *Biochem Soc Symp* 2007;9–22. [PubMed: 17233576]
69. Bosanac I, Alattia JR, Mal TK, Chan J, Talarico S, Tong FK, Tong KI, Yoshikawa F, Furuichi T, Iwai M, Michikawa T, Mikoshiba K, Ikura M. Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand. *Nature* 2002;420:696–700. [PubMed: 12442173]
70. Tu H, Wang Z, Bezprozvanny I. Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region. *Biophys J* 2005;88:1056–1069. [PubMed: 15531634]
71. Taylor CW, da Fonseca PC, Morris EP. IP_3 receptors: the search for structure. *Trends Biochem Sci* 2004;29:210–219. [PubMed: 15082315]
72. Finch EA, Turner TJ, Goldin SM. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* 1991;252:443–446. [PubMed: 2017683]
73. Bezprozvanny I, Watras J, Ehrlich BE. Bell-shaped calcium-response curves of $\text{Ins}(1,4,5)\text{P}_3$ - and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* 1991;351:751–754. [PubMed: 1648178]
74. Sienaert I, Nadif Kasri N, Vanlingen S, Parys JB, Callewaert G, Missiaen L, De Smedt H. Localization and function of a calmodulin-apocalmodulin-binding domain in the N-terminal part of the type 1 inositol 1,4,5-trisphosphate receptor. *Biochem J* 2002;365:269–277. [PubMed: 11955285]
75. Michikawa T, Hirota J, Kawano S, Hiraoka M, Yamada M, Furuichi T, Mikoshiba K. Calmodulin mediates calcium-dependent inactivation of the cerebellar type 1 inositol 1,4,5-trisphosphate receptor. *Neuron* 1999;23:799–808. [PubMed: 10482245]
76. Missiaen L, De Smedt H, Bultynck G, Vanlingen S, Desmet P, Callewaert G, Parys JB. Calmodulin increases the sensitivity of type 3 inositol-1,4, 5-trisphosphate receptors to Ca^{2+} inhibition in human bronchial mucosal cells. *Mol Pharmacol* 2000;57:564–567. [PubMed: 10692497]
77. Adkins CE, Morris SA, De Smedt H, Sienaert I, Torok K, Taylor CW. Ca^{2+} -calmodulin inhibits Ca^{2+} release mediated by type-1, -2 and -3 inositol trisphosphate receptors. *Biochem J* 2000;345 (Pt 2):357–363. [PubMed: 10620513]
78. Jayaraman T, Ondrias K, Ondriasova E, Marks AR. Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation. *Science* 1996;272:1492–1494. [PubMed: 8633244]
79. Yokoyama K, Su I-I, Tezuka T, Yasuda T, Mikoshiba K, Tarakhovsky A, Yamamoto T. BANK regulates BCR-induced calcium mobilization by promoting tyrosine phosphorylation of IP_3 receptor. *EMBO J* 2002;21:83–92. [PubMed: 11782428]
80. Parravinci V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, Saitoh S, Samelson LE, O'Shea JJ, Rivera J. Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. *Nat Immunol* 2002;3:741–748. [PubMed: 12089510]
81. Zweifach A, Lewis RS. Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. *Proc Natl Acad Sci U S A* 1993;90:6295–6299. [PubMed: 8392195]
82. Zweifach A, Lewis RS. Slow calcium-dependent inactivation of depletion-activated calcium current. Store-dependent and -independent mechanisms. *J Biol Chem* 1995;270:14445–14451. [PubMed: 7540169]
83. Zweifach A, Lewis RS. Calcium-dependent potentiation of store-operated calcium channels in T lymphocytes. *J Gen Physiol* 1996;107:597–610. [PubMed: 8740373]
84. Christian EP, Spence KT, Togo JA, Dargis PG, Patel J. Calcium-dependent enhancement of depletion-activated calcium current in Jurkat T lymphocytes. *J Membr Biol* 1996;150:63–71. [PubMed: 8699480]
85. Fasolato C, Hoth M, Penner R. A GTP-dependent step in the activation mechanism of capacitative calcium influx. *J Biol Chem* 1993;268:20737–20740. [PubMed: 8407897]
86. Fasolato C, Hoth M, Matthews G, Penner R. Ca^{2+} and Mn^{2+} influx through receptor-mediated activation of nonspecific cation channels in mast cells. *Proc Natl Acad Sci U S A* 1993;90:3068–3072. [PubMed: 7681994]
87. Kerschbaum HH, Cahalan MD. Single-channel recording of a store-operated Ca^{2+} channel in Jurkat T lymphocytes. *Science* 1999;283:836–839. [PubMed: 9933165]

88. Braun FJ, Broad LM, Armstrong DL, Putney JW Jr. Stable activation of single Ca^{2+} release-activated Ca^{2+} channels in divalent cation-free solutions. *J Biol Chem* 2001;276:1063–1070. [PubMed: 11042187]
89. Hardie RC, Minke B. Novel Ca^{2+} channels underlying transduction in *Drosophila* photoreceptors: implications for phosphoinositide-mediated Ca^{2+} mobilization. *Trends Neurosci* 1993;16:371–376. [PubMed: 7694408]
90. Zhu X, Jiang M, Peyton M, Boulay G, Hurst R, Stefani E, Birnbaumer L. trp, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. *Cell* 1996;85:661–671. [PubMed: 8646775]
91. Wes PD, Chevesich J, Jeromin A, Rosenberg C, Stetten G, Montell C. TRPC1, a human homolog of a *Drosophila* store-operated channel. *Proc Natl Acad Sci U S A* 1995;92:9652–9656. [PubMed: 7568191]
92. Putney JW, Bird GS. Cytoplasmic calcium oscillations and store-operated calcium influx. *J Physiol* 2008;586:3055–3059. [PubMed: 18388136]
93. Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. *Physiol Rev* 2007;87:165–217. [PubMed: 17237345]
94. Worley PF, Zeng W, Huang GN, Yuan JP, Kim JY, Lee MG, Muallem S. TRPC channels as STIM1-regulated store-operated channels. *Cell Calcium* 2007;42:205–211. [PubMed: 17517433]
95. Ambudkar IS, Ong HL. Organization and function of TRPC channelosomes. *Pflugers Arch* 2007;455:187–200. [PubMed: 17486362]
96. Birnbaumer L. The TRPC class of ion channels: a critical review of their roles in slow, sustained increases in intracellular Ca^{2+} concentrations. *Annu Rev Pharmacol Toxicol* 2009;49:395–426. [PubMed: 19281310]
97. Putney JW Jr. Inositol lipids and TRPC channel activation. *Biochem Soc Symp* 2007;37–45. [PubMed: 17233578]
98. Lopez JJ, Salido GM, Pariente JA, Rosado JA. Interaction of STIM1 with endogenously expressed human canonical TRP1 upon depletion of intracellular Ca^{2+} stores. *J Biol Chem* 2006;281:28254–28264. [PubMed: 16870612]
99. Mehta D, Ahmed GU, Paria BC, Holinstat M, Voyno-Yasenetskaya T, Tiruppathi C, Minshall RD, Malik AB. RhoA interaction with inositol 1,4,5-trisphosphate receptor and transient receptor potential channel-1 regulates Ca^{2+} entry. Role in signaling increased endothelial permeability. *J Biol Chem* 2003;278:33492–33500. [PubMed: 12766172]
100. Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH, Dehoff MH, Schwarz MK, Seeburg PH, Muallem S, Worley PF. Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. *Cell* 2003;114:777–789. [PubMed: 14505576]
101. Vazquez G, Wedel BJ, Trebak M, St JB, Putney JW Jr. Expression level of the canonical transient receptor potential 3 (TRPC3) channel determines its mechanism of activation. *J Biol Chem* 2003;278:21649–21654. [PubMed: 12686562]
102. Dietrich A, Mederos YS, Gollasch M, Gross V, Storch U, Dubrovskaya G, Obst M, Yildirim E, Salanova B, Kalwa H, Essin K, Pinkenburg O, Luft FC, Gudermann T, Birnbaumer L. Increased vascular smooth muscle contractility in TRPC6^{-/-} mice. *Mol Cell Biol* 2005;25:6980–6989. [PubMed: 16055711]
103. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Curr Biol* 2005;15:1235–1241. [PubMed: 16005298]
104. Roos J, DiGregorio PJ, Jeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* 2005;169:435–445. [PubMed: 15866891]
105. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006;441:179–185. [PubMed: 16582901]
106. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 2006;312:1220–1223. [PubMed: 16645049]

107. Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD. Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *Proc Natl Acad Sci U S A* 2006;103:9357–9362. [PubMed: 16751269]
108. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 2006;281:20661–20665. [PubMed: 16766533]
109. Mercer JC, Dehaven WI, Smyth JT, Wedel B, Boyles RR, Bird GS, Putney JW Jr. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. *J Biol Chem* 2006;281:24979–24990. [PubMed: 16807233]
110. Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol* 2006;8:771–773. [PubMed: 16733527]
111. Scharenberg AM, Humphries LA, Rawlings DJ. Calcium signalling and cell-fate choice in B cells. *Nat Rev Immunol* 2007;7:778–789. [PubMed: 17853903]
112. Clapham DE. Calcium signaling. *Cell* 2007;131:1047–1058. [PubMed: 18083096]
113. Frischauf I, Schindl R, Derler I, Bergsmann J, Fahrner M, Romanin C. The STIM/Orai coupling machinery. *Channels (Austin)* 2008;2:261–268. [PubMed: 18769136]
114. Potier M, Trebak M. New developments in the signaling mechanisms of the store-operated calcium entry pathway. *Pflugers Arch* 2008;457:405–415. [PubMed: 18536932]
115. Luik RM, Lewis RS. New insights into the molecular mechanisms of store-operated Ca^{2+} signaling in T cells. *Trends Mol Med* 2007;13:103–107. [PubMed: 17267286]
116. Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol* 2007;7:690–702. [PubMed: 17703229]
117. Oh-Hora M, Rao A. Calcium signaling in lymphocytes. *Curr Opin Immunol* 2008;20:250–258. [PubMed: 18515054]
118. Vig M, Kinet J-P. Calcium signaling in immune cells. *Nature Immunol* 2009;10:21–27. [PubMed: 19088738]
119. Cahalan MD, Zhang SL, Yeromin AV, Ohlsen K, Roos J, Stauderman KA. Molecular basis of the CRAC channel. *Cell Calcium* 2007;42:133–144. [PubMed: 17482674]
120. Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-Hora M, Neems DS, Hogan PG, Rao A. Biochemical and functional characterization of Orai proteins. *J Biol Chem* 2007;282:16232–16243. [PubMed: 17293345]
121. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006;443:230–233. [PubMed: 16921383]
122. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 2006;443:226–229. [PubMed: 16921385]
123. Mignen O, Thompson JL, Shuttleworth TJ. Orai1 subunit stoichiometry of the mammalian CRAC channel pore. *J Physiol* 2008;586:419–425. [PubMed: 18006576]
124. Ji W, Xu P, Li Z, Lu J, Liu L, Zhan Y, Chen Y, Hille B, Xu T, Chen L. Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc Natl Acad Sci U S A* 2008;105:13668–13673. [PubMed: 18757751]
125. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R. CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. *Curr Biol* 2007;17:794–800. [PubMed: 17442569]
126. Parvez S, Beck A, Peinelt C, Soboloff J, Lis A, Monteilh-Zoller M, Gill DL, Fleig A, Penner R. STIM2 protein mediates distinct store-dependent and store-independent modes of CRAC channel activation. *FASEB J* 2008;22:752–761. [PubMed: 17905723]
127. Ong HL, Cheng KT, Liu X, Bandyopadhyay BC, Paria BC, Soboloff J, Pani B, Gwack Y, Srikanth S, Singh BB, Gill D, Ambudkar IS. Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. *J Biol Chem* 2007;282:9105–9116. [PubMed: 17224452]

128. Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong DL, Birnbaumer L. Orai proteins interact with TRPC channels and confer responsiveness to store depletion. *Proc Natl Acad Sci U S A* 2007;104:4682–4687. [PubMed: 17360584]
129. Ma HT, Peng Z, Hiragun T, Iwaki S, Gilfillan AM, Beaven MA. Canonical transient receptor potential 5 channel in conjunction with Orai1 and STIM1 allows Sr^{2+} entry, optimal influx of Ca^{2+} , and degranulation in a rat mast cell line. *J Immunol* 2008;180:2233–2239. [PubMed: 18250430]
130. Jardin I, Lopez JJ, Salido GM, Rosado JA. Orai1 mediates the interaction between STIM1 and hTRPC1 and regulates the mode of activation of hTRPC1-forming Ca^{2+} channels. *J Biol Chem* 2008;283:25296–25304. [PubMed: 18644792]
131. Cheng KT, Liu X, Ong HL, Ambudkar IS. Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. *J Biol Chem* 2008;283:12935–12940. [PubMed: 18326500]
132. Liao Y, Erxleben C, Abramowitz J, Flockerzi V, Zhu MX, Armstrong DL, Birnbaumer L. Functional interactions among Orai1, TRPCs, and STIM1 suggest a STIM-regulated heteromeric Orai/TRPC model for SOCE/Icrac channels. *Proc Natl Acad Sci U S A* 2008;105:2895–2900. [PubMed: 18287061]
133. Wissenbach U, Philipp SE, Gross SA, Cavalie A, Flockerzi V. Primary structure, chromosomal localization and expression in immune cells of the murine ORAI and STIM genes. *Cell Calcium* 2007;42:439–446. [PubMed: 17659338]
134. Dehaven WI, Smyth JT, Boyles RR, Putney JW Jr. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem* 2007;282:17548–17556. [PubMed: 17452328]
135. Gross SA, Wissenbach U, Philipp SE, Freichel M, Cavalie A, Flockerzi V. Murine ORAI2 splice variants form functional Ca^{2+} release-activated Ca^{2+} (CRAC) channels. *J Biol Chem* 2007;282:19375–19384. [PubMed: 17463004]
136. Parker NJ, Begley CG, Smith PJ, Fox RM. Molecular cloning of a novel human gene (D11S4896E) at chromosomal region 11p15.5. *Genomics* 1996;37:253–256. [PubMed: 8921403]
137. Williams RT, Manji SS, Parker NJ, Hancock MS, Van SL, Eid JP, Senior PV, Kazenwadel JS, Shandala T, Saint R, Smith PJ, Dziadek MA. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem J* 2001;357:673–685. [PubMed: 11463338]
138. Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell* 2007;131:1327–1339. [PubMed: 18160041]
139. Stathopulos PB, Li GY, Plevin MJ, Ames JB, Ikura M. Stored Ca^{2+} depletion-induced oligomerization of STIM1 via the EF-SAM region: an initiation mechanism for capacitive Ca^{2+} entry. *J Biol Chem* 2006;281:35855–35862. [PubMed: 17020874]
140. Zheng L, Stathopulos PB, Li GY, Ikura M. Biophysical characterization of the EF-hand and SAM domain containing Ca^{2+} sensory region of STIM1 and STIM2. *Biochem Biophys Res Commun* 2008;369:240–246. [PubMed: 18166150]
141. Stathopulos PB, Zheng L, Ikura M. Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. *J Biol Chem* 2009;284:728–732. [PubMed: 19019825]
142. Smyth JT, Dehaven WI, Bird GS, Putney JW Jr. Role of the microtubule cytoskeleton in the function of the store-operated Ca^{2+} channel activator STIM1. *J Cell Sci* 2007;120:3762–3771. [PubMed: 17925382]
143. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD. STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane. *Nature* 2005;437:902–905. [PubMed: 16208375]
144. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* 2006;174:815–825. [PubMed: 16966423]
145. Spassova MA, Soboloff J, He LP, Xu W, Dziadek MA, Gill DL. STIM1 has a plasma membrane role in the activation of store-operated Ca^{2+} channels. *Proc Natl Acad Sci U S A* 2006;103:4040–4045. [PubMed: 16537481]

146. Li Z, Lu J, Xu P, Xie X, Chen L, Xu T. Mapping the interacting domains of STIM1 and Orai1 in Ca^{2+} release-activated Ca^{2+} channel activation. *J Biol Chem* 2007;282:29448–29456. [PubMed: 17702753]
147. Dziadek MA, Johnstone LS. Biochemical properties and cellular localisation of STIM proteins. *Cell Calcium* 2007;42:123–132. [PubMed: 17382385]
148. Smyth JT, Dehaven WI, Bird GS, Putney JW Jr. Ca^{2+} -store-dependent and -independent reversal of Stim1 localization and function. *J Cell Sci* 2008;121:762–772. [PubMed: 18285445]
149. Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, Eder P, Schindl R, Hesch C, Polzinger B, Fritsch R, Kahr H, Madl J, Gruber H, Groschner K, Romanin C. Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. *J Biol Chem* 2008;283:8014–8022. [PubMed: 18187424]
150. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, Romanin C. A cytosolic homomerization and a modulatory domain within STIM1 C-terminus determine coupling to ORAI1 channels. *J Biol Chem* 2009;284:8421–8426. [PubMed: 19189966]
151. Varnai P, Toth B, Toth DJ, Hunyady L, Balla T. Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1-Orai1 complex. *J Biol Chem* 2007;282:29678–29690. [PubMed: 17684017]
152. Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S. STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat Cell Biol* 2007;9:636–645. [PubMed: 17486119]
153. Zeng W, Yuan JP, Kim MS, Choi YJ, Huang GN, Worley PF, Muallem S. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol Cell* 2008;32:439–448. [PubMed: 18995841]
154. Lopez JJ, Jardin I, Bobe R, Pariente JA, Enouf J, Salido GM, Rosado JA. STIM1 regulates acidic Ca^{2+} store refilling by interaction with SERCA3 in human platelets. *Biochem Pharmacol* 2008;75:2157–2164. [PubMed: 18439569]
155. Soboloff J, Spassova MA, Hewavitharana T, He LP, Xu W, Johnstone LS, Dziadek MA, Gill DL. STIM2 is an inhibitor of STIM1-mediated store-operated Ca^{2+} Entry. *Curr Biol* 2006;16:1465–1470. [PubMed: 16860747]
156. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol* 2008;9:432–443. [PubMed: 18327260]
157. Pedersen PL, Carafoli E. Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* 1987;12:146–150.
158. Niggli V, Sigel E. Anticipating antiport in P-type ATPases. *Trends Biochem Sci* 2008;33:156–160. [PubMed: 18343670]
159. Obara K, Miyashita N, Xu C, Toyoshima I, Sugita Y, Inesi G, Toyoshima C. Structural role of counter-transport revealed in Ca^{2+} pump crystal structure in the absence of Ca^{2+} . *Proc Natl Acad Sci U S A* 2005;102:14489–14496. [PubMed: 16150713]
160. Strehler EE, Treiman M. Calcium pumps of plasma membrane and cell interior. *Curr Mol Med* 2004;4:323–335. [PubMed: 15101689]
161. Means S, Smith AJ, Shepherd J, Shadid J, Fowler J, Wojcikiewicz RJ, Mazel T, Smith GD, Wilson BS. Reaction diffusion modeling of calcium dynamics with realistic ER geometry. *Biophys J* 2006;91:537–557. [PubMed: 16617072]
162. Schatzmann HJ. ATP-dependent Ca^{++} -extrusion from human red cells. *Experientia* 1966;22:364–365. [PubMed: 5961668]
163. Shull GE, Greeb J. Molecular cloning of two isoforms of the plasma membrane Ca^{2+} -transporting ATPase from rat brain. Structural and functional domains exhibit similarity to Na^{+} , K^{+} - and other cation transport ATPases. *J Biol Chem* 1988;263:8646–8657. [PubMed: 2837461]
164. Verma AK, Filoteo AG, Stanford DR, Wieben ED, Penniston JT, Strehler EE, Fischer R, Heim R, Vogel G, Mathews S. Complete primary structure of a human plasma membrane Ca^{2+} pump. *J Biol Chem* 1988;263:14152–14159. [PubMed: 2844759]

165. Di Leva F, Domi T, Fedrizzi L, Lim D, Carafoli E. The plasma membrane Ca^{2+} ATPase of animal cells: structure, function and regulation. *Arch Biochem Biophys* 2008;476:65–74. [PubMed: 18328800]
166. Harteneck C. Function and pharmacology of TRPM cation channels. *Naunyn Schmiedebergs Arch Pharmacol* 2005;371:307–314. [PubMed: 15843919]
167. Bautista DM, Hoth M, Lewis RS. Enhancement of calcium signalling dynamics and stability by delayed modulation of the plasma-membrane calcium-ATPase in human T cells. *J Physiol* 2002;541:877–894. [PubMed: 12068047]
168. Belan PV, Gerasimenko OV, Tepikin AV, Petersen OH. Localization of Ca^{2+} extrusion sites in pancreatic acinar cells. *J Biol Chem* 1996;271:7615–7619. [PubMed: 8631796]
169. Lee MG, Xu X, Zeng W, Diaz J, Kuo TH, Wuytack F, Racymaekers L, Muallem S. Polarized expression of Ca^{2+} pumps in pancreatic and salivary gland cells. Role in initiation and propagation of $[\text{Ca}^{2+}]_i$ waves. *J Biol Chem* 1997;272:15771–15776. [PubMed: 9188473]
170. Brini M. Plasma membrane Ca^{2+} -ATPase: from a housekeeping function to a versatile signaling role. *Pflugers Arch* 2009;457:657–664. [PubMed: 18548270]
171. Jousset H, Frieden M, Demaurex N. STIM1 knockdown reveals that store-operated Ca^{2+} channels located close to sarco/endoplasmic Ca^{2+} ATPases (SERCA) pumps silently refill the endoplasmic reticulum. *J Biol Chem* 2007;282:11456–11464. [PubMed: 17283081]
172. Yu R, Hinkle PM. Rapid turnover of calcium in the endoplasmic reticulum during signaling. Studies with cameleon calcium indicators. *J Biol Chem* 2000;275:23648–23653. [PubMed: 10811650]
173. Roderick HL, Lechleiter JD, Camacho P. Cytosolic phosphorylation of calnexin controls intracellular Ca^{2+} oscillations via an interaction with SERCA2b. *J Cell Biol* 2000;149:1235–1248. [PubMed: 10851021]
174. López JJ, Jardin I, Bobe R, Pariente JA, Enouf J, Salido GM, Rosado JA. STIM1 regulates acidic Ca^{2+} store refilling by interaction with SERCA3 in human platelets. *Biochem Pharmacol* 2008;75:2157–2164. [PubMed: 18439569]
175. Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM. SERCA pump activity is physiologically regulated by presenilin and regulates amyloid β production. *J Cell Biol* 2008;181:1107–1116. [PubMed: 18591429]
176. Yamasaki-Mann M, Demuro A, Parker I. cADPR stimulates SERCA activity in *Xenopus* oocytes. *Cell Calcium* 2009;45:293–299. [PubMed: 19131109]
177. Lytton J. $\text{Na}^+/\text{Ca}^{2+}$ exchangers: three mammalian gene families control Ca^{2+} transport. *Biochem J* 2007;406:365–382. [PubMed: 17716241]
178. Aneiros E, Philipp S, Lis A, Freichel M, Cavalié A. Modulation of Ca^{2+} signaling by $\text{Na}^+/\text{Ca}^{2+}$ exchangers in mast cells. *J Immunol* 2005;174:119–130. [PubMed: 15611234]
179. Chernaya G, Vazquez M, Reeves JP. Sodium-calcium exchange and store-dependent calcium influx in transfected chinese hamster ovary cells expressing the bovine cardiac sodium-calcium exchanger. Acceleration of exchange activity in thapsigargin-treated cells. *J Biol Chem* 1996;271:5378–5385. [PubMed: 8621391]
180. Brini M, Manni S, Carafoli E. Recombinant expression of the plasma membrane $\text{Na}^+/\text{Ca}^{2+}$ exchanger affects local and global Ca^{2+} homeostasis in Chinese hamster ovary cells. *J Biol Chem* 2002;277:38693–38699. [PubMed: 12145298]
181. Alfonso A, Lago J, Botana MA, Vieytes MR, Botana LM. Characterization of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger on rat mast cells. Evidence for a functional role on the regulation of the cellular response. *Cell Physiol Biochem* 1999;9:53–71. [PubMed: 10393999]
182. Rumpel E, Pilatus U, Mayer A, Pecht I. Na^+ -dependent Ca^{2+} transport modulates the secretory response to the $\text{Fc}\epsilon$ receptor stimulus of mast cells. *Biophys J* 2000;79:2975–2986. [PubMed: 11106605]
183. Marx J. Alzheimer's disease. Fresh evidence points to an old suspect: calcium. *Science* 2007;318:384–385. [PubMed: 17947560]
184. Tanzi RE, Bertram L. Alzheimer's disease: the latest suspect. *Nature* 2008;454:706–708. [PubMed: 18685694]

185. Spasic D, Tolia A, Dillen K, Baert V, De Strooper B, Vrijens S, Annaert W. Presenilin-1 maintains a nine-transmembrane topology throughout the secretory pathway. *J Biol Chem* 2006;281:26569–26577. [PubMed: 16846981]
186. Cowburn RF, Popescu BO, Ankarcrona M, Dehvari N, Cedazo-Minguez A. Presenilin-mediated signal transduction. *Physiol Behav* 2007;92:93–97. [PubMed: 17568632]
187. Hass MR, Sato C, Kopan R, Zhao G. Presenilin: RIP and beyond. *Semin Cell Dev Biol* 2008;20:201–210. [PubMed: 19073272]
188. Green KN, LaFerla FM. Linking calcium to Ab and Alzheimer's disease. *Neuron* 2008;59:190–194. [PubMed: 18667147]
189. Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL. Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc Natl Acad Sci U S A* 1994;91:534–538. [PubMed: 8290560]
190. Yoo AS, Cheng I, Chung S, Grenfell TZ, Lee H, Pack-Chung E, Handler M, Shen J, Xia W, Tesco G, Saunders AJ, Ding K, Frosch MP, Tanzi RE, Kim TW. Presenilin-mediated modulation of capacitative calcium entry. *Neuron* 2000;27:561–572. [PubMed: 11055438]
191. Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De SB, Yu G, Bezprozvanny I. Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* 2006;126:981–993. [PubMed: 16959576]
192. Nelson O, Tu H, Lei T, Bentahir M, De SB, Bezprozvanny I. Familial Alzheimer disease-linked mutations specifically disrupt Ca^{2+} leak function of presenilin 1. *J Clin Invest* 2007;117:1230–1239. [PubMed: 17431506]
193. Cheung KH, Shineman D, Muller M, Cardenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foskett JK. Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP_3 receptor channel gating. *Neuron* 2008;58:871–883. [PubMed: 18579078]
194. Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP. Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J Biol Chem* 2000;275:18195–18200. [PubMed: 10764737]
195. Dreses-Werringloer U, Lambert JC, Vingtdoux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, Pasquier F, Galimberti D, Scarpini E, Mann D, Lendon C, Campion D, Amouyel P, Davies P, Foskett JK, Campagne F, Marambaud P. A polymorphism in CALHM1 influences Ca^{2+} homeostasis, $\text{A}\beta$ levels, and Alzheimer's disease risk. *Cell* 2008;133:1149–1161. [PubMed: 18585350]
196. Bertram L, Schjeide BM, Hooli B, Mullin K, Hiltunen M, Soininen H, Ingelsson M, Lannfelt L, Blacker D, Tanzi RE. No association between CALHM1 and Alzheimer's disease risk. *Cell* 2008;135:993–994. [PubMed: 19070563]
197. Treiman M, Caspersen C, Christensen SB. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+} -ATPases. *Trends Pharmacol Sci* 1998;19:131–135. [PubMed: 9612087]
198. Rasmussen U, Brøgger Christensen S, Sandberg F. Thapsigargin and thapsigarginic acid, two new histamine liberators from *Thapsia garganica* L. *Acta Pharm Suec* 1978;15:133–140. [PubMed: 79299]
199. Patkar SA, Rasmussen U, Diamant B. On the mechanism of histamine release induced by thapsigargin from *Thapsia garganica* L. *Agents Actions* 1979;9:53–57. [PubMed: 88885]
200. Ali H, Christensen SB, Foreman JC, Pearce FL, Piotrowski W, Thastrup O. The ability of thapsigargin and thapsigarginic acid to activate cells involved in the inflammatory response. *Br J Pharmacol* 1985;85:705–712. [PubMed: 2411328]
201. Thastrup O, Dawson AP, Scharff O, Foder B, Cullen PJ, Drbak BK, Bjerrum PJ, Christensen SB, Hanley MR. Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. *Agents Actions* 1989;27:17–23. [PubMed: 2787587]
202. Oberhauser AF, Monck JR, Balch WE, Fernandez JM. Exocytotic fusion is activated by Rab3a peptides. *Nature* 1992;360:270–273. [PubMed: 1331813]
203. Sagara Y, Inesi G. Inhibition of the sarcoplasmic reticulum Ca^{2+} transport ATPase by thapsigargin at subnanomolar concentrations. *J Biol Chem* 1991;266:13503–13506. [PubMed: 1830305]

204. Toyoshima C, Nomura H. Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* 2002;418:605–611. [PubMed: 12167852]
205. Xu C, Ma H, Inesi G, Al-Shawi MK, Toyoshima C. Specific structural requirements for the inhibitory effect of thapsigargin on the Ca^{2+} ATPase SERCA. *J Biol Chem* 2004;279:17973–17979. [PubMed: 14970206]
206. Wootton LL, Michelangeli F. The effects of the phenylalanine 256 to valine mutation on the sensitivity of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) Ca^{2+} pump isoforms 1, 2, and 3 to thapsigargin and other inhibitors. *J Biol Chem* 2006;281:6970–6976. [PubMed: 16410239]
207. Goeger DE, Riley RT. Interaction of cyclopiazonic acid with rat skeletal muscle sarcoplasmic reticulum vesicles. Effect on Ca^{2+} binding and Ca^{2+} permeability. *Biochem Pharmacol* 1989;38:3995–4003. [PubMed: 2532015]
208. Seidler NW, Jona I, Vegh M, Martonosi A. Cyclo-piazonic acid is a specific inhibitor of the Ca^{2+} -ATPase of sarcoplasmic reticulum. *J Biol Chem* 1989;264:17816–17823. [PubMed: 2530215]
209. Uyama Y, Imaizumi Y, Watanabe M. Effects of cyclopiazonic acid, a novel Ca^{2+} -ATPase inhibitor, on contractile responses in skinned ileal smooth muscle. *Br J Pharmacol* 1992;106:208–214. [PubMed: 1387024]
210. Ma H, Zhong L, Inesi G, Fortea I, Soler F, Fernandez-Belda F. Overlapping effects of S3 stalk segment mutations on the affinity of Ca^{2+} -ATPase (SERCA) for thapsigargin and cyclopiazonic acid. *Biochemistry* 1999;38:15522–15527. [PubMed: 10569935]
211. Moncoq K, Trieber CA, Young HS. The molecular basis for cyclopiazonic acid inhibition of the sarcoplasmic reticulum calcium pump. *J Biol Chem* 2007;282:9748–9757. [PubMed: 17259168]
212. Oka T, Hori M, Ozaki H. Microtubule disruption suppresses allergic response through the inhibition of calcium influx in the mast cell degranulation pathway. *J Immunol* 2005;174:4584–4589. [PubMed: 15814680]
213. Takahashi S, Kinoshita T, Takahashi M. Adenophostins A and B: potent agonists of inositol-1,4,5-trisphosphate receptor produced by *Penicillium brevicompactum*. Structure elucidation. *J Antibiot (Tokyo)* 1994;47:95–100. [PubMed: 8119867]
214. Sureshan KM, Trusselle M, Tovey SC, Taylor CW, Potter BV. 2-Position base-modified analogues of adenophostin A as high-affinity agonists of the D-myo-inositol trisphosphate receptor: in vitro evaluation and molecular modeling. *J Org Chem* 2008;73:1682–1692. [PubMed: 18247493]
215. Takahashi M, Tanzawa K, Takahashi S. Adenophostins, newly discovered metabolites of *Penicillium brevicompactum*, act as potent agonists of the inositol 1,4,5-trisphosphate receptor. *J Biol Chem* 1994;269:369–372. [PubMed: 8276820]
216. Morris SA, Nerou EP, Riley AM, Potter BV, Taylor CW. Determinants of adenophostin A binding to inositol trisphosphate receptors. *Biochem J* 2002;367:113–120. [PubMed: 12088506]
217. Rosenberg HJ, Riley AM, Laude AJ, Taylor CW, Potter BV. Synthesis and Ca^{2+} -mobilizing activity of purine-modified mimics of adenophostin A: a model for the adenophostin-Ins(1,4,5)P₃ receptor interaction. *J Med Chem* 2003;46:4860–4871. [PubMed: 14584937]
218. Hirota J, Michikawa T, Miyawaki A, Takahashi M, Tanzawa K, Okura I, Furuichi T, Mikoshiba K. Adenophostin-mediated quantal Ca^{2+} release in the purified and reconstituted inositol 1,4,5-trisphosphate receptor type 1. *FEBS Lett* 1995;368:248–252. [PubMed: 7628615]
219. Huang Y, Takahashi M, Tanzawa K, Putney JW Jr. Effect of adenophostin A on Ca^{2+} entry and calcium release-activated calcium current (I_{crac}) in rat basophilic leukemia cells. *J Biol Chem* 1998;273:31815–31821. [PubMed: 9822648]
220. Mak DO, McBride S, Foscett JK. ATP-dependent adenophostin activation of inositol 1,4,5-trisphosphate receptor channel gating: kinetic implications for the durations of calcium puffs in cells. *J Gen Physiol* 2001;117:299–314. [PubMed: 11279251]
221. Bird GS, Takahashi M, Tanzawa K, Putney JW Jr. Adenophostin A induces spatially restricted calcium signaling in *Xenopus laevis* oocytes. *J Biol Chem* 1999;274:20643–20649. [PubMed: 10400696]
222. Broad LM, Armstrong DL, Putney JW Jr. Role of the inositol 1,4,5-trisphosphate receptor in Ca^{2+} feedback inhibition of calcium release-activated calcium current I_{crac} . *J Biol Chem* 1999;274:32881–32888. [PubMed: 10551852]

223. Parekh AB, Riley AM, Potter BV. Adenophostin A and ribophostin, but not inositol 1,4,5-trisphosphate or manno-adenophostin, activate the Ca^{2+} release-activated Ca^{2+} current, I_{CRAC} , in weak intracellular Ca^{2+} buffer. *Biochem J* 2002;361:133–141. [PubMed: 11742538]
224. Bootman MD, Collins TJ, Mackenzie L, Roderick HL, Berridge MJ, Peppiatt CM. 2-Aminoethoxydiphenyl borate (2-APB) is a reliable blocker of store-operated Ca^{2+} entry but an inconsistent inhibitor of InsP_3 -induced Ca^{2+} release. *FASEB J* 2002;16:1145–1150. [PubMed: 12153982]
225. Gafni J, Munsch JA, Lam TH, Catlin MC, Costa LG, Molinski TF, Pessah IN. Xestospongins: potent membrane permeable blockers of the inositol 1,4,5-trisphosphate receptor. *Neuron* 1997;19:723–733. [PubMed: 9331361]
226. Maruyama T, Kanaji T, Nakade S, Kanno T, Mikoshiba K. 2APB, 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release. *J Biochem* 1997;122:498–505. [PubMed: 9348075]
227. Oka T, Sato K, Hori M, Ozaki H, Karaki H. Xestospongins C, a novel blocker of IP_3 receptor, attenuates the increase in cytosolic calcium level and degranulation that is induced by antigen in RBL-2H3 mast cells. *Br J Pharmacol* 2002;135:1959–1966. [PubMed: 11959799]
228. De Smet P, Parys JB, Callewaert G, Weidema AF, Hill E, De Smedt H, Erneux C, Sorrentino V, Missiaen L. Xestospongins C is an equally potent inhibitor of the inositol 1,4,5-trisphosphate receptor and the endoplasmic-reticulum Ca^{2+} pumps. *Cell Calcium* 1999;26:9–13. [PubMed: 10892566]
229. Castonguay A, Robitaille R. Xestospongins C is a potent inhibitor of SERCA at a vertebrate synapse. *Cell Calcium* 2002;32:39–47. [PubMed: 12127061]
230. Solovyova N, Fernyhough P, Glazner G, Verkhatsky A. Xestospongins C empties the ER calcium store but does not inhibit InsP_3 -induced Ca^{2+} release in cultured dorsal root ganglia neurones. *Cell Calcium* 2002;32:49–52. [PubMed: 12127062]
231. Ozaki H, Hori M, Kim YS, Kwon SC, Ahn DS, Nakazawa H, Kobayashi M, Karaki H. Inhibitory mechanism of xestospongins-C on contraction and ion channels in the intestinal smooth muscle. *Br J Pharmacol* 2002;137:1207–1212. [PubMed: 12466229]
232. Jaimovich E, Mattei C, Liberona JL, Cardenas C, Estrada M, Barbier J, Debitus C, Laurent D, Molgo J. Xestospongins B, a competitive inhibitor of IP_3 -mediated Ca^{2+} signalling in cultured rat myotubes, isolated myonuclei, and neuroblastoma (NG108-15) cells. *FEBS Lett* 2005;579:2051–2057. [PubMed: 15811317]
233. Ma HT, Patterson RL, van Rossum DB, Birmbaumer L, Mikoshiba K, Gill DL. Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. *Science* 2000;287:1647–1651. [PubMed: 10698739]
234. Ma HT, Venkatachalam K, Li HS, Montell C, Kurosaki T, Patterson RL, Gill DL. Assessment of the role of the inositol 1,4,5-trisphosphate receptor in the activation of transient receptor potential channels and store-operated Ca^{2+} entry channels. *J Biol Chem* 2001;276:18888–18896. [PubMed: 11259416]
235. Prakriya M, Lewis RS. Potentiation and inhibition of Ca^{2+} release-activated Ca^{2+} channels by 2-amino-ethoxydiphenyl borate (2-APB) occurs independently of IP_3 receptors. *J Physiol* 2001;536:3–19. [PubMed: 11579153]
236. Broad LM, Braun FJ, Lievreumont JP, Bird GS, Kurosaki T, Putney JW Jr. Role of the phospholipase C-inositol 1,4,5-trisphosphate pathway in calcium release-activated calcium current and capacitative calcium entry. *J Biol Chem* 2001;276:15945–15952. [PubMed: 11278938]
237. Iwasaki H, Mori Y, Hara Y, Uchida K, Zhou H, Mikoshiba K. 2-Aminoethoxydiphenyl borate (2-APB) inhibits capacitative calcium entry independently of the function of inositol 1,4,5-trisphosphate receptors. *Receptors Channels* 2001;7:429–439. [PubMed: 11918346]
238. Bakowski D, Glitsch MD, Parekh AB. An examination of the secretion-like coupling model for the activation of the Ca^{2+} release-activated Ca^{2+} current I_{CRAC} in RBL-1 cells. *J Physiol* 2001;532:55–71. [PubMed: 11283225]
239. Xu SZ, Zeng F, Boulay G, Grimm C, Harteneck C, Beech DJ. Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. *Br J Pharmacol* 2005;145:405–414. [PubMed: 15806115]

240. Delmas P, Wanaverbecq N, Abogadie FC, Mistry M, Brown DA. Signaling microdomains define the specificity of receptor-mediated InsP₃ pathways in neurons. *Neuron* 2002;34:209–220. [PubMed: 11970863]
241. Trebak M, Bird GS, McKay RR, Putney JW Jr. Comparison of human TRPC3 channels in receptor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. *J Biol Chem* 2002;277:21617–21623. [PubMed: 11943785]
242. Lee YM, Kim BJ, Kim HJ, Yang DK, Zhu MH, Lee KP, So I, Kim KW. TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in murine stomach. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G604–G616. [PubMed: 12631560]
243. Togashi K, Inada H, Tominaga M. Inhibition of the transient receptor potential cation channel TRPM2 by 2-aminoethoxydiphenyl borate (2-APB). *Br J Pharmacol* 2008;153:1324–1330. [PubMed: 18204483]
244. Li M, Jiang J, Yue L. Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7. *J Gen Physiol* 2006;127:525–537. [PubMed: 16636202]
245. Zhang SL, Kozak JA, Jiang W, Yeromin AV, Chen J, Yu Y, Penna A, Shen W, Chi V, Cahalan MD. Store-dependent and -independent modes regulating Ca²⁺ release-activated Ca²⁺ channel activity of human Orai1 and Orai3. *J Biol Chem* 2008;283:17662–17671. [PubMed: 18420579]
246. Peinelt C, Lis A, Beck A, Fleig A, Penner R. 2-Aminoethoxydiphenyl borate directly facilitates and indirectly inhibits STIM1-dependent gating of CRAC channels. *J Physiol* 2008;586:3061–3073. [PubMed: 18403424]
247. Dehaven WI, Smyth JT, Boyles RR, Bird GS, Putney JW Jr. Complex actions of 2-aminoethoxydiphenyl borate on store-operated calcium entry. *J Biol Chem* 2008;283:19265–19273. [PubMed: 18487204]
248. Schindl R, Bergsmann J, Frischauf I, Derler I, Fahrner M, Muik M, Fritsch R, Groschner K, Romanin C. 2-Aminoethoxydiphenyl borate alters selectivity of Orai3 channels by increasing their pore size. *J Biol Chem* 2008;283:20261–20267. [PubMed: 18499656]
249. Missiaen L, Callewaert G, De Smedt H, Parys JB. 2-Aminoethoxydiphenyl borate affects the inositol 1,4,5-trisphosphate receptor, the intracellular Ca²⁺ pump and the non-specific Ca²⁺ leak from the non-mitochondrial Ca²⁺ stores in permeabilized A7r5 cells. *Cell Calcium* 2001;29:111–116. [PubMed: 11162848]
250. Peppiatt CM, Collins TJ, Mackenzie L, Conway SJ, Holmes AB, Bootman MD, Berridge MJ, Seo JT, Roderick HL. 2-Aminoethoxydiphenyl borate (2-APB) antagonises inositol 1,4,5-trisphosphate-induced calcium release, inhibits calcium pumps and has a use-dependent and slowly reversible action on store-operated calcium entry channels. *Cell Calcium* 2003;34:97–108. [PubMed: 12767897]
251. Wu J, Takeo T, Suga S, Kanno T, Osanai T, Mikoshiba K, Wakui M. 2-Aminoethoxydiphenyl borate inhibits agonist-induced Ca²⁺ signals by blocking inositol trisphosphate formation in acutely dissociated mouse pancreatic acinar cells. *Pflugers Arch* 2004;448:592–595. [PubMed: 15197598]
252. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218–230. [PubMed: 16470226]
253. Kuehn HS, Gilfillan AM. G protein-coupled receptors and the modification of FcεRI-mediated mast cell activation. *Immunol Lett* 2007;113:59–69. [PubMed: 17919738]
254. Choi OH, Kim JH, Kinet JP. Calcium mobilization via the sphingosine kinase in signalling by the FcεRI antigen receptor. *Nature* 1996;380:634–636. [PubMed: 8602265]
255. Melendez AJ, Khaw AK. Dichotomy of Ca²⁺ signals triggered by different phospholipid pathways in antigen stimulation of human mast cells. *J Biol Chem* 2002;277:17255–17262. [PubMed: 11856736]
256. Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, Rivera J. The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis. *Immunity* 2007;26:287–297. [PubMed: 17346996]
257. Olivera A, Urtz N, Mizugishi K, Yamashita Y, Gilfillan AM, Furumoto Y, Gu H, Proia RL, Baumruker T, Rivera J. IgE-dependent activation of sphingosine kinase 1 and 2 and secretion of

- sphingosine-1-phosphate requires Fyn kinase and contributes to mast cell responses. *J Biol Chem* 2006;281:2515–2525. [PubMed: 16316995]
258. Melendez A, Floto RA, Gillooly DJ, Harnett MM, Allen JM. FcγRI coupling to phospholipase D initiates sphingosine kinase-mediated calcium mobilization and vesicular trafficking. *J Biol Chem* 1998;273:9393–9402. [PubMed: 9545263]
 259. Ryu SD, Lee HS, Suk HY, Park CS, Choi OH. Cross-linking of FcεRI causes Ca²⁺ mobilization via a sphingosine kinase pathway in a clathrin-dependent manner. *Cell Calcium* 2008;45:99–108. [PubMed: 18675457]
 260. Beaven MA. Division of labor: Specialization of sphingosine kinases in mast cells. *Immunity* 2007;26:271–273. [PubMed: 17376388]
 261. Meyer zu Heringdorf D. Lysophospholipid receptor-dependent and -independent calcium signaling. *J Cell Biochem* 2004;92:937–948. [PubMed: 15258917]
 262. Mitra P, Oskeritzian CA, Payne SG, Beaven MA, Milstien S, Spiegel S. Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. *Proc Natl Acad Sci USA* 2006;103:16394–16399. [PubMed: 17050692]
 263. An S, Bleu T, Zheng Y. Transduction of intracellular calcium signals through G protein-mediated activation of phospholipase C by recombinant sphingosine 1-phosphate receptors. *Mol Pharmacol* 1999;55:787–794. [PubMed: 10220556]
 264. Sato K, Kon J, Tomura H, Osada M, Murata N, Kuwabara A, Watanabe T, Ohta H, Ui M, Okajima F. Activation of phospholipase C-Ca²⁺ system by sphingosine 1-phosphate in CHO cells transfected with Edg-3, a putative lipid receptor. *FEBS Lett* 1999;443:25–30. [PubMed: 9928946]
 265. Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. *Biochim Biophys Acta* 2006;1758:2016–2026. [PubMed: 16996023]
 266. Rivera J, Proia RL, Olivera A. The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat Rev Immunol* 2008;8:753–763. [PubMed: 18787560]
 267. Melendez AJ. Allergy therapy: the therapeutic potential of targeting sphingosine kinase signalling in mast cells. *Eur J Immunol* 2008;38:2969–2974. [PubMed: 18924207]
 268. Reddy S, Aggarwal BB. Curcumin is a non-competitive and selective inhibitor of phosphorylase kinase. *FEBS Lett* 1994;341:19–22. [PubMed: 7511111]
 269. Chuang FY, Sassaroli M, Unkeless JC. Convergence of Fcγ receptor IIA and Fcγ receptor IIIB signaling pathways in human neutrophils. *J Immunol* 2000;164:350–360. [PubMed: 10605030]
 270. Alemany R, Meyer Zu HD, van Koppen CJ, Jakobs KH. Formyl peptide receptor signaling in HL-60 cells through sphingosine kinase. *J Biol Chem* 1999;274:3994–3999. [PubMed: 9933590]
 271. Meyer zu Heringdorf D, Lass H, Alemany R, Lase KT, Neumann E, Zhang C, Schmidt M, Rauen U, Jakobs KH, van Koppen CJ. Sphingosine kinase-mediated Ca²⁺ signalling by G-protein-coupled receptors. *EMBO J* 1998;17:2830–2837. [PubMed: 9582276]
 272. Ghosh TK, Bian J, Gill DL. Sphingosine 1-phosphate generated in the endoplasmic reticulum membrane activates release of stored calcium. *J Biol Chem* 1994;269:22628–22635. [PubMed: 8077214]
 273. Meyer zu Heringdorf D, Liliom K, Schaefer M, Danneberg K, Jaggar JH, Tigyi G, Jakobs KH. Photolysis of intracellular caged sphingosine-1-phosphate causes Ca²⁺ mobilization independently of G-protein-coupled receptors. *FEBS Lett* 2003;554:443–449. [PubMed: 14623109]
 274. Beaven MA. Our perception of the mast cell from Paul Ehrlich to now. *Eur J Immunol* 2009;39:11–25. [PubMed: 19130582]
 275. Pacher P, Csordás P, Schneider T, Hajnóczky G. Quantification of calcium signal transmission from sarco-endoplasmic reticulum to the mitochondria. *J Physiol* 2000;529(Pt 3):553–564. [PubMed: 11118489]
 276. Csordás G, Hajnóczky G. Sorting of calcium signals at the junctions of endoplasmic reticulum and mitochondria. *Cell Calcium* 2001;29:249–262. [PubMed: 11243933]
 277. Csordás G, Hajnóczky G. Plasticity of mitochondrial calcium signaling. *J Biol Chem* 2003;278:42273–42282. [PubMed: 12907683]
 278. Vig M, Dehaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, Hutchings AB, Jouvin MH, Putney JW, Kinet JP. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of

- store-operated calcium release-activated calcium channels. *Nat Immunol* 2008;9:89–96. [PubMed: 18059270]
279. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol* 2008;9:81–88. [PubMed: 18059272]
 280. Calloway N, Vig M, Kinet JP, Holowka D, Baird B. Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca^{2+} stores and on electrostatic interactions. *Mol Biol Cell* 2009;20:389–399. [PubMed: 18987344]
 281. Mathes C, Fleig A, Penner R. Calcium release-activated calcium current (I_{CRAC}) is a direct target for sphingosine. *J Biol Chem* 1998;273:25020–25030. [PubMed: 9737958]
 282. Bradding P. Mast cell ion channels. *Chem Immunol Allergy* 2005;87:163–178. [PubMed: 16107771]
 283. Wischmeyer E, Lentes KU, Karschin A. Physiological and molecular characterization of an IRK-type inward rectifier K^{+} channel in a tumour mast cell line. *Pflugers Arch* 1995;429:809–819. [PubMed: 7603835]
 284. Labrecque GF, Holowka D, Baird B. Characterization of increased K^{+} permeability associated with the stimulation of receptors for immunoglobulin E on rat basophilic leukemia cells. *J Biol Chem* 1991;266:14912–14917. [PubMed: 1831198]
 285. Narenjkar J, Assem SK, Ganellin CR. Inhibition of the antigen-induced activation of RBL-2H3 cells by cetiedil and some of its analogues. *Eur J Pharmacol* 2004;483:107–116. [PubMed: 14729097]
 286. Duffy SM, Berger P, Cruse G, Yang W, Bolton SJ, Bradding P. The K^{+} channel iK_{CA1} potentiates Ca^{2+} influx and degranulation in human lung mast cells. *J Allergy Clin Immunol* 2004;114:66–72. [PubMed: 15241346]
 287. Cruse G, Duffy SM, Brightling CE, Bradding P. Functional $\text{K}_{\text{CA3.1}}$ K^{+} channels are required for human lung mast cell migration. *Thorax* 2006;61:880–885. [PubMed: 16809411]
 288. Vennekens R, Olausson J, Meissner M, Bloch W, Mathar I, Philipp SE, Schmitz F, Weissgerber P, Nilius B, Flockerzi V, Freichel M. Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated non-selective cation channel TRPM4. *Nat Immunol* 2007;8:312–320. [PubMed: 17293867]
 289. Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca^{2+} -activated non-selective cation channel mediating cell membrane depolarization. *Cell* 2002;109:397–407. [PubMed: 12015988]
 290. Takezawa R, Cheng H, Beck A, Ishikawa J, Launay P, Kubota H, Kinet JP, Fleig A, Yamada T, Penner R. A pyrazole derivative potently inhibits lymphocyte Ca^{2+} influx and cytokine production by facilitating transient receptor potential melastatin 4 channel activity. *Mol Pharmacol* 2006;69:1413–1420. [PubMed: 16407466]
 291. Grand T, Demion M, Norez C, Mettey Y, Launay P, Becq F, Bois P, Guinamard R. 9-Phenanthrol inhibits human TRPM4 but not TRPM5 cationic channels. *Br J Pharmacol* 2008;153:1697–1705. [PubMed: 18297105]
 292. Ridgway EB, Gilkey JC, Jaffe LF. Free calcium increases explosively in activating medaka eggs. *Proc Natl Acad Sci U S A* 1977;74:623–627. [PubMed: 322135]
 293. Jaffe L. On the conservation of fast calcium wave speeds. *Cell Calcium* 2002;32:217–229. [PubMed: 12379182]
 294. Rintoul GL, Baimbridge KG. Effects of calcium buffers and calbindin-D28k upon histamine-induced calcium oscillations and calcium waves in HeLa cells. *Cell Calcium* 2003;34:131–144. [PubMed: 12810055]
 295. Hillson EJ, Hallett MB. Localised and rapid Ca^{2+} micro-events in human neutrophils: conventional Ca^{2+} puffs and global waves without peripheral-restriction or wave cycling. *Cell Calcium* 2007;41:525–536. [PubMed: 17324458]
 296. Mackenzie L, Bootman MD, Berridge MJ, Lipp P. Predetermined recruitment of calcium release sites underlies excitation-contraction coupling in rat atrial myocytes. *J Physiol* 2001;530:417–429. [PubMed: 11158273]
 297. Tovey SC, de SP, Lipp P, Thomas D, Young KW, Missiaen L, De SH, Parys JB, Berridge MJ, Thuring J, Holmes A, Bootman MD. Calcium puffs are generic InsP_3 -activated elementary calcium

- signals and are downregulated by prolonged hormonal stimulation to inhibit cellular calcium responses. *J Cell Sci* 2001;114:3979–3989. [PubMed: 11739630]
298. Berridge MJ. Calcium microdomains: organization and function. *Cell Calcium* 2006;40:405–412. [PubMed: 17030366]
 299. Millard PJ, Gross D, Webb WW, Fewtrell C. Imaging asynchronous changes in intracellular Ca^{2+} in individual stimulated tumor mast cells. *Proc Natl Acad Sci U S A* 1988;85:1854–1858. [PubMed: 3162312]
 300. Ryan TA, Millard PJ, Webb WW. Imaging $[\text{Ca}^{2+}]_i$ dynamics during signal transduction. *Cell Calcium* 1990;11:145–155. [PubMed: 2141302]
 301. Kim TD, Eddlestone GT, Mahmoud SF, Kuchty J, Fewtrell C. Correlating Ca^{2+} responses and secretion in individual RBL-2H3 mucosal mast cells. *J Biol Chem* 1997;272:31225–31229. [PubMed: 9395446]
 302. Narenjkar J, Marsh SJ, Assem ES. The characterization and quantification of antigen-induced Ca^{2+} oscillations in a rat basophilic leukaemia cell line (RBL-2H3). *Cell Calcium* 1999;26:261–269. [PubMed: 10668564]
 303. Kotturi MF, Hunt SV, Jefferies WA. Roles of CRAC and Ca_v -like channels in T cells: more than one gatekeeper? *Trends Pharmacol Sci* 2006;27:360–367. [PubMed: 16766050]
 304. Engelke M, Engels N, Dittmann K, Stork B, Wienands J. Ca^{2+} signaling in antigen receptor-activated B lymphocytes. *Immunol Rev* 2007;218:235–246. [PubMed: 17624956]
 305. MacGlashan D Jr. IgE receptor and signal transduction in mast cells and basophils. *Curr Opin Immunol* 2008;20:717–723. [PubMed: 18822373]
 306. Kalesnikoff J, Galli SJ. New developments in mast cell biology. *Nat Immunol* 2008;9:1215–1223. [PubMed: 18936782]
 307. Ozawa K, Szallasi Z, Kazanietz MG, Blumberg PM, Mischak H, Mushinski JF, Beaven MA. Ca^{2+} -dependent and Ca^{2+} -independent isozymes of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells: Reconstitution of secretory responses with Ca^{2+} and purified isozymes in washed permeabilized cells. *J Biol Chem* 1993;268:1749–1756. [PubMed: 8420951]
 308. Hirasawa N, Santini F, Beaven MA. Activation of the mitogen-activated protein kinase/cytosolic phospholipase A_2 pathway in a rat mast cell line. Indications of different pathways for release of arachidonic acid and secretory granules. *J Immunol* 1995;154:5391–5402. [PubMed: 7730640]
 309. Chang WC, Nelson C, Parekh AB. Ca^{2+} influx through CRAC channels activates cytosolic phospholipase A_2 , leukotriene C_4 secretion, and expression of c-fos through ERK-dependent and -independent pathways in mast cells. *FASEB J* 2006;20:2381–2383. [PubMed: 17023391]
 310. Hartmann K, Henz BM, Kruger-Krasagakes S, Kohl J, Burger R, Guhl S, Haase I, Lippert U, Zuberbier T. C_3a and C_5a stimulate chemotaxis of human mast cells. *Blood* 1997;89:2863–2870. [PubMed: 9108406]
 311. Wodnar-Filipowicz A, Moroni C. Regulation of interleukin 3 mRNA expression in mast cells occurs at the posttranscriptional level and is mediated by calcium ions. *Proc Natl Acad Sci U S A* 1990;87:777–781. [PubMed: 2105489]
 312. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of $\text{Fc}\epsilon\text{RI}$ or to calcium ionophores. *Nature* 1989;339:64–67. [PubMed: 2469965]
 313. Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, Dvorak AM, Galli SJ, Dorf ME. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J Exp Med* 1989;170:245–257. [PubMed: 2473161]
 314. Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. $\text{Fc}\epsilon\text{RI}$ and Toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood* 2006;107:610–618. [PubMed: 16174756]
 315. Thompson HL, Burbelo PD, Yamada Y, Kleinman HK, Metcalfe DD. Mast cells chemotax to laminin with enhancement after IgE-mediated activation. *J Immunol* 1989;143:4188–4192. [PubMed: 2592771]

316. Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H₄ receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther* 2003;305:1212–1221. [PubMed: 12626656]
317. Hundley TR, Gilfillan AM, Tkaczyk C, Andrade MV, Metcalfe DD, Beaven MA. Kit and FcεRI mediate unique and convergent signals for release of inflammatory mediators from human mast cells. *Blood* 2004;104:2410–2417. [PubMed: 15217825]
318. Benyon RC, Robinson C, Church MK. Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. *Brit J Pharmacol* 1989;97:898–904. [PubMed: 2474353]
319. van Haaster CM, Engels W, Lemmens PJ, Hornstra G, van der Vussie GJ, Heemskerk JW. Differential release of histamine and prostaglandin D₂ in rat peritoneal mast cells: roles of cytosolic calcium and protein tyrosine kinases. *Biochim Biophys Acta* 1995;1265:79–88. [PubMed: 7857988]
320. Chang WC, Di CJ, Nelson C, Parekh AB. All-or-none activation of CRAC channels by agonist elicits graded responses in populations of mast cells. *J Immunol* 2007;179:5255–5263. [PubMed: 17911611]
321. Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃R is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem* 1993;268:16887–16890. [PubMed: 8349579]
322. Wei C, Wang X, Chen M, Ouyang K, Song LS, Cheng H. Calcium flickers steer cell migration. *Nature* 2008;457:901–905. [PubMed: 19118385]
323. Mazel T, Raymond R, Raymond-Stintz M, Jett S, Wilson BS. Stochastic modeling of calcium in 3D geometry. *Biophys J* 2009;96:1–16. [PubMed: 18849411]

FIGURE 1.

Model for the regulation of calcium signaling in mast cells circa 1995. Cross-linking of the high-affinity receptor for IgE (FcεRI) by antigen (Ag) results in production of IP₃ by PLCγ and release of Ca²⁺ from an IP₃/thapsigargin-sensitive pool in ER. Depletion of this pool coincides with influx of external Ca²⁺ through the channel(s) that can convey other divalent metal ions such as Sr²⁺. For this reason we have left the channel unidentified even though the Ca²⁺-conducting current, *I*_{CRAC}, had been characterized in mast cells. The putative *I*_{CRAC} channel would not account for the nonselective influx mechanism in mast cells. Influx is dependent on maintenance of an electrochemical gradient across the plasma membrane, presumably through extrusion of K⁺, and enables replenishment of the ER pool. In addition, at elevated [Ca²⁺]_i, Ca²⁺ accumulates in a mitochondrial pool of high capacity. Uptake and subsequent release of Ca²⁺ from mitochondria attenuates and prolongs the elevation of [Ca²⁺]_i. Uptake into both pools and efflux of Ca²⁺ from cells are all ATP-dependent processes. None of the Ca²⁺ pumps had been identified in mast cells but were assumed to include SERCA (for uptake into ER) and PMCA (for Ca²⁺ efflux) by analogy with processes in other types of cells and because thapsigargin, a SERCA-pump inhibitor, inhibits Ca²⁺ uptake into ER resulting in spontaneous leakage of Ca²⁺ from ER (dashed arrow). (See text for details and references.)

FIGURE 2.

Time line for observations that helped define mechanisms for calcium signaling in mast cells. The citations noted are discussed in detail in the text. The time line terminates with the discovery of Orai1 as the core protein for Ca^{2+} -specific CRAC channels. However, the early studies of Foreman and colleagues^{6,7} and later studies by others amply demonstrated that Sr^{2+} is taken up and could substitute for Ca^{2+} in supporting histamine release in mast cells, and recent studies support the concept that TRPC channels, in concert with Orai1 and STIM1, account for Sr^{2+} as well as Ca^{2+} uptake in mast cells and other types of cells (see Sections I, VII, and X.B). Work on this aspect of calcium signaling is still in progress and, for this reason, is not included in the time line.

FIGURE 3.

Current model for calcium signaling in mast cells. The model also depicts sites of action of pharmacologic probes such as Adenophostin, 2-APB, and xestospongin, which either activate or inhibit IP3R as indicated (see Section IX). Following release of Ca²⁺ from ER stores through IP3R (most likely IP3R1 or IP3R2), the ER Ca²⁺ sensor, STIM1, aggregates in close proximity to the Ca²⁺-channel protein, Oral1, to activate *I*_{CRAC}. However, in RBL-2H3 and some other types of cells, TRPC proteins interact with the Oral1/STIM1 complex to create a less specific cation channel that can conduct Sr²⁺, as described for Figure 1. This influx is blocked by La³⁺ (not shown) and 2-APB. Ca²⁺ influx is associated with membrane depolarization in part due to influx of Na⁺ via TRPM4, which acts as a negative regulator of mast cell activation. Cells are repolarized through extrusion of K⁺ through the Ca²⁺-activated K⁺ channel, iK_{Ca3}, which is essential for continued Ca²⁺ influx. Associated with the increase in [Ca²⁺]_i, is replenishment of ER stores through SERCA (SERCA 2b or SERCA3), which is inhibited by thapsigargin or cyclopiazonic acid and uptake into mitochondria through the ruthenium red-sensitive MCU channel. Uptake is also blocked by the mitochondrial respiratory inhibitors, oligomycin and antimycin A. As [Ca²⁺]_i subsides, Ca²⁺ is released from mitochondria through an Na⁺/Ca²⁺ exchanger to further modulate the calcium signal. The increase in [Ca²⁺]_i is counteracted by the extrusion of Ca²⁺ through PMCA (isoform not yet identified) and Na⁺/Ca²⁺ exchangers. Both K⁺-dependent (NCKX1 and NCKX3) and K⁺-independent (NCX3) exchangers have been identified in mast cells (see Section VIII.B). A candidate for the “leak” channel (dotted green line) that is unmasked by blocking SERCA with thapsigargin is presenilin (see Section VIII.C). See Section X for other details and references.