Chapter 6
Phospholipase A_2 as a Molecular Determinant of Store-Operated Calcium Entry

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Abstract Activation of phospholipases A_2 (PLA_2) leads to the generation of biologically active lipid products that can affect numerous cellular events. Ca^{2+}-independent PLA_2 ($iPLA_2$), also called group VI phospholipase A_2, is one of the main types forming the superfamily of PLA_2. Beside of its role in phospholipid remodeling, $iPLA_2$ has been involved in intracellular Ca^{2+} homeostasis regulation. Several studies proposed $iPLA_2$ as an essential molecular player of store operated Ca^{2+} entry (SOCE) in a large number of excitable and non-excitable cells. $iPLA_2$ activation releases lysophosphatidyl products, which were suggested as agonists of store operated calcium channels (SOCC) and other TRP channels. Herein, we will review the important role of $iPLA_2$ on the intracellular Ca^{2+} handling focusing on its role in SOCE regulation and its implication in physiological and/or pathological processes.

Keywords Phospholipases A2 • SOCE • TRP channels • Lysophospholipids • STIM1 • Orai1

Abbreviations

AA	Arachidonic acid
$AdPLA_2$	Adipose-specific PLA_2
ARC	Arachidonic acid-regulated calcium channels
BEL	Bromoenol lacotone
CaM	Calmodulin
$cPLA_2$	Cytosolic PLA_2

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DAG	Diacylglycerol
ER	Endoplasmic reticulum
$iPLA_2$	Calcium-independent PLA_2
LA	Lysophasphatidyl acid
$LyPLA_2$	Lysosomal PLA_2
OAG	1-oleoyl-2-acetyl-sn-glycerol
PAF-AH	Platelet-activating factor acetylhydrolases
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PS	Phosphatidylserine
ROC	Receptor operated channels
SMC	Smooth muscle cell
$sPLA_2$	Secretory PLA_2
SOCC/SOCE	Store operated Ca^{2+} channels/entry

6.1 Classification of Phospholipase A_2

The phospholipase A_2 superfamily enzymes are characterized by their ability to catalyze the hydrolysis of glycerophospholipids at the sn-2 position and generate several classes of bioactive lipids, fatty acids and lysophospholipids [1]. Six main families of phospholipases have defined physiological implications. They comprise secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), lysosomal PLA_2, adipose-specific PLA_2 ($AdPLA_2$); and two major Ca^{2+}-independent groups, calcium-independent PLA_2 ($iPLA_2$) and platelet-activating factor acetylhydrolases (PAF-AH). This subdivision was based on their structures, catalytic mechanisms, localizations and evolutionary relationships, and they are collectively identified as groups, using roman numerals (i.e. Group I to Group XVI), with capital letters to distinguish individual sub-families [2]. Many of PLA_2 have contrasted role in cell signaling that involve intracellular Ca^{2+} homeostasis regulation.

6.1.1 Secretory PLA_2 ($sPLA_2$)

The secretory PLA_2s (belonging to Groups I, II, III, V, IX, X and XII in mammalians) were the first type of PLA_2 enzymes discovered. They were identified in organisms such as snakes and scorpions; in components of pancreatic juices; arthritic synovial fluid; and in many different mammalian tissues [3]. Most $sPLA_2$ isoforms are calcium-dependent, and require millimolar concentrations of the ion to function optimally [2, 4, 5]. Consequently, $sPLA_2$s typically function at the external side of the cell hydrolyzing a wide variety of phospholipids [2, 6]. $sPLA_2$ hydrolyzes the sn-2 ester bond in the glyceroacyl phospholipids presents in lipoproteins and cell

membranes, inducing structural and functional changes and forming arachidonic acid (AA), lysopholipids and non-esterified fatty acids with direct proinflammatory effects [7, 8]. In general, $sPLA_2$ isoforms have solid preference for negatively charged phospholipid head groups, in particular phosphatidylserine (PS), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) [9]. Recent studies have suggested that some $sPLA_2$ isoforms can modify cell functions by binding to receptors and other proteins [5].

6.1.2 Cytosolic PLA_2 ($cPLA_2$)

The $cPLA_2$ family (also named Group IVA–F) is one of the major PLA_2 that contains six isoforms, ranging in size from 60 to 85 kDa, which are generally localized in the cytosol. They are active in the presence of mM levels of Ca^{2+} and, with the exception of $cPLA_2\gamma$ (Group IVC), contains in their N-terminals a C2 domain for the binding of two Ca^{2+} ions as well as two conserved phosphorylation sites. $cPLA_2$ family members have a catalytic domain characterized by a three-layer architecture employing a conserved Ser/Asp catalytic dyad, instead of the classical catalytic triad, that is similar in structure to that of $iPLA_2$ [10, 11]. The first group IV $cPLA_2$ (Group IVA) was firstly identified in human platelets in 1986 [12] and was cloned and sequenced 5 years later [13, 14]. $cPLA_2$ is perhaps the far most widely studied cytosolic enzyme and, besides transacylase activity, is also known to have PLA_2 and lysophospholipase activities [15]. $cPLA_2$ is activated by several different mechanisms, and is recruited to the membrane by a Ca^{2+} dependent translocation of the C2 domain. A recent work has localized the lipid binding surface of the enzyme in the presence of Ca^{2+} [16].

From the different PLA_2s, $cPLA_2$ is the only one described to have a preference for AA in the sn-2 position of phospholipids [10, 14]. Upon activation and translocation to intracellular membranes, $cPLA_2$ generates and releases AA from membrane phospholipids leading to an active lipoxygenase and cyclooxygenase metabolism [17]. AA, which acts as precursor for the generation of eicosanoids, is a key player in the prostanoid signaling cascades and therefore its activation is important for regulating various physiological and pathological processes including immune and inflammatory-related processes [2, 18, 19]. Furthermore, AA is also considered as an agonist that induces cytosolic Ca^{2+} entry through cationic channels called arachidonic acid-regulated calcium channels (ARC) [20, 21].

6.1.3 PAF Acetyl Hydrolase/Oxidized Lipid ($PAF\text{-}AH/LpPLA_2$)

Platelet activating factor (PAF) acetylhydrolases (AH) (PAF-AH, Group VIIA and B, and VIIIA and B) have low molecular weight (26–45 kDa) and represent a unique group of acyl hydrolases with a catalytic serine that is capable of releasing acetate

from the sn-2 position of PAF, a 1-*O*-alkyl-PC [22]. However, they can also catalyze the release of oxidized acyl groups from the sn-2 position of PC and PE, not just PAF [2, 4, 23]. Its active site is composed of a serine, histidine, and aspartic acid hydrolase triad, unlike all other PLA_2s, which have dyads [24]. There are four members of this family that specifically catalyze these reactions; one of them is a secreted protein (GVIIA PLA_2), known as plasma-type PAF-AH or "lipoproteinassociated PLA_2" ($LpPLA_2$), that has generated interest as a therapeutic target for atherosclerosis [22, 26–29]. On the other hand, $LpPLA_2$ is a potent phospholipid activator that is secreted by multiple inflammatory cells including monocytes/macrophages, T lymphocytes and mast cells [30, 31]. This enzyme was cloned from human plasma in 1995 and was shown to have anti-inflammatory activity in vivo [25]. The $LpPLA_2$ role in cytosolic Ca^{2+} regulation is still unknown.

6.1.4 Lysosomal PLA_2 ($LyPLA_2$)

Lysosomal PLA_2 was purified from bovine brain as an enzyme that esterifies an acyl group with the hydroxyl group in the C-1 position of ceramide using phospholipids as the acyl group donor, so the enzyme was first named 1-O-acylceramide synthase (ACS). The protein possesses Ca^{2+} independent PLA_2 and transacylase activities. Hiraoka et al. [32] proposed that the hydrolyzed acyl group is transferred through an enzyme-acyl intermediate to ceramide or water, resulting either in the production of either 1-Oacyl- ceramide (ACS activity) or the release of free fatty acids (PLA_2 activity). In terms of catalytic activity, Ly-PLA_2 specifically prefers PC and PE head groups at pH 4.5 in a Ca^{2+}-independent manner. Ly-PLA_2 is ubiquitously expressed in diverse cell types, but highly expressed in alveolar macrophages. In fact, it plays a role in surfactant metabolism, and specifically in the phospholipid catabolism of pulmonary surfactant [33, 34].

6.1.5 Adipose Specific PLA_2 ($AdPLA_2$)

Duncan et al. [35] discovered recently a novel intracellular PLA_2, highly and differentially expressed only in adipocytes and induced during preadipocyte differentiation, that releases sn-2 fatty acid from phospholipids in a Ca^{2+}-dependent manner. This recently discovered enzyme named adipose-specific PLA_2 ($AdPLA_2$, Group XVI), has a molecular weight of 18 KDa. It is found abundantly in white adipose tissue, 40–150 times higher that found in liver. The enzyme is not an acyltransferase, but it functions entirely as a phospholipase, producing lysophosphatidylcholine and AA from the phospholipids. In addition, Duncan and colleagues studied the properties of AdPLA and found its optimal pH was 8.0, requiring cysteine and histidine residues at the active site, with maximal enzymatic activity in the presence of 1.0 mM Ca^{2+} [35]. $AdPLA_2$ have been also implicated in energy regulation as it modultes the release of fatty acids, from stored triglycerides in white adipose

Table 6.1 Isoforms of calcium-independent (Group VI) PLA2

PLA_2 family	Group	Source	MW (KDa)	Alternate name
$iPLA_2$	VIA-1	Human/murine	84–85	$iPLA_2\alpha$
	VIA-2	Human/murine	88–90	$iPLA_2\beta$
	VIB	Human/murine	88–91	$iPLA_2\gamma$
	VIC	Human/murine	146	$iPLA_2\delta$, neuropathy target esterase (NTE)
	VID	Human	53	$iPLA_2\varepsilon$, adiponutrin
	VIE	Human	57	$iPLA_2\zeta$, TTS-2.2
	VIF	Human	28	$iPLA_2\eta$, GS2

tissue, which will be later used as energy source by other tissues. $AdPLA_2$ has been also proposed to play a major role in the supply of AA for prostaglandin E2 (PGE2) synthesis in white adipose tissue [36]. Thus, AdPLA is considered a major regulator of adipocyte lipolysis and is crucial for the development of obesity, although it seems possible that AdPLA could promote obesity through a mechanism distinct from PGE2 signaling [37].

6.1.6 Calcium Independent PLA_2 ($iPLA_2$)

The Ca^{2+} independent PLA_2s are members of the GVI family of PLA_2 enzymes. Currently, six isoforms of $iPLA_2$ (Group VIA–F) have been identified as shown in Table 6.1. While their catalytic sites are similar to that of $cPLA_2$, they do not require Ca^{2+} for catalytic activity and they are generally larger in size, with moleculear weights ranging from 55 to 146 kDa except for Group VIF PLA_2 (~28 kDa). $iPLA_2$s are localized either in the cytosol, the endoplasmic reticulum (ER) or in the mitochondrial membrane [38]. $iPLA_2$ are entirely involved in lipid remodeling, in the Land's Cycle, and also mediate cell growth signaling [2, 4]. Members of this family share a protein domain initially discovered in patatin, the most abundant protein of the potato tuber.

In the next part of this chapter, we will go through $iPLA_2$ classification, regulation, and its role in intracellular Ca^{2+} regulation.

6.2 Sub-classification of $iPLA_2$

6.2.1 GVIA PLA_2 ($iPLA_2\alpha$ and $iPLA_2\beta$)

Many new $iPLA_2$ (GVI PLA_2) members have been identified in the last years, but the first member and the best characterized of this family is the GVIA PLA_2, which was purified from macrophages in 1994 [39, 40]. GVIA PLA_2 is expressed in

multiple different splice variants [41] and, similar to cPLA$_2$ (GIV PLA$_2$), it catalyzes the cleavage of the sn-2 ester bond. However, it does not show specificity for AA in the sn-2 position and is fully active in the absence of Ca^{2+}. The GVIA PLA$_2$ also possesses sn-1 lysophospholipase and transacylase activity [41]. The enzyme has a conserved glycine-rich nucleotide-binding motif (GXGXXG) proximal to the catalytic site and it is activated several-fold by ATP [42]. The N-terminal domain of GIVA PLA$_2$ is composed of seven to eight ankyrin repeats, which are responsible for protein-protein interaction between monomers [43]. It is thought that ankyrin repeats enable the oligomeration of Group VIA monomers required for catalytic activity [39]. In fact, the active form of Group VIA PLA$_2$ is a tetramer [39].

Several splice variants of GVIA PLA$_2$ have been identified [39, 44]. Group VIA-1 or iPLA$_2$α, and Group VIA-2 or iPLA$_2$β [44–48], for example, comprise two catalytically active forms of this enzyme [44–48]. Both isoforms are similar in size, 85 and 88 KDa respectively, and contain eight N-terminal ankyrin repeats and a consensus lipase motif (GXS^{465}XG), whereas in GVIA-2 PLA$_2$ the 8 ankyrin repeats are interrupted by an insertion of 54 amino acids and they exhibit a glutamate residue at position 450, while the corresponding position in Group VIA-1 is glutamine.

Three additional splice variants of GVIA iPLA$_2$ have been identified: Group VIA-3 (also known as iPLA$_2$-2); Group VIA Ankyrin-1 (or Ankyrin-iPLA$_2$-1), and Group VIA Ankyrin-2 (or Ankyrin-iPLA$_2$-2). The GroupVIA-3 splice variant encodes an iPLA$_2$ that is identical to Group VIA-2 PLA$_2$ (iPLA$_2$β) at the N-terminus, that retains the GTS^{519}TG active site and that has a truncated C-terminus. However, it is not known whether Group VIA-3 encodes a functional phospholipase A$_2$. Group VIA Ankyrin-1 is identical to Group VIA-2 at the N-terminus but it ends prior to the GTS^{519}TG active site with a three amino acid modification at the C-terminus; it does not encode a functional PLA$_2$ enzyme [46]. Similar to Group VIA Ankyrin-1, Group VIA Ankyrin-2 also lacks the GTS519TG active site and additionally present with a 73 amino-acids shorter N-terminus and a 50-amino-acid variation at the C-terminus. Group VIA ankyrin-1 and Group VIA ankyrin-2 may act as negative regulators of Group VIA-1 and Group VIA-2 by precluding catalytically active tetramer aggregation [39, 46]. Processes in which GVIA PLA$_2$ has been implicated include phospholipids remodeling, AA release causing eicosanoid formation, protein expression, acetylcholine-mediated endothelium-dependent relaxation of the vasculature, secretion, and apoptosis. iPLA$_2$ plays also an important role in lymphocyte proliferation and in Ca^{2+} signaling regulated by calmodulin (CaM) and by a Ca^{2+} influx factor as detailed below [41, 49–52].

6.2.2 GVIB PLA$_2$ or iPLA$_2$ɣ

The iPLA$_2$ɣ called also GVIB PLA$_2$ have been less studied. It has been involved in the release of AA that leads to eicosanoid formation [53, 54]. iPLA$_2$ɣ contains the consensus lipase motif (GXSXG), a C-terminal peroxisome localization

signal (SKL), and a glycine-rich nucleotide binding loop motif (GXGXXG). Interestingly, the nucleotide-binding motif commences 34 amino acids upstream of the putative active Ser, which is closely identical to the location of the nucleotide binding loop motif of Group VIA (35 amino acids upstream) [53]. A recent study demonstrated that $iPLA_2\gamma$ is responsible for the release of AA and prostaglandin E2 (PGE2) and inflammatory mediators in cardiac myocytes infected by Chagas' disease parasite [55]. Previously, $iPLA_2\gamma$ was also suggested as a critical participant in the Ca^{2+}-induced opening of the mitochondrial permeability transition pore (mPTP) in Liver [56].

6.2.3 GVIC, GVID, GVIE, GIVF PLA_2s

Different Ca^{2+}-independent lipases have been identified newly, and classified according to the terminology of the Group system GVIC, GVID, GVIE and GIVF PLA_2s. The GVIC PLA_2 enzyme has some sequence similarity to GVIA PLA_2 and might play a role in membrane homeostasis. This enzyme was previously known as NEST, the recombinantly expressed esterase domain of NTE (neuropathy target esterase), a membrane protein expressed in neurons of human and mice with physiological function elusive [57, 58] that possesses PLA_2 and lysophospholipase activities [59]. NEST might slowly hydrolyze the fatty acid in the sn-2 position of PC and subsequently, in a fast reaction, release the fatty acid in the sn-1 position.

The genes for the three other enzymes have also been identified before. Although, there was no catalytic activity attributed to corresponding proteins. The enzymes were shown to hydrolyze both LA and AA at the sn-2 position in the absence of free Ca^{2+} [60], thus these three enzymes might play a role in the regulation of triacylglycerol homeostasis which implicates the control of energy metabolism in adipocytes. Besides, PLA_2 activity, these enzymes possess high triacylglycerol lipase and acylglycerol transacylase activities and all of them were inhibited by bromoenol lacotone (BEL) at sub-micromolar levels [60].

6.3 Regulation of $iPLA_2$

6.3.1 ATP and PKC

The $iPLA_2$ protein contains a lipase consensus sequence and a putative ATP-binding motif. ATP has been reported to stimulate $iPLA_2$ activity in rat islets [61], murine P388D1 cells [45], but not to affect the $iPLA_2$ activity of Chinese Hamster Ovary cells [44]. In an early study, Ackerman et al. discovered that both Triton X-100 and ATP enhanced the activity of $iPLA_2$ in P388D1 cells [39]. The enzyme activity was 1.2–6 fold higher in mixed micelles when assayed in the presence of ATP and other

di- or triphosphate nucleotides [39]. In other study, ATP stimulation of an $iPLA_2$ isoform was demonstrated in human pancreatic islet [42]. Interestingly, this same group demonstrated that ATP does not directly activate but rather protects $iPLA_2$ from a loss of its activity [61]. On the other hand, there is no consensus regarding the role of PKC in $iPLA_2$ activation [62]. An early study showed that the activation of PKCα ultimately provoked AA release via $iPLA_2$. This AA release was markedly inhibited by BEL or $iPLA_2$ antisense oligonucleotide [63]. Interestingly, we demonstrated that both diacylglycerol analog 1-oleoyl-2-acetyl-sn-glycerol (OAG) and store depletion with thapsigargin produced a PKCε-dependent activation of $iPLA_2\beta$ in proliferating but not in confluent aortic SMC [64].

6.3.2 Ca^{2+}/Calmodulin Regulation of $iPLA_2$

The first evidence of $iPLA_2$ modulation by CaM came from the observation that Ca^{2+} addition to the cytosol of cardiac myocytes inhibited $iPLA_2$ activity induced by ischemia. This inhibition was demonstrated to be due to CaM [65]. In fact, molecular and structural studies showed that in the absence of CaM, the active site of $iPLA_2$ interacts with the CaM-binding domain, resulting in a catalytically competent enzyme, whereas reversible disruption of this interaction through the binding of CaM abrogates this interaction, resulting in a loss of $iPLA_2$ activity [65–67]. $iPLA_2$ was shown to form a catalytically inactive ternary complex with CaM-Ca^{2+} that could be reversibly dissociated by chelation of Ca^{2+} ion with EGTA to regain full catalytic activity. Although $iPLA_2$ activity is independent of Ca^{2+}, it is able to inhibit the $iPLA_2$ activity by Ca^{2+}-activated CaM and this inhibition is apparently due to the binding to the IQ motif. In fact, the dissociation of CaM from $iPLA_2$ is the main mechanism that changes the Ca^{2+}-independent enzyme into an enzyme that is sensitive to modification in intracellular Ca^{2+} ion homeostasis. Moreover, conformational changes provoked in CaM using agents that inhibited the interaction of CaM with its target proteins resulted in $iPLA_2$ activation. Wolf et al. in 1997 have shown that W7, CaM antagonist, activated $iPLA_2$ in A-10 smooth muscle cells (SMC) [68]. Smani and colleagues also demonstrated that CaM inhibition with calmidazolium and a membrane-impermeable CaM inhibitory peptide, promoted $iPLA_2$ activation in SMC and RBL cell line [69]. Later on, compelling evidences have shown that store depletion with thapsigargin or cyclopiazonic acid stimulated $iPLA_2$ activation through displacement of inhibitory CaM [68–70].

6.3.3 Chemical Inhibition of $iPLA_2$

The most important inhibitor for $iPLA_2$ is BEL, which has specificity 1,000 times higher for $iPLA_2$ over other PLA_2 isoforms [41]. BEL is a suicidal substrate for $iPLA_2$ that is widely used as an irreversible mechanism-based, time- and temperature-dependent, inhibitor. For cell-based studies, it has been described previously that

high concentrations of BEL (25 μM) partially inhibit the magnesium-dependent phosphatidate phosphohydrolase (PAP-1), which converts phosphatidic acid to diacylglycerol (DAG) [71, 72]. To some extent it is possible to identify promiscuous effects of BEL on $iPLA_2$ and PAP-1 by performing experiments with BEL and propranolol in parallel [71, 73]. The latter compound inhibits PAP-1 and not $iPLA_2$. Others and we confirmed that $iPLA_2$ activation induced by Ca^{2+} release from the store is inhibited by BEL [68, 70, 73, 74]. Importantly, Jenkins et al. [75] demonstrated that the commonly used BEL is composed of two enantiomers with different specificity for $iPLA_2$ isoforms. S-BEL has higher specificity to $iPLA_2\beta$, and R-BEL is more specific to $iPLA_2\gamma$, which allowed identifying the type of $iPLA_2$ involved in several different cellular processes. Indeed, we confirmed that S-BEL, but not R-BEL, selectively inhibited $iPLA_2\beta$ activity stimulated by intracellular store depletion in SMC and RBL, indicating that S-BEL is a valuable tool to determine the role of $iPLA_2\beta$ in intracellular signaling processes [64, 76].

6.4 $iPLA_2$ Role in the Ca^{2+} Signaling Network

As described above, for long time $iPLA_2$'s main role was especially related to cellular phospholipids remodeling [41]. However, different reports have demonstrated that the specific beta isoform of $iPLA_2$ ($iPLA_2\beta$) is involved in several agonist-stimulated signaling cascades. $iPLA_2$ has several unique features which confused researchers for many years. One of them relies on its activation independently of the presence or absence of Ca^{2+}. $iPLA_2$ is able to function in the presence of strong Ca^{2+} chelators as BAPTA. At the same time $iPLA_2$ is able to bind the Ca^{2+}–CaM complex. Interestingly, conditions for $iPLA_2$ activation are similar to those described for store operated calcium entry (SOCE). In fact, $iPLA_2$ can be activated by depletion of intracellular Ca^{2+} stores caused by vasopressin or by thapsigargin, an inhibitor of Sarco/Endoplamic reticulum Ca^{2+}-ATPase pump [68, 77, 78].

6.4.1 Overview of the Store Operated Ca^{2+} Channels Signaling Pathway

To increase cytoplasmic Ca^{2+} concentration, Ca^{2+} is either released from intracellular stores or enters into the cell by crossing the plasma membrane through ion channels. Store operated Ca^{2+} channels (SOCC) and receptor operated channels (ROC) are considered the main route for Ca^{2+} entry in non-excitable cells, but they also exist in excitable cells such as skeletal muscle, neurons or smooth muscle [79]. In excitable cells, Ca^{2+} entry is achieved largely through opening of voltage and/or voltage independent channels ROC or SOCC that are responsible of SOCE [80]. The concept of SOCE activation seems to be simple: basically upon depletion of ER

stores, a signal is produced that activates specific Ca^{2+}-conducting channels SOCC, in plasma membrane that allows Ca^{2+} entry into the cell. SOCC role was originally linked only to refilling the intracellular store. However, now it's widely agreed that these channels provide a sustained Ca^{2+} influx for a variety of important functions in eukaryotic cells. Among those functions are exocytosis, vascular contraction and relaxation, Ca^{2+} oscillations, gene transcription, regulation of enzymatic activity, cell proliferation and apoptosis [80, 81].

6.4.1.1 Mechanism of SOCE Activation: Emerging Role of STIM1 and Orai1

One of the most intriguing mysteries of the store-operated pathway is the mechanism of its activation. Questions of how do the stores communicate with the plasma membrane channels and which is the signal produced by the stores upon their depletion, have been a matter of intense investigation for long time. Hypotheses presented can be mainly grouped into two main categories: those that propose the generation of a diffusible molecule with ability to induce SOCC opening, and those that assume a physical interaction between channel subunit and an element of the ER (for review see [80, 82]). Soon after the identification of SOCE Robin Irvine proposed a physical or conformational coupling between elements in the ER and SOCC in the plasma membrane [83], as a mechanism that resembles the classical excitation-contraction coupling between ryanodine receptors and dihydropyridine receptors in the skeletal muscle [84]. Consequently, most of the early studies focused on the association between inositol-triphosphate receptors (IP_3R) and the subunit channel suggested to form SOCC. This hypothesis received support from studies demonstrating that, under resting conditions; TRPC1, TRPC3 and TRPC6 can be co-immunoprecipitated with IP_3R [85, 86]. However, the major challenge for this model came from the studies in triple IP_3R knockout DT40 cells, in which SOCE seemed completely normal [87–89]. Importantly, in 2005 and 2006 the Ca^{2+} sensor of the ER was identified as the STIM1 (Stromal Interaction Molecule-1) protein, and Orai1 was identified as the structural subunit of the channel conducting the Ca^{2+} selective CRAC [90–92]. Several reports have showed that upon Ca^{2+} depletion, STIM1 lose Ca^{2+} from its EF hand, oligomerize and accumulate into punctate structures in the ER membrane located in close proximity (10–25 nm) to the plasma membrane. Furthermore, STIM1 and Orai1 have been reported to accumulate and colocalize in punctate structures along the plasma membrane and to associate by a reversible and physical coupling mechanism upon depletion of the intracellular Ca^{2+} stores which support the conformational coupling model (for review see [93]). While direct coupling of ER-resident STIM1 to PM-resident Orai1 is considered as the most straightforward mechanism for signal transduction, there is a growing body of evidence for the presence of additional structural and/or functional linker(s) between STIM1 and Orai1. Indeed, Balla's group suggested the presence of additional molecular components within the STIM1-Orai1 complex [94]; meanwhile Rosado and colleagues nicely showed that both STIM1 and Orai1

also co-immunoprecipitate with other TRPC channels when stores are depleted [95–97]. Recently, we have demonstrated that store depletion stimulated STIM1 and $iPLA_2\beta$ colocalization required for SOCE in coronary artery [98].

6.4.1.2 Calcium Influx Factor and SOCE Activation

The other hypothesis focuses on diffusible messengers generated upon intracellular stores depletion. Different signaling molecules have been reported to play an essential role in the activation of SOCE in different cell types, including cGMP [99], tyrosine kinases [100], and small GTP-binding proteins [101], among others. However, special efforts were dedicated to the still uncharacterized molecule known as Ca^{2+} influx factor (CIF) by Victoria Bolotina's group. Refined CIF extract was obtained from different cell lines, including human platelets, which stimulated an extracellular Ca^{2+} influx and I_{CRAC} (CRAC current) sensitive to the well-known SOCC inhibitors [76]. Interestingly, soon after STIM1 discovery, Bolotina and coworkers presented compelling evidences demonstrating that CIF production is tightly linked with STIM1 expression and requires the functional integrity of glycosylation sites in its intraluminal SAM domain [102]. In this study, authors demonstrated that upon store depletion, CIF is produced before STIM1 accumulation in puncta and activation of SOCE. Authors showed that lack of STIM1 in the rare neuronal cell line (NG115-401L), which features virtually no SOCE responses [103], or STIM1 downregulation in cells transfected with siRNAs, dramatically impaired active CIF production confirming CIF and STIM1 relationship [102]. Unfortunately, the molecular identity of CIF is still unknown, although its presence and its biological activity were detected by numerous groups in a wide variety of cell types ranging from yeast to human (for review see [51, 79]). Previously, we have characterized in our earliest studies that $iPLA_2\beta$ is the physiological target of CIF and the mechanism of CIF-induced activation of SOCE was depicted as illustrated in Fig. 6.1 [69, 76, 104].

6.4.2 Essential Role of $iPLA_2$ in Store Operated Calcium Entry

In the last 1990s, several works established an interesting scenario for $iPLA_2$ activation, showing that it could be activated by depletion of Ca^{2+} stores caused by vasopressin or by thapsigargin in A10 SMC line [68, 77]. A10 cells stimulation with thapsigargin induced release of AA that was directly correlated to thapsigargin-induced depletion of intracellular Ca^{2+} stores [68]. Next, $iPLA_2\beta$, and not $iPLA_2\gamma$, was identified as the mediator of vasopressin-induced AA release in SMC [75]. Therefore, the role of $iPLA_2\beta$ in SOCE activation was explored and the first evidence of $iPLA_2$ requirement for SOCE activation was obtained by studying SOCE in primary culture of SMC as a model for excitable cells, and RBL cells as a model for non-excitable cells. The functional inhibition of $iPLA_2$ with BEL prevented the

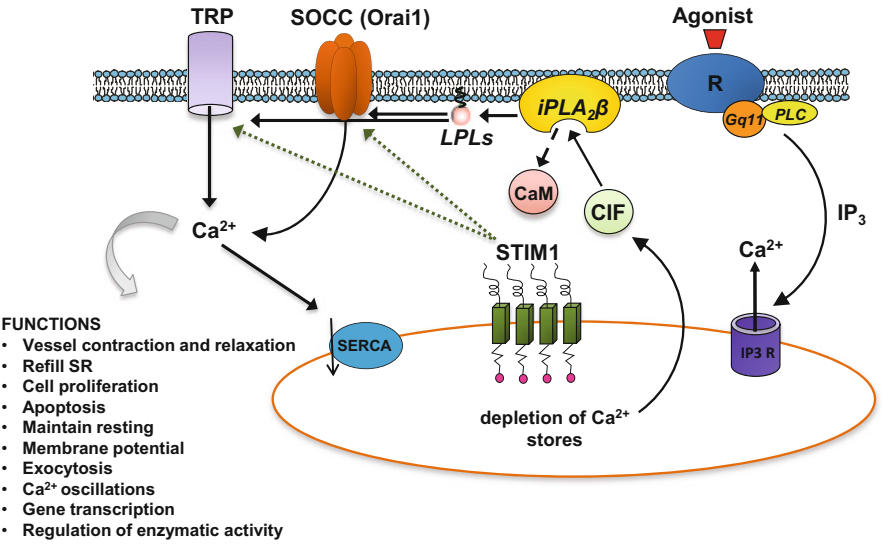

Fig. 6.1 Mechanism suggested for store operated Ca^{2+} entry (*SOCE*), involving Ca^{2+} independent phospholipase $A_2\beta$ (*iPLA*$_2$) pathway. We propose that agonist binding to R, promotes IP_3-induced Ca^{2+} release, activation/translocation of STIM1, Calcium Influx Factor (*CIF*) release, displacement of calmodulin (*CaM*), activation of $iPLA_2$, lysophospholipids (*LPLs*) production, and activation of store-operated Ca^{2+} channels (*SOCC*) (Orai1) and other transient receptor potential channels (*TRP*). The subsequent Ca^{2+} entry is responsible for a large number of functions as indicated

activation of single SOCC in SMC, and whole cell CRAC currents in RBL induced by TG and/or BAPTA-induced depletion of intracellular stores. In addition, molecular inhibition using antisense against $iPLA_2$, or its functional blocking with BEL impaired dramatically SOCE, while Ca^{2+} release from the stores was not affected, which confirmed the novel role of $iPLA_2$ in SOCE pathway [78]. Furthermore, the use of S-BEL enantiomer confirmed that $iPLA_2\beta$ is the isoform responsible of SOCE in RBL [76] and SMC [64]. Interestingly, cell dialysis with recombinant $iPLA_2\beta$ could substitute the endogenous $iPLA_2\beta$ and rescue activation of I_{CRAC} in the cells in which endogenous $iPLA_2\beta$ was knocked down [76]. One of the most important features of $iPLA_2$ is that it exists in a complex with CaM, which keeps it in a catalytically inactive state; and removal of CaM results in $iPLA_2$ activation [66]. Therefore, the inhibition of CaM was found to mimic the effects of thapsigargin-induced SOCE as it activated $iPLA_2$; it evoked a 2APB and BEL-sensitive Ca^{2+} influx; and finally it stimulated single SOCC in SMC [69]. Similar effect of CaM inhibition was also observed in astrocytes [70] and in rat cerebellar granule [105].

The role of $iPLA_2\beta$ in SOCE was further confirmed by us and by many other investigators in a growing number of cell types, including platelets, Jurkat T lymphocytes [69, 78], RBL-2H3 [104], neuroblastoma/glioma [70], keratinocytes [106], skeletal muscle [107], fibroblasts [108], prostate cancer cells [109] and others. In all these studies molecular knock-down and/or functional inhibition of $iPLA_2\beta$ caused full impairment of SOCE. Strikingly, genetic screening of Drosophila

melanogaster performed by Vig et al. indicated that not only STIM1 and Orai1, but also an orthologue of iPLA$_2$β encoded by the CG6718 gene, are gene products with a great impact on SOCE activation [110]. Recently, we have demonstrated that agonist-induced coronary artery contraction involved the activation of SOCE by STIM1, Orai1 and iPLA$_2$ [98]. We have shown that on cells stimulation, STIM1 colocalized with iPLA$_2$β in submembrane compartments suggesting their functional communication and we confirmed that lysophopholipids, product of iPLA$_2$, stimulated an Orai1- but not STIM1- dependent SOCE, suggesting that the functional role of iPLA$_2$β is downstream of STIM1 and upstream of Orai1 in coronary SMC. The complex relationships between the components of the CRAC channel, namely Orai1, STIM1, and iPLA$_2$β in the SOCE pathway have been detailed in a previous review [79].

6.4.3 *iPLA$_2$ and Lysophospholipids Activation of Store Operated Calcium Entry*

Afterwards, numerous studies focused on the molecular mechanism of iPLA$_2$-dependent signal transduction. Several works from Bolotina's lab in the first decade of this centry, provided compelling evidences demonstrating that SOCC can be activated by CIF produced upon depletion of Ca^{2+} stores in the ER, and it in turn, can displace the inhibitory CaM from iPLA$_2$β. The early studies have shown that CIF activated single SOCC in inside-out membrane patches [111], and the channels remained active even after the membrane patches were excised and CIF was washed away [112], indicating the presence of an additional cascade of plasma membrane-delimited reactions that might be involved in CIF-induced activation of SOCC. In 2004, a major finding has been described by Smani et al. demonstrating that CIF extract can displace inhibitory CaM from iPLA$_2$β leading to lysophopholipids production and the activation of SOCC in membrane-delimited manner in SMC [69]. By contrast, CIF dialysis of RBL cells transfected with antisense to iPLA$_2$β failed to activate I_{CRAC}, confirming the need of functional iPLA$_2$β to stimulate SOCE [76]. Furthermore, the exogenous application of lysophopholipids but not AA, products of iPLA$_2$β activation, were able to stimulate SOCE in intact cells and single SOCC in inside-out membrane patches in SMC [69, 74, 98]. Further studies have confirmed that lysophospholids evoked SOCE in different cell lines such as astrocyte [70], rat cerebellar granule neurons [105], skeletal muscle [113], and keratinocytes [114], among others cells. Thus, several independent works established the need of active iPLA$_2$β, and lysophospholipids to stimulate SOCE in a wide range of cells.

However and independently of its role in SOCE signaling, few reports have shown that iPLA$_2$ might activate some TRP channels. Works from Prevarskaya's lab demonstrated that iPLA$_2$β activated both SOCC and TRPM8 channels [109, 115], and AL-Shawaf and colleagues showed recently that lysophosphatidylcholine and AA generated by iPLA$_2$ are involved in TRPC5 activation by sphingosine-1-phosphate [116].

6.5 Significant Potential as Targets for Novel Therapeutics Strategy

The role of iPLA$_2$β, and the consequent activation of SOCE in several physio- and pathological processes have been largely studied. For example, iPLA$_2$β-dependent activation of vascular reactivity was demonstrated in aorta, cerebral, mesenteric, carotid and coronary arteries [98, 117, 118]. Furthermore, iPLA$_2$β-induced SOCE seems involved in SMC proliferation [119] and in HEK cells migration [120]. Molecular knockdown of Orai1, STIM1 or iPLA$_2$β caused a similar reduction in velocity and distance in migrating HEK cells. Previously, Vanden Abeele et al. demonstrated that iPLA$_2$β activated SOCE in LNCaP prostate cancer proliferative cells [109], and they further showed that iPLA$_2$β is also implicated in the lysophospholipid-dependent gating of TRPM8, a cold sensor [115]. On the other hand, Boittin and Reugg published several interesting studies highlighting the involvement of iPLA$_2$-dependent activation of SOCC in dystrophic muscle fibers [109]. They found that iPLA$_2$ is mainly localized in the vicinity of the sarcolemma, suggesting a close proximity with SOCC, which may be located on the sarcolemma and/or in the T-tubular membranes. These authors have also demonstrated that lysophosphatidylcholine acts downstream of iPLA$_2$ and directly activates SOCC in dystrophic fibers [107, 113]. Interestingly, recent studies have determined that iPLA$_2$ can be targeted by secondary signaling pathway to potentiate or inhibit SOCE such as PKCε [64], and Urocortin through cyclic AMP in SMC [98], in skeletal muscle [121], and in hepatoma carcinoma cell lines [122]. These few examples confirm the important role of iPLA$_2$ and SOCE in several physiological and pathological processes and confirm it as a valuable therapeutic target.

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References

1. Burke JE, Dennis EA (2009) Phospholipase A2 biochemistry. Cardiovasc Drug Ther 23(1):49–59
2. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G (2011) Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev 111(10):6130–6185
3. Six DA, Dennis EA (2000) The expanding superfamily of phospholipase A 2 enzymes: classification and characterization. Biochim Biophys Acta 1488(1):1–19
4. Murakami M, Taketomi Y, Miki Y, Sato H, Hirabayashi T, Yamamoto K (2011) Recent progress in phospholipase A 2 research: from cells to animals to humans. Prog Lipid Res 50(2):152–192
5. Chakraborti S (2003) Phospholipase A 2 isoforms: a perspective. Cell Signal 15(7):637–665

6. Lambeau G, Gelb MH (2008) Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 77:495–520
7. Camejo G (2010) Lysophospholipids: effectors mediating the contribution of dyslipidemia to calcification associated with atherosclerosis. Atherosclerosis 211(1):36–37
8. Rodriguéz-Lee M, Bondjers G, Camejo G (2007) Fatty acid-induced atherogenic changes in extracellular matrix proteoglycans. Curr Opin Lipidol 18(5):546–553
9. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, Gelb MH (2002) Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. J Biol Chem 277(50):48535–48549
10. Kramer RM, Sharp JD (1995) Recent insights into the structure, function and biology of cPLA2. Agents Actions Suppl 46:65–76
11. Kramer RM, Sharp JD (1997) Structure, function and regulation of Ca2+-sensitive cytosolic phospholipase A2 (cPLA2). FEBS Lett 410(1):49–53
12. Kramer RM, Checani GC, Deykin A, Pritzker CR, Deykin D (1986) Solubilization and properties of Ca2+-dependent human platelet phospholipase A 2. Biochim Biophys Acta 878(3):394–403
13. Kramer RM, Roberts EF, Manetta J, Putnam JE (1991) The Ca2(+)-sensitive cytosolic phospholipase A2 is a 100-kDa protein in human monoblast U937 cells. J Biol Chem 266(8):5268–5272
14. Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Knopf JL (1991) A novel arachidonic acid-selective cytosolic PLA2 contains a Ca2+-dependent translocation domain with homology to PKC and GAP. Cell 65(6):1043–1051
15. Reynolds LJ, Hughes LL, Louis AI, Kramer RM, Dennis EA (1993) Metal ion and salt effects on the phospholipase A2, lysophospholipase, and transacylase activities of human cytosolic phospholipase A2. Biochim Biophys Acta 1167(3):272–280
16. Burke JE, Hsu YH, Deems RA, Li S, Woods VL, Dennis EA (2008) A phospholipid substrate molecule residing in the membrane surface mediates opening of the lid region in group IVA cytosolic phospholipase A2. J Biol Chem 283(45):31227–31236
17. Leslie CC (1997) Properties and regulation of cytosolic phospholipase A2. J Biol Chem 272(27):16709–16712
18. Kita Y, Ohto T, Uozumi N, Shimizu T (2006) Biochemical properties and pathophysiological roles of cytosolic phospholipase A2s. Biochim Biophys Acta 1761(11):1317–1322
19. Kudo I, Murakami M (2002) Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 68–69:3–58
20. Mignen O, Shuttleworth TJ (2000) I(ARC), a novel arachidonate-regulated, noncapacitative Ca(2+) entry channel. J Biol Chem 275(13):9114–9119
21. Mignen O, Thompson JL, Shuttleworth TJ (2009) The molecular architecture of the arachidonate-regulated Ca2+-selective ARC channel is a pentameric assembly of Orai1 and Orai3 subunits. J Physiol 587(17):4181–4197
22. Khakpour H, Frishman WH (2009) Lipoprotein-associated phospholipase A2: an independent predictor of cardiovascular risk and a novel target for immunomodulation therapy. Cardiol Rev 17(5):222–229
23. Stephens JWW, Myers W (1898) The action of cobra poison on the blood: a contribution to the study of passive immunity. J Pathol Bacteriol 5(3):279–301
24. Tjoelker LW, Eberhardt C, Unger J, Le Trong H, Zimmerman GA, McIntyre TM, Gray PW (1995) Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad. J Biol Chem 270(43):25481–25487
25. Tjoelker LW, Wilder C, Eberhardt C, Stafforinit DM, Dietsch G, Schimpf B, Gray PW (1995) Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature 374(6522):549–553
26. Chen CH (2004) Platelet-activating factor acetylhydrolase: is it good or bad for you? Curr Opin Lipidol 15(3):337–341
27. Packard CJ (2009) Lipoprotein-associated phospholipase A2 as a biomarker of coronary heart disease and a therapeutic target. Curr Opin Cardiol 24(4):358–363

28. Tsimikas S, Tsironis LD, Tselepis AD (2007) New insights into the role of lipoprotein (a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol 27(10):2094–2099
29. Wilensky RL, Macphee CH (2009) Lipoprotein-associated phospholipase A2 and atherosclerosis. Curr Opin Lipidol 20(5):415–420
30. Stafforini DM, Satoh K, Atkinson DL, Tjoelker LW, Eberhardt C, Yoshida H, Prescott SM (1996) Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. J Clin Invest 97(12):2784–2791
31. Min JH, Wilder C, Aoki J, Arai H, Inoue K, Paul L, Gelb MH (2001) Platelet-activating factor acetylhydrolases: broad substrate specificity and lipoprotein binding does not modulate the catalytic properties of the plasma enzyme. Biochemistry 40(15):4539–4549
32. Hiraoka M, Abe A, Shayman JA (2002) Cloning and characterization of a lysosomal phospholipase A2, 1-O-acylceramide synthase. J Biol Chem 277(12):10090–10099
33. Hiraoka M, Abe A, Lu Y, Yang K, Han X, Gross RW, Shayman JA (2006) Lysosomal phospholipase A2 and phospholipidosis. Mol Cell Biol 26(16):6139–6148
34. Abe A, Poucher HK, Hiraoka M, Shayman JA (2004) Induction of lysosomal phospholipase A2 through the retinoid X receptor in THP-1 cells. J Lipid Res 45(4):667–673
35. Duncan RE, Sarkadi-Nagy E, Jaworski K, Ahmadian M, Sul HS (2008) Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). J Biol Chem 283(37):25428–25436
36. Jaworski K, Ahmadian M, Duncan RE, Sarkadi-Nagy E, Varady KA, Hellerstein MK, Sul HS (2009) AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. Nat Med 15(2):159–168
37. Sanchez-Alavez M, Klein I, Brownell SE, Tabarean IV, Davis CN, Conti B, Bartfai T (2007) Night eating and obesity in the EP3R-deficient mouse. Proc Natl Acad Sci 104(8):3009–3014
38. Cummings BS, McHowat J, Schnellmann RG (2002) Role of an endoplasmic reticulum Ca2+-independent phospholipase A2 in oxidant-induced renal cell death. J Pharmacol Exp Ther 283(3):492–498
39. Ackermann EJ, Kempner ES, Dennis EA (1994) Ca2+-independent cytosolic phospholipase A2 from macrophage-like P388D1 cells Isolation and characterization. J Biol Chem 269(12):9227–9233
40. Schaloske RH, Dennis EA (2006) The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 1761(11):1246–1259
41. Winstead MV, Balsinde J, Dennis EA (2000) Calcium-independent phospholipase A2: structure and function. Biochim Biophys Acta 1488(1):28–39
42. Ma Z, Wang X, Nowatzke W, Ramanadham S, Turk J (1999) Human pancreatic islets express mRNA species encoding two distinct catalytically active isoforms of group VI phospholipase A2 (iPLA2) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA2 gene on chromosome 22q13 1. J Biol Chem 274(14):9607–9616
43. Sedgwick SG, Smerdon SJ (1999) The ankyrin repeat: a diversity of interactions on a common structural framework. Trends Biochem Sci 24(8):311–316
44. Tang J, Kriz RW, Wolfman N, Shaffer M, Seehra J, Jones SS (1997) A novel cytosolic calcium-independent phospholipase A2 contains eight ankyrin motifs. J Biol Chem 272(13):8567–8575
45. Balboa MA, Balsinde J, Jones SS, Dennis EA (1997) Identity between the Ca2+-independent phospholipase A2 enzymes from P388D1 macrophages and Chinese hamster ovary cells. J Biol Chem 272(13):8576–8580
46. Larsson PK, Claesson HE, Kennedy BP (1998) Multiple splice variants of the human calcium-independent phospholipase A2 and their effect on enzyme activity. J Biol Chem 273(1):207–214
47. Ma Z, Ramanadham S, Wohltmann M, Bohrer A, Hsu FF, Turk J (2001) Studies of insulin secretory responses and of arachidonic acid incorporation into phospholipids of stably trans-

fected insulinoma cells that overexpress group VIA phospholipase A2 (iPLA2beta) indicate a signaling rather than a housekeeping role for iPLA2beta. J Biol Chem 276(16):13198–13208
48. Larsson Forsell PK, Kennedy BP, Claesson HE (1999) The human calcium-independent phospholipase A2 gene multiple enzymes with distinct properties from a single gene. Eur J Biochem 262(2):575–585
49. Akiba S, Sato T (2004) Cellular function of calcium-independent phospholipase A2. Biol Pharm Bull 27(8):1174–1178
50. Balsinde J, Balboa MA (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A2 in activated cells. Cell Signal 17(9):1052–1062
51. Bolotina VM, Csutora P (2005) CIF and other mysteries of the store-operated Ca2+-entry pathway. Trends Biochem Sci 30(7):378–387
52. Turk J, Ramanadham S (2004) The expression and function of a group VIA calcium-independent phospholipase A2 (iPLA2β) in β-cells can. J Physiol Pharmacol 82(10):824–832
53. Mancuso DJ, Jenkins CM, Gross RW (2000) The genomic organization, complete mRNA sequence, cloning, and expression of a novel human intracellular membrane-associated calcium-independent phospholipase A2. J Biol Chem 275(14):9937–9945
54. Murakami M, Masuda S, Ueda-Semmyo K, Yoda E, Kuwata H, Takanezawa Y, Kudo I (2005) Group VIB Ca2+-independent phospholipase A2γpromotes cellular membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A2. J Biol Chem 280(14):14028–14041
55. Sharma J, Eickhoff CS, Hoft DF, Ford DA, Gross RW, McHowat J (2013) The absence of myocardial calcium-independent phospholipase A2γ results in impaired prostaglandin E2 production and decreased survival in mice with acute Trypanosoma cruzi Infection. Infect Immun 81(7):2278–2287
56. Moon SH, Jenkins CM, Kiebish MA, Sims HF, Mancuso DJ, Gross RW (2012) Genetic ablation of calcium-independent phospholipase A(2)γ (iPLA(2)γ) attenuates calcium-induced opening of the mitochondrial permeability transition pore and resultant cytochrome c release. J Biol Chem 287(35):29837–29850
57. Glynn P (1999) Neuropathy target esterase. Biochem J 344:625–631
58. Glynn P (2005) Neuropathy target esterase and phospholipid deacylation. Biochim Biophys Acta 1736(2):87–93
59. Van Tienhoven M, Atkins J, Li Y, Glynn P (2002) Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. J Biol Chem 277(23):20942–20948
60. Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW (2004) Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 279(47):48968–48975
61. Ma Z, Ramanadham S, Kempe K, Chi XS, Ladenson J, Turk J (1997) Pancreatic islets express a Ca2+-independent phospholipase A2 enzyme that contains a repeated structural motif homologous to the integral membrane protein binding domain of ankyrin. J Biol Chem 272(17):11118–11127
62. Akiba S, Mizunaga S, Kume K, Hayama M, Sato T (1999) Involvement of group VI Ca2+-independent phospholipase A2 in protein kinase C-dependent arachidonic acid liberation in zymosan-stimulated macrophage-like P388D1 cells. J Biol Chem 274:19906–19912
63. Akiba S, Ohno S, Chiba M, Kume K, Hayama M, Sato T (2002) Protein kinase Cα-dependent increase in Ca2+-independent phospholipase A2 in membranes and arachidonic acid liberation in zymosan-stimulated macrophage-like P388D 1 cells. Biochem Pharmacol 63(11):1969–1977
64. Smani T, Patel T, Bolotina VM (2008) Complex regulation of store-operated Ca2+ entry pathway by PKC-ε in vascular SMCs. Am J Physiol Cell Physiol 294(6):C1499–C1508

65. Wolf MJ, Gross RW (1996) The calcium-dependent association and functional coupling of calmodulin with myocardial phospholipase A2. Implications for cardiac cycle-dependent alterations in phospholipolysis. J Biol Chem 271(35):20989–20992
66. Wolf MJ, Gross RW (1996) Expression, purification, and kinetic characterization of a recombinant 80-kDa intracellular calcium-independent phospholipase A2. J Biol Chem 271:30879–30885
67. Jenkins CM, Wolf MJ, Mancuso DJ, Gross RW (2001) Identification of the calmodulin-binding domain of recombinant calcium-independent phospholipase A2β. Implications for structure and function. J Biol Chem 276(10):7129–7135
68. Wolf MJ, Wang J, Turk J, Gross RW (1997) Depletion of intracellular calcium stores activates smooth muscle cell calcium-independent phospholipase A 2. A novel mechanism underlying arachidonic acid mobilization. J Biol Chem 272(3):1522–1526
69. Smani T, Zakharov SI, Csutora P, Leno E, Trepakova E, Bolotina VM (2004) A novel mechanism for the store-operated calcium influx pathway. Nat Cell Biol 6(2):113–120
70. Singaravelu K, Lohr C, Deitmer JW (2006) Regulation of store-operated calcium entry by calcium-independent phospholipase A2 in rat cerebellar astrocytes. J Neurosci 26(37):9579–9592
71. Balsinde J, Dennis EA (1996) Bromoenol lactone inhibits magnesium-dependent phosphatidate phosphohydrolase and blocks triacylglycerol biosynthesis in mouse P388D1 macrophages. J Biol Chem 271(50):31937–31941
72. Balboa MA, Balsinde J, Dennis EA (1998) Involvement of phosphatidate phosphohydrolase in arachidonic acid mobilization in human amnionic WISH cells. J Biol Chem 273(13):7684–7690
73. Johnson CA, Balboa MA, Balsinde J, Dennis EA (1999) Regulation of cyclooxygenase-2 expression by phosphatidate phosphohydrolase in human amnionic WISH cells. J Biol Chem 274(39):27689–27693
74. Smani T, Domínguez-Rodríguez A, Hmadcha A, Calderón-Sánchez E, Horrillo-Ledesma A, Ordóñez A (2007) Role of Ca2+-independent phospholipase A2 and store-operated pathway in urocortin-induced vasodilatation of rat coronary artery. Circ Res 101(11):1194–1203
75. Jenkins CM, Han X, Mancuso DJ, Gross RW (2002) Identification of calcium-independent phospholipase A2 (iPLA2)β, and not iPLA2γ, as the mediator of arginine vasopressin-induced arachidonic acid release in A-10 smooth muscle cells. Enantioselective mechanism-based discrimination of mammalian iPLA2s. J Biol Chem 277(36):32807–32814
76. Csutora P, Zarayskiy V, Peter K, Monje F, Smani T, Zakharov SI, Bolotina VM (2006) Activation mechanism for CRAC current and store-operated Ca2+ entry calcium influx factor and Ca2+-independent phospholipase A2β-mediated pathway. J Biol Chem 281(46):34926–34935
77. Nowatzke W, Ramanadham S, Ma Z, Hsu FF, Bohrer A, Turk J (1998) Mass spectrometric evidence that agents that cause loss of Ca2+ from intracellular compartments induce hydrolysis of arachidonic acid from pancreatic islet membrane phospholipids by a mechanism that does not require a rise in cytosolic Ca2+ concentration. Endocrinology 139(10):4073–4085
78. Smani T, Zakharov SI, Leno E, Csutora P, Trepakova ES, Bolotina VM (2003) Ca2+-independent phospholipase A2 is a novel determinant of store-operated Ca2+ entry. J Biol Chem 278(14):11909–11915
79. Bolotina VM (2008) Orai, STIM1 and iPLA2β: a view from a different perspective. J Physiol 586(13):3035–3042
80. Parekh AB, Putney JW (2005) Store-operated calcium channels. Physiol Rev 85(2):757–810
81. Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodeling. Nat Rev Mol Cell Biol 4(7):517–529
82. Rosado JA, Redondo PC, Sage SO, Pariente JA, Salido GM (2005) Store-operated Ca2+ entry: vesicle fusion or reversible trafficking and de novo conformational coupling? J Cell Physiol 205(2):262–269

83. Irvine RF (1990) 'Quanta' Ca2+ release and the control of Ca2+ entry by inositol phosphates a possible mechanism. FEBS Lett 263(1):5–9
84. Block BA, Imagawa T, Campbell KP, Franzini-Armstrong C (1988) Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. J Cell Biol 107(6):2587–2600
85. Boulay G, Brown DM, Qin N, Jiang M, Dietrich A, Zhu MX, Birnbaumer L (1999) Modulation of Ca2+ entry by polypeptides of the inositol 1,4,5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca2+ entry. Proc Natl Acad Sci 96(26):14955–14960
86. Rosado J, Sage S (2000) Coupling between inositol 1, 4, 5-trisphosphate receptors and human transient receptor potential channel 1 when intracellular Ca2+ stores are depleted. Biochem J 350(3):631–635
87. Sugawara H, Kurosaki M, Takata M, Kurosaki T (1997) Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. EMBO J 16(11):3078–3088
88. Prakriya M, Lewis RS (2001) Potentiation and inhibition of Ca2+ release-activated Ca2+ channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP3 receptors. J Physiol 536(1):3–19
89. Bakowski D, Glitsch MD, Parekh AB (2001) An examination of the secretion-like coupling model for the activation of the Ca2+ release-activated Ca2+ current ICRAC in RBL1 cells. J Physiol 532(1):55–71
90. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Rao A (2006) A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441(7090):179–185
91. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Stauderman KA (2005) STIM1, an essential and conserved component of store-operated Ca2+ channel function. J Cell Biol 169(3):435–445
92. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Cahalan MD (2005) STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. Nature 437(7060):902–905
93. Berna-Erro A, Redondo PC, Rosado JA (2012) Store-operated Ca2+ entry. Adv Exp Med Biol 740:349–382
94. Várnai P, Tóth B, Tóth DJ, Hunyady L, Balla T (2007) 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 282(40):29678–29690
95. Albarran L, Lopez JJ, Dionisio N, Smani T, Salido GM, Rosado JA (2013) Transient receptor potential ankyrin-1 (TRPA1) modulates store-operated Ca(2+) entry by regulation of STIM1-Orai1 association. Biochim Biophys Acta 1833(12):3025–3034
96. Jardin I, Lopez JJ, Salido GM, Rosado JA (2008) Orai1 mediates the interaction between STIM1 and hTRPC1 and regulates the mode of activation of hTRPC1-forming Ca2+ channels. J Biol Chem 283(37):25296–25304
97. Jardin I, Gomez L, Salido G, Rosado J (2009) Dynamic interaction of hTRPC6 with the Orai1-STIM1 complex or hTRPC3 mediates its role in capacitative or non-capacitative Ca2+ entry pathways. Biochem J 420:267–276
98. Domínguez-Rodríguez A, Díaz I, Rodríguez-Moyano M, Calderón-Sánchez E, Rosado JA, Ordóñez A, Smani T (2012) Urotensin-II signaling mechanism in rat coronary artery role of STIM1 and Orai1-dependent store operated calcium influx in vasoconstriction. Arterioscler Thromb Vasc Biol 32(5):1325–1332
99. Pandol SJ, Schoeffield-Payne MS (1990) Cyclic GMP mediates the agonist-stimulated increase in plasma membrane calcium entry in the pancreatic acinar cell. J Biol Chem 265(22):12846–12853

100. Rosado J, Graves D, Sage S (2000) Tyrosine kinases activate store-mediated Ca2+ entry in human platelets through the reorganization of the actin cytoskeleton. Biochem J 351(2):429–437
101. Bird GS, Putney JW (1993) Inhibition of thapsigargin-induced calcium entry by microinjected guanine nucleotide analogues. Evidence for the involvement of a small G-protein in capacitative calcium entry. J Biol Chem 268(29):21486–21488
102. Csutora P, Peter K, Kilic H, Park KM, Zarayskiy V, Gwozdz T, Bolotina VM (2008) Novel role for STIM1 as a trigger for calcium influx factor production. J Biol Chem 283(21):14524–14531
103. Bose DD, Rahimian R, Thomas DW (2005) Activation of ryanodine receptors induces calcium influx in a neuroblastoma cell line lacking calcium influx factor activity. Biochem J 386(2):291–296
104. Zarayskiy VV, Monje F, Peter K, Csutora P, Khodorov B, Bolotina VM (2007) Store-operated Orai1 and IP3 receptor-operated TRPC1 channel: separation of the siamese twins. Channels 1(4):246–252
105. Singaravelu K, Lohr C, Deitmer JW (2008) Calcium-independent phospholipase A2 mediates store-operated calcium entry in rat cerebellar granule cells. Cerebellum 7(3):467–481
106. Ross K, Whitaker M, Reynolds NJ (2007) Agonist-induced calcium entry correlates with STIM1 translocation. J Cell Physiol 211(3):569–576
107. Boittin FX, Petermann O, Hirn C, Mittaud P, Dorchies OM, Roulet E, Ruegg UT (2006) Ca2+-independent phospholipase A2 enhances store-operated Ca2+ entry in dystrophic skeletal muscle fibers. J Cell Sci 119(18):3733–3742
108. Martínez J, Moreno JJ (2005) Role of Ca2+-independent phospholipase A2 and cytochrome P-450 in store-operated calcium entry in 3T6 fibroblasts. Biochem Pharmacol 70(5):733–739
109. Vanden Abeele F, Lemonnier L, Thébault S, Lepage G, Parys JB, Shuba Y, Skryma R, Prevarskaya N (2004) Two types of store-operated Ca2+ channels with different activation modes and molecular origin in LNCaP human prostate cancer epithelial cells. J Biol Chem 279(29):30326–30337
110. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kinet JP (2006) CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312(5777):1220–1223
111. Trepakova ES, Csutora P, Hunton DL, Marchase RB, Cohen RA, Bolotina VM (2000) Calcium influx factor directly activates store-operated cation channels in vascular smooth muscle cells. J Biol Chem 275(34):26158–26163
112. Trepakova ES, Gericke M, Hirakawa Y, Weisbrod RM, Cohen RA, Bolotina VM (2001) Properties of a native cation channel activated by Ca2+ store depletion in vascular smooth muscle cells. J Biol Chem 276(11):7782–7790
113. Boittin FX, Shapovalov G, Hirn C, Ruegg UT (2010) Phospholipase A 2-derived lysophosphatidylcholine triggers Ca 2+ entry in dystrophic skeletal muscle fibers. Biochem Biophys Res Commun 391(1):401–406
114. Jans R, Mottram L, Johnson DL, Brown AM, Sikkink S, Ross K, Reynolds NJ (2013) Lysophosphatidic acid promotes cell migration through STIM1-and Orai1-mediated Ca2+(i) mobilization and NFAT2 activation. J Invest Dermatol 133(3):793–802
115. Vanden Abeele F, Zholos A, Bidaux G, Shuba Y, Thebault S, Beck B, Flourakis M, Panchin Y, Skryma R, Prevarskaya N (2006) Ca2+-independent phospholipase A2-dependent gating of TRPM8 by lysophospholipids. J Biol Chem 281(52):40174–40182
116. AL-Shawaf E, Tumova S, Naylor J, Majeed Y, Li J, Beech DJ (2011) GVI phospholipase A2 role in the stimulatory effect of sphingosine-1-phosphate on TRPC5 cationic channels. Cell Calcium 50(4):343–350
117. Guo Z, Su W, Ma Z, Smith GM, Gong MC (2003) Ca2+-independent phospholipase A2 is required for agonist-induced Ca2+ sensitization of contraction in vascular smooth muscle. J Biol Chem 278(3):1856–1863

118. Park KM, Trucillo M, Serban N, Cohen RA, Bolotina VM (2008) Role of iPLA2 and store-operated channels in agonist-induced Ca2+ influx and constriction in cerebral, mesenteric, and carotid arteries. Am J Physiol Heart Circ Physiol 294(3):H1183–H1187
119. Yang B, Gwozdz T, Dutko-Gwozdz J, Bolotina VM (2012) Orai1 and Ca2+-independent phospholipase A2 are required for store-operated Icat-SOC current, Ca2+ entry, and proliferation of primary vascular smooth muscle cells. Am J Physiol Cell Physiol 302(5):C748–C756
120. Schäfer C, Rymarczyk G, Ding L, Kirber MT, Bolotina VM (2012) Role of molecular determinants of store-operated Ca2+ entry (Orai1, phospholipase A2 group 6, and STIM1) in focal adhesion formation and cell migration. J Biol Chem 287(48):40745–40757
121. Reutenauer-Patte J, Boittin FX, Patthey-Vuadens O, Ruegg UT, Dorchies OM (2012) Urocortins improve dystrophic skeletal muscle structure and function through both PKA-and EPAC-dependent pathways. Am J Pathol 180(2):749–762
122. Zhu C, Sun Z, Li C, Guo R, Li L, Jin L, Li S (2014) Urocortin affects migration of hepatic cancer cell lines via differential regulation of cPLA2 and iPLA2. Cell Signal 26(5):1125–1134