Ca\(^{2+}\)-independent Phospholipase A\(_2\) Is a Novel Determinant of Store-operated Ca\(^{2+}\) Entry*

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Store-operated cation (SOC) channels and capacitative Ca\(^{2+}\) entry (CCE) play very important role in cellular function, but the mechanism of their activation remains one of the most intriguing and long lasting mysteries in the field of Ca\(^{2+}\) signaling. Here, we present the first evidence that Ca\(^{2+}\)-independent phospholipase A\(_2\) (iPLA\(_2\)) is a crucial molecular determinant in activation of SOC channels and store-operated Ca\(^{2+}\) entry pathway. Using molecular, imaging, and electrophysiological techniques, we show that directed molecular or pharmacological impairment of the functional activity of iPLA\(_2\) leads to irreversible inhibition of CCE mediated by nonselective SOC channels and by Ca\(^{2+}\)-release-activated Ca\(^{2+}\) (CRAC) channels. Transfection of vascular smooth muscle cells (SMC) with antisense, but not sense, oligonucleotides for iPLA\(_2\) impaired thapsigargin (TG)-induced activation of iPLA\(_2\) and TG-induced Ca\(^{2+}\) and Mn\(^{2+}\) influx. Identical inhibition of TG-induced Ca\(^{2+}\) and Mn\(^{2+}\) influx (but not Ca\(^{2+}\) release) was observed in SMC, human platelets, and Jurkat T-lymphocytes when functional activity of iPLA\(_2\) was inhibited by its mechanism-based suicidal substrate, bromoenol lactone (BEL). Moreover, irreversible inhibition of iPLA\(_2\) impaired TG-induced activation of single nonselective SOC channels in SMC and BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid)-induced activation of whole-cell CRAC current in rat basophilic leukemia cells. Thus, functional iPLA\(_2\) is required for activation of store-operated channels and capacitative Ca\(^{2+}\) influx in wide variety of cell types.

Activation of specific Ca\(^{2+}\)-conducting channels in plasma membrane is triggered by depletion of intracellular Ca\(^{2+}\) stores in a wide variety of cell types, but the exact molecular mechanism of such communication remains controversial (1–3). Two types of SOC channels have been described so far, but they have distinct biophysical properties, but are activated under the same conditions, by depletion of intracellular Ca\(^{2+}\) stores with agonists, inhibitors of sarco-endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and/or strong Ca\(^{2+}\) chelators. First, Ca\(^{2+}\)-release-activated Ca\(^{2+}\)-selective (CRAC)\(^{1}\) channels have been found (4) and extensively described on the level of whole-cell current in a variety of nonexcitable cells, including Jurkat T-lymphocytes and RBL cells (Refs. 5–8, and for review, see Ref. 9). Second, nonselective SOC channels of small, but resolvable 3 pS conductance has been found and described on single channel and whole-cell current levels by us (10, 11) and by others (12–18) in vascular SMC and human platelets. Despite a great physiological importance of both types of SOC channels, the molecular mechanism of their activation is still unresolved.

In reviewing Ca\(^{2+}\)-independent processes that can be activated by the depletion of intracellular Ca\(^{2+}\) stores in the presence of strong Ca\(^{2+}\) chelators, the Ca\(^{2+}\)-independent phospholipase A\(_2\) (iPLA\(_2\)) attracted our attention as it is activated under the same conditions as those known to trigger activation of SOC channels and CCE. Indeed, it has been recently shown that iPLA\(_2\) can be activated by depletion of Ca\(^{2+}\) stores (19, 20) caused by vasopressin, as well as by thapsigargin (TG, an inhibitor of SERCA (21)), both in the presence and absence of intracellular BAPTA (19). In the field of store-operated channels iPLA\(_2\) has not yet been considered as a potentially important element in signal transduction, but a significant role of iPLA\(_2\) in remodeling of cellular phospholipids (22, 23), and growing evidence of its involvement in a variety of agonist-triggered signaling cascades (see Ref. 24 for review) suggests that it may be a multifaceted enzyme, with multiple physiologically important functions in numerous cell types and tissues.

In this study we sought to determine whether iPLA\(_2\) could be involved in activation of SOC channels and CCE, and we obtained very intriguing and largely unexpected results. Here we provide the first experimental evidence that iPLA\(_2\) is a crucial molecular determinant in activation of SOC channels and capacitative Ca\(^{2+}\) influx in a variety of cell types, including SMC, platelets, Jurkat T-lymphocytes, and RBL cells.

**MATERIALS AND METHODS**

**Cells**—Primary culture SMC from mouse (mSMC) and rabbit (rSMC) aorta were prepared as described previously (10, 25). Human platelets were prepared from the platelet-rich plasma as described previously (26). Jurkat T-lymphocytes and RBL cell lines were kept in culture using standard technique.

**Electrophysiology**—Single channel currents were recorded in mSMC in cell-attached or inside-out configuration using an Axopatch 200B amplifier as described previously (10). Pipettes with tip resistance of 10–20 MΩ were coated with Sylgard. Data were digitized at 5 kHz and filtered at 1 kHz. Representative single channel current traces were additionally filtered at 500 Hz for better visual resolution of small conductance (3 pS) channels. Open channel probability (P\(_{\text{open}}\)) was analyzed.

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\(^{§}\) The abbreviations used are: CRAC, Ca\(^{2+}\)-release-activated Ca\(^{2+}\); RBL, rat basophilic leukemia; SMC, smooth muscle cells; SOC, store-operated cation; iPLA\(_2\), Ca\(^{2+}\)-independent phospholipase A\(_2\); TG, thapsigargin; TEA, tetraethylammonium; BEL, bromoenol lactone; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid tetrasodiumacetoxymethyl ester; PBS, phosphate-buffered saline; 2-APB, 2-aminoethoxydiphenyl borate; PAP, phosphatidate phosphohydrolase; CCE, capacitative Ca\(^{2+}\) entry.
lyzed and plotted over time to illustrate the time course of the activity of all 3-pS channels present in membrane patch. Single channel currents were recorded at +100 mV applied to the membrane (−100 mV applied to the pipette), and upward current deflections represent channel openings in all the representative traces. The recording time was limited by the lifetime of the patch, which usually lasted only 5−10 min. Previously we have shown (10) that native 3-pS SOC channels are the same in mSMC and rSMC. They conduct equally well Na	extsuperscript{+}, Ca	extsuperscript{2+}, and Mn	extsuperscript{2+}, so in patch clamp studies Na	extsuperscript{+} was used as charge carrier, and pipette and bath solutions had the same composition (in mM): 140 NaCl, 1 MgCl	extsubscript{2}, 10 TEA, 10 HEPES (pH 7.4). Experiments were done at 20–22 °C.

Whole-cell currents were recorded in RBL cells using standard whole-cell (dialysis) patch clamp technique. An Axopatch 200B amplifier was used; data were digitized at 5 kHz and filtered at 1 kHz. Pipettes were used with tip resistance of 2–4 MΩ. After breaking into the cell, holding potential was 0 mV, and ramp depolarizations (from −100 to +100 mV, 200 ms) were applied every 5 s. The time course of CRAC current development was analyzed at −80 mV in each cell (amplitude was expressed in pA/pF). The maximum current density (in pA/pF) at −80 mV was determined after 10 min of cell dialysis and summarized for all the cells tested (with S.E. shown in the figures). Representative IV relationships are shown during ramp depolarization after 10 min of cell dialysis. Passive leakage current with zero reversal potential (at the moment of breaking into the cell) was subtracted (it was usually higher in cells pretreated with BEL for 10 min). Intracellular (pipette) solution contained (in mM): 145 cesium glutamate, 3 MgCl	extsubscript{2}, 10 BAPTA, 5 TEA, 10 HEPES (pH 7.2). Extracellular solution was (in mM): 20 CaCl	extsubscript{2}, 1 MgCl	extsubscript{2}, 130 NaCl, 3 CsCl, 5 HEPES (pH 7.4). In some experiments BEL (20 μM) was added to the pipette solution. Experiments were done at 20–22 °C.

**Mn	extsuperscript{2+} Influx**—The rate of Mn	extsuperscript{2+} influx-induced fura-2 quenching was used to estimate divalent cation influx into SMC, as described previously (25). The rate of influx was estimated from the slope during the initial 3 mg/min. The summary data are shown without subtraction of the basal influx that was negligible (0.09 ± 0.02, n = 15).

**Molecular Studies**—Membrane proteins were extracted from cultured rSMC collected from P100 dish in Tris buffer (50 mM Tris-HCl (pH 7.5)) with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 1 mM EDTA), then sonicated four times for 10 s with 15-s breaks between cycles, spun down at 50,000 × g for 10 min at 4 °C, and the supernatant was collected for further centrifugation at 100,000 × g for 60 min at 4 °C. The pellet was re-suspended in the Tris buffer (with protease inhibitors). The amount of protein in membrane fractions was determined, and the samples were aliquoted (50 μg each), frozen, and stored at −80 °C until later use.

Western blots were done using standard methods: samples were loaded on a 7.5% SDS-polyacrylamide gel for electrophoresis. Proteins were transferred to nitrocellulose membranes in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) at 40 V overnight. The membrane was incubated in 3% PBS (PBS containing 0.35% Tween 20) for 1 h. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (anti-rabbit IgG) for 1 h in PBST with 3% milk. The signal was detected by enhanced chemiluminescence (ECL) reagent (Pierce) and exposed to X-ray film.

Polyclonal antibody directed against a unique domain in the iPLA	extsubscript{2}A isoform (Cayman Chemicals) was used to confirm protein expression in plasma membrane fraction from rSMC and mSMC. A 20-base-long antisense corresponding to the conserved nucleotides 59–78 in iPLA	extsubscript{2}A sequence was utilized (ASGVI-18; 5′-fluorescein-CTCTTACCCGGGAATGCTG-3′ (27)). As a control, the sense compliment of ASGVI-18 was used (SGV-18; 5′-fluorescein-ACCCATTCCCGGT-GAAGGG-3′). Both ASGVI-18 and SGVI-18 contained phosphorothioate linkages to limit their degradation and were labeled with fluorescein, and its presence in the cells was verified by imaging (excitation at 480 nm, emission at 515 nm) before the experiments. rSMC were transfected on days 6–7 (60–70% confluence) using Lipofectamine plus (Invitrogen) and following the standard protocols. The transfection rate was more than 70% in SMC.

**iPLA	extsubscript{2} Activity**—The activity of iPLA	extsubscript{2} was determined using the following assay. Briefly, after specific treatments (as described under “Results”) rSMC and RBL cells were collected (using rubber policeman to lift them up), sonicated, and centrifuged at 20,000 × g for 20 min at 4 °C. The supernatant was removed and kept on ice. The amount of protein was determined, and the assay was performed the same day using a modified kit originally designed for cPLA	extsubscript{2} (cPLA	extsubscript{2} assay kit, Cayman Chemicals). To detect the activity of iPLA	extsubscript{2} and not cPLA	extsubscript{2}, the phospholipase activity was assayed by incubating the samples with the substrate, arachidonylthio-PC (1-hexadecyl-2-arachidonoyl-3-deoxy-sn-glycerol-3-phosphocholine) for 1 h at 20 °C in a modified Ca	extsuperscript{2+}-free buffer of the following composition (in mM): 4 EGTA, 160 HEPES (pH 7.4), 300 NaCl, 8 Triton X-100, 60% glycerol, and 2 mg/ml of BSA. The reaction was stopped by addition of 5.5′-dithio-bis(nitrobenzoic acid) for 5 min, and the absorbance was determined at 405 nm using a standard plate reader. The activity of iPLA	extsubscript{2} was expressed in units = absorbance/mg of protein.

**Drugs and Treatments**—BEL (or HELSS) was purchased from Calbiochem and Sigma. The iPLA	extsubscript{2} polyclonal antisera was from Cayman Chemicals. The iPLA	extsubscript{2} sense and antisense oligonucleotides were from Sigma. All other drugs were from Sigma. Please, notice that inhibition of iPLA	extsubscript{2} with BEL is irreversible, requires basal activity of this enzyme, and strongly depends on temperature, time of treatment, and concentration used. When applied to the intact cells it also needs time to permeate into the cell. In most cell types the optimal conditions for BEL treatment (to ensure complete inhibition of iPLA	extsubscript{2}) are the following: intact cells need to be pretreated (in bath solution not containing BSA or serum) for 20–100 μM at 0–37 °C for 10 min. Then BSA can be washed away before the beginning of the experiments.

**Statistical Analysis**—Group data are presented as mean ± S.E. Single or paired Student’s t test was used to determine the statistical significance of the obtained data. The significance between multiple groups was evaluated using analysis of variance. The difference was considered significant at p < 0.05 and is marked by ∗ in the figures.

**RESULTS**

First, we confirmed that iPLA	extsubscript{2} is present and functionally active in aortic SMC. Fig. 1A shows that iPLA	extsubscript{2}A isoform can indeed be detected in both rabbit (rSMC) and mouse (mSMC) aortic SMC by Western blotting with a specific polyclonal antibody. Next we tested whether depletion of Ca	extsuperscript{2+} stores increases the activity of iPLA	extsubscript{2}. To effectively deplete Ca	extsuperscript{2+} stores, we used a combined BAPTA and TG treatment. rSMC were first loaded with BAPTA-AM (20 μM for 20 min, which we have previously shown to effectively buffer intracellular Ca	extsuperscript{2+} in these cells (28)), and then TG (5 μM) was applied for 10 min. After this treatment, we found the activity of iPLA	extsubscript{2} to increase 2.6-fold in comparison with basal level in untreated cells (Fig. 1B), which confirmed that depletion of Ca	extsuperscript{2+} stores causes activation of iPLA	extsubscript{2}.

To test the potential role of iPLA	extsubscript{2} in activation of store-operated channels and CCE pathway in SMC, two independent approaches (molecular and pharmacological) were used. First, rSMC were transfected with antisense or sense oligonucleotides (labeled with fluorescein) that were specifically designed for the iPLA	extsubscript{2}A isoforms (27). As expected, antisense decreased the level of the iPLA	extsubscript{2}A protein expression, which we measured after 36 h after transfection (Fig. 1D). Importantly, transfection of rSMC with antisense prevented activation of iPLA	extsubscript{2} by depletion of Ca	extsuperscript{2+} stores; TG/BAPTA treatment caused a significant increase in iPLA	extsubscript{2} activity in rSMC transfected with sense, but not antisense oligonucleotides (Fig. 1E). At the same time, transfection of rSMC with antisense, but not sense, oligonu-
cleotides for iPLA2 significantly reduced TG-induced Ca\textsuperscript{2+} influx (Fig. 2, A and B). TG-induced Mn\textsuperscript{2+} influx (Fig. 2, C and D), which is considered a more direct measure of ion channel- mediated cation influx in intact cells, was also inhibited in rSMC treated with antisense, but not sense, oligonucleotides. These results for the first time demonstrated that activation of CCE in SMC is dependent on the functional expression of iPLA2.

To obtain additional and independent evidence that can confirm the central role of iPLA2 in activation of CCE, we tested whether mechanism-based inhibition of iPLA2 enzymatic activity could mimic the effects of iPLA2 antisense transfection. BEL, a suicidal substrate for iPLA2, is widely used as an irreversible mechanism-based (time- and temperature-dependent) inhibitor with a specificity $-1,000$ times higher for iPLA2 over other PLA2 isoforms (24, 29). When applied to the intact cells for 30 min at 37–40 °C, 20–100 μM BEL is known to completely inhibit iPLA2 activity (EC\textsubscript{50} = 7 μM) (22), and it is considered to be the most specific inhibitor of iPLA\textsubscript{2}-dependent processes in a variety of cell types. In SMC, we confirmed that pretreatment with BEL (25 μM for 30 min at 37 °C followed by 20 min washing prior to the experiments) prevented activation of iPLA2 by depletion of Ca\textsuperscript{2+} stores (Fig. 1B) and used BEL as an additional tool to study the role of iPLA2 in activation of CCE. Fig. 3 shows the examples of TG-induced 2-aminoethoxy- diphenyl borate (2-APB)-sensitive Ca\textsuperscript{2+} (Fig. 3A) and Mn\textsuperscript{2+} influx (Fig. 3C) in single rSMC. Pretreatment of rSMC with BEL (25 μM for 15–30 min at 37 °C followed by a 10–30-min wash) significantly inhibited both, TG-induced Ca\textsuperscript{2+} (Fig. 3, A and B) and Mn\textsuperscript{2+} influx (Fig. 2, C and D), which was identical to the effects of rSMC transfection with iPLA2 antisense (Fig. 2, A–D). Thus, inhibition of iPLA2 with BEL mimicked the effects of SOC transfection with specific antisense to iPLA2. Identical effects of the molecular and pharmacological inhibition of iPLA2 on CCE not only established the crucial role of iPLA2 in this process, but also confirmed the use of BEL as a tool for functional inhibition of iPLA2 in our further studies of iPLA2-dependent activation of SOC channels.

The effect of BEL on capacitative Ca\textsuperscript{2+} influx was dose-dependent. Fig. 4A shows that pretreatment of rSMC with 10–25 μM BEL (30 min at 37 °C) produced significant inhibition of TG-induced Ca\textsuperscript{2+} influx. The same inhibitory effect was also observed in mSMC in which BEL (25 μM) reduced TG-induced Ca\textsuperscript{2+} influx from Δr = 2.0 ± 0.2 (n = 20) in control cells to 0.3 ± 0.1 (n = 15). It is important that the effect of BEL was irreversible and specific to Ca\textsuperscript{2+} influx. BEL did not affect TG-induced Ca\textsuperscript{2+} release from the stores (Fig. 4A), as indicated by similar increases in intracellular Ca\textsuperscript{2+} (recorded in the absence of extracellular Ca\textsuperscript{2+}) in control and BEL-treated cells. Similarly, BEL-induced inhibition of TG-induced Ca\textsuperscript{2+} influx, but not Ca\textsuperscript{2+} release, was also observed in human platelets (Fig. 4B), which are known to have store-operated cation influx that is similar to SMC (11, 26, 30, 31). To eliminate the potential role of magnesium-dependent phosphatidate phosphohydrolase (PAP-1) (32) in the effect of BEL on CCE, propranolol (50–100 μM for 30 min, which is widely used to inhibit PAP-1 (33), but not iPLA2) was tested. Propranolol did not inhibit TG-induced Ca\textsuperscript{2+} influx in both SMC (Fig. 4A) and human platelets (Fig. 4B), which confirmed that BEL-induced inhibition of CCE is mediated by iPLA2 and not PAP-1.

To confirm that the BEL-induced inhibition of Ca\textsuperscript{2+} and Mn\textsuperscript{2+} influx indeed resulted from inability of SOC channels to get activated by TG, single SMC channels were recorded in cell-attached membrane patches. Fig. 4C shows that treatment of control SMC with TG (1 μM for 10–20 min) resulted in activation of specific 3-pS nonselective cation SOC channels (described previously in detail (10)), which could be recorded in cell-attached membrane patches (in six out of seven experiments). Consistent with Ca\textsuperscript{2+} and Mn\textsuperscript{2+} influx studies, TG failed to activate single SMC channels (Fig. 4D) in SMC in which iPLA2 was inhibited with BEL (five out of five experiments). Rare (1–2/min) openings of 3-pS channels (similar to what is observed in normal resting SMC (10)) could still be detected in cell-attached membrane patches from BEL-pretreated SMC (Fig. 4D), suggesting that BEL does not affect the presence of SOC channels, but inhibits their TG-induced activation. Control experiments showed that BEL pretreatment did not affect the activity of voltage-gated Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels in SMC (data not shown), indicating specificity of BEL-induced inhibition of iPLA2 on activation of SOC channels.

To determine whether the role of iPLA2 in CCE is specific for vascular SMC in which capacitattive Ca\textsuperscript{2+} influx is known to be mediated by nonselective cation channels (10, 12–18), or may be a general phenomenon, we also tested the effects of iPLA2 inhibition by BEL in Jurkat T-lymphocytes and in RBL cells. In these cells CCE is known to be mediated by highly Ca\textsuperscript{2+}- selective CRAC channels (5–8), which have biophysical properties (9) distinct from nonselective SOC channels in SMC, but may be guided by the same store-dependent mechanism. Fig. 5, A and B, show that in Jurkat cells inhibition of iPLA2 with BEL impaired TG-induced Ca\textsuperscript{2+} influx, but did not affect Ca\textsuperscript{2+} re-
conditions as in C

induced inhibition of iPLA2 on the whole-cell CRAC current
activation of CRAC channels, we tested the effect of BEL-
/H9262
fluorescence upon 100
and then Ca2+
°
min at 37
pretreated with BEL (25
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/C
before Mn2+
experiment.

D
changes in intracellular Ca2+

lease, which was identical to SMC and human platelets (Fig. 4,
A and B).

To further confirm that iPLA2 is involved in store-dependent
activation of CRAC channels, we tested the effect of BEL-
induced inhibition of iPLA2 on the whole-cell CRAC current
that develops during cell dialysis with BAPTA in RBL cells.
Similar to SMC, pretreatment of intact RBL cells with BEL (25
µM for 30 min at 37 °C) prevented activation of iPLA2 upon
depletion of Ca2+ stores with TG (Fig. 1C). Fig. 6, A and B,
show the time courses of the development of CRAC current (at
−80 mV) and corresponding IV relationships in representative
control RBL cells and in cells pretreated with BEL (30 µM for 30
min at 37 °C). In all nine control cells tested, a typical CRAC
current developed reaching −2.00 ± 0.15 pA/pF (n = 9) after 10
min of cell dialysis with BAPTA. Contrary to control, in seven
out of nine cells pretreated with BEL, activation of CRAC
channels was dramatically impaired, and CRAC current was
hardly distinguishable (−0.32 ± 0.14 pA/pF, n = 7). We also
tested the effects of acute intracellular application of BEL on
the whole-cell CRAC current during cell dialysis (by including
BEL into the patch pipette). In these experiments we could not
expect to achieve complete inhibition of iPLA2 in all the cells
tested. Indeed, BEL is known to be a mechanism-based inhibi-
tor that is effective at physiological temperature (37–40 °C)
when iPLA2 is normally functional. At room temperature (20–
22 °C, when enzymatic activity of iPLA2 is significantly slow),
higher concentrations and longer treatment could be required for the mechanism based suicidal substrates to effectively inhibit the enzyme activity. Thus, during patch clamp experiments, when BEL was included in the patch pipette and applied inside the cell during its dialysis, we could not expect reliable inhibition of iPLA2 in all the cells during a short 10–15-min recording of CRAC current at room temperature (20–22 °C), which were the time and temperature limitations in our patch clamp experiments. Under such nonoptimal conditions (low temperature and short time) one could expect that the effect of BEL may vary from cell to cell, ranging from full inhibition of CRAC current to no effect. The results of the experiments (Fig. 6, C and D) confirmed these expectations. After 10 min of cell dialysis with BAPTA-containing solution, CRAC current (at −80 mV) developed in all 14 control cells tested, and on average its density was −2.4 ± 0.3 pA/pF (n = 14). When BEL (20 μM) was present in the pipette, the total of 12 cells tested could be divided into three groups: (a) in four cells BEL completely prevented the development of CRAC current, and after 10 min of cell dialysis the inward current was negligible (−0.2 ± 0.1 pA/pF), (b) in four cells CRAC current developed, but was significantly smaller than control (−1.1 ± 0.3 pA/pF), and (c) in four other cells maximum CRAC current density was about the same (or even higher) as in control (∼2.8 ± 0.6 pA/pF). Fig. 6, C and D, show and compare the time course of development and IV relationships of the CRAC current in control cells and in three (a–c) groups of RBL cells acutely treated with BEL. These results confirmed that functional iPLA2 is indeed required for activation of CRAC channels in RBL cells.

Thus, inhibition of expression or functional activity of iPLA2 leads to the impairment of store-dependent activation of SOC channels and capacitative Ca2+ influx in a variety of cell types.

Discussion

These studies demonstrate that iPLA2 is a crucial molecular determinant in activation of store-operated channels and capacitative Ca2+ influx in different cell types. This result is somewhat unexpected, because iPLA2 has never before been considered as an important component of store-operated Ca2+...
influx pathway in any of the models proposed so far (see Refs. 34 and 35 for recent review of the existing models).

Our results provide strong evidence that activation of capacitative Ca\(^{2+}\) influx mediated by two types of SOC channels (CRAC in RBL cells and Jurkat T-lymphocytes and nonselective SOC in SMC and platelets) require the presence and functional activity of iPLA\(_2\). Molecular (antisense) and functional (BEL) inhibition of iPLA\(_2\) produced identical results: in both cases, TG-induced Ca\(^{2+}\) influx was dramatically and irreversibly impaired, while Ca\(^{2+}\) release from the stores was not affected. Functional inhibition of iPLA\(_2\) with BEL also prevented activation of single 3-pS SOC channels and whole-cell CRAC currents upon TG and/or BEL-induced depletion of intracellular stores, which provided a convenient pharmacological tool for future studies of the mechanism of iPLA\(_2\) involvement in CCE.

Although independent lines of evidence presented here show the crucial role of iPLA\(_2\) in the activation of SOC channels and capacitative Ca\(^{2+}\) influx, the exact location of iPLA\(_2\) in this pathway, and the molecular mechanism of iPLA\(_2\)-dependent signal transduction, remain to be determined. It is not clear whether iPLA\(_2\) is involved in originating the signal from endoplasmic reticulum upon depletion of Ca\(^{2+}\) stores or if it is localized in the plasma membrane and is involved in accepting the signal and/or mediating signal transduction to the SOC channels. In view of the fact that iPLA\(_2\) was detected in plasma membrane fraction of SMC (Fig. 1, A and C), it can be speculated that it may be co-localized with SOC channels in plasma membrane. Our recent studies showed activation of single SOC channels in excised membrane patches by the calcium influx factor (CIF) (36) produced upon depletion of Ca\(^{2+}\) stores, and we would like to speculate that iPLA\(_2\) may be directly involved in membrane-delimited activation of SOC channels by CIF. Single SOC channels in excised membrane patches from SMC (10) could be an excellent experimental model to address this possibility.

Arachidonic acid, a main product of all types of PLA\(_2\), including iPLA\(_2\), does not seem to be involved in direct activation of SOC channels and CCE (37, 38). It is now well established that arachidonic acid has its own specific target, so called ARC channel (39), which may be responsible for a part of agonist-induced Ca\(^{2+}\) influx in some cells. Contrary to SOC channels it is not regulated by intracellular Ca\(^{2+}\) stores and has biophysical, pharmacological, and functional properties that are significantly different from CRAC and nonselective SOC channels (38, 40). Alternatively, the products of arachidonic acid degradation may play some role in the store-operated pathway. Recently, it has been shown that inhibition of the lipoxygenase (but not cyclo-oxygenase) family of enzymes reduces CRAC current in RBL cells (41), but the exact mechanism of such effect is not clear. Little is presently known of the potential role of other products of iPLA\(_2\). Thus, it remains unclear which products of iPLA\(_2\) could be involved in regulation of CCE and SOC channels.

The importance of iPLA\(_2\) in cellular function is not limited to its housekeeping role in phospholipid remodeling (maintenance of membrane integrity) that involves generation of lysophospholipid acceptors for incorporation of arachidonic acid into phospholipids. Other important signaling functions of iPLA\(_2\) have also been suggested (see Refs. 24 and 42 for the most recent review), including its role in agonist-induced stimulation of smooth muscle (43) and endothelial cells (44, 45), in lymphocyte proliferation (46), and in endothelium-dependent vascular relaxation (44). We believe that introduction of iPLA\(_2\) as a novel determinant in the store-operated Ca\(^{2+}\) influx pathway may open many new directions for studying physiological and pathological functions in excitable and nonexcitable cells. It may also shed some new light on the still mysterious mechanism of activation of store-operated channels.

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