Calcium Influx Factor Directly Activates Store-operated Cation Channels in Vascular Smooth Muscle Cells*

Elena S. Trepakova‡, Peter Csutora§, Dacia L. Hunton§, Richard B. Marchase§, Richard A. Cohen‡, and Victoria M. Bolotina‡‡

From the ‡Vascular Biology Unit, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02118 and the §Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Recently, we described a novel 3-pS Ca\(^{2+}\)-conducting channel that is activated by BAPTA and thapsigargin-induced passive depletion of intracellular Ca\(^{2+}\) stores and likely to be a native store-operated channel in vascular smooth muscle cells (SMC). Neither Ca\(^{2+}\) nor inositol 1,4,5-trisphosphate or other second messengers tested activated this channel in membrane patches excised from resting SMC. Here we report that these 3-pS channels are activated in inside-out membrane patches from SMC immediately upon application of Ca\(^{2+}\) influx factor (CIF) extracted from mutant yeast, which has been previously shown to activate Ca\(^{2+}\) influx in *Xenopus* oocytes and Ca\(^{2+}\) release-activated Ca\(^{2+}\) current in Jurkat cells. In bioassay experiments depletion of Ca\(^{2+}\) stores in permeabilized human platelets resulted in the release of endogenous factor, which activated 3-pS channels in isolated inside-out membrane patches excised from SMC and exposed to permeabilized platelets. The same 3-pS channels in excised membrane patches were also activated by acid extracts of CIF derived from human platelets with depleted Ca\(^{2+}\) stores, which also stimulated Ca\(^{2+}\) influx upon injection into *Xenopus* oocytes. Specific high pressure liquid chromatography fractions of platelet extracts were found to have CIF activity when injected into oocytes and activate 3-pS channels in excised membrane patches. These data show for the first time that CIF produced by mammalian cells and yeast with depleted Ca\(^{2+}\) stores directly activates native 3-pS cation channels, which in intact SMC are activated by Ca\(^{2+}\) store depletion.

Depletion of intracellular Ca\(^{2+}\) stores activates store-operated (capacitative) Ca\(^{2+}\) influx in a variety of nonexcitable cells (1–5). However, the nature of the store-operated channels (SOC)\(^1\) in different cells as well as the mechanism of their activation remain obscure. Two major types of SOC have been described so far: native Ca\(^{2+}\) release-activated Ca\(^{2+}\) selective (CRAC) channels found in some nonexcitable cells (6, 7) and certain members of the TRP channel family (8–10). Recently, we found a novel Ca\(^{2+}\)-conducting channel that is likely to be a native store-operated channel responsible for capacitative Ca\(^{2+}\) influx and contraction in vascular smooth muscle cells (SMC).\(^2\) Poor cation selectivity of this 3-pS channel distinguishes it from the CRAC channel (6, 7), and although it resembles some channels from the TRP family, none of them described so far has such low single channel conductance (8–10). These 3-pS nonelective cation channels rarely opened in resting SMC but were activated upon depletion of intracellular Ca\(^{2+}\) stores induced by either chelation of intracellular Ca\(^{2+}\) with BAPTA or by thapsigargin (TG)-induced inhibition of sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA). Neither excision of membrane patches from resting (untreated) SMC nor a variety of second messengers (including Ca\(^{2+}\), InsP\(_3\), InsP\(_4\), GTP\(_S\), cAMP, cGMP, ATP, and ADP) activated these channels in inside-out membrane patches.

These native 3-pS channels provide a powerful new tool to address the mechanism of activation of these channels in SMC and specifically the link to depletion of intracellular Ca\(^{2+}\) stores. In previous work, partially purified extracts of calcium influx factor (CIF) from *pmr1* yeast, which are genetically deficient in SERCA (and therefore depleted in organelar Ca\(^{2+}\)), were shown to evoke Ca\(^{2+}\) influx when injected into *Xenopus* oocytes and to activate I\(_{\text{CRAC}}\) when introduced via a patch pipette into Jurkat cells (12). Here we tested if the 3-pS channels from SMC could be activated directly by this putative CIF (12–15) or if conformational coupling with InsP\(_3\) receptors in the stores is required for their activation (16–18). We also addressed the question of whether or not the channel is already present in the plasma membrane of resting SMC or if the channel is delivered and incorporated into the membrane only upon depletion of the stores (19, 20).

We show that the 3-pS channels are immediately activated in inside-out membrane patches by the putative CIF extracted from either SERCA-deficient *pmr1* yeast or from human platelets with depleted Ca\(^{2+}\) stores. This is the first evidence of direct activation of native store-operated channels in inside-out membrane patches by a putative CIF that was chemically extracted or obtained in bioassay from different cell types following depletion of their Ca\(^{2+}\) stores.

\(^*\) This work was supported by National Institutes of Health Grants HL54150, HL07224, HL55993, HL07918, and DK55647 and the Juvenile Diabetes Foundation International. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^‡\) To whom correspondence should be addressed: Vascular Biology Unit, Boston University School of Medicine, 650 Albany St., X-704, Boston, MA 02118. Tel.: 617-638-7118; Fax: 617-638-7113; E-mail: vbolotina@med-medi1.bu.edu.

\(^‡‡\) The abbreviations used are: SOC, store-operated channels; SMC, smooth muscle cells; TG, thapsigargin; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase; GTP\(_S\), guanosine 5′-O-(thiotriphosphate); CIF, Ca\(^{2+}\) influx factor; BAPTA, 1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CRAC, Ca\(^{2+}\) release-activated Ca\(^{2+}\) channel; TRP, transient receptor potential; InsP\(_3\), inositol 1,4,5-trisphosphate; InsP\(_4\), inositol 1,3,4,5-tetrakisphosphate; pSi, psicosiements; HPLC, high pressure liquid chromatography.

\(^1\) The abbreviations used are: SOC, store-operated channels; SMC, smooth muscle cells; TG, thapsigargin; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase; GTP\(_S\), guanosine 5′-O-(thiotriphosphate); CIF, Ca\(^{2+}\) influx factor; BAPTA, 1,2-bis-(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CRAC, Ca\(^{2+}\) release-activated Ca\(^{2+}\) channel; TRP, transient receptor potential; InsP\(_3\), inositol 1,4,5-trisphosphate; InsP\(_4\), inositol 1,3,4,5-tetrakisphosphate; pSi, psicosiements; HPLC, high pressure liquid chromatography.

\(^2\) E. S. Trepakova, M. Gericke, R. M. Weisbrod, R. A. Cohen & V. M. Bolotina, submitted for publication.
Experiment 1. CIF from pmr1 yeast activates single channel currents in inside-out patches from SMC. a, the open probability (NP) of single channels in an inside-out membrane patch from SMC before and after application of CIF (open bar). Below, part of the original trace of single channel outward current (at +100 mV applied to the inside of membrane patch) is shown. The closed state of the channel is marked by 0, and open states are labeled as 1, 2, 3, and 4 at the beginning of the original trace. b, amplitude histogram (from the event list) of a single channel current after CIF application (from experiment shown in a). The numbers above each peak represent the amplitude of the corresponding current level. c, example of single channel currents activated by CIF in an inside-out membrane patch (under symmetrical 140 mM NaCl conditions) recorded at indicated potentials applied to the inside of the membrane. The closed state of the channel is shown by 0, and the open state is shown as 1 at the beginning of the original traces. Upward deflections correspond to outward and downward deflections to inward single channel current. The panels on the right represent all-points histograms calculated for each trace. d, voltage dependence of the open channel probability (NP) in inside-out membrane patches from TG-activated mouse SMC. Summary data from eight experiments. e, current-voltage relationship for the CIF-activated single channel current. Standard bath solution contained 140 mM NaCl and no Ca<sup>2+</sup>. Pipette solutions contained: 140 mM NaCl (●), 140 mM NaCl, 1 mM CaCl<sub>2</sub> (○), 140 mM NaCl, 10 mM CaCl<sub>2</sub> (▲). Each point is an average of three to seven experiments.

Experimental Procedures

SMC Preparation—SMC were isolated from mouse thoracic aorta as described previously<sup>2</sup> using collagenase (4 mg/ml), elastase (2 mg/ml), and trypsin inhibitor (2 mg/ml), (1-h treatment at 37°C), placed on coverslips in 1% Dulbecco modified Eagle medium (Life Technologies, Inc.) supplied with 1% fetal bovine serum (Sigma), and kept at 37°C in 5% CO<sub>2</sub> for 2–5 days. Under these conditions, SMC attached to the coverslips and spread slightly attaining a spindle-like shape but did not divide. These SMC stained positively for α-actin.

Electrophysiology—Single channel currents were recorded in inside-out membrane patches from resting (unstimulated) SMC as recently described<sup>21</sup> using a low noise Axopatch 200B amplifier (Axon Instruments). To improve the signal-to-noise ratio, pipettes were coated with Sylgard (Dow Corning Corp.) and polished to a resistance of 10–20 MΩ (when filled with high sodium pipette solution). pCLAMP 6 software (Axon Instruments) was used for data acquisition and analysis. Data were filtered at 1 KHz and stored for later analysis. Representative traces of single channel currents were later additionally filtered at 100–200 Hz for better visual resolution of a 3-pS single channel on the figures. Liquid junction potential was corrected. Experiments were conducted at 20–22°C.

The open channel probability (NP) was analyzed and plotted over time to illustrate the time-course of channel activity. The amplitude of single channel currents was analyzed using all-point histograms or amplitude histograms obtained from the event list, because both methods gave the same single channel current amplitude in our experiments as was described before.<sup>2</sup>

Standard bath solution contained: 140 mM NaCl, 2.8 mM KCl, 2 mM MgCl<sub>2</sub>, 5.5 mM glucose, 10 mM HEPES (pH 7.4). Pipette solution contained: 140 mM NaCl, 10 mM tetraethylammonium chloride, 0.2 mM EGTA (pH 7.4). In some experiments Ca<sup>2+</sup> (1 or 10 mM) was added to the pipette or bath solutions (instead of EGTA). The pipette solutions contained 100 μM niflumic acid and 100 nM iberiotoxin to suppress Ca<sup>2+</sup>-dependent Cl<sup>−</sup> and K<sup>+</sup> currents, respectively. In control experiments neither of these inhibitors affected 3-pS channels or TG-induced Ca<sup>2+</sup> influx in SMC.<sup>2</sup>

CIF Preparations—Acid extracts from wild type and pmr1 mutant yeast were prepared as described previously<sup>12</sup>. Human platelets were obtained from the regional Red Cross following 5–7 days of storage. Unstimulated platelets were kept at room temperature. The Ca<sup>2+</sup> stores in platelets were depleted by either TG (2 μM, 20 min), overnight hypothermic treatment (4°C), or by a combination of both stimuli. Acid extracts from platelets were diluted 1:30 and applied at a final concentration corresponding to about 1.7 × 10<sup>5</sup> platelets/ml. Anion exchange HPLC purification of the crude extracts was performed using a Keystone Scientific Partisil 10 SAX column eluted with a linear salt and pH gradient from 5 to 750 mM (NH₄)H₂PO₄, and from pH 2.8 to 3.7, respectively.

Xenopus Oocyte Assay—CIF activities were assayed by microinjection of extracts into fura-2 loaded albino Xenopus laevis oocytes as described<sup>12</sup>. Changes in intracellular Ca<sup>2+</sup> were measured on an Olympus IX70 inverted microscope through a 10x UplanAPO objective, NA = 0.17, equipped with a rapid excitation filter changer alternating between 340 and 380 nm (Ludl Electronics) and a CCD camera (Sensys, Photometrics). Changes in fluorescence were analyzed in a 600 × 400 μm area of the oocyte close to the injection site. Data are expressed as the 340/380 nm ratio.

Bioassay of CIF from Permeabilized Platelets—Human platelets were concentrated from platelet-rich plasma and filtered through Sepharase-2B gel into regular HEPES buffer: 137 mM NaCl, 2.7 mM...
KCl, 1 mM MgCl₂, 3.3 mM NaH₂PO₄, 5.5 mM glucose, 3.8 mM HEPES (pH 7.4), 0.1 mM aspirin, 6 milliunits/ml apyrase. Filtered platelets were transferred into an intracellular solution containing: 110 mM KCl, 10 mM NaCl, 1 mM KH₂PO₄, 5 mM KH₂CO₃, 20 mM HEPES (pH 7.1), 2 mM Mg-ATP, ATP-regenerating system (creatine phosphate 5 mM creatine phosphokinase 15 units/ml) and permeabilized with 50 μg/ml saponin for 10 min, washed, and resuspended in saponin-free intracellular solution. Within the next 30 min the suspension of permeabilized platelets was added to the chamber (at a concentration of ~1.75 × 10⁹ platelets/ml) containing an inside-out membrane patch excised from SMC. Before application to the membrane patches, each preparation of permeabilized platelets was tested for TG (1 μM)-induced emptying of their stores which was monitored as described earlier (22).

Platelet Lysate—Approximately 10¹¹ platelets (filtered as described above) were exposed to +4 °C for 12 h, then concentrated into 3 ml of standard bath solution used for patch-clamp (see above), and sonicated in a Branson sonifier at 40 watts, 4 times for 5 s (at +4 °C). Lysate was centrifuged at +4 °C for 25 min at 20,000 × g. The supernatant was collected and used during the day. It was applied to inside-out membrane patches at a concentration corresponding to ~2.5 × 10⁷ cells/ml.

Drugs—All the drugs were from Sigma.

Statistics—The data are presented as mean ± S.E. with n showing the number of experiments in the text. Statistical significance was assessed using the t-test and ANOVA (analysis of variance). Values of p < 0.05 were considered to be significant.

RESULTS
To assess the possible mechanism of activation of the 3-pS nonselective cation channels, the inside-out membrane patches with little or no single channel activity were excised from resting SMC. Application of CIF extracts from genetically modified (SERCA-deficient) pmr1 yeast (12) at a final concentration corresponding to ~4.5 × 10⁷ cells/ml to the cytoplasmic side of membrane patch immediately activated 3-pS channels in 16 of 18 experiments (Fig. 1). Although the single channel currents were very small (about 0.3 pA at 100 mV), they could be clearly resolved (Fig. 1a). The histograms of the current amplitude (Fig. 1b) revealed the presence of several (two to five) channels with the same current amplitude in each membrane patch. Fig. 1c shows an example of CIF-activated single channel currents in an inside-out membrane patch recorded at different membrane potentials with all-point histograms showing single channel current amplitudes. CIF-activated channels had the same electrophysiological characteristics as those previously described for the native 3-pS channel activated by BAPTA and TG in intact SMC.² The I/V relationship of the CIF-activated channel (Fig. 1d) was linear with a slope corresponding to ~3.3 ± 0.4 pS (n = 7) in the absence of extracellular Ca²⁺, and 3.2 ± 0.2 pS (n = 6) and 3.1 ± 0.4 pS (n = 3) in the presence of 1 and 10 mM of Ca²⁺, respectively. The dependence on membrane potential of the Nₚ₀ of CIF-activated channels, which did not change at negative, but significantly increased at high positive membrane potentials (Fig. 1d), was identical to that of native store-operated 3-pS channels.² Thus, CIF extracts activated 3-pS channels with the properties indistinguishable from the channels activated by TG and/or BAPTA in intact SMC. In contrast, similar extracts from wild-type yeast, which do not produce CIF (12), did not activate 3-pS channels in inside-out membrane patches (in four out of four experiments).

We also sought a relatively abundant mammalian source for CIF preparations. In recent publications we described capacitative Ca²⁺ influx in human platelets and showed its SERCA-dependent regulation by nitric oxide (22). Because of these results and the availability of relatively large quantities of platelets, we initiated a series of experiments to determine if a CIF similar to that derived from yeast (12) could also be produced by human platelets and if it also activates 3-pS channels in excised membrane patches from SMC.

The first series of experiments was designed to bioassay CIF produced by human platelets during depletion of their Ca²⁺ stores. The cytoplasmic side of an inside-out membrane patch from SMC was exposed to suspension of permeabilized platelets (Fig. 2a), and the activity of the 3-pS channel was monitored before and during TG-induced depletion of the platelets’ Ca²⁺ stores. Fig. 2b shows that the addition of CIF to permeabilized platelets activated 3-pS channels in the isolated patch (in five of six experiments), and at the peak of activity Nₚ₀ reached 0.23 ± 0.06 (at ~100 mV) and 0.011 ± 0.004 after addition of platelets. However, the subsequent addition of TG to permeabilized platelets activated 3-pS channels in the isolated patch (in five of six experiments), and at the peak of activity Nₚ₀ reached 0.23 ± 0.06 (at ~100 mV, n = 5). The time course and peak activity of the channels in this bioassay system were similar to the time course of TG-induced channel activation in cell-attached patches in intact SMC.² The channel could be blocked by a high concentration of La³⁺ (2 mM) applied from the cytoplasmic side (n = 5, Fig. 2b). The addition of TG to inside-out patches in the absence of platelets

Fig. 2. CIF released from permeabilized platelets directly activates single 3-pS channels in an inside-out membrane patch from SMC. a, schematic illustration of the experiment shown in b (see description under “Experimental Procedures.”) b, the plot of open channel probability (NP₀) in an inside-out membrane patch excised from the resting SMC during the course of the experiment (at ~100 mV applied to the inside of membrane). Suspension of permeabilized platelets (at a final concentration of ~1.75 × 10⁹ cells/ml) was applied to the cytoplasmic side of a membrane patch followed by TG (2 μM) and La³⁺ (2 mM) as indicated by the vertical arrows. Traces below the graph show actual single channel current recordings (at an expanded time scale) before and 4 min after application of TG. The closed state of the channel is shown by 0, and the open state is shown as 1 at the beginning of the original traces. Downward deflections correspond to an inward single channel current. The panel on the right represents an all-points current histogram at the peak of channel activity c, the plot of NP₀ in inside-out membrane patch (at 100 mV) before and after application of a lysate of cold-activated platelets (equivalent to ~2.5 × 10⁷ cells/ml). Traces below the graph show actual single channel current recordings (at an expanded time scale). The closed state of the channel is shown by 0, and open states are shown as 1 and 2 at the beginning of the trace. Upward deflections correspond to outward single channel currents. The panel on the right represents all-points current histogram at the peak of channel activity.
did not produce activation of 3-pS channels (n = 12). Thus, it appears that upon depletion of Ca\(^{2+}\) stores, a factor is produced and released from the platelets through their permeabilized plasma membrane that is capable of activating 3-pS channels in isolated membrane patches.

In another series of experiments, to avoid the use of TG, platelets were activated by exposure to low temperature, and the lysates prepared from the platelets were applied to inside-out membrane patches from SMC. Application of the lysate at concentration equivalent to ~1.7 × 10\(^8\) cells/ml to inside-out membrane patches immediately activated 3-pS channels in five of seven membrane patches from resting SMC. Fig. 3c shows that partially purified acid extracts from activated platelets utilized in Fig. 3 were subjected to anion exchange HPLC. The absorbance at 262 nm of the column effluent is shown in Fig. 4a. Initially, pooled fractions were tested for activity in the oocyte assay, and eventually two half-minute fractions (no. 49 and 50 shown in the inset in Fig. 4a) were found to activate Ca\(^{2+}\) influx in oocytes (Fig. 4b). Fraction no. 51 was found to have no activity in the oocyte assay (Fig. 4b). When these same fractions were applied to

![Image](http://www.jbc.org/Downloadedfrom)

**FIG. 3.** CIF extracts purified from activated platelets activate Ca\(^{2+}\) influx in Xenopus oocytes and single channel currents in inside-out membrane patches from SMC. a, pseudo-colored ratiometric images of an albino Xenopus oocyte preloaded with fura-2 following injection at t = 0 of 14 nl of crude acid extract prepared from cold- and TG-treated human platelets. Starting concentration of crude extract corresponded to ~5 × 10\(^8\) platelets/ml. Increases in the 340/380 nm ratio relative to those at the time of injection are shown. External Ca\(^{2+}\) was 5 mM. The asterisk in the first frame denotes the point of injection. Data are representative of 10 experiments with independent preparations. b, changes in intracellular Ca\(^{2+}\) (shown as fura-2 340/380 nm fluorescence ratio) in Xenopus oocytes following injection of extracts prepared from platelets: trace 1, treated with 2 µM TG for 20 min; trace 2, exposed to cold overnight; traces 3 and 4, exposed to cold and then treated with TG; trace 5, left untreated and kept at room temperature. All the traces except trace 4 were recorded in the presence of 5 mM Ca\(^{2+}\) in the bath. c, the plot of NP\(_{\text{out}}\), in inside-out membrane patch (at −100 mV) before and after application of the same acid extracts (at final concentrations corresponding to ~1.7 × 10\(^8\) cells/ml) from nontreated resting platelets (no. 5) and from platelets treated with cold and TG (2 µM for 20 min) (no. 1) as those described in 6. All-point current histogram at the peak of channel activity is shown on the right.
inside-out membrane patches isolated from SMC, fraction no. 51 caused no activation of 3-pS channels (Fig. 4c). However, the subsequent addition of fraction no. 50 immediately activated 3-pS channels, and their activity was so high that up to three single channel current levels could be seen even at negative membrane potentials (Fig. 4, c and d, in five of six experiments).

**DISCUSSION**

This study demonstrates for the first time that CIF from mammalian and yeast cells with depleted Ca\(^{2+}\) stores directly activates native 3-pS cation channels, which in intact SMC are activated by Ca\(^{2+}\) store depletion. The results presented here allow us to address several important issues related to the mechanism of store-operated Ca\(^{2+}\) influx in vascular SMC and possibly in other cell types.

Strong evidence was obtained that finalized the proof that the 3-pS channel in vascular SMC is a native store-operated channel. Indeed, this Ca\(^{2+}\)-conducting channel is activated in intact SMC following depletion of their stores (achieved by intracellular Ca\(^{2+}\) chelation with BAPTA or TG-induced inhibition of SERCA-dependent Ca\(^{2+}\) uptake).\(^2\) Demonstration of the direct activation of 3-pS channels by CIF produced upon depletion of intracellular Ca\(^{2+}\) stores (but not other known second messengers) establishes a store-dependent mechanism for 3-pS channel activation in SMC.

Our experiments also directly demonstrate that a native SOC is constitutively present in plasma membrane of SMC. For its activation it does not need to be delivered to or incorporated into the plasma membranes following depletion of the stores, as was previously proposed (19, 20). Indeed, 3-pS channels were inactive in silent membrane patches isolated from resting SMC, but up to five channels were immediately activated when CIF was applied to the inside of the membrane patches. Interestingly, after activation in intact SMC, 3-pS channels remain active even when excised from SMC, implying that activation of the channels results in a stable modification rather than in an easily reversible interaction that is common to a variety of second messengers. Excision from the cell may also eliminate the mechanisms responsible for channel deactivation. Similarly persistent activity has been recently reported for a store-operated current (I\(_{\text{SOC}}\)) in macro patches excised from *Xenopus* oocytes (19).

Several attractive models for store-dependent regulation of plasma membrane channels have been proposed and supported by strong experimental evidence in nonexcitable cells (for recent reviews see Refs. 4, 5, and 24), although all models still face unresolved questions and the mechanism of SOC regulation remains obscure.

Our study for the first time provided solid evidence for a CIF-mediated mechanism of store-operated Ca\(^{2+}\) entry at the level of single store-operated channels activated by CIF in isolated membrane patches. CIF with identical biological activities was found to be produced upon store depletion in both yeast and human platelets. Importantly, CIF activates single SOC in isolated membrane patches independently of whether it is released by permeabilized platelets during store depletion or chemically extracted from platelets after store depletion. The results with permeabilized platelets dismiss the concern about the possible induction of nonphysiological activity as a result of chemical extraction. They also clearly show, at least for the native SOC from SMC, that the direct physical coupling of this SOC to intracellular Ca\(^{2+}\) stores during their depletion is not required for channel activation. The question that remains open is whether the mechanism of SOC regulation is the same for different store-operated channels and different cell types.

Strong arguments against a CIF-based model of SOC activation have been previously raised by some experimental data that were apparently inconsistent with a freely diffusible and long lived messenger traveling from Ca\(^{2+}\) stores to the plasma membranes (19, 25, 26). Other recent data have provided compelling evidence for the importance of secretory processes and vesicle docking and/or membrane fusion in the activation of store-operated Ca\(^{2+}\) influx (19, 27). In addition, direct interaction between the InsP\(_{3}\) receptor and the TRP3 channel has been described (11, 16–18) and calls into question the role of a diffusible CIF in activation of store-operated channels (24). Although multiple mechanisms of activation of Ca\(^{2+}\) influx in different cell types are possible, it remains attractive to con-
CIF Directly Activates Store-operated Channels

1. Putney, J. W., Jr. (1986) *Cell Calcium* 7, 1–12
2. Berridge, M. J. (1985) *Biochem. J.* 232, 1–11
3. Clapham, D. E. (1993) *Cell* 80, 259–268
4. Parekh, A. B. & Penner, R. (1997) *Physiol. Rev.* 77, 901–930
5. Barritt, G. J. (1999) *Biochem. J.* 337, 153–169
6. Kershaw, H. H. & Cahalan, M. D. (1999) *Science* 283, 836–839
7. Hoth, M., Faselato, C. & Penner, R. (1999) *Ann. N. Y. Acad. Sci.* 707, 198–209
8. Friel, D. D. (1996) *Cell* 85, 617–619
9. Montell, C. (1997) *Mol. Pharmacol.* 52, 755–763
10. Zhu, X. & Birnbaumer, L. (1998) *Neurosci. Lett.* 13, 211–217
11. Boulay, G., Brown, D. M., Qin, N., Jiang, M., Dietrich, A., Zhu, M. X., Chen, Z., Birnbaumer, M., Mikoshiba, K. & Birnbaumer, L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 14555–14560
12. Cautera, P., Su, Z., Kim, H. Y., Bagrim, A., Cunningham, K. W., Nuccitelli, R., Keizer, J. E., Hanley, M. R., Blasuck, J. E. & Marchase, R. B. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 121–126
13. Randriamampita, C. & Tsien, R. Y. (1993) *Nature* 364, 809–814
14. Parekh, A. B., Terlau, H. & Stuhmer, W. (1993) *Nature* 364, 814–818
15. Kim, H. Y., Thomas, D. & Hanley, M. R. (1995) *J. Biol. Chem.* 270, 9706–9708
16. Putney, J. W., Jr. (1997) *Cell Calcium* 21, 257–261
17. Kiselyov, K., Xu, X., Mozhayeva, G. N., Kuo, T., Pessah, I., Mignery, G. A., Zhu, X., Birnbaumer, L. & Muallem, S. (1998) *Nature* 396, 478–482
18. Kiselyov, K., Mignery, G. A., Zhu, M. X. & Muallem, S. (1999) *Mol. Cell* 4, 423–429
19. Yao, Y., Ferrer-Montiel, A. V., Montal, M. & Tsien, R. Y. (1999) *Cell* 98, 475–485
20. Somasundaram, B., Norman, J. C. & Mahaut-Smith, M. P. (1995) *Biochem. J.* 309, 725–729
21. Hirakawa, Y., Gericke, M., Cohen, R. A. & Bolotina, V. M. (1999) *Am. J. Physiol.* 277, H1732–H1744
22. Trepakova, E. S., Cohen, R. A. & Bolotina, V. M. (1999) *Circ. Res.* 84, 201–209
23. Oliver, A. E., Tablin, F., Walker, N. J. & Crowe, J. H. (1999) *Biochem. Biophys. Acta* 1416, 349–360
24. Putney, J. W., Jr. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 14668–14671
25. Petersen, C. C. H. & Berridge, M. J. (1996) *Pflugers Arch.* 432, 286–292
26. Jacomi, M., Pyle, J., Bortolon, R., Ou, J. & Clapham, D. E. (1997) *Curr. Biol.* 7, 599–602
27. Patterson, R. L., van Rossum, D. B. & Gill, D. L. (1999) *Cell* 98, 487–499

Acknowledgment—We thank Dr. I. Medina for critical reading of the manuscript.
Calcium Influx Factor Directly Activates Store-operated Cation Channels in Vascular Smooth Muscle Cells
Elena S. Trepakova, Peter Csutora, Dacia L. Hunton, Richard B. Marchase, Richard A. Cohen and Victoria M. Bolotina

J. Biol. Chem. 2000, 275:26158-26163.
doi: 10.1074/jbc.M004666200 originally published online June 12, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M004666200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 27 references, 10 of which can be accessed free at http://www.jbc.org/content/275/34/26158.full.html#ref-list-1