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Store depletion triggers the calcium release-activated calcium current (I_{CRAC}) in macrovascular endothelial cells: a comparison with Jurkat and embryonic kidney cell lines

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Abstract In endothelial cells, different types of Ca^{2+} conductances have been described, but none of them has been clearly identified as I_{CRAC}, the Ca^{2+} release-activated Ca^{2+} current originally described in mast and lymphoma cells. Here we show that in bovine pulmonary artery endothelial cells (CPAE) depletion of intracellular Ca^{2+} stores by inositol 1,4,5-trisphosphate ($InsP_3$), Ca^{2+} ionophores and Ca^{2+} pump inhibitors activates a Ca^{2+}-selective conductance in the presence of the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA). The current shows inward rectification, a highly positive reversal potential and is blocked by micromolar concentrations of La^{3+}. The conditions used in studies of endothelial cells were also employed in those of HEK-293, an embryonic kidney cell line commonly used to express putative store-operated channels, and Jurkat cells, the reference cell model. Similar to CPAE, HEK cells also have an I_{CRAC}-like current. At 0 mV holding potential the estimated current density is –0.1 and –0.2 pA/pF in CPAE and HEK cells respectively, i.e. 15 and 30% of that measured in Jurkat cells. As shown in studies of Jurkat cells, larger Na^+ currents are detectable in CPAE and HEK cells following store depletion in Ca^{2+}- and Mg^{2+}-free medium. The current carried by Na^+ ions is similarly blocked by micromolar La^{3+}, is inwardly rectifying and has a positive reversal potential.

Key words Calcium influx · CPAE cells · Calcium-release activated (CRAC) current · Endothelium · HEK-293 · Jurkat · Patch-clamp · Store-operated channels (SOC)

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Introduction

The free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a primary role in the control of cell life and death. At rest, the average $[Ca^{2+}]_i$ is kept very low (100–200 nM) by means of active uptake mechanisms inside the internal stores and extrusion processes across the plasma membrane. Complex patterns of cytosolic Ca^{2+} spikes and waves are generated by tight control of Ca^{2+} release from internal stores and Ca^{2+} influx across the plasma membrane [20]. Different signalling molecules are known to selectively activate conductances that are permeable to Ca^{2+} ions. These Ca^{2+} pathways, activated by the agonist directly or through the production of second messengers, are variably distributed among different cell types and tissues [6]. However, the type of Ca^{2+} entry pathway that is triggered by empty stores is shared by a much larger number of eukaryotic cells, probably because the requirement of filled stores is mandatory for cell survival. Since the time that this phenomenon was originally described and named "capacitative Ca^{2+} influx" [21], a growing number of Ca^{2+} conductances have been proposed as store-operated channels (SOCs) and only recently they have been gathered together in a well-established family [3].

The first described store-operated current is I_{CRAC}, a current with a very low Ca^{2+} conductance (10–20 fS) and a Ca^{2+} selectivity comparable to that of the voltage-operated-Ca^{2+} channels [10, 11, 28]. In lymphoma and mast cells this current has been thoroughly characterized in terms of its electrophysiological properties and mechanisms of regulation [10–12, 18, 28–30]. However, its precise mechanism of activation remains elusive [1–3, 6].

In endothelial cells (ECs) different types of depletion-activated currents have been reported ([4, 8, 9, 13, 14, 22, 23] for a review see [16]), but none of them has been unambiguously identified as I_{CRAC}. Furthermore, because of a lower Ca^{2+} selectivity over monovalents and a higher Ca^{2+} conductance, these channels more closely resemble those linked to the products of the transient receptor

potential (*trp*) and the transient receptor potential-like (*trpl*) genes, originally identified in *Drosophila* [1–3]. *trp* is considered a prototype gene for SOCs and proteins homologous to Trp have been found in different organisms ranging from worms to humans [1–3]. Trp-dependent conductances with different Ca^{2+} selectivities can be activated by Ca^{2+} release from internal stores, following expression in *Xenopus* oocytes, Sf9, COS, CHO and HEK cells [1–3, 19, 24, 26, 27]. Except for *Xenopus* oocytes, in which an endogenous I_{SOC} with a high degree of similarity to I_{CRAC} has been demonstrated [25], very little is known about the endogenous currents that are activated by empty stores in the other cell models.

Given that Ca^{2+} signalling in vascular endothelial cells (ECs) plays a pivotal role in many different processes, ranging from blood clotting, angiogenesis and the immune response, to control of vessel permeability and tone, it is important to establish, first, what types of Ca^{2+} channels are present in the plasma membrane of ECs and, second, how a particular channel is selectively recruited in response to a defined stimulus [16]. The aim of the present study was to verify if a CRAC-like conductance is present in vascular ECs. Therefore, the store-operated Ca^{2+} current was studied in the bovine pulmonary artery endothelial cells, CPAE, and, for comparison, in two other cell lines: the Jurkat cells, as a "positive" control for I_{CRAC} [28] and the embryonic kidney cells, HEK-293, as a hypothetical "negative" control, given that this cell line is largely employed in transfection studies, aimed at unravelling the molecular structure of SOCs [2, 19, 24].

Materials and methods

Cell culture

Bovine pulmonary artery endothelial cells CPAE (ATCC CCL 209) and embryonic kidney cells HEK-293 were grown in DMEM containing 10% human serum, 2 mM L-glutamine, 2 U/ml penicillin and 2 mg/ml streptomycin at 37°C in a humidity-controlled incubator with 10% CO_2. Jurkat cells were grown in RPMI 1640 containing 10% human serum, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 5 mM $NaHCO_3$, 2 mM L-glutamine, 2 U/ml penicillin and 2 mg/ml streptomycin at 37°C in a humidity-controlled incubator with 5% CO_2.

For experiments CPAE and HEK cells were detached by exposure to 0.05% trypsin in Ca^{2+}- and Mg^{2+}-free phosphate-buffered solution, reseeded on gelatin-coated coverslips and used within 36–48 h. Jurkat cells (about 10^5) in complete culture medium were briefly centrifuged, resuspended in the standard recording medium and left to adhere to the coverslip before starting perfusion.

Patch-clamp experiments

The whole-cell configuration of the patch-clamp technique was used to study single cells under voltage-clamp. Currents were monitored with an EPC-7 (List-Elektronic) patch-clamp amplifier and sampled at 10 kHz (1024 points per record, filtered at 1 kHz). Voltage ramps of 50 ms duration, from –120 to +40 mV (for Jurkat cells, to avoid activation of outward currents) or from –100 to +100 mV (for CPAE and HEK cells), were delivered every 2 s from a holding potential of 0 mV. Cell surface area was estimated from the cell capacitance, as indicated by the analog compensation circuit of the EPC-7 amplifier. Experiments were carried out at room temperature (20–23°C).

Solutions

During current recordings cells were constantly perfused via a multichannel pipette, allowing rapid exchange of bath solutions. The standard extracellular solution was a modified Krebs medium containing (mM): 150 NaCl, 6 CsCl, 20 $CaCl_2$, 50 mannitol, 10 glucose, 10 HEPES, pH 7.4 with NaOH. When $CaCl_2$ was omitted (Ca^{2+}-free solutions) hypertonicity was maintained by increasing the mannitol concentration to 100 mM. The osmolality of these solutions, as measured with a vapour osmometer (Wescor 5500, Schlag, Gladbach, Germany) was 390±5 mosmol kg^{-1}. The standard pipette solution contained (mM): 145 glutamic acid, 8 NaCl, 2 $MgCl_2$, 10 HEPES, pH 7.2 with CsOH. 0.5 Na_2ATP and 12 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, Cs salt (CsBAPTA) were added freshly every day from 100 mM stock buffered solutions. When required the $[Ca^{2+}]_i$ was buffered between 50 and 100 nM with Ca^{2+}-free and Ca^{2+}-saturated CsBAPTA (10 mM final concentration).

Results

To understand if endothelial and embryonic kidney cells are endowed with the same type of current originally described in mast and Jurkat cells, conditions were chosen to simulate as closely as possible those employed with those cell models.

The driving force for Ca^{2+} was magnified by using an external Ca^{2+} concentration of 20 mM, known to produce maximal Ca^{2+} currents [11]. Outward K^+ currents were blocked by internal Cs^+, whereas a possible contamination by inward rectifying K^+ channels was excluded by substituting external K^+ with Cs^+. Because of the well-known inhibition of I_{CRAC} by Ca^{2+} entry [10, 11], incoming Ca^{2+} was highly buffered with 12 mM BAPTA. Mast and Jurkat cells are small and round shaped, with an average diameter of between 5 and 10 μm. In these cells, access to the internal "milieu" can be reached rather quickly, following patch rupture. A good access to the cell is required to obtain a substantially high amount of Ca^{2+} buffer during current activation. HEK cells, and ECs in particular, are large and flat. Cells were therefore used within 36–48 h from plating, and small, isolated cells were chosen. Small, nonconfluent ECs have a higher imput resistance compared to large confluent cells [16], a condition that favours the detection of small currents, such as I_{CRAC}.

It has recently been shown that CPAE cells possess a volume-sensitive anion conductance, active under isotonic conditions [15]. The current is blocked by cell shrinkage; therefore, to avoid any contribution from this type of current, mannitol was routinely included in the external solution (see Materials and methods and [15]).

As shown in Fig. 1 (left panels), when the above-mentioned conditions were employed, a small inward current similarly developed in Jurkat (top panel) and CPAE (middle panel) cells clamped at 0 mV holding po-

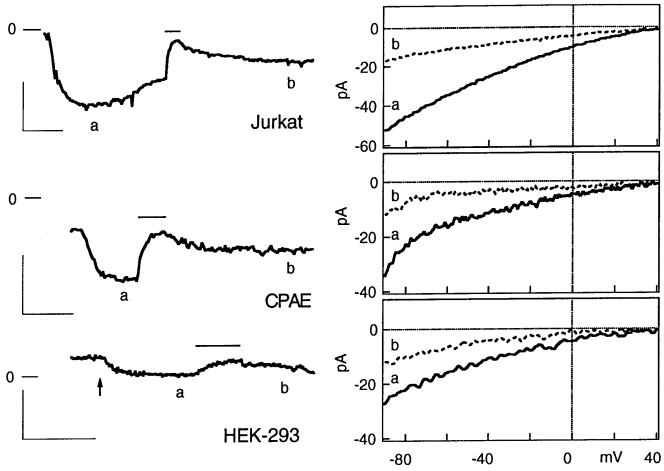

Fig. 1 Activation of Ca^{2+}-release-activated Ca^{2+} current (I_{CRAC}) in Jurkat, CPAE and HEK-293 cells. *Left panels*: time course of I_{CRAC} activation at 0 mV holding potential for the three different cell types. Cells were continuously perfused with the external standard solution containing 20 mM $CaCl_2$. Where indicated (*horizontal bar*) 10 μM $LaCl_3$ was applied with the same external solution. Jurkat cells (*top panel*) and CPAE cells (*middle panel*) were dialysed with the standard internal solution containing 12 mM BAPTA and 1 μM inositol 1,4,5-trisphosphate ($InsP_3$). HEK-293 cells (*bottom panel*) were dialysed with the standard internal solution containing 10 mM BAPTA, buffered with $CaCl_2$ to reach a final concentration of 50–100 nM. Current was triggered by puff application (*arrow*) of 1 μM ionomycin close to the cell. *Calibration bars*: 10 pA and 1 min. *Right panels*: the subtracted *I*/*V* relationships, obtained as described in Materials and methods, are shown at the peak current (*a*) and after recovery from perfusion with 10 μM La^{3+} (*b*)

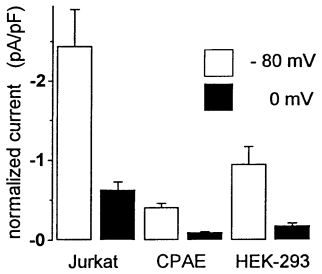

Fig. 2 Average current density in Jurkat, CPAE and HEK-293 cells. Cells were voltage-clamped at 0 mV holding potential. I_{CRAC} was activated as shown in Fig. 1 or by application of 50 μM 2,5-di(*tert*-butyl)-1,4-benzohydroquinone (*tBHQ*). Current values were measured at 0 and –80 mV from the instantaneous *I*/*V* relationships, obtained at the peak current as shown in Fig. 1 (*right panels*), and were normalized to the cell capacitance

tential. Inositol 1,4,5-trisphosphate ($InsP_3$,1 μM) was included in the patch pipette to obtain faster activation of the current. The presence of the buffer itself was indeed sufficient to activate the current, albeit with variable delays (data not shown).

The bottom panel of Fig. 1 (left) shows the activation of I_{CRAC} in HEK-293 cells, triggered by puffing ionomycin, a Ca^{2+} ionophore, close to the cell. To avoid spontaneous activation of the current, before ionomycin application, $[Ca^{2+}]_i$ was buffered at about 50–100 nM, in the presence of a high concentration of the Ca^{2+} chelator BAPTA (see Materials and methods). This protocol has been previously shown to prevent passive store depletion in rat basophilic leukaemia cells, and was effective also in HEK cells, as long as ionomycin was applied within 5 min.

The corresponding right panels of Fig. 1 show fast current/voltage (*I*/*V*) relationships, 50 ms long, recorded at 0.5 Hz while holding the cells at 0 mV. The two traces, shown in each panel, correspond to subtracted ramps obtained at the current peak (a), and during recovery from a short application of 10 μM La^{3+} (b). In this figure, a few ramps, recorded during La^{3+} application, were used for background subtraction. In previous studies, background ramps were routinely chosen before current development. When the two procedures are compared, currents obtained with the background in La^{3+} are reduced slightly in size, likely due to the fact that at this concentration La^{3+} can be easily washed away but does not cause a full block, and have a slightly less positive reversal potential, maybe due to a concomitant blockage of a small contaminating leak current. For ECs this latter procedure is, however, much more reliable since transient leak currents often accompany patch rupture, and initial ramps are frequently useless. Nonetheless the subtracted ramps, shown in Fig. 1, still reverse at high positive potentials (>40 mV). Both procedures were routinely employed.

I/*V* relationships, similar to those shown in Fig. 1, were obtained by perfusion with 2,5-di(*tert*-butyl)-1,4-benzohydroquinone (tBHQ) (50 μM), an inhibitor of sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs). When tBHQ was perfused from the beginning of the cell recording, the current reached its maximum within 3–5 min (not shown, but see also Fig. 3A).

Figure 2 compares the average current densities for the three cell types, measured at two different potentials, 0 and –80 mV, from *I*/*V* relationships obtained as described in Fig. 1. Compared to Jurkat cells, the current density at 0 mV is about 15% in CPAE cells (–0.10±0.04 pA/pF, mean±SEM, n=11), and 30% in HEK-293 cells (–0.18±0.03 pA/pF, mean±SEM, n=8)[1].

With a mean cell size of 36.4±1.9 and 19.3±2.6 pF, for CPAE and HEK cells respectively, a total current of –3.4 to –3.6 pA could be measured at 0 mV holding potential. Similarly, in Jurkat cells, a total current of about –5 pA was measurable with an average cell size of 8.4±1.1 pF (n=10).

In addition to high Ca^{2+} selectivity, inward rectification and high sensitivity to La^{3+}, another property of I_{CRAC} is its peculiar permeability to monovalent cations.

[1] For CPAE cells, currents activated by a low dose of $InsP_3$ or by perfusion with tBHQ were pooled together since maximal current activation was reached within a few minutes from cell recording and no substantial difference was observed between the two procedures. For HEK cells, currents obtained with a low dose of $InsP_3$, or ionomycin added within 50–100 s from cell recording, were also pooled together. The corresponding values were in fact 0.9±0.4 and 1.0±0.4 pA/pF at –80 mV, for $InsP_3$- and ionomycin-activated currents respectively

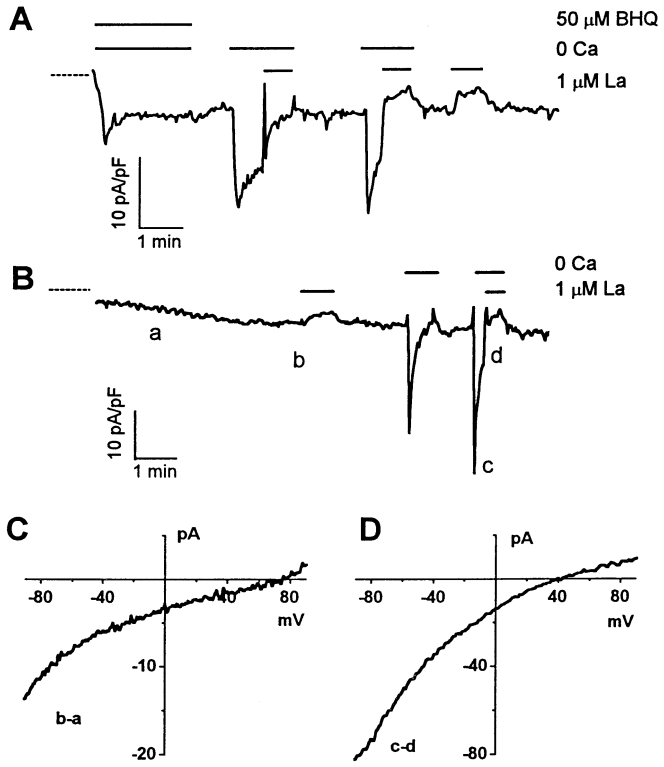

Fig. 3A–D Na^+ current following store depletion in CPAE cells. CPAE cells were voltage-clamped as described in Fig. 1 (*middle panels*). During current recordings cells were constantly perfused via a multichannel pipette, allowing rapid exchange of bath solutions. **A** The internal solution contained 12 mM BAPTA, in the absence of InsP_3. The cell was initially perfused with a divalent-free solution containing 50 μM tBHQ. The bath solution was then alternately switched from the standard high-Ca^{2+}-containing solution (20 mM $CaCl_2$) to the divalent-free solution (indicated as "*0 Ca*"). Currents were blocked by 1 μM La^{3+} added to either the standard, or the divalent-free bath solution. **B** The internal solution contained 12 mM BAPTA, in the presence of 1 μM InsP_3. Other conditions as in *panel* **A**. **C** *I*/*V* relationship in high-Ca^{2+} medium, obtained by subtracting ramps recorded before current activation (*a*), from ramps recorded at the peak Ca^{2+} current (*b*), as shown in *panel* **B**. **D** *I*/*V* relationship with the preparation bathed in divalent-free medium, obtained by subtracting ramps recorded in the same medium containing La^{3+} (*d*) from ramps at the peak Na^+ current (*c*) as shown in *panel* **B**. The trace is representative of 5 similar experiments

The phenomenon has been thoroughly investigated by Lepple-Wienhues and Cahalan [12].

In media containing millimolar amounts of Mg^{2+}, I_{CRAC} is practically impermeant to monovalent cations. In the virtual absence of divalent cations, large linear currents are instantaneously recorded when the medium is exchanged from a high-Ca^{2+} to a divalent-free medium containing ethylenebis(oxonitrilo)tetraacetate (EGTA) [11, 12]. However, in the absence of Mg^{2+} when the external Ca^{2+} concentration is reduced to micromolar levels, I_{CRAC} transiently decreases and then increases to reach a much larger peak, which is followed by a slow deactivation [12]. CPAE cells were challenged either from the beginning of the cell recording with an isotonic, divalent-free solution (contaminant, micromolar Ca^{2+}) containing 50 μM tBHQ (Fig. 3A), or with the divalent-free solution after full development of the current induced by InsP_3 (1 μM) in the high-Ca^{2+} medium (Fig. 3B). In this latter panel, the La^{3+}-sensitive current, which was maximally activated in the high-Ca^{2+} medium, was transiently reduced by the initial decrease in $[Ca^{2+}]_o$, when switching to a divalent-free solution, and was then followed by Na^+ permeation, as soon as divalent-free conditions were attained. The bottom panels of Fig. 3 show the *I*/*V* relationships in high-Ca^{2+} (C) and Ca^{2+}-free (D) medium obtained from the current trace shown in panel B. The current recorded in the absence of divalent ions becomes larger after full depletion of the stores, is blocked by micromolar La^{3+} concentrations, has a positive reversal potential and is inwardly rectifying (Fig. 3D). On average, currents in Na^+ reversed at 30±7 mV (*n*=5, mean±SEM). When the peak current in Na^+ is compared to the maximal current in Ca^{2+}, a ratio of 7.9±2.1 is estimated for currents recorded at 0 mV holding potential. Similar results have been obtained in studies of HEK-293 cells (data not shown). On average, in these cells, the Na^+ current reversed at 16±3 mV and the Na^+/Ca^{2+} ratio for currents measured at 0 mV was 6.1±1.6 (*n*=4).

Discussion

The main finding of this study is the detection of an I_{CRAC}-like conductance in the vascular endothelium cell line CPAE and in the embryonic kidney cell line HEK-293, using conditions similar to those previously employed in studies of mast and lymphoma cells [10–12, 18, 28–30]. The smaller current density, respectively 15 and 30% of that in Jurkat cells, is consistent with modest (if compared to these latter cells) Ca^{2+} influx and Ca^{2+} "plateaus", recorded upon full depletion of Ca^{2+} stores by SERCA inhibitors [6, 8]. It remains to be established whether a lower current density reflects a reduced number of channels, or a cell-specific channel modulation. However, a likely underestimation of the current density, mainly due to the background subtraction in La^{3+} and/or to the slow activating procedures, cannot be excluded. On the other hand, a larger surface to volume ratio, characteristic of ECs, may be also responsible for the reduced channel density. It is worth mentioning that clones of Jurkat cells, with normal store content but a reduced rate of refilling, have been shown to express 10–30% of the current expressed in the parental cell line [5]. The large surface to volume ratio of ECs is also believed to be responsible for the slightly different shape of the *I*/*V* curve shown in Fig. 1 (middle panel), which is mainly due to suboptimal compensation of the capacitative transients.

The inward rectification, the highly positive reversal potential, and the high sensitivity to La^{3+} point to a highly selective Ca^{2+} current, similar to I_{CRAC}. Moreover, at 0 mV holding potential, currents carried by Ba^{2+} (10 mM) were undetectable (not shown), consistent with

the highest permeability of I_{CRAC} being for Ca^{2+} ions, and the fact that Ba^{2+} is a poor substitute for Ca^{2+}. On the other hand, a procedure which allows the larger Na^+ current typical of I_{CRAC} [12] to be revealed helps to unmask the low level of expression of this type of current in CPAE and HEK cells, and therefore is a powerful tool with which to detect this current type in cell systems from which it was originally excluded.

There are numerous implications of these findings. They will be briefly summarized here. First, these data confirm that I_{CRAC} is a common pathway for Ca^{2+} entry that is triggered simply by depletion of intracellular Ca^{2+} stores, and is shared by many eukaryotic cells. Apart from rat mast cells and human lymphoma cells, in which I_{CRAC} was originally described [10, 28], I_{CRAC}-like currents have also been detected with a similar procedure in hepatocytes, fibroblasts, thyrocytes, HL-60 cells, pancreatic acinar cells and *Xenopus* oocytes [6, 25]. Second, the use of HEK-293 cells, as a "negative" control, was surprisingly misleading, since in this cell type, a current with properties similar to I_{CRAC} is also measurable under the conditions employed in the study of Jurkat and CPAE cells. Indeed, this finding extends the minor observation, frequently reported in transfection studies, that the current induced by overexpression of *trp/trpl* genes never resembles the small endogenous current activated by store depletion [2, 19]. The presence of a I_{CRAC}-like current in HEK cells must be taken into consideration, since assembly of exogenous proteins with endogenous CRAC components may not be excluded a priori, and may be able to modify the overall current properties. Moreover, some components of the Trp/Trpl family are Ca^{2+}-activated channels [27] that can be triggered following previous activation of an endogenous I_{CRAC} current. Finally, these findings are particularly relevant to ECs, in which the presence of I_{CRAC} has been widely debated [16]. These data, in fact, confirm theoretical calculations based on the rate of Ca^{2+} influx induced by store depletion, at varying driving forces, under unbuffered intracellular conditions. Accordingly, in perforated, voltage-clamped ECs, a current density of about –0.01 pA/pF at –80 mV has been calculated from reconstructed *I*/*V* relationships, which show typical inward rectification and a positive reversal potential [17]. Such a type of reconstruction is consistent with the present findings, since a reduction in current size of at least one order of magnitude is expected under unbuffered conditions.

However, many questions remain to be answered. In vascular ECs different Ca^{2+} conductances have been described to occur following agonist stimulation [4, 8, 13–17, 22, 23]. Given that Ca^{2+} fluxes play a pivotal role in most of the EC's functions [16], it will be interesting to find out which channel type contributes most to store refilling upon different depletion protocols or stimuli. Moreover, the detection of an I_{CRAC}-like current reported here concerns small, non-confluent ECs, and it remains to be established whether a similar current is expressed at higher or lower levels in confluent ECs as well.

Unfortunately, as yet, no specific drug is available to block I_{CRAC}. On the other hand, it has been clearly demonstrated that different K^+ and Cl^- channel inhibitors are also effective blockers of I_{CRAC} and capacitative Ca^{2+} entry [7, 9]. Therefore, the specific contribution of this influx pathway must be carefully considered to fully understand the physiological and pathological aspects of the vascular tissue.

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References

1. Berridge MJ (1995) Capacitative calcium entry. Biochem J 312:1–11
2. Birnbaumer L, Zhu X, Jiang M, Boulay G, Peyton M, Vannier B, Brown D, Platano D, Sadeghi H, Stefani E, Birnbaumer M (1997) On the molecular basis and regulation of cellular capacitative calcium entry: roles for Trp proteins. Proc Natl Acad Sci USA 93:15195–202
3. Clapham DE (1996) TRP is cracked but is CRAC TRP? Neuron 16:1069–1072
4. Davis MJ, Sharma NR (1997) Calcium-release-activated calcium influx in endothelium. J Vasc Res 34:186–195
5. Fanger CM, Hoth M, Crabtree GR, Lewis RS (1995) Characterization of T cell mutants with defects in capacitative calcium entry: genetic evidence for the physiological roles of CRAC channels. J Cell Biol 131:655–667
6. Fasolato C, Innocenti B, Pozzan T (1994) Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels? Trends Pharmacol Sci 15:77–83
7. Franzius D, Hoth M, Penner R (1994) Non-specific effects of calcium entry antagonists in mast cells. Pflügers Arch 428:433–438
8. Gericke M, Droogmans G, Nilius B (1993) Thapsigargin discharges intracellular calcium stores and induces transmembrane currents in human endothelial cells. Pflügers Arch 422: 552–557
9. Gericke M, Oike M, Droogmans G, Nilius B (1994) Inhibition of capacitative Ca^{2+} entry by a Cl^- channel blocker in human endothelial cells. Eur J Pharmacol 269:381–384
10. Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355: 353–356
11. Hoth M, Penner R (1993) Calcium release-activated calcium current in rat mast cells. J Physiol (Lond) 465:359–386
12. Lepple-Wienhues A, Cahalan D (1996) Conductance and permeation of monovalent cations through depletion-activated Ca^{2+} channels (I_{CRAC}) in Jurkat T cells. Biophys J 7:787–794
13. Lückhoff A, Clapham DE (1992) Inositol 1,3,4,5-tetrakisphosphate activates an endothelial Ca^{2+}-permeable channel. Nature 355:356–358
14. Mendelowitz D, Bacal K, Kunze DL (1992) Bradykinin-activated calcium influx pathway in bovine aortic endothelial cells. Am J Physiol 439:H942–H943
15. Nilius B, Prenen J, Szuecs G, Wei L, Tanzi F, Voets T, Droogmans G (1997) Calcium-activated chloride channels in bovine pulmonary artery endothelial cells. J Physiol (Lond) 498:381–396
16. Nilius B, Viana F, Droogmans G (1997) Ion channels in vascular endothelium. Annu Rev Physiol 59:145–170
17. Oike M, Gericke M, Droogmans G, Nilius B (1994) Calcium entry activated by store depletion in human umbilical vein endothelial cells. Cell Calcium 16:367–376

18. Parekh AB, Penner R (1995) Depletion-activated calcium current is inhibited by protein kinase in RBL-2H3 cells. Proc Natl Acad Sci USA 92:7907–7911
19. Philipp S, Cavalie A, Freivhel M, Wissenbach U, Zimmer S, Trost C, Marquart A, Murakami M, Flockerzi V (1996) A mammalian capacitative calcium entry channel homologous to Drosophila TRP and TRPL. EMBO J 15:6166–6171
20. Pozzan T, Rizzuto R, Volpe P, Meldolesi J (1994) Molecular and cellular physiology of intracellular calcium stores. Physiol Rev 74:595–636
21. Putney JW Jr (1990) Capacitative calcium entry revisited. Cell Calcium 11:611–624
22. Vaca L, Kunze DL (1994) Depletion of intracellular Ca^{2+} stores activates a Ca^{2+}-selective channel in vascular endothelium. Am J Physiol 267:C920–C925
23. Vaca L, Kunze DL (1995) $InsP_3$-activated Ca^{2+} channels in the plasma membrane of cultured vascular endothelial cells. Am J Physiol 269:C733–C738
24. Xu XZ, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. Cell 89:1155–1164
25. Yao Y, Tsien R (1997) Calcium current activated by depletion of calcium stores in *Xenopus* oocytes. J Gen Physiol 109:703–715
26. Zitt C, Zobel A, Obukhov AG, Harteneck C, Kalkbrenner F, Luckhoff, A, Schultz G (1996) Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. Neuron 16:1189–119
27. Zitt C, Obukhov AG, Strübing C, Zobel A, Kalkbrenner F, Lückhoff A, Schultz G (1997) Expression of TRPC3 in Chinese hamster ovary cells results in calcium-activated cation currents not related to store depletion. J Cell Biol 138:1333–1342
28. Zweifach A, Lewis RS (1993) Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. Proc Natl Acad Sci USA 90:6295–6299
29. Zweifach A, Lewis RS (1995) Slow calcium-dependent inactivation of depletion-activated calcium current. Store-dependent and -independent mechanisms. J Biol Chem 270:14445–14451
30. Zweifach A, Lewis RS (1996) Calcium-dependent potentiation of store-operated calcium channels in T-lymphocytes. J Gen Physiol 107:597–610