

Depletion of intracellular calcium stores activates an outward potassium current in mast and RBL-1 cells that is correlated with CRAC channel activation

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Abstract Highly Ca^{2+} selective Ca^{2+} channels activated by store depletion have been recently described in several cell types and have been termed CRAC channels (for calcium release-activated calcium). The present study shows that following store depletion in mast and RBL-1 cells, monovalent outward currents could be recorded if the internal solution contained K^+ but not Cs^+ . The activation of the outward K^+ current correlated with the activation of I_{CRAC} , in both time and amplitude, suggesting that the K^+ current might be carried by CRAC channels. The amplitude of the outward current was increased if external Ca^{2+} was reduced or replaced by external Ba^{2+} . The outward K^+ conductance might have a physiological role in maintaining the driving force for Ca^{2+} entry during the activation of CRAC channels.

Key words: Store-operated channel; Calcium release-activated calcium channel; Potassium conductance; Barium permeation; Mast cell; RBL-1 cell

1. Introduction

Based on strong evidence from intracellular Ca^{2+} measurements, $^{45}\text{Ca}^{2+}$ flux measurements, and Mn^{2+} quenching experiments, Putney [26] developed the idea of the capacitative Ca^{2+} entry mechanism which relates the activation of Ca^{2+} entry to the filling state of intracellular Ca^{2+} stores. Capacitative Ca^{2+} currents activated by depletion of internal Ca^{2+} stores have been demonstrated in mast cells [13–15], RBL-2H3 cells [6,15,23,30], and Jurkat T-cells [3,5,15,19,25,31,32] as well as in a large variety of other non-excitable cell types [2,7]. In the following, SOC (store-operated channel) refers to the whole family of these channels whereas CRAC (calcium release-activated calcium) channel refers to the one originally described in mast cells which is characterized by a very high selectivity of Ca^{2+} over other cations [14,15] and its very low unitary conductance in the fS range [14,31].

In the present study, depletion-activated monovalent outward currents in mast cells and RBL-1 cells are reported in the presence of physiological external Ca^{2+} and Mg^{2+} concentrations: K^+ but not Cs^+ permeates a depletion-activated channel which is likely to be identical with CRAC channels. The activation of this conductance correlates with the activation of CRAC channels, in both time and amplitude. The

amplitude of the outward current was modulated by the extracellular divalent concentration: it was increased if external Ca^{2+} was reduced or replaced by external Ba^{2+} .

2. Materials and methods

Mast cells from rat peritoneum and RBL-1 cells (a rat basophilic leukemia cell line from the American Type Culture Collection, Rockville, MD 20852-1776, USA, ATCC 1378) were obtained and cultured as described [6,13,14]. The different culture media (modified medium M199 for mast cells, Dulbecco's modified Eagle's medium for RBL-1 cells) were supplemented with fetal calf serum (10%), NaHCO_3 (45 mM), glucose (2.5–5 mM), streptomycin (0.12 mg ml⁻¹), and penicillin (0.64 mg ml⁻¹).

For experiments, coverslips with cells were transferred to the recording chamber and kept in a Ringer's solution. Experiments were performed at room temperature (22–26°C) in the tight-seal whole-cell configuration of the patch-clamp technique using Sylgard-coated patch pipettes with resistances of 2–4 M Ω . Series resistances were in the range of 3–8 M Ω and were not compensated. High-resolution membrane currents were recorded using an EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany) controlled by the 'E9SCREEN' software on an Atari computer. All voltages were corrected for a liquid junction potential of 8 mV between external and internal solutions. High-resolution currents were low-pass filtered at 3 kHz and acquired at a sampling rate of 10 kHz, while a charting program on another computer synchronously recorded at low resolution (2 Hz) the holding potential, holding current (low-pass filtered at 500 Hz), fura-2 fluorescence, and timing of solution changes. All values are given as mean \pm standard deviation (number of experiments). Voltage ramps were of 50 ms duration, covering a range of –100 mV to +100 mV or +100 mV to –100 mV. No difference in currents was observed between the two ramp protocols indicating that the outward conductance was not gated by the incoming Ca^{2+} . All current traces shown are corrected by leak traces taken before activation of I_{CRAC} . Capacitance and series resistance were cancelled before each voltage ramp using the automatic neutralization routine of the EPC-9. The holding potential was usually kept at 0 mV, because Ca^{2+} influx at this potential is moderate enough to be buffered effectively by the intracellularly supplied BAPTA or EGTA.

The standard intracellular solution contained (in mM): K-glutamate or Cs-glutamate 145, NaCl 8, MgCl_2 1, Mg-ATP 0.5, HEPES- (KOH or CsOH) 10, ethylene glycol bis-*N,N,N',N'*-tetraacetic acid (EGTA) or 1,2-bis (2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) 10, fura-2 pentapotassium salt 0.1, inositol 1,4,5-trisphosphate (InsP_3 , Amersham) 0.01–0.03, pH 7.2. The standard external solution contained (in mM): CsCl or NaCl 140, KCl 2.8, different concentrations of CaCl_2 or BaCl_2 , MgCl_2 2, glucose 11, HEPES- (CsOH or NaOH) 10, pH 7.2. In all experiments with RBL-1 cells, the external solution contained at least 0.5 mM Ba^{2+} , which is sufficient to block K^+ channels; 0.5 mM Ba^{2+} does not affect I_{CRAC} significantly [14]. The free Ca^{2+} concentration of the external solutions with no additional Ca^{2+} added was measured to be 20 μM using the fluorescent dye Mag-fura (Molecular Probes). Solution changes were made by puffing solutions via the wide-tipped application pipette onto the cell to maximize speed. The solution exchange was completed within a few seconds. The pressure application did not cause artifacts since application of bath solution had no effect.

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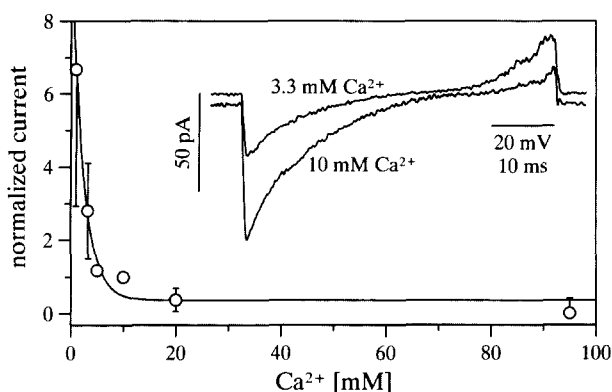


Fig. 1. K^+ outward current activated by pool depletion is shown as a function of $[Ca^{2+}]_o$. The experiments were performed with mast cells using standard internal K-glutamate and standard external solutions (10 mM Ca^{2+} , 140 mM Na^+ , 2.8 mM K^+). I_{CRAC} was activated by internally dialyzing the cells with $InsP_3$ (10 μ M) and EGTA or BAPTA (10 mM). The external Ca^{2+} concentration was changed to 3.3 mM after I_{CRAC} was fully activated. The inset shows the voltage ramp currents (after subtraction of linear leak currents) in 10 and 3.3 mM Ca^{2+} . The plot shows the outward currents at +82 mV membrane potential as a function of $[Ca^{2+}]_o$ (normalized to the currents in 10 mM Ca^{2+}). An exponential function was fitted to the data ($I/I_{10Ca} = 0.36 + 9.77 \times \exp -([Ca^{2+}]_o/2.29 \text{ mM})$).

3. Results

It has been shown that different maneuvers that deplete Ca^{2+} stores lead to the activation of I_{CRAC} in mast cells [13–15], RBL-2H3 cells [6,15,23,30] and RBL-1 cells [15]. As shown in Fig. 1, in addition to the activation of inward I_{CRAC} an outward current could be observed in mast cells following store depletion. The outward current was strongly dependent on $[Ca^{2+}]_o$. Only a very small outward component could be observed at 10 mM or higher external Ca^{2+} concentrations but this component was significantly enlarged when changing to lower, more physiological Ca^{2+} concentrations. The inset of Fig. 1 shows an example of an experiment in which $[Ca^{2+}]_o$ was reduced from 10 to 3.3 mM which resulted in a reduction of the inward current and in an increase of outward current. This outward component was observed with K-glutamate-based internal solutions but not with Cs-glutamate ($n=52$, compare also Fig. 2), suggesting that the current was mainly carried by K^+ . However, the current was not affected by replacing external Na^+ by K^+ ($n=5$) suggesting that K^+ and Na^+ were equally permeant. Changes in outward currents were not observed in response to a change of $[Ca^{2+}]_o$ between 1 and 10 mM if I_{CRAC} was not previously activated (control conditions: no $InsP_3$ and/or no EGTA/BAPTA in the internal solution, $n=10$). Since application of ionomycin also activated the outward K^+ current (and of course also the inward Ca^{2+} current) it is very likely that the outward current is activated by store depletion and not by a different mechanism, e.g. by $InsP_3$ itself.

In the next series of experiments I_{CRAC} was activated in 10 mM Ba^{2+} solution (free $[Ca^{2+}]_o \approx 20 \mu$ M). Under these conditions a simultaneous and correlating activation in time and amplitude of inward and outward currents could be obtained in case the internal solution was K-glutamate-based and not Cs-glutamate-based (compare Fig. 2B). In the latter case only the activation of the inward current could be measured. No outward current was detectable. The K^+ outward current was

much bigger with 10 mM Ba^{2+} in the external solution than with 10 mM Ca^{2+} , indicating that the outward current is modulated by external divalent cations. Fig. 2A shows pooled data from mast and RBL-1 cells in which I_{CRAC} was activated using the internal K^+ (open circles) or Cs^+ (open diamonds) based internal solutions. The outward current at +82 mV is plotted versus the inward current at –82 mV membrane potential indicating the correlation of the current in amplitudes. Fig. 2B depicts the activation time course of inward and outward currents in a single mast cell (left) and RBL-1 cell (right) with K^+ internal solution (open circles) and with Cs^+ internal solution (open diamonds). In all cases ($n=19$), inward and outward current developed in parallel. No difference in currents was observed when voltage ramps were applied from +100 mV to –100 mV (and not from –100 mV to +100 mV as routinely done) indicating that the outward conductance was not gated by the incoming Ca^{2+} which enters the cell at negative membrane potentials. The ramp currents after the amplitude of I_{CRAC} reached steady state in the corresponding cells are shown in Fig. 2C. These traces illustrate the pronounced N-shape of the current-voltage relationship under these conditions.

Previously we showed that I_{CRAC} in mast cells was reduced by $9 \pm 2\%$ ($n=4$) when replacing external Na^+ and K^+ by *N*-methyl-D-glucamine (membrane potential –40 mV [14]). A reduction of $10.9 \pm 6.1\%$ ($n=6$) was also seen at more negative potentials (–82 mV) indicating the voltage independence of this reduction. The same was found when external Na^+ was replaced by external Cs^+ (data not shown): in mast cells the current was reversibly reduced by $5.4 \pm 2.1\%$ ($n=3$, –82 mV) and in RBL-1 cells by $10.4 \pm 2.8\%$ ($n=3$, –82 mV). Similar current reductions occurred in the whole negative voltage range. Replacing external Na^+ by external K^+ or vice versa did not change the amplitude of I_{CRAC} in both cell types ($n=11$).

4. Discussion

The main result of this study is that depletion of intracellular Ca^{2+} stores activates a K^+ outward conductance correlated with the activation of CRAC channels. The amplitude of the outward current is modulated by the external divalent concentration: it was increased if external Ca^{2+} was reduced or replaced by external Ba^{2+} . The outward current was suppressed if the internal solution contained Cs^+ instead of K^+ . A store depletion-activated K^+ conductance has been previously found in *Xenopus* oocytes [22] and in MDCK cells [4] but was not investigated in further detail.

The activation of the outward current at very positive potentials and the reduction of inward current through CRAC channels after replacing Na^+ and/or K^+ by Cs^+ or *N*-methyl-D-glucamine can be explained in three different ways. (1) Even in the presence of millimolar external Ca^{2+} concentrations, CRAC channels carry a small amount of inward Na^+ and outward K^+ current but no Cs^+ current. (2) A second depletion activated channel is responsible for the monovalent inward and outward currents. (3) CRAC channels contain a binding site that can be occupied by monovalent cations that influences Ca^{2+} influx. A binding site with low affinity for Na^+ has been postulated by Kostyuk et al. [17] for VOC channels. This third possibility, however, only explains the reduction of the inward current but not the appearance of

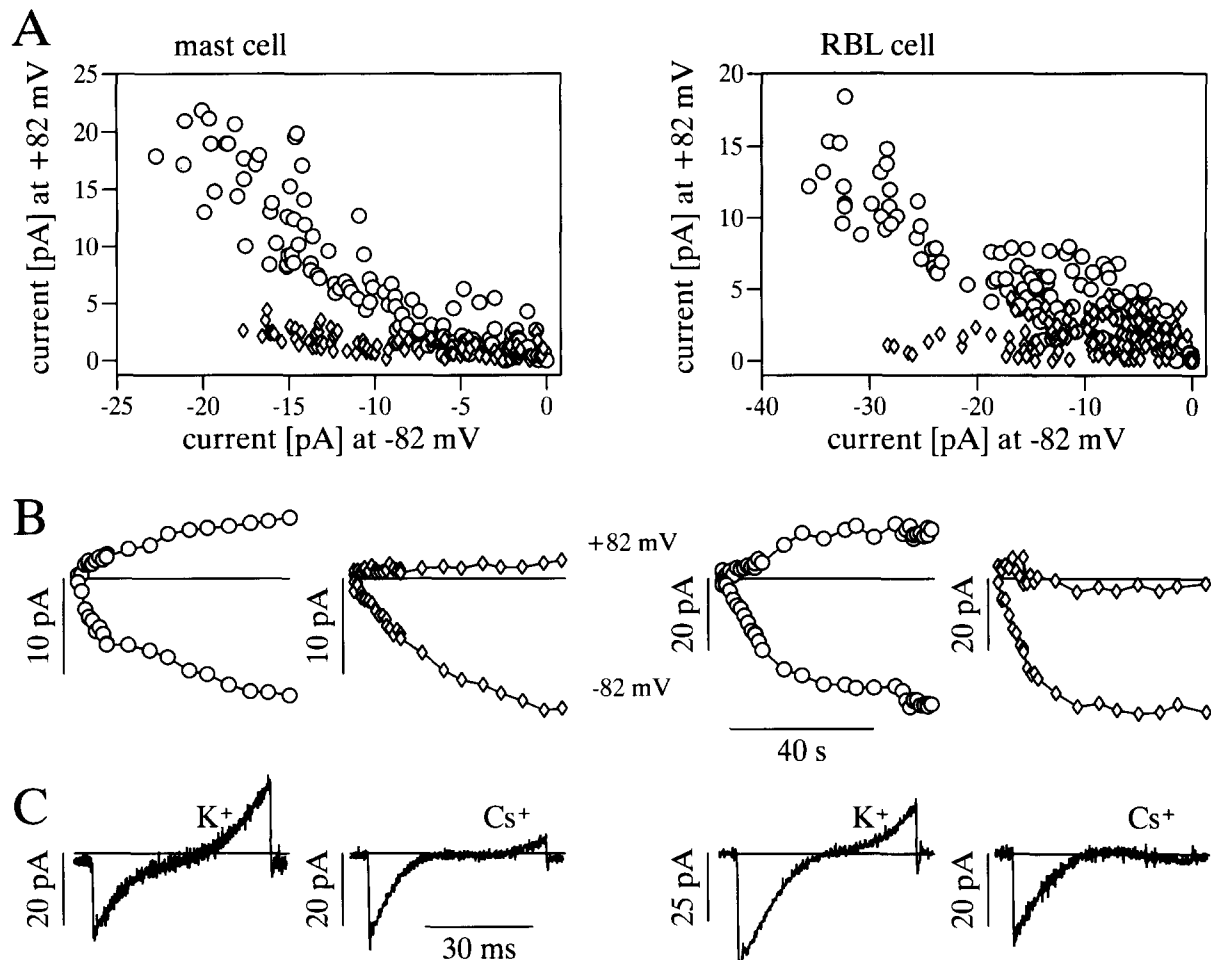


Fig. 2. Ba^{2+} and K^{+} currents through CRAC channels. CRAC currents were activated by $InsP_3$ and EGTA or BAPTA in 10 mM $[Ba^{2+}]_o$ in mast or RBL-1 cells. The experiments were carried out in K-glutamate (open circles) or Cs-glutamate (open diamonds) containing internal solution. The external solution contained 10 mM Ba^{2+} , 140 mM Na^{+} , and 2.8 mM K^{+} . A: Outward currents at +82 mV are plotted versus inward currents at -82 mV (8 pooled mast cells, 9 pooled RBL-1 cells). B: Activation of CRAC currents is shown for single cells at -82 and +82 mV. C: Leak-corrected ramp currents after complete activation of I_{CRAC} are shown for the corresponding cells in B.

the outward current. Hence, one has to postulate in this case that inward and outward current are carried by two different depletion activated channels. An argument against this possibility is the finding that the amplitude of the monovalent inward current carried by Na^{+} (5–10% of the inward current in 10 mM external Ca^{2+}) matches the amplitude of the monovalent outward current under these conditions.

The correlation of the time course of activation and the relative amplitudes between the outward current and the inward current through CRAC channels favors the possibility that CRAC channels carry both the inward Ca^{2+} or Ba^{2+} current and the outward K^{+} current. The final proof or disproof of this postulate has to await cloning of the channel gene or the availability of a highly specific channel blocker. Up to now, the commonly used Ca^{2+} influx blockers have been found to be nonspecific and therefore not of use in distinguishing between different channels [9].

Monovalent currents activated by store depletion have also been measured in the complete absence of extracellular divalent cations in mast cells as well as in Jurkat T-cells. In mast cells, it was concluded that the permeability for Na^{+} and K^{+} through CRAC channels was similar [14] whereas Na^{+} permeation was greater than K^{+} permeation in Jurkat T-cells

[19]. Furthermore, it was shown that Na^{+} and K^{+} permeate much better than Cs^{+} [19,25] in good agreement with the permeability of the monovalent outward conductance described in the present study. If the monovalent outward current in the presence of divalent cations and the monovalent inward current in the absence of all divalent cations were carried by the same channel, it could be concluded that the relative permeability ratios for monovalent cations do not depend on the extracellular absence or presence of divalent cations. This finding would have the interesting implication that the 'selectivity filter' for monovalent cations is not influenced by divalent cations. Of course, the absolute amplitude of monovalent currents strongly depends on the concentration and type of divalent cations present in the extracellular solution. Lepple-Wienhues and Cahalan [19] reported two strong pieces of evidence that monovalent currents are indeed carried by CRAC channels in the complete absence of divalent cations. (1) By switching back and forth between extracellular Ca^{2+} and divalent free solutions they showed that Ca^{2+} and Na^{+} currents developed in parallel. (2) The slow inactivation of Na^{+} currents correlated with a reduced subsequent amplitude of the Ca^{2+} current. The observed following increase of Ca^{2+} currents is probably mediated by a recently described

positive feedback effect of $[Ca^{2+}]_o$ on CRAC channels [3,32]. The slow inactivation of Na^+ currents [14,19] in the absence of extracellular divalent cations and the slow inactivation of Ba^{2+} currents in the presence of 2 mM extracellular Mg^{2+} [15] could both be explained by the reverse of the $[Ca^{2+}]_o$ -dependent positive feedback mechanism.

Better permeation of K^+ and Na^+ compared to Cs^+ in the presence of external divalent cations was also reported for various VOC channels [10,12]. However, CRAC channels behave differently from VOC channels in terms of monovalent permeation in the following ways. (1) Outward Cs^+ currents through VOC channels have been measured in different cell types [8,10,12,18] whereas CRAC channels seemed not to carry any Cs^+ current. (2) Only the removal of external Ca^{2+} was necessary to obtain huge monovalent currents through VOC channels [10,12] whereas all divalent cations (including Mg^{2+}) had to be removed to allow large monovalent inward currents through CRAC channels [14,19].

Recently, a lot of interest has focused on the question whether the trp (transient receptor potential) channel from *Drosophila* photoreceptors or one of its mammalian homologues is similar or identical to the CRAC channel [1,2,7]. It has been suggested that trp is a store-operated Ca^{2+} channel and it has been shown that trp channels expressed in Sf9 cells or *Xenopus* oocytes could be activated by thapsigargin [24,27,29]. However, if one compares the data published on permeation and selectivity for both channel types, it becomes clear that CRAC and trp are not identical. The Ca^{2+} selectivity over monovalent cations for CRAC channels in mast, RBL, and Jurkat T-cells was found to be 1000/1 or greater [14,15] whereas for trp it is about 40/1 [11]. Comparing the endogenous insect SOC channels with expressed trp channels in Sf9 cells, Vaca et al. [29] showed that this SOC channel and the trp channel were clearly different regarding the permeation properties of Ca^{2+} , Ba^{2+} , Mg^{2+} , and Na^+ . Different permeation properties were also reported for store operated channels in a human epidermal cancer cell line (A431) [20], in vascular endothelium [28], in HT₂₉ colonic carcinoma cells [16], and in MDCK cells [4]. Selectivity and permeation properties have so far been proven the best tools to distinguish different members of the SOC family.

Since the monovalent currents in this study were measured in the presence of physiological extracellular Ca^{2+} and Mg^{2+} concentrations, there is a potential physiological role of the depletion activated K^+ conductance. The outward K^+ current might inhibit the depolarization of the cell due to Ca^{2+} entry, therefore working as a positive feedback mechanism. The outward current was measured at very positive, probably unphysiological potentials, however it might be masked by the Ca^{2+} inward current at more physiological potentials like 0 mV which is probably close to the resting potential of mast cells. Consistent with that hypothesis is the finding that net inward CRAC currents at 0 mV are about 10% bigger if the internal solution contained Cs^+ but not K^+ [15]; outward K^+ currents would decrease the net CRAC inward current. This role of the

K^+ outward conductance is particularly interesting in mast cells since they apparently do express functional K^+ channels which could serve as a regulator of membrane potential.

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