Automated Patch-Clamp Technique: Increased Throughput in Functional Characterization and in Pharmacological Screening of Small-Conductance Ca\textsuperscript{2+} Release-Activated Ca\textsuperscript{2+} Channels

RIKKE L. SCHRØDER,1 SØREN FRIIS,1 MORTEN SUNESEN,1 CHRIS MATHES,2 and NIELS J. WILLUMSEN3

The suitability of an automated patch clamp for the characterization and pharmacological screening of calcium release–activated calcium (CRAC) channels endogenously expressed in RBL-2H3 cells was explored with the QPatch system. CRAC currents (I\textsubscript{CRAC}) are small, and thus precise recordings require high signal-to-noise ratios obtained by high seal resistances. Automated whole-cell establishment resulted in membrane resistances of 1728 ± 226 MΩ (n = 44). CRAC channels were activated by a number of methods that raise intracellular calcium concentration, including EGTA, ionomycin, Ins(1,4,5)P\textsubscript{3}, and thapsigargin. I\textsubscript{CRAC} whole-cell currents ranged from 30 to 120 pA with rise times of 40 to 150 s. An initial delay in current activation was observed in particular when I\textsubscript{CRAC} was activated by passive store depletion using EGTA. Apparent rundown of I\textsubscript{CRAC} was commonly observed, and the current could be reactivated by subsequent addition of thapsigargin. I\textsubscript{CRAC} was blocked by SKF-96365 and 2-APB with IC\textsubscript{50} values of 4.7 ± 1.1 μM (n = 9) and 7.5 ± 0.7 (n = 9) μM, respectively. The potencies of these blockers were similar to values reported for I\textsubscript{CRAC} in similar conventional patch-clamp experiments. The study demonstrates that CRAC channels can be rapidly and efficiently targeted with automated patch-clamp techniques for characterization of physiological and pharmacological properties. (Journal of Biomolecular Screening 2008:638-647)

Key words: automated patch clamp, QPatch, intracellular calcium stores, CRAC channels

INTRODUCTION

CALCIUM RELEASE–ACTIVATED CALCIUM (CRAC) CHANNELS constitute a group of low-conductance store-operated channels that are highly Ca\textsuperscript{2+} selective and, upon activation, give rise to a characteristic inward-rectifying membrane current (I\textsubscript{CRAC}). The functional CRAC channel is formed by multimerization of the 4-transmembrane CRACM1 protein (denoted Orai1 by Feske et al.1), and the exact number of transmembrane units to form the functional channel has yet to be elucidated.2 The ion selectivity of CRAC is determined by specific glutamate and aspartate residues at the outer mouth of the channel, as for other Ca\textsuperscript{2+}-selective channels.3,4 CRAC channels are widely distributed in nonexcitable vertebrate cells, including T lymphocytes, mast cells, macrophages, and fibroblasts (see Parekh4), and also in neuroblastoma cells.5 In addition, I\textsubscript{CRAC}–like currents have been demonstrated in invertebrates, Drosophila S2 cells,6 and Caenorhabditis elegans.7 Activation of CRAC channels is triggered by any procedure that reduces the concentration of free Ca\textsuperscript{2+} inside intracellular endoplasmic reticulum (ER) Ca\textsuperscript{2+} stores. In vivo, this is caused by activation of Ca\textsuperscript{2+} channels in the ER membrane in response to receptor binding of the intracellular messenger Ins(1,4,5)P\textsubscript{3}. The resultant inward Ca\textsuperscript{2+} flow across the plasma membrane is mediated by CRAC channels, which partially inactivate.8 The signaling pathway comprises 2 recently identified integral membrane proteins: (1) STIM1, which is located in the ER membrane and acts as a sensor of intraluminal ER Ca\textsuperscript{2+} concentration,9 and (2) Orai1, which is located in the plasma membrane and is considered a CRAC channel subunit.1

The occurrence of CRAC channels in T lymphocytes and their demonstrated role in immunodeficiency10 direct interest toward pharmacological interaction with CRAC channel function. One example is the role of CRAC channels in severe combined immune deficiency (SCID) syndrome. SCID is caused by a heterogeneous group of genetic disorders, one of which is associated with a missense mutation in human Orai1.1 Furthermore, CRAC channels have been linked to Alzheimer’s disease (see Parekh and Putney8). Therefore, CRAC channels most likely have implications for pathophysiology and consequently may become...
important targets for future drug development. Due to an extremely low single-channel conductance (estimated to ~10 fS by noise analysis\textsuperscript{11}), patch-clamp studies of CRAC channels are conducted in the whole-cell configuration.

We have employed the automated QPatch patch-clamp system to examine the basic functional properties of CRAC expressed in cultured RBL-2H3 cells. Specifically, the aim was to explore whether an automated higher throughput patch-clamp technique constitutes an efficient tool for the characterization of essential physiological CRAC channel properties, as well as for CRAC channel compound screening. Targeting these aims included characterizing the pharmacological effects on whole-cell CRAC currents of a number of compounds known to activate CRAC channels by reducing the free Ca\textsuperscript{2+} concentration in the intracellular ER stores (EGTA, Ins(1,4,5)P\textsubscript{3}, ionomycin, and thapsigargin) or to inhibit the channels (SKF-96365, 2-APB). Potency values for these I\textsubscript{CRAC} blockers are similar to reported values from conventional patch-clamp experiments.

The QPatch system enables simultaneous execution of 16 or 48 independent I\textsubscript{CRAC} patch-clamp experiments in a highly uniform and reproducible manner, and the QPatch can be operated continuously and unattended for several hours.\textsuperscript{12,13} This article describes experiments conducted with the 16-channel QPatch patch-clamp system. Depending on the applied voltage and compound application protocols, QPatch throughput is at least 10- to 30-fold higher than the manual patch clamp. Although a conventional patch-clamp experiment takes ~30 min for 1 cell (and hence 1 IC\textsubscript{50} value), the QPatch can obtain 8 to 12 IC\textsubscript{50} values in the same period. Moreover, the QPatch can operate for several hours into the evening, extending its throughput capability. A recent case study with voltage-gated calcium channels confirmed that the QPatch 16 increases throughput compared with the conventional patch clamp by ~10-fold.\textsuperscript{14}

**MATERIALS AND METHODS**

**Cells**

RBL-2H3 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco-BRL, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were grown at 37 °C in 5% CO\textsubscript{2} to approximately 80% confluence and subsequently split. Cells used for experiments on the QPatch also had a confluence of approximately 80%. It was previously observed that if the overall confluence of cells was higher or much lower than this, it was difficult to get high-resistance seals. Prior to the QPatch experiments, cells were washed with phosphate-buffered saline (PBS) and loosened from the bottom of the culture flask with trypsin. To obtain optimal isolated cells in suspension for experiments, we kept the trypsinized cells in the incubator at 37 °C and 5% CO\textsubscript{2} for approximately 2 min. The morphology of the cells was visually inspected with a microscope. If the cells appeared round and single celled, they were detached from the culture flask by gently tapping the sides. If the morphology of the cells was not round, the cells were returned to the incubator for a few more minutes. Following this treatment, the cells easily separated from each other after resuspension in the serum-free QPatch storage medium containing CHO-S-SFM II (Gibco-BRL, Life Technologies, Carlsbad, CA) supplemented with 25 mM HEPES, 0.04 mg/mL soybean trypsin inhibitor, and 1% penicillin/streptomycin. The cell suspension was transferred to the cell tank stirrer on the QPatch platform and was used for up to 5 h after harvest.

**Solutions and chemicals**

The extracellular solution contained (in mM) the following: 140 NaCl, 2.8 KCl, 10 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, 10 CsCl, 10 HEPES, and 10 mM glucose. The pH was adjusted to 7.4 with NaOH, and the osmolarity was adjusted to 340 to 350 mOsm with sucrose. The intracellular solution contained (in mM) the following: 145 glutamate, 8 NaCl, 1 MgCl\textsubscript{2}, 10 HEPES, and 10 EGTA or 10 BAPTA. Just before an experiment, 4 mM Na\textsubscript{2}ATP was added, and the pH was adjusted to 7.2 with CsOH. The osmolarity was adjusted to 330 to 340 mOsm with sucrose.

When D-myo-inositol 1,4,5-tris-phosphate (Ins(1,4,5)P\textsubscript{3}) was used as an activator, 20 μM was added to the intracellular solution on the day of use. The CRAC channel activators Ins(1,4,5)P\textsubscript{3}, ionomycin, and thapsigargin and the I\textsubscript{CRAC} channel blockers 2-aminoethyl diphenylborinate (2-APB) and 1-[β-(3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-imidazole hydrochloride 1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole (SKF-96365) were purchased from Sigma (St. Louis, MO). Stock solutions of activators and blockers were prepared in DMSO and diluted to the final concentration in the extracellular solutions. The final DMSO concentration was 0.1%.

**Electrophysiology**

All experiments were carried out with the QPatch 16, which performs 16 parallel and independent patch-clamp recordings on a disposable QPlate. For details on QPatch operation and QPlate properties, see Willumsen\textsuperscript{13} and Mathes.\textsuperscript{12} Prior to these patch-clamp experiments, cells were prepared automatically by the QPatch. Cells in the storage medium were transferred from the QPatch cell tank stirrer to an onboard mini centrifuge (QFuge). Cells were spun down, the storage medium was removed, and the pellet was washed with extracellular solution. The washing step was repeated, and the cell pellet was finally resuspended in the extracellular solution. Positioning of the cells on the measurement site in the QPlate was done by application of negative hydrostatic pressure. Gigaseal and whole-cell configurations were achieved according to the settings specified in the assay. For these experiments, suction pulses and suction ramps were used to establish
the whole configuration. Once the whole-cell configuration was established, the membrane resistance value \( R_{\text{mem}} \) was continuously checked. If \( R_{\text{mem}} \) fell outside a reasonable value range (user defined), the experiment was discontinued. Cells were held at a holding potential of 0 mV. Every 3 or 6 s, a voltage ramp from \(-100\) to \(+100\) mV (100 ms duration) was executed. This voltage protocol was used for all experiments. The series resistance \( R_s \) and the capacitance of the cell \( C_{\text{slow}} \) were measured just after whole-cell establishment and typically before execution of the voltage protocol. The QPatch system uses a 16-channel parallel amplifier (Sophion Bioscience, Ballerup, Denmark) for recordings of whole-cell currents. Data were filtered at 1 kHz and sampled at 5 kHz. Due to high gigaseal resistances, leak currents were small, and leak subtraction was generally omitted. All experiments were done at room temperature. The solution exchange time for the QPatch system was 60 to 80 ms. The different liquid applications (saline solutions, compounds, and reference compounds) were performed sequentially at a volume of 5 μL per application according to the application protocol specified in the assay.

Data analysis and presentation

Data were analyzed using the QPatch assay software (Sophion Bioscience). Data are presented as mean ± standard error of the mean (SEM), unless otherwise noted; \( n \) indicates the number of individual cells used to obtain the data.

Current versus time (I-t) plots were made by measuring the current at \(-80\) mV and plotting this current as a function of time. The time for experiment start is the time when the QPlate barcode is registered by the QPatch. Thus, on the I-t plots, the absolute time from experiment start is indicated. A break-in period is shown as the first indicated period in the I-t plots. The break-in period started upon establishment of the whole-cell configuration. Throughout the break-in period, the voltage protocol was continuously applied, and the developing ICRAC was recorded in the presence of the extracellular solution. From the time course, the initial delay of current was measured as the time difference from the first data point to the data point where the current started continuously to increase. The time to full activation was determined from the I-t plots as the time window for onset of current development to stable maximal current. All figures shown represent screen shots of original figures generated by the QPatch assay software.

RESULTS

To measure the small inward-rectifying CRAC whole-cell current, one must obtain high seal and whole-cell resistances (i.e., >1 GΩ). Prior to the study, growth conditions and assay protocol settings had been optimized to yield RBL-2H3 cells suited for patch-clamp studies on the QPatch (see Materials and Methods). As a consequence, the success rate for obtaining high-resistance seals was high, and the average seal resistance was \( 2535 \pm 323 \) MΩ \((n = 144, \text{data obtained from 9 different QPlates})\). It was observed that there was a positive correlation between the final concentration of cells applied to the QPlate and the high-resistance seal success rate (i.e., higher density of cells increased the number of gigaseals; data not shown). Guided by this observation, we prepared the cells to a final optimal concentration of \( \approx 5 \times 10^6 \) cells per mL, and a 5-μL cell suspension was applied to each measurement site. Figure 1 summarizes the statistics from a typical QPlate. The average membrane resistance just after establishment of the whole-cell configuration (R whole cell) was \( 3308 \pm 535 \) MΩ \((n = 111, \text{data from 9 QPlates from different experimental days})\). In general, nearly 80% of the cells that reached the user-defined seal criteria successfully reached the whole-cell configuration. For the rest of the cells, it was either not possible to establish the whole-cell configuration or the \( R_{\text{mem}} \) was lower than the discard level.

CRAC currents activate by mechanisms that cause release of Ca\(^{2+}\) from internal stores. A common way to activate I\(_{\text{CRAC}}\) experimentally is to passively deplete ER Ca\(^{2+}\) stores with a Ca\(^{2+}\) ion chelator or by activating D-myo-inositol trisphosphate (Ins(1,4,5)P\(_3\)) receptors located on the ER. The I\(_{\text{CRAC}}\) current recorded with the QPatch has several key properties that are also observed with traditional manual whole-cell patch-clamp recordings. Figure 2A shows CRAC current activated by passive depletion of Ca\(^{2+}\) from stores using 10 mM EGTA in the intracellular solution. CRAC currents from these experiments were characterized by an initial delay of 10 to 60 s (mean value 29 ± 3 s, \( n = 32 \)) followed by a steep rise in current, which lasted 45 to 150 s prior to full activation (mean value 80 ± 5 s, \( n = 32 \)). A typical time course of the normalized current is seen in Figure 2B. At \(-80\) mV, the EGTA-induced current level was in the range of 3 to 5 pA/pF (data not shown). The experiments demonstrate that I\(_{\text{CRAC}}\) is activated directly in response to passive depletion of the intracellular Ca\(^{2+}\) stores. Figure 2C shows a characteristic offline-corrected current sweep where the current recorded just after breakthrough to the whole-cell configuration (break-in period) was subtracted from the maximal EGTA-induced current response. Characteristic biophysical features of I\(_{\text{CRAC}}\) such as inward rectification, small inward current amplitude, and the negligible current at 0 mV are clearly demonstrated.

Another approach to activate I\(_{\text{CRAC}}\) is to deplete the intracellular Ca\(^{2+}\) stores by application of an intracellular messenger. We used the second-messenger molecule Ins(1,4,5)P\(_3\) in the intracellular solution, which binds to the InsP\(_3\) receptor on the membrane of the ER and opens Ca\(^{2+}\) channels, causing release of Ca\(^{2+}\) into the cytoplasm. In these experiments, the current developed almost instantaneously with no delay phase (Fig. 3). The time course for current activation was 50 to 150 s (mean value 75 ± 29 s, \( n = 14 \)). The activated current was blocked by the Ins(1,4,5)P\(_3\)-receptor antagonist 2-APB, which is reported to inhibit I\(_{\text{CRAC}}\) in RBL cells, Jurkat T cells, and Drosophila S2 cells at concentrations >10 μM.\(^{6,15-17}\)
Activation of I_{CRAC} by combined application of 10 mM BAPTA and 10 μM Ins(1,4,5)P_3 in the intracellular medium is demonstrated in Figure 4A. Immediately after QPatch established a whole-cell recording configuration, the inward-rectifying I_{CRAC} current developed and reached its maximum value within 100 s. To monitor the activation time course of I_{CRAC}, we plotted the current value at –80 mV against time (Fig. 4B). The QPatch was also used to record I_{CRAC} in cells where the ER stores were passively depleted with 15 mM BAPTA in the intracellular solution (Fig. 4C). Note the slower ICRAC activation kinetics with passive depletion compared with the Ins(1,4,5)P_3 receptor activation in panel B.

CRAC is a highly selective Ca^{2+} channel. The channel is permeable to monovalent cations only when all divalent cations are removed from the extracellular medium. We used this property of the channel to confirm the I_{CRAC} current recorded by QPatch. First, Na^+ ions were replaced with an equal molar concentration of NMDG-Cl in the external medium. Shown in Figure 4D, the I_{CRAC} current was still present even in the absence of extracellular Na^+ ions. The average I_{CRAC} current in NaCl (n = 9) or NMDGCl (n = 7) external medium was 2.2 ± 0.2 (SD) and 2.4 ± 0.3 pA/pF, respectively. I_{CRAC} is blocked in rank order by Ba^{2+} < Cd^{2+} < Gd^{3+} ions.

We tested the inhibition of I_{CRAC} by Ba^{2+}, Cd^{2+}, or Gd^{3+} by including 1 mM of each blocker in the extracellular medium. Figure 4E shows an example of the reversible inhibition of I_{CRAC} by 1 mM Cd^{2+}. The partial return of I_{CRAC} during the saline wash is likely due to the incomplete washout of Cd^{2+} ions. Figure 4F summarizes the rank-order block of I_{CRAC} by Ba^{2+} < Cd^{2+} < Gd^{3+} ions.

The Ca^{2+} ionophore ionomycin is a classic tool to deplete Ca^{2+} stores by making the ER membrane permeable to Ca^{2+}. After a short period (~100 s) of dialysis of the cell with standard intracellular solution, ionomycin was applied extracellularly. This increased the current to maximal level within 1 min (Fig. 5A). Following activation of the current, the CRAC channel blocker SKF-96365 was added, and the ionomycin-induced current was almost completely abolished (Fig. 5B).

The final approach used to activate CRAC channels was to apply the SERCA pump inhibitor, thapsigargin. Ca^{2+} pump inhibition prevents reuptake of Ca^{2+} into the intracellular stores, causing store depletion and subsequent activation of CRAC channels in the plasma membrane. Figure 5C shows an

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<th>R seal (MΩ)</th>
<th>R wc (MΩ)</th>
<th>Rs (MΩ)</th>
<th>Cslow (pF)</th>
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FIG. 1. QPlate statistics. The table lists for each QPlate position (A1 to H2) the electrical resistance at each measurement site (R chip), the cell-attached configuration resistance (R seal), the whole-cell resistance (R wc), the series resistance value (Rs), the estimated cell capacitance value (Cslow), and the whole-cell duration. The histogram below illustrates that the whole-cell resistances (green bars) were generally higher than the cell-attached resistances (blue bars).
example where CRAC channels were activated by extracellular application of thapsigargin. The effect of thapsigargin was measured after an initial dialysis with 10 mM EGTA for approximately 100 s. In these experiments, the delay before current activation was almost eliminated upon application of thapsigargin, as described by Fierro and Parekh. The current peaked within 40 to 100 s (mean \(65 \pm 21\) s, \(n = 6\)) and was blocked by application of 2-APB (Fig. 5C). In some experiments, full current activation was obtained during the initial dialysis with the internal buffer solution, and application of thapsigargin did not increase the current amplitude (data not shown). In a few \(I_{\text{CRAC}}\) activation experiments, it was observed that the current inactivated or “ran down” substantially following maximal activation (Fig. 5D).

The number of known blockers of \(I_{\text{CRAC}}\) current is limited. We tested the effect of SKF-96365 on \(I_{\text{CRAC}}\) and estimated the IC\(_{50}\) value from the current block. The current was activated by passive store depletion using EGTA. SKF-96365 was added in 4 increasing concentrations (from 0.1 to 100 \(\mu\)M in 10-fold increments), which blocked the current with increasing potency.
FIG. 3. I-t plot from $I_{\text{CRAC}}$ activated by Ins(1,4,5)P$_3$ (20 μM) and EGTA (10 mM) in the intracellular solution (top bar) and subsequently inhibited by 2-aminoethyl diphenylborinate (2-APB; 30 μM). The current was measured at −80 mV and plotted as a function of time.

Previous experiments have shown that full inhibitory effect at each concentration is first obtained after an additional application. Consequently, each concentration of SKF-96365 was applied twice, and the effect was measured at the end of the second application period. Current recordings from a representative experiment are shown in Figure 6. From the I-t plot (Fig. 6A), the current block was fitted by a Hill equation to determine the IC$_{50}$ value (Fig. 6B). The mean IC$_{50}$ value was 4.7 ± 1.1 μM ($n = 9$). We were not able to test the effect of 300 μM because the compound precipitated at this concentration (sonication, vortexing, heating, and increased DMSO concentration could not bring it into solution). Because 100 μM of SKF-96365 blocks the current close to initial background level, full compound effect is most likely obtained with this concentration.

The effect of the IP$_3$ receptor antagonist 2-APB was also tested systematically. This compound has previously been shown to modulate $I_{\text{CRAC}}$ by a mechanism independent of interaction with IP$_3$ receptors. $^{17}$ 2-APB was tested at 4 different concentrations (applied extracellularly). The CRAC current was activated by Ins(1,4,5)P$_3$ and BAPTA in the intracellular solution. Application of 3.3 μM 2-APB paradoxically increased the current, whereas 10, 30, and 100 μM blocked the current (Fig. 6D). The inhibitory effect of 100 μM 2-APB was comparable to the effect observed at 30 μM (results not shown). From the I-t plot, the current block was fitted to a Hill fit, and the IC$_{50}$ value was determined to 7.5 ± 0.7 μM ($n = 9$). In control experiments, the vehicle (DMSO) was added and had no modulatory effect on the current (data not shown).

DISCUSSION

CRAC channels have been found in a number of nonexcitable vertebrate cells, including T lymphocytes and other blood cell types. CRAC channels are activated by depletion of intracellular ER Ca$^{2+}$ stores. In many cell types, store depletion is a fundamental element in the signaling pathways associated with cellular processes such as cell proliferation, motility, and secretion, and it is believed that CRAC channels possess the potential of becoming an important target for pharmacological agents, especially in the therapeutic area of immunology. $^{20}$ The very low conductance of CRAC channels prohibits direct single-channel patch-clamp studies, whereas whole-cell studies are feasible (see Parekh and Putney$^{8}$).

In the present study, we have explored whether CRAC channels may be targeted with automated patch-clamp technology. The QPatch 16 patch-clamp system, which was primarily developed to increase experimental throughput in commercial ion channel drug screening, was used for all experiments. Here we employ the system in a basic scientific study of ion channel properties. Importantly in this regard, once an experimental protocol has been defined and specified in the software, the automated patch-clamp system operates unmanned for hours, and data are retrieved, processed, and presented in a user-defined format.

CRAC channels were activated by 4 different procedures: (1) Ins(1,4,5)P$_3$, which is the natural physiological activator and activates Ca$^{2+}$ channels in the ER membrane; (2) EGTA, which chelates cytosolic Ca$^{2+}$, causing Ca$^{2+}$ to be depleted from the stores; (3) ionomycin, which is a Ca$^{2+}$ ionophore that is inserted in the ER membranes, causing store depletion; and (4) thapsigargin, which inhibits the Ca$^{2+}$ ATPase in the ER membrane, inhibiting Ca$^{2+}$ reuptake into the stores. All these procedures led to activation of CRAC channels (Figs. 2-6). The resulting $I_{\text{CRAC}}$ could be inhibited by CRAC inhibitors (2-APB or SKF-96365), as discussed below. Generally, $I_{\text{CRAC}}$ activation was faster when elicited with Ins(1,4,5)P$_3$ than with passive store depletion (Figs. 2-4).

The most striking observed difference for activation of $I_{\text{CRAC}}$ was the time delay before current activation. Using passive depletion with EGTA to activate $I_{\text{CRAC}}$ resulted in a distinct time delay (mean value 29 ± 3 s, $n = 32$) before onset of current development. This delay was negligible for experiments with Ins(1,4,5)P$_3$, ionomycin, or thapsigargin compared with experiments using EGTA, as reported by others. $^{19}$

Rundown or inactivation was a characteristic feature of $I_{\text{CRAC}}$ activation. It ranged from negligible (e.g., in Fig. 2B) to almost
complete elimination of I_{CRAC} (e.g., in Fig. 5D) and could be a consequence of CRAC desensitization or replenishment of Ca^{2+} in the stores. The fact that the current could be reactivated by thapsigargin suggests that the observed inactivation is a consequence of partial store refilling. Another interpretation of the thapsigargin enhancement of I_{CRAC} in these experiments is incomplete activation of I_{CRAC} plus Ca^{2+}-dependent inactivation of the current. This interpretation would be consistent with the previously reported observation that interfering with mitochondrial Ca^{2+} uptake “unmasked” putative Ca^{2+}-dependent inactivation of I_{CRAC}.21

The effects of 2 CRAC inhibitors, SKF-96365 and 2-APB, were characterized. SKF-96365 yielded regular concentration-response relationships (Fig. 6A,B). The mean estimated IC_{50} value of 4.7 ± 1.1 μM (n = 9) is similar to previously published values.22,23 The action of 2-APB was complex. Application of a low concentration of 2-APB (3.3 μM) increased rather than reduced the inward steady current level. This behavior is in accordance with observations previously reported from mammalian and Drosophila S2 cells.6,17 The mean estimated IC_{50} value was 7.5 ± 0.7 μM (n = 9), which is similar to the values reported in the literature (~10 μM).5,23 The high concentrations

**FIG. 4.** Activation time course for I_{CRAC}. (A) The activation of I_{CRAC} with an intracellular solution containing 10 μM Ins(1,4,5)P3 and 10 mM BAPTA. The first 14 ramp traces are superimposed. The time constants for the 1st and 14th traces are indicated. (B) I-t plot from the current values at –80 mV for all 224 traces recorded in panel A. (C) The activation time course of I_{CRAC} with an intracellular medium containing 15 mM BAPTA to passively deplete endoplasmic reticulum stores. (D) The I_{CRAC} current recorded in a cell bathed in an external medium with no Na^+ ions. NaCl was replaced with NMDG-Cl. I_{CRAC} was activated by passive depletion with 15 mM BAPTA in the internal buffer solution. (E) The block of I_{CRAC} with 1 mM CdCl₂; 15 mM BAPTA was used to activate I_{CRAC} by passive depletion. (F) The blocking efficiency of I_{CRAC} by 1 mM BaCl₂, CdCl₂, or GdCl₃. Seven to 8 cells (±SD) were tested for each cation.
of 2-APB (≥10 μM) always blocked the current but never induced a transient enhancement of the current, as has been reported from other studies. These discrepancies among observations indicate that some of the 2-APB effects may be dependent on the experimental conditions.

We conclude that CRAC channels can be targeted successfully with automated patch-clamp technology. Due to the small magnitude of the CRAC currents, it is unlikely that automated high-throughput patch-clamp systems based on sub-gigaohm seals may be suitable for assessment of drug effects on CRAC channels.

In this article, essential physiological properties were demonstrated with a high degree of confidence, and drug-screening protocols were executed that led to IC_{50} values similar to literature values based on the conventional patch clamp. The advantages of using the automated patch clamp for the recordings were: (1) highly increased experimental throughput, (2) uniform and reproducible execution of experiments, and (3) unbiased interpretation of data automatically analyzed by the system software based on predefined criteria. The results demonstrate that the automated patch clamp constitutes a powerful tool for pharmacological characterization of CRAC channels and thus may...
become instrumental in the development of novel compounds aiming at the treatment of diseases involving store-operated Ca\textsuperscript{2+} channels.

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Address correspondence to:

Chris Mathes, Ph.D.
Sophion Bioscience A/S
Baltorpvej 154
DK-2750 Ballerup, Denmark

E-mail: cma@sophion.com