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Electrophysiological characterization of I_{CRAC} in rat basophilic leukemia cells (RBL-2H3) using Automated Patch Clamp

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Introduction

Rat basophilic leukaemia (RBL) cells endogenously express calcium-release-activated-calcium (CRAC) channels (1). CRAC channels are activated by depletion of intracellular calcium stores, via the involvement of STIM-1 (stromal interaction molecule) sensing the depletion of the stores and travelling to the cell membrane activating the channel (2, 3). Several approaches can be used to deplete calcium stores ultimately leading to activation of I_{CRAC}. The present work was to develop assays using different depletion strategies on the QPatch automated patch clamp platform.

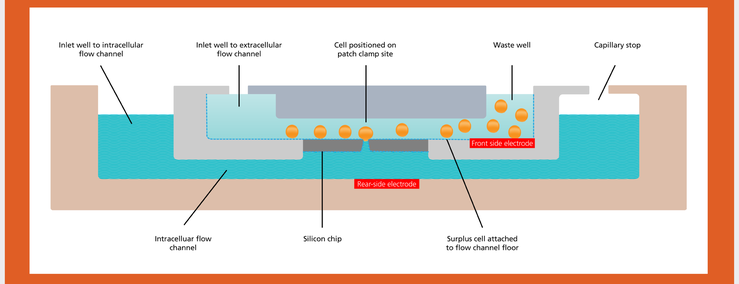
Summary

Endogenously expressed Icrac can reliably recorded on the QPatch automated patch clamp platform. It was possible to activate I_{CRAC} using different strategies

1. Activation by passive depletion,
2. Activation with IP3,
3. Activation with ionomycin,
4. Activation with thapsigargin

Methods

Electrophysiology: All experiments were carried out on QPatch 48 (Sophion Bioscience A/S). Complete solution exchanges were accomplished using the microfluidic flow channels of the QPlate consumable as shown below.



Solutions: The intracellular solution contained (in mM): 145 Glutamate*, 8 NaCl, 1 MgCl2, 10 HEPES, 10 BAPTA (or EGTA). pH was adjusted to 7.2 with CsOH and osmolarity was adjusted with sucrose to 320 mOsm.

*L-Glutamic acid (G8415 from SIGMA).

When IP3 was used as the activator 20 µM was added to the intracellular solution on the day of experiments.

The extracellular solution contained (in mM): 140 NaCl, 2.8 KCl, 10 CaCl2, 2 MgCl2, 10 CsCl, 10 HEPES, 10 mM glucose. pH was adjusted with NaOH to 7.4 and osmolarity was adjusted with sucrose to 350 mOsm.

Blockers, 2APB, SKF-96365 and YM-58483 (3,5-Bis(trifluoromethyl)pyrazole derivative (BTP2) were dissolved in the extracellular solution.

Cells: RBL-2H3 cells from ATCC were grown according to the SOP from Sophion Bioscience.

Voltage protocol: 100 ms long voltage ramps from -100 to +100 mV were applied with an intersweep interval of either 3 or 6 s. Cells were held at 0 mV between the sweeps.

Analysis: All analysis was performed using the Sophion Assay Software. I_{CRAC} was evaluated at -80 mV

References

1. Hoth M, Penner R: Depletion of intracellular calcium stores activates a calcium current in mast cells, Nature, 1992, vol. 355, 353-356.
2. Zhang SL, Ying Y, Roos J, Ashot Kozak J, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD: STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane, Nature, 2006, Vol 437(6), 902-905.
3. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP: CRACM1 is a Plasma Membrane Protein Essential for Store-Operated Ca^{2+} Entry, Science, 2006, vol. 312, 1220-3.

Results and discussion

Activation of I_{CRAC} by passive depletion

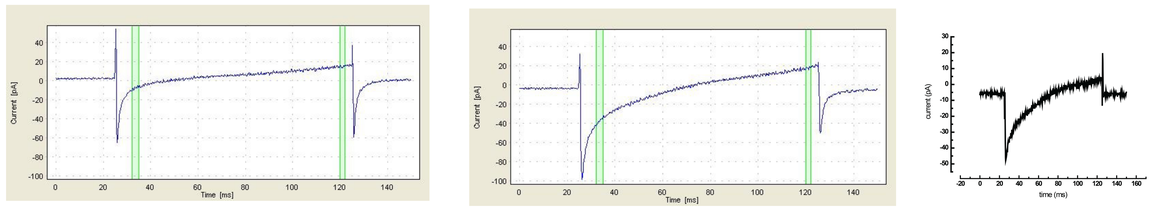


Fig. 1: **I_{CRAC} activation via passive depletion in RBL-2H3 cells:** Passive depletion of internal Ca^{2+}-stores takes advantage of the constant leakage of Ca^{2+} from the endoplasmatic reticulum (ER). The Ca^{2+} is chelated by EGTA or BAPTA and will thus not be available to return to the ER via the Ca^{2+}-ATPase in the ER membrane. Left: Representative current trace before activation of I_{CRAC}. Middle, fully activated I_{CRAC} (passive depletion with 10 mM EGTA). Right: Leak subtracted I_{CRAC}, the inactivated and the activated current traces, where the inactivated sweep was subtracted from the activated.

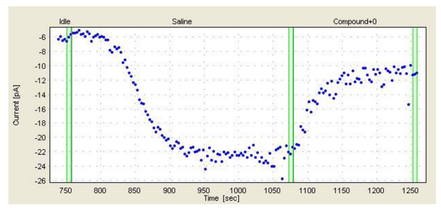


Fig. 2: Typical time course of I_{CRAC} activation with passive depletion (10 mM EGTA), followed by addition of 30 µM 2-APB (compound+0). Shown data was extracted at V=-80mV.

Activation of I_{crac} with IP3

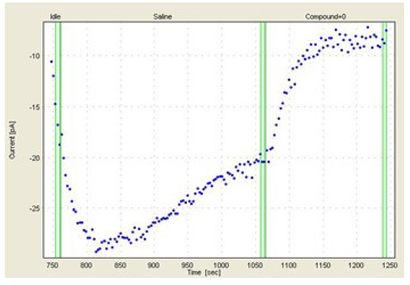


Fig. 3: **Activation of I_{CRAC} with IP3**: Stimulation of IP3-specific receptors in the ER membrane results in the release of Ca^{2+} from the stores which results in the activation of I_{CRAC}. Typical current over time plot recorded from RBL-2H3 cells. 20 µM IP3 was added to the internal solution. Immediate activation of I_{CRAC} was observed after whole cell rupture. Cells were exposed to 30 µM 2-APB at the end of the experiment.

Activation of I_{crac} with ionomycin

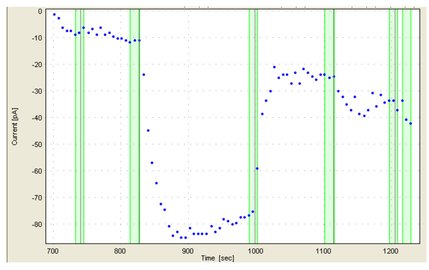


Fig. 4: **Activation of I_{CRAC} using ionomycin**: Externally applied ionomycin leads to incorporation of ionophore into the ER membrane. The Ca^{2+}-permeability of ionomycin results in a depletion of the ER store and eventually an activation of I_{CRAC}.

Typical current response to 4 µM ionomycin. The current was blocked with 100 µM the I_{CRAC} blocker SKF 96365.

Activation of I_{crac} with thapsigargin

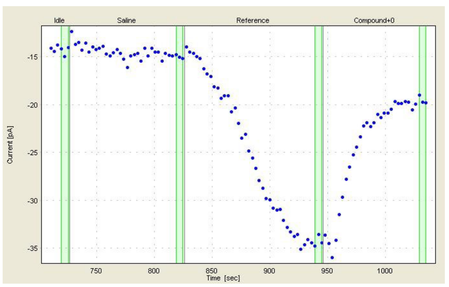


Fig. 5: **Activation of I_{CRAC} using thapsigargin**: External application of the SERCA inhibitor thapsigargin results in a block of the Ca^{2+} reuptake. This in turn depletes the Ca^{2+}-stores and hence results in activation of I_{CRAC}. A typical current over time plot shows the response to externally applied thapsigargin (2 µM). Following activation, the current was blocked with 30 µM 2APB.

IC_{50} determination for YM-58483

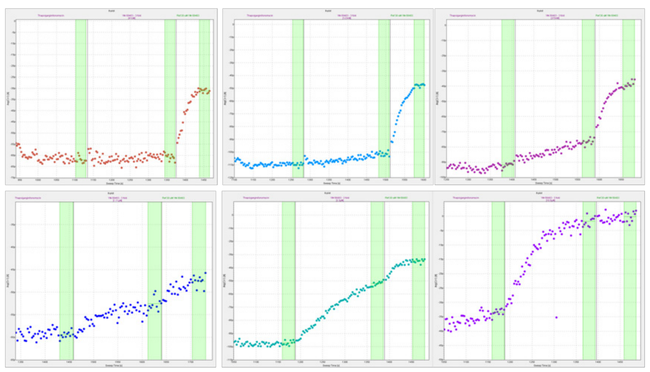


Fig. 6: **I_{CRAC} inhibition by YM-58483**: YM-58483 (3,5-Bis(trifluoromethyl)pyrazole derivative (BTP2) inhibits Ca^{2+} influx by the concerted actions of store-operated Ca^{2+} channels and Ca^{2+}-activated cation channels. I_{CRAC} was activated using 2 µM thapsigargin followed by different concentrations of YM-58483. 30 µM 2APB were used at the end of the experiment as positive control. Shown are six representative current versus time plots (I-T) for increasing concentrations of YM-58483 on different cells. The highest concentration was 30 µM, testing in a 3-fold dilution. The green vertical bars indicate the steady-state current and the points used to calculate the IC_{50} value in the Hill fit.

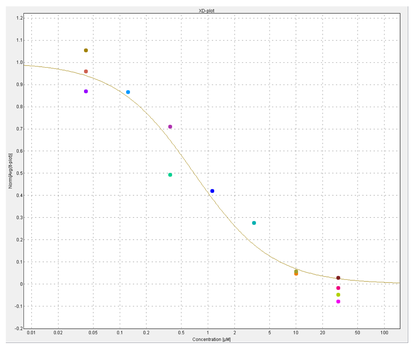


Fig. 7: **Concentration-response curve for YM-58483.** The IC_{50} value of YM-58483 was determined to 587 nM.

Pos.	Primed	Cell att…	Giga…	Whol…	R chip [MΩ]	R seal [MΩ]	R whole-cell…	WC duratio…	Completed …
A1	✔	✔	✔	✔	2.21	30000	15408.3	1082	1
A2	✔	✔	✔	✔	2.1	18137.3	19387.3	1096	1
B1	✔	✔	✔	✔	2.04	19097.9	3443.2	1104	1
B2	✔	✔	✔	✔	2.25	145.2	437.7	1167	1
C1	✔	✔	✔	✔	2.22	21453.1	12082.2	1127	1
C2	✔	✔	✔	✔	2.22	8967.4	3578.5	1135	1
D1	✔				2.51	0	0	0	0
D2	✔	✔	✔	✔	2.01	17898.2	17533.6	1136	1
E1	✔	✔	✔	✔	2.19	77359.3	13497.7	1159	1
E2	✔	✔	✔		2.03	31.5	16.1	0	0
F1	✔	✔	✔	✔	2.16	11333.6	26213.4	1136	1
F2	✔	✔	✔	✔	1.98	6507.2	5500.4	1168	1
G1	✔	✔	✔	✔	2.07	9831.6	9295.6	1188	1
G2	✔	✔	✔	✔	2	2675.4	2267.8	1169	1
H1	✔	✔	✔	✔	2.02	30000	15924.8	1198	1
H2	✔	✔	✔	✔	1.97	11381.7	9277	1199	1
Total	16	15	15	14					14
Succes…	100 %	94 %	94 %	88 %					

Fig. 8: **Performance overview of a typical QPatch experiment.**