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Development of an Automated Patch Clamp Assay for recording STIM1/Orai1 – mediated currents using Qube 384

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Introduction

Calcium (Ca^{2+}) is an important second messenger that is involved in critical processes in cell homeostasis and signal transduction. The ratio between extra- and intracellular Ca^{2+} contributes to setting the plasma membrane potential. Intracellularly, Ca^{2+} is stored in organelles such as Mitochondria and the Endoplasmic reticulum (ER). Depletion of these stores triggers the opening of Ca^{2+} - permeable channels that allow entry of Ca^{2+} into the cell along the electrochemical gradient. This process is called store-operated calcium entry (SOCE). Activation of SOCE happens as a secondary result of ER depletion (ref). ER is being depleted by an inositol-triphosphate (IP3) pathway and phospholipase C cascade, via the IP3 receptor. SOCE can experimentally be activated by elevating intracellular levels of IP3 in the presence of a Ca^{2+} chelator.

The activation of SOCE can directly be measured as a change in clamp current when performing patch-clamp experiments. In this study the automated patch clamp system, Qube 384 was used to record Icrac. Qube 384 is a second-generation automated patch clamp device capable of testing thousands of compounds per day whilst providing true giga-ohm seal quality data. Using the Qube 384 in a drug discovery cascade enables acquisition of mode of action data simultaneous with hit detection during the primary screen, thereby minimizing the need for extended follow-up validations studies.

In this study, we present an assay for STIM1/Orai1. Firstly, we present that Orai1 current can be activated either by exchange of the intracellular solution or alternative by de facto caging an agonist in the IC in the on-cell configuration. Secondly, we pharmacologically validate the assay using the trivalent lanthanide cations blocker lanthanum (La). With this, we demonstrate that Qube 384 also is a versatile patch clamp system, even though it is fully automated and can be running unattended.

Conclusion

In this study, we used the automated patch clamp set-up Qube 384, to develop an assay for measuring the current running through a calcium release-activated channel (Icrac). Icrac is now known to be mediated by two proteins (Orai1 and STIM1), where Orai1 is located at the cell membrane and STIM1 in the membrane of the endoplasmic reticulum (ER).

The automated patch clamp system Qube 384 operates with pre-defined plans (Figure 3 & 7), where it is easy to control whole-cell formation, protocols etc. The agonist (IP3) was added by utilizing two different methods, in two separate experiments sets. Either by the exchange of the intracellular solution or by adding the agonist in the priming internal solution and monitoring the current after rupturing of the membrane. When activating current by exchanging the intracellular solution, the consumable (QChip) must be removed from the amplifier preventing the first seconds after activation to be recorded. This time span immediately after activation can be recorded using the break-in during the experiment approach.

Icrac expression levels were low in the used cells resulting in a relatively small assay window. Nevertheless, the presented assay is well-suited to investigate Icrac function.

References

1) Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: Role in proliferation and migration - May 2009 The FASEB Journal 23(8):2425-37

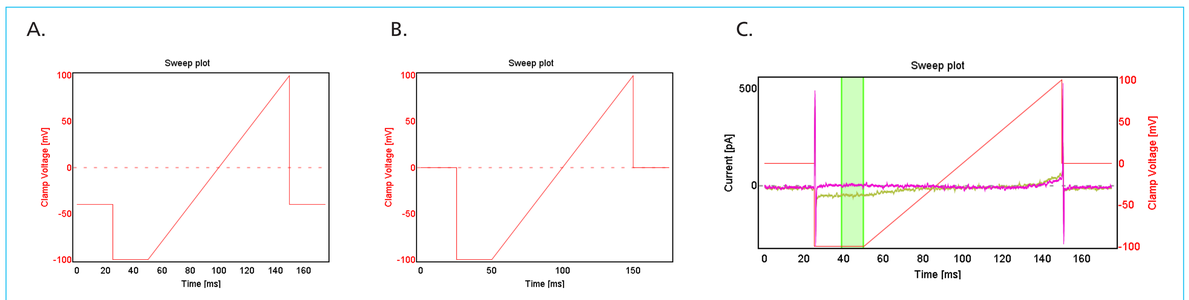

Fig. 1: Voltage protocol and representative calcium current traces. A) Voltage ramp for measuring the calcium current in on cell mode. A depolarization from -40 mv to -100 mV, followed by ramp to +100 mV. B) Experiment voltage protocol. Same as figure 1a, except with different holding potential. C) Example raw current traces recorded from the same well on a single-hole QChip.

Fig. 2: Qube 384 with stacker

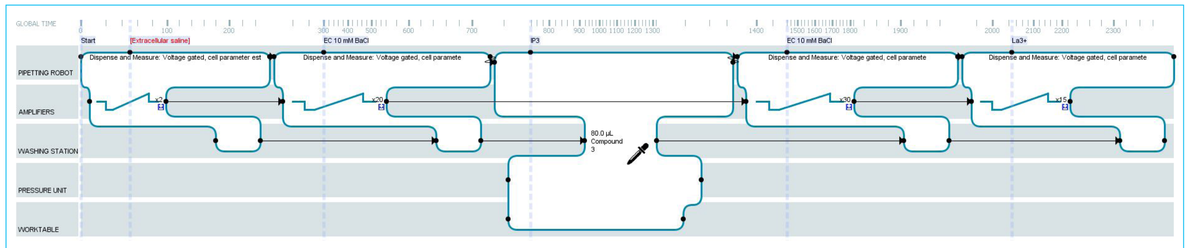

Fig. 3: Experimental set up in Sophion Viewpoint software. At the beginning of each block (liquid period), a defined solution was added to the cells. Following this addition, a voltage protocol was executed. Saline was added during the first liquid periods with different Barium concentration as indicated to foster seal formation. The liquid addition was followed by an application of a voltage protocol. The middle block is the exchange of the intracellular solution. Compound effects were assessed during the two last periods.

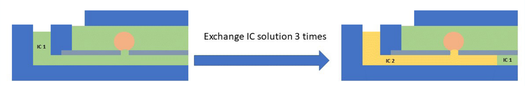

Fig. 4: Schematic representation of the internal solution exchange. In the present study, this feature was used to introduce IP3 as the agonist.

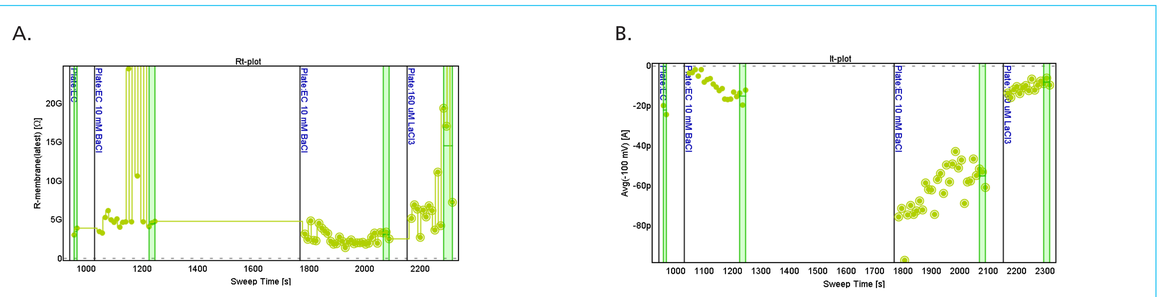

Fig. 5: A) Total resistance and B) current from an example consumable site plotted versus experiment time. For protocol see figure 3. First, two liquid periods contain an exchange of barium concentration for improvement of sealing. Middle liquid period (without data) is when the intracellular solution is being exchanged. Last two liquid period is when the compound is being tested.

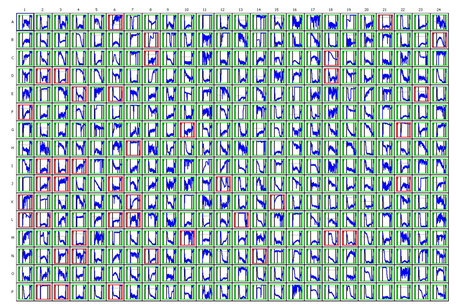

Fig. 6: Plate view showing 384 IT plots recorded from a single hole QChip. Experiments with resistance < 100 MOhm in whole-cell configuration are indicated by a red frame.

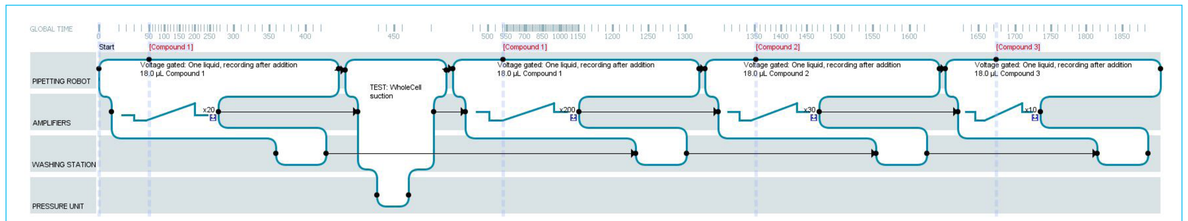

Fig. 7: Experimental set up in Sophion Viewpoint software. The membrane was ruptured during the second block to gain whole-cell access using two pressure pulses. Compound effects were assessed during the three last periods.

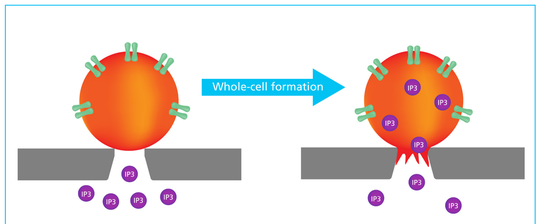

Fig. 8: Schematic representation of the QChip flow channel during rupture of the membrane.

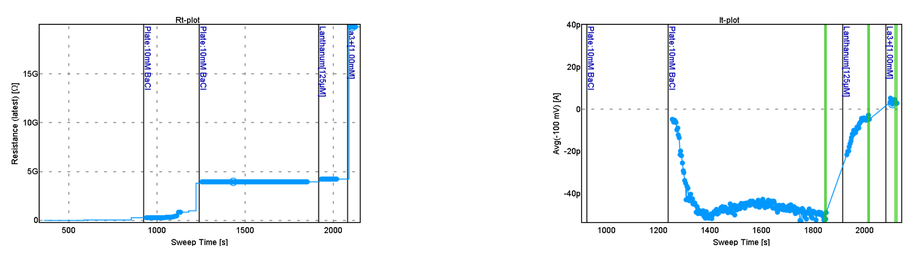

Fig. 9: A) Representative total resistance and B) current versus experiment time plot. The experimental set up is shown in figure 7. The first liquid period contains exchange of barium concentration for improvement of sealing. Whole-cell access is gained during the second liquid period using two pressure pulses. Following membrane rupture. IP3 is entering the cell and activating Icrac. Compound effects were assessed during the last two liquid periods.

Table 1: Schematic overview of result from the assays. Calcium current specified as the current which can be blocked by lanthanum.

	Exchange Intracellular Solution	Whole cell caging of Agonist
Success rate (R_{mem}>100 MΩ)	91 %	89 %
Cells with Ca^{2+} current	54 %	74 %
Average current level	47 ± 2 pA	46 ± 2 pA

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