Application Report:

HEK-hKir2.1 on Patch

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Sophion Bioscience A/S, Baltorpvej 154, DK-2750 Ballerup, Danmark
Phone: + 45 44 60 88 00 Fax: +45 44 60 88 99 E-mail: info@sophion.dk www.sophion.com

hKir2.1 optimized for QPatch

Introduction

The aim of this report is to demonstrate the performance of HEK-hKir2.1 from CreaCell on QPatch HT. The cell line was characterized in terms of biophysical properties such as IV-relationship, effect of extracellular K^+, effect of Ba^{2+} and pharmacologically properties of the channel.

Materials & Methods

Cells

HEK-hKir2.1 cells were grown and harvested according to the SOP's specified from CreaCell (CreaCell.com) and modified for use on QPatch by Sophion Bioscience.

Cell handling on the QPatch HT

HEK-hKir2.1 cells were harvested and placed in the cell-containing facility on the QPatch, the QStirrer. Here, the cells were kept stirred in serum-free medium for up to 4 hours. When an experiment is started on the QPatch, the pipettes pick up 1.5 ml cells from the QStirrer, and transfer them to the centrifuge unit on the platform, the QFuge. The cell pellet is washed twice by the QPatch and resuspended in a user-defined volume of extracellular Ringer's solution ranging from 200-500 µl, depending on cell density.

Ringer's solutions

Extracellular Ringers solution (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose. pH=7,4 (w. NaOH), 305 mOsm (w. sucrose)

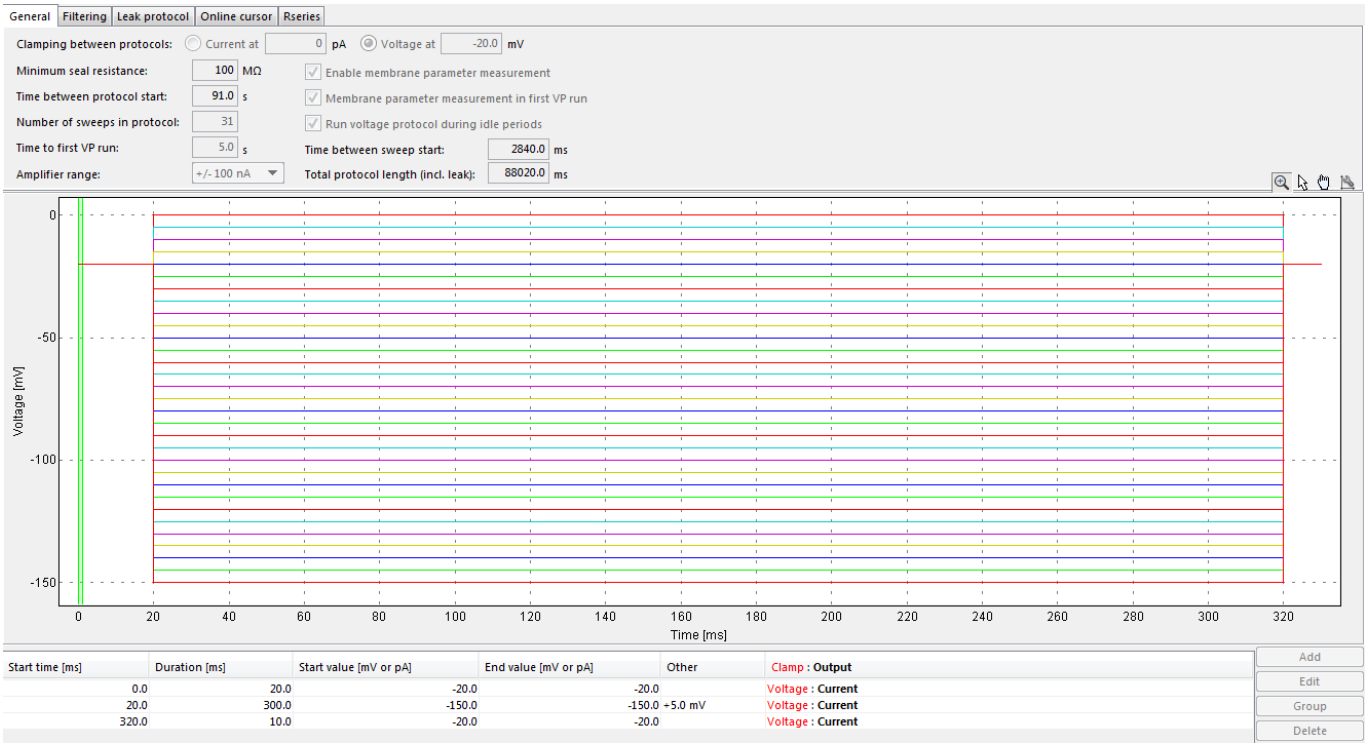
Intracellular Ringers solution (in mM): 5.374 $CaCl_2$, 1.75 $MgCl_2$, 10 EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP. pH=7.2 (w. KOH), 295 mOsm (w. sucrose)

Protocol set-up

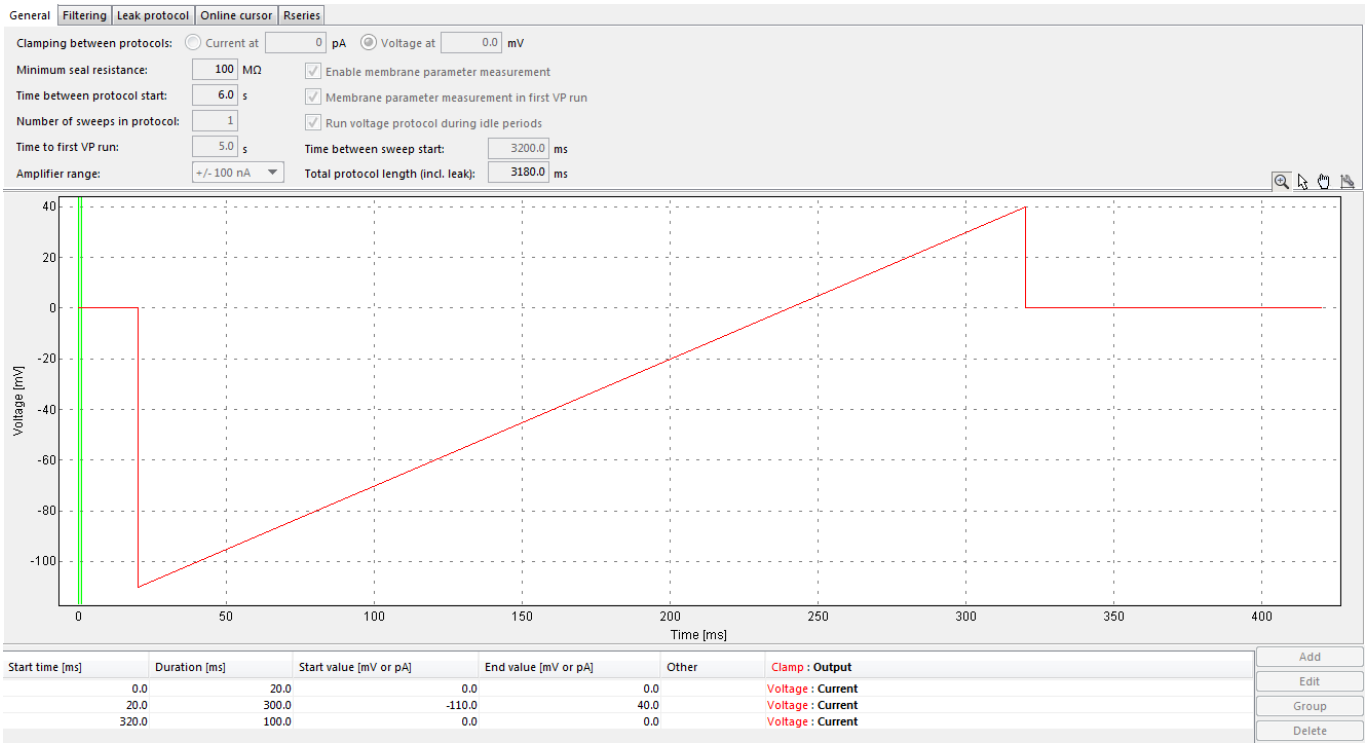
Voltage protocols

For experiments with HEK-hKir2.1 the following protocols were used. 1) Step IV protocol from -150 mV to 0 mV in 5 mV steps, Vhold = -20 mV, 2) Ramp IV protocol from -110 mV to +40 mV, Vhold= 0 mV, 3) short pulse from -20 mV to -110 mV for 300 ms, Vhold = -20 mV and 4) Long ramp protocol with a 500 ms step at -120 mV.

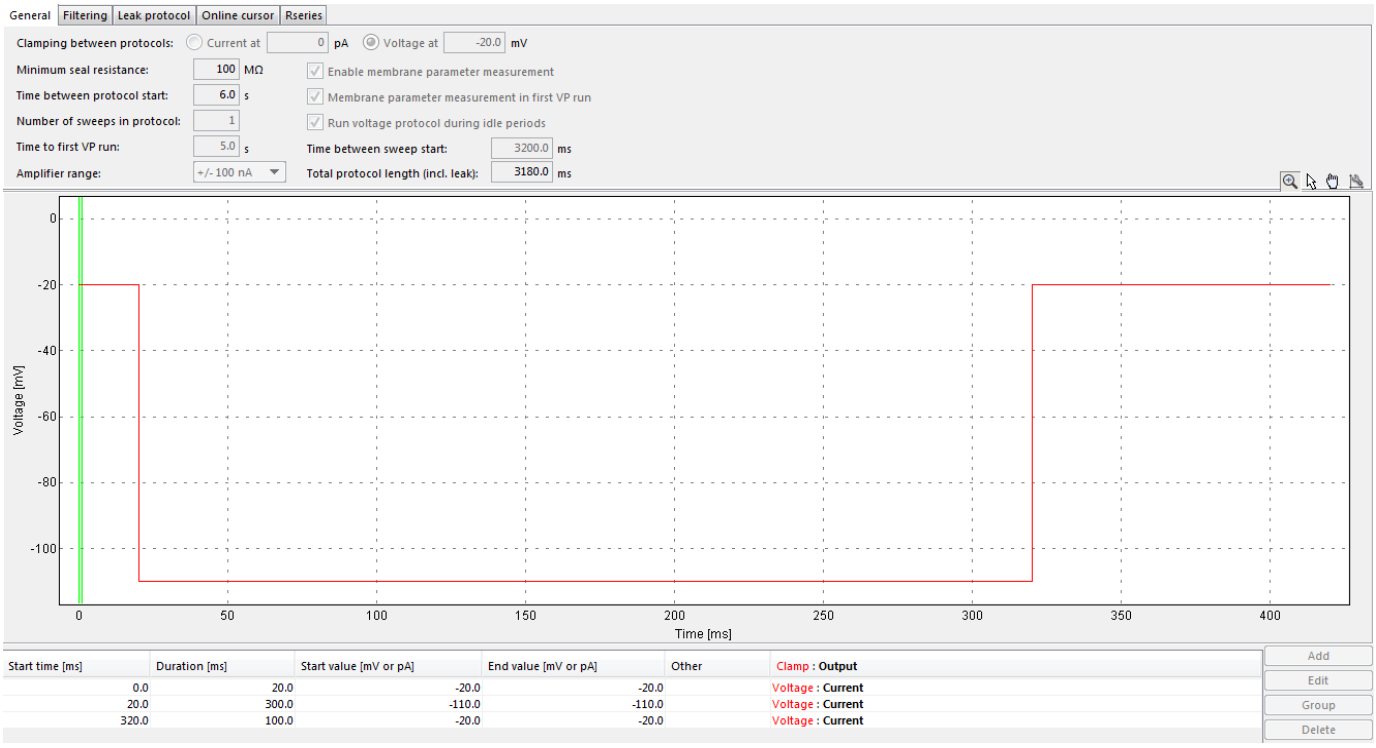
#1 Step IV protocol



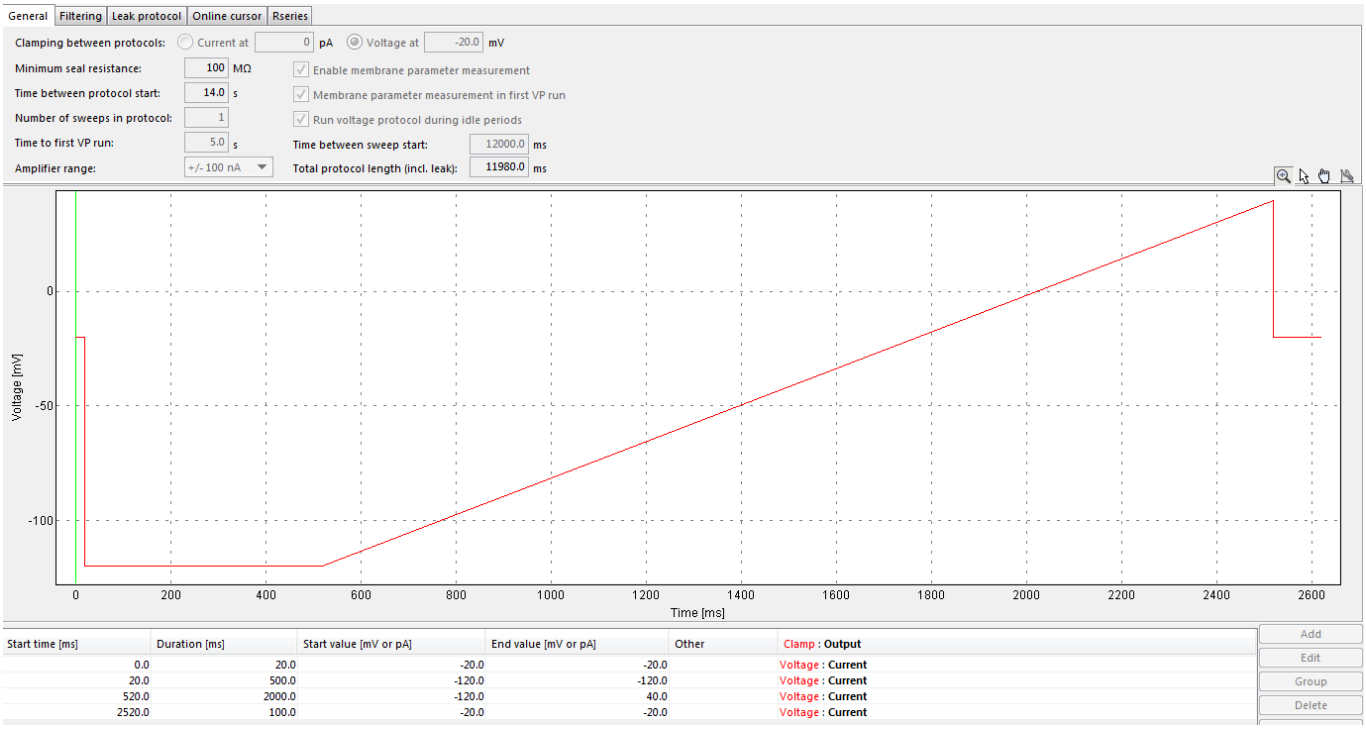
#2 Ramp IV protocol



#3 Short Pulse protocol



#4 Long Ramp protocol



Results

Experiments were carried out using four different protocols in order to study 1) IV-relationship, 2) effect of extracellular K^+ and 3) effect of the modulator chloroethylclonidine (CEC).

The IV-relationships for was studied by applying a step protocol from -150 mV to 0 mV with a holding potential at -20 mV, where the hKir2.1 channels are non-conducting. Stepping to potential negative to the reversal potential result in a large inward current. At potential more positive than Erev result in a small outward current. Raw current traces from the current-voltage relationship experiment are shown in figure 1a. The corresponding IV curve is shown in figure 1b. The reversal potential is observed at Erev=-80.2±0.54 mV, n=8 which corresponds to the theoretical Erev that is -85.9 mV for this channel under these specific experimental conditions. The maximal inward current response Imax=-2824.23±234.24 pA, n=8 was observed at a potential of Vmin=-138.15±1.96 mV, n=8. The small inward current response was observed at Imin=143.81±12.01 pA, n=8 at a potential of Vmax=-62.16±0.99 mV, n=8.

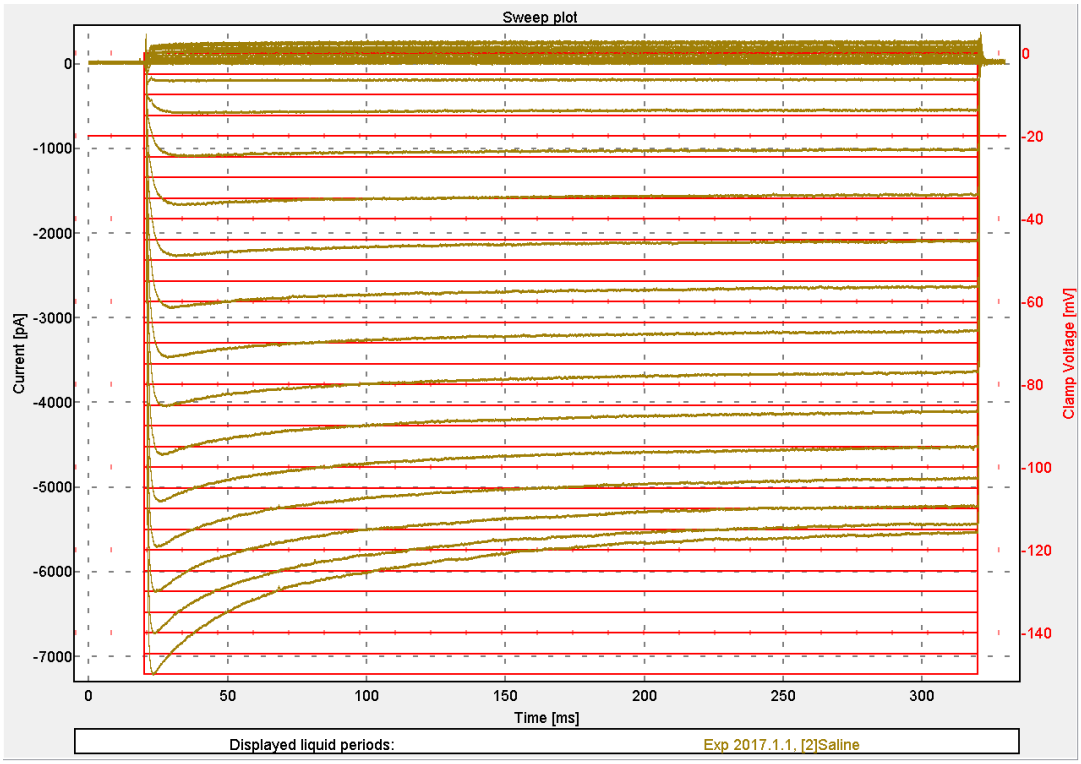


Fig. 1a. Raw hKir2.1 current traces during a step voltage protocol from -150 mV to 0 mV with 5 mV step increment.

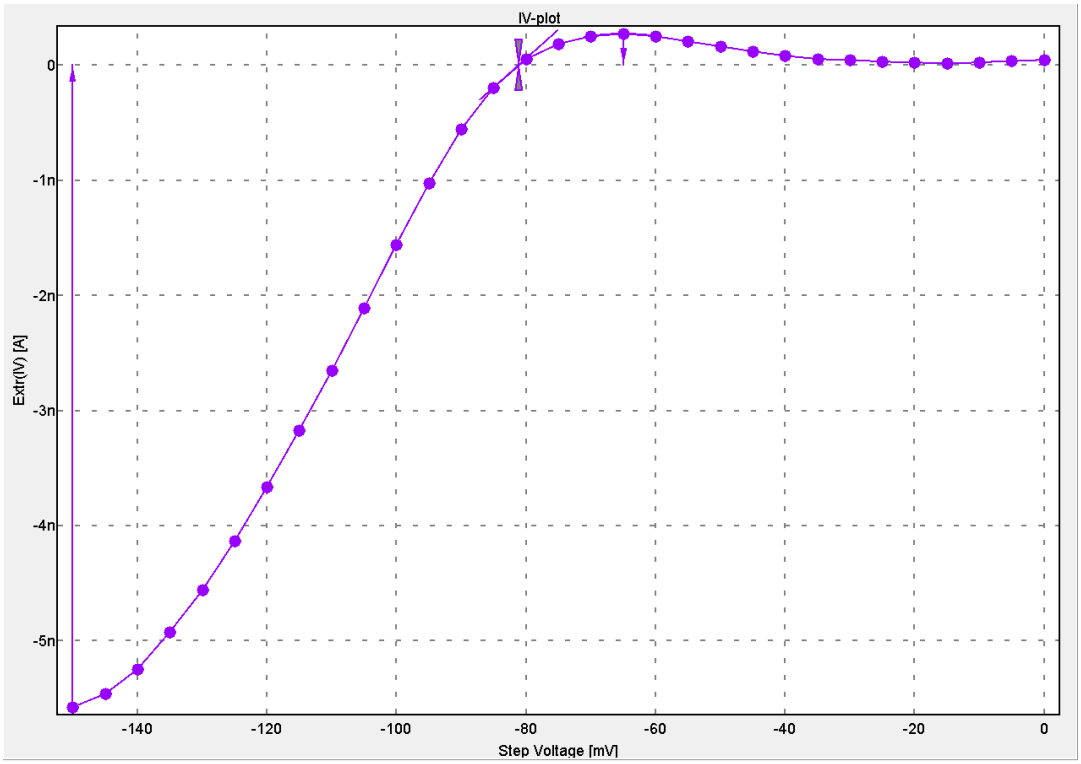


Fig. 1b. IV curve showing a strong inward current a low potentials and a small outward current at potentials positive to Erev.

Effect of external K^+

In the next section, we show the effect of increasing K^+ in the extracellular solution. By increasing the K^+-concentration from 4 mM to 8 and 16 mM K^+ in the extracellular solution, an increase in the conductance is observed in both the inward (negative to Erev) and the outward (positive to Erev) direction in hKir2.1 (Fig. 2). At the same time, a parallel change in Erev was observed in accordance to Nernst equation for 4 mM K^+=-84.2 mV, 8 mM K^+=-69.0 and 16 mM K^+=-55.7 mV (theoretical; 4 mM K^+=-85.9 mV, 8 mM K^+=-68.4 mV and 16 mM K^+=-50.9 mV)[2].

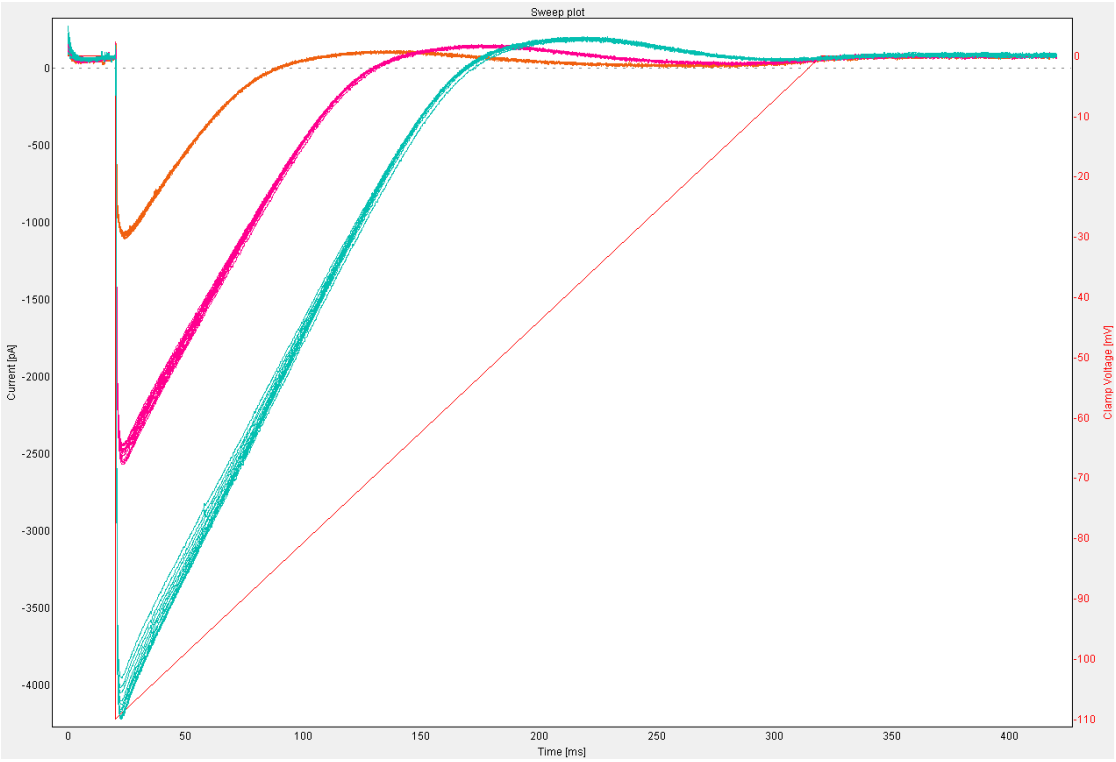


Fig. 2 Effect of extracellular K^+. Raw current traces at 4 (orange), 8 (pink) and 16 mM (blue) external K^+.

Effect of Ba^{2+} on hKir2.1

The hKir2.1 channel is blocked by external Ba^{2+} in a voltage-dependent manner. Fig. 3a shows the effect of 5 increasing concentrations of external Ba^{2+}, starting from 100 mM in a 10-fold dilution. The corresponding Hill fit is shown in Fig. 3b. IC_{50} is estimated to 5.12±0.42 μM, n=10, which corresponds well to literature values[1].

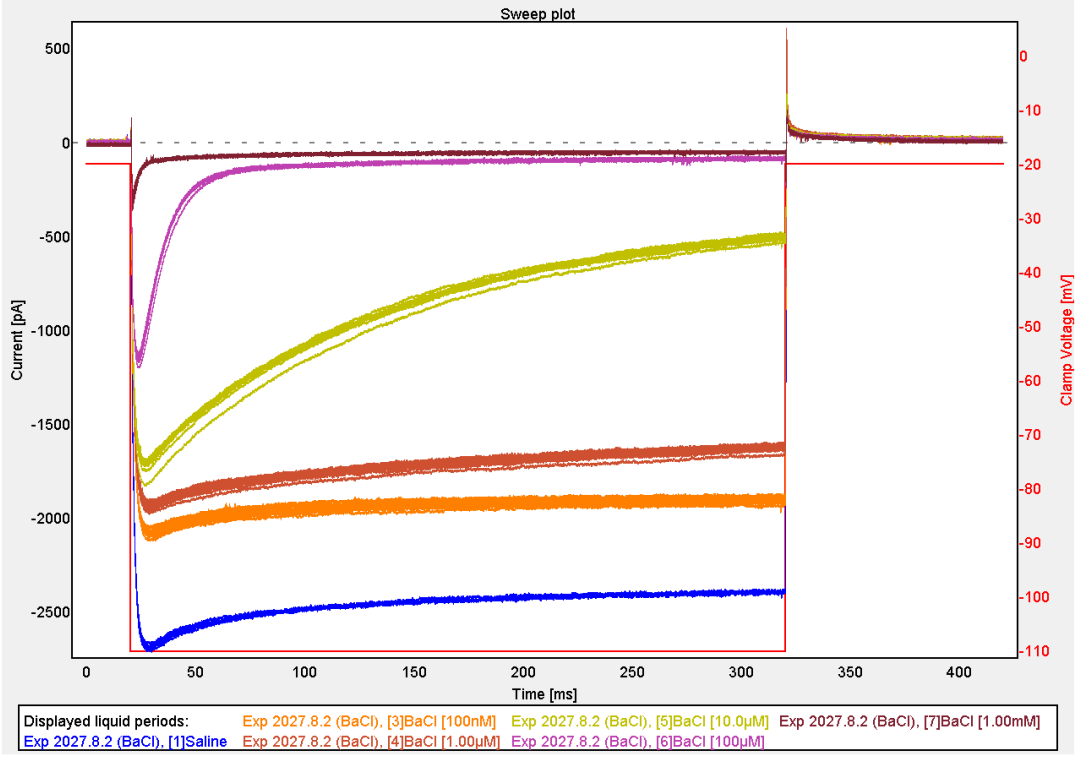


Fig. 3a. Raw current traces from application of 5 increasing concentrations of extracellular Ba^{2+}.

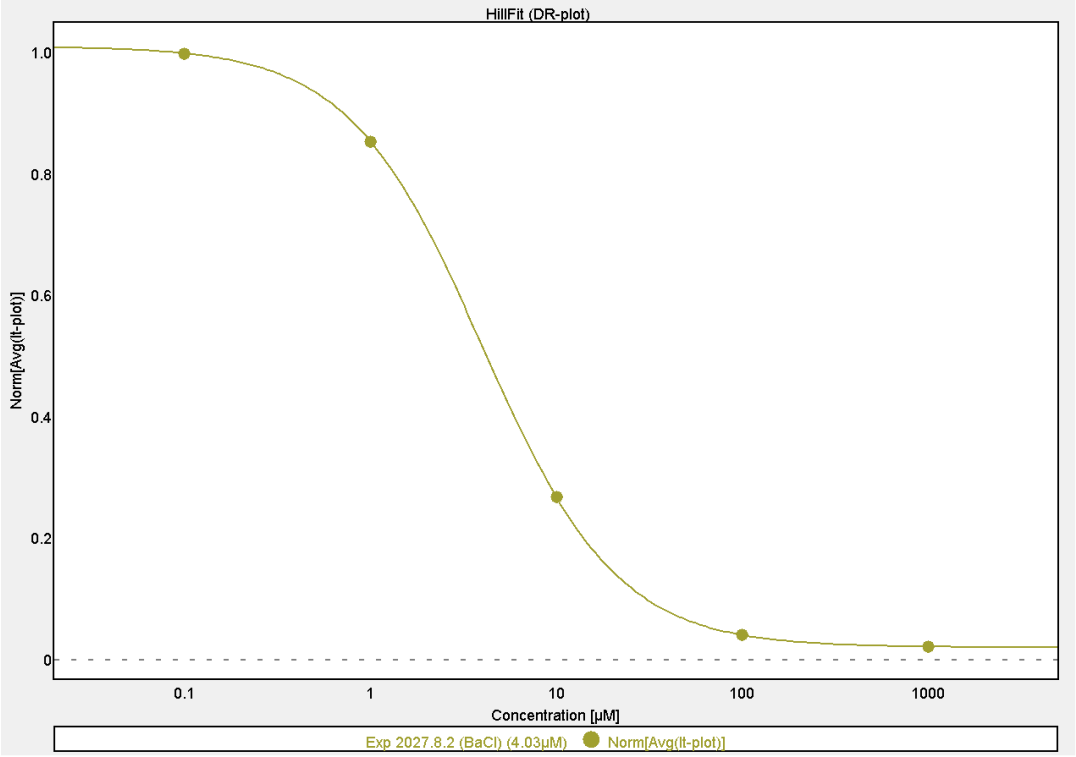


Fig. 3b. Normalized Hill fit showing the dose-dependency of Ba^{2+} on hKir2.1.

Pharmacological effect of Chloroethylclonidine (CEC)

In the next section we will show data from pharmacological voltage-dependent block of hKir2.1 by CEC. The experiment was performed by application of extracellular solution with four increasing concentrations of CEC: 15.6 μM, 63 μM, 250 μM and 1 mM. Raw current traces are shown in Fig. 4.

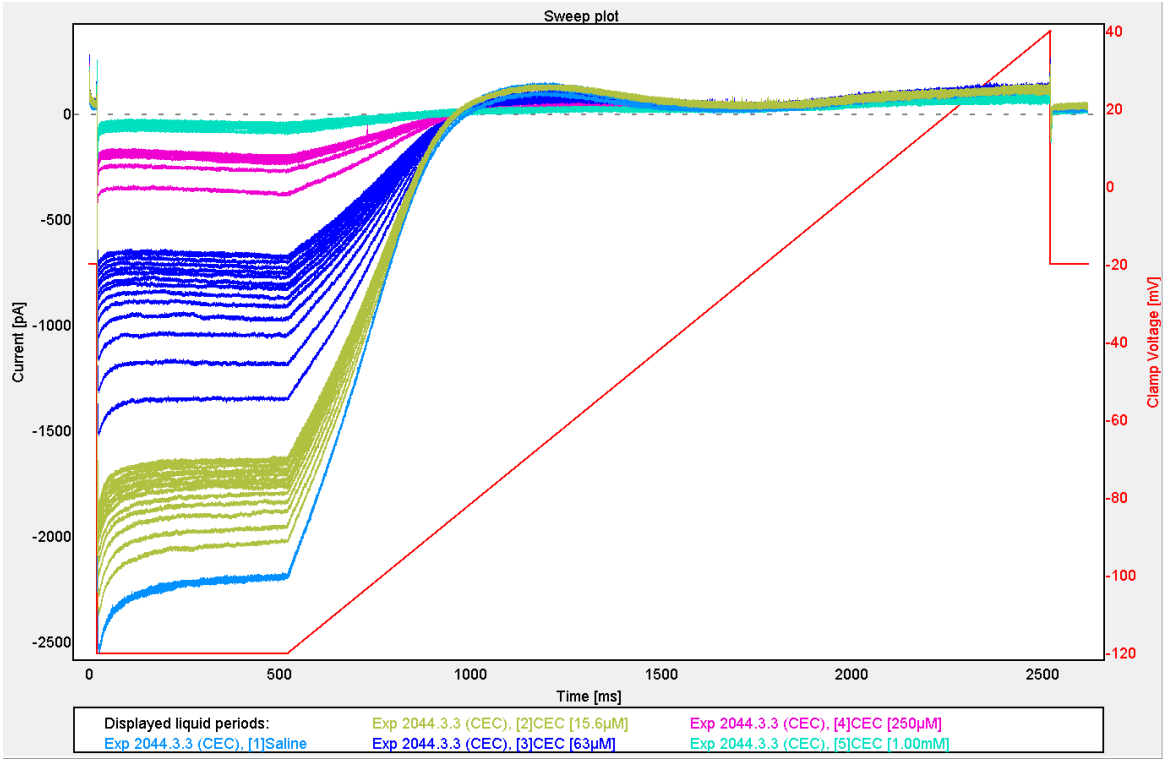


Fig. 4. Raw current traces of hKir2.1 with four increasing concentrations of CEC applied.

Fig. 5a shows the time-current plot and Fig. 5b the corresponding Hill fit. In average the IC_{50} for CEC was estimated to IC_{50}=31.03±0.97, n=11.

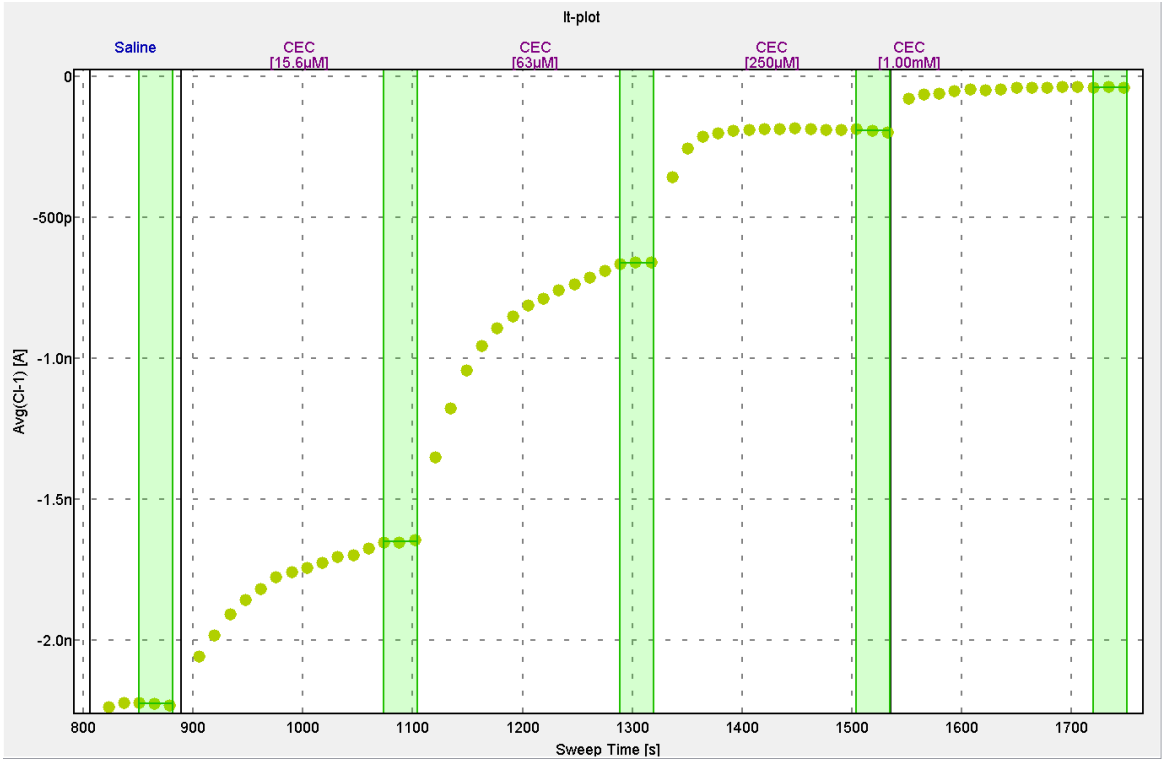


Fig. 5a. Time-current plot from a single cell with four increasing concentrations of CEC.

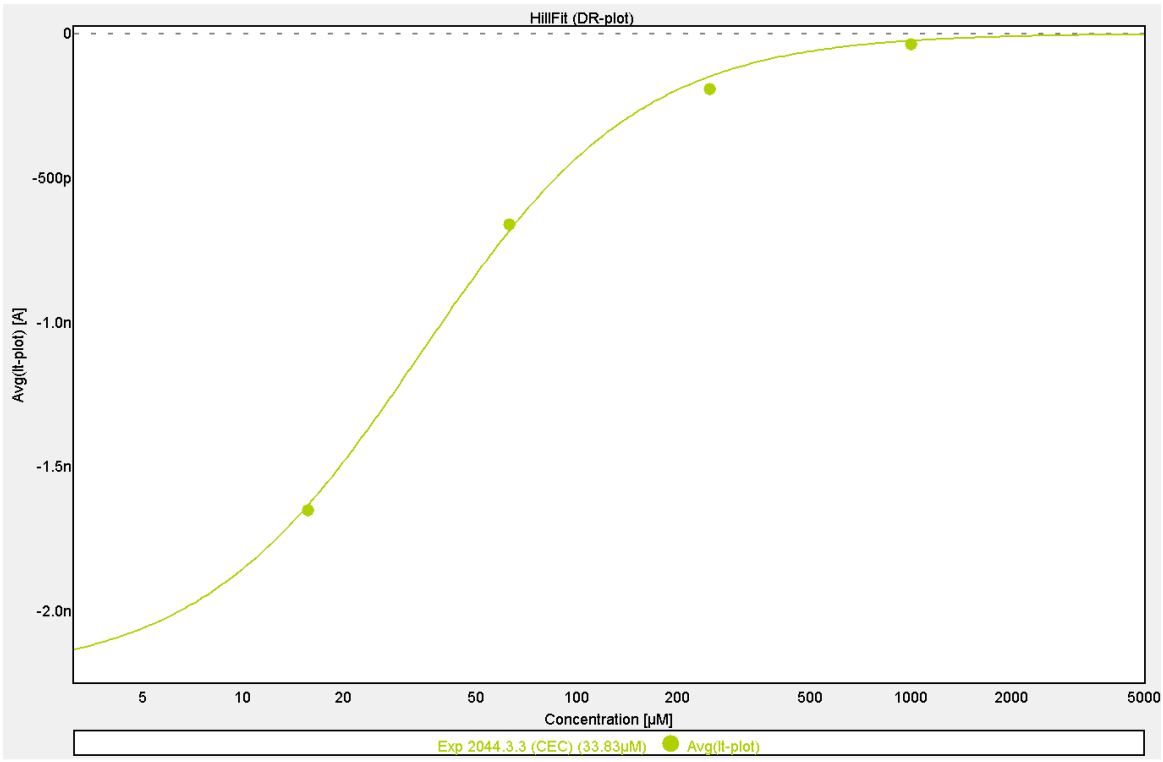


Fig 5b. Hill fit showing four concentrations of CEC.

Experimental statistics

QPlate statistics

In a typical experiment with HEK-hKir2.1 cells the overall performance is shown from the QPlate statistics in Figure 6. Data shows that 81% of the experiments were completed. 85% of the cells had true giga-seals.

QPlate '01065535032273'

Used in job: #2044 - HLO_hKir2.1 CreaCell - CEC 4-pt DR

Start of use: 2012-05-15 12:51:51

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A3	✓	✓	✓	✓	1.72	825.1	993.6	974	1
B3	✓	✓	✓	✓	1.70	1213.8	1928.8	977	1
C3	✓	✓	✓		1.69	299.2	16.1	0	0
D3	✓				1.70	0.0	0.0	0	0
E3	✓	✓	✓	✓	1.68	720.2	1832.2	972	1
F3	✓	✓	✓	✓	1.69	382.4	1232.3	1031	1
G3	✓	✓	✓	✓	1.72	1882.5	163.0	979	1
H3	✓	✓	✓	✓	1.70	887.6	3052.3	973	1
A4	✓	✓	✓	✓	1.67	1678.4	5200.3	962	1
B4	✓	✓	✓	✓	1.70	1030.5	4445.8	985	1
C4	✓	✓	✓		1.71	756.8	0.0	0	0
D4	✓	✓	✓	✓	1.71	1659.2	1347.3	987	1
E4	✓	✓	✓	✓	1.69	688.4	6060.0	990	1
F4	✓	✓	✓	✓	1.67	610.6	1152.1	965	1
G4	✓	✓	✓	✓	1.69	3449.6	4974.7	982	1
H4	✓	✓	✓	✓	1.72	3666.8	18622.3	986	1
Total	16	15	15	13					13
Success rate	100 %	94 %	94 %	81 %					

Fig. 6 QPlate statistics showing success rates for cell attachment to the QPlate orifice, seal quality, whole-cell success rates and number of completed experiments in single hole mode.

The data in this report was based on experiments on HEK-hKir2.1 from CreaCell (CreaCell.com)

References

[1]Preisig-Muller R, Schlichthorl G, Goerge T, Heinen S, Bruggemann A, Rajan S, Derst C, Veh RW & Daut J (2002). Heteromerization of Kir2.x potassium channels contributes to the phenotype of Andersen's syndrome. *Proc Natl Acad Sci USA* **99**, 7774–7779.

[2]Hsueh-Kai Chang, Jay-Ron Lee, Tai-An Liu, Ching-Shu Suen, Jorge Arreola, and Ru-Chi Shieh (2010). The Extracellular K^+ Concentration Dependence of Outward Currents through Kir2.1 Channels Is Regulated by Extracellular Na^+ and Ca^{2+}. *J. Biol. Chem.* ***285***, 23115–23125

Materials & Methods

Conclusion

We have demonstrated the functionality of HEK-hKir2.1 on the QPatch. Biophysical characteristics of the hKir2.1 channels were studied from high resistance whole cell recordings in IV- and dose-response experiments and the values found on the QPatch correspond well to published literature values.