SOPHION BIOSCIENCE

Application Report

HEK-hKir2.1 tested on QPatch

The biophysical characteristics of the hKir2.1 channel were studied from high resistance whole cell recordings in IV- and dose-response experiments and the values correspond to literature values.

Introduction

The aim of this report is to demonstrate the performance of HEK-hKir2.1 from CreaCell on QPatch. 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.

Results and discussion

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 E_{rev} 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 E_{rev}=-80.2±0.54 mV, n=8 which corresponds to the theoretical E_{rev} that is -85.9 mV for this channel under these specific experimental conditions. The maximal inward current response I_{max}=-2824.23±234.24 pA, n=8 was observed at a potential of V_{min}=-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 V_{max}=-62.16±0.99 mV, n=8.

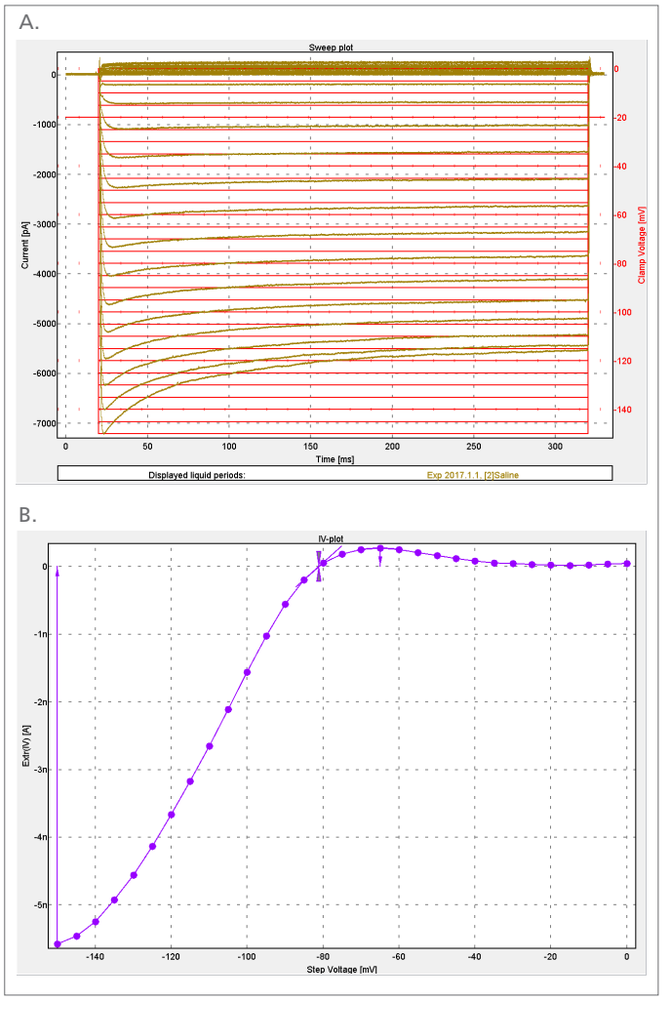


Fig. 1 : A. Raw hKir2.1 current traces during a step voltage protocol from -150 mV to 0 mV with 5 mV step increment. B. IV curve showing a strong inward current a low potential and a small outward current at potentials positive to Erev.

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 E_{rev}) and the outward (positive to E_{rev}) direction in hKir2.1 (Figure 2). At the same time, a parallel change in E_{rev} was observed in accordance to N_{ernst} 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, 1).

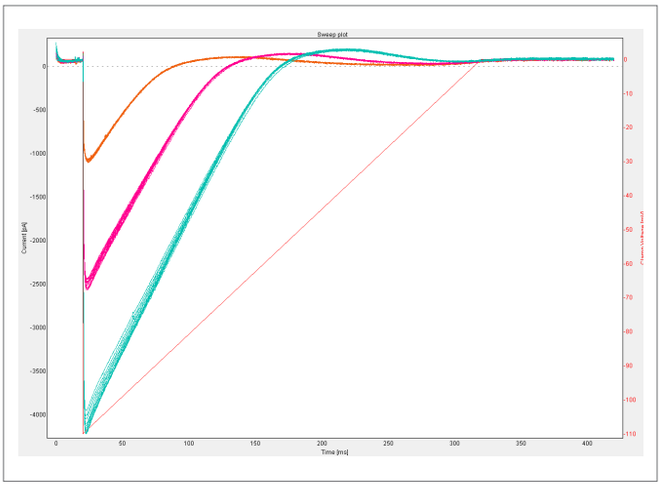


Fig. 2: Effect of extracellular K^+. Raw current traces at 4 (orange), 8 (pink) and 16 mM (blue) external K^+.

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

The hKir2.1 channel is blocked by external Ba^{2+} in a voltage-dependent manner. Figure 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 Figure 3b. IC_{50} is estimated to 5.12±0.42 µM, n=10, which corresponds well to literature values (2).

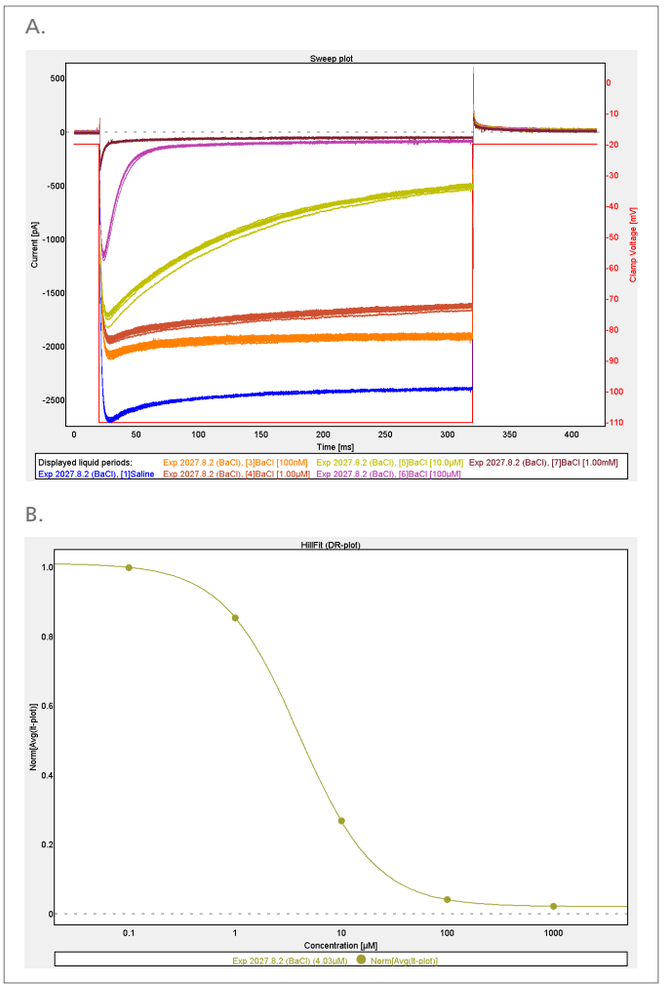


Fig. 3: A. Raw current traces from application of 5 increasing concentrations of extracellular Ba^{2+}. B. Normalized Hill fit showing the dose-dependency of Ba^{2+} on hKir2.1.

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 Figure 4.

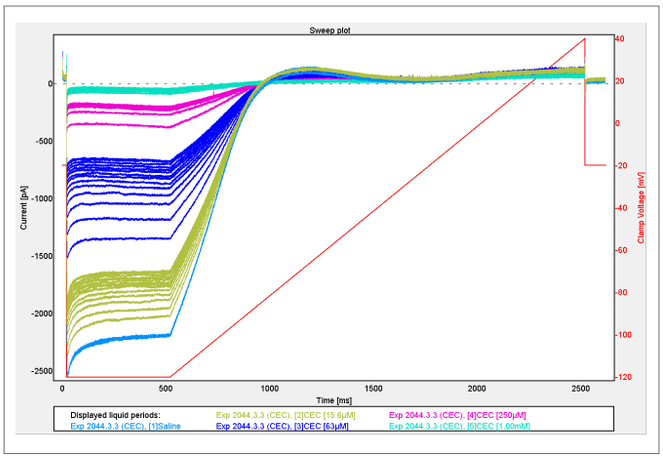


Fig. 4: Raw current traces of hKir2.1 with four increasing concentrations of CEC applied.

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

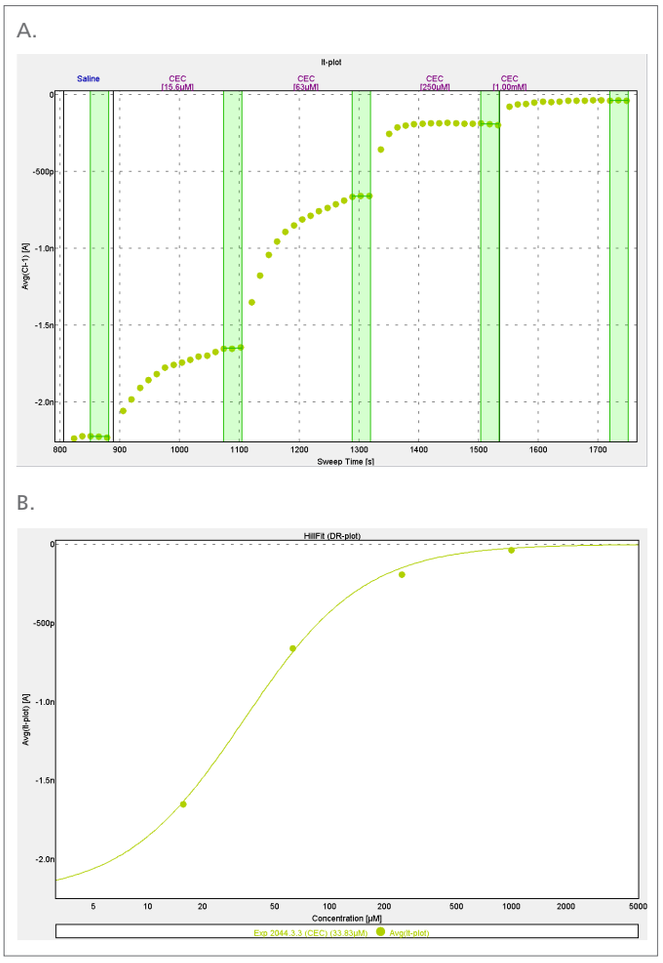


Fig. 5: A. Time-current plot from a single cell with four increasing concentrations of CEC.. B. Hill fit showing four concentrations of CEC.

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.

Methods

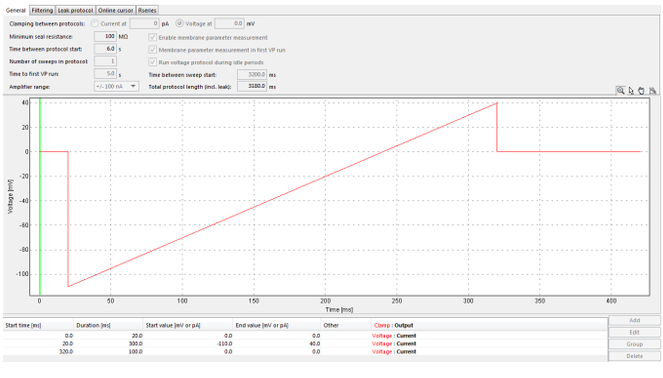
HEK-hKir2.1 cells were grown and harvested according to the SOP's specified from CreaCell (CreaCell.com) and modified for use on QPatch by Sophion Bioscience.

Voltage protocols

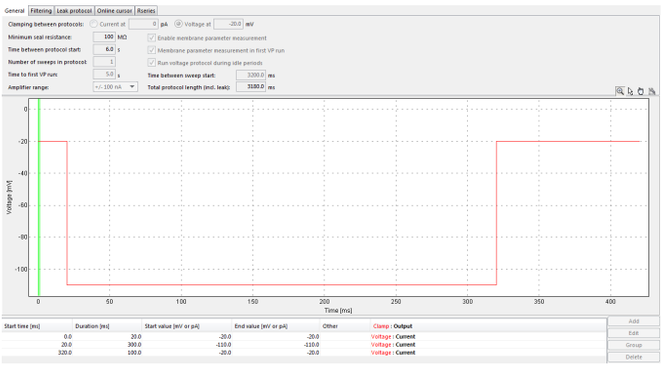
For experiments with HEK-hKir2.1 the following protocols were used. 1) Step IV protocol from -150 mV to 0 mV in 5 mV steps, V_{hold} = -20 mV, 2) Ramp IV protocol from -110 mV to +40 mV, V_{hold}= 0 mV, 3) short pulse from -20 mV to -110 mV for 300 ms, V_{hold} = -20 mV and 4) Long ramp protocol with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

#1 Step IV protocol

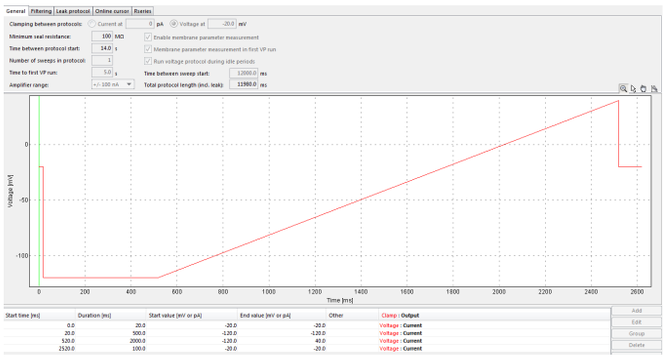
#2 Ramp IV protocol



#3 Short pulse protocol



#4 Long ramp protocol



References:

1. 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 Ca2+. J. Biol. Chem. 285, 23115–23125.
2. 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.

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