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Application Report

CHO-KvLQT1/minK on QPatch

We demonstrate that the CHO-KvLQT1/minK application can be efficiently run on QPatch with high throughput while maintaining high data quality that matches literature values for the given test compounds

Summary

The human KCNQ1 gene encodes the pore-forming unit of the voltage-gated potassium channel, while KCNE1 encodes for the regulatory minK subunit. The complex is responsible for repolarizing the heart action potential, and in this study we validate that the CHO-KvLQT1/mink application can be efficiently run on QPatch with high throughput, while maintaining high data quality.

Introduction

The slowly activating, delayed rectifier K^+ channel is among other activities important for regulating the repolarization phase of cardiac action potentials. The KvLQT1/minK channel consists of two transmembrane proteins. Mutations in the genes KCNQ1/KCNE1 coding for KvLQT1/minK are associated with predisposition to deafness, cardiac arrhythmia syndromes including long QT syndrome, atrial fibrillation and sudden infant death syndrome.

Experiments were performed to validate the CHO-KvLQT1/minK cells line on the QPatch. The cell line was characterized in terms of biophysical properties such as IV-relationship, activation, inactivation together with the pharmacological properties of the channel. Furthermore, the current stability and current expression was evaluated. This ion channel is known for having notorious run-down, but by using this QPatch-optimized cell line in combination with the experimental conditions described here, stable current recordings can be obtained.

Results and discussion

Electrophysiological recordings on KvLQT1/minK channels are often associated with run-down problems. By using the QPatch system and the conditions cited in this report, we overcame the run-down problems.

Figure 1 (top) shows raw traces of KvLQT1/minK with four subsequent additions of extracellular solution measured on a single cell. The corresponding current-time plot data is shown in Figure 1 below.

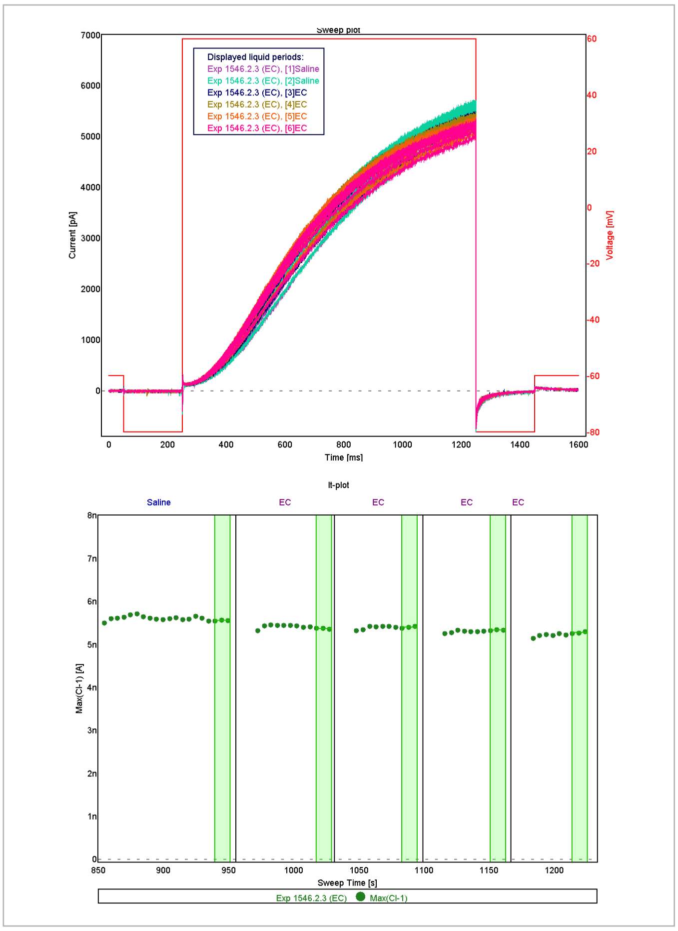

Fig. 1: Raw traces of KvLQT1/minK recordings of 4 consecutive application of extracellular solution on a single cell (top). Bottom: corresponding current-time plot.

Experiments were conducted to evaluate the IV-relationship of the KVLQT1/minK channel. Figure 2 (top) shows the currents elicited at potentials ranging from -80 mV to +40 mV in a representative experiment. The corresponding IV plot is shown in Figure 2 (bottom).

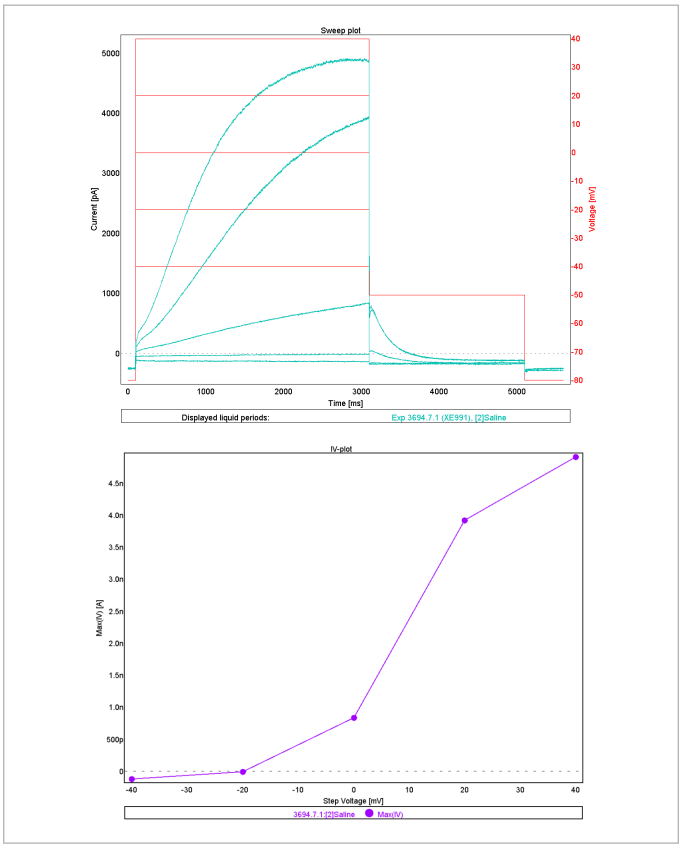

Fig. 2: IV-relationship of the KvLQT1/minK channel. Top: Raw data sweeps elicited using an IV-protocol ranging from -80 mV to +40 mV. The voltage step protocol is shown in red. Bottom: corresponding IV-curve measured at the end of the step protocol.

Experiments were performed to evaluate the pharmacological properties of the KvLQT1/minK channel. The response of KVLQT1/minK to a known blocker XE-991 was tested. Figure 3 (top) shows the IT-plot of the peak amplitude to four concentrations of XE-991 (0.01, 0.1, 1, 10μM). Figure 3 (bottom) shows the corresponding Hill fit. The resulting IC_{50} for XE-991= 0.96±0.4 μM, n=7 (literature value 1-6 μM).

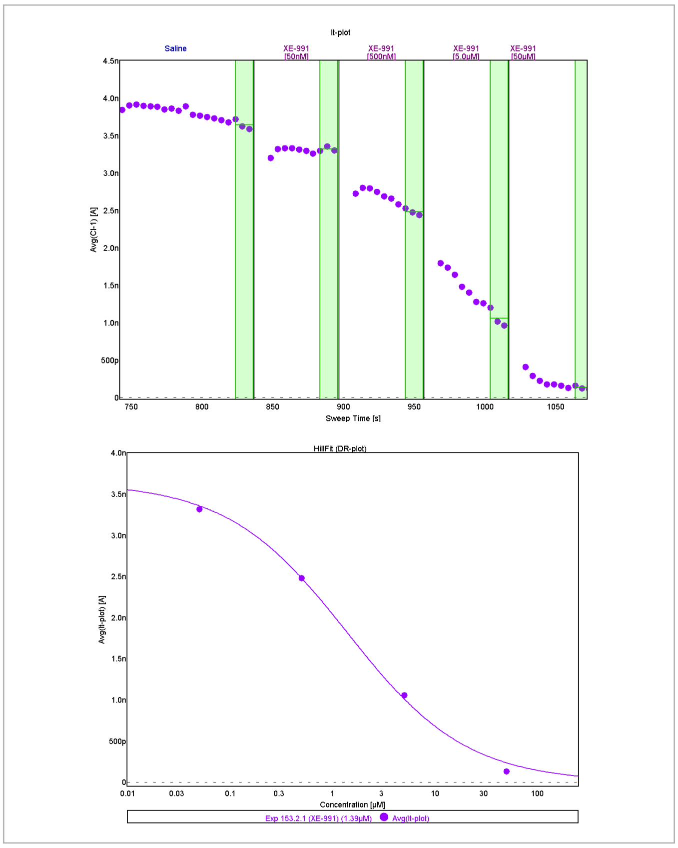

Fig. 3: Block of KvLQT1/minK current with XE-991. Top: IT-plot showing the current amplitude in response to four increasing concentrations of XE-991. Bottom: Corresponding Hill fit

Next, the effect on the blocker bepridil was tested on the KvLQT1/minK currents. Figure 4 (top) shows the current–time plot of the peak amplitude in response to four increasing concentrations of bepridil (0.05, 0.5, 5, 50 μM). Figure 4 (bottom) shows the corresponding Hill fit. The resulting IC_{50} = 8.96±1.0 μM, n=8 (literature value 5.3-10.5 μM).

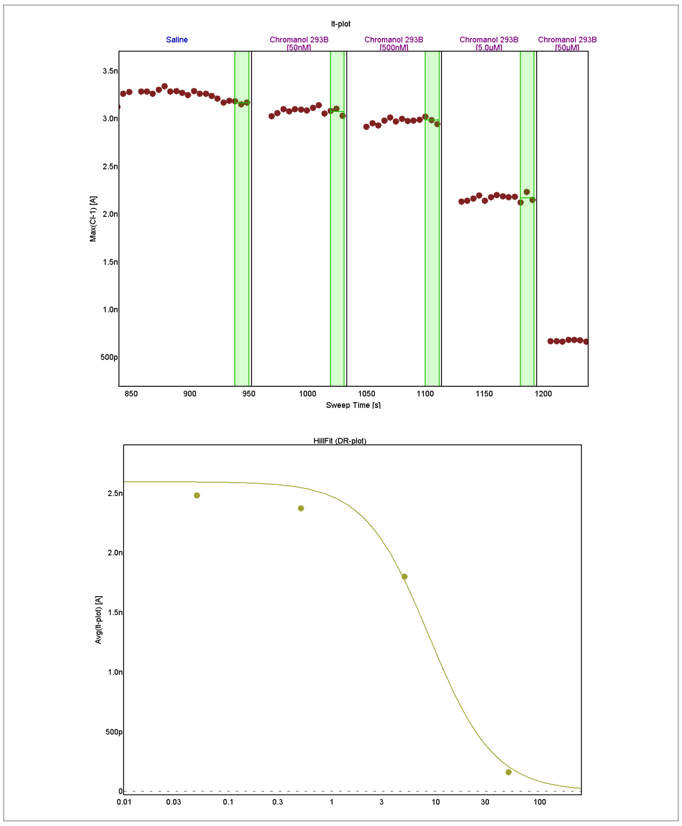

Fig. 4: Block of KvLQT1/minK current with bepridil. Top: IT-plot showing the current amplitude in response to four increasing concentrations of bepridil. Bottom: Corresponding Hill fit.

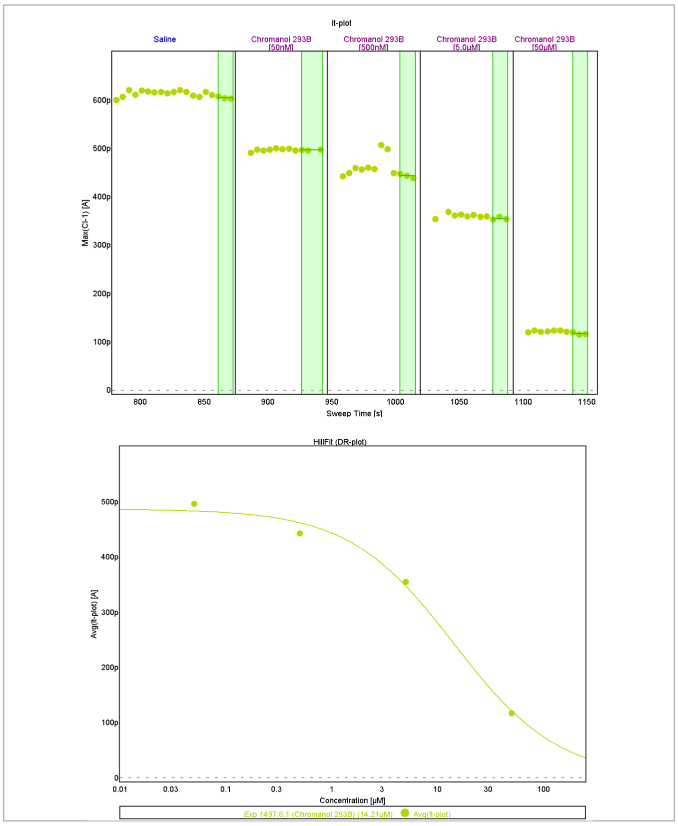

Fig. 5: Block of KvLQT1/minK current with chromanol 293B. Top: IT-plot showing the current amplitude in response to four increasing concentrations of chromanol 293B. Bottom: Corresponding Hill fit.

Furthermore, experiments were performed to evaluate chromanol 293B on KvLQT1/minK currents. Figure 5 (top) shows the current–time plot of the peak amplitude to in relation to four concentrations of chromanol 293B (0.05, 0.5, 5, 50 μM). Figure 5 (bottom) shows the corresponding Hill fit. The resulting IC_{50} for chromanol 293B= 10.6±1.1 μM, n=13 (literature value 10-12.4 μM)

	XE-991 [μM]	Bepridil [μM]	Chromanol 293 [μM]
IC_{50}	0.96±0.4, n=7	8.96±1.0 n=8	10.6±1.1, n=13

The average peak amplitude was 2.80±0.46 nA, n=28

QPlate '01065535028240'

Used in job: #1551 - HLO_KvLQT1/minK DR_1 rerun 1

Start of use: 2012-01-25 13:30:53

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
C4	✓	✓	✓	✓	1.69	2826.1	1847.1	544	1
D4	✓	✓	✓	✓	1.68	1828.3	2423.2	127	0
E4	✓	✓	✓	✓	1.70	3004.5	2075.0	544	1
F4	✓	✓	✓	✓	1.72	2120.7	1497.9	79	0
G4	✓	✓	✓	✓	1.69	2240.6	301.5	82	0
H4	✓	✓	✓	✓	1.71	1177.6	548.6	77	0
A5	✓	✓	✓	✓	1.70	1400.5	1339.3	547	1
B5	✓	✓	✓	✓	1.67	1954.2	1537.1	545	1
C5	✓	✓	✓	✓	1.67	2005.3	1724.7	554	0
D5	✓	✓	✓	✓	1.64	1039.1	1053.0	554	0
E5	✓	✓	✓	✓	1.66	1222.3	1376.0	575	0
F5	✓	✓	✓		1.72	1176.8	9.9	0	0
G5	✓	✓	✓	✓	1.72	1952.5	612.0	555	0
H5	✓	✓	✓	✓	1.69	2133.1	1424.5	555	0
A6	✓	✓	✓	✓	1.69	1833.6	1203.3	553	0
B6	✓	✓	✓	✓	1.68	3055.1	1474.8	551	0
C6	✓	✓	✓	✓	1.70	1733.4	1138.0	552	0
D6	✓	✓	✓	✓	1.70	2294.7	1296.1	553	0
E6	✓	✓	✓	✓	1.73	1565.4	1060.1	553	0
F6	✓	✓	✓	✓	1.72	2826.6	1930.6	553	0
G6	✓	✓	✓	✓	1.71	2397.4	1503.8	549	0
H6	✓	✓	✓	✓	1.68	2660.4	1272.3	552	0
Total	47	47	46	45					20
Success rate	98 %	98 %	96 %	94 %					

Fig. 6: QPlate overview showing overall success rate for KvLQT1/minK experiments in single-hole.

Conclusion

We have demonstrated the functionality of CHO-KvLQT1/minK on QPatch. Biophysical- and pharmacological properties was studied in high resistance whole-cell recordings in IV- and dose-concentration experiments.

We conclude that the CHO-KvLQT1/minK application can be efficiently run on QPatch with high throughput while maintaining high data quality, which match literature values for the given test compounds.

Methods

Cells

CHO cells stably expressing KVLQT1/minK were obtained from B'SYS. Cells were cultured and harvested for QPatch experiments as described in the Sophion SOP.

QPatch

All experiments were performed using the QPatch single-hole and multi-hole technologies and CHO cells expressing the KvLQT1/minK were kept in culture medium in the QStirrer for up to four hours.

References:

1. S. Y. M. Yeung, I. A. Greenwood 2005. Electrophysiological and functional effects of the KCNQ channel blocker XE991 on murine portal vein smooth muscle cells. Br J Pharmacol. 146 (4): 585-595
2. A. R. Mackie, K. L. Byron 2008. Cardiovascular KCNQ (K_V7) potassium channels: Physiological regulators and new therapeutic intervention. Mol Pharmacol fast forward. 74 (5): 1171-1179

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