SOPHION BIOSCIENCE

Application Report

HEK293-K_{Ca}1.1 for QPatch

Calcium-activated ion channels on QPatch. In this study several intracelluar solutions have been used in one experiment on the voltage-dependent large-conductance Ca^{2+} activated K^+ channels, often referred to as BK channels

Summary

- A solid assay of KCa1.1 (BK) on QPatch with biophysical and pharmacological characteristics as described in the literature.
- Fluoride-free internal solutions allow for precise clamping of the cytosolic calcium concentration
- QPatch offers the possibility to test up to 8 different intracellular solutions on one QPlate.

Introduction

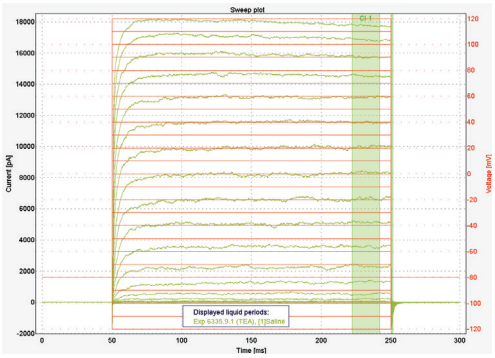
Performing automated patch clamp experiments with different intracellular solutions is somehow difficult. It is possible to test eight different solutions in one experiment using QPatch in combination with the multi well reservoir.

In this study the multi-well reservoir has been used for experiments on the voltage-dependent large-conductance Ca^{2+}-activated K^+ channel, often referred to as BK channel or K_{Ca}1.1. A human embryonic kidney cell line (HEK293) stably expressing BK channel was used.

BK channels are large conductance calcium- and voltage-gated potassium channels, which allow potassium flux when activated with intracellular calcium and/or membrane potential (Strøbæk et al., 1996).

Results

Four intracellular Ringer's with different concentrations of calculated free calcium were tested on the HEK-BK cell line (10. 3. 0.3 and 0.01 µM free calcium). Seals were obtained in physiological Ringer's solution. The main cation of the extracellular solution was exchanged during the experiment in the following sequence: Na^+ —> K^+ —> Na^+ —>TEA^+ —> Na^+ .



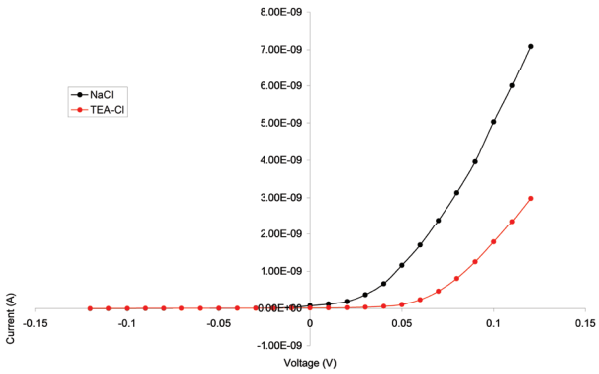


Fig. 1: Top: Raw sweep in physiological Ringer's solutions (asymmetrical K^+ gradient), and bottom: IV relation in control situation (black) and with 5 mM TEA-Cl (red)

The raw current traces from the BK channel are shown in Figure 1 (top). In Figure 1 (bottom) it can be seen that the current can be partly blocked with 5 mM tetraethyl ammonium chloride (TEA-Cl) as expected.

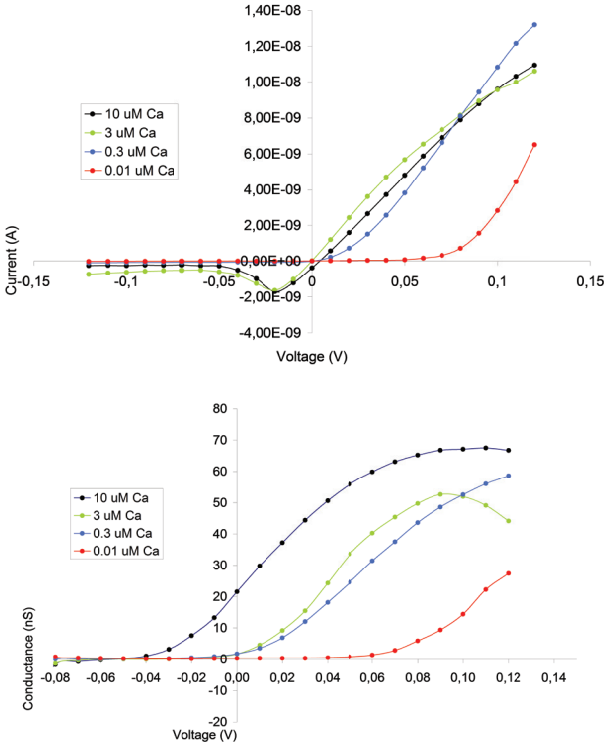


Fig. 2: Top: IV relations with different concentrations of intra cellular calcium in symmetrical Ringers solution with symmetrical K+ gradients. Bottom: conductance/voltage (GV) plot relations with different concentrations of intra cellular calcium in physiological Ringers solutions. (asymmetrical)

Figure 2 (top) shows the calcium, and voltage dependence of BK channels. The channel showed already activity at negative potentials in the presence of high concentration of internal calcium. As a result of this, an inward current was observed in these high calcium conditions at negative potentials using a symmetrical K^+ gradient (= calculated reversal potential for K^+ = 0 mV) The GV - plot in Figure 2 (bottom) was recorded in physiological Ringer solution (asymmetrical K^+ gradient). As expected, increasing concentrations of internal calcium shifts the GV curve towards more negative potentials.

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Conclusion

In these studies four different concentrations of free intracellular calcium was were simultaneously tested simultaneously on HEK293 cells expressing the BK channel using QPatch. The obtained data is in accordance with previously published data (Strøbæk et al., 1996).

Controlling free calcium in electrophysiological experiments is a great task, since current change so rapid with changing calcium concentrations. It is crucial to tightly control intracellular calcium levels in a BK channel assay as open probability of the channel critically depends on free cytosolic calcium. Using different Ringer's solutions in one job is unique for QPatch and shows the flexibility of QPatch. Using QPatch with different intracellular Ringer's solutions it is possible to track the different Ringer's solutions through the whole data analysis and QPatch thereby delivers a final report on which Ringer's performed best. The feature is very useful for Ringer's solutions optimization and as it was used in this study, for testing different compositions of intracellular Ringer's solutions.

Methods

Ringer's solution

Intracellular solutions:

In mM (all four solutions, pH=7.2, mOsm ≈ 295)

10 nM free calcium: 0.965 $CaCl_2$, 1.785 $MgCl_2$, 30/10 KOH/EGTA, 120 KCl, 10 M HEPES

300 nM free calcium: 5.17 $CaCl_2$, 1.42 $MgCl_2$, 30/10 KOH/EGTA, 110 KCl, 10 M HEPES

3 µM free calcium: 1.085 $CaCl_2$, 5.54 $MgCl_2$, 3/1 KOH/EGTA, 27/9 KOH/NTA, 120 KCl, 1 M HEPES

10 µM free calcium: 1.37 $CaCl_2$, 5.41 $MgCl_2$, 3/1 KOH/EGTA, 27/9 KOH/NTA,120 KCl, 1 M HEPES

Extracellular solution:

In mM: 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 145 NaCl, 4 KCl, 10 Glucose. pH 7.4; mOsm ≈ 310

Cells were cultured according to the Sophion standard HEK SOP. Passaged three times a week and harvested with trypsin and transferred to a serum-free media for experiments.

The voltage protocol used was an IV protocol (-120 to +120 mV), with a holding potential at -80 mV (Figure 1, top).

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