SOPHION BIOSCIENCE

Application Report

Large molecules: Scorpion toxin block of BK channels on Qube 384

Using Automated Patch Clamp recordings 'challenging' ChTx peptide toxin pharmacology was efficiently determined

Summary

- Qube 384 is capable of performing pharmacology measurements with large molecules.
- Low volume microfluidic application ensures that expensive and scarce molecules like antibodies and toxins can be efficiently tested.
- Key pharmacology metrics defined in these assays are comparable to values reported in the literature:

	IC_{50}	
Channel/peptide toxin	Qube	Literature [1]
BK/charybdotoxin	61 nM	50 nM

Introduction

Currently, more than 90% of approved drugs are small molecules, but large molecules (>1.000 Da, also known as biologics) are rapidly rising in prominence and importance in drug discovery and already constitute the lion's share of the top 10 selling drugs worldwide.

Large molecules have gained attention due to their mode of action, often achieving greater target specificity and potency than small molecule drugs. This, however, comes at a cost: usually, they are expensive and scarce and can have unwanted polyreactivity ("stickiness"). In addition, they are more sensitive to their environment, as their three-dimensional structure is key to their function and relies on other, weaker interactions than covalent bonds.

While automated patch clamp systems have been used for small molecule drug discovery and characterization for the past 20 years, the use of APC systems for large molecule characterization have been scarcer. For this case study, we chose to use Charybdotoxin to modulate BK channels to highlight the performance and functionality of Qube 384, working with larger molecules.

BK channels have important roles in physiology and pathophysiology (e.g. neuronal excitability, neurotransmitter release and cardiovascular function[2]). The activation of BK channels is both voltage and Ca^{2+} dependent, with the maximal sensitivity to Ca^{2+} changes in the concentration range of 0.5 to 1.8µM intracellular calcium[3].

Using fluoride in the intracellular solution to enhance the seal quality would cause the calcium to precipitate and hamper the BK channel recordings. Hence, a fluoride-free intracellular solution was used, still obtaining a high success rate.

The Qube can apply compounds with high accuracy, and low volumes (18 µL per application) as each recording site is supported by built-in microfluidic channels. This accommodates the testing of expensive and scarce molecules like antibodies and toxins, in this case, ChTx.

Leiurus quinquestriatus hebraeus also known as the death stalker.
Photo credit: Arie van der Meijden / University of Porto

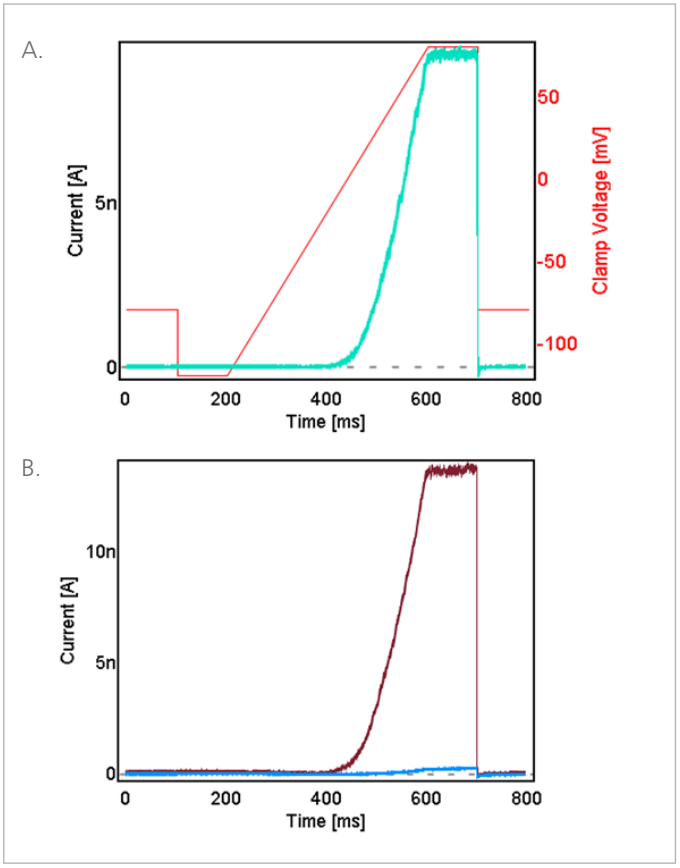

Fig. 1: Raw data traces of BK currents. A) Cells were held at -80 mV, stepped to -120 mV for 100 ms and then ramped to +80 mV within 400 ms. After 100 ms at +80 mV, the cells were clamped to -80 mV again (voltage protocol shown in red). B) The current response of BK channels before (brown) and after (blue) application of 30 mM TEA.

Results

BK currents

HEK cells expressing the human BK (K_{Ca}1.1) channel were measured on the Qube 384. During the experiment, cells were depolarized, as shown in Fig.1A. Currents were measured before and after whole-cell suction application, as the whole-cell formation allows calcium to enter the cells, thus activating the BK channels. The cells showed a median current of 9.9 nA with a median sealing resistance of 1.5 GΩ. Full current block was achieved by 30 mM TEA (Fig.1B).

Large molecules compound handling and application

The scorpion toxin Charybdotoxin (ChTx, Alomone Labs) is a peptide with a molecular weight of 4.3 kDa. It was stored in small aliquots at -20°C at ≥1000-fold concentrated, and the final solution was made on the day of the experiment at room temperature. BK channels were blocked by ChTx with an IC_{50} value of 61 nM (Fig.2). BK channels are both voltage and calcium-sensitive, and the IC_{50} value will be dependent on intracellular calcium concentration and subunit composition[1,4].

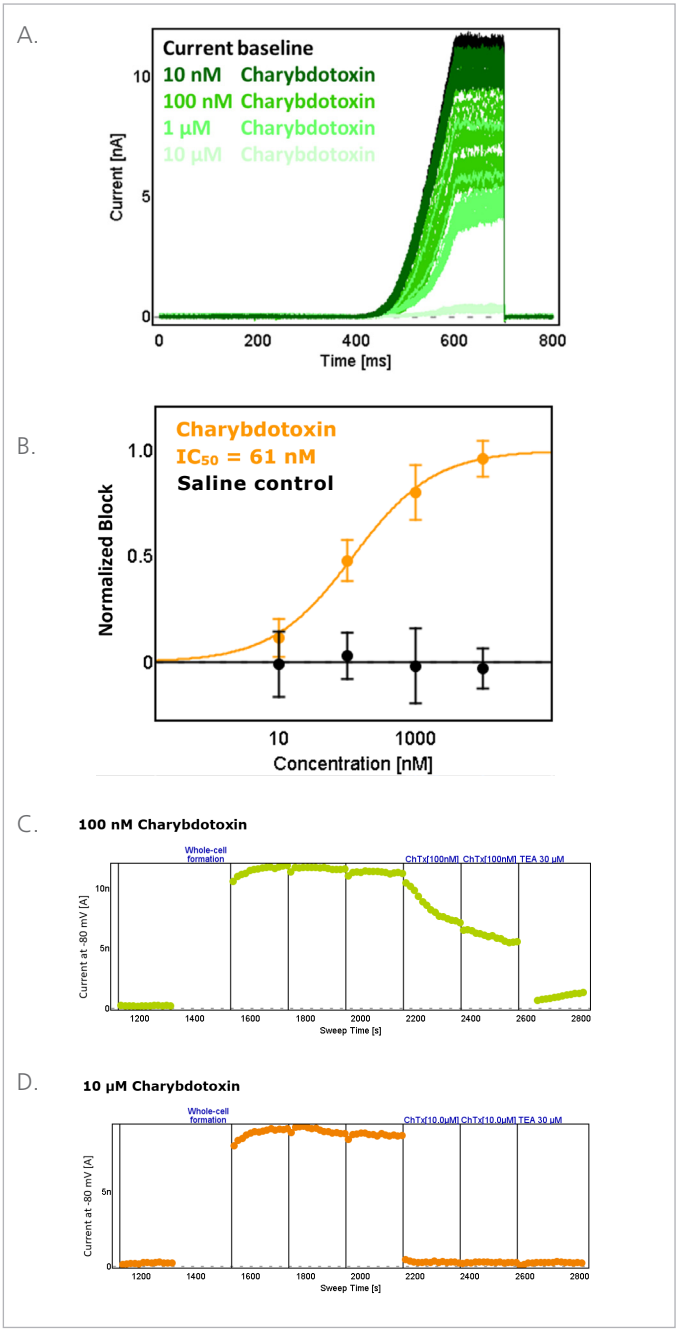

Fig. 2: BK channels were blocked by ChTx. A) Raw data traces of BK current after ChTx addition (Different cells with similar current baseline were used to illustrate different blocking kinetics of ChTx). B) Dose-response plots fitted with the Hill equation: Normalized BK current in saline control conditions (black) or exposed to different concentrations of ChTx in a non-cumulative manner (orange). The current was normalized to the baseline current and the current after adding 30 mM TEA. Values are average ± SD. (C and D) Time course of ChTx block at 100 nM and 10 uM ChTx (for C and D, respectively). Plotted is the current size (measured at +80 mV) versus time during the time span of the experiment. Vertical bars mark the liquid periods: After whole-cell formation, calcium enters the cells, and the BK channels open. The current is allowed to stabilize over three saline additions, after which ChTx is added twice, followed by a full block by 30 uM TEA. At the end of each of the liquid periods, three current values were averaged for subsequent calculations.

Methods

Saniona kindly provided HEK-BK cells; growth and harvest were performed according to Sophion standard procedures. After harvest, the cell suspensions were prepared by the Qube's automated cell preparation unit (two washes for 300 s at 50 g). Whole-cell suction was applied after seal resistance establishment (contact your application scientist for information on solutions and the intra-experimental whole-cell suction).

BK cells were depolarized using the same ramp 3x10 times, followed by 2x10 ramps in compound solution (ChTx or saline control), and concluded by ten ramps in 30 mM TEA with an inter-sweep interval of 15 seconds (idle sweeps activated).

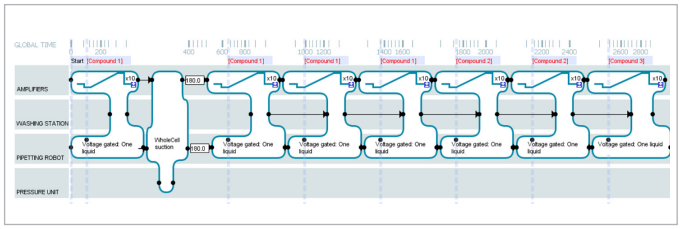

Fig. 3: Example of a BK pharmacology measurement setup as seen in the Sophion Viewpoint software. The first liquid addition (left block) contains ten ramps before the whole-cell suction and is followed by 3x10 ramps in saline (third-fifth block) and 2x10 ramps in compound/negative control solution (sixth-seventh block). Lastly, ten ramps in reference solution are applied to the cells (right block).

Quality control filters

For BK cells, using the criteria

- Capacitance post whole-cell suction > 4 pF
- Membrane resistance > 100 MΩ
- Maximum current > 500 pA
- Minimum current reduction by 30 mM TEA > 50%,

Success rates above 75%.

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Large molecules and APC in the literature:

For more information about the use of QPatch and Qube 384 for peptide/toxin characterization, also see below an incomplete list of references, contact our application scientists or visit our publication database at https://sophion.com/knowledge-center/publications/

1. Agwa AJ, Blomster L V, Craik DJ, King GF, Schroeder CI. Efficient Enzymatic Ligation of Inhibitor Cystine Knot Spider Venom Peptides: Using Sortase A To Form Double-Knottins That Probe Voltage-Gated Sodium Channel Na(V)1.7. Bioconjug Chem. 2018 Oct;29(10):3309–19.
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Author:

Melanie Schupp, Application Scientist

Kim Boddum, Research Scientist