Sophion
[Together with Biolin Scientific]

Qube as a tool for assay optimization of CiPA cells & protocols by using multiple IC, EC solutions and hERG, $Na_V1.5$ and $Ca_V1.2$ on the same QChip

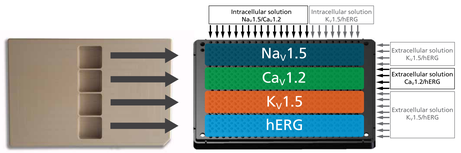
Melanie Schupp, Anders Lindqvist, Pia Bladsgaard Andersen, Weifeng Yu

Introduction

The Comprehensive in vitro Proarrhythmia Assay (CiPA) proposal provides an attractive perspective for increasing the efficacy of the drug development process. A detailed electrophysiological analysis of $Na_V1.5$ (peak and late currents), $K_V4.3$ (Ito), hERG (IKr), K_VLQT1/minK (IKs), $Ca_V1.2$ and Kir2.1 (IK1) upon addition of a potential drug is a major part of assessing the drug's proarrhythmic risk. Gaining a better understanding of the complex relationship between QT-elongation and the occurence of Torsades des Pointes (TdP), potentially enables compounds with properties that today are considered as problematic to be further developed. High throughput screening (HTS) supports this quest and is furthermore of great importance for discovering pharmacologically active substances and understanding ion channels.

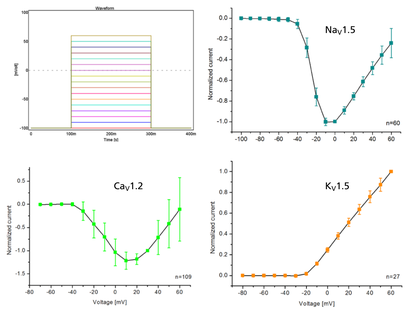
Qube is a giga-seal automated patch clamp (APC) instrument, providing 384 amplifiers for the consumable QChip 384 with its integrated electrodes. The QChip has built-in microfluidic flow channels that ensure a fast and complete exchange of liquid for reliable measurements on ligand-gated ion channels and sequential additions to the same site. Here, four different cell lines expressing the cardiac ion channels $Na_V1.5$, $Ca_V1.2$, $K_V1.5$ and hERG, were transferred from a cell clone cell transfer plate (ccCTP) onto the same QChip. A range of pharmacological substances and voltage protocols were applied to address the suitability of Qube for measuring different cell populations in parallel.

[Fig. 1]: Qube setup. With 384 parallel measurement sites and 10–15 minutes per plate run, the Qube enables testing of more than 1,500 compounds on different cells in one hour. With full automation features for unattended operation – a plate stacker and a cell preparation unit –, the system provides walk-away functionality for more than 6,000 compounds before user interventions is needed.

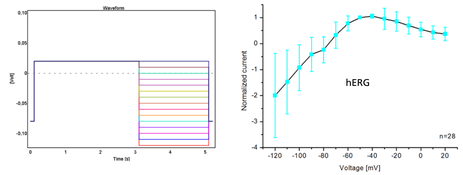


[Fig. 2]: Transfer of cells from the four-hole cell clone cell transfer plate (ccCTP) to the QChip 384. The ccCTP enables the experimenter to work with a varying number of cell clones. Between one and 16 different cell / channel types can thus be investigated simultaneously on one QChip. Here, 4 cell types expressing the ion channels $Na_V1.5$, $Ca_V1.2$, $K_V1.5$ and hERG were transferred and measured in the QChip. The extracellular solutions and intracellular solutions were different across the plate (see Fig. 2).

IV Curves

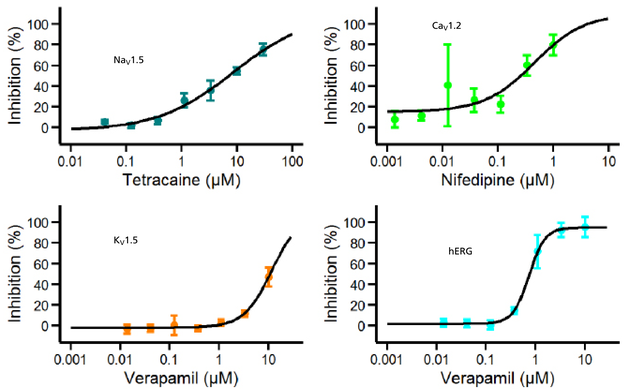


[Fig. 7]: IV protocol and normalized IV curve for $Na_V1.5$, $Ca_V1.2$ and $K_V1.5$. Cells were depolarized for 200 ms from -100 to +60 mV in steps of 10 mV (upper left). The average peak current for each voltage step was normalized to the maximal response for $Na_V1.5$ (upper right), $Ca_V1.2$ (lower left) and $K_V1.5$ (lower right).



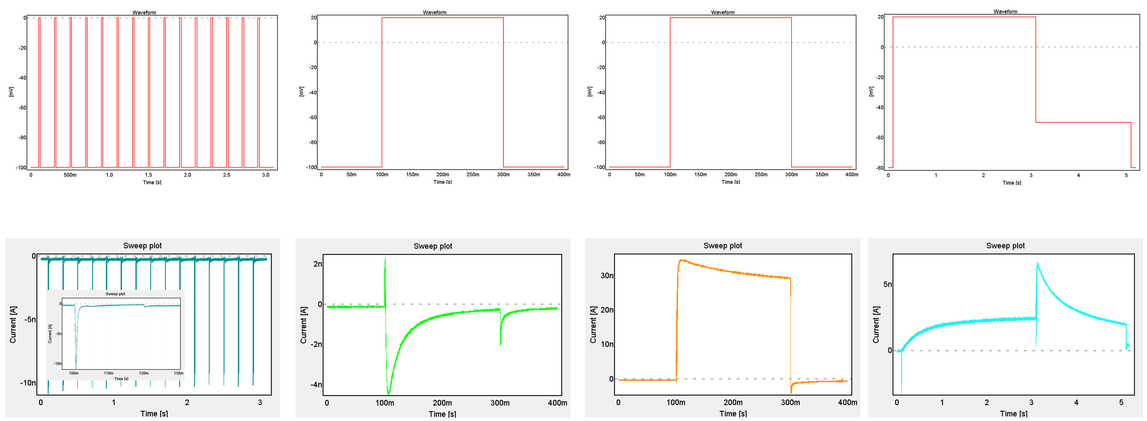
[Fig. 8]: IV protocol and normalized IV curve for hERG. Cells were depolarized for 3 s from -80 to +20 mV, followed by 2 s at a voltage between -120 mV and +20 mV in steps of 10 mV (left). The average peak tail current for each voltage step was normalized to the maximal response (right).

Pharmacology of $Na_V1.5$, $Ca_V1.2$, $K_V1.5$ and hERG



[Fig. 10]: Dose response relationships for tetracaine, nifedipine and verapamil acting on $Na_V1.5$ (upper left), $Ca_V1.2$ (upper right), $K_V1.5$ (lower left) and hERG (lower right). Corresponding pIC_{50} values are 5.1 ($Na_V1.5$), 6.3 ($Ca_V1.2$), 4.9 ($K_V1.5$) and 6.1 (hERG), n=2-4 wells per data point.

Electrophysiological stimulation of different ion channels



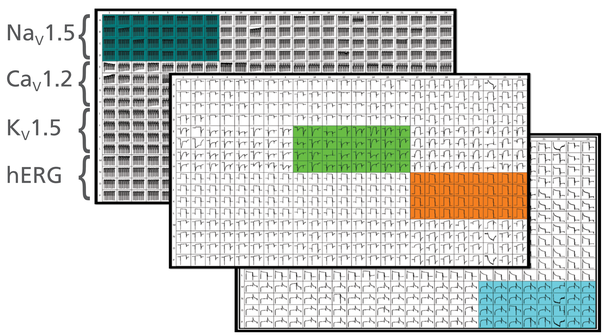
[Fig. 3]: $Na_V1.5$ protocol and electrophysiological response. Cells were depolarized for 20 ms from -100 mV to 0 mV in a 5 Hz stimulation train (left). The depolarization results in a $Na_V1.5$ current (right).

[Fig. 4]: $Ca_V1.2$ protocol and electrophysiological response. Cells were depolarized for 200 ms from -100 mV to 20 mV (left). The depolarization results in a $Ca_V1.2$ current (right).

[Fig. 5]: $K_V1.5$ protocol and electrophysiological response. Cells were depolarized for 200 ms from -100 mV to 20 mV (left). The depolarization results in a Kv1.5 current (right).

[Fig. 6]: hERG protocol and electrophysiological response. Cells were depolarized for 3 s from -80 mV to 20 mV, followed by 2 s at -50 mV before returning to -80 mV (left).

Parallel measurements of different ion channel currents



[Fig. 9]: Parallel measurement of 4 different cell lines. $Na_V1.5$ (olive), $Ca_V1.2$ (green), $K_V1.5$ (orange) and hERG (cyan) were measured by applying the according voltage protocols. Quarters containing the matching intracellular and extracellular solution for the according ion channel are colored. The voltage protocol for $Na_V1.5$ was followed by the identical $Ca_V1.2$/$K_V1.5$ protocol and concluded with stimulating the hERG channel.

Conclusion

We tested the parallel investigation of four different cell lines stably expressing the ion channels $Na_V1.5$, $Ca_V1.2$, $K_V1.5$ and hERG on the Qube. Cells were measured in whole cell voltage clamp mode and the current response towards voltage step protocols and different ion channel blockers was observed. Considering the pharmacological responses and the emerging IC_{50} values, we can conclude that the Qube is able to reliably measure different cell types with differing voltage and application protocols on QPlate. In addition, the IV curves for $Na_V1.5$, $Ca_V1.2$, $K_V1.5$ and hERG further confirm the Qube as a precise mean to analyze the behavior of multiple ion channels. If the CiPA proposal is carried out, the Qube will be able to serve as a next-generation APC instrument, supporting high quality data for a safe and swift drug development process.

Materials and methods

Cells

Human $Na_V1.5$ and $Ca_V1.2$ channels were measured in stably expressing HEK293 cells, human $K_V1.5$ and hERG channels were expressed in CHO cells. Cells were harvested in trypsin or detachin, washed in extracellular solution and transferred to the ccCTP.

Extracellular solutions

For $Na_V1.5$, $K_V1.5$ and hERG (in mM): 2 $CaCl_2$, 1 $MgCl_2$,10 HEPES, 4 KCl, 145 NaCl, 10 Glucose. The osmolarity was adjusted to 305 mOsm with sucrose, pH 7.4.
For $Ca_V1.2$ (in mM): 10 $CaCl_2$, 2 $MgCl_2$,10 HEPES, 4 KCl, 145 NaCl, 10 Glucose. The osmolarity was adjusted to 305 mOsm with sucrose, pH 7.4.

Intracellular solutions

For $Na_V1.5$ and $Ca_V1.2$ (in mM): 140 CsF, 1/5 EGTA/ CsOH, 10 HEPES, 10 NaCl. The osmolarity was adjusted to 320 mOsm with sucrose, pH 7.3.
For $K_V1.5$ and hERG (in mM): 120 KF, 20 KCl, 10 HEPES, 10 EGTA. The osmolarity was adjusted to 300 mOsm with sucrose, pH 7.2.

Compounds

Tetracaine, nifedipine and verapamil (all Sigma-Aldrich) were dissolved in DMSO to a final concentration of ≤0.3% DMSO. Tetracaine was applied to HEK-$Na_V1.5$ cells in 3-fold dilutions ranging from 30 µM to 41.2 nM, nifedipine was applied to HEK-$Ca_V1.2$ cells in 3-fold dilutions ranging from 1 µM to 1.37 nM and verapamil was applied to CHO- $K_V1.5$/hERG DUO cells in 3-fold dilutions ranging from 30 µM to 41.2 nM.

Data aquisition

Electrophysiological measurements were planned and performed with the ViewPoint Software. Sampling was done at 25000 Hz with a cut-off frequency of 1000 Hz (Bessel filter).

Data analysis

Recorded whole-cell current traces were stored in an integrated database (Oracle). IV-relationships for current activation and concentration-dependent drug effects (Hill fit and IC_{50}) were analyzed using the Sophion Analyzer Software.