SOPHION BIOSCIENCE

Application Report

Keep it simple – Ready-To-Use $Ca_V1.2$ cells on Qube 384

High throughput screening on $Ca_V1.2$ channels without the need for cell culture

Summary

CiPA recommended $Ca_V1.2$ assay protocols were run on Qube 384 using Ready-To-Use (RTU) $Ca_V1.2$ cells from NMI TT with ~80% success rate, low run-down and reliable pharmacology. We demonstrate the quality and versatility of ready-to-use $Ca_V1.2$ cells, but also that RTU cells can be a valuable addition to the laboratory tool pack to 1) decrease variation from cell culture and 2) decrease dependence on skilled cell culture personnel.

- Ready-to-use cells easily ignites your experiment within minutes
- Up to 82% success rate
- Reliable pharmacology including safety pharmacology recommended by CiPA
- Rundown of only +0.2% ± 0.06% per minute

Introduction

The voltage-sensitive L-type Ca^{2+}-channel (LTCC) $Ca_V1.2$ is widely expressed in vascular smooth muscle tissue and the heart muscle[1-3]. The opening of the channels leads to an increase of intracellular calcium, which acts as second messenger and thereby affects a variety of cellular processes[4] including heart muscle contraction and neurotransmitter release. $Ca_V1.2$ is, therefore, an important target in e.g. safety pharmacology screening. The channels are known to require a large depolarization for their activation and once activated they display a long-lasting current flow, which typically can be blocked by low micromolar concentrations of e.g. dihydropyridines, phenylalkylamines and benzothiazepines[5,6].

In this study, currents from freshly thawed ready-to-use CHO-h$Ca_V1.2$ were recorded on the high-throughput platform Qube 384 in multi-hole mode (10 patch holes per well). Success rates, rundown, sealing properties and the pharmacological effects of three compounds were determined.

Results

Calcium currents and $Ca_V1.2$ hallmarks

Cells were clamped to -100 mV and calcium currents were evoked by application of a depolarization step to +20 mV for 200 ms every 15 seconds (Fig.1, top). Alternatively, the cells were stimulated with the CiPA-recommended voltage protocol (Fig.1, bottom), with 30 seconds between sweep starts.

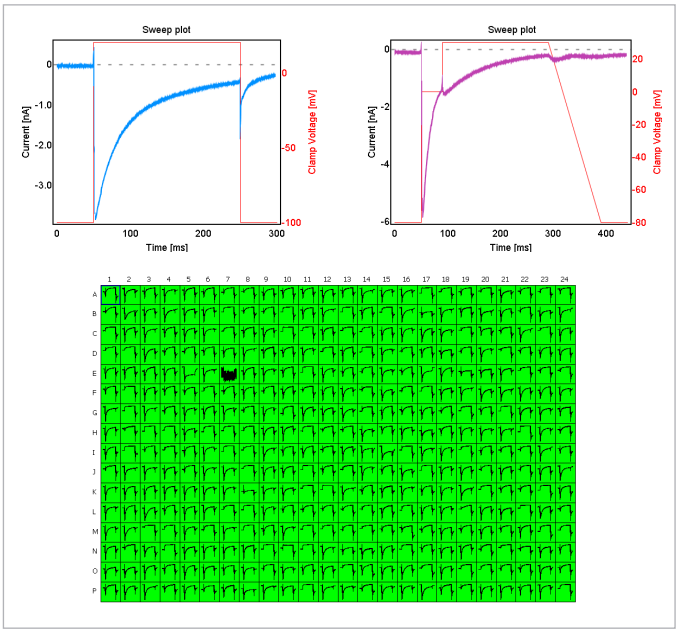

Fig. 1: Raw data traces and voltage protocols. Top: The left y-axis displays current, the right y-axis the clamp voltage. Top left: Recording of a calcium current (blue) in response to a depolarization step from -100 mV to +20 mV for 200 ms (red) in $Ca_V1.2$-expressing cells (multi-hole, 10 patch holes/well). Top right: Recording of a calcium current (purple) in response to a depolarization according to the CiPA protocol. Bottom: Plate view of the calcium currents after the one step depolarization.

Measurements performed on a multi-hole QChip had success rates of between 75 and 82% with the following success criteria:

- Current < -0.5 nA per well
- Capacitance > 4 pF per cell
- Resistance > 200 MΩ per cell

On a typical QChip 384 with the CiPA-recommended voltage protocol, cells passing the success criteria showed a peak calcium current of -2.0 ± 0.1 nA per well. The average whole-cell resistance, throughout the experiment, was 425 ± 10 MΩ per cell and the average capacitance 7.0 ± 0.1 pF per cell (all mean ± SEM). With a stimulation frequency of 0.07 Hz over 25.5 minutes, there was an average rundown/runup of +0.2% ± 0.06% per minute (mean ± SEM). Comparing the rundown within a typical experiment setup of baseline vs. compound application after 13.5 minutes, -1.1% ± 0.6% rundown occurred (mean ± SEM).

Pharmacology

For the screening of both use-dependent and use-independent compounds, one of the two different voltage protocols were used per experiment. For taking use-dependent compounds like verapamil and diltiazem into account, the CiPA-recommended voltage protocol was modified such as that the holding potential was raised from -80 to -60 mV or -50 mV.

1) CiPA-recommended (but with a V_{hold} of -60 mV)
- 50 ms at -60 mV
- 40 ms at 0 mV
- 200 ms at +30 mV
- 75 ms ramp from +30 mV to -60 mV
- 50 ms at -60 mV

2) CiPA-recommended (but with a V_{hold} of -50 mV)
- 50 ms at -50 mV
- 40 ms at 0 mV
- 200 ms at +30 mV
- 75 ms ramp from +30 mV to -50 mV
- 50 ms at -50 mV

20 stimuli were executed, the first set without, the second one with compound (Fig.2). The time between sweep starts was 5 or 15 seconds. Nifedipine, diltiazem or verapamil were applied in 5 different concentrations (single dose per well). The current amplitude of the last saline addition and the 3 µM reference addition served as baseline/full response (0% vs 100% block respectively) for normalization. Using -50 instead of -60 mV holding potential shifted the dose-response curve for diltiazem and verapamil but not for nifedipine when using 15 seconds in between sweeps (Fig.3). Choosing 5 instead of 15 seconds for the time between sweep starts did not change the compound effect (compare verapamil data at a holding potential of -50 mV in Fig.3 and 4).

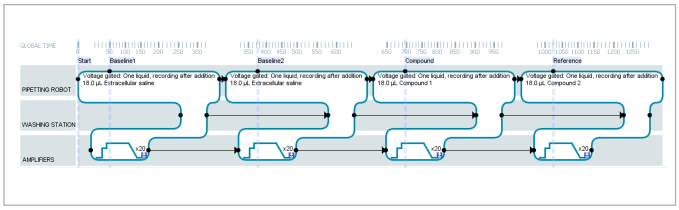

Fig. 2: Experimental setup for $Ca_V1.2$ pharmacology measurements as seen in the Sophion Viewpoint software. The first saline addition (Baseline 1) contains 20 repetitions of the voltage protocol and was repeated (Baseline 2). Then followed 20 more depolarizations in the presence of test compound before 3 µM nifedipine was added as reference.

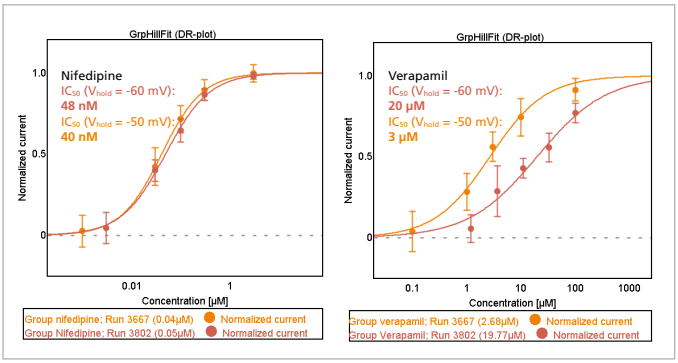

Fig. 3: Dose-response curves for nifedipine and verapamil using a holding potential of -50 or -60 mV and 15 seconds in between sweeps. The Hill-fit was applied to the data points, values are mean ± SD.

The data was reproducible in-between days for all compounds (Fig.4) and the rundown using 0.3% DMSO was constant at 1±12% and 0±14% comparing baseline vs compound current amplitude.

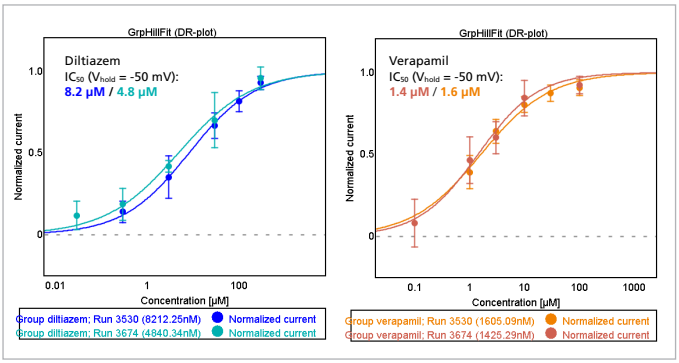

Fig. 4: Dose-response curves for diltiazem and verapamil using a holding potential of -50 mV and 5 seconds in between sweeps. Experiments were executed on different days. The Hill-fit was applied to the data points, values are mean ± SD.

The experiments confirmed the use-dependent nature of diltiazem and verapamil. It also shows, that for detecting use-dependent compounds on $Ca_V1.2$, a holding potential of -50 mV rather than -60 or -80 mV should be applied.

This assay also demonstrated the quality and versatility of ready-to-use $Ca_V1.2$ cells.

Methods

Cells

Experiments in this study were performed using CHO-h$Ca_V1.2$ RTU cells (kindly provided by NMI TT Pharmaservices, Reutlingen, Germany). They express the human CACNA1C, CACNB2 and CACNA2D genes using the proprietary IonGate expression system. The cells can be obtained by contacting acCELLerate GmbH, Osterfeldstraße 12-14, 22529 Hamburg, Germany, accellerate.me.

Cell culture

No cell culture was needed for these frozen, ready-to-use CHO-h$Ca_V1.2$ cells. Before job execution, they were thawed and brought into solution according to the protocol provided by NMI TT.

Experimental setup

Whole-cell protocol:
A two-second suction pulse from -10 mbar to -250 mbar was followed by 10 seconds at -10 mbar and thereafter a two-second suction pulse from -10 mbar to -350 mbar was applied. For more parameters, see Fig.5.

One step depolarization voltage protocol:
Cells were held at -100 mV holding potential and depolarized for 200 ms to +20 mV.

CiPA voltage protocol:
Cells were held at -60 mV or -50 mV holding potential and then depolarized for 40 ms to 0 mV, followed by 200 ms at +30 mV and a ramp from +30 mV to -60 or -50 mV within 75 ms.

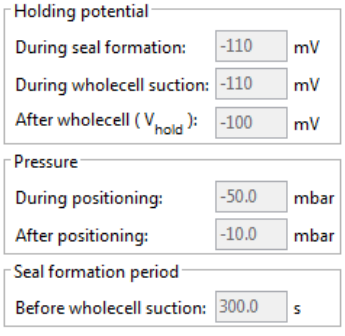

Fig. 5: Detailed parameters of the whole-cell protocol.

References:

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