SOPHION BIOSCIENCE

Application Report

hCa$_V$1.2 recordings using QPatch

A reproducible high-throughput assay with high success rates for biophysical and pharmacological studies of the Ca$_V$1.2 channel

Summary

The development of screening assays for the Ca$_V$1.2 channel has been challenging due to the tendency of the channel to exhibit declining current levels (rundown) during the experiment[1,2]. Here we report a robust Ca$_V$1.2 assay yielding high success rates, low rundown and reliable pharmacology.

- Pharmacology and current-voltage relationship in accordance with literature values
- Success rates of up to **98%**
- Stable currents with rundown as low as **2% per min**

Introduction

The L-type voltage-gated calcium channel Ca$_V$1.2 is expressed in various mammalian tissues, including heart, smooth muscle and brain[3,4,5], and its dysfunction has been implicated in a range of cardiovascular and neurological diseases[6,7]. Opening of the Ca$_V$1.2 channel results in an increase in the intracellular concentration of calcium ions (Ca^{2+}), affecting a variety of cellular processes including muscle contraction, hormone secretion and neuronal transmission[7,8,9], thus rendering the channel an important pharmacological target.

Ca$_V$1.2 channels require a strong depolarization to be activated, they display relatively long-lasting activity and can be blocked by low micromolar concentrations of e.g. dihydropyridines, phenylalkylamines and benzothiazepines[8,9]. Following activation, the channel displays both Ca^{2+}- and voltage-dependent inactivation[10].

In this study, current traces from HEK-hCa$_V$1.2 cells were recorded using QPatch employing both single-hole and multi-hole (10 patch holes per well) QPlates. The current-voltage relationship and pharmacology of three different compounds were determined with high success rates and low rundown.

Results and discussion

The following experiments were carried out:

- Recordings of Ca^{2+} currents
- Current-voltage relationship of Ca$_V$1.2 channels
- Pharmacology of Ca$_V$1.2 channels
- Rundown analysis

1. Recordings of Ca^{2+} currents

Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (Fig. 1).

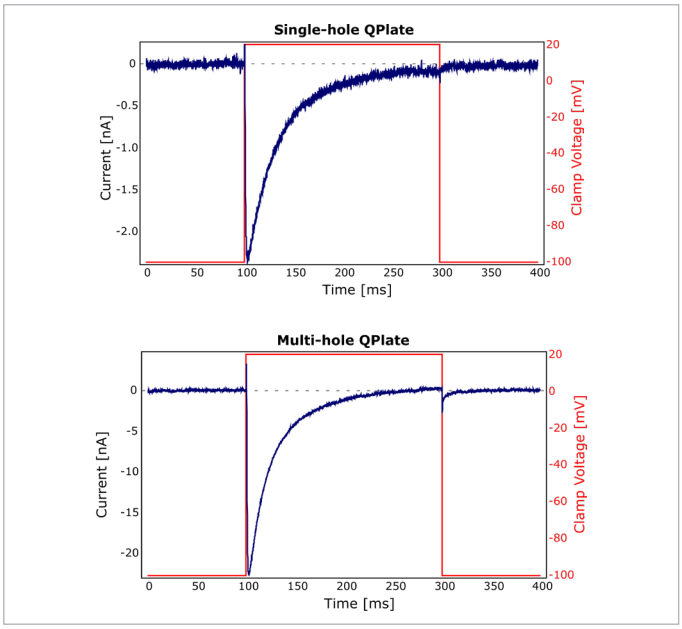

Fig. 1: Representative recordings of Ca^{2+} current (blue) on a single-hole (top) and multi-hole (bottom) QPlate following a 200 ms depolarization from -100 mV to 20 mV (red) in Ca$_V$1.2 expressing cells.

The criteria for a successful recording were (average of first 10 sweeps):

Single-hole QPlate:
I_{peak} < -100 pA
$R_{membrane}$ > 100 MΩ per cell
C_{total} > 4 pF per cell

Multi-hole QPlate:
I_{peak} < -500 pA
$R_{membrane}$ > 100 MΩ per cell
C_{total} > 4 pF per cell

Where I_{peak} is the peak current, $R_{membrane}$ is the membrane resistance and C_{total} is the total capacitance.

The success rates were up to **71%** and **98%**, for single-hole and multi-hole QPlates, respectively. The mean success rates and peak currents were (58 ± 8) % and (1.2 ± 0.3) nA for single-hole QPlates (***N*** = 6 QPlates), and (88 ± 5) % and (14 ± 3) nA for multi-hole QPlates (***N*** = 6 QPlates). The success rates and peak currents started declining at cell passage numbers above 20.

2. Current-voltage relationship of $Ca_V1.2$ channels

The current-voltage relationship was quantified on single-hole and multi-hole QPlates by applying a depolarization step protocol from -100 mV to +60 mV for 200 ms in increments of 10 mV. The time between sweeps was 15 s (Fig. 2).

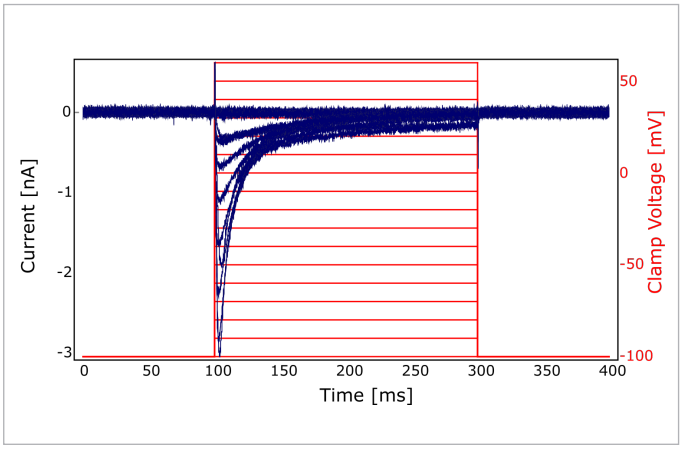

Fig. 2: Representative measurement of current-voltage relationship using a single-hole QPlate. Ca^{2+} current response of a cell expressing $Ca_V1.2$ channels (blue) following a depolarization step protocol in 10 mV increments from -100 mV to +60 mV (red).

The peak current was extracted at each voltage step, normalized to the peak current at 0 mV and plotted as a function of voltage (Fig. 3).

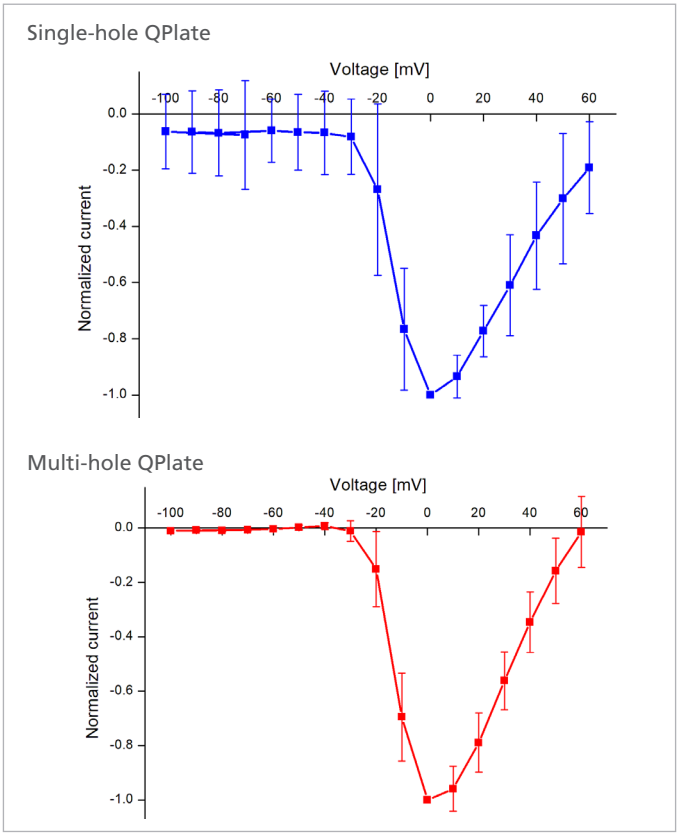

Fig. 3: Current-voltage plots of normalized current versus voltage [mV] for a single-hole QPlate (top) and a multi-hole QPlate (bottom). Displayed is the averaged normalized current ± SD.

3. Pharmacology of $Ca_V1.2$ channels

The $Ca_V1.2$ pharmacology was quantified on single-hole and multi-hole QPlates. Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (see Fig. 1).

After 10 initial depolarization steps (15 s between sweeps), a voltage protocol of 10 depolarization trains (5 pulses at 2.5 Hz and 30 s between sweeps) was applied three times. First, the baseline was established (0.1% DMSO) (Fig. 4A). Subsequently, one of the three compounds, diltiazem, nifedipine or verapamil, was applied in 5 concentrations (non-cumulative). The concentration range was a 4-fold dilution with the highest concentration being:

- Diltiazem 100 µM
- Nifedipine 3 µM
- Verapamil 50 µM

Finally, 10 µM nifedipine was applied as a positive control.

The peak current of the last pulse of the depolarization train (ROI in Fig. 4 top) was extracted for the compound (I_{comp}) and normalized to the corresponding peak current of the negative (I_-) and positive (I_+) controls (Fig. 4 middle), according to:

$$\text{Normalized current} = \frac{I_{comp} - I_-}{I_+ - I_-}$$

The normalized current was plotted as a function of compound concentration (Fig. 4 bottom). The IC_{50} values were extracted by fitting the Hill equation to the data and listed in Table 1 together with literature values.

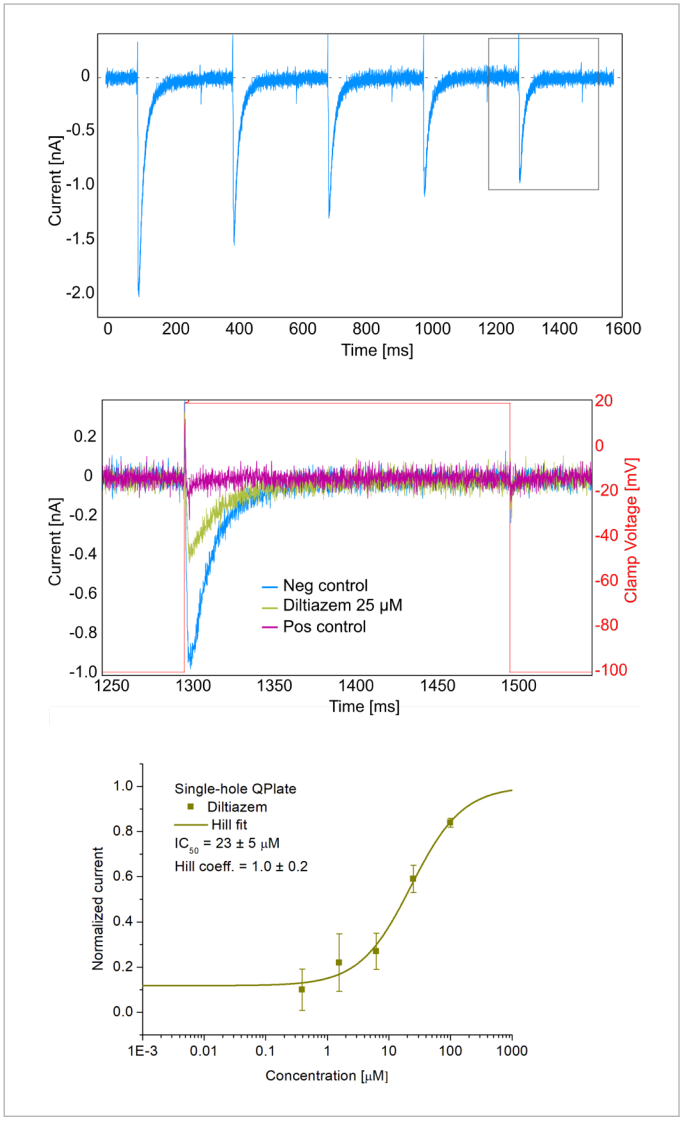

Fig. 4: Figure 4: $Ca_V1.2$ pharmacology measurement using a **single-hole QPlate**. Top: Representative $Ca_V1.2$ current trace upon the application of a depolarization train (5 pulses from –100 mV to +20 mV at 2.5 Hz). Middle: Peak current of the last depolarization of the train (ROI in top graph) displayed for the negative control (0.1 % DMSO, blue), upon the addition of 25 µM diltiazem (green), and for the positive control (10 µM nifedipine) (pink). Bottom: Dose-response relationship of diltiazem. Each data point is the average ± SD of at least 5 cells and the IC_{50} value and Hill coefficient were estimated by fitting the Hill equation.

Table 1: Potency of the three tested compounds. IC_{50} values for the listed compounds, quantified on single-hole and multi-hole QPlates, together with literature values.

Compound	IC_{50} (µM) Single-hole	N_{QPlate}	IC_{50} (µM) Multi-hole	N_{QPlate}	IC_{50} (µM) Literature values
Diltiazem	23 ± 5	4	21 ± 5	4	6 - 250[11,12]
Nifedipine	0.32 ± 0.03	4	0.11 ± 0.08	4	0.06 - 0.10[1,11]
Verapamil	12 ± 2	4	5.7 ± 0.6	4	2.7 - 5.3[1,11]

The IC_{50} values were in good agreement with IC_{50} values measured on other $Ca_V1.2$ cell lines in-house (11 µM ± 3 µM, 0.052 µM ± n/a µM, 5 µM ± 2 µM for diltiazem, nifedipine and verapamil, respectively).

4. Rundown analysis

A concern with $Ca_V1.2$ assays is the rundown of the current under continuous stimulation. Here, the rundown was quantified using single-hole and multi-hole QPlates. Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (see Fig. 1). After 10 initial depolarization steps (15 s between sweeps), an additional 50 sweeps of single depolarization steps were applied (15 s between sweeps), followed by 25 depolarization trains that contained 5 pulses at 2.5 Hz (30 s between sweeps).

The rundown was quantified for the second voltage protocol by comparing the peak currents evoked by the 1st and 50th depolarization (Fig. 5, top):

$$\text{Rundown (\% per min):} \quad \frac{I\text{peak}_1 - I\text{peak}_{50}}{I\text{peak}_1 \cdot \Delta t}$$

where Δt is the time between sweep 1 and 50 in minutes. The mean rundown was (3.0 ± 0.1) % per min and (1.7 ± 0.2)% per min for multi-hole and single-hole QPlates, respectively.

The rundown was also quantified for the third voltage protocol, by comparing the 1st and 25th train depolarization (see Fig. 5, bottom). For the first depolarization within the train the mean rundown was (4.1 ± 0.1) % per min and (3.5 ± 0.2) % per min for multi-hole and single-hole QPlates, respectively.

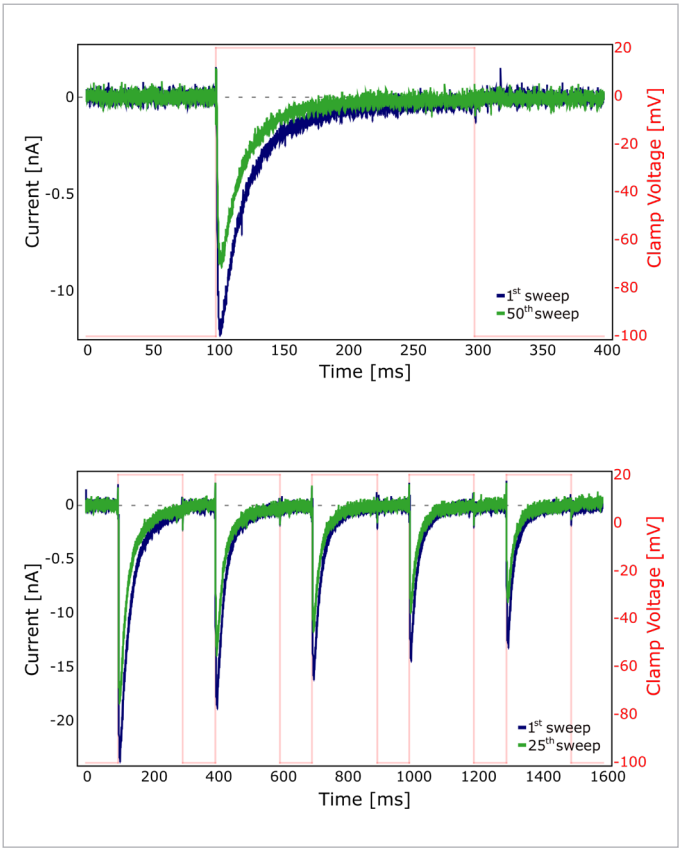

Fig. 5: Example current traces used for quantifying rundown. Top: 1st (blue) and 50th (green) depolarization in voltage protocol 2. Bottom: 1st (blue) and 25th (green) depolarization train in voltage protocol 3. Rundown was quantified for the first depolarization of the train.

Conclusion

$Ca_V1.2$ Ca^{2+} currents were recorded using the QPatch on both single-hole and multi-hole QPlates. The reported current-voltage relationship and pharmacology experiments were in accordance with literature values and highly reproducible. The current levels were stable over time with rundown as low as **~2% per min** and high success rates were achieved (up to **71%** and **98%** for single-hole and multi-hole QPlates, respectively) with cells at passage numbers below 20.

Methods

Experiments in this study were performed on HEK-h$Ca_V1.2$ cells, kindly provided by SB Drug Discovery. The cell line is stably transfected with the $Ca_V1.2$ channel and cultured in 30 µM verapamil according to SB Drug Discovery guidelines. HEK-h$Ca_V1.2$ cells were harvested according to Sophion standard procedures.

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Author:

Kadla R Rosholm, Application scientist

Sophion Bioscience A/S, Baltorpvej 154, 2750 Ballerup, Denmark
Phone: +45 4460 8800, E-mail: info@sophion.com

sophion.com