SOPHION BIOSCIENCE

Application Report

$Ca_V1.2$ on Qube 384 - pharmacology, stability and CiPA

High throughput screening of $Ca_V1.2$ pharmacology with success rates of 95%

Summary

- Success rates of 95%
- Reference pharmacology in accordance with literature values
- Rundown of 0.3 - 0.8% 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 CHO-h$Ca_V1.2$ were recorded on the high-throughput platform Qube 384 in multi-hole mode (10 patch holes per well) at both a customer's site and at Sophion. Success rates, rundown, sealing properties and the pharmacological effects of two compounds were determined.

Results and discussion

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 left). Alternatively, the cells were stimulated with the CiPA voltage protocol (Fig.1, top right), with 30 seconds between sweep starts.

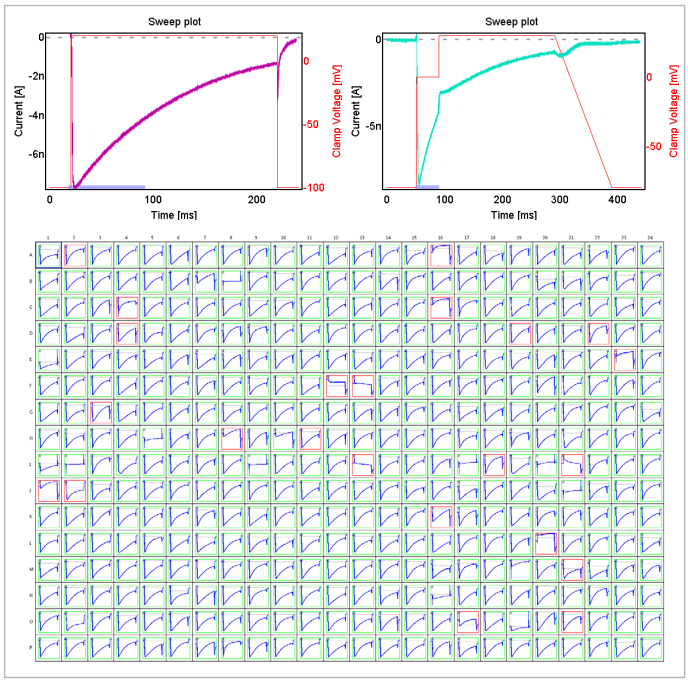

Fig. 1: Raw data traces and voltage protocols. Top: The left y-axis displays current and the right y-axis the holding potential. The purple bar on the bottom of the x-axis (time) shows the cursor interval of peak current detection. Top left: Recording of a calcium current (purple) following a standard depolarization 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 (turquoise) following a depolarization according to the CiPA protocol. Bottom: Plate view of the calcium currents after a standard depolarization. Red squares indicate a site that has failed the success criteria.

Measurements were performed on multi-hole QChips and had success rates of routinely 95% with the following success criteria:

- Current < -1 nA per well
- Capacitance > 6 pF per cell
- Resistance > 200 MΩ per cell

On a typical QChip 384 with a standard voltage protocol, cells passing the success criteria showed a peak calcium current of

5.7±0.1 nA per well. The average whole-cell resistance over the course of the experiment was 548 ± 13 MΩ per cell (mean ± SEM) and the average capacitance 13.6 ± 0.1 pF per cell. With a stimulation frequency of 0.07 Hz over 12.25 minutes, there was an average rundown of 0.8% per minute.

Using the CiPA protocol, cells showed a peak calcium current of 6.1±0.1 nA per well. The average whole-cell resistance over the course of the experiment was 756 ± 15 MΩ per cell (mean ± SEM) and the average capacitance 12.3 ± 0.1 pF per cell. With a stimulation frequency of 0.03 Hz over 50 minutes, there was an average rundown of 0.28% per minute.

Current-voltage relationship

A step protocol from -100 mV up to +60 mV was applied in 10 mV steps. The maximum current was on average elicited at a voltage of -13 ± 7 mV (± SD). An IV-curve on average current at each potential (± SEM) is shown in fig. 2.

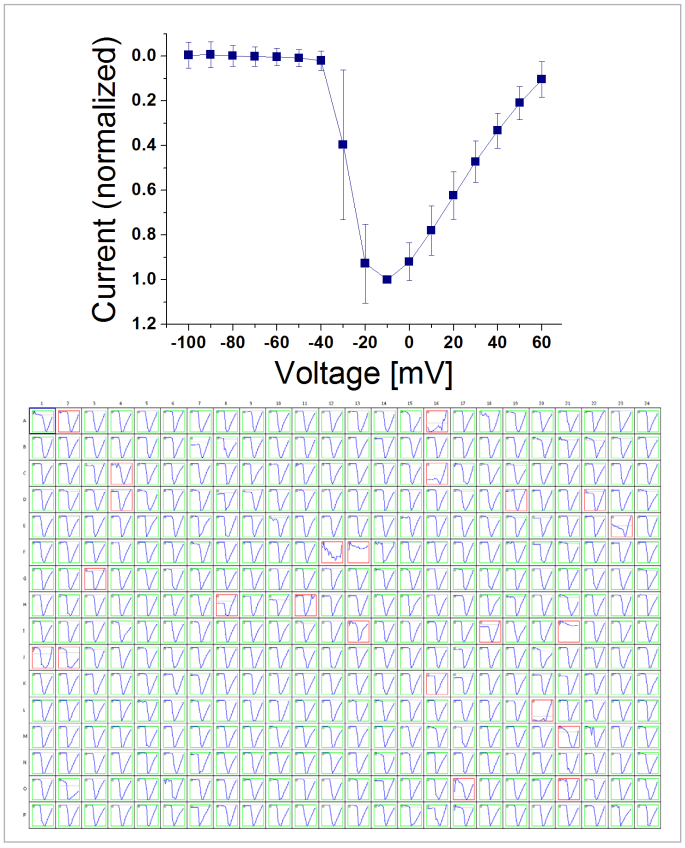

Fig. 2: Top: Normalized current-voltage relationship of $Ca_V1.2$. Error bars display the SEM of the average values. Bottom: Plate view of IV curves for each position. Currents were recorded on a multi-hole QChip 384.

Pharmacology

After 10 activation sweeps with a standard depolarization, 10 train stimuli of 5 pulses at 2.5 Hz each were executed, the first set without, the second one with compound (Fig.3). The time between sweep starts was 15 seconds. Diltiazem and verapamil were applied in 7 different concentrations in a cumulative manner (single dose per well). The highest concentration of both compounds was 100 µM. Each lower concentration was a serial 3-fold dilution with 0.1% DMSO in each concentration.

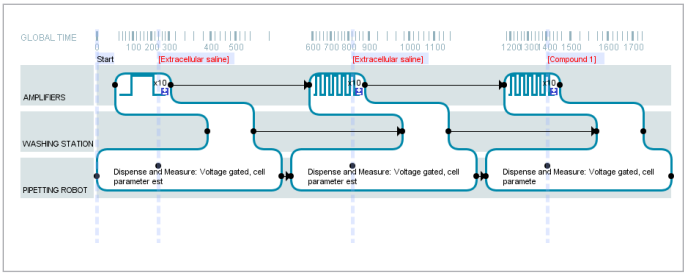

Fig. 3: Experimental setup for $Ca_V1.2$ pharmacology measurements as seen in the Sophion Viewpoint software. The first liquid addition (left block) contains 10 single depolarizations and is followed by 10 train depolarizations in saline (middle block). Lastly, 10 train stimulations in compound solution are applied to the $Ca_V1.2$-expressing cells (right block).

The current amplitude of the *last* depolarization step of the train in the presence of compound [Compound1] was normalized to the *last* depolarization step of the train in control conditions, [Extracellular saline].

Also, the *first* depolarization step of the stimulation train in the compound period [Compound 1] was normalized to the *first* stimulation step in the second saline period [Extracellular saline] (Fig.3). To point out potential use-dependent mode of action, the first and the fifth normalized current were compared to each other (Fig.4). This confirmed that diltiazem had no effect and verapamil exhibited a slight block at its highest concentration when analyzing the first peak of the train stimulation, whereas there was a potent effect of both compounds on the 5th peak. The Hill fits to these concentration-dependent effects are shown in Fig.4 and results are in line with the strong use-dependency of verapamil and diltiazem (Tab.1).

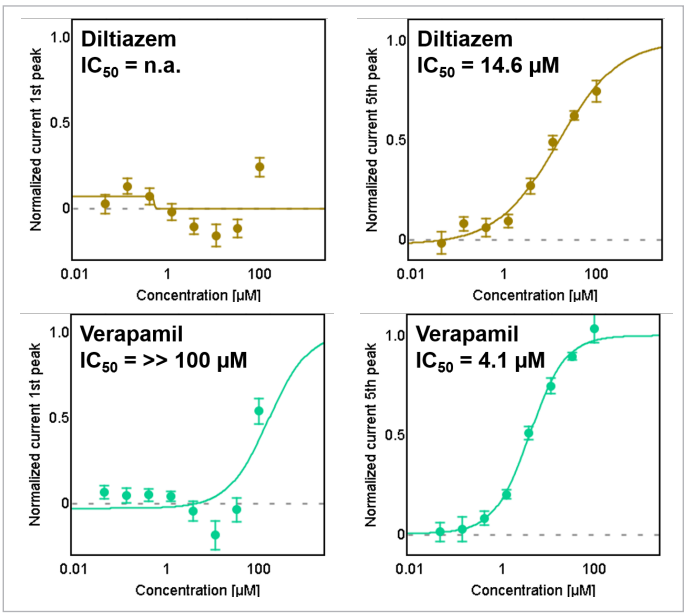

Fig. 4: Dose-response curves for diltiazem and verapamil. The current amplitude at the end of a 2.5 Hz stimulation train (right panel), or at the beginning of the train (left panel) was compared in the presence of compound vs. the 0.1% DMSO control. The Hill-fit was applied to the data points, error bars are ± SEM.

Table 1: IC_{50} values of two reference compounds

Compound	Stimulation	Qube 384	Literature
Dilitazem	IC_{50} at 1st train stimulation	>> 100 µM	33 µM[7]
	IC_{50} at 5th train stimulation	15 µM	
Verapamil	IC_{50} at 1st train stimulation	>> 100 µM	2 µM[8]
	IC_{50} at 5th train stimulation	4 µM	

Conclusion

The CHO-hCa_V1.2 cell line routinely shows success rates of 95% in experiments performed using the Qube 384 and is suitable for designing robust assays. The pharmacology experiments reliably confirm use-dependent action for the compounds diltiazem and verapamil, while the rundown was as low as 0.3-0.8% per minute.

Methods

Cells

Experiments in this study were performed using CHO-hCa_V1.2 cells (kindly provided by Charles River Laboratories, Cleveland, OH). The cells express the human CACNA1C, CACNB2 and CACNA2D genes.

Cell culture

CHO-hCa_V1.2 cells were induced with tetracycline 48 hours before the experiment and transferred to 30˚C 24 hours previous to the job execution. Cells were harvested with Detachin, spun down and resuspended in serum-free medium with HEPES. For more information, contact your application scientist.

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.

Standard voltage protocol:
Cells were held at -100 mV holding potential and were depolarized for 200 ms to +20 mV. Train stimulations repeated the single depolarization steps 5 times at 2.5 Hz.

CiPA voltage protocol:
Cells were held at -80 mV holding potential and were depolarized for 40 ms to 0 mV, followed by 200 ms at +30 mV and a ramp from +30 mV to -80 mV within 100 ms.

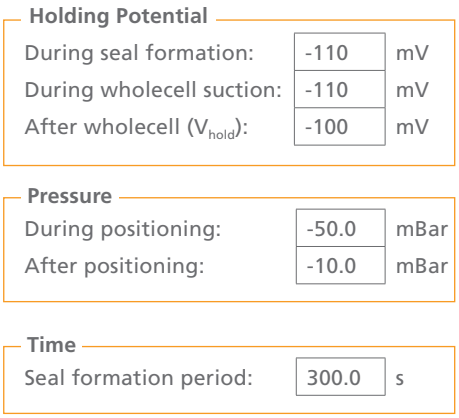

Fig. 5: Detailed parameters of the whole-cell protocol.

References:

1. Sinnegger-Brauns MJ, Hetzenauer A, Huber IG et al. 2004. Isoform-specific regulation of mood behavior and pancreatic β cell and cardiovascular function by L-type Ca2+ channels. J Clin Invest; 113:1430-9
2. Moosmang S, Schulla V, Welling A et al. 2003. Dominant role of smooth muscle L-type calcium channel CaV1.2 for blood pressure regulation. EMBO J; 22:6027-34
3. Zhang J, Berra-Romani R, Sinnegger-Brauns MJ et al. 2007. Role of CaV1.2 L-type Ca2+ channels in vascular tone: effects of nifedipine and Mg2+. Am J Physiol Heart Circ Physiol; 292:H415-25
4. Tsien RW, Tsien RY 1990. Calcium channels, stores, and oscillations. Annu Rev Cell Biol; 6:715–760
5. Reuter H 1983. Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature; 301: 569–574
6. Reuter H 1979. Properties of two inward membrane currents in the heart. Annu Rev Physiol; 41: 413–424
7. Catterall WA, Perez-Reyes E, Snutch TP et al. 2005. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev; 57(4):411-25
8. Kuryshev YA, Brown AM, Emir Duzic E et al. 2014. Evaluating State Dependence and Subtype Selectivity of Calcium Channel Modulators in Automated Electrophysiology Assays. Assay Drug Dev Technol; 12(2): 110–119

Acknowledgement:

Cells kindly provided by Charles River Laboratories

Author:
Melanie Schupp, Application scientist

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

sophion.com