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\textsuperscript{2+}-channel (LTCC) Ca\textsubscript{V}1.2 is widely expressed in vascular smooth muscle tissue and the heart muscle\textsuperscript{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\textsuperscript{4} including heart muscle contraction and neurotransmitter release. Ca\textsubscript{V}1.2 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\textsuperscript{5,6}.

In this study, currents from CHO-hCa\textsubscript{V}1.2 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\textsubscript{V}1.2 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.
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 [Compound1] 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).
The CHO-hCaV1.2 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-hCaV1.2 cells (kindly provided by Charles River Laboratories, Cleveland, OH). The cells express the human CACNA1C, CACNB2 and CACNA2D genes.

#### Cell culture

CHO-hCaV1.2 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.

### References:


### Table 1: IC50 values of two reference compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stimulation</th>
<th>Qube 384</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dilitazem</strong></td>
<td></td>
<td></td>
<td>33 μM²</td>
</tr>
<tr>
<td>IC50 at 1st train stimulation</td>
<td>&gt;&gt; 100 μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50 at 5th train stimulation</td>
<td>15 μM</td>
<td></td>
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<tr>
<td><strong>Verapamil</strong></td>
<td></td>
<td></td>
<td>2 μM²</td>
</tr>
<tr>
<td>IC50 at 1st train stimulation</td>
<td>&gt;&gt; 100 μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50 at 5th train stimulation</td>
<td>4 μM</td>
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</tbody>
</table>

### 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.

| Holding Potential | | Pressure | | Time |
|-------------------|-----------------|----------|----------|
| During seal formation: | -110 mV | During wholecell suction: | -110 mV | After wholecell (V<sub>hold</sub>): | -100 mV | Seal formation period: | 300.0 s |

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

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