Preliminary Investigations into Cav1.2 Ion Channel Cell Line: Potential of Calcium Imaging and Electrical Stimulation

NMI Natural and Medical Sciences Institute
Natural and Medical Sciences Institute

NMI Natural and Medical Sciences Institute at the University of Tübingen
Markwiesenstrasse 55
72770 Reutlingen, Germany
Contact: ayla.aksoyaksel@nmi.de

Ayla Aksoy-Aksel, Timm Danker, Udo Kraushaar

NMI Natural and Medical Sciences Institute at the University of Tübingen
72770 Reutlingen, Germany

Introduction

The voltage-gated L-type calcium channels (Cav1.2) are expressed in multiple tissues, including heart, smooth muscle, and neurons. They are therapeutic targets in cardiac arrhythmia and hypertension. Due to their key role in shaping the cardiac action potential, they are also part of the CiPA-compliant panel of the human ion channel to be tested for cardiac safety.

The CHO cell line with inducible expression of CaV1.2, proprietary cell line by NMI TT, provides an important tool routinely utilized by major pharma companies for automated patch-clamp screens.

The approach described here combines intracellular calcium imaging with electric field stimulation (EFS) option integrated to the Functional Drug Screening System FDSS /µCELL (Hamamatsu Photonics).

We employed that configuration to stimulate Cav1.2 channel cell line and simultaneously detect the transient intracellular calcium changes with calcium sensitive fluorescent dye. Furthermore, initial method validation has been performed by application of Cav1.2 channel activators and inhibitors prior to recordings.

Cav1.2 Cell Line Electrophysiological Investigations

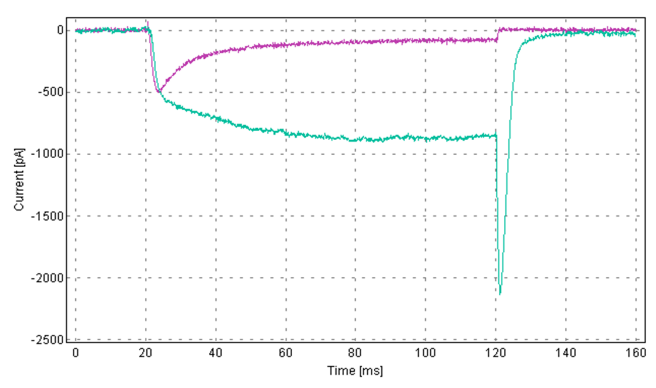


Fig.1: Pharmacological validation: The known Cav1.2 activator FPL64176 enhances Cav1.2 currents by altering inactivation and recovery from inactivation kinetics. Purple: unaltered Cav1.2 currents, green: after application of FPL64176

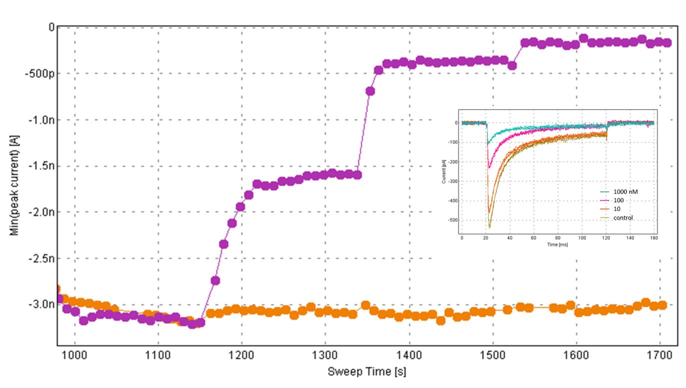


Fig.2: Pharmacological validation: Inhibition of Cav1.2 currents by the inhibitor Nifedipine (purple).
Inset: Representative traces of calcium currents with different Nifedipine concentrations.
Note: run-down free control condition (orange).

Calcium Responses to Different Concentrations of Extracellular Calcium and Potassium

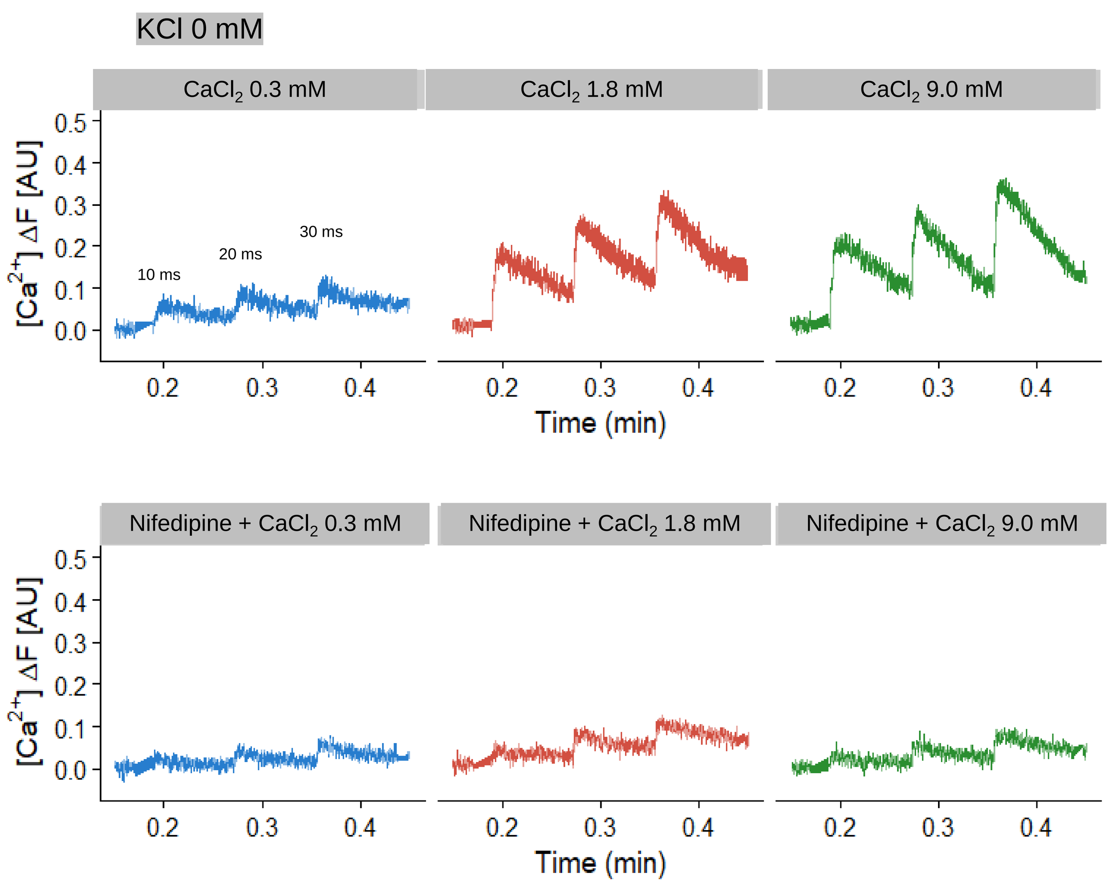


Fig.3: Concentration of the extracellular calcium affects the signal amplitude, in the absence of KCl (0 mM). Nifedipine (20 µM) inhibits the signal for all conditions. Stimulation parameters for all conditions are: 0.2 Hz, 30 V, 3 pulses (10, 20, 30 ms respectively). The signal is normalized to the initial intensity, average of 4-wells for each condition.

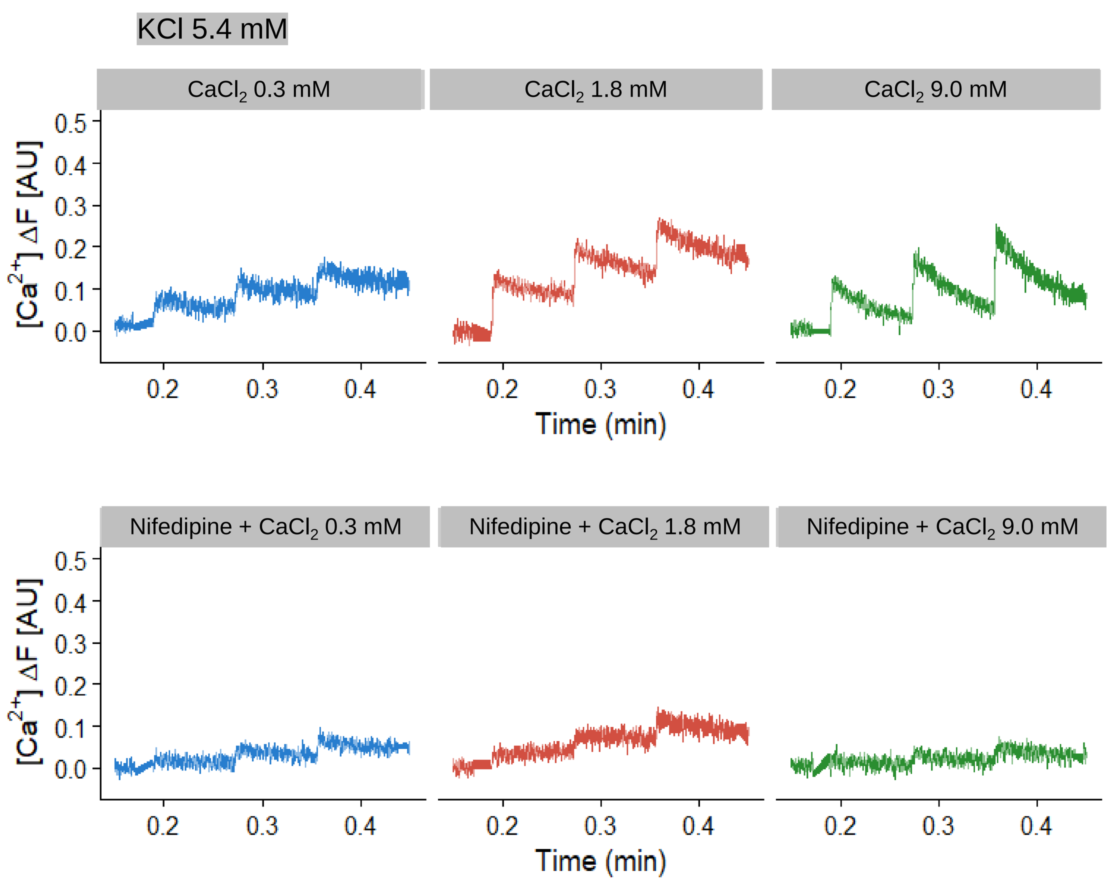


Fig.4: Concentration of the extracellular calcium affects the signal amplitude, also in the presence of KCl (5.4 mM). Nifedipine (20 µM) inhibits the signal for all conditions. Stimulation parameters for all conditions are: 0.2 Hz, 30 V, 3 pulses (10, 20, 30 ms respectively). The signal is normalized to the initial intensity, average of 4-wells for each condition.

Calcium Responses to Cav1.2 Channel Activators or Inhibitors

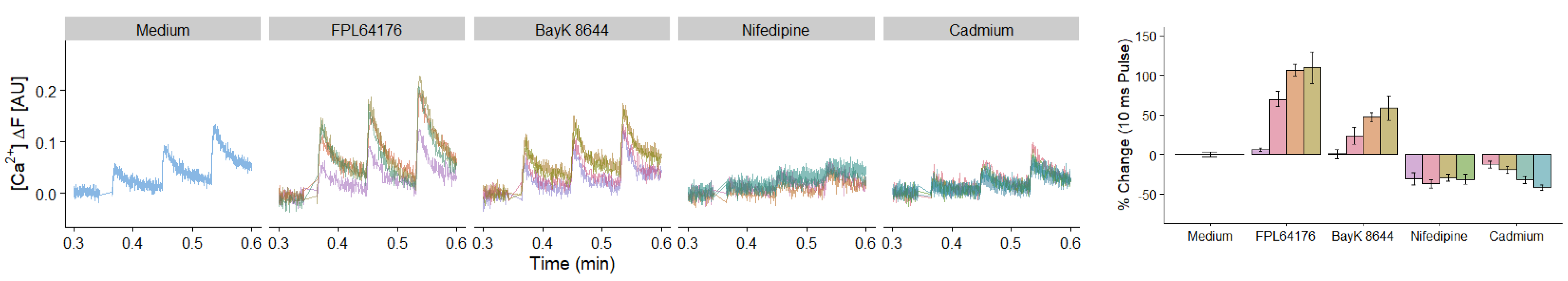
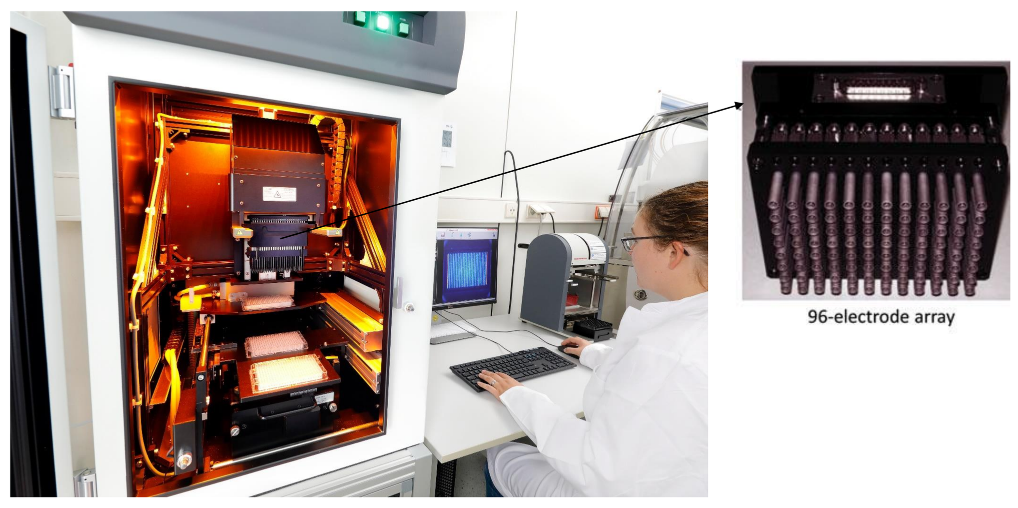


Figure 5: Cav1.2 channel activators increase the calcium signal, whereas inhibitors reduce the calcium signal. $CaCl_2$ and KCl concentrations for all conditions are 1.8 mM and 2.5 mM, respectively. Stimulation parameters for all conditions are: 0.2 Hz, 30 V, 3 pulses (10, 20, 30 ms respectively). The signal is normalized to the initial signal intensity, average of 4-wells for each condition. Right: Change (% of Medium) of the 10 ms signal upon application of compounds (4 concentrations were applied for each compound, all in µM: FPL64176: 0.05, 0.5, 2.5, 5.0; BayK 8644: 0.05, 0.5, 2.5, 5.0; Nifedipine: 0.05, 0.5, 5.0, 10.0; Cadmium chloride: 0.5, 5.0, 50.0, 100.0.

EFS head, illuminator optics, and sensor camera for FDSS/µCELL

Integrated stimulation unit with the FDSS / µCELL systems.



Materials and Methods

Cells: CaV1.2 proprietary cell line by NMI TT, cultured CHO cells expressing hCav1.2 / β2 / α2δ1 channel were used.

Electrophysiology: Whole-cell patch-clamp experiments were performed on QPatch 16X. Fig1-2 show data obtained in multhole mode of the Qpatch (Xmode). Data analysis was accomplished with the QPatch Assay Software.

Recording solution Extracellular (in mM): NaCl 145, KCl 4, HEPES 10, $CaCl_2$ 10, pH 7.4 (NaOH) ~315 mOsm. Intracellular (in mM): CsCl 140, EGTA 10, HEPES 10, MgATP 4, pH7.2 (KOH) ~295 mOsm.

Calcium imaging: cells were plated in a 96-well black walled plate suitable for calcium imaging (17000 cells per well cultivated for 3 days). Cells were loaded with 1-2 µM Cal520/AM (AAT Bioquest) for 45 minutes at 37°C.

Recording solution (in mM): NaCl 121, $MgCl_2$ 0.8, $NaHCO_3$ 6, D-Glucose 5.5, HEPES 25, pH 7.4 (NaOH); to this solution different concentrations of KCl and $CaCl_2$ were added as indicated. All compounds were applied 5-10 min prior to start of the measurements.

Fluorescent images were sampled at 0.01 s and 4x4 binning for the 96-well plate format was used to generate the calcium signals.

Results

CHO based cell line that stably expresses all 3 subunits of the human cardiac L-type Ca channel (hCav1.2 / β2 / α2δ1) has been used to test EFS stimulation unit with FDSS/µCELL equipment.

- Signal amplitude increases with increased extracellular calcium concentration
- Extracellular KCl concentration has less effect compared to the $CaCl_2$ concentration
- Addition of selective Cav1.2 channel inhibitor, Nifedipine, reduced the signal from all tested conditions
- Cav1.2 channel activators increased the signal compared to the control condition

Summary and Outlook

Cav1.2 cell line is easy to maintain in cell culture and shows reliable responses with both patch clamp and EFS stimulation systems. The measured signal can be optimized for the EFS stimulation system by varying the duration of the pulse or changing the composition of the extracellular solution towards higher calcium and lower potassium concentration. The calcium imaging responses were further validated by selective Cav1.2 channel activator or inhibitors.

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