Application Note

Channel: Cav1.3
Cells: HEK293
Tools: SyncroPatch96®

Characterization of Cav1.3 on Nanion's SyncroPatch96®

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Introduction

HEK-cells expressing Cav1.3 channels were investigated with the SyncroPatch96.
Cav1.3 is an L-type, high voltage activated (HVA) calcium channel found in certain neuronal dendrites.

Four L-type voltage gated Ca channels (LTCC) isoforms are known (Cav1.1, Cav1.2, Cav1.3, and Cav1.4). All of them are characterized by high sensitivity to dihydropyridine (DHP) Ca^{2+} channel modulators, although differences in their voltage-dependence of DHP block exist. Furthermore, they own different biophysical and pharmacological properties and different cellular distribution. The unique DHP sensitivity distinguishes them from other voltage-gated Ca^{2+} channel types. Gating differences are intrinsic properties of their pore-forming α-1 subunits which assemble with accessory α-2-δ and β-subunits (in some tissues also a γ-subunit) to form a functional channel.

Results

In the experiments presented here, the stability of current amplitude is shown, which is a prerequisite to perform reliable pharmacological experiments. Furthermore, inhibitors and a potentiator were added to the cells, and the corresponding IC_{50} or EC_{50} values were calculated.

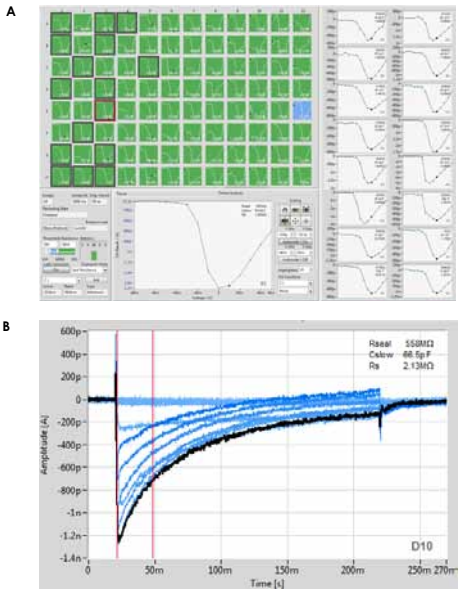

Fig.1 IV relation of Cav1.3 currents. A, Screenshot of SyncroPatch96 software with highlighted IV curves and **B**, exemplary raw data trace.

Figure 1 shows representative current voltage relation curves and an exemplary raw data trace which represents a summarized current of 4 cells, since recordings were performed on a 4-hole NPC96 patch clamp chip. External solution contained 5 mM Ca^{2+}. In general, for HEK293, CHO or other cell lines, more than 50 % of the individual cells reach a seal resistance above 1 GΩ, more than 80 % of cells reach a seal resistance above 500 MΩ. Seals remain stable throughout the experiment, thus complete IC_{50}s can be generated from one cell.

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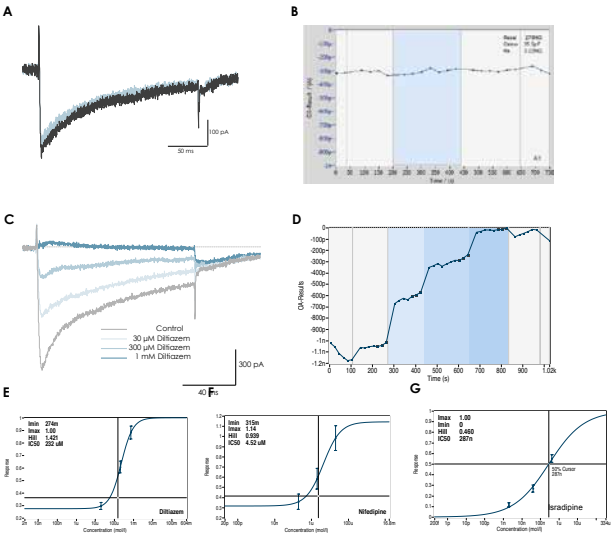

Fig. 2 Pharmacology on Cav1.3 as recorded on the SyncroPatch96. A Raw data traces of one exemplary in the presence of control solution or application of DMSO control. Traces at beginning (t = 0 min., black trace) and at the end of control experiment (t = 15 min., blue). **B** Timeplot of current amplitude. Blue area represents the presence of DMSO control. **C** Cav1.3 current in increasing concentrations of Diltiazem and corresponding timeplot, shades of blue represent increasing concentrations (**D**). **E-G** Mean concentration response curves for Diltiazem, IC_{50} = 232 µM (n = 13); for Nifedipine IC_{50} = 4.5 µM (n = 12) and Isradipine, IC_{50} = 287 nM (n=14).

Pharmacological experiments were performed using the inhibitors Diltiazem, Nifedipine and Isradipine (Fig. 2). Control applications for current amplitude stabilization were performed, followed by the application of 3 increasing inhibitor concentrations and a subsequent washout.

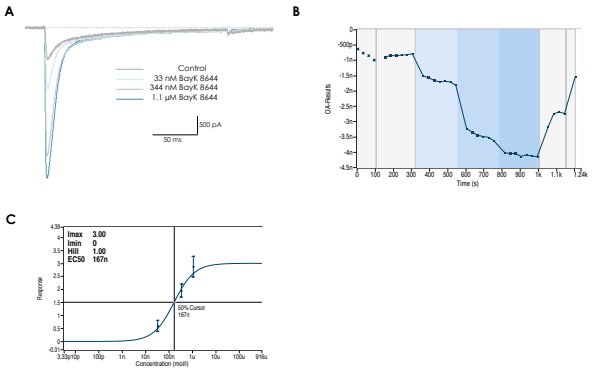

Fig. 3 Cav1.3 currents can be potentiated by S(-)BayK 8644. A Raw data traces of Cav1.3 currents in the presence of increasing S(-)BayK 8644 concentrations and corresponding timeplot of current amplitude (**B**). **C** EC_{50} = 167 nM (n = 12).

Figure 3 shows raw data traces of an exemplary cell in the presence of increasing concentrations of S(-) BayK 8644.

The SyncroPatch96 is a high throughput and highly reliable automated patch clamp device for recording voltage-gated or ligand-gated channels. Importantly, the ability to perform cumulative concentration response curves on single cells drastically reduces the consumable cost.

User-friendly software, excellent success rates, multiple additions of compound to each cell and powerful and automated analysis result in high quality, reliable data at an increased throughput with an economical cost per data point.

Methods

Cells

HEK293 Cav1.3 (origin confidential) cells were cultured and harvested according to Nanion's standard cell culture protocol.

Cell Culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch®.

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