Application Note

Channel: $Ca_V3.2$
Cells: HEK293
Tools: SyncroPatch® 384PE

High Throughput Pharmacology of $Ca_V3.2$ Channels on Nanion's SyncroPatch® 384PE

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Cells kindly provided by Millipore.

Summary

The $Ca_V3.2$ channel is one of the three low voltage activated (LVA) T-type calcium channels. The LVA currents differ from the high voltage activated (HVA) calcium currents in their activation and inactivation kinetics. LVA currents are activated at lower voltages (typically activating above -50 mV and peaking at around -20 mV), they display faster inactivation, slower deactivation and a smaller conductance of Ba^{2+} ions as compared with the HVA currents[1]. The $Ca_V3.2$ channel contains the α1H subunit, encoded by the CACNA1H gene on the human chromosome 16p13.3[2].

T-type channels are expressed in a wide variety of organs throughout the human body, including nervous tissue, heart, kidney, smooth muscle, and many endocrine organs. They have been implicated in a variety of physiological processes including neuronal firing, smooth muscle contraction and hormone secretion. More recently, $Ca_V3.2$ has been shown to play a role in nociception and pain[3,4,5].

Here we present high quality data with reliable pharmacology on $Ca_V3.2$ expressing HEK cells at a high throughput collected on the SyncroPatch® 384PE. Current-voltage plots and concentration response curves for the compounds nitrendipine, nifedipine, mibefradil and amiloride are shown. The IC_{50} values for these compounds are within the expected range[2,6,7] and success rates of up to 79% for completed experiments were recorded.

Results

For the evaluation of the performance of HEK cells expressing $Ca_V3.2$ cells, Seal Resistance values were determined from one experiment and are shown in Fig. 1 at the start and end of the experiment.

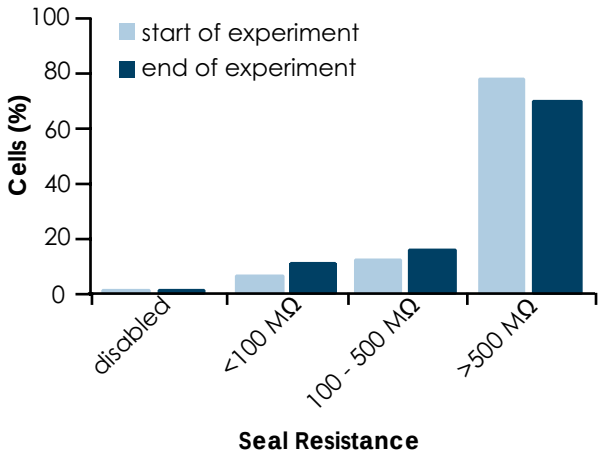

Figure 1: Statistics of $Ca_V3.2$ expressing HEK293cells recorded on one NPC-384 chip on the SyncroPatch® 384PE. Success rate (seal resistance) of individual HEK cells on the SyncroPatch® 384. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue).

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Currents mediated by $Ca_V3.2$ could be reliably recorded on the SyncroPatch® 384PE with a high success rate. Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch® 384PE during an experiment recording the current-voltage relationship of $Ca_V3.2$ expressed in HEK cells. In this experiment, 83% of cells had a seal resistance > 500 MΩ with a further 11% with a seal resistance > 100 MΩ.

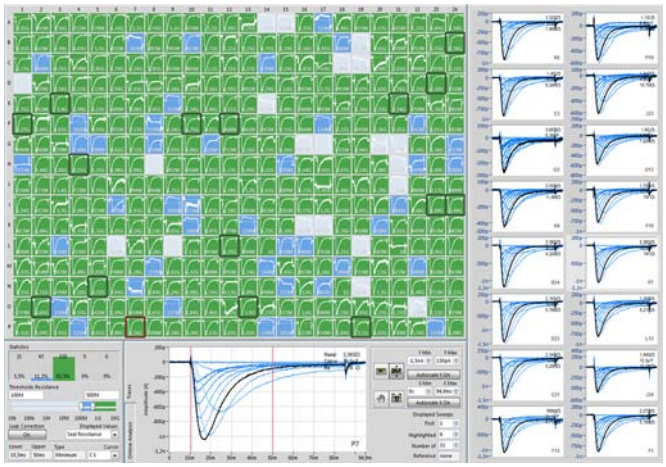

Figure 2: Typical recording from $Ca_V3.2$ expressed in HEK cells on the SyncroPatch® 384PE. The screenshot shows the data acquisition and analysis software used on the SyncroPatch® 384PE. Three hundred and eighty-four small color-coded pictures shown in the upper left part from A1 to P24 display 384 recordings. Wells are color-coded based on seal resistance (green: Rmemb > 500 MΩ, blue: Rmemb = 100 - 500 MΩ, light blue or grey: Rmemb < 100 MΩ or disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. The experiment shows the current voltage relationship elicited by depolarizing steps from -60 mV to 40 mV in 10 mV increments from a holding potential of -120 mV. The black trace highlights the maximum current response followed by a step to -10 mV.

Figure 3 shows the current-voltage relationship for an exemplar $Ca_V3.2$-expressing HEK cell and block of the current by nitrendipine (50 µM). Figure 3B and 3D show the online analysis for the current-voltage relationship and the pharmacology experiment, respectively. In Panel D, the vertical lines and color-coded regions show addition and incubation of compound. The grey region shows incubation in vehicle (1% DMSO, 0.01% Pluronic) and the blue region incubation in 50 µM nitrendipine. Each cell was exposed to a single concentration of nitrendipine and the concentration response curve calculated across the whole plate. The average concentration response curves for four calcium channel blockers are shown in Figure 4. Only cells which satisfied certain quality control criteria (Rmemb, Rseries, Cm and peak current amplitude) were included in the analysis.

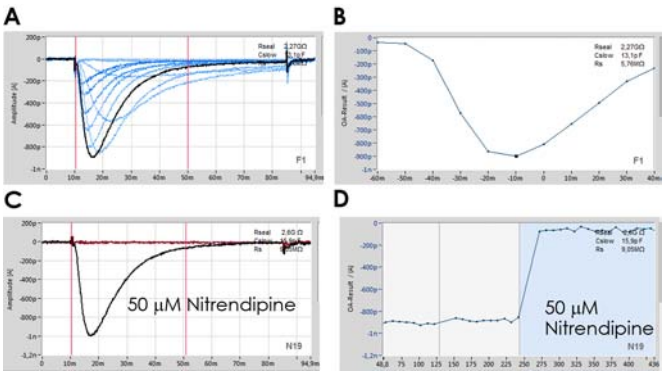

Figure 3: IV and inhibition of $Ca_V3.2$ channels expressed in HEK cells on the SyncroPatch® 384PE. A $Ca_V3.2$-mediated current responses to a voltage step protocol (see Fig. 2). **B** The corresponding current voltage relationship from (A) shows the typical IV curve of $Ca_V3.2$ channels[1,2]. **C** Current response of an exemplar cell elicited using a voltage step to -10 mV for 75 ms from a holding potential of -120 mV. The red trace shows inhibition by 50 µM nitrendipine, the cursors indicate the maximum peak amplitude used for plotting the online analysis timeplot. **D** Corresponding online analysis showing peak amplitude plotted against time. The online analysis is color-coded, the grey region indicates the application of control solution including vehicle (1% DMSO, 0.01% Pluronic) and the blue region indicates application of 50 µM nitrendipine.

A summary of IC_{50} values and success rates for the 4 compounds is shown in Table 1. The estimated IC_{50} values agree well with those found in the literature[2,6,7]. The success rates for completed experiments were between 69 and 79%.

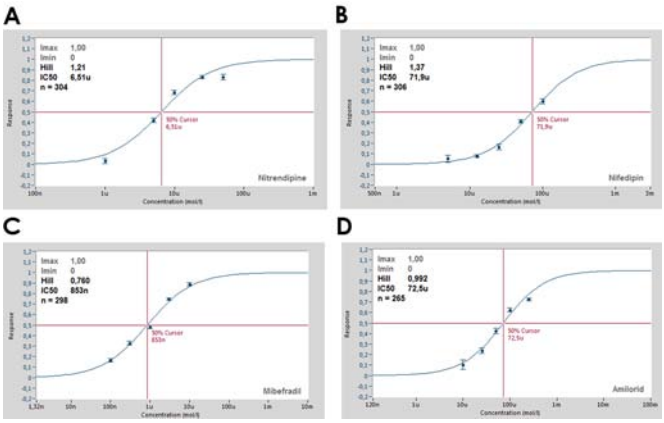

Figure 4: Average concentration response curves for 4 different calcium channel blockers on the SyncroPatch® 384PE. The concentration response curves were constructed across the whole plate as shown in Figure 5. The SyncroPatch® 384PE analysis software (DataControl® 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation. A summary of the IC_{50} values and success rates is shown in Table 1.

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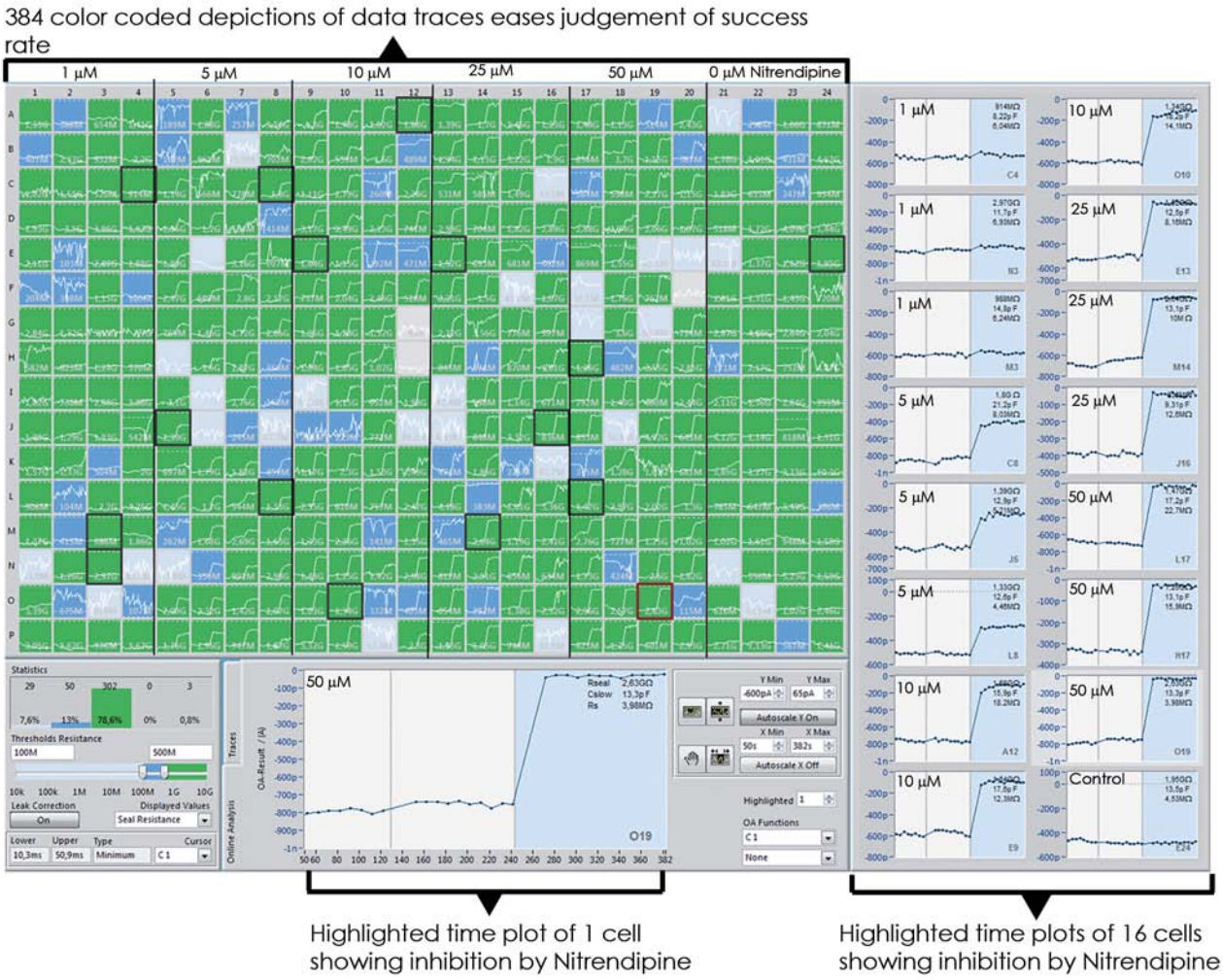

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of $Ca_V3.2$ expressing HEK cells as recorded on one NPC-384 patch clamp chip. A single compound (in this case nitrendipine) was applied to individual wells at varying concentrations (including control wells) across the plate. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Depending on the seal resistance, pictures are green (Rmemb > 500 MΩ), blue (Rmemb = 100 – 500 MΩ), light blue or grey (Rmemb < 100 MΩ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of $Ca_V3.2$ channels from the test pulse to -10 mV during application of control solution including vehicle (1% DMSO, 0.01% Pluronic; grey region) and inhibition by nitrendipine (blue region).

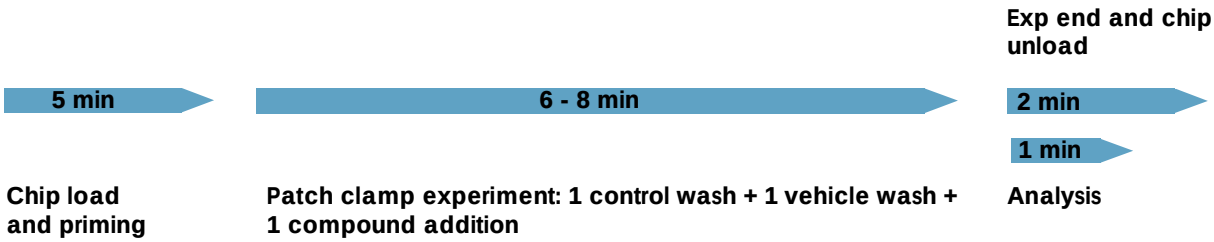

Figure 6: Timeline of an experiment on the SyncroPatch® 384PE. The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single point concentration response curve on $Ca_V3.2$-mediated currents took approximately 13-15 min.

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Compound	IC_{50} (µM)	Success rate (%)	Literature range (µM)
Nitrendipine	6.5 (304)	79	9[6]
Nifedipine	71.9 (306)	79	21[6]
Mibefradil	0.85 (298)	77	1.2 - 1.4[2,7]
Amiloride	72.5 (265)	69	167[7]

Table 1: IC_{50} values for nitrendipine, nifedipine, mibefradil and amiloride on $Ca_V3.2$-mediated currents recorded on the SyncroPatch® 384PE. Shown are IC_{50} values (number of cells shown in brackets), success rate for completed experiments and the expected literature IC_{50} values. All IC_{50} values recorded on the SyncroPatch® 384PE agree well with the literature values[2,6,7].

Figure 5 shows a screenshot of the SyncroPatch® 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. Here, online analysis is chosen and the graphs represent current amplitude versus time. An individual well can be highlighted to monitor progression of the experiment. In the Online Analysis view, the time points at which solution additions have been made are indicated by vertical lines, as well as different background colors. In this example, grey shows incubation in control (vehicle; 1% DMSO, 0.01% Pluronic) solution and blue is nitrendipine.

In conclusion, $Ca_V3.2$ expressed in HEK cells can be recorded on the SyncroPatch® 384PE with high success rates for completed experiments (typically >70%). The timeline of each experiment was about 13-15 minutes (start – end) and included wash with vehicle followed by incubation in 1 concentration of blocker. The current-voltage relationship of $Ca_V3.2$ recorded on the SyncroPatch® 384PE is in good agreement with the literature[1,2]. The IC_{50}'s calculated using the SyncroPatch® 384PE's analysis software, DataControl® 384, of four calcium channel blockers were in good agreement with the literature[2,6,7].

The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording $Ca_V3.2$ currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

HEK293 cells expressing $Ca_V3.2$.

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® 384PE. A voltage step protocol from -120 mV (holding potential) to -10 mV for 75 ms was applied to the cells every 10 s for pharmacology experiments. Peak amplitude at -10 mV was used for analysis. The current-voltage relationship was elicited by depolarizing steps from -60 mV to 40 mV in 10 mV increments from a holding potential of -120 mV.

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