Application Note

Channel: hTRPA1
Cells: HEK293
Tools: SyncroPatch® 384PE

High Throughput Activation and Block of hTRPA1 on Nanion's SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells were supplied by EMD Millipore, USA

Summary

Transient receptor potential (TRP) channels have become important potential targets in drug discovery for the treatment of, for example, pain, respiratory diseases such as asthma, cancer and immune disorders, multiple kidney diseases and skeletal disorders[1].

The transient receptor potential ankyrin 1 (TRPA1), a member of the TRP family of cation channels, plays a predominant role in the sensation of noxious cold[2] and inflammatory pain[3]. The channel is activated by a range of environmental irritants causing pain, pungent compounds found in foods such as garlic, mustard and cinnamon, as well as metabolites produced during oxidative stress[4]. Consistent with its proposed function in nociception, TRPA1 has been shown to be expressed in sensory neurons of the dorsal root ganglion (DRG) and trigeminal ganglion, both of which transmit painful responses[2]. Thus, within drug development, much attention is paid to the TRPA1 channel. Preclinical data and data from a recent human genetic study[5] highlight TRPA1 antagonists as a promising new approach for the treatment of acute and chronic pain. Indeed, a TRPA1 antagonist has shown positive results in a proof-of-concept study for diabetic neuropathic pain[6].

The most challenging aspects involved in the screening of the TRPA1 channel are the channel's mechanosensitivity, fast desensitization and activity dependence on intracellular calcium. Here, we present high quality data with reliable pharmacology on hTRPA1 expressing HEK cells collected on the SyncroPatch® 384PE. Data is presented showing activation of the TRPA1 channel by SCMA and inhibition by A-967079.

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Results

For the evaluation of the performance of hTRPA1 (HEK293) cells, Seal Resistance, Cslow and the Series Resistance (Rseries) were determined from one experiment (Fig. 1) .

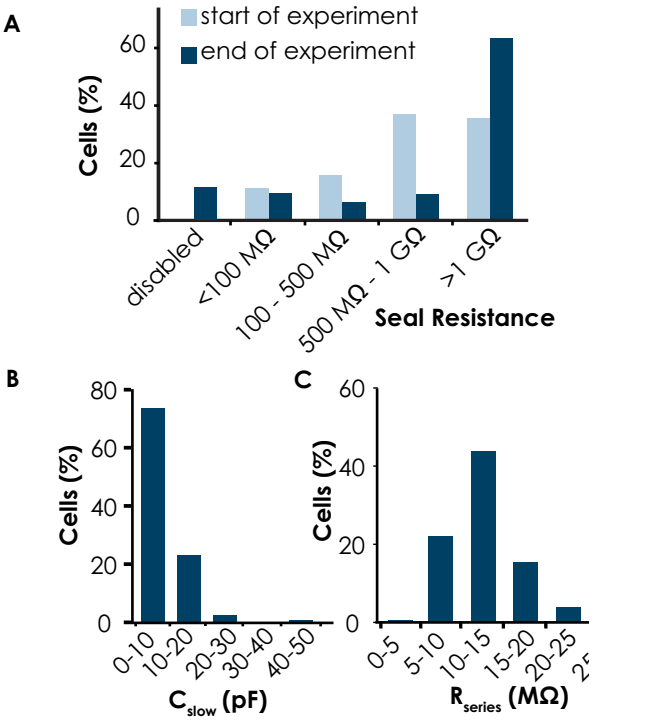

Figure 1: Statistics of TRPA1 expressing HEK293 cells recorded on one NPC384 chip on the SyncroPatch® 384 PE
A Success rate (seal resistance) of individual HEK 293 cells on the SyncroPatch® 384. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B** Bar graph of cell capacitance (Cslow) and **C** Series Resistance (Rseries) values for HEK293 cells expressing TRPA1.

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Currents mediated by hTRPA1 could be reliably and sustainably activated by the agonist Supercinnamaldehyde (SCMA; 5 µM) and blocked by A-967079 in a concentration-dependent manner (Fig. 2). TRPA1 currents were first activated by SCMA (white region), a single concentration of A-967079 was applied to each cell (blue region) and finally a high concentration of A-967079 (grey region) was applied to fully block the current.

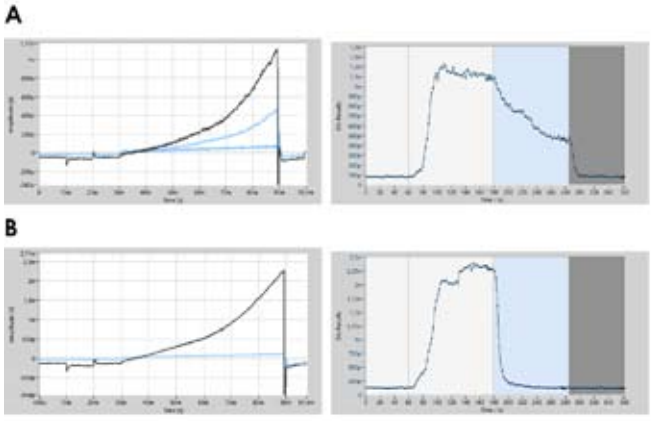

Figure 2: Activation of TRPA1 by SCMA and concentration-dependent block by A-967079
Ramp recordings (left) and snapshots of the corresponding time course of the current amplitude at 60 mV (right) of exemplar HEK cells expressing hTRPA1. **A** Current responses after exposure to 5 µM SCMA (left panel: black trace; right panel: white region), block by 15 nM A-967079 (left panel: light blue trace; right panel: blue region)) and full block by 5 µM A-967079 (left panel: dark blue trace; right panel: grey region). **B** Current responses after exposure to 5 µM SCMA (left panel: black trace; right panel: white region), block by 500 nM A-967079 (left panel: light blue trace; right panel: blue region)) and full block by 5 µM A-967079 (left panel: dark blue trace; right panel: grey region).

Each cell was exposed to a single concentration of A-967079 and the concentration response curve calculated across the whole plate. The average concentration response curve for 242 cells is shown in Figure 3. The calculated IC_{50} was 6.6 nM (n = 242) which is similar to that reported in the literature (67 nM)[7] . Only cells which returned to the baseline (recorded at the start of the experiment) upon application of the full-block concentration of A-967079 (5 µM) were included in the analysis. In this experiment, 242 cells were used for the concentration response curve giving a success rate of 63% for completed experiments.

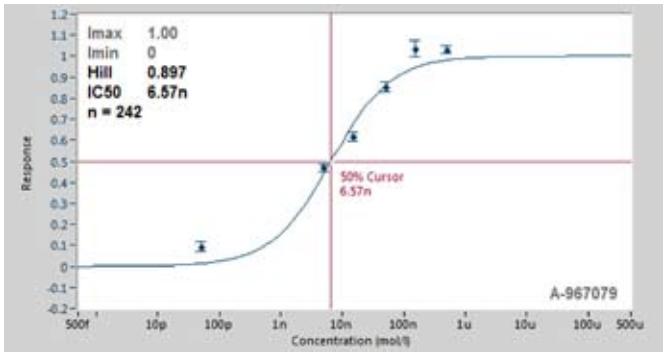

Figure 3: Average concentration response curve for A-967079
The concentration response curve was constructed across the whole plate. Only cells which returned to baseline after the full block concentration of A-967079 were used for analysis giving an IC_{50} = 6.6 nM (n = 242) and a success rate of 63% for completed experiments.

TRPA1 has been shown to be mechanosensitive[8] and we observed activation of TRPA1 when control solution was added to the cells at a speed of 5 µl/s (Fig. 4). This mechanical activation was also observed during application of antagonist. To avoid this mechanical activation of TRPA1 during activation with SCMA the solution was added using a very slow speed (2.5 µl/s) and the travelling speed of the pipette was also adjusted to minimise pressure changes at the cell when the pipette enters the well.

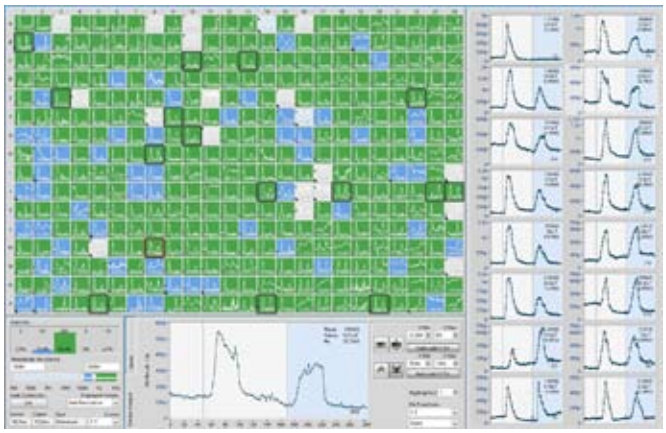

Figure 4: TRPA1 is also mechanosensitive.
Screenshot of the PatchControl 384 software during an experiment activating TRPA1 using control solution added at a speed of 5 µl/s. This effect was not blocked by A-967079 (blue region).

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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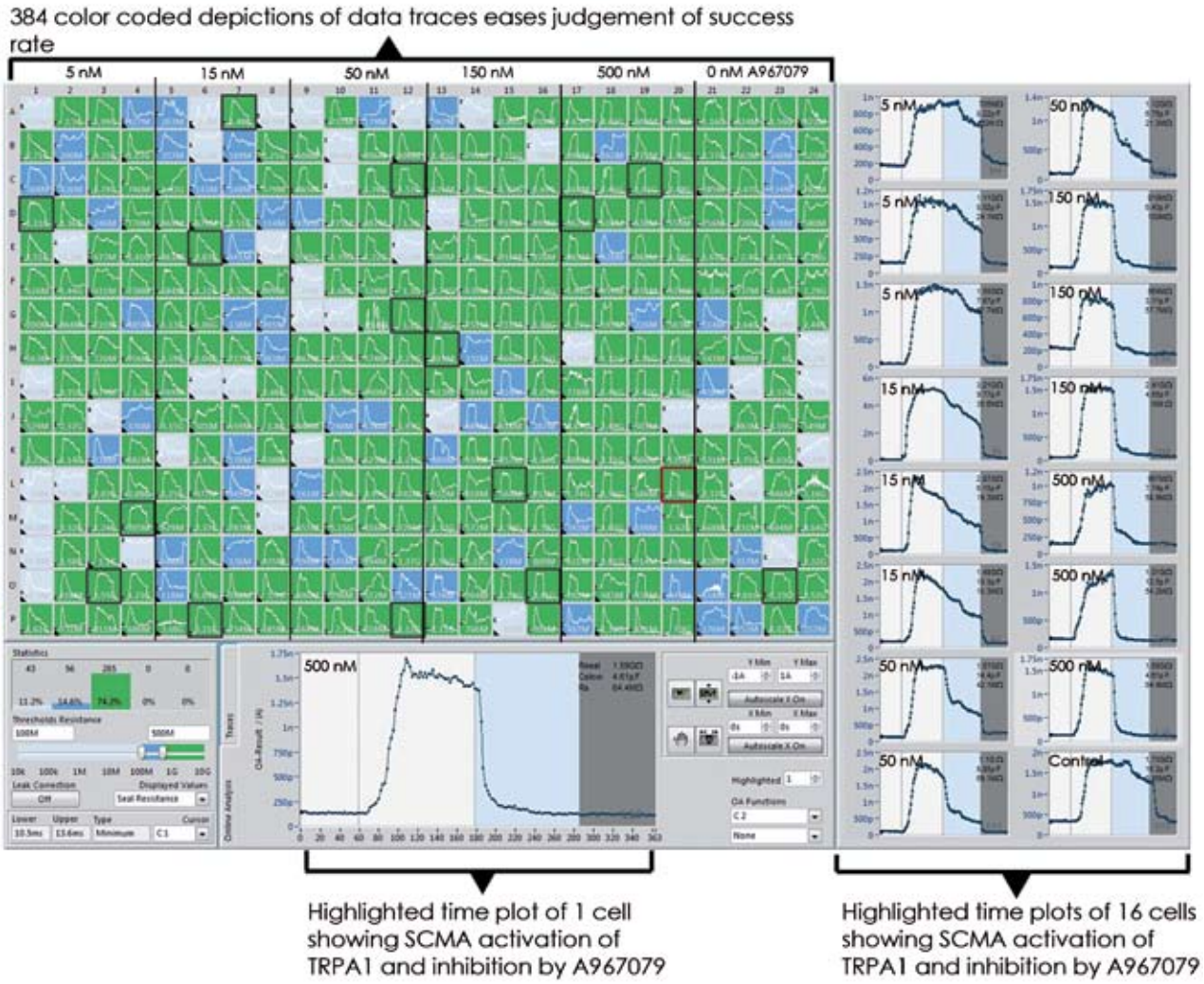

Figure 5:
Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of hTRPA1 expressing HEK293 cells as recorded on one NPC-384 patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen 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 SCMA (5 µM) activation (white region), block by A-967079 (blue region) at the indicated concentrations and full block (grey region) using 5 µM A-967079. One minute of baseline current was recorded prior to application of SCMA. Only cells which returned to baseline after the full block concentration of A-967079 were used for analysis.

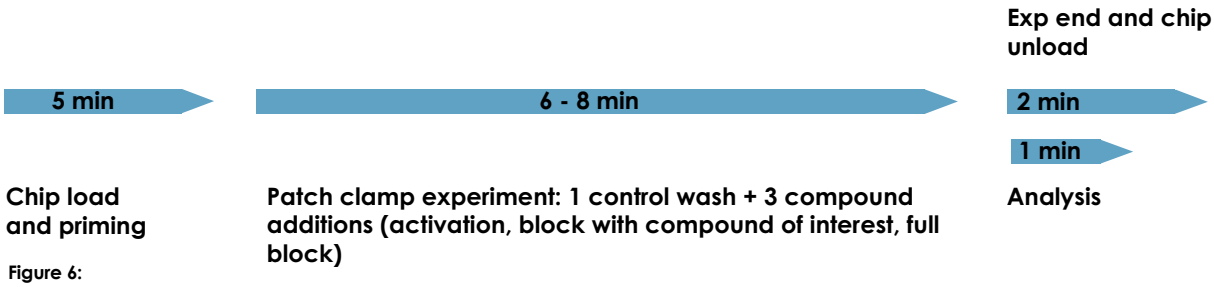

Figure 6:
The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single point concentration response curve plus activation of TRPA1 with SCMA and full block at the end of the experiment took approximately 13-15 min.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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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. In this case, online analysis is chosen and the graphs represent current amplitude plotted against time. An individual well can be highlighted to monitor the 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 case, white shows activation of TRPA1 by SCMA, blue shows inhibition by the indicated concentration of the TRPA1 antagonist A-967079 and grey shows full block by 5 µM A-967079.

In conclusion, hTRPA1 expressed in HEK293 cells can be recorded on the SyncroPatch® 384PE with a good success rate. The timeline of each experiment was about 13-15 minutes (start – end) and included wash, activation with SCMA, time to reach a stable current, single compound concentration application and full block, all performed on each individual cell, drastically reducing the consumable cost per data point to < $0.60.

The activation properties of hTRPA1 recorded on the SyncroPatch® 384PE are in excellent agreement with those reported in the literature[9]. As expected, hTRPA1 could be activated by SCMA[10] and inhibited by A-967079 in a concentration-dependent manner with an IC_{50} similar to that reported in the literature[7]. As has been previously reported[8], we found that TRPA1 was mechano sensitive and could be activated by pipetting control solution at a speed of 5 µl/s. However, due to the flexibility of the experimental settings of the SyncroPatch® 384PE, the application speed and the travelling speed of the pipette can be adjusted to ensure that the mechanical stimulation is eliminated.

The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording hTRPA1 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

1. Clapham, D. 2003. Nature. 426: 517-524
2. Story G.M., *et al*. 2003. Cell. 112(6): 819-29.
3. Bautista D., *et al.* 2006. Cell. 124: 1269–1282
4. Bautista D., *et al.*, 2005. Natl. Acad. Sci. U.S.A.,102 (34): 12248-52
5. Kremeyer, B., *et al.* 2010. Neuron. 66: 671-680
6. http://www.prnewswire.com/news-releases/glenmarks-trpa1-antagonist-grc-17536-shows-positive-data-in-a-proof-of-concept-study-275445961.html
7. Chen, J., *et al.* 2011. Pain. 152(5): 1165-72
8. Brierley, S.M., *et al.* 2011. J. Physiol. 589(14): 3575-93
9. Bandell, M., *et al.* 2004. Neuron. 41 (6): 849-57
10. Macpherson, M.J., *et al.* 2007. Nature. 445: 541-45

Methods

Cells

PrecisION - hTRPA1 expressing HEK293 cells (Item Number CYL3066) were supplied by EMD Millipore, USA.

Cell culture

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

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE. A voltage ramp protocol from -50 mV to +60 mV in 60 ms was applied to the cells every 2 s. Current amplitude at 60 mV was used for analysis. Solutions were applied to the cell at a speed of 2.5 µl/s.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de