

Activation and inhibition of TREK-1 on Nanion's SyncroPatch® 384PE

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Summary

The TWIK-related K⁺ channel (TREK-1) is a two pore domain (K2P) K⁺ channel encoded by the KCNK2 gene¹. The protein is a homodimer, each subunit comprised of transmembrane (TM) helical regions (M1-M4), two pore domains (P1 & P2), an extracellular region with 2 helices, and intracellular N and C termini^{1,2}. The pore helices and pore loops form the K⁺ selectivity filter¹. First discovered in 1996², it plays an important physiological role in background K⁺ conductance² and thus plays a major role in regulating resting membrane potential. TREK-1 widely is expressed throughout the CNS and spinal cord, particularly in the cortex, hippocampus, thalamus, hypothalamus, cerebellum and basal ganglia^{2,3}. Additionally, TREK-1 is expressed in high levels in small and medium sized dorsal root ganglion (DRG) neurons⁴. It is also expressed in other regions such as lung, heart, kidney, skeletal muscle² and human myometrium where it is up-regulated during pregnancy⁵ and may play a role in maintaining a negative membrane potential prior to labor. TREK-1 is modulated by a variety of different physical and chemical stimuli including mechanical (stretch)^{4,6,7}, temperature⁴, intracellular acidosis⁷, poly-unsaturated fatty acids^{6,8} and phospholipids^{4,6,8}. It has also been shown to be opened by volatile anaesthetics^{9,10} and is likely to be an important target for these agents¹⁰. TREK-1 has been proposed to play a pivotal role in cerebral ischemia¹⁰, epilepsy¹⁰, depression¹¹, pain perception¹² and temperature sensing⁴ and is an interesting therapeutic target.

Human TREK-1 expressed in HEK cells were recorded on the SyncroPatch 384PE with good success rates. hTREK-1 was activated by BL-1249 and blocked by THA.

Results

For the evaluation of the performance of hTREK-1 (HEK293) cells, Seal Resistance (R_{series}) at the start and end of one experiment using single hole chips was determined (Fig. 1). HEK293 cells expressing hTREK-1 could be captured to the patch clamp aperture with a high success rate (>93% of cells had R_{Seal} > 500 MΩ at the start of the experiment).

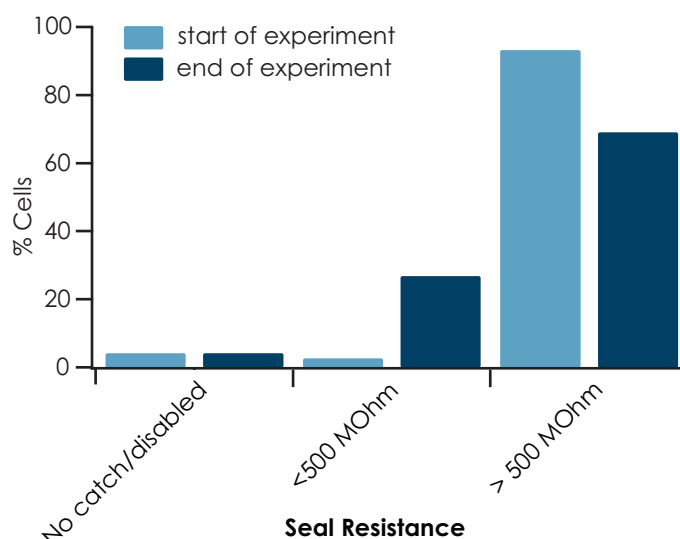


Figure 1: Statistics of hTREK-1 expressing HEK293 cells recorded on one NPC-384 chip on the SyncroPatch® 384PE. Success rate (seal resistance) of individual HEK cells on the SyncroPatch® 384PE. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue).

Application Note

BL-1249 is a K⁺ channel opener with bladder relaxant properties¹³. It is proposed to exert its effects via activation of TREK-1¹³. Expression of TREK-1 is relatively high in bladder smooth muscle cells versus human aortic cells and BL-1249 has been shown to act as a potassium channel activator exhibiting bladder versus vascular selectivity both *in vitro* and *in vivo*. K⁺ channel openers

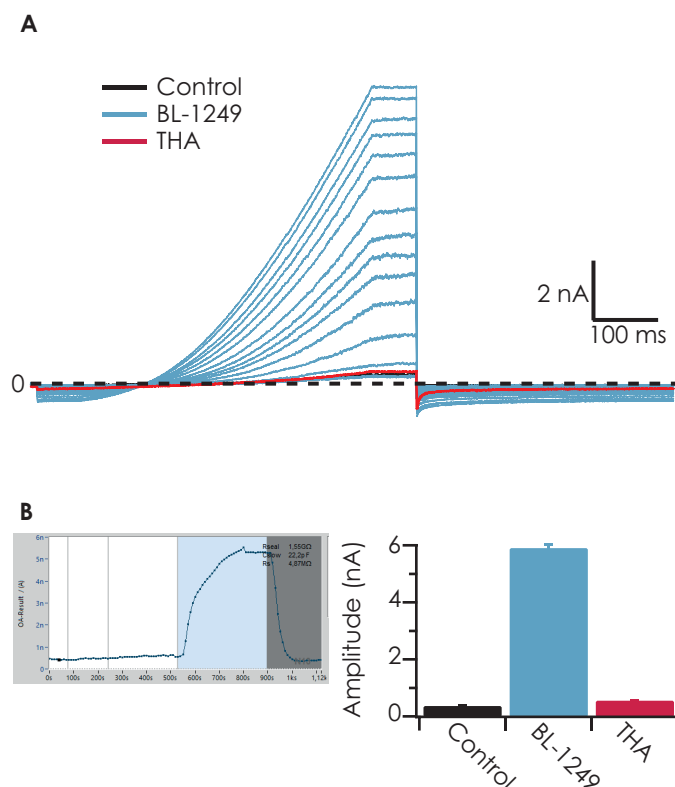


Figure 2: Activation and inhibition of hTREK-1. **A** Raw traces of an exemplar cell in control conditions (black), in the presence of the activator, BL-1249 (10 μ M; blue) and in the presence of the inhibitor, THA (10 μ M; red). Multiple traces in the presence of the activator are included to show development of activation over time **B** Online analysis (left; current amplitude at 60 mV) is plotted versus time and application of BL-1249 is shown in blue and THA shown in grey. A bar graph of the amplitude values for an average of 368 cells is shown on the right in control (black), BL-1249 (blue) and THA (red). Shown are average $\pm$ S.E.M. (10 μ M; blue) and in the presence of the inhibitor, THA (10 μ M; red).

are potential therapeutic targets for treating the overactive bladder and because of its preferential expression in bladder smooth muscle cells, TREK-1 is an attractive target for the treatment of this condition, without significant effects on blood pressure¹³. Figure 2 shows current responses of an exemplar cell to a voltage ramp protocol from -120 mV to 60 mV over 450 ms in control (black) conditions and after activation with 10 μ M BL-1249 (blue). As expected, activation of TREK-1 results in an outwardly rectifying current². Several traces in the presence of BL-1249 are included in the figure from the start to end of incubation to show development of current activation. Quaternary ammonium ions, e.g. tetrahexylammonium (THA) have been shown to bind to the pore of TREK-1 with high affinity and block the channel¹⁴. Figure 2 shows subsequent block of BL-1249-activated hTREK-1 by 10 μ M THA (red). As expected, THA at this concentration completely blocked the hTREK-1-mediated current. Figure 2B shows the online analysis values for the positive current of an example cell and the corresponding amplitudes for an average of 368 cells. The outward current activated by BL-1249 was 5.9 ± 0.1 nA compared with 352 ± 15 pA in control conditions and 540 ± 20 pA after block with THA.

Figure 3 shows a screenshot of the SyncroPatch[®] 384 software during an 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 baseline amplitudes in control solution, blue indicates BL-1249 (10 μ M) and grey shows inhibition by THA (10 μ M). A single concentration of BL-1249 and THA were used in this experiment but different concentrations of activator or inhibitor could be used to investigate concentration response relationships.

Application Note

384 color coded depictions of data traces eases judgement of success rate

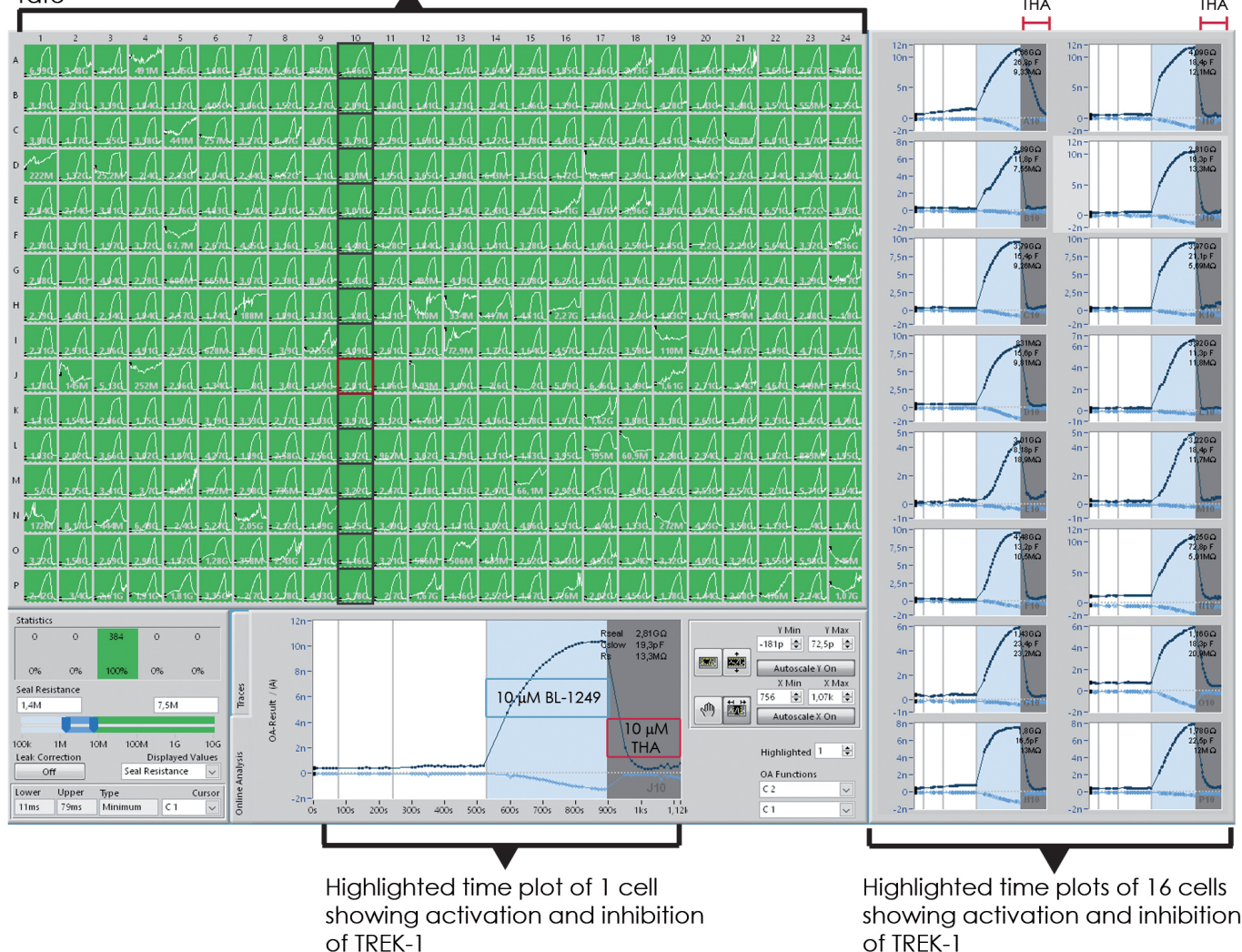


Figure 3: Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of hTREK-1 expressing HEK293 cells as recorded on one NPC-384 (single-hole) patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of hTREK-1 in control solution (white region), after activation with 10 μ M BL-1249 (blue region) and following block with 10 μ M THA. Almost 5 minutes of baseline current was recorded prior to application of BL-1249.

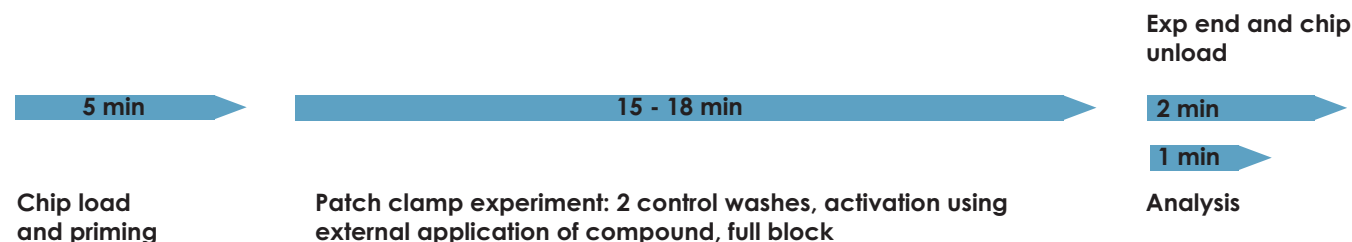


Figure 4: The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single addition of activator plus full block of hTREK-1 took approximately 22-25 min.

Application Note

In conclusion, hTREK-1 expressed in HEK293 cells can be recorded on the SyncroPatch® 384PE with an excellent success rate for RSeal > 500 MΩ at the start of the experiment (>93%). hTREK-1 was activated by BL-1249 and blocked by THA. The timeline of each experiment (Figure 4) was about 22-25 minutes (start – end) and included wash with control solution to establish a stable baseline, activation by external application of BL-1249, time to reach a stable current and full block using THA, all performed on each individual well, drastically reducing the consumable cost per data point to < \$0.60.

The outward rectification properties of hTREK-1 recorded on the SyncroPatch® 384PE, activation and block of the channel are in excellent agreement with the literature^{2,12,13}.

References

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The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording hTREK-1 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.

Methods

Cells

hTREK-1 expressing HEK293 cells were supplied by SB Drug Discovery, Catalog Ref: SB-HEK-TREK1.

Cell culture

Cells were cultured according to the manufacturer's recommendations and harvested according to Nanion's standard protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE using single-hole chips. A voltage ramp protocol from -120 mV to +60 mV over 450 ms, with 70 ms at -120 mV and +60 mV at the start and end of the ramp, respectively, was applied to the cells every 10 s. Intersweep holding potential was -80 mV. Current amplitude at +60 mV was used for analysis. Compounds were applied via the external solution.