Application Note

Activation and Inhibition of TASK-1 on Nanion’s SyncroPatch 384PE

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Summary

The resting membrane potential of excitable cells is determined by leak conductances predominantly mediated by KCNK and two-pore-domain potassium channels (K2P)\(^1\). K2P channels are characterized by the presence of two pore forming regions and four trans-membrane spanning (4TMS) regions in each channel subunit and form functional dimers. These channels are essential for the production of background leak type potassium currents that act to regulate resting membrane potential and levels of cellular excitability\(^1\). The TWIK-related acid-sensitive K\(^+\) channel 1 (K2P3.1 or TASK-1) is a member of the K2P channel family and is encoded by the KCNK3 gene\(^2\). TASK-1 is ubiquitously expressed throughout the CNS\(^3\)-\(^5\) but also in other tissues such as in the heart, adrenal gland, lung, pancreas, kidney, intestine and prostate\(^6\)-\(^8\). TASK-1 has been implicated in atrial fibrillation (AF) pathophysiology and was suggested as an atrial-selective antiarrhythmic drug target\(^9\)-\(^10\). TASK-1 is activated by extracellular acidosis and inhibited by anandamide and by local anesthetics including bupivacaine. Volatile general anesthetics such as halothan and xenon stimulate TASK-1\(^11\).

Here, we demonstrate recordings of human TASK-1 expressed in HEK cells on the SyncroPatch 384PE with good success rates. hTASK-1 was activated by extracellular alkalization and blocked by tetrahexylammonium chloride (THA).

Results

For the evaluation of the performance of TASK-1 expressing HEK293 cells, Seal Resistance (Rseal) at the start and end of one experiment using single hole chips were determined (Fig. 1). Cells were captured with a high success rate (94% of cells had a seal >500 MΩ at the start of the experiment).

![Seal Resistance](image)

Figure 1: Statistics of TASK-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 384. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue).
TASK-1 is sensitive to extracellular pH in the narrow physiological range, activated by extracellular alkalinization and inhibited by acidic pH. Relatively large changes in extracellular pH can take place, for example, during hypoxia, ischemia or during epileptic seizures, pathophysiological conditions in which TASK-1 is proposed to be involved. Under physiological conditions, activation of TASK-1 elicits a typical outward rectifying leak conductance, which is not entirely voltage and time independent. Figure 2 demonstrates the activation of TASK-1 channels by extracellular alkalinization and their current-voltage behavior under physiological potassium gradient conditions.

In a study with TREK-1, it has been shown that quaternary ammonium ions, e.g. tetrahexylammonium (THA) bind to the pore of K2P channels with high affinity and block the channel. Figure 2 shows subsequent block of pH-activated hTASK-1 by 5 μM THA (red). As expected, THA at this concentration blocked the hTASK-1-mediated current. Figure 2B shows the online analysis values for the outward current of an example cell at +60 mV and the corresponding amplitudes depicted as a bar graph for an average of 93 cells (Figure 2C). The outward current activated by external alkalinization with pH 8.9 was 578 ± 40 pA compared with 256 ± 69 pA in control conditions and 333 ± 73 pA after block with THA. THA blocks all K2P channels and is not K2P subtype specific. To isolate hTASK-1 from other K2P channel subtypes in native cells, the novel TASK-1 specific blocker A293 appears to be a useful compound.

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. On the left, the raw trace view is chosen. On the right, the online analysis view is shown representing current amplitude plotted against time for 16 selected wells. 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, light grey indicates activation with different pH (8.2, 8.7 and 8.9) and dark grey shows inhibition by THA (5 μM). A single concentration of THA was used in this experiment but different concentrations of activator or inhibitor could be used to investigate concentration response relationships.
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 hTASK-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 raw traces and online analysis plots of current amplitudes versus time of hTASK-1 after activation with different pH and following block with 5 μM THA (red trace). Two and a half minutes of baseline current was recorded before current activation with increased pH.

Figure 4: Timeline of an experiment on the SyncroPatch 384PE. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for three additions of activator plus full block of TASK-1-mediated currents took approximately 10-12 min.
In conclusion, hTASK-1 expressed in HEK cells can be recorded on the SyncroPatch 384PE with high success rates for RSeal > 500 MΩ at the start of the experiment (typically >90%). hTASK-1 was activated by alkaline pH and inhibited by THA. The timeline of each experiment was about 18-20 minutes (start – end) and included wash with control solution to establish a stable baseline, activation by external application of pH and full block using THA (Figure 4).

The typical outward rectification of hTASK1 mediated currents in asymmetric potassium concentration, the activation by extracellular alkalization and the inhibition by THA recorded on the SyncroPatch 384PE are as expected\(^\text{1,3,12,15}\).

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

References


Methods

Cells

HEK293 cells expressing hK2P3.1 (TASK-1)

Cell culture

Cells were cultured and harvested according to Nanion’s gentle cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion’s standard procedure for the SyncroPatch 384PE. Cells were held at -80 mV. A voltage ramp protocol from -120 mV to +60 mV in 450 ms was applied to the cells every 10 s. Current amplitude at 60 mV was used for analysis. To calculate the pH response curve, cells were activated with increasing pH and data were normalized to the maximum response at pH 8.9. Cumulative pH response curve was calculated and fitted with a Hill equation.