Application Note

Channel: hTRPV4
Cells: CHO
Tools: Port-a-Patch

Activation and inhibition of TRPV4 using different stimuli on the Port-a-Patch

The electrophysiology team at Nanion Technologies GmbH, Munich.

Summary

TRPV4 is a member of the transient receptor potential channel (TRP) family. Transient receptor potential vanilloid type 4 (TRPV4) shares approximately 40% identity with TRPV1 and TRPV2[1] and is a Ca^{2+}-permeable non-selective cation channel[1-3] expressed in a wide range of tissues including neurons of the central and peripheral nervous systems, and in non-neuronal tissue including human T cells, corneal and retinal epithelial cells, endothelial cells of the eye, liver, heart, kidney, synoviocytes, epithelial lining of trachea and lung airways, stellate cells of the pancreas, and many more[2]. TRPV4 is activated by a number of stimuli including endogenous ligands such as lipid arachidonic acid and its metabolites[4], synthetic ligands such as GSK1016790A[5], warm temperature >27-35°C[6,7], hypotonic extracellular solution (cell swelling)[1,8], and mechanical stress[2]. Given its widespread distribution in many organs, this suggests that TRPV4 plays a major role in many physiological processes[2,9]. These include osmoregulation, thermoregulation, Ca^{2+} homeostatus in vascular endothelium, maintaining vascular tone and endothelial cell function, as well as playing roles in the cardiac, respiratory, urinary, skeletal, and digestive systems[2,3,9]. Additionally, TRPV4 is thought to play a role in neuronal excitability in the central nervous system and nociception[2,8]. TRPV4 has been implicated in a number of diseases including neuropathic and inflammatory pain, respiratory and cardiovascular diseases, and cancer[2,3,9].

Here we present data of hTRPV4 collected on the Port-a-Patch using heated solution *via* the External Perfusion System. Heat activated hTRPV4 was blocked by ruthenium red. The TRPV4 specific agonist, GSK1016790A also activated TRPV4 and the response was blocked by ruthenium red.

Results

TRPV4 can be activated by a number of stimuli, including warm temperature >27°C[6,7]. We activated hTRPV4 expressed in CHO cells using heated solution at 45°C. There was some basal activity of TRPV4 at room temperature but the current amplitude increased to 5.7 ± 0.8 nA (n = 6) from control current of 2.7 ± 0.6 nM (n = 6; $p<0.01$, Student's t test). Figure 1 shows the activation of TRPV4 by warm solution from an example cell and the timecourse of the experiment.

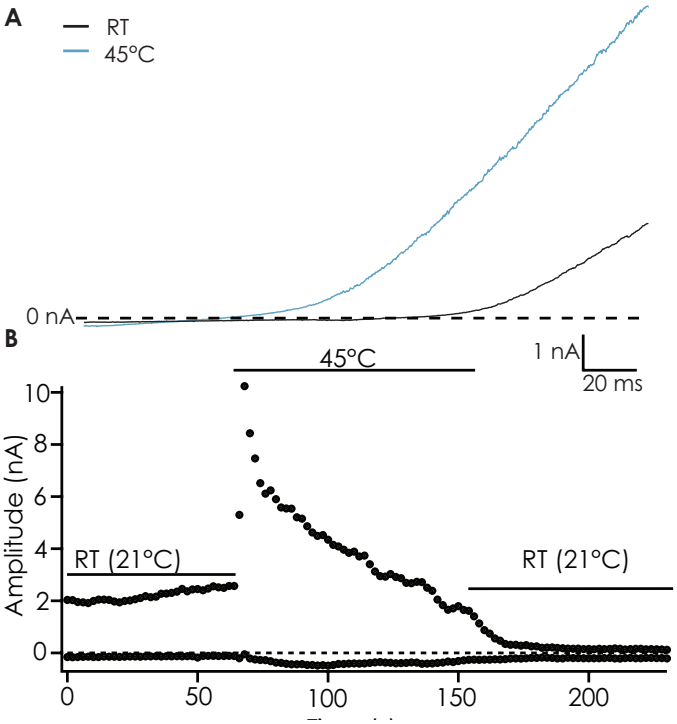

Figure 1: A Whole cell current responses from induced CHO cells expressing TRPV4 to a ramp protocol (-100 mV to +100 mV over 200 ms) at RT (21°C) (black) and 45°C (light blue). TRPV4 was activated at 45°C. Some basal activity was seen in some cells at the start of the experiment. **B** Timecourse of TRPV4 activation with warm solution (45°C).

Application Note

TRPV4 was activated by warm solution and blocked by ruthenium red applied at 45°C. Figure 2 shows the time course of the exeriment and corresponding example traces.

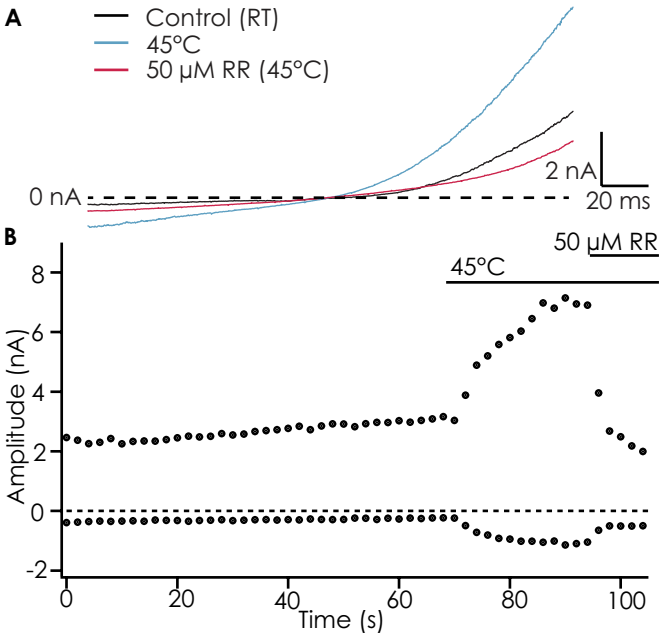

Figure 2: A Traces from an example cell showing basal TRPV4 current (black), activation by warm solution (45°C, light blue) and block by 50 µM ruthenium red (RR; red). **B** Timecourse of the experiment.

TRPV4 was also activated by the specific activator, GSK1016790A. Figure 3 shows the timecourse of the experiment where TRPV4 is activated by GSK1016790A and then blocked by ruthenium red.

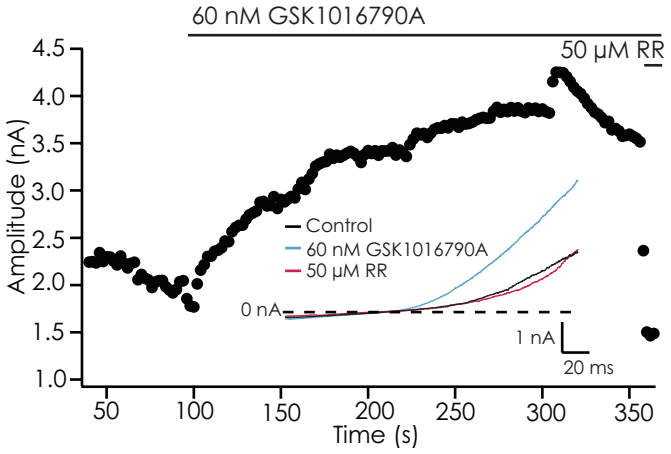

Figure 3: Activation of TRPV4 by GSK1016790A and subsequent block by ruthenium red. Shown is the timecourse of an example cell with corresponding traces.

In conclusion, hTRPV4 expressed in CHO cells can be reliably activated using the External Perfusion System of the Port-a-Patch with Temperature Control which enables heating the external solution up to 50°C. TRPV4 was also activated by GSK1016790A. The current was blocked by ruthenium red regardless of activation stimulus (heat or GSK1016790A). The Port-a-Patch is, therefore, a useful tool for identifying TRPV4 inhibitors as potential therapeutics for a variety of conditions including neupathic and inflammatory pain, respiratory and cardiovascular disease and and some cancers.

References

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Methods

Cells

CHO cells stably expressing hTRPV4.

Cell culture

CHO cells stably expressing hTRPV4 were cultured using standard culture conditions for the Port-a-Patch and induced using 1 mg/ml tetracycline for at least 24-36 hours prior to measurements.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch. Currents were elicited by 200 ms voltage ramps from -100 mV to +100 mV, Vhold = -80 mV. External solution was warmed 45°C using the Temperature Control of the External Perfusion System to activate hTRPV4. Alternatively, hTRPV4 was activated by 60 nM GSK1016790A *via* the external solution. Ruthenium red was made up in external solution and applied at 45°C or co-applied with GSK1016790A at room temperature.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de

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