

Heat activation of TRPV1 on Nanion's Patchliner®

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Summary

The transient receptor potential cation channel, subfamily V, member 1 (TRPV1), is a ligand-gated, non-selective cation channel widely expressed in the peripheral and central nervous system. The TRPV1 channel can be activated by a number of physical and chemical stimuli, including capsaicin (the active ingredient in chili peppers), noxious heat (typically $>42^{\circ}\text{C}$) and low pH. The TRPV1 channel is putatively involved in the perception and transmission of painful stimuli. Importantly, this channel is proposed to underlie many chronic pain states including inflammation, neuropathic pain and cancer pain, amongst others (1). These types of pain states are currently poorly managed by the pain medications available and this has led the pharmaceutical industry to seek novel targets for pain management, such as TRPV1. However, TRPV1 antagonists have so far failed in clinical trials due to an undesirable increase in core body temperature (2) resulting in hyperthermia. From these studies, it is proposed that tonically active TRPV1 channels are involved in maintaining normal body temperature and this could have significant influences on drug design. Finding novel compounds with differing effects on capsaicin activation and heat activation may be crucial in the discovery of lead compounds for the treatment of pain and other disease states.

Here we present data collected on the 4-channel Patchliner® with temperature control showing the potential use of the Patchliner® to record capsaicin and heat activation of TRPV1 channels and subsequent block by ruthenium red (RR).

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Results

Current responses of an individual cell to 200 ms voltage ramps (-100 mV to +100 mV) and activation by application of external solution at increasing temperatures are shown in Figure 1. The solution was heated in the pipette of the Patchliner® and rapidly applied to the cell at a speed of 10 $\mu\text{l/s}$. A temperature response curve could be constructed. Activation began at approximately 42°C . The EC_{50} for temperature was estimated to be approximately 50°C .

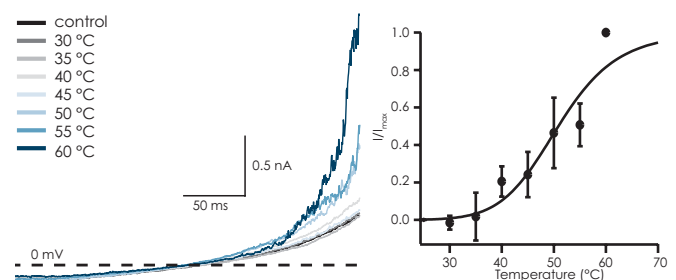


Figure 1: Activation of TRPV1 by increasing temperature. The solution inside the pipette was heated to the temperature indicated and applied to the cell. TRPV1 was activated at temperatures above 42°C . A temperature response plot was constructed and a temperature EC_{50} of approximately 50°C was estimated.

Application Note

Figure 2 shows the activation of TRPV1 channels using either heat (60°C) or capsaicin in the same cell. Following challenge by application of external solution at 60°C, capsaicin (1 µM) was applied at room temperature.

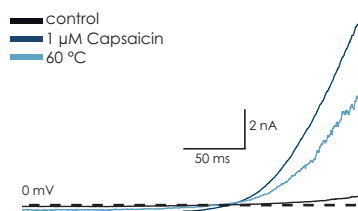


Figure 2: Activation of TRPV1 by capsaicin and temperature in the same cell. TRPV1 could be activated by application of either 1 µM capsaicin or external solution heated to 60°C.

A full concentration response curve to RR at 0.1, 1, 10 and 30 µM could be performed (Fig. 3). RR was first pre-incubated and then co-applied with heat (60°C). A concentration response curve was constructed and the IC_{50} was estimated to be 7.1 ± 1.2 µM ($n = 3 - 6$ per concentration). The concentration response curve of RR for capsaicin activation is also shown for comparison (IC_{50} 1.5 ± 0.2 µM; $n = 3$).

In summary, TRPV1 receptors stably expressed in CHO cells can be reliably activated by noxious temperature

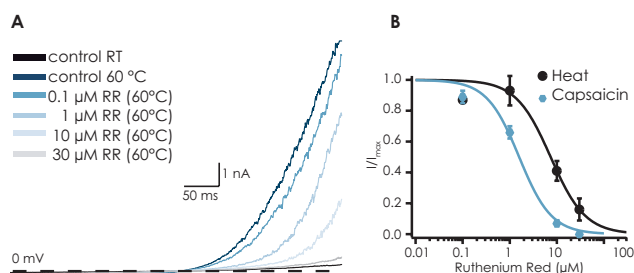


Figure 3: **A** TRPV1 block at 60°C by RR. RR at increasing concentrations (0.1 µM - 30 µM) was pre-incubated and then applied at 60°C. **B** $IC_{50} = 7.1 \pm 1.2$ µM ($n = 3-6$ per concentration); IC_{50} of capsaicin response by RR was 1.5 ± 0.2 µM; $n = 3$.

(>42°C) using the temperature regulation feature of the Patchliner®. Solution can be heated to temperatures up to 60°C in the pipette and rapidly applied to the cell, transiently activating TRPV1 channels. Additionally, a concentration response curve to the TRP channel antagonist ruthenium red is shown here. The ability to separate antagonistic responses of capsaicin and heat activation of TRPV1 may be critical in novel lead compound discovery, and therefore, the Patchliner® provides a viable, higher throughput alternative to conventional patch clamp for the discovery of active TRPV1 lead compounds with a suitable drug profile.

References

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2. Gavva, N.R., Treanor, J.J., Garami, A., Fang, L., Surapaneni, S., Akrami, A., Alvarez, F., Bak, A., Darling, M., Gore, A., Jang, G.R., Kesslak, J.P., Ni, L., Norman, M.H., Palluconi, G., Rose, M.J., Salfi, M., Tan, E., Romanovsky, A.A., Banfield, C., Davar, G. 2008. „Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans.“ *Pain.* 136: 202-210

Methods

Cells

CHO cells stably expressing hTRPV1 were used.

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 Patchliner®. Currents were elicited every second by 200 ms voltage ramps from -100 mV - +100 mV, $V_{hold} = -100$ mV. For heat activation of channels, external solution was heated in the pipette for 90 s and rapidly applied to the cell at a speed of 10 µl/s. Capsaicin was applied at room temperature for approximately 30 s before wash with external solution.

RR was diluted in external solution at the indicated concentrations and pre-incubated for at least 30 s before co-application with heat (60°C) or with 1 µM capsaicin.