

TRPV1 and TRPM8 recorded on Nanion's Port-a-Patch

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Summary

TRPV1 and TRPM8 are members of the transient receptor potential channel (TRP) family. This family was designated TRP because of a spontaneously occurring *Drosophila* mutant lacking TRP that responded to a continuous light with a transient receptor potential.

TRPV1 is mainly expressed in sensory nerves. Its presence is essential for transduction of nociception as well as inflammatory and hypothermic effects of vanilloid compounds. It also contributes to acute thermal nociception and thermal hyperalgesia following tissue injury.

TRPV1 forms a relatively Ca²⁺-selective ion channel with outwardly rectifying properties. It is activated by vanilloids such as capsaicin, by protons, increased temperatures, lipoxygenase products, as well as anandamide (Huang *et al.*, 2002).

Menthol, a secondary alcohol produced by the peppermint herb, *Mentha piperita*, is widely used in the food and pharmaceutical industries as a cooling/soothing compound and odorant. It induces Ca²⁺ influx in a subset of sensory neurons from dorsal root and trigeminal ganglia, due to activation of TRPM8, a Ca²⁺-permeable, cold-activated member of the TRP superfamily of cation channels (McKemy *et al.*, 2002; Peier *et al.*, 2002).

Here we present data of TRPV1 and TRPM8 collected on the Port-a-Patch. Channel activation with capsaicin as well as menthol are shown.

Results

Whole cell current responses of an individual cell expressing the TRPV1 channel is shown in Figure 1. The activator was applied and washed out using Nanion's External Perfusion System.

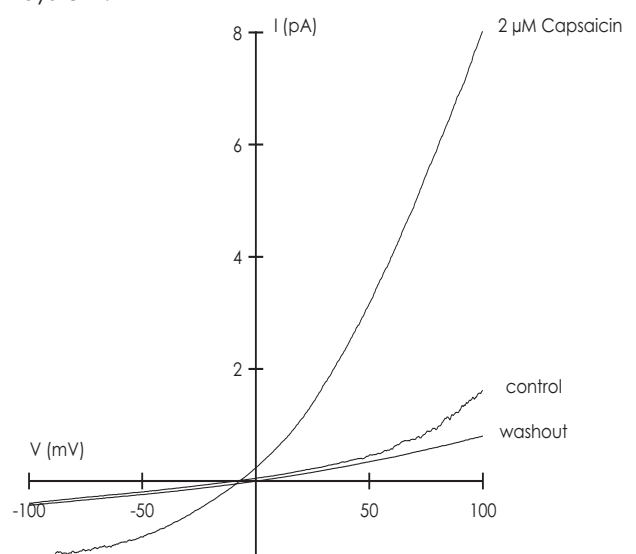


Figure 1: Whole cell current responses from HEK293 cells transiently expressing TRPV1 to a ramp voltage protocol (-100 mV to +100 mV). 2 μ M capsaicin reversibly activated the channel. Data were kindly provided by Dr. David Cohen, Oregon Health & Science University, Portland, USA.

Application Note

Figure 2 shows representative traces from an individual cell stably expressing TRPM8 in the absence and presence of increasing concentrations of menthol. The activator was applied using the Perfusion System. The exchange times for a full solution exchange using the Perfusion System are approx. 150 ms. A full concentration response of menthol ranging from 1 μM to 300 μM was achieved.

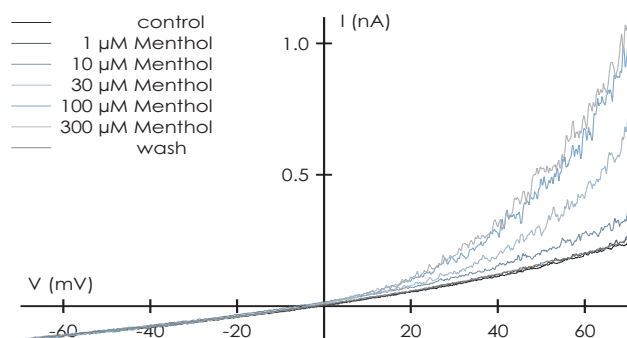


Figure 2:

Increasing concentrations of menthol acting on the TRPM8 channel. After the highest concentration was applied, the activator was washed out again. Ramp voltage protocol was applied from -70 mV to +70 mV within 200 ms.

Another example shows the time-dependent increase of whole cell currents when applying 500 ms pulses from

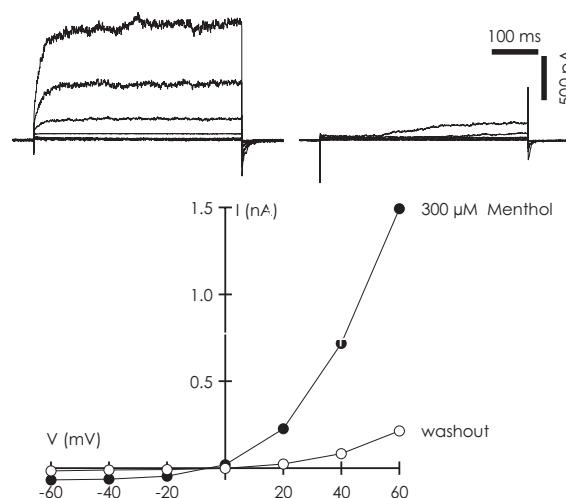


Figure 3:

Current-voltage relation of TRPM8-expressing cells in the presence of 300 μM menthol (top, left) and after washout (top, right). Corresponding IV-curves of the steady state current (below). Voltages were applied from -60 mV to +60 mV for 500 ms.

-60 mV up to +60 mV. Recordings were performed in the presence of 300 μM menthol and after washout (Figure 3).

In conclusion, we have demonstrated stable recordings of the TRP channels TRPV1 and TRPM8 on a planar patch clamp system.

References

1. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002). „An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors“. *Proc. Natl. Acad. Sci. U.S.A.* 99 (12): 8400-5.
2. McKemy DD, Neuhauser WM, Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52-58.
3. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002) A TRP channel that senses cold stimuli and menthol. *Cell* 108: 705-715.

Methods

Cells

HEK293 cells stably expressing TRPM8 (Millipore) were used. HEK293 cells transiently transfected with TRPV1 were kindly provided by Dr. David Cohen, Oregon Health & Science University, Portland, USA.

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 Port-a-Patch. For the application and washout of the activators, Nanion's External Perfusion System was used.