Application Note

Channel: hTRPM8
Cells: CHO
Tools: Patchliner®

TRPM8 activation by menthol and Eucalyptol performed on Nanion's Patchliner®

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Cells kindly provided by Chantest.

Summary

TRPM8 is a member of the transient receptor potential channel (TRP) family. TRPM8 is known to be a thermo-sensitive channel, activated by cold temperatures (below ~25˚C) and ligands such as menthol, Eucalyptol and icilin. It belongs to the melastatin subfamily of TRP channels[1] and shows an outward rectification with a relatively high permeability for calcium ions and little selectivity between monovalent cations. 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 Ca^{2+} influx in a subset of sensory neurons from dorsal root and trigeminal ganglia, where the TRPM8 channel is specifically expressed[2,3].

Here we present data of hTRPM8 collected on the Patchliner®. Cells performed well on the Patchliner® with a success rate of 89% for seal resistance >600 MΩ. Channel activation by the agonists menthol and Eucalyptol is shown.

Cells sealed	RSeal (GΩ)	Whole cell	Current amplitude (pA)
85/96 (89%)	1.6 ± 2.2	79/85 (93%)	876 ± 358

Table 1:
Seal parameters for CHO cells expressing hTRPM8 recorded on the Patchliner®. Cells sealed with a high success rate (89%) with an average seal resistance (RSeal) > 1 GΩ.

Results

For the evaluation of the performance on the Patchliner® of the hTRPM8 (CHO) cells, success rate (cells sealed), seal resistance (RSeal) and average current in the presence of agonist were determined (Table 1).

The whole cell current of an individual cell expressing TRPM8 channel is shown in Figure 1A. The recording shows the activation of the TRPM8 by menthol (1 mM) followed by wash using Nanion's standard external solution. The corresponding time course of the experiment is shown in Figure 1B. The current elicited by menthol was fully reversible upon washout.

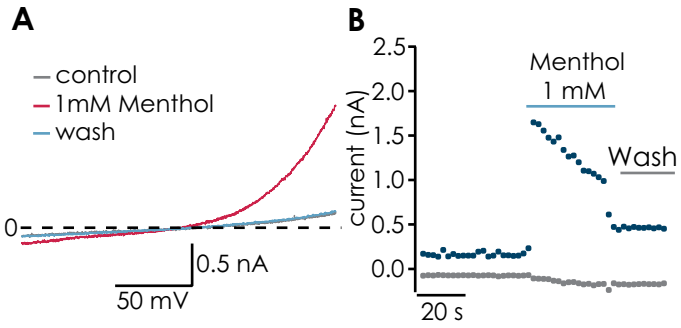

Figure 1:
A Whole cell current responses from induced CHO cells expressing TRPM8 to a ramp protocol (-80 mV to +80 mV over 200 ms) in the presence, absence and after wash of 1 mM menthol. The holding potential was set at -40 mV to eliminate any possible contributions by voltage-dependent sodium channel activity. **B** Time course measurement of TRPM8 activation with menthol

Application Note

The concentration response relationship obtained for menthol using a voltage ramp protocol is shown in Figure 2. The EC_{50} = 148 ± 16 µM (n = 7; Hill coefficient 1.85). This is in good agreement with the literature[4,5].

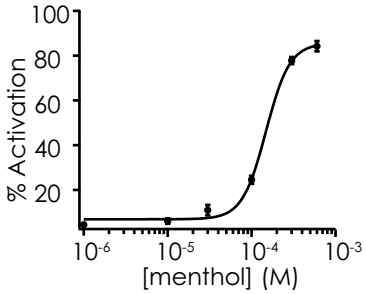

Figure 2:
Concentration response curve for menthol with representative Hill fit. The EC_{50} for menthol was 148 ± 16 µM (n = 7).

Another molecule which is known to activate TRPM8 is Eucalyptol, an essential oil often used in alternative medicine in analgesic and anti-inflammatory remedies. The effect of Eucalyptol on TRPM8 activation was less than that of menthol. TRPM8 activation by Eucalyptol is shown in Figure 3. Whole cell current responses from induced CHO cells expressing TRPM8 to a ramp protocol by 1 mM Eucalyptol followed by a wash are shown. The time course of the experiment showing activation by Eucalyptol and menthol at the same concentration (1 mM) on the same cell is also shown in Figure 3.

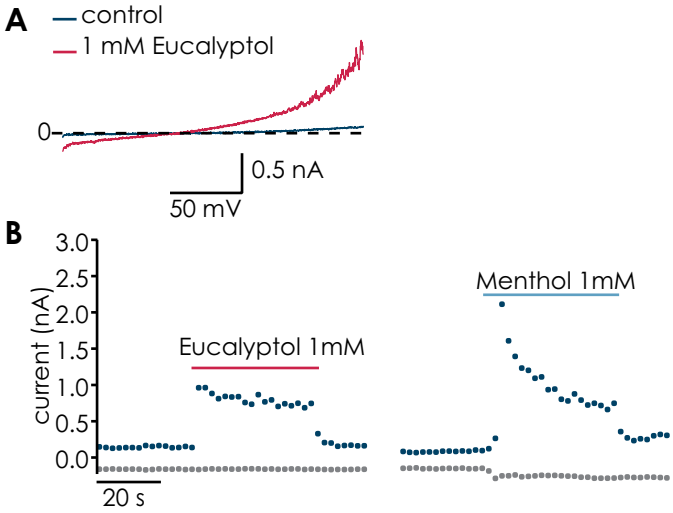

Figure 3:
A Whole cell current responses from induced CHO cells expressing TRPM8 to a ramp protocol (-80 mV to +80 mV over 200 ms). 1 mM Eucalyptol activated the channel. **B** Time course of the experiment. Activation of Eucalyptol and menthol on the same cell. Eucalyptol activated TRPM8 to a lesser degree compared with menthol activation.

In conclusion, hTRPM8 expressed in an inducible CHO cell line provided by Chantest can be reliably recorded on the Patchliner® with activation by menthol[4,5] and Eucalyptol as expected.

References

1. Montell, C., *et al*., 2002. Cell 108: 595–598.
2. McKemy, D.D., et al., 2002. Nature 416: 52-58.
3. Peier, A.M., et al., 2002. Cell 108: 705-715
4. Andersson, D.A., et al., 2004. J. Neurosci. 24(23):5364–5369
5. Zhang, X., et al.,2012. Nat. Cell Biol. 14(8):850-858

Methods

Cells

CHO cells stably expressing hTRPM8 provided by Chantest.

Cell culture

CHO cells stably expressing hTRPM8 under tetracycline induction control were grown at 37°C, 5% CO_2 in a humidified atmosphere in the incubator. hTRPM8 expression was induced by the addition of 1 mg/ml tetracycline at least 24 hours prior to measurements. Patchliner® recordings were performed 24 to 36 hours after induction. Induction time of shorter than 24h, or longer than 36h, led to a lower success rate in terms of currents observed and seal rate, respectively.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. Currents were elicited by 200 ms voltage ramps from -100 mV to +100 mV, Vhold = -40 mV. For agonist activation of channels, menthol and Eucalyptol were diluted in external solution at the indicated concentrations and applied at room temperature for approximately 40 s before wash with external solution.

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