SOPHION BIOSCIENCE | Discover more

Application Report

Measurement of TRPM8 on Sophion´s Automated Patch Clamp systems

Application of temperature-dependent TRPM8 pharmacology

Summary

Transient Receptor Potential Melastatin TRPM8 can be reliably measured on Sophion´s Automated Patch Clamp (APC) systems using stably expressing Human Embryonic Kidney (HEK203) cells. The currents responded well to pharmacological and temperature-controlled modulation. Concentration-response curves could be performed in a cumulative and non-cumulative way as well. The acquired EC_{50} and IC_{50} values of racemic menthol (RM), icilin and capsazepine were reliable, reproducible and comparable to literature values[10,11]. Based on the presented data, Sophion´s medium and high-throughput systems can be successfully used to study TRPM8 channel pharmacology and drug discovery.

Introduction

TRPM8 is a temperature-sensitive ion channel belonging to the TRP channel family[3]. It is primarily known as the principal sensor of cold temperatures and menthol in the human body[4,5]. It is mainly expressed in sensory neurons of the dorsal root ganglia and trigeminal ganglia[4]. They are known as nonselective cation channels mainly conducting Ca^{2+} and Na^{+} ions[6].

TRPM8 has a complex role in pain modulation, especially in cold allodynia (painful response to cold). Overexpression of the channel has been linked with prostate, breast and pancreatic cancers, influencing cell proliferation, migration and survival[7,8,9]. TRPM8 can also play a role in bladder disorders, as well as dry eye disease, regulating tear production[10,11].

Due to its complex role in many disorders, TRPM8 can be an important therapeutic target.

Here, we present a reliable TRPM8-assay expressed in HEK293 using Sophion´s APC systems: the QPatch Compact (QPC), QPatch and the Qube 384. The report highlights temperature-dependent activation of TRPM8 using menthol and icilin and block using capsazepine[5].

Results and discussion

Activation of TRPM8 using racemic menthol

First, we studied the activating effect of the well-known activator menthol on TRPM8 using a 100 ms classical ramp protocol from –80 mV to 80 mV. The outward current could be effectively activated by increasing concentration of racemic menthol (RM) on all APC devices (Figure 1A, B). The potency of the compound was more pronounced at lower temperature (18 °C) (Figure 1C). The EC_{50} values were similar on all 3 systems (Table 1). The effect was stronger both on QPC and QPatch when lower temperature was applied (Table 1). Activation of the inward current by 100 µM -(-) menthol using ligand-gated application on the Qube 384 was also performed. At –50 mV, we saw that inward TRPM8 current could be stably activated (Figure 2A, C). The activating effect was temperature-sensitive, and it was stronger at 18 °C compared to 27 °C (Figure 2B, D).

Activation of TRPM8 using icilin

Next, we assessed the activating effect of icilin using the Qube 384. As expected, the compound effectively activated the outward TRPM8 current (Figure 3A, B). When the temperature was lowered to 18 °C, the activating effect was more pronounced with an EC_{50} value of –6.72 (CI –6.88 to –6.49) at 27 °C vs. –7.45 (CI: –7.54 to –7.36) at 18 °C (Figure 3C).

Effect of capsazepine on TRPM8

As a next step, we checked the blocking effect of capsazepine. As expected, the compound effectively inhibited the outward TRPM8 current both on QPatch and Qube 384 in a concentration-dependent manner (Figure 4A, B). The EC_{50} values of capsazepine were comparable to what is available in the literature[10] (Table 2). Interestingly, there was almost no effect of lower temperature (18 °C) on the efficacy of capsazepine (Figure 4C and Table 2).

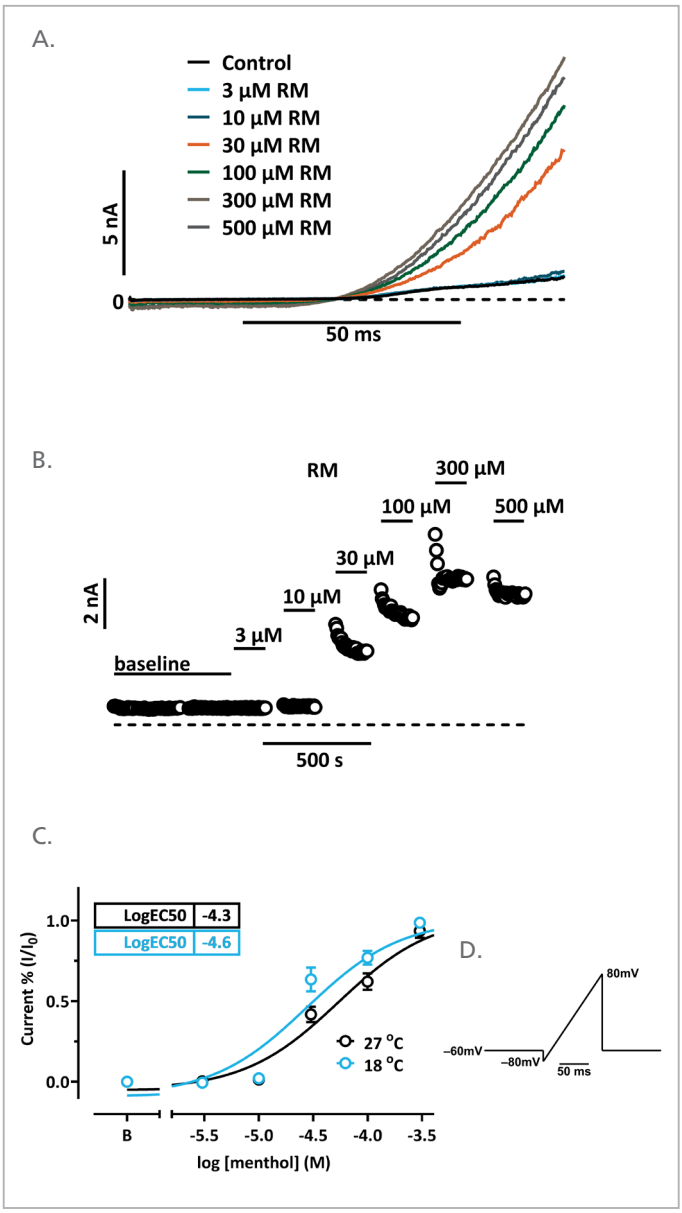

Fig. 1: A: Representative traces of TRPM8 currents recorded on the QPatch system after application of increasing concentrations of RM, compared to baseline (B). **B:** Effect of increasing concentrations of RM on outward TRPM8 currents at +50 mV. **C:** Concentration–response curves for RM at 27 °C and 18 °C, showing a comparison of its temperature-dependent effects. **D**: Currents were recorded using a standard voltage ramp protocol.

Table 1: Log IC_{50} values calculated for capsazepine on the QPatch and Qube 384 at 27 °C and 18 °C.

$logEC_{50}$ values for RM		
	27 °C	18 °C
QPC	-4.3 (CI: -4.6 to -4.0)	-4.8 (CI: -5.1 to -4.5)
QPatch	-4.3 (CI: -4.4 to -4.1)	-4.6 (CI: -4.7 to -4.4)
Qube 384	-4.1 (CI: -4.5 to -3.7)	-

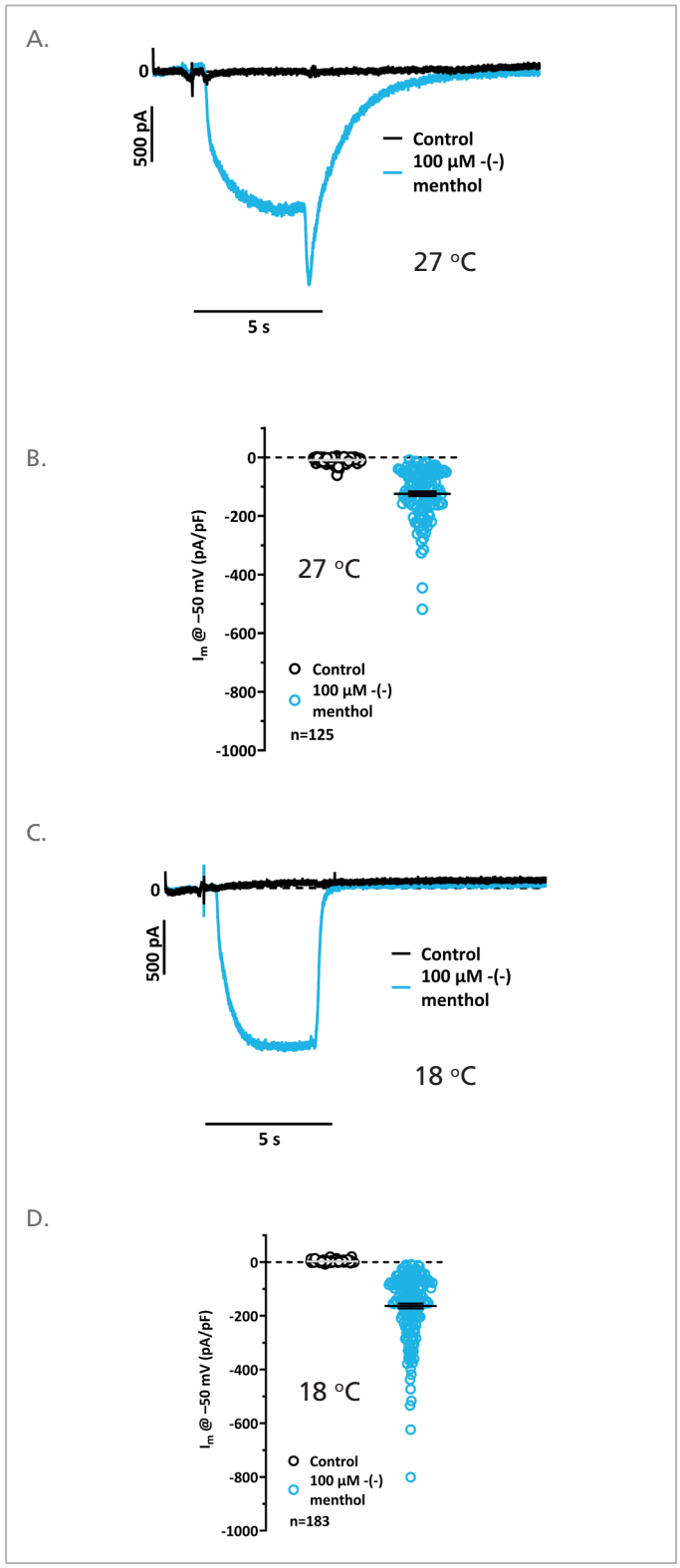

Fig. 2: **A, C**: Original traces of inward TRPM8 currents recorded after activation with 100 µM (–)-menthol at 27 °C (panel, A) and 18 °C (panel, C), respectively. **B, D**: Individual and average current densities measured before and after 100 µM (–)-menthol application at 27 °C (top panel, B) and 18 °C (bottom panel, D). Data are presented as mean ± SEM.

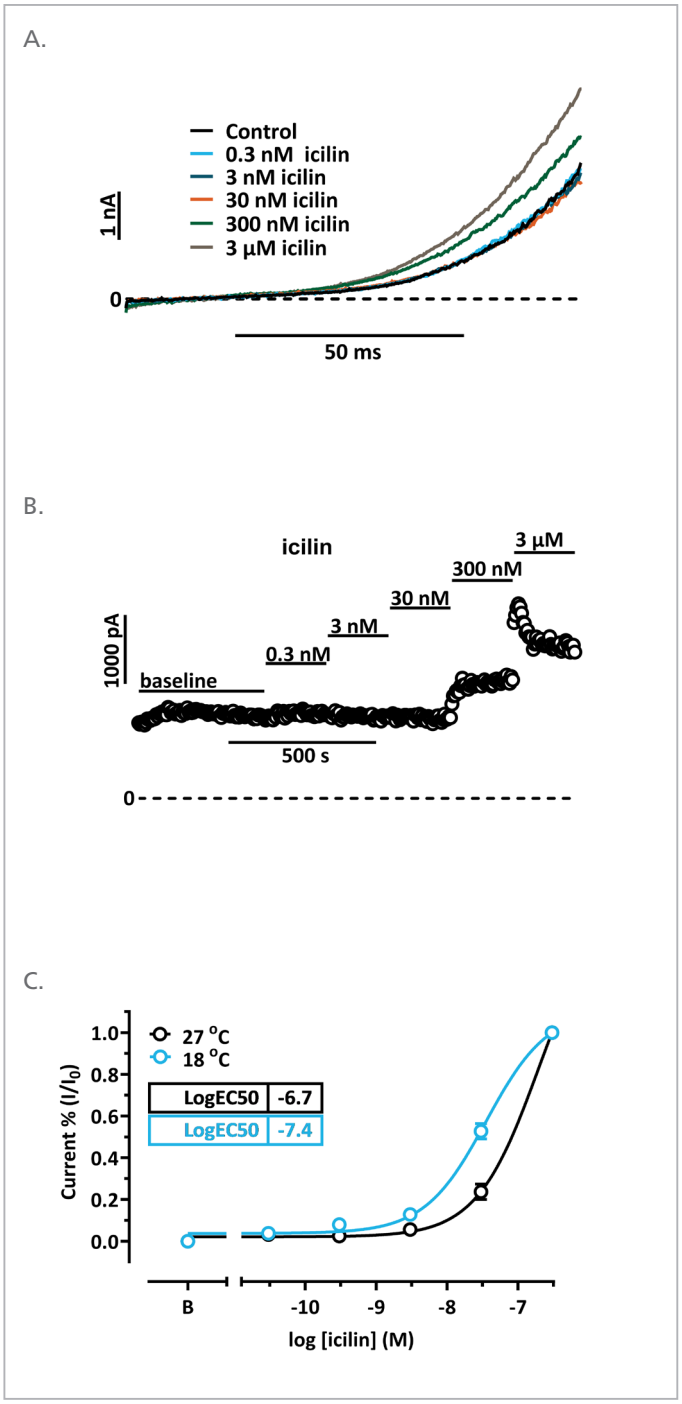

Fig. 3: **A:** Representative traces of TRPM8 currents activated by icilin on the Qube 384 platform, compared to baseline (B). **B:** Effect of increasing concentrations of RM on icilin-activated outward TRPM8 currents at +50 mV. **C:** Concentration–response curves for icilin at 27 °C and 18 °C, showing a comparison of its temperature-dependent effects.

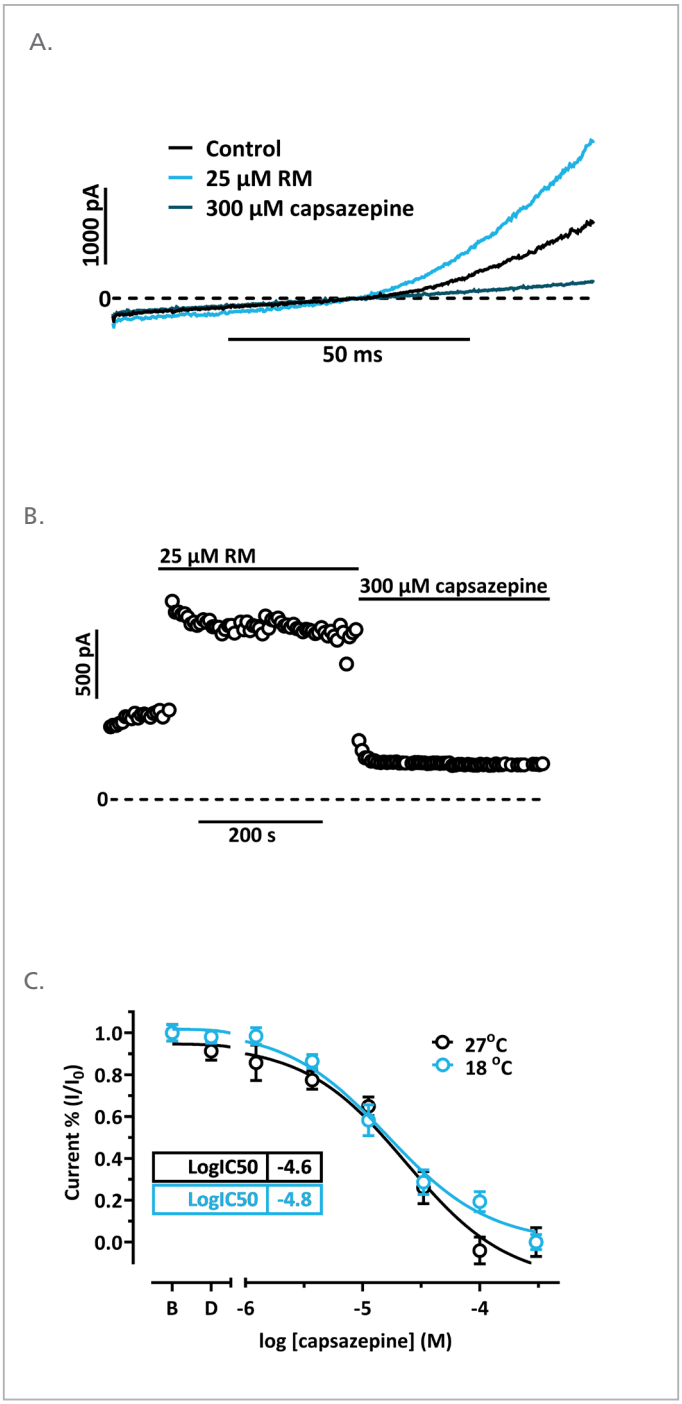

Fig. 4: **A:** Representative traces of TRPM8 currents recorded under control conditions, after activation with 100 µM (–)-menthol, and following application of 300 µM capsazepine on the QPatch system. **B:** Effect of 300 µM capsazepine on menthol-activated outward TRPM8 currents at +50 mV compared to baseline. **C:** Concentration-dependent inhibition of TRPM8 currents by capsazepine, showing no significant difference in potency between 27 °C and 18 °C.

Table 2: Log IC_{50} values calculated for capsazepine on the QPatch and Qube 384 at 27 °C and 18 °C.

logEC$_{50}$ values for capsazepine		
	27 °C	18 °C
QPatch	-5.3 (CI: -5.4 to -5.2)	-5-1 (CI: -5.2 to -5.0)
Qube 384	-4.6 (CI: -4.8 to -4.4)	-4.8 (CI: -5.0 to -4.6)

Methods

For APC recordings, Sophion´s QPatch Compact, QPatch and Qube 384 were used. During the experiments, Sophion´s standard extracellular (EC000) and K-gluconate-containing intracellular (IC000-K-gluconate) recording solution was used. After reaching GΩ seal resistances, a short negative repeated (if necessary) suction was applied to reach whole-cell configuration (WC) on a holding potential of –60 mV. To elicit currents, the holding potential was to –80 mV, and classical ramp protocol was used from –80 mV to 80 mV for 100 ms, with a sweep interval of 5 s. The outward current was analyzed at 50 mV while the inward current was investigated at –50 mV. To study the temperature sensitivity of TRPM8, the measurements were carried out at 27 °C and 18 °C. For pharmacological modulation, racemic menthol, -(-) menthol, icilin and capsazepine were used. For statistical analysis and calculation of EC_{50} and IC_{50} values, the Sophion Analyzer and Graphpad Prism 10 were used.

References

1. Montell C, Birnbaumer L, Flockerzi V. The TRP channels, a remarkably functional family. **Cell**. 2002 Mar 8;108(5):595-8.
2. McKemy, D. D., Neuhausser, W. M., & Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. **Nature**, 416(6876), 52-58.
3. Izquierdo C, Martín-Martínez M, Gómez-Monterrey I, González-Muñiz R. TRPM8 Channels: Advances in Structural Studies and Pharmacological Modulation. **Int J Mol Sci**. 2021;22(16):8502. Published 2021 Aug 7. doi:10.3390/ijms22168502
4. Gees M, Colsoul B, Nilius B. The role of transient receptor potential cation channels in Ca^{2+} signaling. **Cold Spring Harb Perspect Biol**. 2010 Oct;2(10):a003962.
5. Grolez GP, Chinigò G, Barras A, Hammadi M, Noyer L, Kondratska K, Bulk E, Oullier T, Marionneau-Lambot S, Le Mée M, Rétif S, Lerondel S, Bongiovanni A, Genova T, Roger S, Boukherroub R, Schwab A, Fiorio Pla A, Gkika D. TRPM8 as an AntiTumoral Target in Prostate Cancer Growth and Metastasis Dissemination. **Int J Mol Sci.** 2022 Jun 15;23(12):6672.
6. Huang Y, Li S, Jia Z, Zhao W, Zhou C, Zhang R, Ali DW, Michalak M, Chen XZ, Tang J. Transient Receptor Potential Melastatin 8 (TRPM8) Channel Regulates Proliferation and Migration of Breast Cancer Cells by Activating the AMPK-ULK1 Pathway to Enhance Basal Autophagy. **Front Oncol.** 2020 Dec 4;10:573127.
7. Yee NS. TRPM8 Ion Channels as Potential Cancer Biomarker and Target in Pancreatic Cancer. **Adv Protein Chem Struct Biol.** 2016;104:127-155.
8. Aizawa N, Fujita T. The TRPM8 channel as a potential therapeutic target for bladder hypersensitive disorders. J Smooth Muscle Res. 2022;58(0):11-21. Chen, X., Xu, L., Zhang, H., Wen, H., & Yang, F. (2022). Differential Activation of TRPM8 by the Stereoisomers of Menthol. **Frontiers in pharmacology**, 13, 898670.
9. Gallar J, Pflugfelder S, Galor A, Gupta PK, Hamrah P. Corneal sensory nerve regulation of tear production through stimulation of transient receptor potential melastatin 8 (TRPM8) channel: A potential new approach for treating dry eye disease. **Ocul Surf**. 2025 Jul 18;38:142-154. 10.
10. Malkia A, Pertusa M, Fernández-Ballester G, Ferrer-Montiel A, Viana F. Differential Role of the Menthol-Binding Residue Y745 in the Antagonism of Thermally Gated TRPM8 Channels. **Molecular Pain**. 2009;5. 11.
11. Chuang HH, Neuhausser WM, Julius D. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. **Neuron**. 2004 Sep 16;43(6):859-69.

Author:

Andràs Horváth, Application Scientist

Naja Møller Sørensen, Application Scientist