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Measuring TRP channels using automated patch clamp

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Abstract

Introduction: Transient receptor potential channels are a superfamily of non-selective cation channels and can be activated by a wide range of stimuli such as temperature, pharmacological agents, mechanical stress, pH or osmolarity. In this study we investigated the Transient Receptor Potential Vanilloid 3 (TRPV3) and the Transient Receptor Potential Melastatin 8 (TRPM8) channels using Sophion´s Automated Patch Clamp (APC) platforms, the QPatch Compact (QPC), the QPatch and the Qube 384.

Methods: For APC recordings Human embryonic kidney (HEK) cells expressing TRPV3 and TRPM8 channel were used. For TRPV3 pharmacology 2-Aminoethoxydiphenyl borate (2-APB) and ruthenium red (RR), and for TRPM8 modulation racemic menthol (RM), icilin and capsazepine was used. TRPV3 was measured at 25°C and 34°C while TRPM8 was investigated at 27°C and 18°C.

Results: Using QPatch, TRPV3 could be activated by 2-APB in a concentration dependent manner (Fig. 1 A, B). The IC_{50} value was slightly higher at 34°C (43.03 µM) compared to 25°C (46.05 µM) (Fig. 1 C). The activated current could be blocked by RR (Fig. 2 A, B). Inhibition of the current by RR was stronger at 34°C (5.3 µM) compared to 25°C (10.7 µM) (Fig. 2 C). Interestingly the inhibitory effect of RR was stronger when a higher concentration was applied initially (2.1 µM vs 10.7µM) (Fig. 2 D). As expected, TRPM8 could be activated by increasing concentration of RM on all APC platforms (Fig 3 A, B; Fig 5 A, B; Fig 6C). The activating effect was higher at lower temperature of 18°C (Fig. 3C; Fig 5C). The $logEC_{50}$ values were: –4,3 at 27°C vs. –4.55 at 18°C QPC, –4,3 at 27°C vs. –4.8 at 18°C QPatch and –4,3 at 27°C vs. –4.8 at 18°C Qube 384. Like RM, icilin was effectively activating TRPM8 using the Qube 384 ($logEC_{50}$: -6.7 at 27°C vs. -7.44 at 18°C) (Fig A, B). During recordings performed on QPatch and Qube 384, capsazepine effectively inhibited RM-activated TRPM8 in a concentration dependent manner (Fig 4 A, B). Interestingly, the blocking effect of TRPM8 by capsazepine was only slightly different if 27°C and 18°C were applied (Fig 4 C; Fig 6 D). The log IC_{50} values were: –5,3 at 27°C vs. –5.1 at 18°C QPatch and –4,6 at 27°C vs. –4.8 at 18°C Qube 384.

Conclusion: Both pharmacological and temperature-controlled modulation of TRPV3 and TRPM8 could be successfully applied on Sophion´s APC platforms.

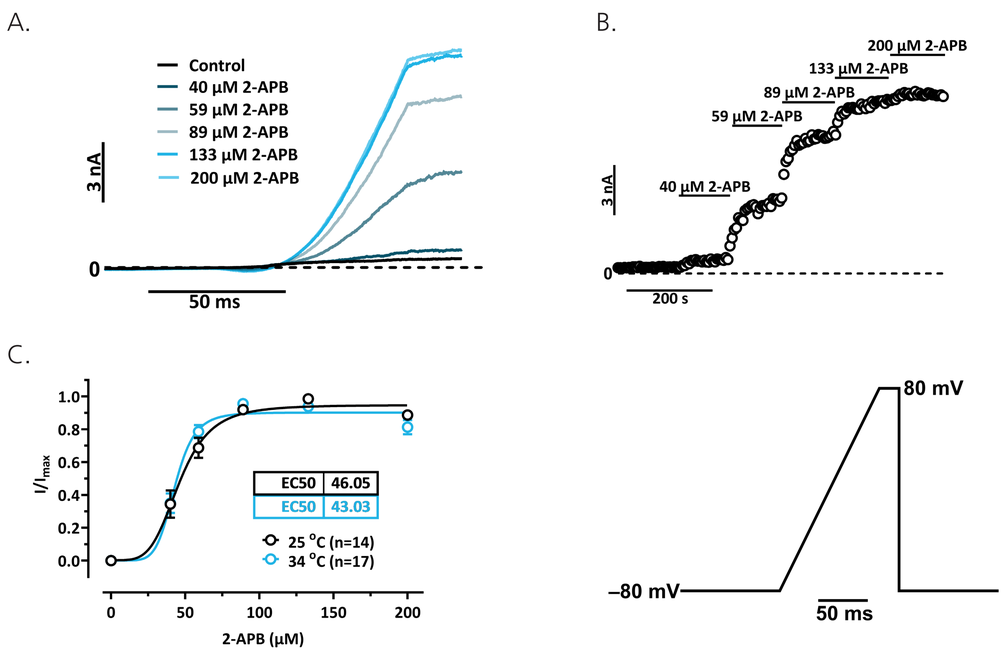

Fig. 1: A: Original traces of TRPV3 after addition of various concentrations of 2-APB. **B:** Effect of increasing concentrations of 2-APB on the outward current at 80 mV. **C**: Comparison of the dose dependent effect of 2-APB at 25°C and 34°C.

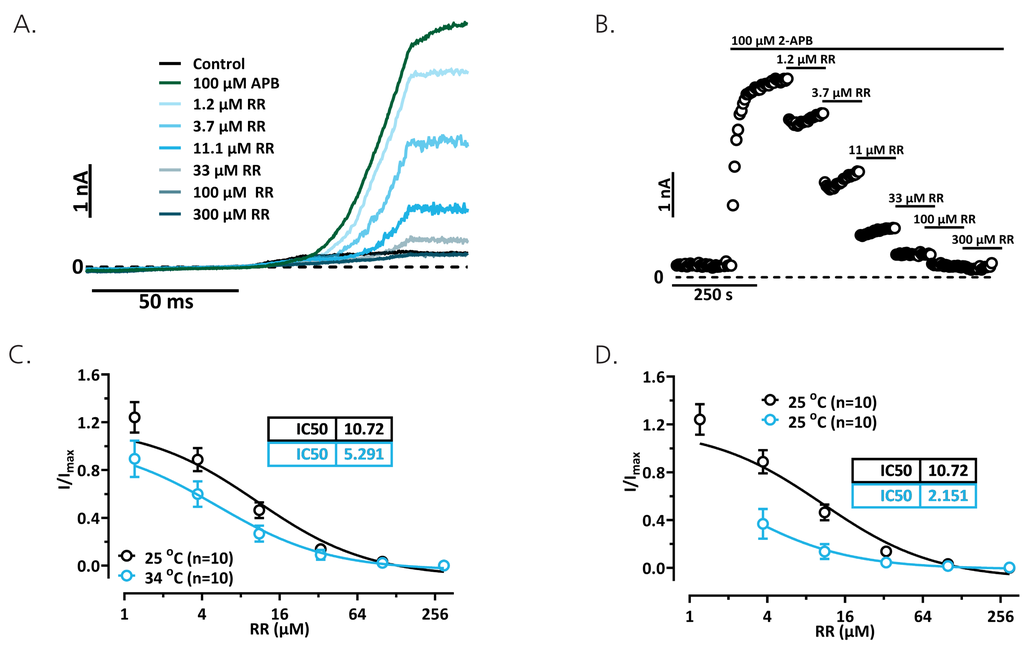

Fig. 2: A: Original traces of 2-APB activated TRPV3 current after addition of multiple concentrations of RR. **B:** Effect of increasing concentrations of RR on 2-APB-activated current measured at 80 mV. **C:** Effect of temperature on the dose-dependent block of 2-APB-activated TRPV3 using RR. **D:** Increase in the blocking effect of RR on 2-APB-activated TRPV3 with larger starting concentration of RR at 25°C.

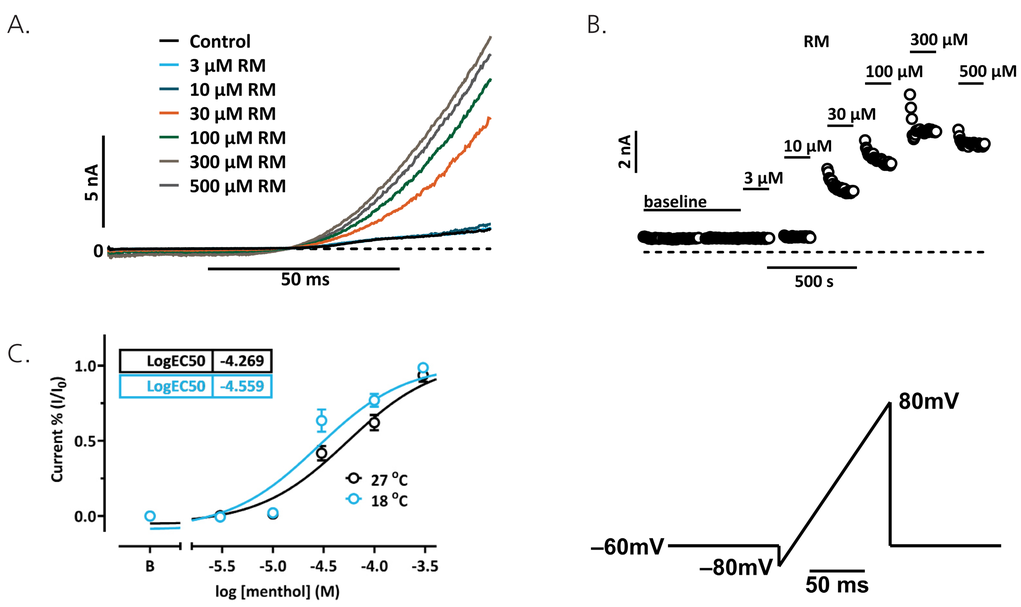

Fig. 3: A: Original traces of TRPM8 after addition of various concentrations of RM on QPatch compared to baseline (B). **B:** Effect of increasing concentrations of RM on the outward current at 50 mV. **C:** Comparison of the concentration-dependent effect of RM at 27°C and 18°C.

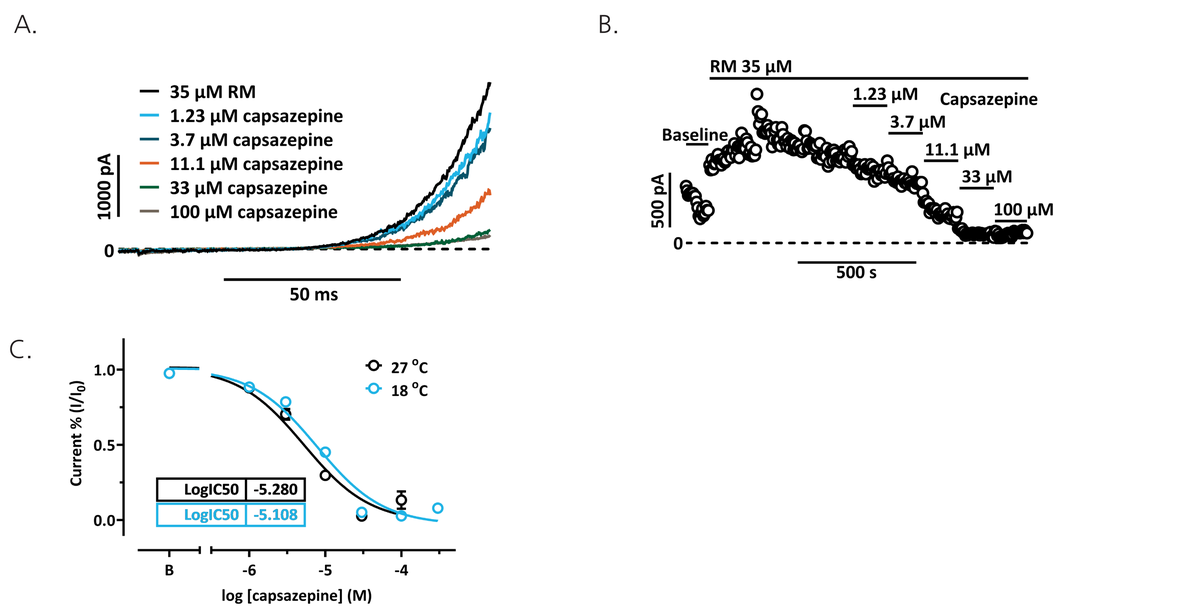

Fig. 4: A: Original traces of RM-activated (35µM) TRPM8 current (B as baseline) after inhibition by multiple concentrations of capsazepine. **B:** Time course-effect of capsazepine on RM-activated current recorded at 50 mV. **C:** Effect of temperature on the concentration-dependent block of RM-activated TRPM8 using capsazepine.

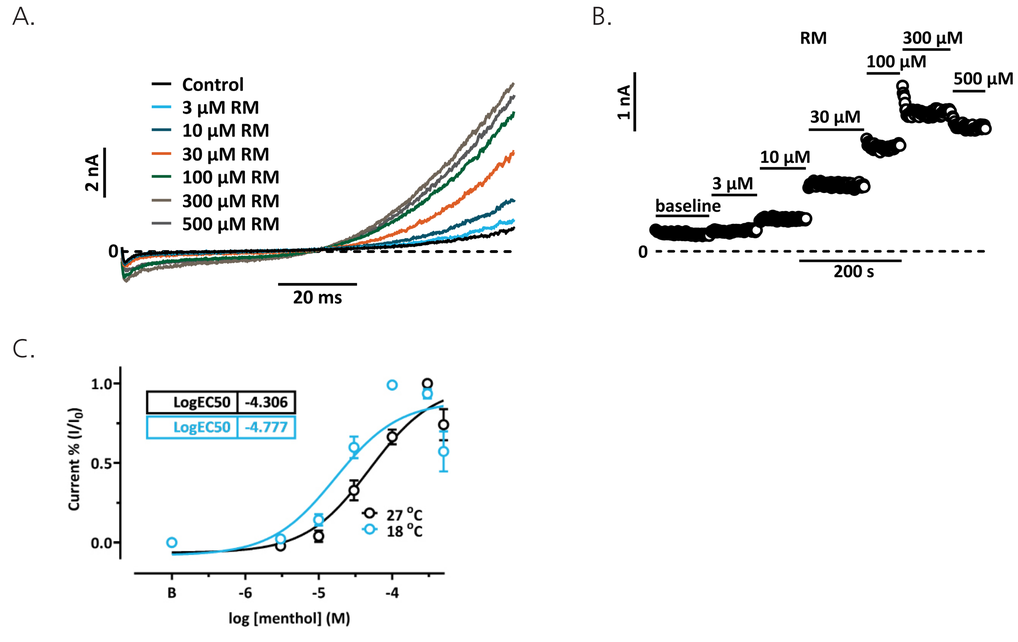

Fig. 5: A: Original recordings of activating TRPM8 by multiple concentrations of RM (compared to baseline (B)) using QPC. **B:** Time course recordings of the outward current activated by RM at 50 mV. **C:** Temperature-sensitive activating effect of RM measuring at 27°C and 18°C.

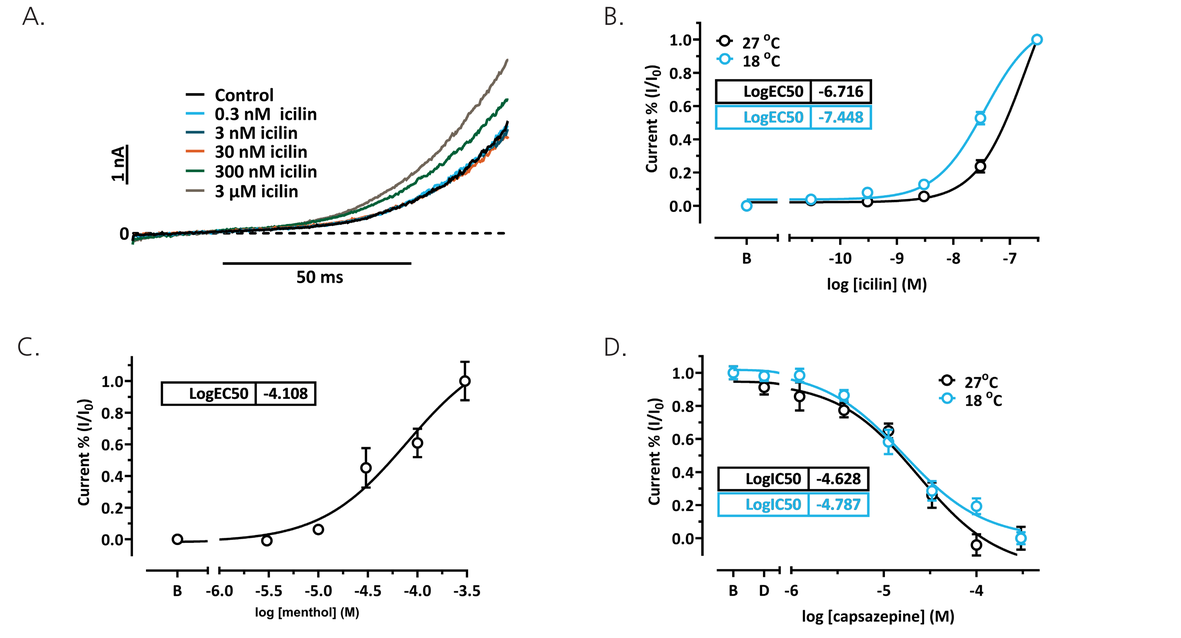

Fig. 6: A: Original traces of TRPM8 after addition of increasing concentrations of icilin compared to baseline (B) using the Qube 384. **B:** Effect of temperature on concentration-dependent activation of TRPM8 outward current by icilin at 50 mV. **C:** concentration-dependent activation of TRPM8 using RM at 18°C. D: Lack of difference in blocking effect of capsazepine on RM-activated TRPM8.

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