SOPHION BIOSCIENCE | Discover more

Application Report

Measuring TRPV3 channel using the QPatch

Pharmacological modulation of TRPV3 using automated patch clamp platforms

Summary

HEK-TRPV3 is stable on QPatch and experiments yielded high success rates, high-quality gigaseals and good recordings of the ion channel current, although TRPV3 can be a challenging channel and more experiments need to be done to fully understand its polymodal nature. The temperature control feature provided more control over the channel as at 34°C data produced were more consistent and with less variation of phenotypes. The half maximal effective and inhibitory concentrations (EC_{50} and IC_{50}) found in this study are comparable to previously reported values.

Introduction

Transient receptor potential cation channel, subfamily V, member 3 (TRPV3) is part of the large family of various non-selective cation channels. It was first described in 2002, and its sequence shows 43% identity to TRPV1 and 41% to TRPV4. Several studies reported expression of the channel in skin, keratinocytes and oral and nasal mucosa[1], suggesting that TRPV3 plays a role in heat sensing[2,3] as well as in hair growth[4], wound healing, itching and pain perception[1,5]. Recent case studies revealed that mutations of TRPV3 are associated with Olmsted Syndrome[6].

The channel could be activated by innocuous temperatures (>33° C) and gives a fast, strong response to noxious hot temperatures. A recent preprint has reported that it could be activated by cold temperature in the presence of tetrahydrocannabivarin. For chemical modulation, the most commonly used activator is 2-Aminoethoxydiphenyl borate (2-APB)[7], while the non-selective blocker ruthenium red can be applied as an inhibitor.

According to its role in various processes in the body, especially pain perception and diseases, TRPV3 has received increasing attention as a therapeutic target.

Here, we present a robust assay measuring TRPV3 channel expressed in human embryonic kidney (HEK) cells on Sophion´s medium throughput automated patch clamp (APC) system the QPatch. The report highlights the concentration-dependent activating effect of 2-APB and the role of moderate temperature (34°C) in activating TRPV3, and we used the non-selective blocker ruthenium red to inhibit the current.

Results and discussion

Pharmacological activation and temperature sensitivity of TRPV3

As expected, TRPV3 responded rapidly to a high concentration (300 µM) of 2-APB producing a large outwardly rectifying current (Fig. 1).

Next, we assessed the potency of 2-APB on TRPV 3 and the effect of moderate temperature. The compound activated a robust outward current in a concentration dependent manner at 25C with an EC_{50} of 46.05 µM, consistent with literature data7. Interestingly moderate temperature resulted in a small change in potency with EC_{50} of 43.03 µM. In line with previous reports, higher concentrations of 2-APB (300 µM) led to decrease in the current amplitude, so those concentrations were excluded from the Hill-fit. The results suggest that innocuous temperature slightly influences the potency of 2-APB on TRPV3 (Fig. 2).

Pharmacological block of TRPV3

Subsequently, we tested the possible blocking effect of the non-selective blocker ruthenium red (RR) on the 2-APB-activated (100 µM) current. In this experimental setting, 2-APB was added twice. Interestingly, the activating effect could not saturate even after repeated additions. At 25°C RR only started to inhibit TRPV3 at higher concentrations with an IC_{50} of 10.72 µM. Conversely, at 34°C activation and saturation of the current by 2-APB, as well as blocking effect of RR were stronger with an IC_{50} of 5.2 µM. Repeating the addition of 100 µM 2-APB three times and using a higher starting concentration of RR increased the blocking effect even at 25°C. The results suggest that higher temperatures are positively affecting the activating efficiency of 2-APB and the blocking effect of RR (Fig. 3).

Discussion

In this study, we present the application of HEK-TRPV3 stably expressing cell line to investigate the effects of pharmacological modulators of TRPV3 at 25°C and 34°C. Initially, pharmacological activation of TRPV3 by 2-APB showed a slight shift in potency with the elevated temperature. It is reported that TRPV3 is sensitive to innocuous temperatures (threshold around 33-35 oC), however a stronger response appears only at using noxious temperature which can explain the moderate increase in the outward current observed during our recordings[2]. Our results also demonstrated that 2-APB-activated TRPV3 current is sensitive to RR. Both the effect of 2-APB and RR were more stable and pronounced at 34°C, suggesting that innocuous temperature favors the stability of recordings as well as the compound effects.

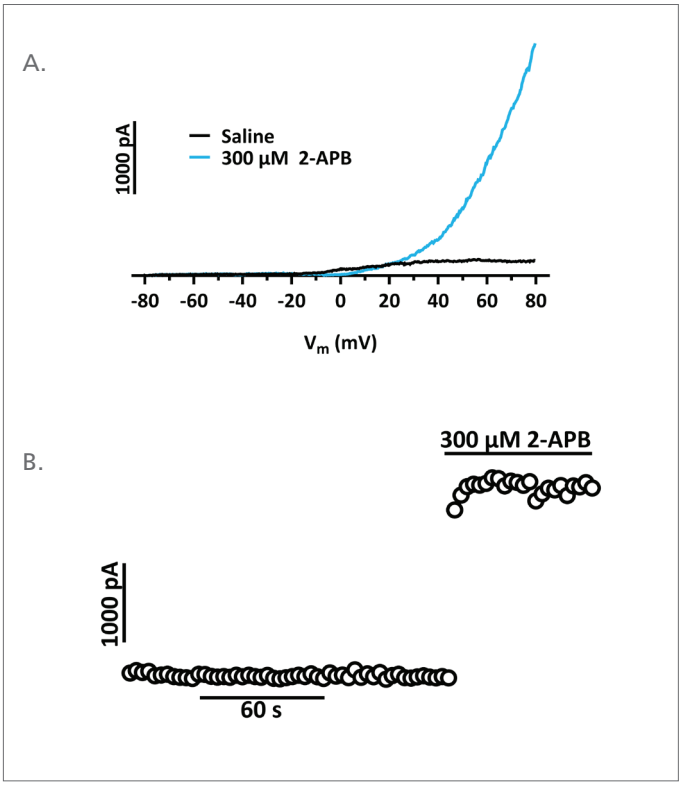

Fig. 1: A: Original recording of TRPV3 before and after 2-APB (200µM). **B**: Time course image of outward, 2-APB-acitvated TRPV3 current measured at 80 mV.

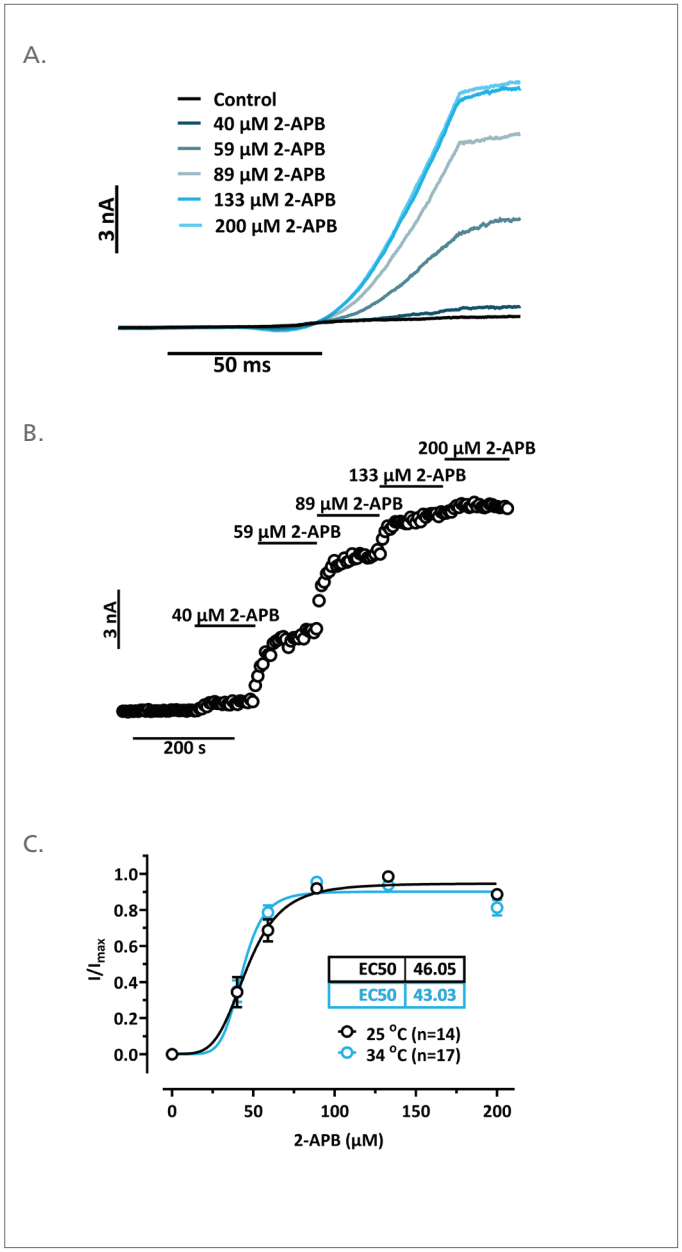

Fig. 2: A: Original traces of TRPV3 after addition of various concentrations of 2-APB. **B**: Effect of increasing concentrations of 2-APB on the outward current at 80 mV. C: Comparison of the dose dependent effect of 2-APB at 25°C and 34°C.

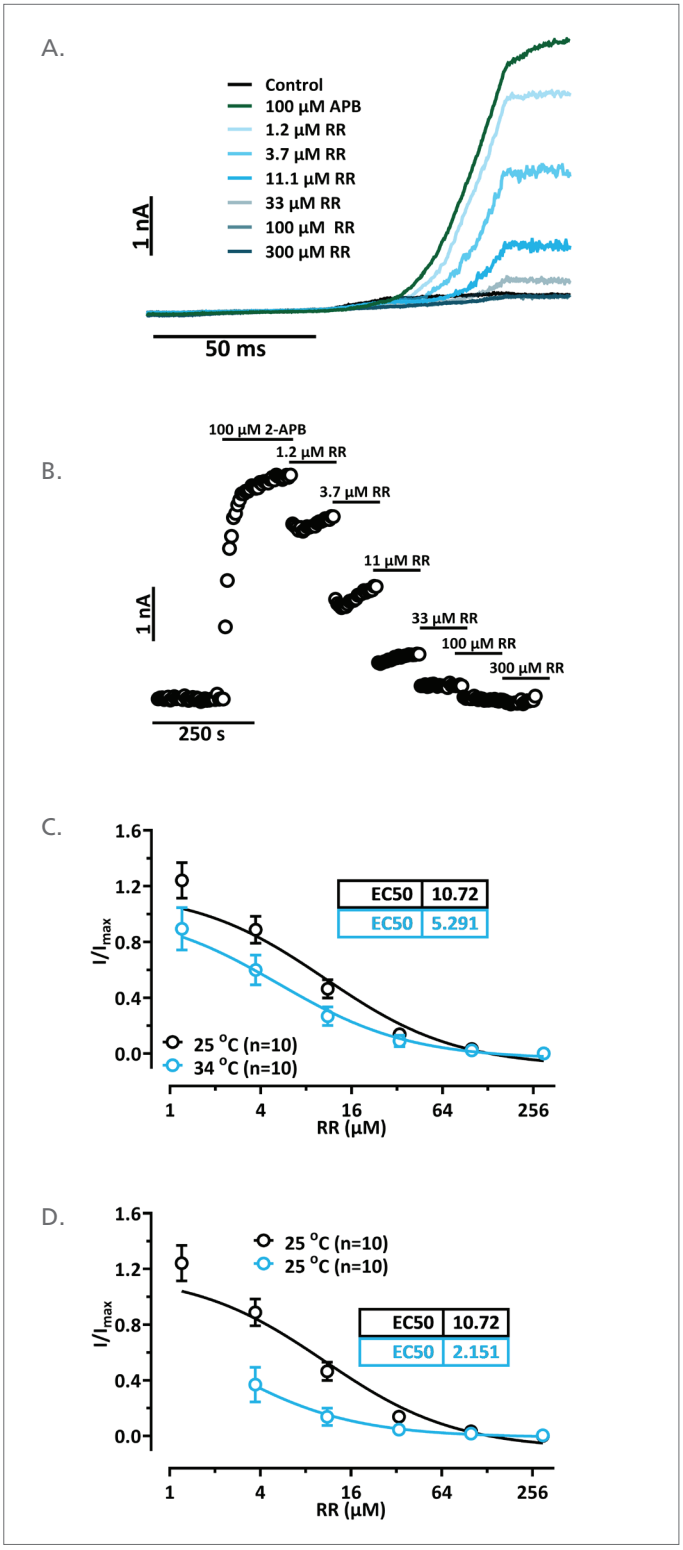

Fig. 3: A. Original traces of 2-APB activated TRPV3 current after addition of multiple concentrations of RR. **B**: Effect of increasing concentrations of RR on 2-APB-activated current measured at 80 mV. **C**: Effect of temperature on the dose-dependent block of 2-APB-activated TRPV3 using RR. **D**: Increase in the blocking effect of RR on 2-APB-activated TRPV3 with larger starting concentration of RR at 25°C.

Conclusion

The presented data is similar to the results published in literature mainly carried out by manual patch clamping. Those values indicate that Sophion´s QPatch is a suitable APC device to study TRPV3 a in higher throughput, reliable and precise way.

Methods

HEK-TRPV3 cell line was kindly provided by B´SYS GmbH (Witterswil, Switzerland)

Cell culturing was carried out according to Sophion´s standard cell culturing protocol.

For APC recordings Sophion´s QPatch 16 and Qube 384 were used. During the experiments Sophion´s standard extracellular (EC000) without Mg^{2+} and K^{+}-free intracellular (IC700) 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 –90 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 the end of the ramp at 80 mV. To study the temperature sensitivity of TRPV3 the measurements were carried out at 25°C and 34°C.

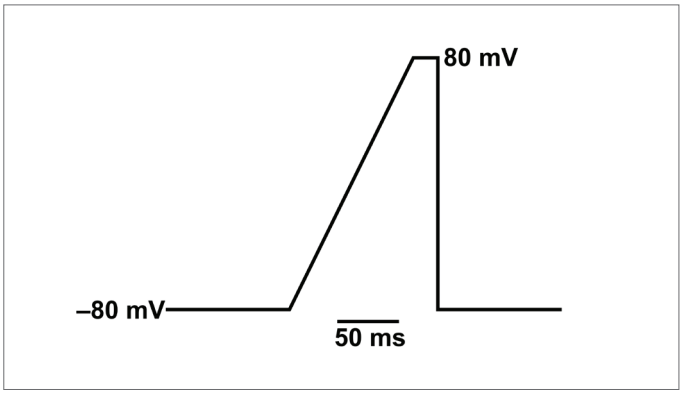

Fig. 4: For pharmacological activation of TRPV3-potentiator 2-Aminoethoxydiphenyl borate (2-APB) was applied in various concentrations. For inhibition, the non-selective blocker ruthenium red was used in 6 concentrations. For statistical analysis the Sophion Analyzer and GraphPad Prism 10 were used. Data were presented as mean±SEM. During dose-response experiments, EC_{50} and IC_{50} values were estimated by application of classical Hill-fit.

References

1. Yang Pu and Zhu, M. X. TRPV3. in *Mammalian Transient Receptor Potential (TRP) Cation Channels: Volume I* (ed. Nilius Bernd and Flockerzi, V.) 273–291 (Springer Berlin Heidelberg, Berlin, Heidelberg, 2014). doi:10.1007/978-3-642-54215-2_11.
2. Peier, A. *et al.* heat-sensitive TRP channel expressed in keratinocytes. *Science (1979)* **296**, 2046–2049 (2002).
3. Moqrich, A. *et al.* Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. doi:10.1126/science.1108609ï.
4. Imura, K. *et al.* Influence of TRPV3 mutation on hair growth cycle in mice. Biochem Biophys Res Commun 363, 479–483 (2007).
5. Lezama-García, K. *et al.* Transient receptor potential (Trp) and thermoregulation in animals: Structural biology and neurophysiological aspects. Animals vol. 12 Preprint at https://doi.org/10.3390/ani12010106 (2022).
6. Zhang, J., Guo, M. Y., Yuan, D. Y., Wei, J. Y. & Cui, H. Erlotinib therapy for Olmsted syndrome with p.L655P missense mutation in the TRPV3 gene: a case report. *Front Med* (Lausanne) **12**, (2025).
7. Chung, M. K., Lee, H., Mizuno, A., Suzuki, M. & Caterina, M. J. 2-Aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. *Journal of Neuroscience* **24**, 5177–5182 (2004).

Author:

András Horváth, Application Scientist