Application Note

Channel: hTRPV3
Cells: HEK293
Tools: Patchliner

Heat activation of TRPV3 on Nanion´s Patchliner

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by EMD Millipore, USA.

Summary

The transient receptor potential cation channel, subfamily V, member 3 (TRPV3), is a ligand-gated, non-selective cation channel first described in 2002[1,2,3]. It exhibits 43% sequence identity to TRPV1[1]. Although TRPV3 has been detected immunologically in the CNS and suggested to be often co-localized with TRPV1[3], it is found more robustly in keratinocytes in skin and, given it's threshold for temperature activation of >34˚C[1,2,3], it has been speculated that TRPV3 may act in co-operation with sensory afferents to perceive warmth and signal elevated temperature. TRPV3 can also be activated by the ligand 2-Aminoethoxydiphenyl borate (2-APB)[4]. The TRPV3 channel, along with other TRPV channels, may play an important role in chronic pain and, therefore, is receiving increasing attention as a potential therapeutic candidate for the treatment of chronic pain.

Here we present data collected on a 4-channel Patchliner® with temperature control showing the potential use of the Patchliner to record TRPV3 currents activated by 2-APB or heat. As previously reported, TRPV3 currents sensitize to repeated applications of 2-APB[4] or heat[1], a phenomenon we also observed. At low concentrations of 2-APB, the currents were primarily outwardly rectifying but at higher concentrations and with prolonged exposure they often became dual rectifying (data not shown). This is also in good agreement with the literature[4]. In contrast, the temperature-activated responses were always outwardly rectifying with little inward current. The inward currents activated by 2-APB could be blocked by ruthenium red (RR) as expected[4].

Results

Current responses of an individual cell to 200 ms voltage ramps (-100 mV to +100 mV) and activation by application of increasing concentrations of 2-APB are shown in Figure 1. A concentration response curve (using amplitude at +90 mV) revealed an EC_{50} for 2-APB activation of 61.1 ± 7.6 µM (n = 11), in good agreement with the literature[4,5]. Interestingly, as previously reported[1,4] TRPV3 currents displayed sensitization to repeated application of 2-APB or heat (Figure 1).

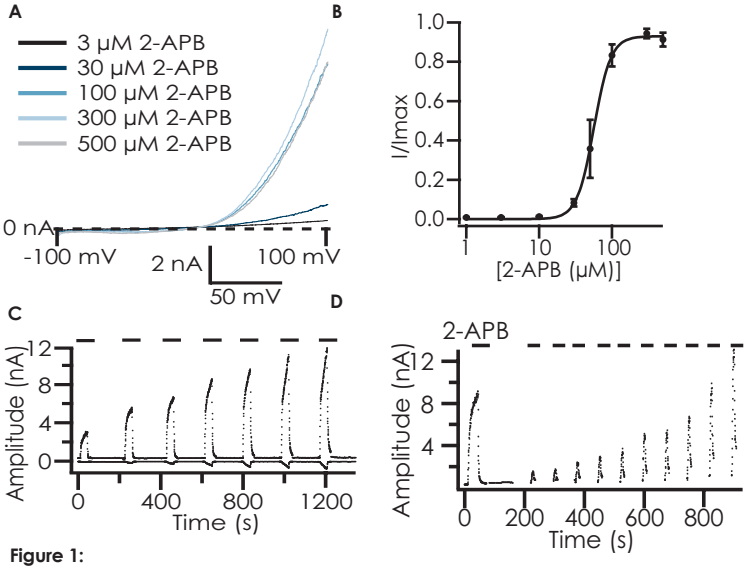

Figure 1:
A Activation of TRPV3 by increasing concentrations of 2-APB. **B** Concentration response curve for 2-APB activation, EC_{50} = 61.1± 7.6 µM (n = 11). **C & D** Repeated exposure of TRPV3 currents to 2-APB (**C**) or temperature (**D**) caused sensitization of currents (lines indicate 2-APB or heat exposure).

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Figure 2 shows the activation of TRPV3 channels by increasing temperature. Outwardly rectifying currents started to activate at 38°C and increased in amplitude as temperatures increased, up to 54°C, in good agreement with the literature[1,2,3]. To rule out the involvement of TRPV1 a control was performed using capsaicin. Currents activated by 100 µM 2-APB were not activated by 1 µM capsaicin (Fig. 2B) as previously reported[1].

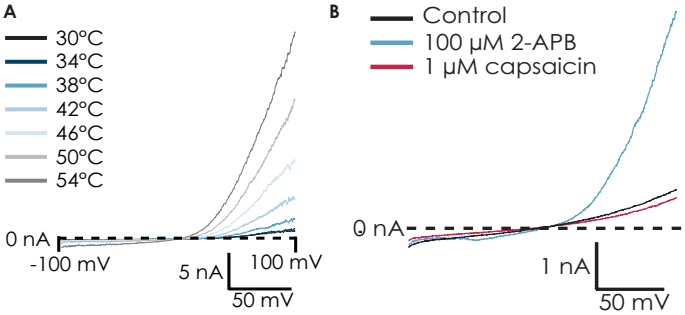

Figure 2:
A Activation of TRPV3 by increasing temperature. The solution inside the pipette was heated to the temperature indicated and applied to the cell. TRPV3 was activated at temperatures of 38°C and above. **B** TRPV3 was activated by 100 µM 2-APB but not 1 µM capsaicin.

A full concentration response curve to RR at 0.1, 1, 3 and 10 µM was performed (Fig. 3) using 100 µM 2-APB as the activator. As expected[4], RR blocked the inward current with an IC_{50} of 2.4 ± 0.4 µM (n = 4).

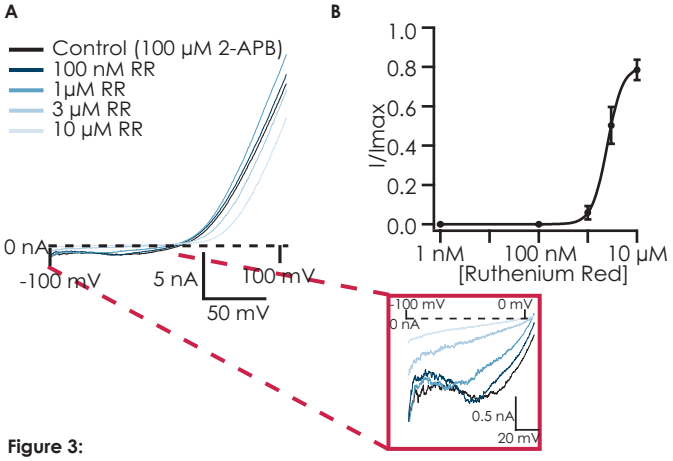

Figure 3:
TRPV3 block of 2-APB response by RR. RR at increasing concentrations (0.1 µM - 10 µM) was pre-incubated and then co-applied with 100 µM 2-APB. As expected[4], inhibition was seen at negative potentials (as seen in inset) whereas little block was seen at positive potentials at these concentrations. **B** Concentration response curve of the inward current (at -50 mV) revealed an IC_{50} = 2.4 ± 0.4 µM (n = 4).

In summary, TRPV3 receptors stably expressed in HEK293 cells can be reliably activated by moderate (≥38°C) to hot temperatures (up to 54°C), or by 2-APB in a concentration-dependent manner. The data shown here agrees well with published literature using conventional patch clamp electrophysiology to study TRPV3[1-5]. Therefore, the Patchliner provides a viable, higher throughput alternative to conventional patch clamp for the discovery of active TRPV3 lead compounds with a suitable drug profile.

References

1. Peier *et al.*, 2002. Science. 296: 2046-2049
2. Xu *et al.*, 2002. Nature. 418: 181-186
3. Smith *et al.*, 2002. Nature. 418: 186-190
4. Chung *et al.*, 2004. J. Neurosci. 24: 5177-5182
5. Hu *et al.*, 2009. PNAS. 106: 1626-1631

Methods

Cells

PrecisION hTRPV3- HEK recombinant cell line (CYL3065) was kindly supplied by EMD Millipore, 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 Patchliner. Currents were elicited by 200 ms voltage ramps from -100 mV to +100 mV, V_{hold} = -20 mV. For heat activation of channels, 100 µl of external solution was heated in the pipette until desired temperature was reached (within 30 s) and rapidly applied to the cell at a speed of 10 µl/s (N.B. a control application of 50 µM 2-APB at room temperature was performed at the start to ensure expression of TRPV3 in each cell prior to heat application). 2-APB was diluted in external solution at the indicated concentrations and applied at room temperature for approximately 35 s before wash with external solution. RR was diluted in external solution at the indicated concentrations and pre-incubated for at least 30 s before co-application with 100 µM 2-APB.

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