Heat activation of TRPV3 on Nanion’s Patchliner®

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

Summary

The transient receptor potential cation channel, subfamily V, member 3 (TRPV3), is a ligand-gated, non-selective cation channel first described in 2002. It exhibits 43% sequence identity to TRPV1. Although TRPV3 has been detected immunologically in the CNS and suggested to be often co-localized with TRPV1, it is found more robustly in keratinocytes in skin and, given its threshold for temperature activation of >34°C, it has been speculated that TRPV3 may act in cooperation with sensory afferents to perceive warmth and signal elevated temperature. TRPV3 can also be activated by the ligand 2-Aminoethoxydiphenyl borate (2-APB). 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 or heat, 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. 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.

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 EC50 for 2-APB activation of 61.1 ± 7.6 µM (n = 11), in good agreement with the literature. Interestingly, as previously reported, 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, EC50 = 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).
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. 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.

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. 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**

5. Hu et al., 2009. PNAS. 106: 1626-1631

**Methods**

**Cells**

HEK293 cells stably expressing hTRPV3 were used.

**Cell culture**

Cells were cultured and harvested according to Nanion’s standard cell culture protocol.