Application Note

Channel: mTRPM7
Cells: HEK293
Tools: Port-a-Patch®

TRPM7 activation by internal sequestering of Mg^{2+} ions recorded on the Port-a-Patch®

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Cells were kindly provided by V. Chubanov & T. Gudermann, LMU, Munich

Summary

Transient receptor potential (TRP) channels are an important class of receptors found widely distributed throughout the mammalian central and peripheral nervous systems. They have been shown to be activated by many stimuli including temperature, mechano-stimulation, divalent cations and pH, amongst others. TRP channels are receiving much attention as potential targets for the treatment of, for example, pain, respiratory diseases such as asthma, cancer and immune disorders (for review see ref. 1).

The TRPM7 receptor is thought to play a role in magnesium homeostasis[1,2,3]. A role for TRPM7 in intracellular pH sensing[4], the pathological response to blood vessel wall injury[5] and cell adhesion[6] has also been suggested. In electrophysiological studies TRPM7 can be recorded using a voltage ramp protocol. It displays a characteristic large outward current with little inward current and can be blocked by the presence of internal Mg^{2+} ions[1,3,7].

Here we present data recorded on the Port-a-Patch® with internal perfusion showing recordings of mouse TRPM7 (mTRPM7) and block of this channel by the internal perfusion of Mg^{2+} ions.

Results

Current responses of a cell induced to express mTRPM7 are shown in Figure 1. Currents were elicited using a voltage ramp from -100 mV to +100 mV over 200 ms. When Mg^{2+} ions were sequestered in the internal solution using internal EGTA or BAPTA, some run-up of the current could be seen at the start of the recording (consistent with the literature[3]) and the recording was stable for several minutes. The shape of the ramp, with little inward current but a large outward current is in good agreement with the literature[1,3,7].

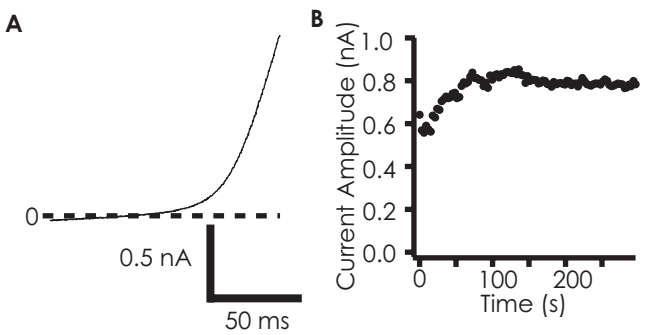

Figure 1:
The mTRPM7 current could be stably recorded on the Port-a-Patch®. Cells were induced to express the mTRPM7 receptor using ponasterone and were recorded using a voltage ramp from -100 to +100 mV over 200 ms (**A**). After a small run-up of the current after whole cell access, the current was stable for almost 5 minutes (**B**).

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Figure 2 shows the current response of a cell induced to express mTRPM7 and block of this current upon internal perfusion of Mg^{2+} ions.

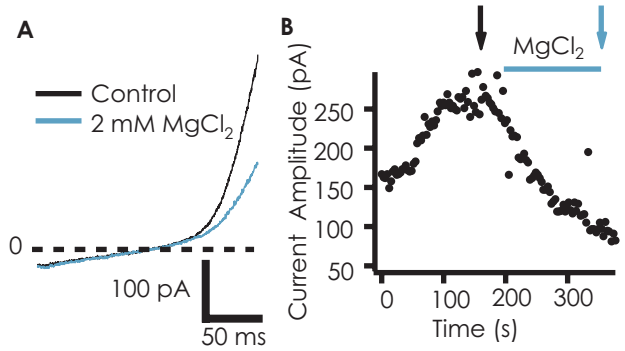

Figure 2:
The mTRPM7 current could be blocked by internal Mg^{2+} ions. **A** mTRPM7 current was recorded using a voltage ramp protocol. After some run-up in the beginning the recording was stable. Using the internal perfusion system from Nanion, Mg^{2+} ions could be perfused in the internal solution and this blocked the current. The arrows shown in the timecourse (**B**) indicate the time points at which the traces were taken for Panel A.

In summary, it is possible to record the TRPM7 current on the Port-a-Patch®. What is more, using the internal perfusion system for the Port-a-Patch®, TRPM7 currents could be blocked by internal perfusion of Mg^{2+} ions. The ease with which the internal solution can be exchanged on the Port-a-Patch® provides a distinct advantage over conventional patch clamp set-ups, where the exchange of the internal solution is somewhat difficult, due to the dimensions of the pipette.

TRPM7 currents could be activated by sequestering Mg^{2+} ions on the inside. In this way, pharmacological agents for activation or inhibition of the TRPM7 channel perfused either internally or externally could be investigated. This may provide further insight into the physiological and pathological roles of TRPM7, as well as the identification of novel agents for activation and antagonism of the TRPM7 channel.

References

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Methods

Cells

HEK293 cells containing an inducible mTRPM7 channel were used.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol. Cells were grown on the dishes for 1 - 3 days and then media was exchanged for media containing 5 µM ponasterone for 24 - 48 hours to induce mTRPM7 expression (as described in ref. 3).

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch®. The internal perfusion system was also used. Currents were elicited every 3 s by 200 ms voltage ramps from -100 mV to +100 mV. Currents were activated or blocked using EGTA/BAPTA or Mg^{2+} ions internally, respectively.

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