Application Note

New frontier: Investigating regulation of lysosomal ion channels at elevated temperatures in the native system

Ion Channels:
TRP ion channels
TMEM175
Cells:
Human LYSO-Preps™ (lysosomes isolated from HEK293 cells)
Tools:
SyncroPatch 384

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Summary

Ion channel activity is modulated by various stimuli including voltage, pH, lipids or neurotransmitters that turn various inputs into electrical signals. In addition, the activity can also be modulated by temperature with the example of the transient receptor potential (TRP) family of ion channels that show a remarkably high sensitivity to changes in temperature and mediate temperature-sensation in animals [1-4].

While temperature dependence has been extensively characterized for ion channels in the plasma membrane, the thermal sensitivity of organellar ion channels remains largely unexplored, mostly due to significant technical barriers, recordings have been restricted to room temperature, potentially masking critical physiological behaviors.

We have recently established high throughput automated patch clamp recordings in collaboration with Oria Bioscience providing highly pure populations of large individual organelles [5-7]. We have now used this approach to characterize isolated lysosomes at room (21°C) and elevated recording temperatures (35°C) with overexpression of transmembrane protein 175 (TMEM175), an endosomal and lysosomal cation channel associated with neurodegenerative disorders, including Parkinson's disease [8]. At 35°C we observed higher basal and agonist induced current densities for TMEM175 in our experiments, whilst apparent affinity (EC_{50}) of the DCPIB/TUG-891 mixture remained unchanged between the two conditions. The results suggest that the recording temperature could be a crucial factor in the dynamic regulation of lysosomal electrophysiology relevant for ion channels, transporters and pumps.

Results

TMEM175 currents were successfully acquired from lysosomes in whole-lysosome configuration with intraluminal pH 7.2 using the SyncroPatch 384. Figure 1A,B show representative recordings at 21°C and 35°C where TMEM175 currents were activated by adding a mixture of the specific activators DCPIB and TUG-891 to the cytoplasmic solution [8]. Subsequently, application of 4-AP lead to full block of the current, indicating that the current is indeed mediated by TMEM175 at both temperatures. We could confirm that capacitance values were not affected by

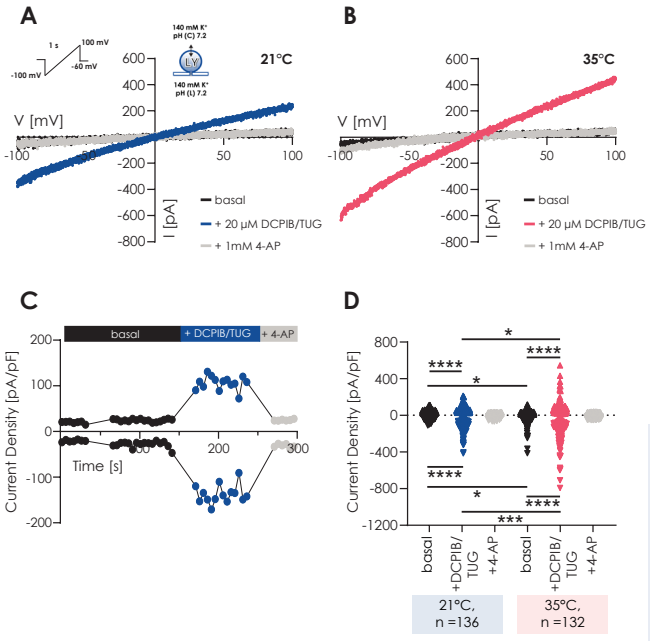

Figure 1: A *Representative current trace from a lysosome isolated from HEK293 cells overexpressing TMEM175, enlarged with Vacuolin, showing activation by DCPIB/TUG-891 and subsequent block by 4-AP at 21°C.* **B** *Corresponding recording at 35°C.* **C** *I–T plot of currents at 100 mV and -100 mV from a single lysosome illustrating activation by DCPIB/TUG-891 and subsequent inhibition by 4-AP at 21°C.* **D** *Comparison of current densities at both voltages at 21°C and 35°C before and after DCPIB/TUG-891 application and after 4-AP block.*

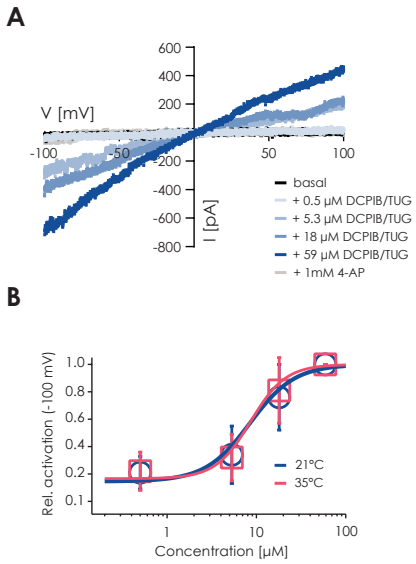

Figure 2: A *Representative current traces from a lysosome isolated from a HEK293 cell overexpressing TMEM175, showing activation by increasing concentrations of a DCPIB/TUG-891 mixture and subsequent block by 4-AP at 21°C.* **B** *Concentration–response analysis from experiments as in A at −100 mV, comparing recordings at 21°C and 35°C, yielding EC_{50} (± SD) values of 8.6 ± 5.8 (n = 50) and 8.7 ± 6.6 (n = 38), respectively.*

elevated temperature with values of 2.5 ± 0.8 pF at 21°C and 2.6 ± 0.9 pF at 35°C, verifying recording stability. Activation with DCPIB and TUG-891 at both temperatures increased inward and outward current density at -100 mV and 100 mV, respectively. Subsequent application of 4-AP induced current block at both voltages (Figure 1C). Basal and agonist induced current density was significantly higher at 35°C vs 21°C suggesting that recording temperature affects TMEM175 channel activity (Figure 1D). Agonist induced effects were also concentration dependent (Figure 2A). Interestingly, following analysis of cumulative 4-pt dose response curves, we could confirm that the apparent affinity of DCPIB/TUG891 was unchanged between both temperatures. Channel activation was comparable with an EC_{50} of 8.6 ± 5.8 µM (n = 50) at 21°C and 8.7 ± 6.6 µM (n = 38) at 35°C (Figure 2B).

Conclusions

To our knowledge, this is the first report of TMEM175 recordings at 35°C in isolated native lysosomes. The results clearly demonstrate the advantage and the broad range of applications enabled by the SyncroPatch 384 combining high-throughput automated lysosomal patch clamp with controlled elevated temperatures opening up new possibilities for studying intracellular ion channels under more physiological conditions.

Methods

Experimental setup

Human LYSO-Preps™ (Ready-to-use enlarged lysosomes) isolated from HEK cells overexpressing TMEM175 channels were provided by Oria Bioscience. Lysosomal currents were recorded using NPC-384T nanoS-type (1x; PN: 22 2111) consumables on the SyncroPatch 384. Human LYSO-Preps™ yield populations of $2x10^7$ lysosomes per sample that were diluted to a density of 200k lysosomes per ml. This was sufficient to execute several full chips (> 5) sequentially on the SyncroPatch 384. We implemented the low cell (lysosome) density approach, using fewer lysosomes and a reduced volume while maintaining a high catch rate. This method allowed us to achieve stable lysosomal seals in 'whole-lysosome' mode, with seal resistances consistently exceeding 0.5 Giga Ohms throughout the experiments.

References

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