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Automated Patch Clamp assay for proton-activated HOTOP1 channel

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Introduction

Manual patch clamp is the gold standard for ion channel studies but is low-throughput and labor-intensive. Automated patch clamp (APC) systems, like the 8-well QPatch Compact (QPC) with rapid solution exchange capabilities, overcome these limitations, enabling efficient study of channels. Central to the QPC's functionality is a microfluidic flow channel that ensures precise and controlled delivery of solutions. This microfluidic design facilitates complete and rapid solution exchange around the cell, a critical feature for accurately studying channels activated by transient stimuli such as protons. OTOP1 is a proton-selective ion channel crucial for acid sensing in tissues like the inner ear and taste buds, acting as a sour taste receptor and ammonium sensor. Characterizing OTOP1's biophysical properties is vital to understand its physiological roles and therapeutic potential.

Conclusion

This study demonstrates the successful implementation of an automated patch clamp assay using the QPatch Compact system to characterize proton-activated hOTOP1 channels. The system enabled rapid and reproducible solution exchange, critical for studying transient proton-evoked responses. Our results confirm that hOTOP1 mediates inward currents in response to acidic pH and ammonium, and that these currents are modulated by extracellular zinc in a concentration-dependent manner. The fitted IC_{50} for Zn^{2+} inhibition aligns with previous findings, validating the assay's reliability. This automated approach offers a scalable and efficient platform for future pharmacological screening and mechanistic studies of proton-sensitive ion channels.

Materials and methods

Automated Patch Clamp: All data were recorded using the QPatch Compact System (Sophion Bioscience A/S). All experiments were conducted using QPlate8X consumables.

Cell preparation: HEK293 cells stably expressing human OTOP1 channels were harvested either using detachin or trypsin. Cells were transferred to serum-free medium (EX-CELL® ACF CHO Medium, Sigma-Aldrich) supplemented with 25 mM HEPES. The cells were washed and resuspended in extracellular buffer immediately before the experiment.

Solutions: Extracellular solution used for priming (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, pH 7.4.

During the experiment, NMDG-based extracellular solutions consisted of (in mM): 160 NMDG, 2 CaCl2, 10 CHES (N-cyclohexyl-2-aminoethanesulfonic acid) for pH 10 / 10 HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid) for pH 7.4 / 10 MES (2-Morpholinoethanesulphonic acid) for pH 6.0 / 10 or 100 Homo-PIPES (homopiperazine-1,4-bis(2-ethanesulfonic acid)) for pH 5.0 and 4.0.

NH_4Cl solution consisted of (in mM): 160 NH_4Cl, 2 $CaCl_2$, 10 HEPES.

pH of solutions was adjusted with NMDG-OH and HCl.

Intracellular solution (in mM): 140 CsF, 1/5 EGTA/CsOH, 10 HEPES, 10 NaCl, pH 7.3

Whole-cell protocol: The whole-cell configuration was established using a standard whole-cell protocol for CHO-cells.

Voltage protocol: A constant holding potential V=-80 mV was applied unless noted otherwise.

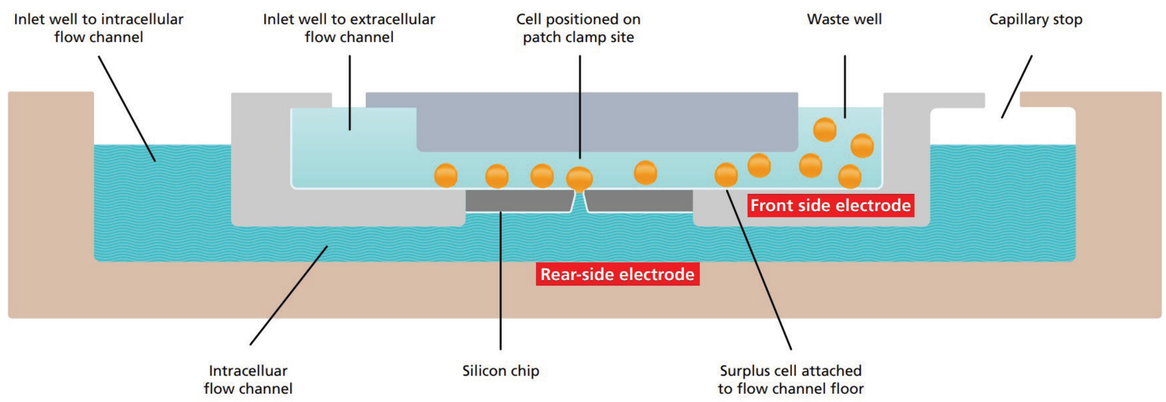

Fig. 1: Schematic representation of a microfluidic flow channel. A cell suspension is injected into the external microfluidic channel, while negative pressure is applied to capture a single cell at the patch hole. A controlled pressure sequence then ruptures the membrane patch at this site, establishing stable electrical access to the cell. Each consumable is equipped with built-in Ag/AgCl electrodes, ensuring precise and reproducible current and voltage recordings.

Results

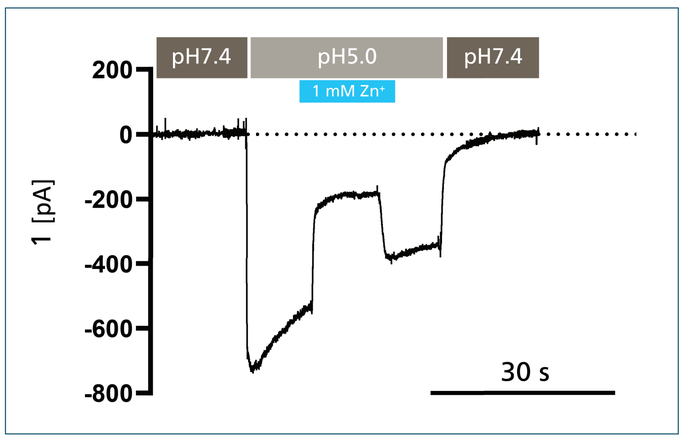

Fig. 2: Acidic pH evokes an inward current inhibited by zinc in HEK293-hOTOP1 cells. Representative current trace recorded from HEK293-hOTOP1 cells. External solution was rapidly exchanged from pH7.4 to pH5.0 using QPC`s microfluidic flow channel. Subsequent application of 1 mM Zn^{2+} resulted in a significant reduction of the acid-evoked current. Wash out of Zn^{2+} recovered most of the acid-activated current.

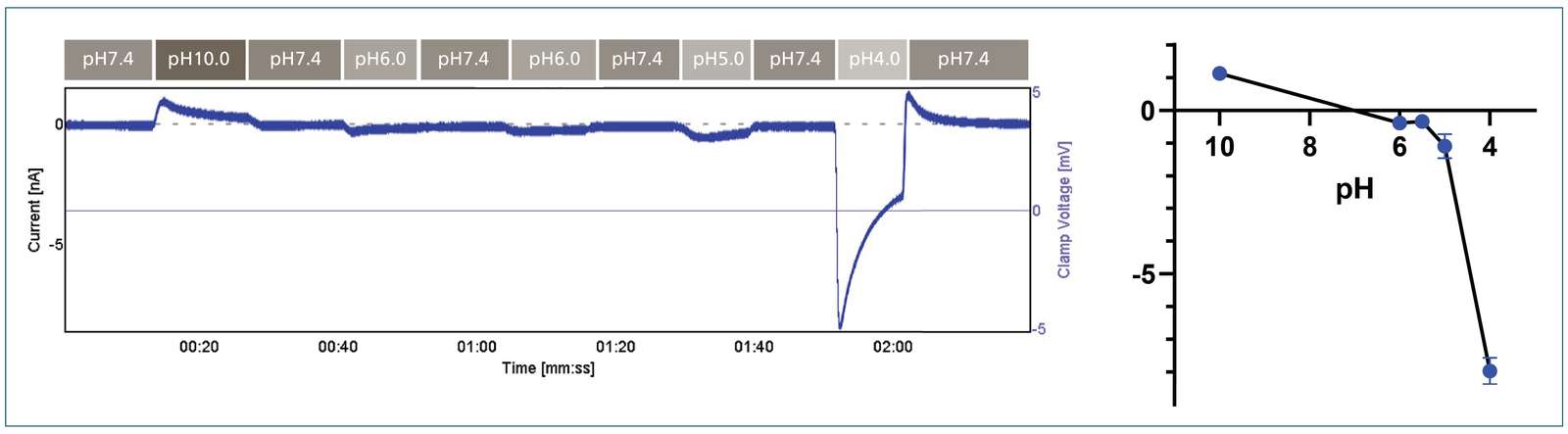

Fig. 3: pH-dependence of hOTOP1 current in HEK293 Cells. Representative current trace **(left)** and average pH-response plot **(right)** illustrating hOTOP1 activity. Cells were voltage-clamped at Vm=0 mV to reduce proton driving force. External solutions with pH values ranging from 10.0 to 4.0 were applied to stimulate the cells. The bottom panel shows the average peak current responses (N=5 cells, mean ± SEM) across various pH stimulations.

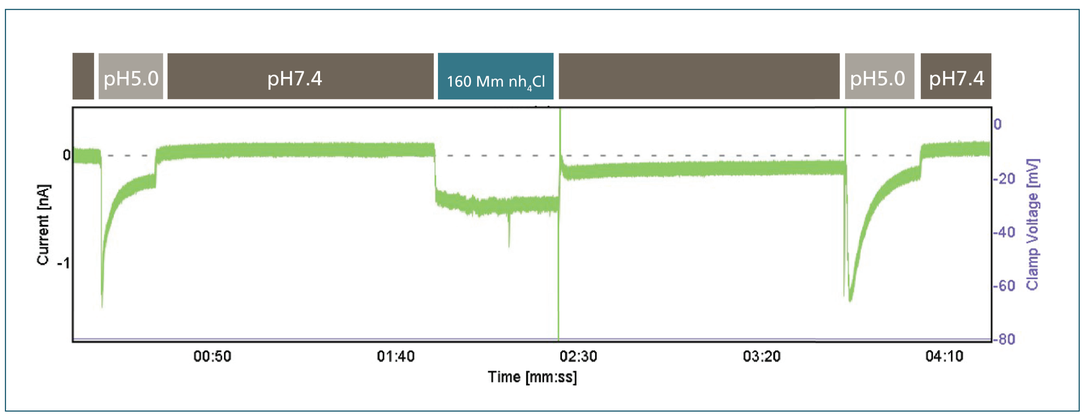

Fig. 4: hOTOP1 mediates ammonium-evoked inward currents. Representative current trace demonstrating the electrophysiological response of hOTOP1-expressing cells to 160 mM NH_4Cl. Prior to and following NH_4Cl stimulation, cells were challenged with a pH 5.0 buffer solution to confirm hOTOP1 functional expression. This data corroborates the initial findings from the Liman lab, which first identified hOTOP1 as the molecular entity responsible for mediating ammoniumevoked inward currents (Liang et al., 2023, *Nature Communications*).

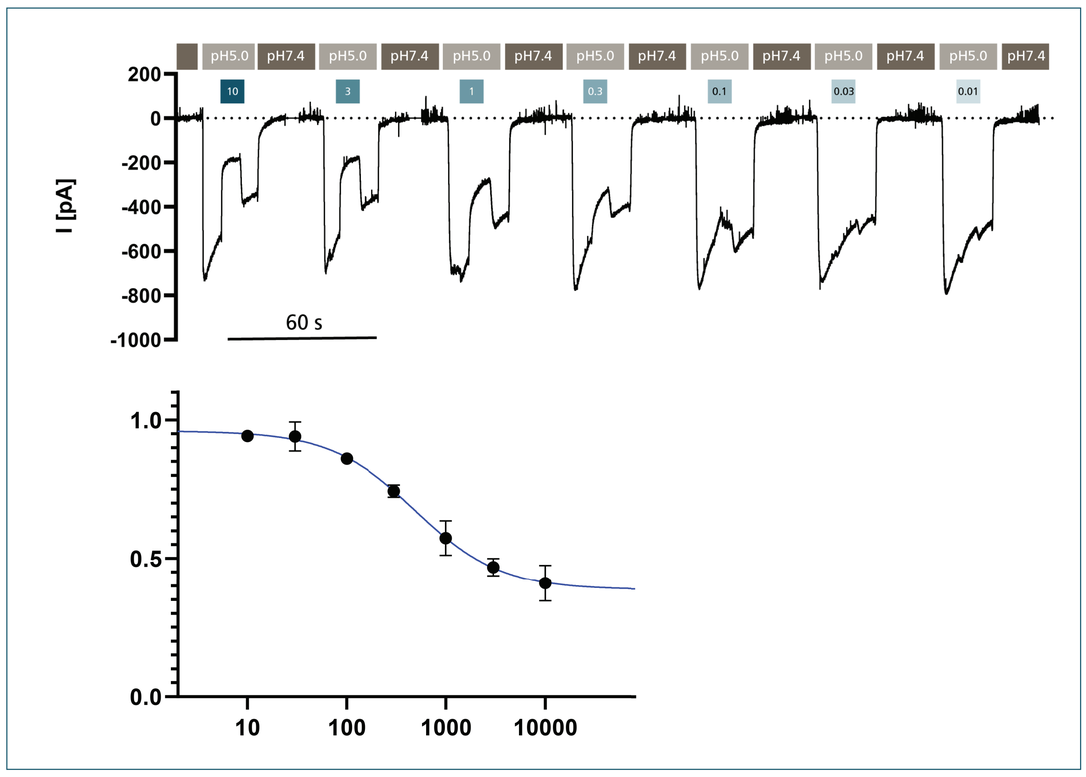

Fig. 5: Concentration-dependent inhibition of hOTOP1 by zinc. (Top) Representative whole-cell current trace demonstrating the inhibitory effect of varying Zn^{2+} concentrations on acid-evoked hOTOP1 currents. Currents were activated by an external solution at pH5.0. Effect of Zn^{2+} was assessed by washing in varying concentrations of Zn^{+} during the pH5.0 stimulation pulse followed by a wash out step. This protocol was repeated for seven different Zn^{2+} concentrations (100 nM to 10 µM). **(Bottom)** Average concentration-response curve (N=6 cells; mean ± SEM) forZn^{2+} inhibition of hOTOP1. Residual current was calculated as the ratio of current immediately prior to Zn^{2+} washout to the current immediately after washout. The fitted IC_{50} for Zn^{2+} was 489 µM (95% CI: 364 to 695 µM).

References

1. Liang, Z., Wilson, C. E., Teng, B., Kinnamon, S. C., & Liman, E. R. (2023). *The proton channel OTOP1 is a sensor for the taste of ammonium chloride*. **Nature Communications, 14,** Article 6163. https://doi.org/10.1038/s41467-023-41637-4

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