

Evidence for Modulation of the Sour Taste Receptor OTOP1 by Compounds Associated with Licorice Aftertaste

Manuel Arcangeletti¹, Irene Riva¹, Menella Valotta¹, Katja Blasi¹, Marcel Winnig¹, Anna Mondini¹, Jean-Francois Rolland¹

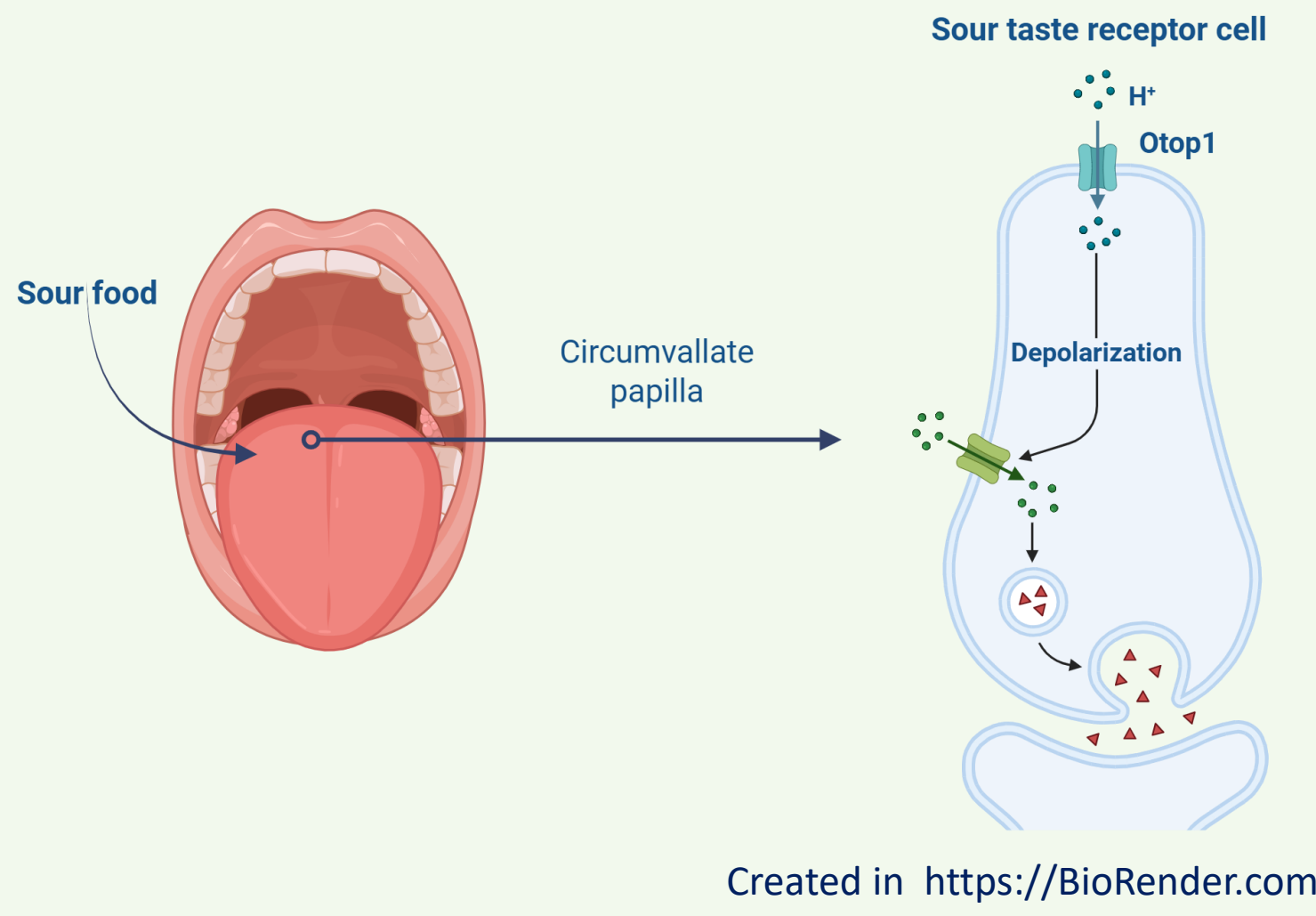
¹Axxam S.p.A. OpenZone, Via Meucci 3, 20091 Bresso (Milan), Italy

Introduction

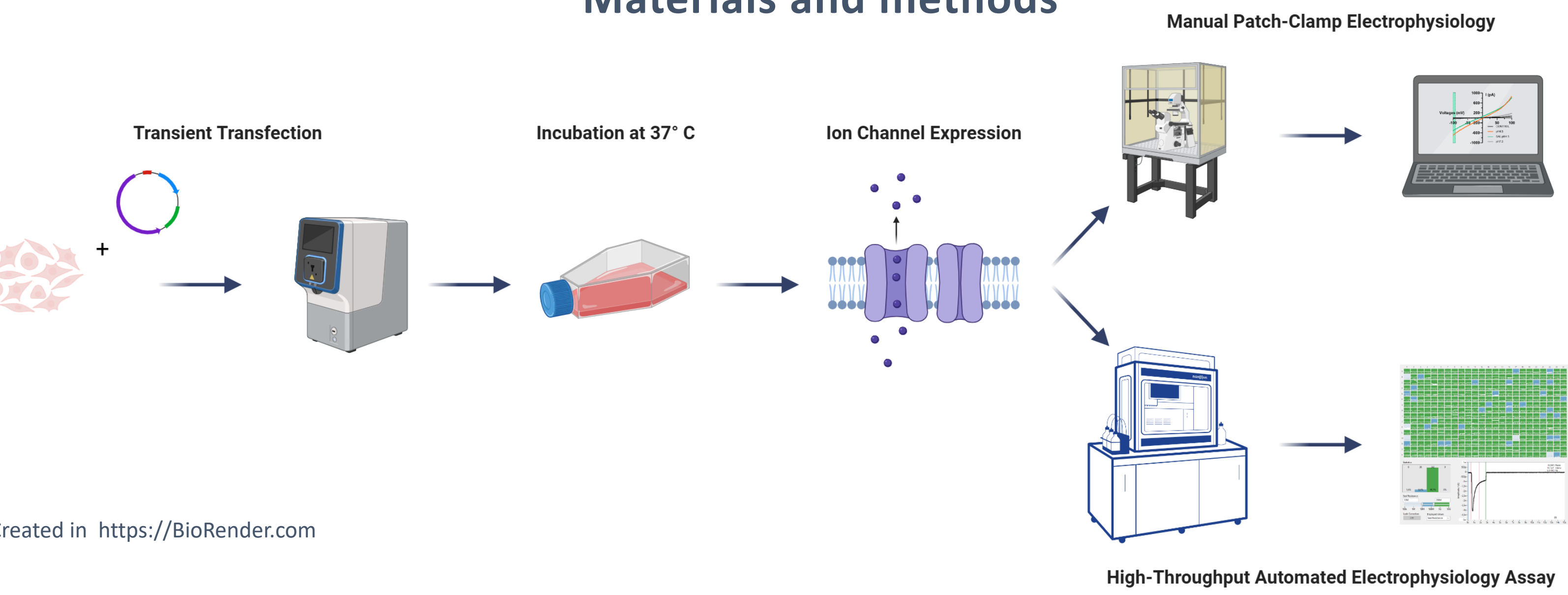
OTOP1 a proton-selective ion channel expressed in taste receptor cells has been identified as a critical molecular sensor for sour taste (Tu et al., 2018; Sladkov & Kolesnikov, 2024). In addition, OTOP1 has recently been reported to act as a sensor for ammonium chloride (NH₄Cl), a tastant described as bitter, salty, and mildly sour, which is a key component of salty licorice (Zhang et al., 2023). The present study illustrates the development of a reliable high throughput automated electrophysiological assay to investigate OTOP1 function and modulation. Human OTOP1 was transiently expressed in CHO cells using MaxCyte electroporation, and recording conditions were optimized on the SyncroPatch 384 platform to achieve stable and reproducible measurements of proton-mediated currents. We then tested a panel of sweet-tasting compounds, selected based on their association with a licorice-like aftertaste. Several compounds exhibited inhibitory effects on OTOP1-mediated proton currents at the SyncroPatch 384. To validate these findings, three representative compounds (one strongly associated with licorice aftertaste, one showing weaker activity and one negative control) were further examined using the golden standard method for electrophysiology i.e. manual patch-clamp confirming the effects observed in the automated assay. Together, these findings establish a robust assay for studying OTOP1 pharmacology and provide evidence that sweet-associated compounds can modulate a key taste receptor involved in sour and ammonium taste, offering new insight into molecular mechanisms underlying complex taste perceptions.

Summary

- OTOP1 is a proton-selective ion channel involved in sour taste and ammonium detection.
- There is a need for robust, high-throughput assays to study taste-related ion channels like OTOP1. This study illustrates the development of an automated electrophysiology assay using the SyncroPatch 384 platform.
- Human OTOP1 was transiently expressed in HEK and CHO cells, and recording conditions were optimized for stable, reproducible proton currents.
- A panel of sweet-tasting, licorice-like compounds was tested and found to inhibit OTOP1-mediated currents.
- Manual patch-clamp validation confirmed the automated assay results. The work establishes a scalable platform for OTOP1 pharmacology and shows that certain sweet compounds can modulate taste-related ion channel activity.

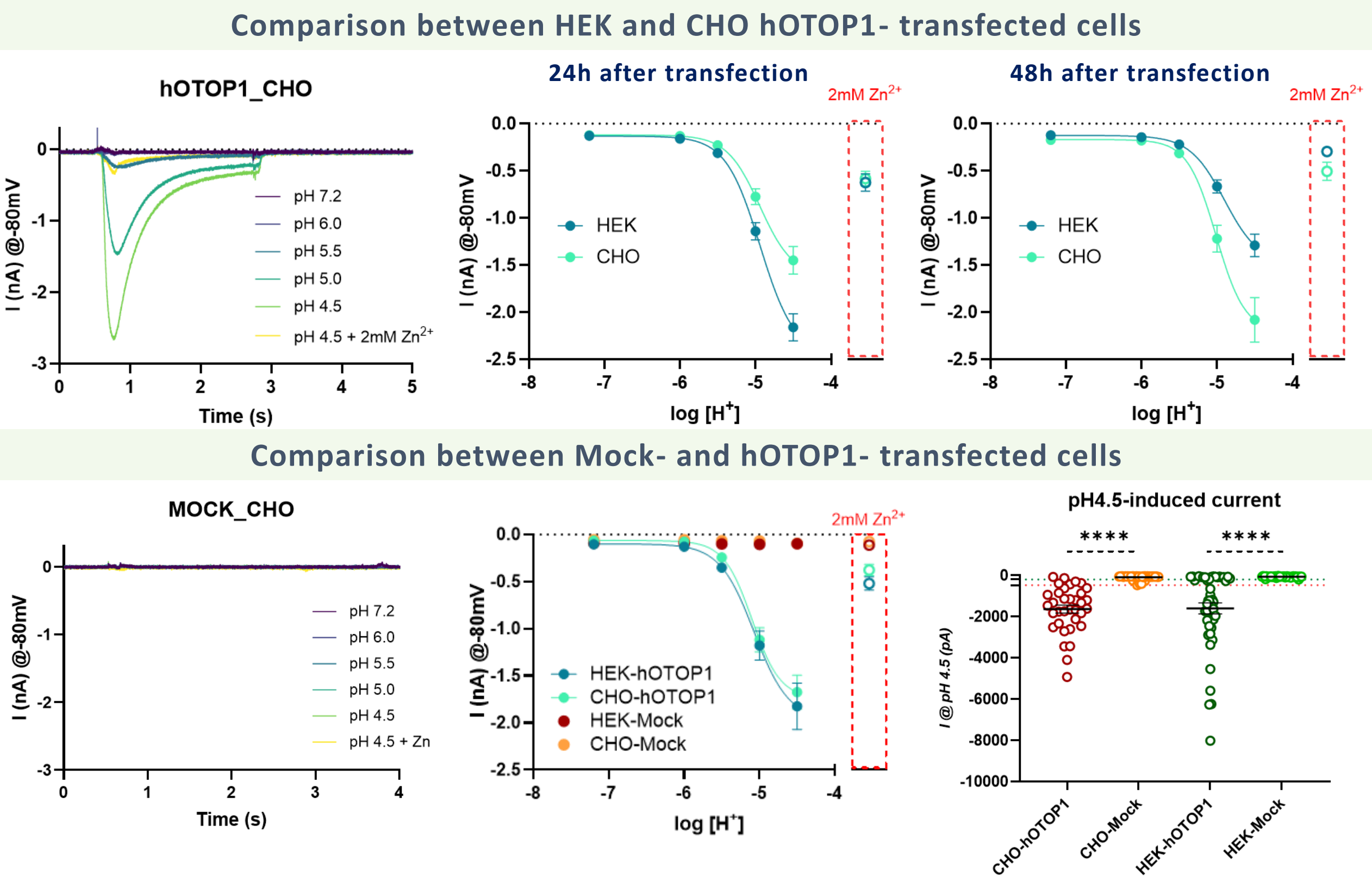


Materials and methods



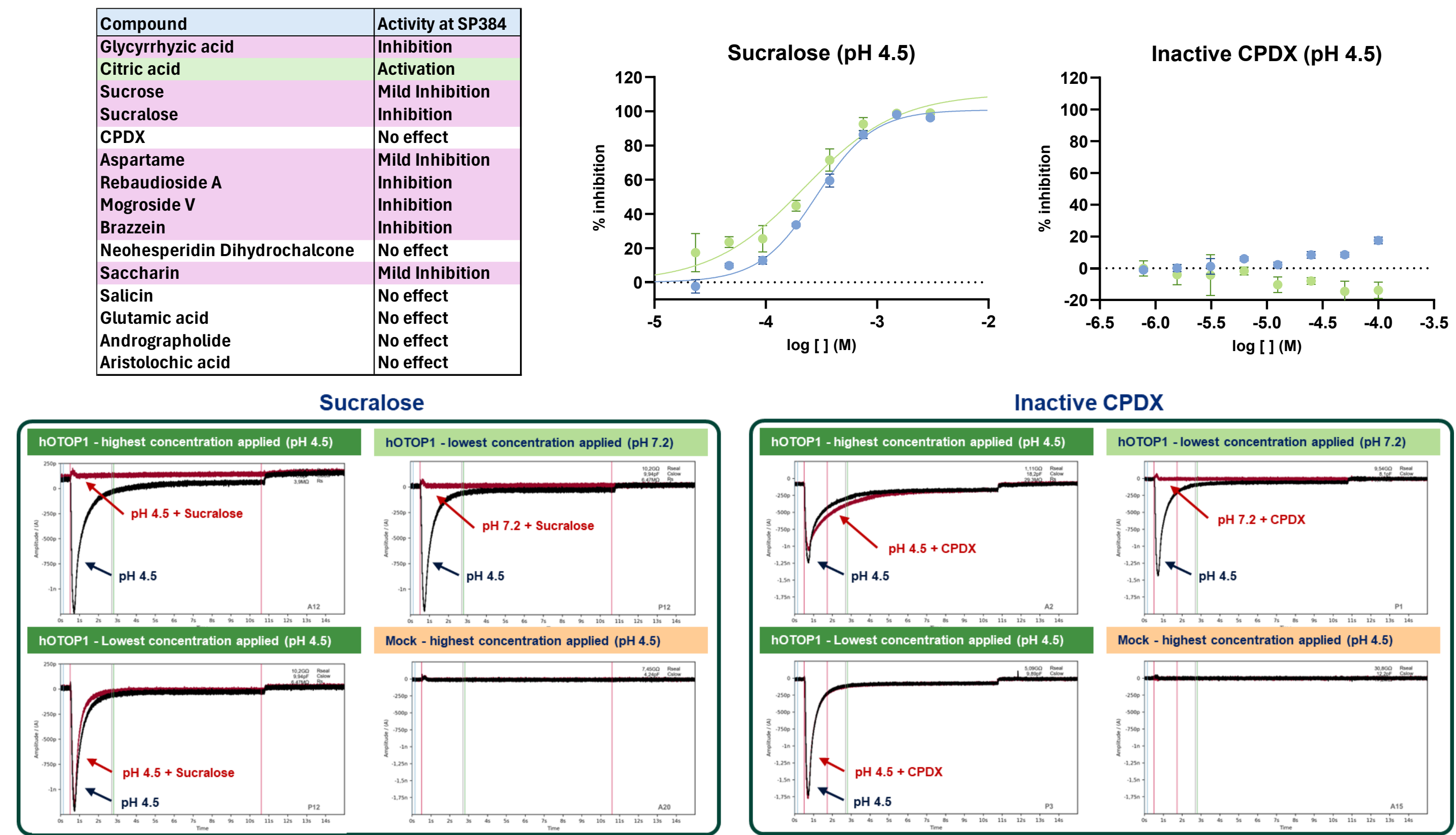
- HEK-293 and CHO cells were transiently transfected with plasmids encoding human OTOP1 (hOTOP1) or an empty plasmid (mock) using MaxCyte electroporation technology.
- Following transfection, cells were incubated for 24 or 48 hours to allow expression and membrane localization of the ion channel. Functional activity was then assessed using either a high-throughput automated electrophysiology platform or manual patch-clamp recordings.

Development of a high-throughput automated electrophysiology assay to investigate OTOP1 activity



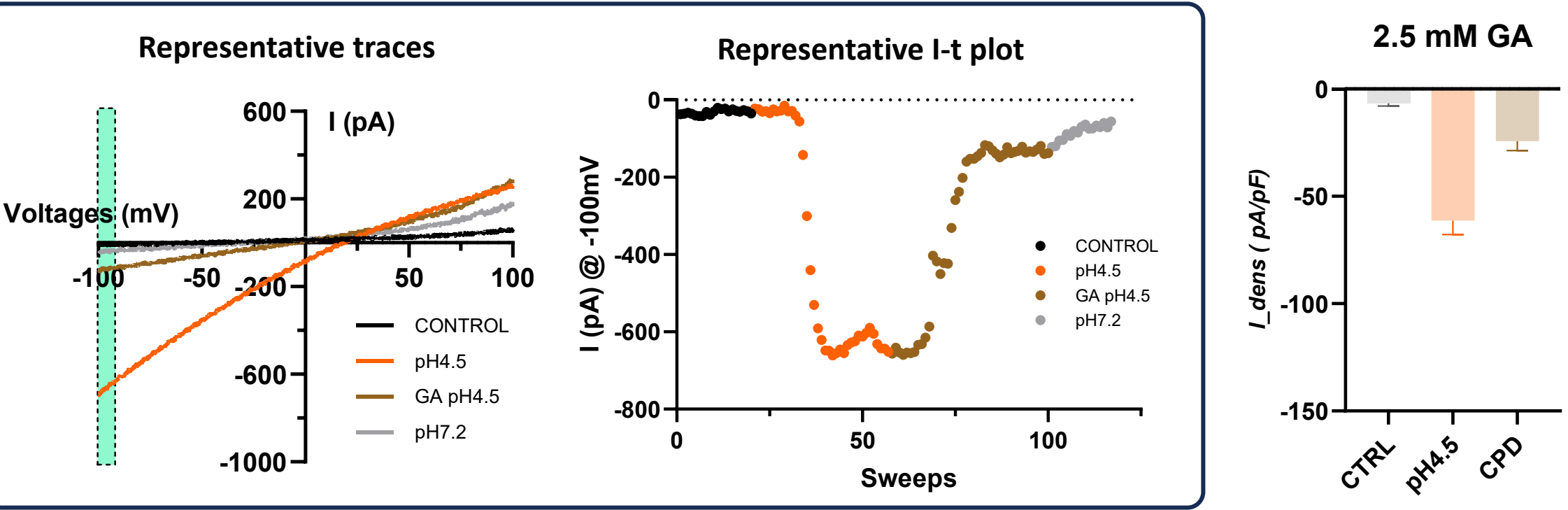
- A high-throughput electrophysiology assay for transiently expressed hOTOP1 was established using the SyncroPatch platform.
- Dose-response (D-R) curves to extracellular pH were generated in both HEK-293 and CHO cells at 24h (upper panel, middle) and 48h post-transfection (upper panel, right).
- Comparable proton currents were observed across both cell lines and time points, indicating consistent assay performance.
- The 48h time point was selected for subsequent experiments due to improved cell recovery post-transfection and overall recording stability.
- CHO cells transfected with hOTOP1 displayed robust proton currents, whereas mock-transfected CHO cells showed no detectable response.
- These results confirm that the measured proton currents are specific to hOTOP1 expression.

Evaluation of a panel of sweet-tasting compounds associated with a licorice-like aftertaste



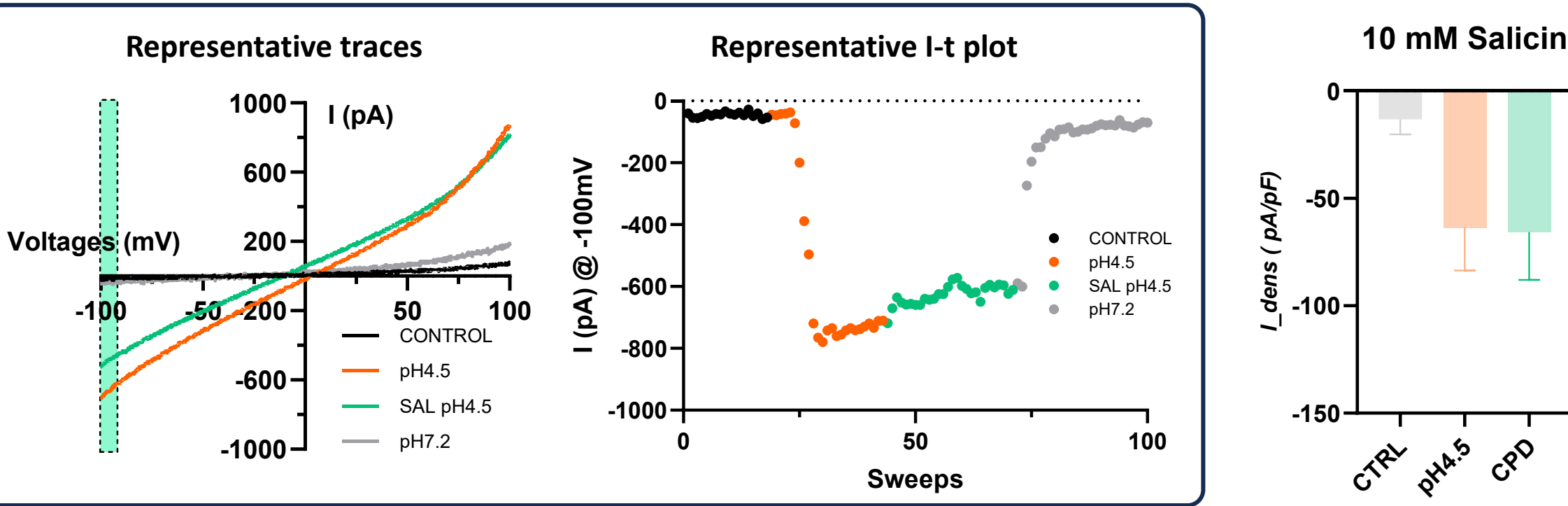
- Fifteen different compounds were evaluated using SyncroPatch384 at both pH 7.2 and pH 4.5 to investigate their effects on channel activity.
- Only Citric acid was found to activate proton current at pH 7.2 (data not shown), while several compounds induced an inhibition in the channel activity (such as sucralose).
- Six compounds did not show any effect on hOTOP1 at the concentration tested (such as CPDX)
- Mock cells, which do not express the channel under investigation, were included as controls to rule out non-specific effects or artifacts.

Glycyrrhizic acid (GA)



- Three compounds were also tested at Manual Patch.
- GA (2.5mM) and Sucralose (not shown) significantly reduced hOTOP1-specific proton current.

Salicin



- In contrast, Salicin (10mM) did not produce any detectable effect on hOTOP1-mediated proton currents.
- These results confirmed what observed at SyncroPatch.

- ## Conclusions

 - A high-throughput electrophysiology assay for transiently expressed hOTOP1 was established using the SyncroPatch 384 platform, and D-R curves to extracellular pH were generated.
 - Sweet-tasting compounds associated with a licorice-like after taste were evaluated using SyncroPatch384 at both pH 7.2 and pH 4.5 to investigate their effects on channel activity. Interestingly, several of them induced an inhibition in channel activity. Citric acid was the only compound tested, showing hOTOP1 activation.
 - Three compounds were tested at manual patch and the results confirmed what observed at the SyncroPatch 384.
- Tu YH, Cooper AJ, Teng B, Chang RB, Artiga DJ, Turner HN, Mulhall EM, Ye W, Smith AD, Liman ER. An evolutionarily conserved gene family encodes proton-selective ion channels. *Science*. 2018 Mar 2;359(6379):1047-1050. doi: 10.1126/science.aao3264. Epub 2018 Jan 25. PMID: 29371428; PMCID: PMC5845439.
 - Sladkov, K.D., Kolesnikov, S.S. Structure and Functions of the OTOP1 Proton Channel. *Biochem. Moscow Suppl. Ser. A* 18, 175–187 (2024). <https://doi.org/10.1134/S1990747824700181>.
 - Liang, Z., Wilson, C.E., Teng, B. et al. The proton channel OTOP1 is a sensor for the taste of ammonium chloride. *Nat Commun* 14, 6194 (2023). <https://doi.org/10.1038/s41467-023-41637-4>.

