Case Study

High-Throughput Electrophysiology Solutions for TRPML Drug Discovery

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Introduction

Transient Receptor Potential (TRP) channels are a diverse family of membrane proteins critical for cellular signalling and ion homeostasis. By regulating membrane excitability and intracellular levels of calcium and other ions, TRP channels influence numerous physiological functions. This family is categorized into several subfamilies, including TRPV, TRPM, TRPA, TRPC, and TRPML, each with unique ion selectivity and functional roles.

Among these, the TRPML subfamily, comprising TRPML1, TRPML2, and TRPML3, is distinct for its permeability to Ca^{2+}, Na^{+}, K^{+}, and trace metals such as Fe^{2+}, Mn^{2+} and Zn^{2+}. Unlike other TRP channels, TRPML channels are located in intracellular compartments such as lysosomes and endosomes, where they regulate ion balance and support membrane trafficking. TRPML1, activated by phosphoinositides, is vital for lysosomal calcium release and overall lysosomal function. Mutations in TRPML1 are linked to mucolipidosis type IV (ML-IV), a neurodegenerative disorder characterized by impaired motor skills.

TRPML channels also play significant roles in immune responses and sensory functions. TRPML2 is predominantly expressed in immune tissues, indicating its involvement in immune regulation, while TRPML3 is found in sensory tissues, including the cochlea and eye, highlighting its contribution to sensory processes. Due to their critical functions, TRPML channels are emerging as promising therapeutic targets for lysosome-related neurodegenerative diseases.

Supporting Your Research

SB Drug Discovery's comprehensive TRP channel discovery platform includes meticulously optimized TRP channel cell lines, including TRPML1, TRPML2, and TRPML3, coupled with robust assays designed to support your drug discovery research. Leveraging both 384-well fluorescence-based and electrophysiology platforms for high-throughput screening and lead optimization, SB Drug Discovery enables rapid hit identification and downstream discovery cascades.

TRP Channel Discovery Platform

- **Comprehensive TRP Channel Expertise:** Broad scientific expertise, optimized TRP channel cell lines and assays, facilitating high-throughput screening and lead optimization.
- **Optimized TRPML Assays:** Tightly controlled assays to ensure reproducibility and reliability, aiding TRPML discovery programs.
- **DMTA Support:** SB Drug Discovery's TRP channel platform supports rapid cycles of design-make-test-analyze to expedite TRP channel drug discovery.

Methods

HEK cells stably expressing TRPML1, TRPML2 or TRPML3, were produced by SB Drug Discovery. Whole-cell patch-clamp experiments were carried out at room temperature using multi-whole chips on the Syncropatch 384i automated electrophysiology platform. Currents are elicited by using repeated ramps from -140 mV to +100 mV over 190 ms, from a holding potential of 0 mV. Data analysis are performed using Data Control 384 V3.2.1 (Nanion) and Prism V10.1 (GraphPad).

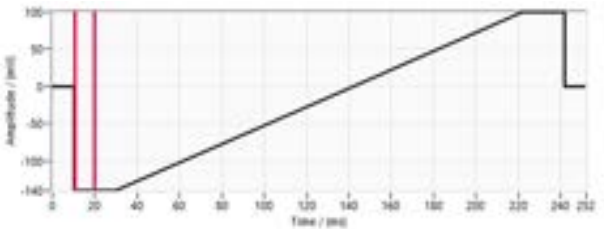

Figure 1. Voltage protocol diagram showing TRPML currents elicited by using repeated voltage ramps, steadily increasing from -140 mV to +100 mV over 190 ms, from a holding potential of 0 mV, repeated every 20 seconds.

Electrophysiology

To validate the TRPML electrophysiology assay and demonstrate its reliability, a series of experiments were conducted using the SyncoPatch 384i automated electrophysiology platform. TRPML1, TRPML2 and TRPML3 channels were assessed on the same electrophysiology plate, with cells displaying stable currents and marked activation/inhibition being classed as useable. The assay achieved a consistently high success rate, with over 80% of cells providing usable data across the three TRPML targets (Figure 2).

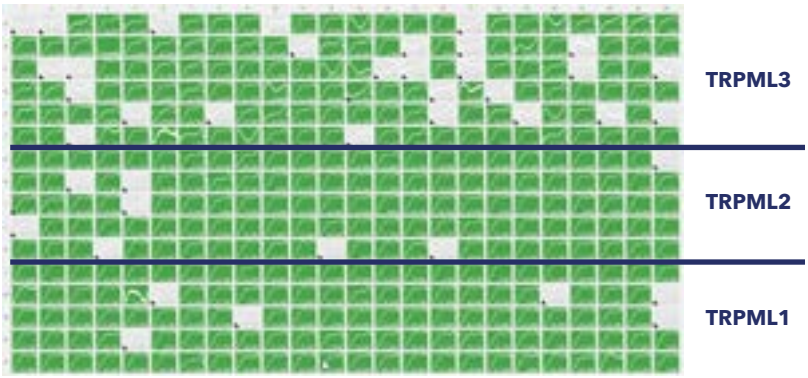

Figure 2. Screenshot from DataControl384 showing TRPML1, TRPML2 and TRPML3 tested within the same experiment. Each green well represents a cell that has successfully completed the experiment and passed the QC criteria. On average, >80% of cells provide useable data for each channel tested.

Subsequent analysis explored the concentration-dependent modulation of TRPML channels using reference agonist ML-SA5. Superimposed current traces revealed a clear dose-dependent effect across a range of concentrations (0.03-3 µM) for all three subtypes (Figure 3).

Comparative pharmacological profiling was conducted to evaluate the selectivity of ML-SA5 across TRPML1, TRPML2 and TRPML3. Consistent with published literature, ML-SA5 EC_{50} values fell between 0.2-0.5 µM for all three subtypes. Additionally, assessment of ML-SA1 and ML-SA5 on TRPML1 provided further insights into agonist activity (Figure 4).

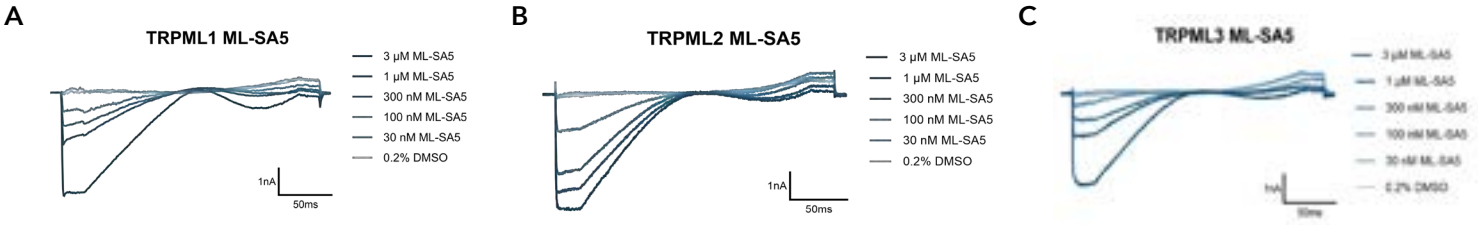

Figure 3. Superimposed current traces showing the effect of increasing concentrations of the agonist, ML-SA5, against TRPML1 (A), TRPML2 (B) and TRPML3 (C).

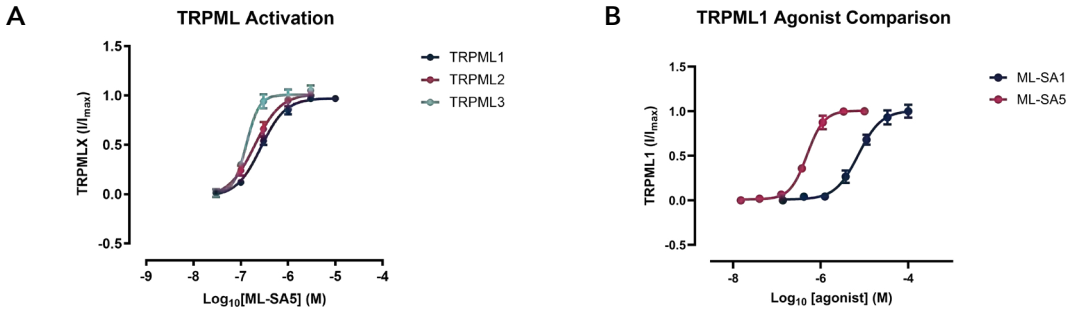

Figure 4. Concentration-response curves comparing the reference agonist ML-SA5 against TRPML1, TRPML2 and TRPML3 channels (A) and comparison of two reference agonists ML-SA1 and ML-SA5 against the TRPML1 channel (B). Data generated using the automated electrophysiology platform.

The reference compound, ML-SI3, has two enantiomers, (1R,2R)-ML-SI3 and (1S,2S)-ML-SI3. From literature, (1R,2R)-ML-SI3 has been reported to inhibit all three TRPML channels, showing greatest effect on TRPML1, with lower affinity for TRPML2 and TRPML3 channels. In contrast, the (1S,2S)-ML-SI3 enantiomer has been shown to activate TRPML2 and TRPML3 channels and inhibit TRPML1. The experiments carried out at SB Drug Discovery confirmed the reported literature (Figures 6 & 7).

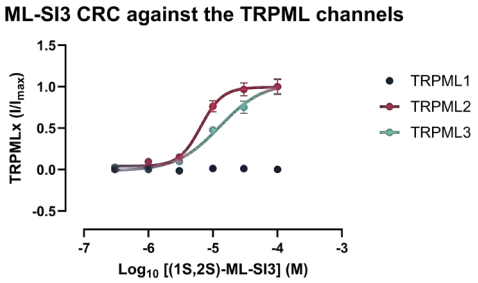

Figure 5. Concentration-response curve of the reference compound, (1S,2S)-ML-SI3, tested in agonist mode, showing a marked concentration-dependent response against the TRPML2 and TRPML3 channels, and no activation on the TRPML1 channel. Data generated using the automated electrophysiology platform.

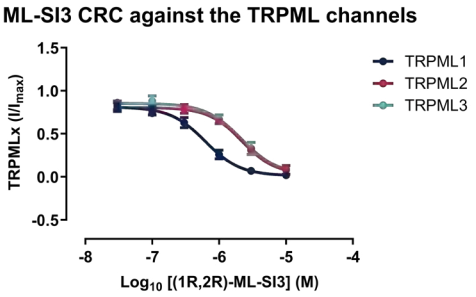

Figure 6. Concentration-response curves of the reference compound, (1R,2R)-ML-SI3, tested in antagonist mode, against the TRPML1, TRPML2 and TPRML3 channels. Data is generated using the automated electrophysiology platform.

Assay reproducibility and performance were assessed across multiple experiments. High success rates and precise EC_{50} determinations for ML-SA5 demonstrated the reliability of the assay (Figure 5).

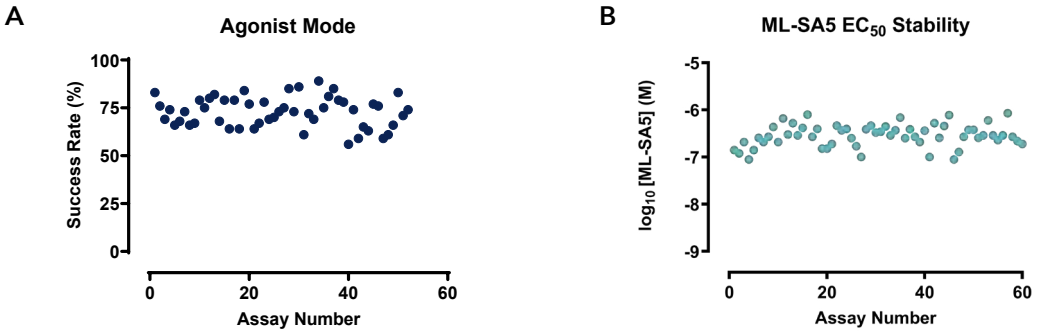

Figure 7. Scatter plots of success rates over multiple assays for agonist mode (average of 73% of cells passing QC) (A) and the $\log_{10} EC_{50}$ values for the reference agonist ML-SA5 (average of -6.55 $\log_{10}$ M) (B). Success criteria include R_{Series} < 20 MΩ, stable currents during the external solution application, marked activation by standard agonist, ML-SA5, and marked inhibition by standard blocker, Lanthanum Chloride.

SB Drug Discovery's TRP channel platform delivers comprehensive solutions for advancing TRPML1, TRPML2 and TRPML3 research. With advanced cell-based assay capabilities and a full suite of technologies, including fluorescence-based assays, solid-supported membrane electrophysiology, manual patch clamp and automated electrophysiology, we provide robust and flexible options to suit diverse project needs. From hit identification through to lead optimization, our platform ensures high-quality, reproducible data to accelerate TRP channel-targeted drug discovery and drive success at every stage of development.

Advance your research with SB Drug Discovery's electrophysiology services. Offering end-to-end ion channel screening capabilities, we deliver the results you need to advance your ion channel drug discovery research.

Contact us today to explore how our Electrophysiology services can accelerate your drug discovery efforts and drive meaningful advancements in therapeutics.

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