Investigating the correlation between thallium flux and automated patch-clamp for ion channel activators

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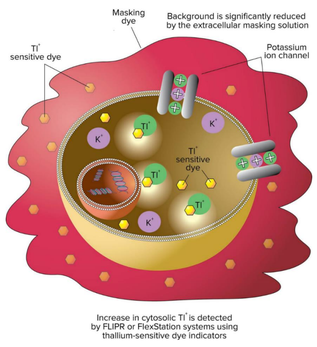
Overview

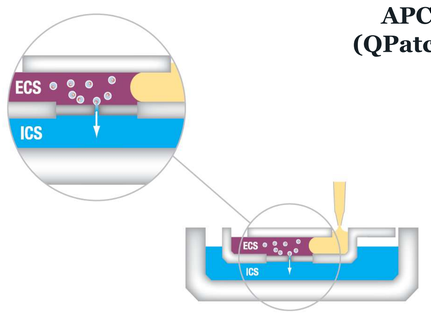
- Ion channels play a key role in regulating resting membrane potential and cell excitability and are attractive targets for therapeutic intervention.
- Thallium (Tl^+) flux assays, which measure the flow of Tl^+ through potassium channels, offer a high throughput method for the identification of potassium channel activators. However, these assays are a surrogate for channel function and it is important to have an appropriate panel of orthogonal and translational electrophysiology assays in place to confirm activity at the channel of interest.
- We used a Tl^+ flux assay to screen a library of 100K compounds and identified 173 'hit' compounds as potential activators of a K2P potassium channel. These compounds were then screened in an automated patch-clamp (APC) assay to confirm activity.
- Compounds with the highest activity in Tl^+ flux were the most likely to confirm in automated patch-clamp, but the correlation between the two systems was not entirely uniform.
- **Aim: to investigate the correlation between thallium flux and automated patch-clamp**

Methods



Thallium Flux

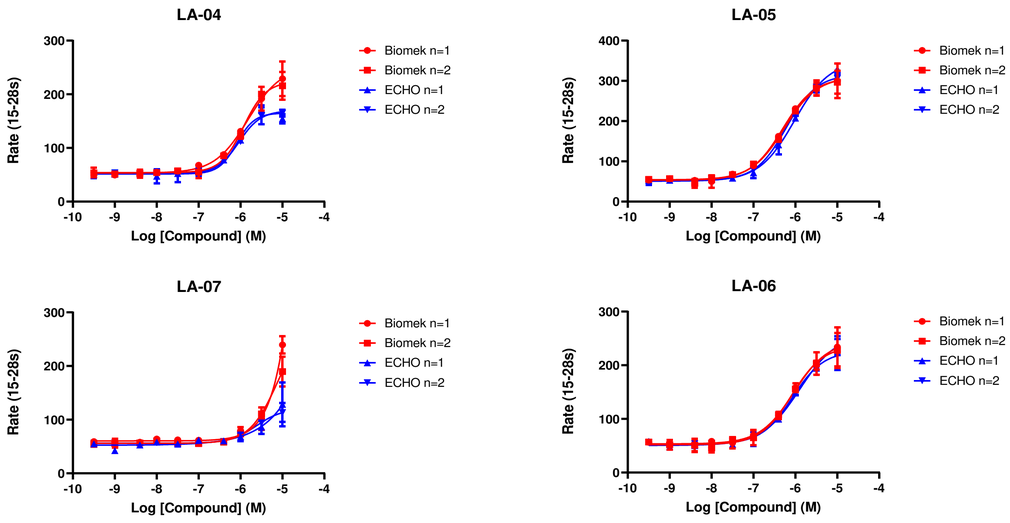
- FLIPR Potassium Assay Kit (Molecular Devices, USA) used according to manufacturers guidelines.
- Tl^+ sensitive dye within the cell detects the flow of Tl^+ through potassium channels.
- Typically, compounds are pre-incubated with cells for 30 minutes prior to Tl^+ addition. Alternative pre-incubation periods were studied.
- 5-10K cells per well. Channel activity is measured as the rate of fluorescence increase following Tl^+ addition.



APC (QPatch)

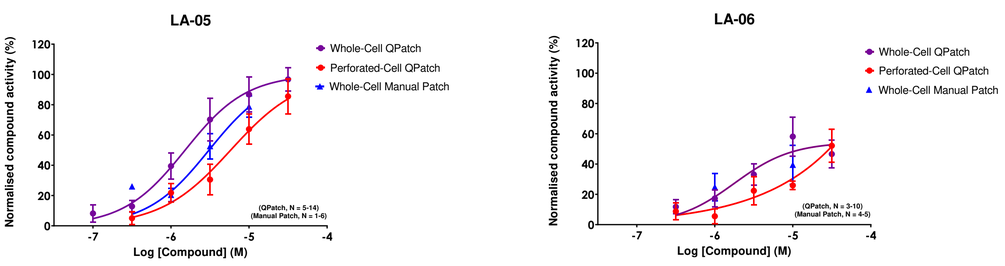
- Cells in extracellular solution (ECS) move through a microfluidic system and suction pulls a single cell to the patch-clamp hole.
- After a gigaseal is formed, the membrane is ruptured, allowing whole-cell electrophysiological recordings to be performed.
- Test compounds are applied to the inlet wells. Continuous recording allows multiple concentrations / compounds to be applied to a single cell.
- Perforated-cell patch-clamp is a variant of traditional patch-clamp, where pores are formed in the membrane, allowing electrical access but maintaining cytoplasmic constituents within the cell.

Liquid Handling



- Dose response curves were prepared using either an acoustic (ECHO, Labcyte) or a tip-based (Biomek FX, Beckman Coulter) dispenser. Compounds were run in Tl^+ flux assays with a 30 minute pre-incubation. Rate (15-28s) denotes the rate of Tl^+ flux. (Data represent mean ± SD, n=2).
- A subset of compounds displayed a reduction in the magnitude of the response when they were handled on the acoustic dispenser, demonstrating that the liquid handling method can influence the response in Tl^+ flux.

Automated and Manual Patch-Clamp



- Whole-cell QPatch, perforated-cell QPatch and whole-cell manual patch-clamp data for LA-05 and LA-06. (Data represent mean ± SEM, n≥1).
- Whole-cell and perforated-cell APC methods produce comparable results for both active and inactive compounds. Manual whole-cell patch-clamp (the gold standard patch-clamp technique), confirmed APC potency and efficacy data for active compounds.

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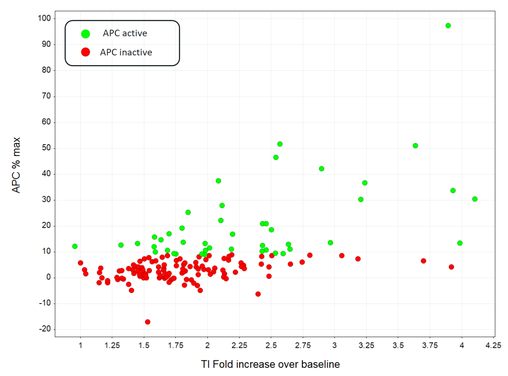
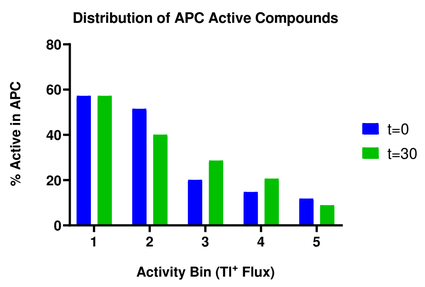
Correlation Between Tl^+ flux and APC

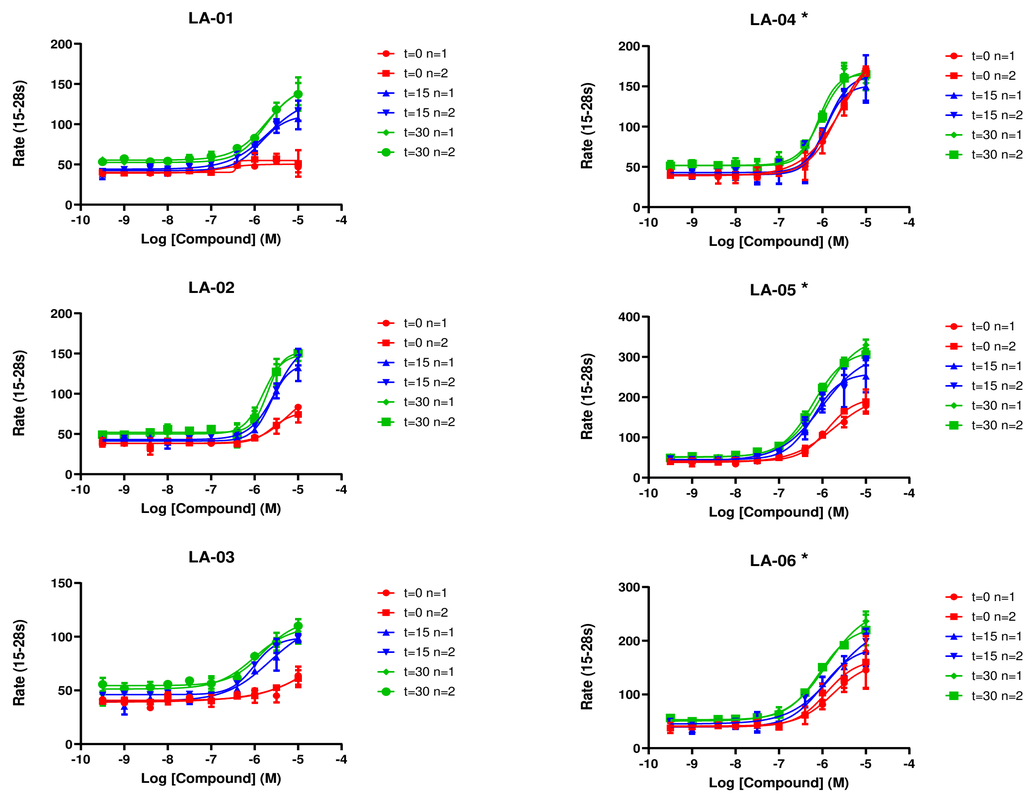


- 173 compounds identified as 'hits' in a Tl^+ flux screen of 100K compounds were screened in APC (QPatch).
- Plot shows correlation between Tl^+ flux and APC data, coloured by activity in APC.
- Tl^+ flux data represents fold-stimulation relative to DMSO control. APC data represents percentage of maximal channel activation.
- Compounds with the highest activity in Tl^+ flux were the most likely to confirm in APC, but the correlation was not entirely uniform.



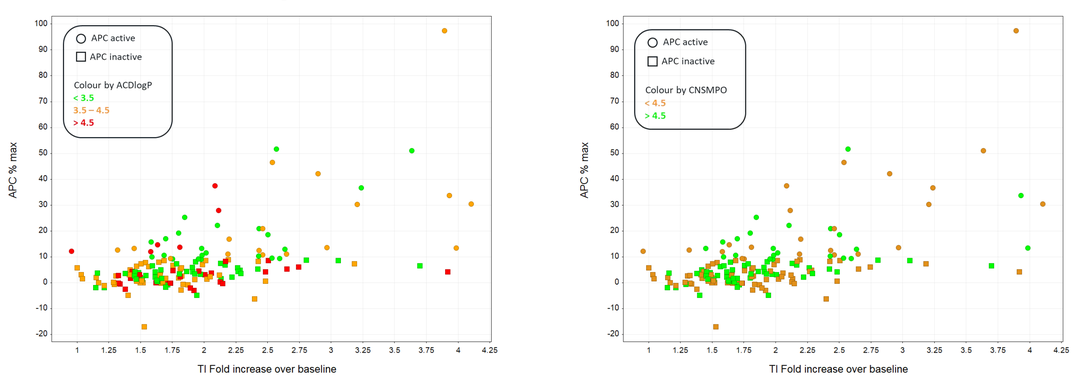
- Compounds were screened in Tl^+ flux, with either a 30 minute (t=30) or no (t=0) pre-incubation.
- Compounds were then binned based on Tl^+ flux activity; Bin 1 highest activity to Bin 5 lowest activity.
- For each bin, the percentage of compounds which were active in APC was then calculated.
- Data confirmed that compounds with the highest activity in Tl^+ flux were the most likely to be active in APC.

Compound Incubation Time



- Compounds were screened in dose response format in the Tl^+ flux assay with either a 30 minute (t=30), a 15 minute (t=15) or no pre-incubation (t=0). Rate (15-28s) denotes the rate of Tl^+ flux. (Data represent mean ± SD, n=2). * denotes compounds which were active in APC.
- A subset of compounds displayed a marked time dependency in the magnitude of their response. The magnitude of the response at t=0 may be indicative of the response in APC for some compounds.

Compound Properties



- Correlation between Tl^+ flux and APC data, coloured by ACDlogP (left) or CNSMPO (right). ACDlogP is a measure of lipophilicity and CNSMPO a score for predicted CNS penetration.
- Neither metric could adequately explain the correlation between the two systems, but phys-chem properties of individual compounds may contribute to differential responses in the two systems.

Conclusions

- **No single factor tested can adequately explain the correlation between the two systems.**
- **Compound incubation time and liquid handling method can influence the response in Tl^+ flux.**
- **Looking at these factors in combination, prior to selecting compounds for electrophysiology, may enable us to prioritise screening outputs.**