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A novel high throughput assay to identify activators of the two-pore-domain potassium channel TRESK

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TRESK, also known TWIK-related spinal cord potassium channel or $K_{2P}18.1$, is a two-pore-domain potassium channel. Mutations in the TRESK gene (*KCNK18*) have been linked to typical migraine. TRESK functions to restore membrane potential, allowing the outward flow of potassium ions as the membrane depolarizes. Relatively restricted protein expression in sensory ganglia, particularly the trigeminal ganglia, suggests a small molecule TRESK activator could act to reduce excitability and neural responsiveness, providing targeted therapeutic benefit in migraine and neuropathic pain.

A cell-based assay was developed to monitor TRESK activity and identify novel activators. Baculovirus transduced U2OS cells were used to configure a high throughput 384-well plate-based fluorescence screen to identify selective activators of TRESK. The FluxOR™ Potassium Ion Channel system (Life Technologies, USA) was used to screen approximately 20000 compounds, including a subset known ion channel pharmacaphores.

Initial screening identified Cloxyquin (5-Chloroquinolin-8-ol) as an activator of TRESK. Average maximal activity was 261% relative to DMSO containing controls. A *p*EC50 of *5.42* +/- 0.0*1* (s.e.m), n=12, was calculated. Cloxyquin was shown to have no activator properties against the two pore potassium channel TREK or un-transfected cells. Importantly, Cloxyquin was shown to activate TRESK in standard whole cell electrophysiology experiments, *p*EC50 = *5.49*, $n \geq 3$.

Several other novel activators of TRESK were also identified. Levosimendan, Carprofen, Rimonabant, Cefatrizine, Etodolac, Deferasirox, Flunixin meglumine, Entacapone and Niflumic acid all activated TRESK at 10µM to greater than 160% of DMSO containing controls (*$p<0.05$).* Subsequent analysis of these compounds showed that whilst they had no effect on un-transfected cells all showed activity at TREK, suggesting a lack of selectivity at two pore potassium channels.

Assessment of putative activators, including proprietary MRCT compounds, using standard whole cell electrophysiology is ongoing. It is hoped that compounds with appropriate TRESK activity and acceptable physical chemical properties could then be used to further elucidate the role of TRESK in behavioral models of migraine and ultimately lead to novel therapeutics.