Characterizing Gi GPCRs using high throughput fluorescence and electrophysiology based assays for drug discovery

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1 Introduction

G protein-gated inwardly rectifying potassium (GIRK) channels are critical regulators of neuronal and cardiac excitability. In the brain, GIRK primarily assembles as a Kir3.1/Kir3.2 heterotetramer, activated by Gβγ subunits released upon stimulation of Gi/o-coupled GPCRs. GIRK activity serves as a direct and physiologically relevant readout of Gi/o signaling; however, conventional approaches-including FRET, BRET, and cAMP assays-are constrained by biological complexity or require artificial adenylate cyclase stimulation.

We describe a Gi/o GPCR assay that enables high-throughput, real-time quantification of GIRK activation using both fluorescence-based and automated electrophysiology approaches. This assay utilizes a HEK293 cell line stably expressing Kir3.1 and Kir3.2, with the flexibility to transiently express a range of Gi/o-coupled GPCRs using just-in-time expression constructs. We could activate GIRK using ML297 and compare the EC_{50} value obtained using fluorescence and automated patch clamp assays. What is more, GIRK channels could be activated using both fluorescence and automated patch clamp assays. This effect was not observed in HEK cells without M2 or GIRK channel expression. This approach provides a physiologically relevant, scalable, and high-throughput method for identifying modulators of Gi/o-coupled GPCRs. Its compatibility with a variety of neurotransmitter receptors, and ability to generate reproducible pharmacological data using fluorescence and electrophysiological measurements, make it a valuable tool for both research and drug discovery efforts targeting Gi/o GPCRs.

2 Activation of GIRK by increasing external K+

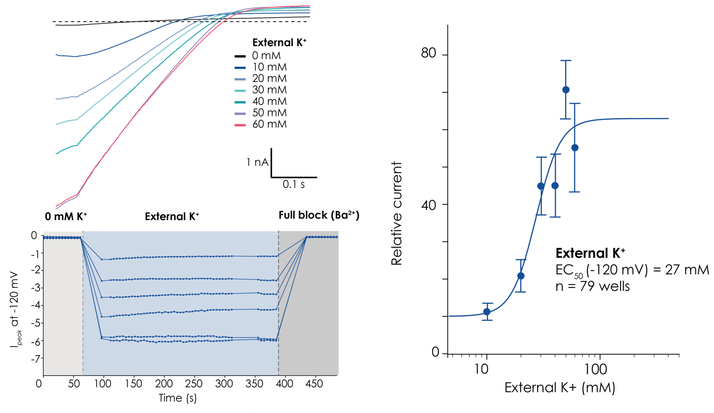

Figure 1: A. Basal activity of Kir3.1/3.2 by increasing concentrations of external K^+. Currents increased after addition of increasing concentrations of K^+ and this was blocked by Ba^{2+}. **B.** Time course of the experiment. **C.** Concentration response curve for external K^+ revealing an EC_{50} for K^+ of 27 mM (n = 79 wells).

3 Effect of Gi GPCR agonism on GIRK activity

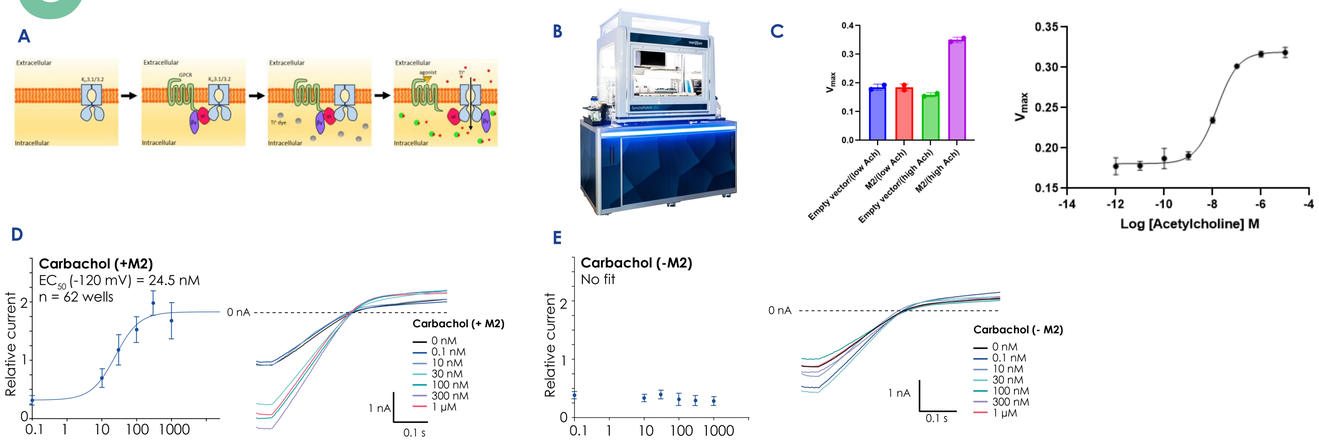

Figure 2: A. Co-expression of Kir3.1/3.2 and GPCRs in HEK cells are then used on the SyncroPatch 384 or fluorescence measurements following addition of a Tl^+ sensitive dye. **B.** The SyncroPatch 384 was used for the automated patch clamp experiments. **C. Fluorescence results:** When Kir3.1/3.2 is co-expressed with M2, acetylcholine activates Kir3.1/3.2 in a dose-dependent manner with an EC_{50} of 15 nM. **D.** When Kir3.1/3.2 is co-expressed with M2, carbachol activates Kir3.1/3.2 in a concentration-dependent manner with an EC_{50} of 24.5 nM (n = 62 wells). The data was normalized to basal current and fully activation using ML297 (trace not shown for simplicity). **E.**. When M2 is not co-expressed with Kir3.1/3.2, no concentration-dependent effect of carbachol could be observed.

4 Enhancement of GIRK by ML297

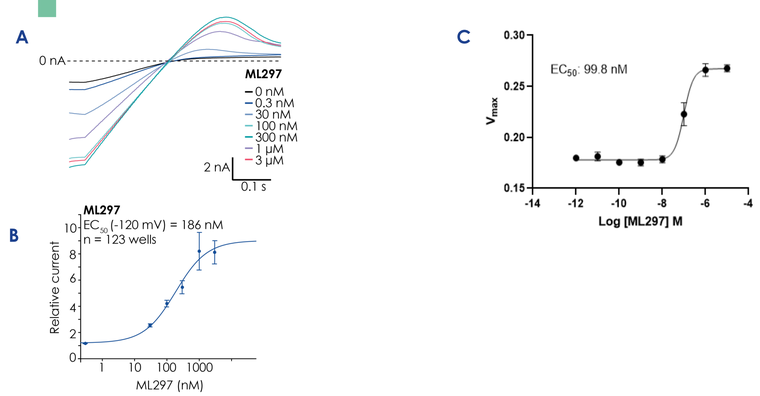

Figure 3: **A.** Concentration-dependent activation of Kir3.1/3.2 using ML297, shown are example average traces in response to increasing concentrations of ML297. **B.** Concentration response curve for ML297 revealing an EC_{50} of 186 nM (n = 123 wells). Responses were normalized to basal current at 0 nM. C. Concentration response curve for ML297 revealing an EC_{50} of 99.8 nM.

5 Summary

- **Kir3.1/3.2** expressed in HEK cells (ION Biosciences) was activated by increase of external K^+ concentration.
- **G-protein coupled activation** could be achieved through co-expression with M2 and activation by an agonist using both the SyncroPatch 384 and fluorescence technique.
- **No concentration-dependent** activation by carbachol was observed when M2 was not present.
- **Kir3.1/3.2** was activated by the agonist **ML297** on the SyncroPatch 384 and using a fluorescence technique.