

Development and validation of ligand-gated ion channel assays using the Qube 384 automated electrophysiology platform

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1 ABSTRACT

Ligand-gated ion channels are of particular interest to the pharmaceutical industry for the treatment of diseases from a variety of therapeutic areas including CNS disorders, respiratory disease and chronic pain. Ligand-gated ion channels have historically been investigated using fluorescence-based and low throughput patch-clamp techniques. However, with the development of the Qube 384 automated patch-clamp system, the rapid exchange of liquid and direct measurement of ion channel currents on a millisecond timescale is now possible at a greater throughput than previously possible. Here, we have used the Qube platform to develop assays against two ligand-gated families: 1) the P2X receptor and 2) the GABA_A receptor families. The P2X family is comprised of 7 family members, which are cation permeable and gated by the binding of extracellular ATP. We have assessed both agonist and antagonist pharmacology of 4 members of the P2X family, P2X₁, P2X₂, P2X₃ and P2X₄, as well as two species homologs, rP2X₃ and gpP2X₃. The GABA_A α1β3γ2 receptor is a chloride permeable ion channel gated by the binding of GABA. We utilized stacked liquid addition to assess the open state kinetics of the channel and to investigate the effects of a positive allosteric modulator on channel function. As such, we have successfully characterized and developed assays for both the P2X receptor and GABA_A receptor families and present EC₅₀/IC₅₀ data for antagonists and positive allosteric modulators.

2 MATERIALS AND METHODS

Cell Culture: HEK-hP2X₁, HEK-hP2X₂, HEK-hP2X₃, HEK-hP2X₄, HEK-rP2X₃, HEK-gpP2X₃, and HEK-GABA_A α1β3γ2 cells were produced at Charles River Laboratories and are commercially available. All cells were grown according to their respective SOPs as developed by Charles River. Cells were kept in a serum-free medium in the cell hotel on the Qube instrument for up to 4 hours during experiment.

Solutions: For both P2X and GABA experiments the following extracellular saline solution was used (mM): 145 NaCl, 4 KCl, 10 HEPES, 10 Glucose, 1 MgCl₂, 2 CaCl₂, pH7.4. For GABA experiments the following intracellular saline solution was used (mM): 20 KCl, 120 KF, 10 HEPES, 10 EGTA, pH7.2. For P2X receptor assays the extracellular saline during wash periods was supplemented with 0.5 U/mL apyrase (Sigma) and the following intracellular saline solution was used (mM): 140 CsF, 10 NaCl, 10 HEPES, 10 EGTA pH 7.3.

Qube experiments: All experiments were carried out using the Qube platform which performs 384 parallel and independent patch-clamp recordings on a disposable, single hole or multi-hole QChip. The holding potentials used for GABA and the P2X receptor family were -70mV and -60mV, respectively.

Analysis: Data analysis was performed using Qube Analyzer software and GraphPad Prism (7.0).

3 RESULTS

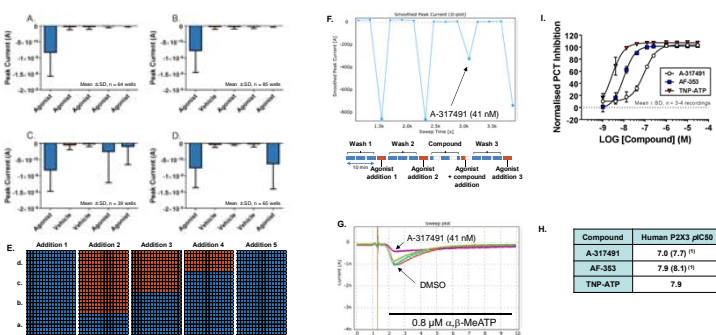


Figure 1. P2X₃ receptor assay development and antagonist pharmacology. A-D. P2X₃ current amplitude in the presence of vehicle/agonist. E. Schematic diagram of compound plates used to investigate P2X₃ desensitization, indicating agonist (blue) and wash (orange) conditions. F. Current/time plot demonstrating inhibition by A-317491 (41 nM). G. Raw current trace of P2X₃ current in the presence of agonist ± A-317491 (41 nM). H. Normalized percent inhibition data with Hill fit in the presence of A-317491 (white), AF-353 (blue) and TNP-ATP (red). I. pIC₅₀ values of each compound compared to literature values (where available).

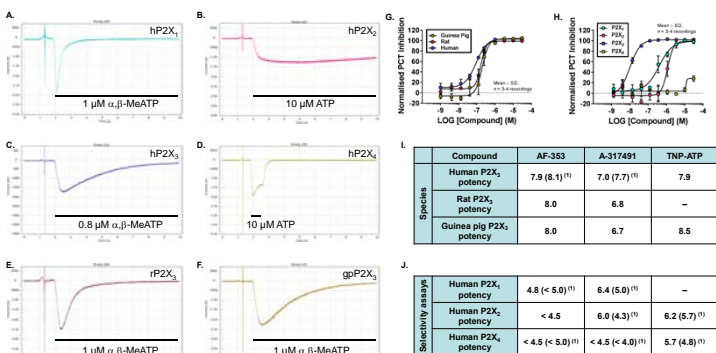


Figure 2. P2X receptor family and P2X₃ species homolog pharmacology. Raw current traces with agonist concentration for A. hP2X₁, B. hP2X₂, C. hP2X₃, D. hP2X₄, E. rP2X₃, F. gpP2X₃, G. Normalized percent inhibition data with Hill fit for hP2X₃, rP2X₃ and gpP2X₃ in the presence of A-317491. H. pIC₅₀ values of A-317491, AF-353 and TNP-ATP in human, rat and guinea pig P2X receptor family members compared to literature values (where available).

4 SUMMARY

Here we report pharmacological data for 4 different members of the P2X receptor family, two P2X₃ species homologs and the GABA_A α1β3γ2 receptor using the automated Qube 384 patch-clamp system. The P2X₃ receptor was shown to undergo rapid desensitization and we have demonstrated that the receptor must undergo three washes with extracellular saline to allow recovery from desensitization and return of the current to its original size. Using this three stage washing protocol, we demonstrated that P2X₃ currents are reproducible after multiple agonist additions and can be inhibited. pIC₅₀ data for three reference antagonists was in accordance with literature values. Furthermore, a similar protocol was adopted for P2X₁, P2X₂, P2X₄, rP2X₃ and gpP2X₃. rP2X₃ and gpP2X₃ demonstrated comparable pIC₅₀ values to that of hP2X₃ and literature values. In accordance with literature values, A-317491 was inactive against P2X₁, was less potent against P2X₁ and P2X₂ compared to hP2X₃.

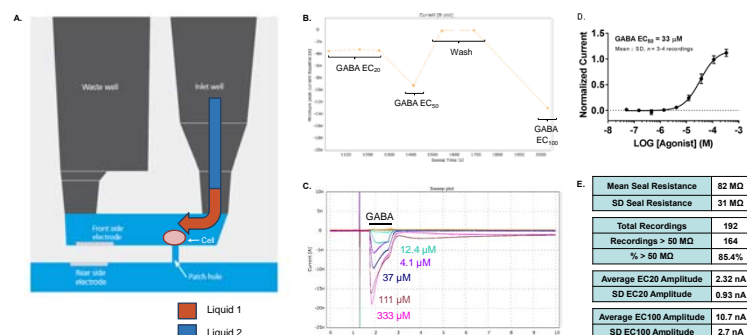


Figure 3. GABA_A α1β2γ₂ assay development and positive allosteric modulator pharmacology. A. Schematic diagram of stacked liquid addition on the Qube. B. Current/time plot demonstrating EC₅₀ and EC₁₀₀ activation by GABA. C. Raw current trace of GABA_A α1β2γ₂ current in the presence of increasing concentrations of diazepam. D. Concentration response curve in the presence of increasing concentrations of GABA. E. Summary of recording parameters taken from 192 experiment wells.

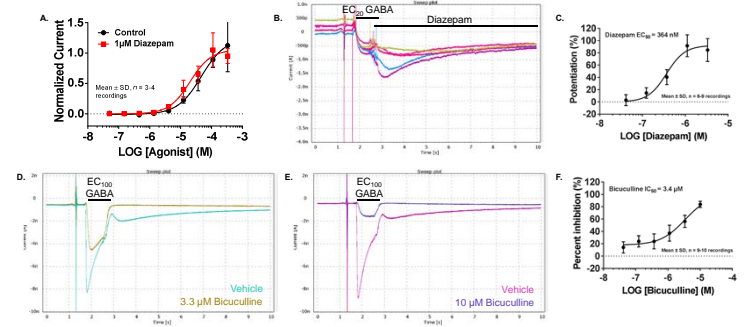


Figure 4. Assessment of positive and negative allosteric modulators using open and closed state conformations. A. GABA EC₅₀ curve ± 1 μM diazepam. B. Raw current traces demonstrating modulation of GABA by diazepam in open state conformation. C. Potentiation of GABA EC₅₀ current by increasing concentrations of GABA. D. Raw current trace demonstration 50% inhibition of GABA EC₁₀₀ current by bicuculline. E. Raw current trace demonstrating 90% inhibition of GABA EC₁₀₀ current by bicuculline. F. Inhibition of GABA EC₁₀₀ current by increasing concentrations of bicuculline.

We have developed a range of assays for investigating the effects of various types of modulators against the GABA_A α1β3γ2 receptor. Using the stacked liquid addition protocol and multi-hole QChips, we demonstrated an 85% success rate (seal resistances >50 MΩ) with an average GABA EC₂₀ response of 2.32 ± 0.93 nA and a GABA EC₅₀ concentration of 33 μM. The GABA EC₅₀ curve demonstrated a leftward shift in the presence of diazepam (EC₅₀ 20 μM with 1 μM diazepam). Using the open state conformation, diazepam potentiated the GABA EC₂₀ current up to approximately 100% with an EC₅₀ of 364 nM. The GABA current was also inhibited by bicuculline giving an IC₅₀ of 3.4 μM when using an EC₁₀₀ concentration of GABA to activate the channel.

⁽¹⁾ Khakh, B.S. & North, A.R. (2012) *Neuron*

Thank you to Antoinette Cooper, Carmela Clark and Suzie Clarke and the cell culture team for their time and expertise.