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Original article

Development of a high-throughput electrophysiological assay for the human *ether-à-go-go* related potassium channel hERG

Daniel J. Gillie [a], Steven J. Novick [b], Brian T. Donovan [c], Lisa A. Payne [d], Claire Townsend [a,*]

[a] Biological Reagents and Assay Development, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA
[b] Quantitative Sciences, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA
[c] Screening and Compound Profiling, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA
[d] Biological Reagents and Assay Development, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA

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ABSTRACT

Introduction: Drug-induced prolongation of the QT interval via block of the hERG potassium channel is a major cause of attrition in drug development. The advent of automated electrophysiology systems has enabled the detection of hERG block earlier in drug discovery. In this study, we have evaluated the suitability of a second generation automated patch clamp instrument, the IonWorks Barracuda, for the characterization of hERG biophysics and pharmacology. **Methods**: All experiments were conducted with cells stably expressing hERG. Recordings were made in perforated patch mode either on a conventional patch clamp setup or on the IonWorks Barracuda. On the latter, all recordings were population recordings in 384-well patch plates. **Results**: HERG channels activated with a $V_{1/2} = -3.2 \pm 1.6$ mV (n = 178) on the IonWorks Barracuda versus -11.2 ± 6.1 mV (n = 9) by manual patch clamp. On the IonWorks Barracuda, seal resistances and currents were stable (<30% change) with up to six cumulative drug additions and 1-min incubations per addition. Over 27 experiments, an average of 338 concentration–response curves were obtained per experiment (96% of the 352 test wells on each plate). HERG pharmacology was examined with a set of 353 compounds that included well-characterized hERG blockers. Astemizole, terfenadine and quinidine inhibited hERG currents with IC_{50} values of 159 nM, 224 nM and 2 μM, respectively (n = 51, 10 and 18). This set of compounds was also tested on the PatchXpress automated electrophysiology system. We determined through statistical methods that the two automated systems provided equivalent results. **Discussion**: Evaluating drug effects on hERG channels is best performed by electrophysiological methods. HERG activation and pharmacology on the IonWorks Barracuda automated electrophysiology platform were in good agreement with published electrophysiology results. Therefore, the IonWorks Barracuda provides an efficient way to study hERG biophysics and pharmacology.

1. Introduction

The human ether-à-go-go related gene (hERG, KCNH2, or Kv11.1) encodes a potassium channel that plays a major role in the repolarization phase of the cardiac action potential (Sanguinetti, Jiang, Curran, & Keating, 1995). Loss-of-function mutations in the hERG gene underlie one of the most common forms of congenital long QT syndrome, LQT2 (Shimizu et al., 2004). This inherited disease is characterized by a prolonged QT interval in the electrocardiogram and severe arrhythmias. Blockade of the hERG channel by drugs can also cause severe arrhythmias and has been a major concern for drug safety since the withdrawal of several drugs from the market in the late nineteen nineties (De Ponti, Poluzzi, & Montanaro, 2000; Redfern et al., 2003). Regulatory agencies require the testing of novel chemical entities against the hERG channel before the start of clinical trials (International Conference on Harmonization, ICH S7B, 2005). The advent of automated electrophysiology technologies for studying hERG has allowed the detection of hERG block earlier in drug discovery.

In this report, we describe a high-throughput electrophysiology assay for hERG that is run on the IonWorks Barracuda, a second generation automated electrophysiology instrument. The IonWorks Barracuda has many technological improvements over its predecessor, the IonWorks Quattro. First, it allows continuous voltage clamp of cells. Second, it is a

Abbreviations: CHO, Chinese hamster ovary; cLogP, calculated logP, where P is the partition coefficient between n-octanol and water; C_m, membrane capacitance; HEK, human embryonic kidney; hERG, human ether-à-go-go related gene; IC_{50}, 50% inhibitory concentration; IW, IonWorks; KCNH2, potassium voltage-gated channel, subfamily H (eag-related), member 2; LQT2, long QT syndrome type 2; PBS, phosphate buffered saline; pIC_{50}, $-\log_{10}(IC_{50})$; PPC, population patch clamp; QT interval, interval between the Q and T waves of the electrocardiogram; R_a, access resistance; R_m, membrane resistance; R_s, series resistance; SD, standard deviation; $V_{1/2}$, voltage for half-maximal effect.

* Corresponding author at: GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA. Tel.: +1 919 483 8113; fax: +1 919 483 6465.
E-mail address: claire.y.townsend@gsk.com (C. Townsend).

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true 384-well platform, with 384 amplifiers allowing simultaneous recordings from an entire 384-well patch plate. Third, up to 8 additions may be performed on each well giving experimenters the possibility to perform cumulative concentration–response curves within a single well. Other improvements include solid silver chloride electrodes and the use of disposable pipette tips for drug additions. We conducted a detailed study evaluating the suitability of the IonWorks Barracuda for use in hERG screening. Examinations of hERG biophysical properties, pharmacology against literature standard blockers and a comparison to other accepted, but lower throughput, automated patch clamp technologies were performed.

2. Methods

2.1. HERG expression

HERG channels were stably expressed in CHO-K1 (Chen et al., 2007) or HEK293 cells (Donovan et al., 2011). Cryo-preserved cells were used in all experiments. Frozen cells were stored in a liquid nitrogen freezer and quickly thawed in Dulbecco's modified Eagle medium F-12 supplemented with 10% fetal bovine serum before plating into tissue culture vessels. Cells were cultured at 37 °C, 5% CO_2 for 2–4 h and then placed at 30 °C, 5% CO_2 for 24–96 h prior to recording hERG currents. For QPatch recordings, CHO-K1 cells were cultured in Iscove's DMEM supplemented with 10% non-heat inactivated fetal bovine serum, 2% HT supplement, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 0.1 mM non-essential amino acids, and 0.4 mg/mL geneticin. Culturing the cells at 30 °C increases functional expression of hERG channels (Chen et al., 2007). All cell culture reagents were from Invitrogen (Carlsbad, CA).

2.2. Electrophysiology solutions

For manual patch clamp and IonWorks Barracuda recordings, the external solution used was a Tyrode's salts buffer supplemented with (in mM) 20, HEPES; and 12, $NaHCO_3$ with pH adjusted to 7.35 using NaOH. The internal solution used was (in mM): 140, KCl; 1, $CaCl_2$; 1, $MgCl_2$; and 20, HEPES; with pH adjusted to 7.35 using KOH. The perforation agent amphotericin B was prepared as a 30 mg/mL stock solution in DMSO and added to the internal solution at a concentration of 0.1 mg/mL on the day of the experiment.

For PatchXpress recordings, the internal solution was (in mM): 130, K-Aspartate; 10, KCl; 10, HEPES; 5, EGTA; 5, MgATP; 0.1, $CaCl_2$ (Ricca Chemical Company, Arlington, TX); and 1, $MgCl_2$; with pH adjusted to 7.2 using KOH. The PatchXpress external solution was (in mM): 140, NaCl (J.T. Baker, Phillipsburg, NJ); 4, KCl; 2, $CaCl_2$; 1, $MgCl_2$; 10, HEPES; and 10, glucose; with pH adjusted to 7.4 using NaOH.

For IonWorks Quattro recordings, the internal solution was (in mM): 140, KCl; 1, $MgCl_2$; 1, $CaCl_2$; 20, HEPES; with pH 7.3 adjusted with KOH. The external solution was PBS containing Ca^{2+} and Mg^{2+} (Invitrogen, Carlsbad, CA).

QPatch assay buffers were as follows (all values in mM). The internal solution consisted of 100, KCl; 30, KF; 10, HEPES; 10, EGTA; 5, MgATP; 1, $CaCl_2$; and 1, $MgCl_2$. The resulting solution was adjusted to pH 7.2 with KOH. The external solution consisted of 145, NaCl (J.T. Baker, Phillipsburg, NJ); 4, KCl; 2, $CaCl_2$; 1, $MgCl_2$; 10, HEPES; and 10, glucose. The resulting solution was adjusted to pH 7.4 with NaOH.

For IonWorks Barracuda and Quattro experiments, compound stocks were made in DMSO and diluted in external solution containing 0.03% Pluronic Acid F-127 (Invitrogen, Carlsbad, CA). Unless otherwise noted, all assay buffer components were from Sigma-Aldrich, Saint-Louis, MO.

2.3. Patch clamp recordings

CHO-K1 hERG cells were cultured as described above in 35-mm plastic culture dishes. Prior to recording hERG currents, culture medium was replaced with external solution and the culture dish was placed onto the microscope stage of a patch clamp set up. All recordings were made in the perforated patch clamp mode with internal solution containing 0.1 mg/mL amphotericin (Rae, Cooper, Gates, & Watsky, 1991). Fire-polished pipettes of G150TF-4 borosilicate glass (Warner Instruments, Hamden, CT) had resistances ranging from 2–4 MΩ. After giga-seal formation, access resistance (R_a) was monitored and current recordings were not started until R_a reached values below 20 MΩ. Currents were filtered at 5 kHz with an Axopatch 200B patch clamp amplifier (Molecular Devices, Sunnyvale, CA). Current recordings were digitized with a Digidata 1320 interface under the control of the pCLAMP10 software (Molecular Devices, Sunnyvale, CA). All recordings were made at room temperature.

To examine the voltage dependence of activation of hERG, currents were elicited by a 2-step voltage protocol consisting of a 5-s family of voltage steps ranging from −80 to +50 mV in 10 mV increments followed by a 1-s repolarization step to −50 mV. Each protocol was delivered once every 15 s. Currents were measured at the peak elicited by the repolarizing step to −50 mV. HERG tail currents were recorded with a 2-step voltage protocol consisting of a 5-s depolarization to +20 mV and a family of 5-s steps to voltages ranging from −130 mV to +10 mV applied in 10 mV increments every 30 s. Currents were measured at the peak of the 2nd step. For both protocols, the holding potential was −80 mV.

2.4. IonWorks Barracuda recordings

2.4.1. Cell preparation

CHO-K1 hERG cells were dissociated from T225 tissue culture flasks by aspirating the culture medium, rinsing the cells with warmed (37 °C) Ca^{2+} and Mg^{2+} free phosphate buffered saline solution (PBS, Invitrogen, Carlsbad, CA) and adding 3.5 mL warmed Versene dissociation buffer (Invitrogen, Carlsbad, CA) per flask. The cells were incubated in Versene for 3 to 5 min in a 37 °C incubator. The flasks were then tapped to dislodge the cells. Warmed cell culture medium (12 mL) was added to each flask and the cells were gently triturated by pipetting up and down 2–3 times. The cells were then allowed to recover for 5–10 min at room temperature. Cells were pelleted at 1000 rpm for 2 min. The supernatant was discarded and the cell pellet was resuspended in external solution at a density of 1.5–2.0 million live cells per mL. Cells were counted on a Vi-CELL cell viability analyzer (Beckman Coulter, Brea, CA). A total of 7.5–10 million cells in 5 mL of external buffer were then loaded into the IonWorks Barracuda to yield 12,000–16,000 cells per well in a population patch clamp plate. This range of cell densities was optimal for seal resistances and pharmacology. Lower cell densities decreased seal resistances and resulted in high numbers of wells rejected for low resistance (<30 MΩ). High cell numbers have been shown to reduce the apparent potency of some hERG blockers on the IonWorks Quattro platform (Bridgland-Taylor et al., 2006). We have observed this phenomenon and have used the lowest number of cells giving acceptable seal resistances (>95% of the wells with resistances above 30 MΩ in baseline recordings).

2.4.2. IonWorks Barracuda setup protocol

All recordings were made at room temperature in the population patch clamp (PPC) mode (Finkel et al., 2006) with 384-well IonWorks Barracuda PPC plates (Molecular Devices, Sunnyvale, CA). Each well first received 8 μL of external solution. Internal solution was circulated under the patch plate and vacuum was applied under the patch plate to get a liquid junction. The resistance of each well was measured in a "hole test" by applying a short pulse to +10 mV from a potential of 0 mV. Then 8 μL of cell suspension was added to each well at a speed of 0.5 μL/s. Cells were allowed to seal on the PPC patch plate substrate for 4 min before perfusion of the amphotericin containing internal buffer under the patch plate. During that time, seal resistances were monitored by applying 60-ms pulses to −70 mV from a holding potential