hERG K⁺ channel currents and pharmacology using the IonFlux system

Introduction

HERG (human ether-a go-go-related gene) K$^+$ channels are strongly expressed in the heart and are responsible for a rapid component (IK$_r$) of the repolarizing currents in the cardiac action potential (Curran '95; Sanguinetti '95). Loss of function mutations affecting hERG are associated with some inherited forms of long QT syndrome (LQTS) and increase the risk for a serious ventricular arrhythmia, *torsade de pointes* (Tanaka '97; Moss '02). HERG K$^+$ channel inhibition by both cardiac and noncardiac drugs has also been identified as the most common cause of acquired, drug-induced LQTS that may lead to sudden cardiac death (Vandenberg, Walker & Campbell '01). In fact, the side effect of hERG K$^+$ channel inhibition is one of the major reasons of drug withdrawal or drug re-labeling in recent years, therefore *in vitro* evaluation of the effects of drugs on hERG channels expressed heterologously in mammalian cells has been recommended as part of the preclinical safety package by the International Conference on Harmonization (ICHS7B Expert Working Group, '02).

The gold standard of evaluating drug effects on hERG K$^+$ current is manual patch-clamp recording. However, this low-throughput, high-cost approach is limiting in safety screening of large numbers of drugs. Recently, automated electrophysiology systems have been developed that can obtain high-throughput recordings and achieve reasonably comparable results with manual patch clamp. The IonFlux™ system developed by Fluxion Biosciences is designed to combine the convenience and throughput of a plate reader with the performance of the traditional patch clamp assay. Here we present results of recordings of hERG currents expressed in mammalian cell cultures and the pharmacological inhibition profiles of a panel of drug compounds using the IonFlux system.

Figure 1. The IonFlux system utilizes a "plate reader" format to simplify workflow and increase throughput. Systems are available with 16 and 64 amplifiers. Throughput of 10,000 data points per day can be achieved.

Materials and Methods

Cells
Chinese hamster ovary (CHO) cells that express hERG channels under G418 selection were utilized (Millipore PrecisION™ hERG-CHO Recombinant Cell Line, Cat# CYL3038). Cells were cultured in Glutamax DMEM/F12 medium (Gibco, Cat# 11320) containing 10% fetal bovine serum, 1% penn-strep, and 500 μg/mL G418 in a humidified, 5% CO$_2$ atmosphere. Cells were either grown at 37°C and then transferred to a 30°C incubator at least 24 hrs before the experiments, or kept at 30°C after passaging. The cultures were never permitted to exceed 90% confluence. For experiments, cells were released from culture flasks using Detachin (Genlantis, San Diego, CA, Cat# T100100) and after washing and gentle trituration, cells were suspended at a concentration of 2 - 5 million cells per ml in extracellular solution (ECS).

Solutions & Compounds
Extracellular solution (ECS) contained (mM): NaCl 145, KCl 4, MgCl$_2$ 1, CaCl$_2$ 2, HEPES 10, dextrose 10 brought to pH 7.4 using NaOH. The intracellular solution contained (mM): KCl 120, HEPES 10, Na$_2$ATP 4, EGTA 10, CaCl$_2$ 5.374, MgCl$_2$ 1.75 brought to pH 7.2 using KOH.

hERG blocker compounds were purchased from Sigma. Compounds were first dissolved in DMSO as high concentration stock solutions (10-50 mM), then diluted in dose series in DMSO before diluted into the final concentrations in the ECS, hence the final concentrations of DMSO were equal in the same dose series (0.1- 0.3%). A negative control of of DMSO solution (0.1 - 0.3%) was always applied before compound applications, and was found not to induce a change in current amplitudes exceeding 10%.

Experimental protocols

Each well of the IonFlux well plate consumable was loaded with 250 µL of internal solution, compound, or cell suspension. IonFlux plates look and handle exactly like standard well plates, but the plate bottom has been replaced by a microfluidic network that connects to the wells in a repeating pattern across the plate. After loading the plate, all flow control steps were controlled by the instrument, including cell trapping, seal formation, whole cell break-in, compound application, and washing.

The IonFlux-16 system includes 16 fully-featured amplifiers. All 16 recording channels are simultaneously applied with the specified voltage command waveforms. The system utilizes 20-cell ensembles for each amplifier channel to improve data consistency and success rates. For recording hERG currents, cell ensembles were voltage clamped at a holding potential of -80 mV, and seal resistances were constantly monitored by a small step to -100 mV. HERG channels were activated at +50 mV (800 ms), and the outward tail current at -50 mV were measured by subtracting a baseline reading at -50 mV before activation from the peak outward tail current at -50 mV after activation. A step to -120 mV (800 ms) after the -50 mV repolarization step was also included for recovery of hERG channels from inactivation (see Figure 2). In experiments studying the current-voltage relationships (IV responses), either the activation step was clamped to voltages between -50 mV and 60 mV in twelve 10 mV increments, or the first repolarization step was clamped to voltages between -120 mV and +50 mV in eighteen 10 mV increments (see Figure 3). The voltage protocol was applied every 6 s. Leak current was compensated online using two pairs of small pulses (-80 mV to -100 mV 50 ms/50 ms). Ionic currents were sampled at 5 kHz, and recorded at room temperature (20 - 23 °C).

For pharmacology studies, after hERG currents were stabilized in the data acquisition phase (~5 min), low to high concentrations of the same compound (including negative controls of 0.1 - 0.3% DMSO solutions) were applied sequentially to the same cell ensembles for 3 - 5 minutes each, either in a cumulative fashion or in an on-off (compound-wash) sequence. During experiments, currents were continuously monitored except during the holding periods when a holding voltage of -80 mV is applied.

Example whole-cell hERG channel currents

Figure 2 shows the waveform of the voltage protocol (top graph) and a typical screenshot of hERG currents during runtime at room temperature (bottom graph), in which the traces for all 16 amplifier channels (16 cell ensembles) were overlayed in one graph. Automatic leak compensation was turned on. Resistances and current amplitudes (ΔI) were measured at the marked cursor positions (pink and light green for resistance measurements, green and blue for current measurements).

Typical currents for a 30-cell ensemble ranged from 2 - 12 nA; current amplitudes are determined by the number of cells in the whole cell configuration and the current per cell. The seal resistance for a 30-cell ensemble measured in parallel ranged from 3 to 40 MΩ, which translates to a seal resistance of 90 – 1200 MΩ per cell ($R_{cell} = R_{ensemble}$ x 30).
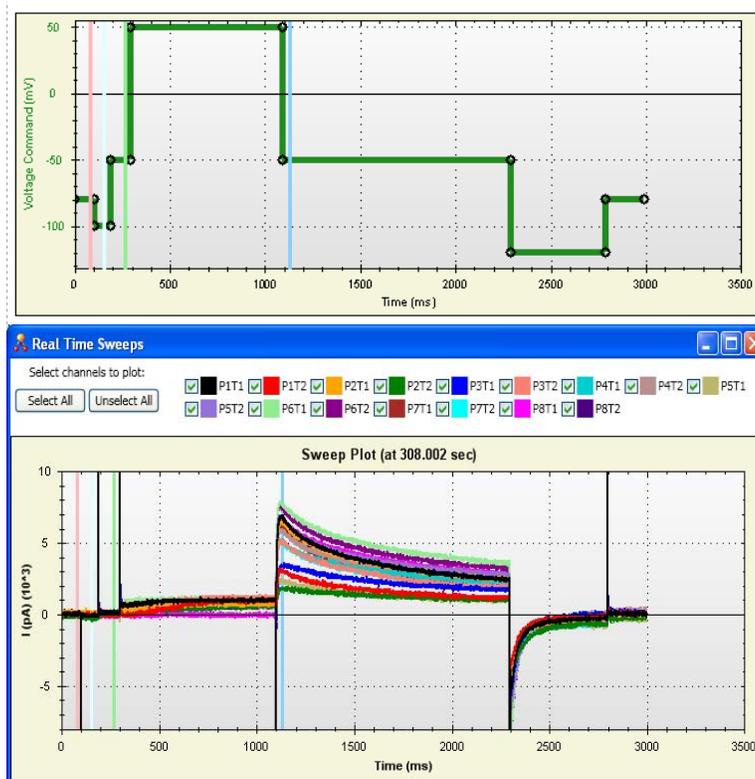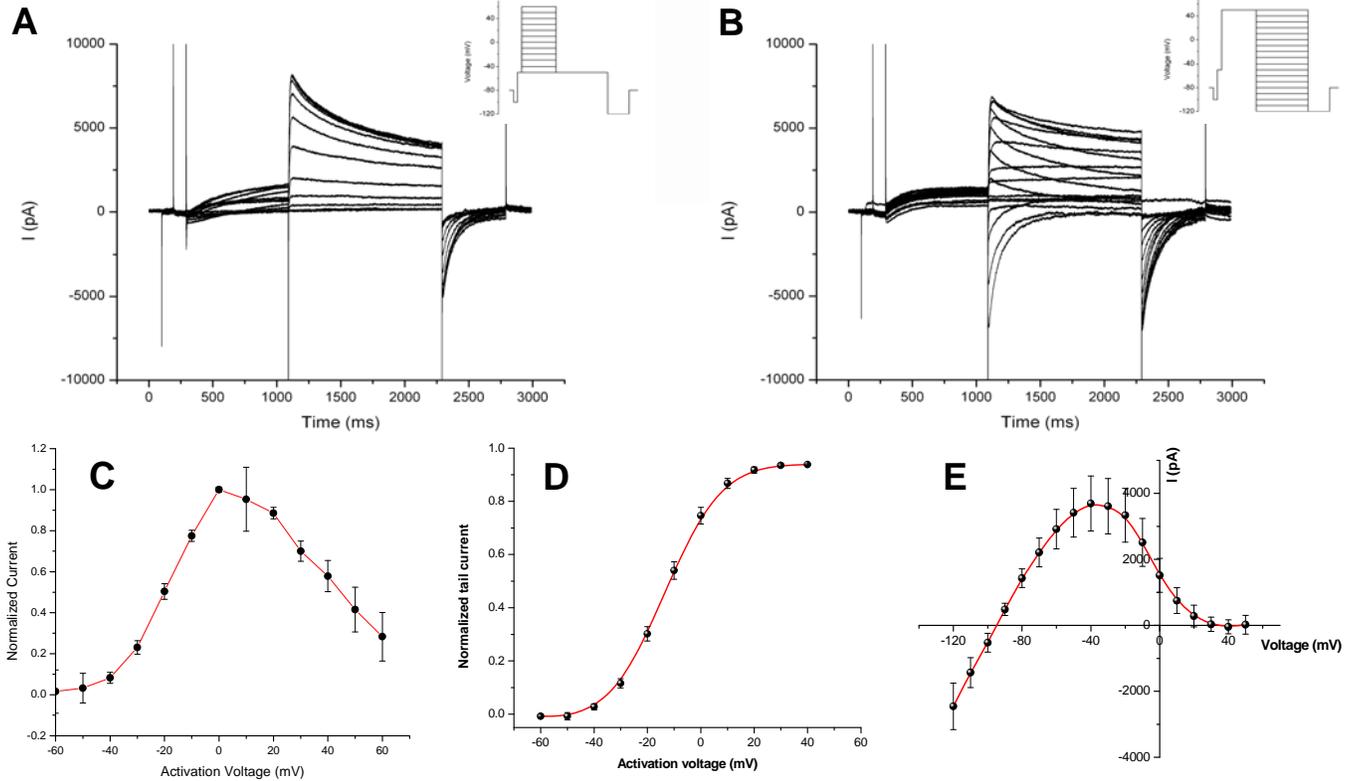

Figure 2. The voltage waveform for the hERG current and a representative screenshot of hERG channel recordings from 16 individual cell ensembles recorded on the same IonFlux plate.

Figure 3. The current-voltage relationships of recorded hERG currents at room temperature. Cells were clamped at a holding potential of -80 mV. (A) shows a representative overlay of recording sweeps at different activation voltages from a cell ensemble. IV plots of the activation current and the normalized tail current measured at -50 mV were shown in (C) and (D) . The fully-activated IV plot for hERG cells is shown in (B) (sweep overlay) and (E) (IV plot).

Voltage dependence of recorded hERG currents

Current-voltage relationships of recorded hERG currents at room temperature are shown in Fig. 3. Cells were clamped at a holding potential of -80 mV. Fig. 3A shows a representative overlay of recording sweeps at different activation voltages from a cell ensemble. Cells were depolarized to voltages between −60 mV and 60 mV for 800 ms to activate hERG currents, then re-polarized to -50 mV for the outward tail current. IV plots of the activation current and the normalized tail current measured at -50 mV were shown in (C) and (D). The Boltzmann function fit indicates a half maximum activation voltage of −6±0.3mV (D). This agrees well with previously presented APC data (PatchXpress, Guo & Guthrie, 2005), and is shifted by approximately 8 mV from the activation voltage measured at 23 °C using manual patch clamp recording (-14mV, Zhou et al., 1998). The fully-activated IV plot for hERG cells is shown in (B) (sweep overlay) and (E) (IV plot), where currents were activated by a depolarizing step to 50 mV, and repolarized to different voltages. The tail current reversed at -94 mV; at more negative voltages, the current became inward. The calculated reversal potential is −90 mV, based on the solutions used.
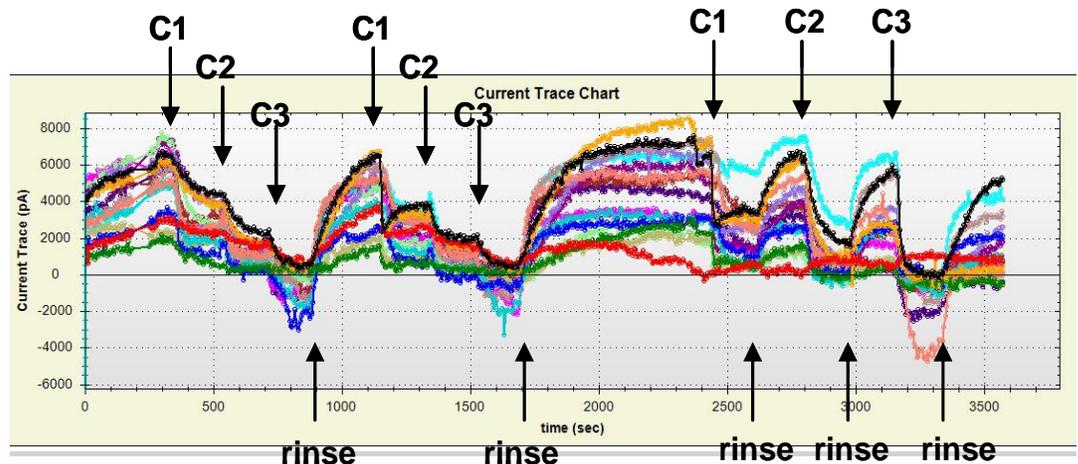
Figure 4. Longevity of hERG recording and two types of compound application sequences. Experimental screen capture showing two cumulative blocker concentration sequences, followed by a protocol during which the blocker is removed before each subsequent concentration is applied.

hERG blocker IC50s

Upon obtaining whole-cell access, hERG currents could be continuously recorded for more than 60 (up to 120) min (see an example in Figure 4). Therefore a couple of different methods could be used to determine IC50 value of a compound using the same cell ensemble.

Each experimental pattern contains up to eight compounds that can be applied to the same cell ensemble. The example traces in Figure 4 illustrate two different dose-response experiment protocols. C1, C2, and C3 were three different concentrations from low to high (can be same or different concentrations in different zones). First, C1, C2, C3 were repeated twice in a cumulative fashion then in an on-off sequence (Fig. 4). Subsequent applications of the compound doses (cumulative or on-off if the block is reversible) induced similar percentage of the hERG current block at each concentration, highlighting the consistency of the IC50 studies using Ionflux-16. In this example, two different compounds (amitriptyline and cisapride) were loaded in different zones of the plate. IC50 values were generated fitting individual cell ensembles and then averaged.

The IC50s of a variety of known hERG blockes with a range of lipophillicity were determined by Hill fits to the data (Figure 5). Results were compared to literature values for each compound in Table 1.

Discussion

The experiments validate the applicability of the IonFlux platform for studying hERG K+ currents and screening compounds for hERG channel inhibition. Ensemble recording from multiple cells offered the advantage of greater success rates and stable currents over a long period of time during continuous recording. The activation and inactivation of the hERG current showed similar voltage-dependences as in manual patch-clamp. Due to the longevity of hERG recording (>60min), the fast application and wash-out of compounds, and the continuous monitoring of voltage-dependent currents during compound application, IC50s of the compounds can be obtained from the same set of cell ensembles in one experiment. The IC50 values obtained using the IonFlux platform agree well with literature values (Redfern '03; Guo & Guthrie '05). Even lipophilic compounds show excellent agreement with literature values: , terfenadine IC50 = 25nM, bepredil IC50 = 54nM and astemizole IC50 = 15nM.

High throughput screening of drug effects on hERG channels is critical in drug safety testing. The compound potency data obtained indicates that IonFlux will be a valuable tool for hERG screening and compound profiling.

References

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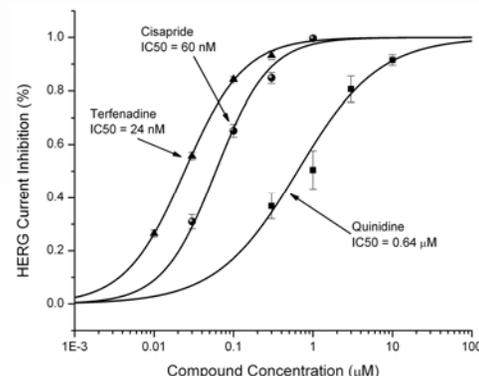Zhou Z, et al. (1998) *Biophys J* 74:230-41

Figure 5. Measured IC50 values for three blockers were obtained by fitting dose-response data with a Hill function. All fitting results are

Compound	cLogP	IonFlux IC50 (µM)	Literature IC50 (µM)
Amitriptyline	4.9	3.4	4.0 – 10
Astemizole	6.1	0.015	0.001-0.026
Bepridil	6.2	0.054	0.29 – 0.55
Cisapride	3.8	0.06	0.005 – 0.070
Diphenhydramine	3.5	8	2.6-3.8
Dofetilide	2.0	0.06	0.006 – 0.158
Droperidol	3.1	0.5	0.10 – 0.85
E4031	1.4	0.06	.01 - 0.13
Haloperidol	3.8	0.79	0.019 – 0.050
Prazosin	2.0	1.7	1.6 – 2.1
Propranolol	2.8	13	10 - 15
Quinidine	2.8	0.64	0.30 – 1.10
Ranolazine	2.2	2.3	11.5-14.6
Terfenadine	6.5	0.024	0.02 – 0.200
Thioridazine	6.0	0.2	0.033 – 1.25
Verapamil	4.5	0.7	0.22 – 0.83

Table 1. A comparison of the IC50 values for a number of known blockers were obtained using the IonFlux system and compared to literature values (right column). The cLogP values are also shown as a measure of compound lipophilicity.

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