Characterization and Functional Validation of CHO hERG with IonFlux HT Single Cell Mode

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 non cardiac 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 single cells mode.

The IonFlux plates are based on the 96 or 384 SBS-standard format. (Left) an IonFlux HT system capable of 64 parallel recording. (Right) the microfluidic network attached to the bottom of a 384 well plate. This plate is used with the IonFlux HT

Methods

Culturing

B'SYS CHO (Chinese Hamster Ovary) hERG human ERG (ether-a-go-go related gene) cells were cultured following B'SYS guidelines (B'SYS GmbH, Switzerland, info@bsys.ch).

In brief, B'SYS CHO hERG Complete Media contained F-12 (HAM) with GlutaMAX (31765-Invitrogen), 10% FBS (F2442 - Sigma), 1.0% Penicilin/Strepomycin (15140-Invitrogen), 100Mg/ml Hygromycin (10689 - Invitrogen), and 100Mg/ml Neomycin (G418; 10131 - Invitrogen).

The cells were incubated in a 30°C incubator for 1-5 days before running experiments to boost channel expression. Cells were detached using Detachin (100077-508 VWR) for 15 minutes at 37°C.

Solutions

Extracellular Ringer's Solution

2 $CaCl_2$, 1 MgCl2, 10 HEPES, 4 KCl, 145 NaCl, *10 Glucose (in mM) pH = 7.4 (with NaOH) 305 mOSM (with sucrose)

Intracellular Ringer's Solution

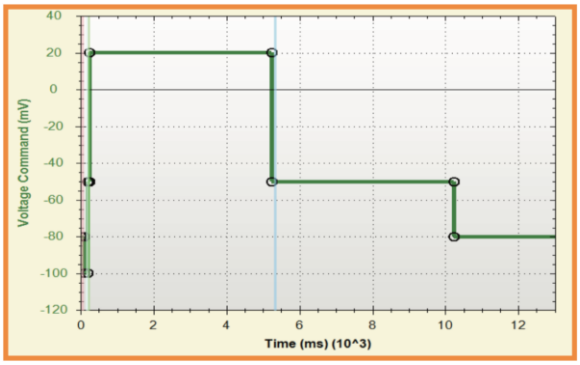
5.374 $CaCl_2$, 1.75 $MgCl_2$, 10 EGTA, 10 HEPES, 120 KCl, *4 Na_2-ATP (in mM) pH = 7.2 (with KOH) 295 mOsm (with sucrose)

Extracellular Ringer's was approximately 10 mOsm higher.

Verapamil was purchased from Sigma. Verapamil was first dissolved in DMSO as a high concentration stock solution (10 mM), then diluted in dose series in DMSO and FL Reagent (Fluxion Biosciences) before being diluted into the final concentrations in the ECS. The final concentration of DMSO was equal in the same dose series (0.1%). A negative control of DMSO solution (0.1%) was always applied before compound applications, and was not found to induce a change in current amplitudes exceeding 10%

Protocols

A sample voltage protocol is shown below (figure 1) for evoking hERG current. Every 15 seconds there was a 5 second depolarizing voltage step (from -80 mV to +20mV) which was followed by a 5 second tail step to -50mV. Example raw currents for one zone (16 traps) are shown below the voltage protocol.



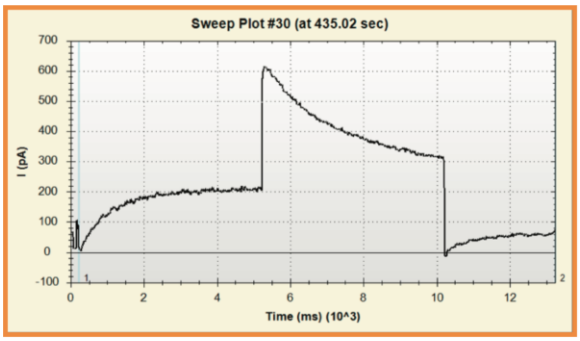


Figure 1: Example of raw hERG current sweeps are shown below the voltage protocol

Results

Success Rate in Single Cell Mode and Stability of Recordings

A sample hERG current trace is shown below in figure 2 for one zone (16 traps) on an IonFlux single cell HT plate. In this example, there were (~69%) successful recordings showing expression levels ranging from at least 200 pA in peak tail current measurements.

B'SYS CHO hERG cells showed stability in current measurements on the IonFlux even up to ~40 minutes of recording. A sample hERG current versus time trace is shown in figure 3. In this trace, current expression ranged from ~100-900 pA with a success rate of ~81%. The majority of currents were stable during the ~40 minutes of recording with minimal run-down observed.

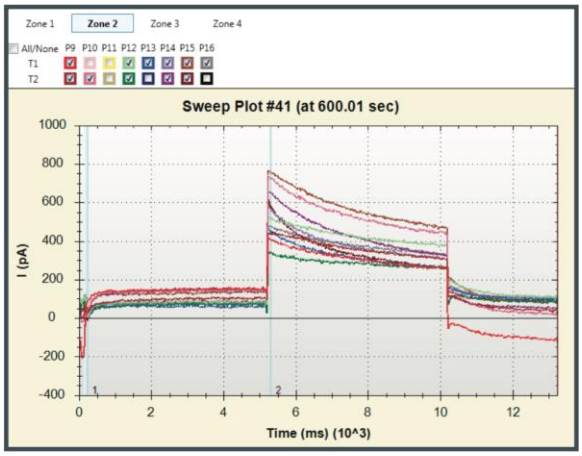


Figure 2: Example of raw superimposed hERG current sweeps are shown for one zone.

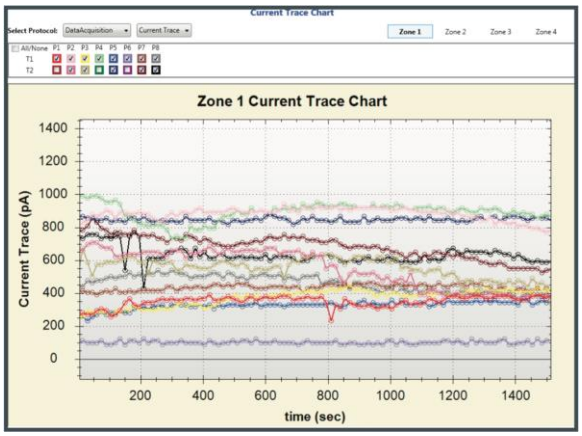


Figure 3: Example of hERG current versus time trace is shown for one zone

The graph below (figure 4) shows a summary of B'SYS CHO hERG+ current percentage success for 5, 10, 15, and 20 minute time points (average +/- SEM). The success rates using a 200 pA threshold were above 50% for all time points tested (N=5 plates).

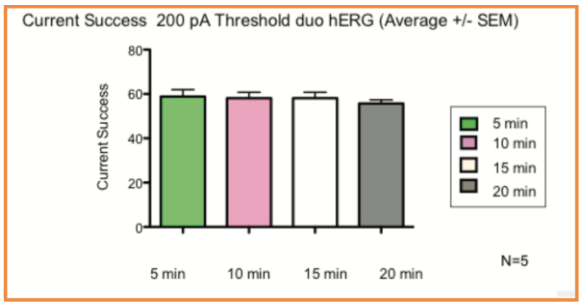


Figure 4: Percentage of hERG current success for 5, 10, 15, and 20 minute time points

Resistance percentage success were measured at 5, 10, 15 and 20 minute time points (see Figure 5 below, (Average +/- SEM). Gigaseal success (with an 800 M Ohm Threshold) were above 50% (N= 5 plates) for all of these time points.

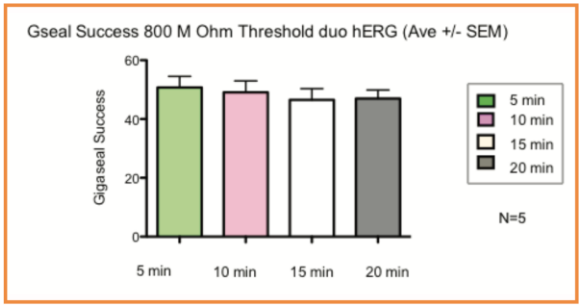


Figure 5: Percentage of hERG Gigaseal current success for 5, 10, 15, and 20 minute time points

In addition, the average resistance values for these same time points are shown in figure 6. The average resistance values were above 1 GigaOhm for all time points.

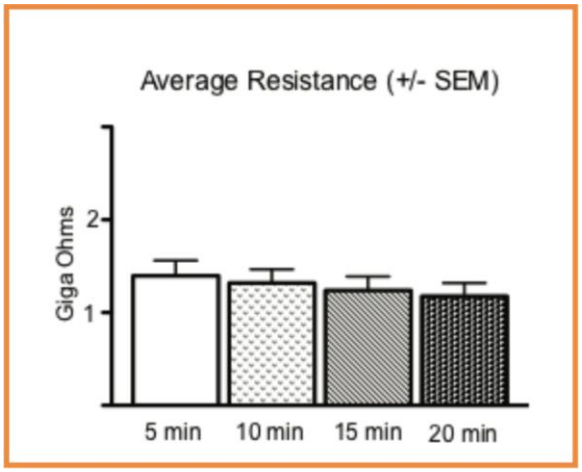


Figure 6: Average resistance values for 5, 10, 15, and 20 minute time points.

hERG Biophysical Characterization

hERG currents were evoked by the following voltage protocol - every 15 seconds, there was a 5 second depolarizing voltage step (from -80mV to +50 mV) followed by a 5 second tail current. A raw current is shown in figure 7.

The hERG steady rate current increased in amplitude until 0mV. At more positive values, the hERG current decreased due to fast inactivation. The I/V relationship was bell shaped (figure 8).

The hERG tail current was stepped from -120mV to -10mV (in 10mV intervals from a holding potential of -80mV. At potentials that were negative to the reversal potential (~-77mV) inward tail currents were observed (figures 9 & 10).

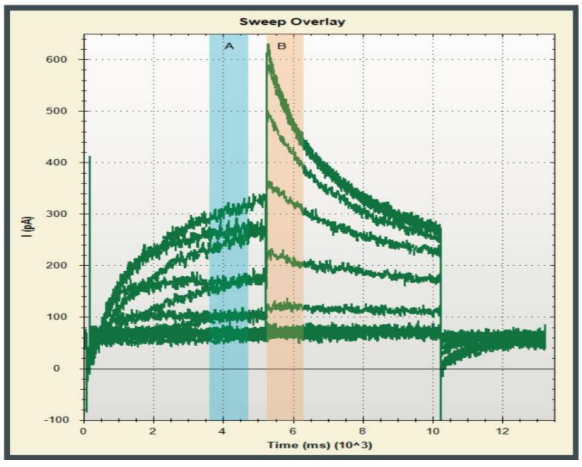


Figure 7: Raw current traces for tail current biophysical characterization. Cursor “A” was used for hERG I/V current characterization whereas cursor “B” was used for tail current characterization

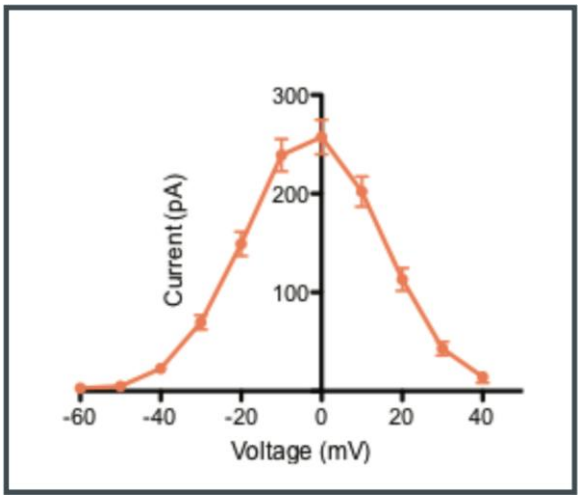


Figure 8: I/V relationship for hERG (mean +/- SEM).

Pharmacology

Verapamil was used to characterize the B’SYS CHO hERG+ pharmacology. The voltage protocol in figure 1 was used for this pharmacological characterization. Verapamil was applied in four increasing concentrations with a 10 fold dilution starting from 0.001 Mm. The compound plate setup is shown below in figure 11.

Example raw current traces from the IonFlux Sweep Overlay window are shown below in figure 12. One trace is shown per application of Verapamil starting with the control (saline) trace. Figure 13 shows an example current versus time plot showing the e ct of increasing applications of Verapamil on hERG current. The steady state values at the end of each application were used for Hill plots.

Pattern Compound	Well	Cell ID	Compound A	Concentration A
P1 - C1	A3	BSYS hERG CHO +	Saline	0
P1 - C2	A4	BSYS hERG CHO +	Verapamil	0.001
P1 - C3	A5	BSYS hERG CHO +	Verapamil	0.01
P1 - C4	A6	BSYS hERG CHO +	Verapamil	0.1
P1 - C5	B6	BSYS hERG CHO +	Verapamil	1
P1 - C6	B5	BSYS hERG CHO +	Verapamil	10

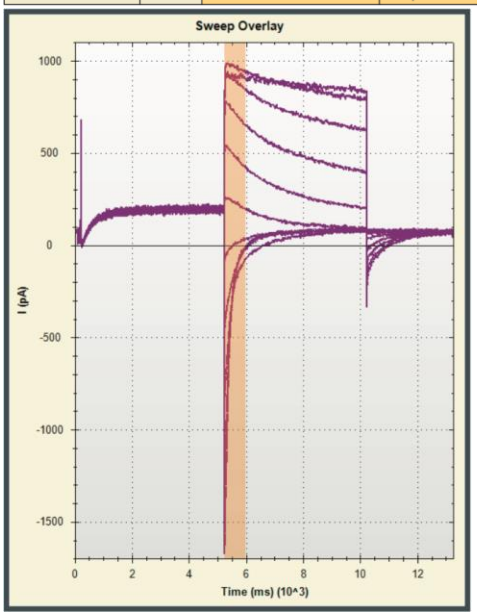


Figure 9: Raw tail current data.

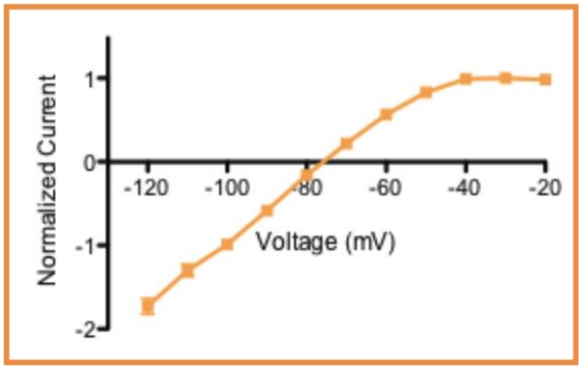


Figure 10: Normalized tail current plotted against voltage. Erev = 77 mV (mean +/- SEM , n=18)

The Hill fit using the average blocker effect in a verapamil 5 point dose response experiment showed an IC50 = 190 nM (N=16). The graph for this t is shown in Figure 14.

Conclusion

CHO hERG cell line was characterized with IonFlux HT single cell plates. The cells showed stable currents with current success above 50%. Run down was minimal for hERG currents. Pharmacology of the hERG current was tested with Verapamil with five increasing concentrations of Verapamil. The 5 dose response experiment for Verapamil showed an IC50 = 190 nM (N=16).

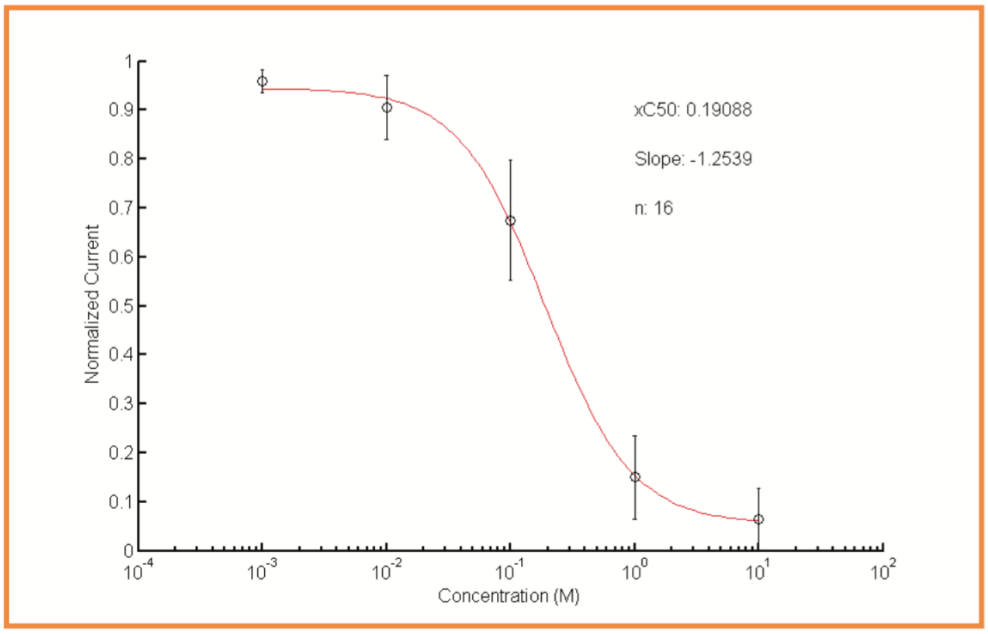


Figure 11: Sample plate compound information for a 4 point dose response Verapamil experiment.

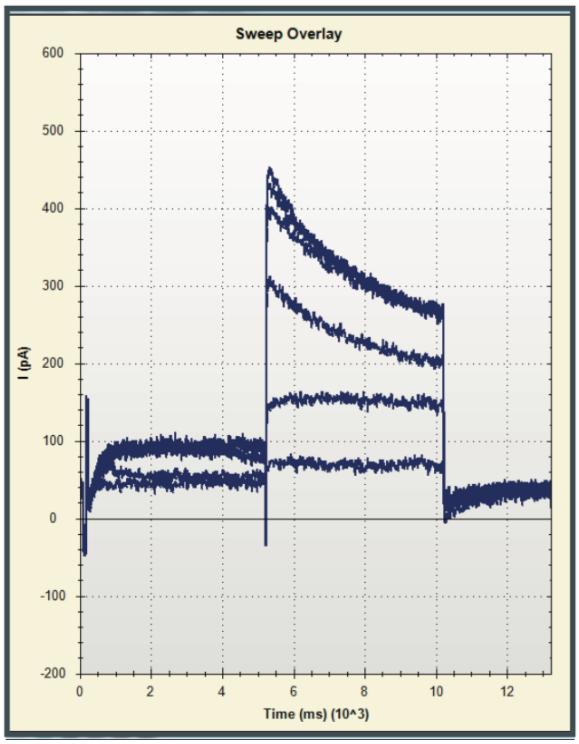


Figure 12: hERG current raw traces. One trace is shown per application of Verapamil (starting with a control saline trace).

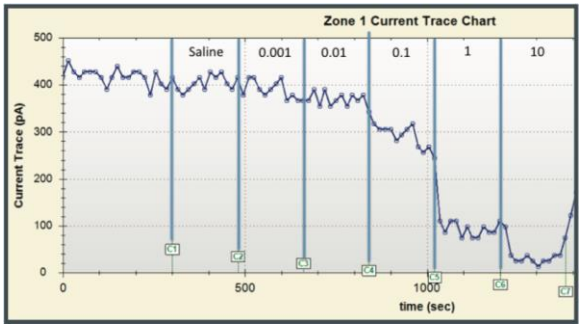


Figure 13: Current versus time plot showing the effect of increasing applications of Verapamil on an example current.

References

Curran ME, et al. (1995). Cell 80, 795-803
Moss AJ, et al. (2002) Circulation 105:794-799
Sanguinetti MC et al. (1995). Cell 81:299-307
Tanaka T, et al.(1997) Circulation 95:565-567
Vandenberg JI, Walker BD, & Campbell TJ. (2001)

Figure 14: Hill plot for the average current in a 5 dose response Verapamil experiment. IC50=190nM (N=16)