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Application Report

Comprehensive *in vitro* Proarrhythmia Assay for CHO hERG on QPatch: Physiological Solutions and Temperature

Assay optimization for increased success rates at elevated temperature (35-37°C) using physiological intracellular solution (seal enhancer free)

Summary

- hERG (K_V11.1) channels play a critical role in cardiac electrophysiology, and off-target interference with hERG channel function can lead to deadly cardiac arrhythmias in the clinic.
- Unintended hERG inhibition is a leading cause of compound attrition and a major liability in drug discovery programs.
- The CiPA initiative (Comprehensive in vitro Proarrhythmia Assay) defines a set of standardized protocols for screening compound effects on hERG channels, including voltage stimulation protocols, recording solutions, and temperature-controlled experiments between 35-37°C.
- Here, we describe an optimized assay for recording CHO hERG on the Sophion QPatch under CiPA-compatible conditions, allowing recordings at 35-37°C for an average of 33.5 ± 2.0 mins.
- Cumulative concentration-response experiments using four hERG inhibitors generated IC_{50} and Hill coefficient values remarkably similar to published manual patch-clamp data, demonstrating the utility of QPatch automated patch-clamp for CiPA-compatible hERG screening at physiological temperature.

Introduction

The human Ether à go go–Related Gene (hERG) encodes the K_V11.1 potassium channel, a critical component of the cardiac rapid delayed rectifier current (I_{Kr}) that governs repolarization of the ventricular action potential[1]. Because I_{Kr} is a major determinant of action potential duration, inhibition of hERG current can delay repolarization and produce QT interval prolongation, potentially leading to torsades de pointes and fatal arrhythmias[2-3]. As a result, hERG liability safety screening has become an essential step in regulatory evaluation for drug discovery[4-6].

Many structurally diverse compounds have been withdrawn or restricted due to unintended hERG inhibition. Early hERG screening helps reduce compound attrition by detecting high-affinity block, state dependent inhibition, or slow unbinding kinetics, all of which increase proarrhythmic risk. Regulatory agencies such as the FDA and ICH require in vitro hERG data as part of preclinical safety pharmacology packages aimed at reducing the likelihood of drug induced long QT syndrome. The CiPA (Comprehensive *in vitro* Proarrhythmia Assay) initiative modernizes proarrhythmia assessment by defining protocols for investigating hERG including a standardized voltage protocol, temperature-controlled experiments (35-37° C), concentration-response measurements for assessing IC_{50}, Hill coefficients, and binding/unbinding kinetics[7-8].

Here, we describe a protocol for using automated whole-cell patch-clamp electrophysiology (Sophion QPatch) to measure hERG under physiological conditions compatible with CiPA methodology. We employ a temperature control unit for recording at high temperature (35-37°C) with a KCl-based physiological intracellular recording solution, and we identify parameters in the automated whole-cell protocol that made significant improvements for experimental longevity at elevated temperature. Finally, we use our approach to measure five-point concentration response curves and calculate IC_{50} and Hill coefficients for four hERG channel inhibitors. Our IC_{50} measurements were remarkably similar to published values from manual patch-clamp experiments, demonstrating the utility of QPatch for generating CiPA and GLP- compatible data.

Results and discussion

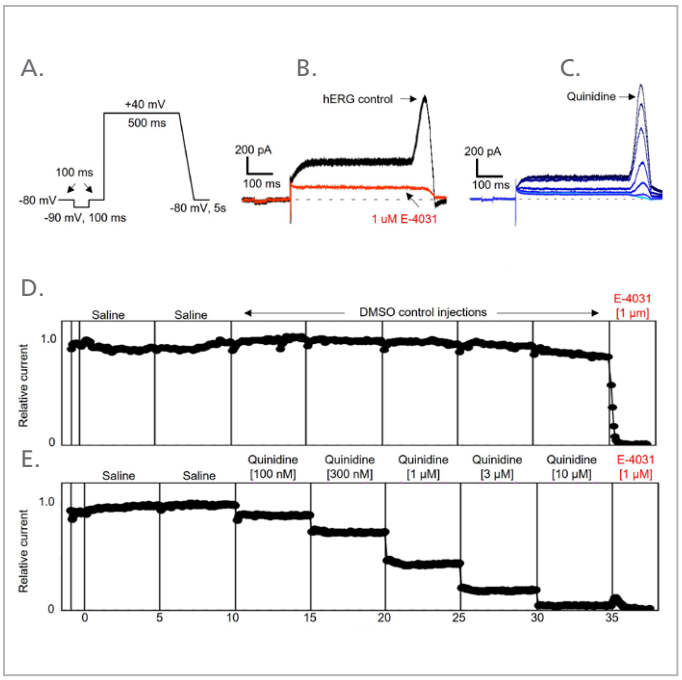

Fig. 1: hERG recordings under CiPA protocol at physiological temperature. **(A)** CiPA aligned voltage protocol consisting of a depolarization to +40 mV (500 ms) followed by a hyperpolarizing ramp to –80 mV. **(B)** Representative hERG currents recorded in control (black) and after application of the reference blocker E 4031 (1 µM, red), confirming current identity. **(C)** Example hERG traces showing concentration dependent inhibition by quinidine. **(D)** Time course of normalized peak hERG current during saline and DMSO control injections, demonstrating current stability prior to reference block. **(E)** Concentration response sequence for quinidine (100 nM–10 µM) showing progressive reduction of peak current, with E 4031 (1 µM) applied at the end of the experiment.

Assay set-up: application and voltage protocols

Whole-cell protocol optimization was performed on the QPatch with Sophion recording solutions EC000 and IC000 to identify key parameters for increasing experimental longevity and success rates at higher temperatures (35-37°C). Through this effort, we improved our whole-cell protocols to achieve high quality recordings of hERG under CiPA conditions (Figure 1). The CiPA voltage stimulation protocol (Figure 1A) was applied every 5 sec, and the peak tail current elicited during the depolarizing ramp (+40 to -80 mV) was monitored over the course of the experiment under control conditions (Figure 1B) or concentration-response experiments (Figure 1C). Liquid additions were performed every 5 mins through the experiment to perfuse either vehicle control (0.1% DMSO) solution or test compounds through the QPlate microfluidic chamber. Current vs. time plots monitoring the peak tail current over time show the stability of control recordings under ideal conditions versus a concentration-response experiment with quinidine (Figure 1D-E). Despite potential stress on the gigaseal from repeated injections, it was possible to maintain the whole-cell seal resistance and recording quality through the experiment, allowing measurement of five cumulative concentrations per cell in concentration-response experiments (Figure 1E). At the end of each experiment, reference inhibitor E-4031 (1 µM) was applied for leak correction, to confirm hERG identity, and to monitor rundown in control recordings.

From replicate experiments on QPatch 48 with single-hole consumables (8 plates, 48 wells per plate, n=384), we formed gigaseals (1.70 ± 0.28 GΩ) in 355 recordings (92.2%), with 299 (77.6%) of total attempted recordings passing quality filters of $R_{membrane}$ >0.1 GΩ and C_{slow} >4 pF (Table 1). For filter-passed recordings, the experiment duration was 33.5 ± 2.0 mins (mean ± SEM), providing a sizeable window for testing multiple cumulative drug concentrations per cell.

Table 1: Summary of experimental replicates at physiological temperature (35°C). Successful WC recordings include wells with $R_{membrane}$ > 0.1 GΩ and C_{slow} > 4 pF. Membrane resistance during gigaseal formation (R_{seal}) and after whole-cell configuration ($R_{whole\text{-}cell}$) and experiment duration is shown as mean ± S.E.M.

JobID	Primed	Cell attach	WC	Filter pass	R seal (GΩ)	R whole-cell (GΩ)	Duration (mins)
1579	48	48	45	39	0.79 ± 0.13	0.67 ± 0.59	41.9 ± 0.9
1580	48	48	44	39	1.17 ± 0.35	0.48 ± 0.04	37.4 ± 2.2
1583	48	47	41	32	1.93 ± 0.27	0.55 ± 0.05	40.4 ± 1.8
1584	48	48	47	40	1.83 ± 0.23	0.91 ± 0.09	27.7 ± 1.8
1585	48	48	41	30	1.25 ± 0.15	0.77 ± 0.06	29.9 ± 2.4
1586 (2 plates)	96	95	91	75	3.23 ± 0.57	1.01 ± 0.09	29.4 ± 2.3
1587	48	47	45	43	2.11 ± 0.62	0.69 ± 0.04	28.1 ± 2.4
Total	384	381 (99.2%)	354 (92.2%)	298 (77.6%)	1.77 ± 0.28	0.73 ± 0.07	33.5 ± 2.0

Whole-cell protocol settings

To improve recording quality at physiological temperature, we identified the following whole-cell protocol parameters as most significant for assay quality: reduced positioning pressure during cell addition, increased negative pressure for gigaseal and whole-cell formation, and increased negative holding pressure for the duration of the experiment (Table 2). These modifications improve recording quality without further addition of components that may interfere with IC_{50} measurements in hERG screening, such as fluoride, pluronic, or BSA. However, these experiments will typically have lower success rates than room temperature or fluoride-based assays, and initial screening under these conditions can be informative for counter-screening before advancing to more difficult CiPA-compatible assays with lower success rates.

Table 2: Whole-cell protocol parameters for recording at 35°C. These parameters were optimized for Sophion CHO-hERG (K_V11.1) stable cell line using Sophion EC000 and IC000 solutions.

Protocol Category	Parameter	Default	High temperature
Gigaseal requirements	Holding pressure	-20 mbar	-35 mbar
Whole-cell requirements	Holding pressure	-20 mbar	-35 mbar
Cell positioning	Positioning pressure	-100 mbar	-70 mbar
Gigaseal method	Allowed pressure (min – max)	-130 to -20 mbar	-130 to -35 mbar

Cell culture optimization

Success rates were also dependent on cell quality. Cells were grown to ~60-70% confluency and split every 2-3 days to prevent overgrowth, which can be detrimental for hERG expression and current density in stable lines. From 298 cells (77.6%) passing recording quality filters, 205 cells expressed hERG current for concentration-response experiments (68.8% of filter-passed cells; 53.4% overall). Therefore it is imperative to ensure the proportion of cells expressing hERG among total filter-passed cells is maximized for high throughput screens. This can be achieved through careful cell culture technique, with some specific tips for preparing cells in automated patch-clamp experiments (see: Sophion cell culturing for automated patch clamp).

Pharmacology: Cumulative concentration-response experiments

To test our assay, we measured four reference hERG blockers (cisapride, dofetilide, quinidine, verapamil) in cumulative concentration-response experiments under high temperature CiPA conditions. Cells were recorded in control with two liquid additions of saline to ensure current stability, followed by five injections of increasing concentrations of inhibitor. Representative current traces from each experiment group are shown in Figure 2A. Cells that passed quality filters were normalized to the peak current in the final saline liquid period before compound addition, and relative inhibition was averaged across recordings and fit with a modified Hill Fit to calculate each IC_{50} and Hill coefficient (Figure 2B; Table 3)[8]. Of the 205 cells that both passed recording quality filters and expressed hERG current, we collected five-point concentration response data from 122 cells. This indicates a 59.5% success rate for completing 30+ min concentration-response experiments at 35-37°C among the cells that expressed hERG current. This demonstrates that despite the challenges posed by elevated temperature, which typically impacts assay duration, cumulative concentration-response relationships were achievable with reasonable success rates.

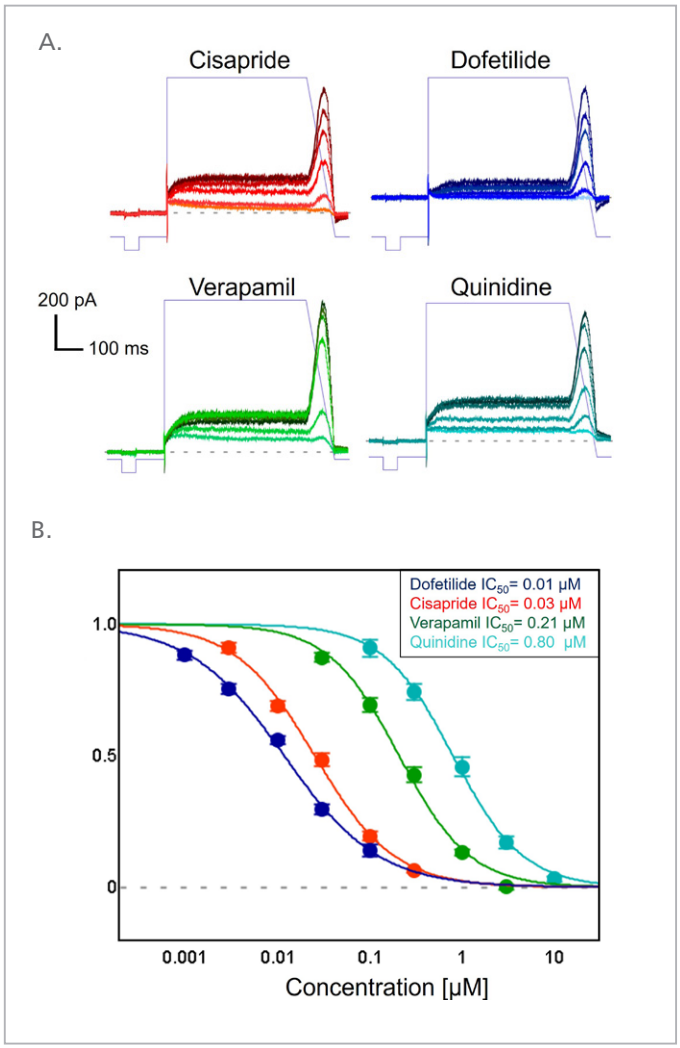

Fig. 2: Concentration–response experiments for four hERG reference compounds at physiological temperature. **(A)** Representative hERG current traces recorded at 35°C using the CiPA voltage protocol (thin blue line) during cumulative concentration–response experiments for cisapride (red), dofetilide (blue), verapamil (green), and quinidine (teal). Increasing concentrations produced a progressive reduction of the peak tail current following repolarization, consistent with concentration dependent block of hERG. **(B)** Normalized peak current plotted against concentration for each compound with group Hill fits. The IC_{50} values were estimated from each Hill fit: dofetilide (0.01 µM), cisapride (0.03 µM), verapamil (0.21 µM), and quinidine (0.80 µM). These values are similar to published manual patch-clamp data (Table 3).

Table 3: Summary of hERG concentration-response experiments at 35-37°C. IC_{50} values were calculated from group Hill fits (Figure 2B). Reference IC_{50} values were obtained from the literature. N values reflect quality-filtered recordings ($R_{membrane}$ >0.1 GΩ and C_{slow} >4 pF) used in the final analysis.

Compound	IC_{50} [μM]	Reference IC_{50} [μM]	Hill Coefficient	n (total=122)
Control	-	-	-	32
Cisapride	0.03	0.010 [9]	1.01	10
Dofetilide	0.01	0.013 [10-11]	0.85	28
Quinidine	0.80	0.8 [12]	1.15	20
Verapamil	0.21	0.25 [13]	1.14	32

Conclusion

In this report, we describe our approach for performing CiPA-compatible hERG assays at physiological temperature on the Sophion QPatch system. By applying a modified whole-cell protocol, it was possible to record CHO-hERG at 35-37° C with a success rate of 77.6%. The elevated temperature challenges the longevity of the experiments; however, it was possible to record for 35-40 mins and conduct cumulative concentration-response experiments in 59.5% of recorded cells. IC_{50} measurements on QPatch were similar to published manual patch-clamp data at physiological temperature, supporting the utility of Sophion QPatch technology as another tool for accurate measurements of hERG cardiac liability.

Methods

Cells culture & preparation: Cells were cultured according to the Sophion SOP. CHO cells heterogeneously expressing hERG (K_V11.1) channel were kindly provided by B'SYS GmbH. The cells were harvested using Detachin™ (Genlantis) and transferred to serum-free medium (EX-CELL® ACF CHO Medium, Sigma-Aldrich) supplemented with 25 mM HEPES, 40 μg/mL trypsin inhibitor, and penicillin/streptomycin. Cells were washed and resuspended in Sophion EC000 extracellular buffer.

Patch clamp experiments: All patch clamp experiments were carried out using the QPatch or Qube system (Sophion Bioscience A/S, Denmark).

Solutions: Extracellular solution (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose; pH 7.4, 300 mOsm. Intracellular solution (in mM): 120 KCl, 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 4 Na_2-ATP; pH 7.2, 295 mOsm.

Compounds: All compounds were dissolved in 100% DMSO and diluted 1000 times in extracellular solution to make working solutions at each test concentration.

Whole-cell protocol: The whole-cell configuration was established using a modified whole-cell protocol for CHO cells (#CHO – High Temperature Assay).

Voltage protocols: The voltage stimulation protocol proposed by the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative was used in this study. The protocol consisted of a +40 mV depolarizing pulse of 500 ms followed by a 100 ms hyperpolarizing ramp down from +40 mV to -80 mV, repeated in 5 sec intervals. Cells were held at -80 mV between sweeps.

Test procedure and temperature control: During the experiment, the temperature at the recording site was clamped using water-circulation temperature control system. The temperature at recording site was held at 35°C during the whole cell process and subsequent experiment. After establishment of a whole-cell configuration, vehicle control solution (0.1% DMSO extracellular solution) was added, and then the voltage protocol was executed to measure the tail current as a baseline. Subsequently, compound solutions were added cumulatively at 5 concentrations, and the tail currents at each concentration were recorded. At the end of experiment, 1 μM E-4031 solution was applied to block the hERG current and used as a reference.

For information on dissociation procedures and solutions contact us at info@sophion.com.

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