Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines

CONTEXT OF USE

As it is anticipated that nonclinical ion channel data will play an important role for regulatory decision-making in drug development programs, standardized protocols are recommended. The following contains detailed voltage protocol recommendations for hERG, $Ca_V1.2$, and $Na_V1.5$ channel studies using patch clamp method. These recommendations are based on current knowledge, and are expected to evolve over time. Therefore, the document is time-stamped for version control. We encourage you to verify with the FDA prior to initiating the studies to ensure that the document you have is up-to-date and to clarify questions including which protocol(s) to test for a specific drug.

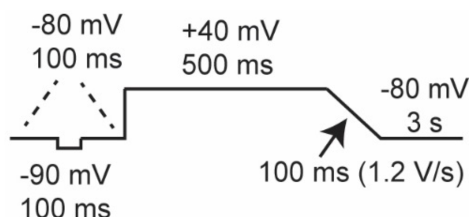
For torsade de pointes risk assessment as defined by the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, drug effects should initially be evaluated on hERG, $Ca_V1.2$ and late $Na_V1.5$ currents. Drug effects on additional currents including but not limited to peak $Na_V1.5$ and $K_V7.1$/minK currents may be requested by the review Division on a case-by-case basis to address cardiac safety concerns.

ION CHANNEL PROTOCOLS

Drug block of ion channels is sensitive to voltage protocol, recording temperature, and additional experimental factors. Therefore, standardized protocols are recommended for each ionic current to allow for data interpretation. Because most ionic currents measured in whole cell configuration exhibit time- and/or activity-dependent change in characteristics following whole cell formation, baseline current stability in control solution must be recorded and achieved prior to drug application for accurate assessment of drug effects.

HERG current protocol to assess IC_{50} only

Data derived from this protocol are intended for safety margin calculation (IC_{50} / free C_{max}). Manual or automated patch clamp experiments on hERG1a-expressing cells may be performed at room temperature, and seal resistance must be >1GΩ. This voltage protocol is approximately 5 s in duration, and is to be repeated every 5 s. The voltage "ramp down" phase is 100 ms in duration, from +40 mV to -80 mV (hence a voltage change of 1.2 V/s). The small hyperpolarizing voltage pulse from -80 to -90 mV is used to calculate input resistance according to Ohm's law. Quality of the recorded cell and ongoing experiment integrity should be reflected in stable holding current (associated with the -80 mV step just prior to the depolarizing voltage step) and input resistance. If high seal resistance is obtained, then holding current and input resistance may be used as indicators of cell health and are expected to remain stable throughout the experiment.



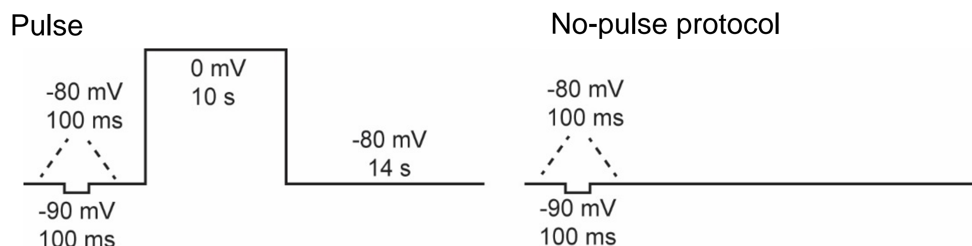
The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 5 KCl, 1 $MgCl_2$*$6H_2O$, 1 $CaCl_2$*H_2O, 12.5 dextrose; pH adjusted to 7.4 with 5 M NaOH; ~280 mOsM. For internal solution, the following is recommended (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 5 EGTA, 1.5 MgATP; pH adjusted to 7.3 with 1 M KOH; ~280 mOsM. The use of these solutions will result in ~15 mV liquid junction potential, and the command voltage step should take this into account. For example, to set the command voltage at -80 mV, -65 mV should be used. Series resistance compensation is recommended but not required for this current and protocol if high GΩ seal and low leak current is achieved. Data should be filtered at 2 kHz and then digitized at 5 kHz. For positive control, 1 μM E-4031 should be applied to some cells to show the % of residual current not attributable to hERG channels evoked by this protocol.

To ensure baseline recording is stable enough for drug application, cells should be presented with this protocol in control solution until hERG current amplitudes for 25 consecutively recorded current traces exhibit <10% difference. Then drugs may be applied as the voltage pulse continues. Drug effect should be monitored until steady state hERG current suppression is obtained, and each cell may be exposed to up to two concentrations of drugs as long as cell properties (as defined by holding current at -80 mV and input resistance) remain stable.

The amplitude of hERG current is defined as the peak outward current measured during the ramp down phase minus the holding current at -80 mV (just prior to the depolarizing step to +40 mV). To quantify drug potency against hERG channels using this protocol, the steady state hERG current amplitude (averaged value from 5 current traces) in drug solution should be divided by the averaged amplitude from the last 5 traces measured in control solution just prior to drug application to calculate the fractional block. Then fractional block is to be plotted against drug concentration tested, and the data fit with the Hill Equation to generate an IC_{50} and the Hill coefficient. In terms of data summary, FDA requires submission of each individual cell's fractional block value to estimate the variability of experimental data and quantify the uncertainty of calculated block potency parameters.

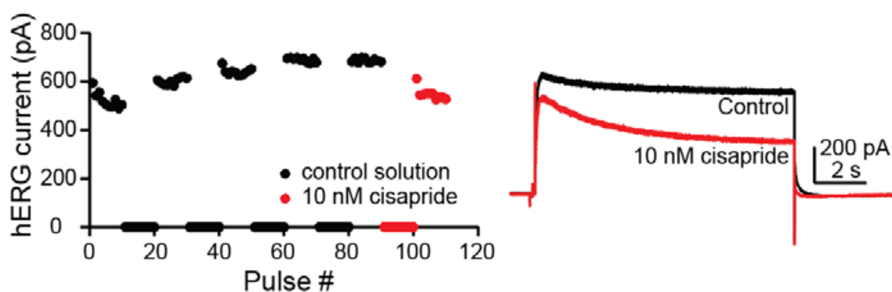
HERG current protocol to assess drug block kinetics and IC_{50} calculation

Manual patch clamp experiments on hERG1a-expressing cells should be performed at 37°C using the "pulse" and "no-pulse" protocol as shown below. Deviation from this recommendation should be discussed with the FDA prior to initiating the studies.



The pulse protocol is used to activate outward current including hERG current, and contains a 10-s depolarizing voltage step from a holding potential of -80 mV to 0 mV. The no-pulse protocol is used to maintain hERG channel closure. Each protocol is approximately 25-s long, and is repeated every 25 s. Both protocols contain a 100 ms hyperpolarizing voltage step from -80 to -90 mV. Current response to this hyperpolarizing step is used to calculate input resistance throughout the experiment.

To ensure baseline recording in control solution is stable enough for drug application, cells should be alternately presented with 10 repeats of the pulse protocol (to measure activity-dependent changes) followed by 10 repeats of the no-pulse protocol (to assess activity-independent changes) in control solution until peak (measured at the beginning of the 10-s depolarizing step) and steady state hERG current (measured at the end of the 10-s depolarizing step) reaches stability, defined as <10% difference in current amplitude for 2 consecutive blocks of hERG current recordings (or 20 hERG current traces). Then individual drugs should be bath-applied during the subsequent 10 repeats of the no-pulse protocol at a flow rate of 2.5-3 mL/min to ensure adequate solution exchange, and drug effects on hERG channels assessed with 10-20 repeats of the pulse protocol. Note that one cell should be exposed to only one drug concentration. The figure below illustrates the time course plot of a representative experiment recorded from one cell, using cisapride as the test compound.

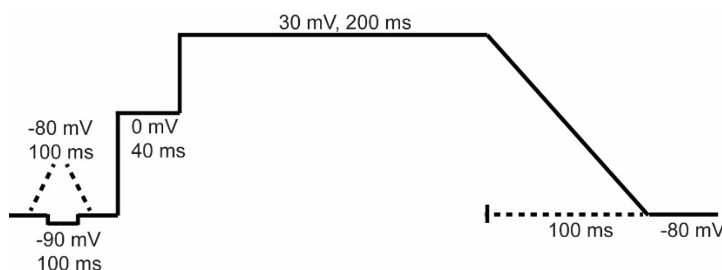


To quantify drug potency against hERG channels, the steady state hERG current amplitude from the last 1-5 hERG current traces (depending on when drug effect reaches steady state) should be compared to the amplitude from the last 5 traces measured in control solution just prior to drug application. The amplitude of hERG current at steady state is defined as the outward current measured at the end of the 0 mV step minus holding current at -80 mV. IC_{50} and Hill coefficient of steady state current block should be reported.

The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 5 KCl, 1 $MgCl_2*6H_2O$, 1 $CaCl_2*H_2O$, 12.5 dextrose; pH adjusted to 7.4 with 5 M NaOH; ~280 mOsM. For internal solution, the following is recommended (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 5 EGTA, 1.5 MgATP; pH adjusted to 7.3 with 1 M KOH; ~280 mOsM. The use of these solutions will result in ~15 mV liquid junction potential, and the command voltage step should take this into account. For example, to set the command voltage at -80 mV, -65 mV should be used. Series resistance compensation is recommended. Data should be filtered at 2 kHz and then digitized at 5 kHz. For positive control, 1 μM E-4031 should be applied to some cells to show the % of residual current not attributable to hERG channels evoked by this protocol.

Ca_V1.2 current

Manual or automated patch clamp experiments on Ca_V1.2 channels expressed in recombinant cell line may be performed at room temperature using the following protocol. Seal resistance must be >1GΩ. As with hERG current recording, the small hyperpolarizing step from -80 to -90 mV allows for input resistance calculation for every recorded current trace. If high seal resistance is achieved, then holding current and input resistance may be used as indicators of cell quality and should remain stable throughout the experiment. This protocol is repeated every 5 s in control solution until Ca_V1.2 current amplitude reached stability for at least 2 minutes. Then drugs may be bath applied as the protocol continues. Each cell may be exposed up to two drug concentrations if cell quality remains stable.

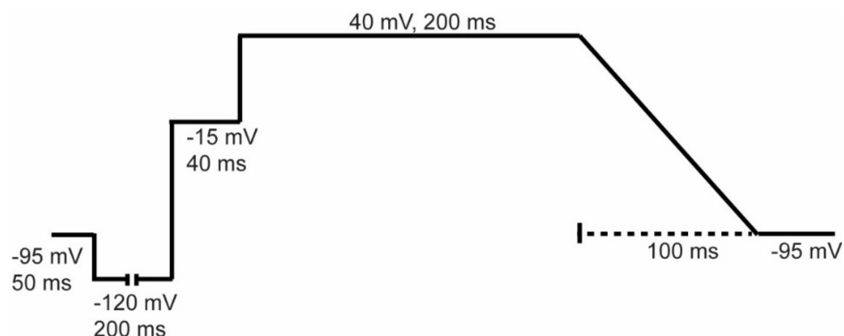


$Ca_V1.2$ current amplitude (leak current-subtracted and not raw value) is measured at two places – as the peak inward current at the 0 mV step and as the peak inward current evoked at the "ramp down" phase (+30 to -80 mV ramp down in 100 ms).

The following external solution is recommended (in mM): 137 NaCl, 10 HEPES, 4 KCl, 1 $MgCl_2*6H_2O$, 1.8 $CaCl_2*H_2O$, 10 dextrose; pH adjusted to 7.4 with 5 M NaOH. Solution flow rate may be set by the sponsor and must be reported. For internal solution, the following is recommended (in mM): 120 Aspartic Acid, 120 CsOH, 10 CsCl, 10 HEPES, 10 EGTA, 5 MgATP, 0.4 TrisGTP; pH adjusted to 7.2 with 5 M CsOH; ~290 mOsM. Liquid junction potential is expected to be ~17 mV and should be accounted. For example, to hold the cell at -80 mV, the command voltage should be -63 mV. Series resistance compensation is required, with % compensation noted at the beginning of the experiment and readjusted as changes occur throughout the experiment (% compensation should be reported). Data should be filtered at 3 kHz and then digitized at 10 kHz.

Late $Na_V1.5$ current

These experiments may be performed at room temperature using manual or automated patch clamp platform. Seal resistance must be >1GΩ. Late $Na_V1.5$ current should be studied using the voltage protocol show below. To induce late $Na_V1.5$ current, 150 nM ATXII should be used.



This protocol is repeated every 10 s until late $Na_V1.5$ current reaches stability for at least 2 minutes. Then the test compound may be applied as the protocol continues. If possible, at the end of the experiment 30 μM TTX should be applied to show that the current measured is mediated by $Na_V1.5$ channels. Late current (leak-subtracted offline and not raw value) is measured at two places – at the inward current at the end of the -15 mV step and as the peak inward current at the "ramp down" phase. If TTX cannot be applied to cells after the test compound, then it should be tested in a subset of cells independently to demonstrate the % inward current that is mediated by $Na_V1.5$ channels.

The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 4 CsCl, 1 $MgCl_2$*6H_2O, 2 $CaCl_2$*H_2O, 10 dextrose; pH adjusted to 7.4 with 5 M NaOH; ~281-287 mOsM. For internal solution, the following is recommended (in mM): 130 CsCl, 7 NaCl, 1 $MgCl_2$*6H_2O, 5 HEPES, 5 EGTA, 5 MgATP, 0.4 TrisGTP; pH adjusted to 7.2 with 5 M CsOH; ~290 mOsM. Series resistance compensation is required, with % compensation noted at the beginning of the experiment and readjusted as changes occur throughout the experiment (% compensation should be reported). Data should be filtered at 3 kHz and then digitized at 10 kHz.

Peak Na_V1.5 current

These experiments may be performed at room temperature using manual or automated patch clamp platform. Peak Na_V1.5 current is studied using the same voltage protocol and internal/external solutions as the late Na_V1.5 current. ATXII is not used in these experiments. Note that peak Na_V1.5 current data should not be derived from the same cells/recordings as the late Na_V1. 5 current experiments as the presence of ATXII in the latter complicates data interpretation. Peak Na_V1.5 current should be recorded in control solution until it reaches stability for at least 2 minutes. Then the test compound should be applied as the protocol continues. If possible, at the end of the experiment 30 μM TTX should be applied to show that the current measured is mediated by Na_V1.5 channels. If TTX cannot be applied to cells after the test compound, then it should be tested in a subset of cells independently as positive control. Peak Na_V1.5 current is measured as the inward current at the -15 mV step. Absolute inward current amplitude may be used here to quantify drug effects. For these experiments, data should be filtered at 5 kHz and then digitized at 20 kHz.

Recommended reference drugs

Reference drugs should be included for each current to establish assay sensitivity and accuracy. Multiple nominal concentrations should be included to allow for estimation of the drug binding kinetics and potencies (IC_{50}/Hill coefficient) for hERG channels, and potencies for Ca_V1.2, and peak and late Na_V1.5 currents. If the drug binding kinetics and/or potencies (IC_{50}s/Hill coefficients) of the reference drugs are sufficiently different from the historical values obtained in-house for the development of CiPA, then additional drugs may be requested to aid data interpretation. For the hERG current protocol used to assess block kinetics and IC_{50}s, a minimum of two reference drugs is needed to evaluate the assay's performance in estimating the kinetics of block achieved by drugs with distinct physiochemical and electrophysiological properties. We recommend astemizole (1, 3, 10, and 30 nM) and verapamil (30, 100, 300, and 1000 nM) for hERG current assessed with this protocol. For the hERG current protocol used to assess IC_{50} only, we recommend verapamil (30, 100, 300, and 1000 nM). For Ca_V1.2 current, we recommend verapamil (30, 300, 3000, and 30000 nM). For peak Na_V1.5 current, we recommend flecainide (1000, 3000, 10000, 30000 nM). For late Na_V1.5 current, we recommend ranolazine (1000, 5000, 30000, and 100000 nM). For each concentration, 4 – 7 cells are recommended to replicate the assay to aid in evaluation of reproducibility.

DATA SUBMISSION

The electronic common technical document (eCTD) is CDER's standard format for electronic regulatory submissions (http://www.fda.gov/ectd). We encourage you to consult with the FDA about the current supported data formats for electrophysiology recordings prior to initiating data transfer. Regulatory submission will include data that allow for reconstruction of the original electrophysiology recordings (unaltered, i.e., no baseline zeroing) Additionally, the cell line used, its source, and exact ion channel-related proteins expressed (both alpha and auxiliary subunits if any) must be documented in the assay report for each current assessed.

REFERENCES

Li Z, Dutta S, Sheng J, Tran PN, Wu W, Chang K, Mdluli T, Strauss DG and Colatsky T. Improving the In Silico Assessment of Proarrhythmia Risk by Combining hERG (Human Ether-a-go-go-Related Gene) Channel-Drug Binding Kinetics and Multichannel Pharmacology. *Circ Arrhythm Electrophysiol*. 2017;10:e004628.

Sheng J, Tran PN, Li Z, Dutta S, Chang K, Colatsky T and Wu WW. Characterization of loperamide-mediated block of hERG channels at physiological temperature and its proarrhythmia propensity. *Journal of pharmacological and toxicological methods*. 2017.