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, methods for data quality assessment, and data analysis plans to quantify drug effects are recommended. The following contains detailed voltage protocol recommendations for hERG, CaV1.2, and NaV1.5 channel studies using patch clamp method to support an evaluation of torsade de pointes risk using the Comprehensive in vitro Proarrhythmia Assay (CiPA). 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: 1) ensure that the document you have is up-to-date; 2) clarify which protocol(s) to test for a specific drug; and 3) address additional questions.

Note that drug effects on additional cardiac ionic currents may be requested by the review Division on a case-by-case basis to address cardiac safety concerns. With the exception of the hERG protocol, protocols to study other ionic currents are not routinely requested by the review division.

ION CHANNEL PROTOCOLS TO ASSESS IC_{50}

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 and data quality assessment. Data quality here is defined by cell health, recording quality, and stability of ionic current measured for the duration of individual experiments. Cell health and recording quality are defined by passive membrane properties including holding current and input resistance measured at rest or holding potential. 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. Recording temperature should be done at physiological temperature (~37°C) or as close to physiological temperature as possible unless stated otherwise.

HERG current protocol

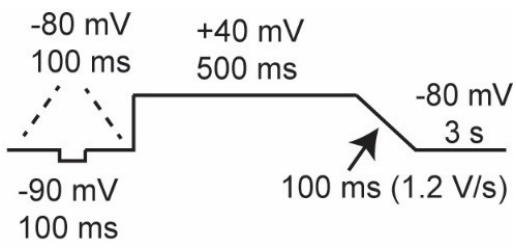
Data derived from this protocol are used to understand the relationship between drug potency on affecting hERG current and therapeutic exposure level. Seal resistance should be ≥1GΩ. This voltage protocol is approximately 5 s in duration, and is to be repeated every 5 s. The voltage “ramp down” phase 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 following initial whole cell dialysis period for the remaining duration of the experiment.

The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 5 KCl, 1MgCl2*6H2O, 1 CaCl2*H2O, 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. If possible, 1 µM E-4031 should be applied to the recorded cells at the end of the experiments to show the % of residual current not attributable to hERG channels evoked by this protocol.

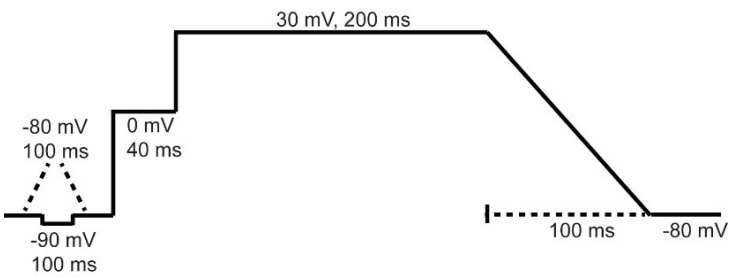
To ensure the baseline recording is stable enough for drug application, cells should be presented with this protocol in control solution until hERG current amplitudes for 25 consecutively recorded current traces exhibit <10% difference. Then drugs may be applied as the voltage pulse continues. Drug effect 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. It is recommended that at least 4 concentrations are tested for each drug if possible, and that % current inhibition associated with these concentrations range from ~20% to 80 % inhibition to allow better estimation of the concentration associated with 50% current inhibition (IC50).

HERG current is measured as the peak outward current during the ramp down phase. To quantify drug potency against hERG channels using this protocol, the steady state hERG current amplitude (averaged value from 5 consecutive 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 addition, a table with each individual cell's fractional block value to estimate the variability of experimental data and quantify the uncertainty of calculated block potency parameters should be provided.



Ca$_V$1.2 current

Adequate voltage control and series resistance compensation is necessary for these experiments. 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 CaV1.2 current amplitude reached stability for at least 30 traces. 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.

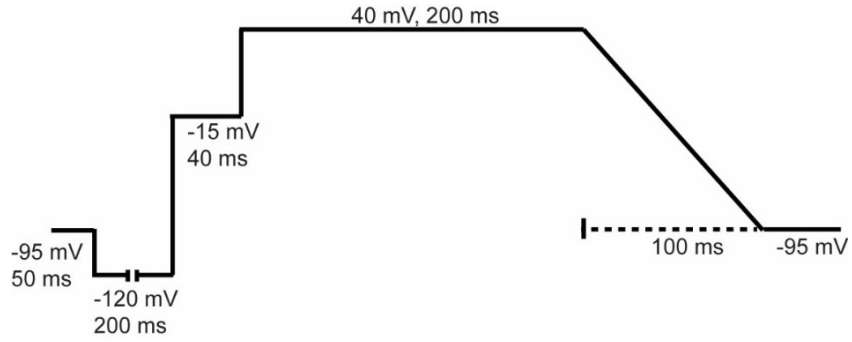


$Ca_V1.2$ current amplitude (leak current-subtracted and not raw value) is measured at two places – as the peak inward current at the 0 mV step and as the peak inward current evoked at the "ramp down" phase (+30 to -80 mV ramp down in 100 ms). If possible, at the end of the experiment 100 µM verapamil should be applied to show that the current measured is mediated by CaV1.2 channels.

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

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 maintains stability for at least 12 consecutive traces. 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 MgCl2*6H2O, 2 CaCl2*H2O, 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 MgCl2*6H2O, 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$_V$1.5 current

Adequate voltage control and series resistance compensation is necessary for these experiments. Peak NaV1.5 current is studied using the same voltage protocol and internal/external solutions as the late NaV1.5 current. Note that peak NaV1.5 current data should not be derived from the same cells/recordings as the late NaV1. 5 current experiments as the presence of ATXII in the latter complicates data interpretation. Therefore, ATXII is not used in peak NaV 1.5 current experiments. Peak NaV1.5 current should be recorded in control solution until current. 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 NaV1.5 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 NaV1.5 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 tested to allow for estimation of drug potencies against ion channel-of-interest. Drug concentrations should span from achieving 20% to 80% inhibition if possible. For the hERG current, we recommend cisapride, terfenadine, or dofetilide. For CaV1.2 currents, we recommend verapamil. For peak NaV1.5 current, we recommend flecainide. For late NaV1.5 current, we recommend ranolazine. For each concentration, 4 – 7 cells are recommended to replicate the assay to aid in evaluation of data 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

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. doi: 10.1016/j.vascn.2017.08.006.

Wu M, Tran PN, Sheng J, Randolph AL, and Wu WW. Drug potency on inhibiting late Na^+ current is sensitive to gating modifier and current region where drug effects were measured. *Journal of pharmacological and toxicological methods*. 2019. doi: 10.1016/j.vascn.2019.106605.