# 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, the use of standardized protocols, methods for data quality assessment, and data analysis plans to quantify drug effects are recommended. The following contains a description of best practice considerations for ion channel studies based on ICH S7B draft Q&As section 2.1 (dated 27 August 2020) and detailed voltage protocol recommendations for hERG, $Ca_V1.2$, and $Na_V1.5$ channel studies using patch clamp method to support an integrated risk assessment of the potential of a drug to induce torsade de pointes and/or other types of arrhythmias. 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 Agency 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 evaluated using additional protocols or on additional cardiac ionic currents not mentioned in this document may be requested by the review division on a case-by-case basis to address cardiac safety concerns.

## BEST PRACTICE CONSIDERATIONS

### *Positive and negative controls*

Positive controls should be included for each current to establish assay sensitivity and accuracy. Multiple nominal concentrations (4) 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. For the hERG current, we recommend cisapride, terfenadine, or dofetilide. For $Ca_V1.2$ currents, we recommend verapamil. For peak $Na_V1.5$ current, we recommend flecainide. For late $Na_V1.5$ current, we recommend ranolazine. For each concentration, the number of cells should be based on the data variability. A general recommendation is to obtain 4 – 7 cells to facilitate evaluation of data reproducibility.

Vehicle (negative) controls should be included in the study to provide an assessment of recording stability and level of background current. Therefore, duration of the vehicle control experiments should be that of test article experiments, and a full blocker should be added at the end of the experiment. For hERG current, E4031 (0.5 – 1 μM) is recommended; $Ca_V1.2$, verapamil (100 μM); and $Na_V1.5$, tetrodotoxin or TTX (30 μM). The vehicle should include all non-compound materials in the test article solution such as solubilizing agents, impurities, and preservatives if possible.

### *Recording temperature*

The effects of some drugs on cardiac ion channels are temperature-sensitive. Patch clamp experiments on cells overexpressing ion channels should be performed at physiological temperature (37°C) or near physiological temperature (35 – 37°C). Bath temperature should be measured and monitored by a thermistor placed inside of the cell chamber. Temperature should be recorded for the duration of the experiment along with the current traces by connecting the output of the temperature controller to the digitizer.

### *Recording quality*

Seal resistance should be high enough so that the leak conductance at all voltages specified by the voltage protocol and series resistance do not compromise voltage control. If series

resistance compensation is implemented, then % compensation applied at the beginning of the experiment and any subsequent readjustment to accommodate series resistance change should be reported.

A sufficient number of traces should be recorded in control solution to illustrate that steady state level is achieved prior to drug application. Likewise, drug effect should be monitored until steady state is reached. These aspects of recording quality can be inspected by constructing current amplitude vs. time (I-T) plots for individual cells in the assessment of drug effects. Thus, all raw and unaltered waveforms should be included in the submission (see “Data Submission” below).

### *Concentration verification and sample collection method*

The concentrations of drug to which the cells were exposed should be verified by a validated analytical method. Solution samples should be collected from the cell chamber to allow for an understanding of what drug concentrations the recorded cells were exposed to. In terms of sample collection method, samples may be collected during *real* experiments (collected after steady state inhibition is reached) or *satellite* experiments. In a satellite experiment, the electrophysiology perfusion apparatus and temperature controller should be set up as that in a real experiment, and drug perfusion duration should approximate the real experiment prior to sample collection. Sample collection details are expected in the nonclinical study report.

Emerging data seen by the Agency show that deviations between nominal and actual concentrations occur for a range of molecules in a drug- and perfusion system-dependent manner. Therefore, details regarding how the drug is perfused in the real experiment as well as duration of drug perfusion should be documented in the nonclinical study report. These details include a description of the perfusion system (e.g. constant flow, recirculating, or static in which drug is directly applied to the cell chamber, etc). Both nominal and measured concentrations should be reported and used to construct the concentration-inhibition graphs for estimation of $IC_{50}$ and Hill coefficients. When measured concentrations are used to estimate drug potency, describe whether exposures to individual cells are adjusted (using concentrations recovered from real samples), or nominal concentrations are adjusted using an average % deviation (using concentrations recovered from satellite samples).

### *Voltage control for recording $Na_V1.5$ and $Ca_V1.2$ currents*

At physiological temperature, peak $Na_V1.5$ and $Ca_V1.2$ currents occur extremely fast. Therefore, it is essential to demonstrate adequate voltage control in these experiments. Before starting the pharmacology experiment, the current-voltage (I-V) relationship (e.g. from -60 mV to 40 mV in 5 mV increment) should be established in the recorded cell to rule out voltage escape. Cells with inadequate voltage control, as demonstrated by a non-graded increase in current amplitude in response to voltage step increments should not be used in pharmacology experiments.

### *Data summary and reporting*

The primary endpoints are half inhibitory concentration or $IC_{50}$ value (reported in both micromolar and ng/mL units) and the Hill coefficient. The 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, along with the mean values of $IC_{50}$ and the Hill coefficient (and appropriate measures of data variability). If ≥50% current inhibition cannot be achieved (e.g. solubility issue, disruption of recording, etc), then the reason should be explicitly stated in the nonclinical study report.

To demonstrate recording quality in the experiment, the study report should contain example

traces of specific currents and examples of time-course plot of current amplitude, input resistance, and holding current for individual cells, in the absence and presence of test drug. If time-dependent changes such as current run-up or run-down in baseline condition were corrected for drug inhibition estimation, the correction method applied should be described. Current subtraction method used - using a full blocker at the end of the experiment or calculated passive/leak current - should also be described.

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.

### *Data Submission*

The electronic common technical document (eCTD) is CDER's standard format for electronic regulatory submissions (http://www.fda.gov/ectd).

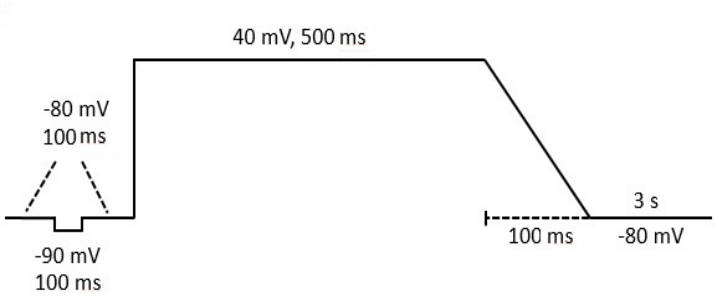
We recommend submitting the following information for each ion channel experiment:

1. Raw and unaltered electrophysiology records (e.g. no baseline subtraction or zeroing of baseline). The file format for the raw electrophysiology records should be in xls, xlsx or xpt format, and contain at a minimum information about time, voltage and current signals (note specific units for these signals).
2. An overview file, e.g. in xls, xlsx, xpt or txt, describing the experimental conditions for each of the raw electrophysiology records. The description should include at a minimum the name of the file, temperature of the recording, when drugs and at what concentrations were added, current subtraction method used, and other information relevant to interpret the results.

## ION CHANNEL PROTOCOLS TO ASSESS DRUG POTENCY ($IC_{50}$)

This section provides recommendations of standardized voltage protocols and internal/external solutions for each ionic current. The following recommendations only include information specific to that protocol. Best practice recommendations stated above should be followed as well 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 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, 1 $MgCl_2$*$6H_2O$, 1 $CaCl_2$*$2H_2O$, 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. Data should be filtered at 2 kHz and then digitized at 5 kHz. It is recommended that 0.5 - 1 μM E-4031 to be applied to all recorded cells at the end of the experiments to show the % of residual current not attributable to hERG channels evoked by this protocol. The residual current in E-4031 may be used for subtraction to isolate the hERG current.

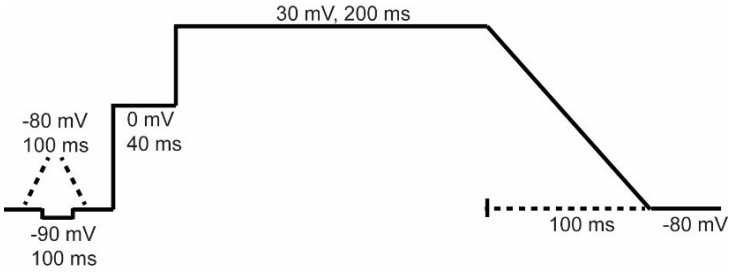
To ensure the baseline recording is stable enough for drug application, cells should be presented with this protocol in control solution until hERG current amplitudes for 25 consecutively recorded current traces exhibit <10% difference. Then drugs may be applied as the voltage pulse continues. Drug effect 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.

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.

### *$Ca_V1.2$ 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 $Ca_V1.2$ currents reach stability. The definition of stability should be provided in the study report. 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.



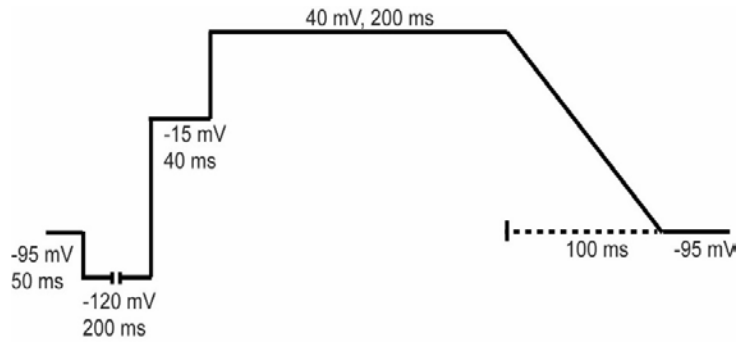

$Ca_V1.2$ current amplitude (leak current-subtracted or verapamil subtracted and not raw value) is measured at two places – as the peak inward current at the 0 mV step and as the peak inward current evoked at the "ramp down" phase (+30 to -80 mV ramp down in 100 ms). If possible, at the end of the experiment,100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

Two methods may be used to quantify $Ca_V1.2$ current amplitude. First is the leak subtraction method. The 10 mV hyperpolarizing step may be used to calculate leak current for subtraction, and this method is suitable for calculating peak current at the 0 mV step and the inward current at ramp down phase. Second is the verapamil subtraction method. This is applicable only for cells with very stable passive membrane properties throughout the recording period, and is useful to calculate ramp current amplitude. Because peak current may not be completely eliminated in the presence of verapamil, peak current should be measured using the leak subtraction method.

The following external solution is recommended (in mM): 137 NaCl, 10 HEPES, 4 KCl, 1 $MgCl_2*6H_2O$, 1.8 $CaCl_2*2H_2O$, 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. 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 shown below. To induce late $Na_V1.5$ current, 150 nM ATX-II should be used.




This protocol is repeated every 10 s until late $Na_V1.5$ currents reach stability (i.e., current amplitude for 25 consecutively recorded current traces exhibit < 10% difference). Then the test compound may be applied as the protocol continues. It is recommended that, at the end of the experiment, 30 µM tetrodotoxin (TTX) should be applied to show that the current measured is mediated

by $Na_V1.5$ channels. Late current 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.

Similar to $Ca_V1.2$ peak and ramp current, late $Na_V1.5$ current may be calculated by subtracting leak current or TTX-insensitive current from the inward current.

The following external solution is recommended (in mM): 130 NaCl, 10 HEPES, 4 CsCl, 1 $MgCl_2$*$6H_2O$, 2 CaCl2*$2H_2O$, 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. Data should be filtered at 3 kHz and then digitized at 10 kHz.

### *Peak $Na_V1.5$ current*

Adequate voltage control and series resistance compensation is necessary for these experiments. Peak $Na_V1.5$ current is studied using the same voltage protocol and internal/external solutions as the late $Na_V1.5$ current. 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. Therefore, ATX-II is not used in peak $Na_V$ 1.5 current experiments. Peak $Na_V1.5$ current should be recorded in control solution until current reaches the steady state. Then the test compound should be applied as the protocol continues. it is recommended that, 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 if input resistance is high, holding current is small, and the ratio of peak inward current to holding current is small. For these experiments, data should be filtered at 5 kHz and then digitized at 20 kHz.

## REFERENCES

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