The Comprehensive* in Vitro Proarrhythmia Assay

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Outline

• Cellular electrophysiology
• Cardiac arrhythmias like Torsade de Pointes
• CiPA
  • Basis for proarrhythmia assessment
  • Project team
Cellular electrophysiology...in 5 minutes

• Cells have energy-dependent machinery that keeps \([K^+]_i > [K^+]_o\) and \([Na^+, Ca^{++}]_i < [Na^+, Ca^{++}]_o\). Everything else is passive!

• At rest, permeability to \(K^+\) is low, but much higher than it is to \(Na^+\) or \(Ca^{++}\), so a few positive charges leave the cell (no anions!), making it a little negative (-60 to -90 mV), at which point the impact of the negative potential just offsets the tendency for \(K^+\) to move down the concentration gradient.
Action potential

• When the cell is depolarized, Na⁺ permeability/conductance increases relative rapidly, moving positive charges into the cell—positive feedback!
• In the cardiac myocyte, Ca²⁺ conductance also increases, a little more slowly.
• Slower still, Na⁺ channels close (inactivate) and K⁺ channels open, tending to drive the cell back towards the resting potential, turning off Ca²⁺ channels, and relieving the inactivation of now-closed Na⁺ channels, and finally mostly turning off the K⁺ channels.
• Equilibrium is then restored.
• The entire ballet is choreographed by how the individual channel types respond to the membrane potential—and how quickly they respond.
Cardiac action potential

- Because the heart is the most important organ in the body, cardiac myocytes have lots of different K channel types...
Torsade de pointes and similar

• Mostly attributable to effects on IKr.
• You can go decades with impaired IKr function (and long QT) without dying!
• Impaired IKr function allows regenerative activity—another action potential—to happen sooner than it should, but as long as the whole heart is more or less doing the same thing, nothing really bad happens.
• Arrhythmia requires some part of the heart to be partly electrically decoupled from other parts, so that the abnormal action potential has some place to go, setting up a circuit of uncoordinated activity that does not sustain blood flow.
Basis for CiPA

• You cannot fully characterize the electrical coupling in the heart and predict in whom or when an arrhythmia will occur.
• You can fully characterize a drug’s effects on the various ion channel types and predict the vulnerability to arrhythmia.
  • IKr block does not cause arrhythmia! Regenerative activity requires an intact INa and ICa.
  • Drugs that block one of these over the same concentration range as they block IKr may not be proarrhythmic.
• So, vulnerability to proarrhythmia could be assessed using
  • Cardiac myocytes expressing the normal complement of channels at their proper density – not readily available
  • Studies of drug effects on each channel type – what CiPA proposes
CiPA

• Characterization of drug effects on ion channels
• Reconstruction of channel effects on the cardiac action potential
• Sanity check
Characterizing drug effects on ion channels

• Some mammalian cells have few channels of their own, but can be made to express/overexpress individual human channel types. These are available for all the channel types in the human ventricular myocyte.

• Voltage clamp experiments allow you to dissect out effects of voltage and time – and the effects of a drug – on a channel type’s openings.

• Some drugs exert simple channel block. Others only exert effects when a channel is in a certain state (“use dependence”).

• High-throughput systems allow parameterization with narrow confidence limits
Reconstruction

• Cell model is collection of channel models with appropriate current densities
• Reconstruction does not involve guessing at unknown parameters; everything determined by voltage clamp assays
• Interrogate the action potential model for degree of regenerative activity, as a function of heart rate, etc.
• But...
  • Maybe you didn’t assay all the currents
  • Maybe you missed a novel use-dependent property
Sanity check

• Two ways to look for missed drug effects
  • Isolated human or stem-cell-derived human cardiac myocytes
    • Imperfect phenotype \rightarrow \text{ calibration with selective blockers}
    • Comparison with appropriately parameterized myocyte model
  • ECG
    • Different channel blocking activities result in different patterns on the ECG
CiPA Project Team – 1/2

• Channel Work Stream
  • Develop channel-specific voltage clamp protocols
  • Led by electrophysiology group in Safety Pharmacology Society

• In Silico Work Stream
  • Voltage clamp data analysis
  • Reconstruction
  • Proarrhythmia metric
  • Led by Tom Colatsky/FDA and academic electrophysiology community

• Myocyte Work Stream
  • Protocols for handling myocytes
  • Recording technology
  • Led by ILSI-HESI
CiPA Project Team – 2/2

• Compound Selection Work Stream
  • Pick drugs for engineering efforts
  • Pick drugs for validation/calibration
  • Led by Cardiac Safety Research Consortium

• Qualification Work Stream
  • What will ICH need to see

• Steering Committee
  • Communications among work streams
  • Whine about rate of progress
Summary

• Science well established; engineering not so much
• Human ion channels; human myocytes \( \rightarrow \) translation
• Consideration of drug effects on multiple ion channels will provide a higher degree of specificity than do current non-clinical and clinical approaches
• Should correct poor labeling for approved drugs, increase safe compounds entering the drug development pipeline