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

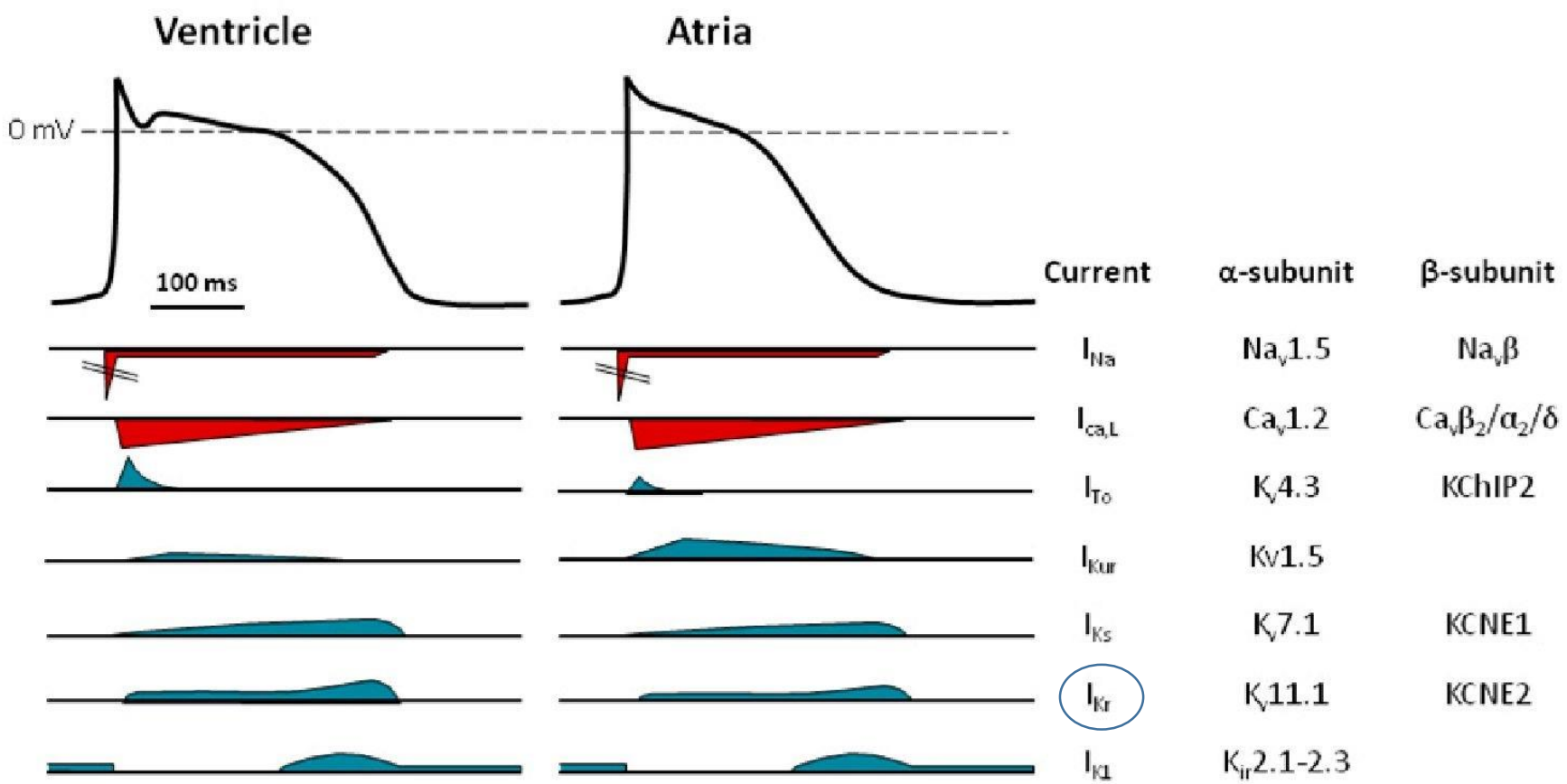
- Cells have energy-dependent machinery that keeps $[K^+]_i > [K^+]_o$ and $[Na^+, Ca^{++}]_i < [Na^+, Ca^{++}]_o$. Everything else is passive!
- At rest, permeability to K^+ is low, but much higher than it is to Na^+ or Ca^{++}, so a few positive charges leave the cell (no anions!), making it a little 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, Ca^{++} 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 Ca^{++} 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 → 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 → 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