From single myocyte to whole-heart; the intricate dance of electrophysiology and modeling

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Contraction of the heart is triggered by an electrical signal, initiated in the pacemaker cells, that propagates along the conduction system and generates an action potential in the myocardium. The waveform of the cardiac action potential is shaped by the activity of numerous types of ion channels and transporters. Many of these transport systems function differently under pathological conditions, leading to altered action potentials and consequently less efficient excitation-contraction coupling along with an increased risk for arrhythmias. It is therefore very important to characterize the various ion transport pathways in conditions as close to in vivo as possible.

Hodgkin and Huxley were the first to clearly demonstrate the complex interaction of ionic transport mechanisms using a computer model over 60 years ago[1]. Introduction of the single cell patch-clamp technique by Neher and Sakmann more than 30 years ago[2] facilitated the biophysical characterization of individual ion transporters and a deeper understanding of how their activity integrates to produce the overall electrical signal of a cardiac myocyte. In parallel, more and more complex mathematical models of myocyte electrophysiology were developed, which complemented experimental findings and clinical observations to add novel insight into electrical activity of the heart. However, isolated myocytes lack the electrical, metabolic and mechanical coupling within the myocardial synctium. Such coupling modulates action potentials and ion currents in individual cells and is therefore an essential part of electrical signaling in the whole heart. To capture this feedback, we need to advance from experiments and modeling on single cells to the tissue level. Two articles in this issue of the journal provide exciting advancements in this direction.

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First, Ramos-Franco et al. introduce their novel loose-patch photolysis (LPP) technique that allows the simultaneous recording of transmembrane ion currents and membrane potentials in whole hearts.[3] The membrane current is measured with a giant patch pipette loosely attached to the heart. To eliminate the large leak current due to the poor electrical seal between the pipette and the tissue, a second patch system clamps the bath in which the heart is immersed to the same voltage as the tissue. A fiber optic positioned inside the giant patch pipette is used to measure optically the local action potential in the same spot where membrane currents are recorded. The third component of the system consists of a flash-photolysis system used for photolysis of specific activators or inhibitors of different ion channels and transporters. Thus, LPP allows the measurement of specific ion currents during a normal, physiological, action potential in an intact perfused heart.

The first feature revealed by this new technique was the strong electrotonic coupling imposed by the tissue. In their experiments, Ramos-Franco et al. first inhibited L-type Ca^{2+} channels by perfusing the heart with nifedipine. Then, they removed the Ca^{2+} influx inhibition locally, near the patch pipette, by inducing photolysis of nifedipine. The efficiency of photolysis was confirmed by a vigorous and sustained increase in the amplitude of local Ca^{2+} transients. However, when comparing the local action potential before and after nifedipine photolysis, the authors noticed that re-activation of L-type Ca^{2+} channels had practically no effect on the local action potential. Thus, at the whole heart level, the effect of a local increase in an inward conductance on membrane potential is cancelled by the electrical coupling with the neighboring tissue. In these experiments, the local area for recording and photolysis had a diameter of 200-250 μm (25-50 myocytes). An intriguing application of this innovative technique would be to determine the conditions (size of the tissue in which an inward current is activated, current density, and the relationship between them) needed to overcome the electrical sink of the rest of the tissue. Such experiments may help solve the controversy regarding the source-sink relationship needed to trigger focal arrhythmias.

Ramos-Franco et al. further report that during a physiological action potential in the intact mouse heart, photolysis of nifedipine induces a biphasic membrane current that displays an early fast component and a late slower component. Pharmacological analysis revealed that the early current is mediated by L-type Ca^{2+} channels while the late component is caused by activation of the Na^+/Ca^{2+} exchanger following Ca^{2+} release from the sarcoplasmic reticulum. Simultaneous measurements of the membrane current and action potential allowed the authors to uncover that most of Ca^{2+} influx occurs during the rapid repolarization of the membrane (Phase 1 of the action potential) when L-type channels deactivate. This conclusion was supported by the observation that slowing down the rate of Phase-1 repolarization by partially blocking the transient outward current (I_{to}) significantly prolonged the time to peak and the half duration of the early current. Thus, at whole heart level, Ca^{2+}-induced Ca^{2+} release is triggered during Phase 1 of the action potential, which leads to activation of the Na^+/Ca^{2+} exchanger in Phase 2. This result implies that Ca^{2+}-induced Ca^{2+} release contributes to but is not the result of Phase 2. Further studies should be aimed at determining to what extent this timing holds in hearts from higher mammals that have smaller I_{to}, and consequently more pronounced action potential plateau, than mouse hearts.

As any new technique, loose-patch photolysis has some limitations, most notable being its reliance on the availability of light-sensitive blockers or activators of membrane currents and the fact that signals can be recorded only from the epicardium. Nevertheless, LPP is a very intriguing technical innovation that should provide exciting insights into excitation-contraction coupling at the whole-heart level. Of particular interest is identifying how intact-heart excitation-contraction coupling is altered in different etiologies of heart disease.

In the second paper, Zhou et al. are presented with the clinical observation that there are two types of action potential duration restitution curves in patients.[4] Both types show alternans at high pacing frequencies, but as the rate is further increased, the alternans disappear in one group while they become more pronounced in the other. The latter case would result in greater heterogeneity in repolarization and could be potentially arrhythmogenic. Furthermore, if more regions displayed alternans, there would be a higher propensity for activity to break down into fibrillation. Since further experimentation was constrained by the use of human subjects, the authors turned to computer simulations in order to elucidate the mechanisms responsible for their findings.

The quality of the solution obtained from computer simulations depends on the fidelity of the underlying mathematical model. Several representations of human ventricular cells have been developed with each model being based on a particular data set with a specific set of observations targeted.[5-7] This can lead to quite different behaviors between models.[8] The choice of model to use, therefore, depends on the particular question being asked. The one chosen by the authors was the O'Hara and Rudy ionic model,[9] which is one of the most comprehensive today, being able to reproduce a wide variety of phenomena.

The authors followed an approach in which they did not try to fit a particular action potential, but did a comprehensive search of the parameter space, varying only channel conductances. This is a powerful technique since it does not tie behavior to particular cells which may have been chosen by chance or bias, and in the past, has led to large differences in model behavior for the same tissue. The analysis showed that in parameter space, the behavior of the ionic model tended to cluster in phenotypical groups. Indeed, there is a great deal of variability between cells found within the same heart, so it should not be surprising that there is no one-cell-fits-all model.

The study also further highlights the role of emergent behavior when nonlinear components are coupled together. We can study channels and mechanisms in great detail in isolated preparations. However, there is always uncertainty in the relative quantity of components when assembled into the whole, and this can dictate functioning. This can, for example, explain species specific differences wherein similar components may be expressed in several species, but the different ratios cause significant differences in behavior. In the paper presented, there appear to be two competing mechanisms of calcium regulation that result in either the eye or pitchfork restitution curves. In one type of cell, the strong L-type calcium current serves to raise intracellular $[Ca^{2+}]_i$ so that SERCA activity is increased to restore calcium balance. The interactions of the calcium handling components are complex and depend exquisitely on the relative strength of each.

This leaves the door open, of course, for further tissue level simulations to examine exactly how this type of heterogeneity in action potential duration at rapid rates can lead to reentry. There is still a leap from single cell to tissue behavior, as there is from channel to cell level behavior. Questions to be addressed are how regions large enough to exhibit one particular type of alternans manifest since electrotonic interaction tends to lead to an average behavior. Another issue to consider is the mechanism by which this type of heterogeneity leads to arrhythmia. These types of simulations are to be built on top of what has been discovered by this study, assigning distributions of the cell types and performing stimulation protocols to elicit reentry. Eventually, the loop must be closed and these mechanisms should be tested experimentally in intact animal hearts. The loose patch photolysis technique developed by Ramos-Franco et al.[3] could directly test the modeling prediction of Zhou et al.[4] that large L-type Ca^{2+} current conductance is responsible for the disappearance of repolarization alternans with increasing the pacing rate in whole-hearts.

The two studies highlighted in this commentary represent a major leap towards understanding the dynamic relationship between electrical activity of single myocytes and that of the intact heart. They further emphasize that synergy between modelling and experimentation is key to unraveling the mechanisms that underlie the normal and pathological function of the whole-heart.

Acknowledgments

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