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How does the shape of the cardiac action potential control calcium signaling and contraction in the heart?

Luis F. Santana, **Edward P. Cheng**, and **W. Jonathan Lederer***
Department of Physiology & Biophysics University of Washington Box 357290 Seattle, WA 98195

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The function of the heart is to pump blood through the pulmonary and systemic vasculature. To do this, the atria and ventricles must contract in a precise sequence in response to a conducted electrical signal, the action potential (AP).

The cardiac AP originates in the sinoatrial node and is conducted to the atria and ventricles through electrical synapses: the gap junctions. The AP morphology varies with species, heart rate, location within the heart, developmental stage, and in response to neurohormones and drugs. Unlike the brief APs of skeletal muscle and neurons, which typically last ≈3-5 ms, the cardiac action potential is 100's of milliseconds long and has five distinct phases (Figure 1). One of these components, sometimes considered one of little importance, the "rapid repolarization" period (also called "phase 1") is the focus of a clever paper presented in the current issue of *Journal of Molecular and Cellular Cardiology* [1].

In Fig. 1, we use a color-coded human AP to illustrate its five phases. Phase 0 (red) is the leading edge of the AP and corresponds to the period of rapid depolarization (depolarization rate ≈ 250 V/s) from the diastolic membrane potential of ≈ −90 mV to +50 mV. It is produced primarily by the activation of cardiac Na^+ channels with a contribution from cardiac Ca^{2+} channels. Phase 1 (blue), also referred as the "notch", is produced by termination of the depolarizing inward Na^+ current due to inactivation and the concomitant activation of the transient outward K^+ (I_{to}) and Na^+/Ca^{2+} exchanger (I_{NCX}) currents. This results in a transient repolarization period (i.e. the notch) as the membrane potential goes from ≈ +50 to +30 mV (or more negative). Phase 2 (green) is the long (hundreds of milliseconds) plateau phase of the action potential during which the membrane potential changes little. This is produced by the balance of small, but non-inactivated Na^+ and L-type Ca^{2+} current (I_{Ca}) components and hyperpolarizing K^+ currents. In phase 3 (orange), reductions of Ca^{2+} and Na^+ currents and increasing K^+ current contribute to the repolarization of the myocyte. Towards the end of phase 3 the repolarizing actions of these K+ currents are opposed by an inward I_{NCX} produced by the Na^+/Ca^{2+} exchanger operating in its Ca^{2+} extrusion mode. Phase 4 (black) is the diastolic membrane potential of quiescent ventricular myocytes of (≈ −90 mV). The detailed molecular identities of the membrane

* Center for Biomedical Engineering and Technology University of Maryland at Baltimore 725 W. Lombard Street Baltimore, MD 21201(santana@uw.edu and jlederer@umaryland.edu) .

currents that contribute to each phase depend on the cardiac tissue type and anatomic location of the tissue as well as the specie and developmental age of the animal [2]. Similarly, the exact AP morphology depends on these same variables.

The coupling of cardiac membrane depolarization to Ca^{2+} release from the sarcoplasmic reticulum (SR) in myocytes (i.e. excitation-contraction (EC) coupling) takes place during the initial phases of the action potential when the L-type Ca^{2+} channels open. EC coupling is dependent on the positioning of the L-type Ca^{2+} channels within 15 nm of the junctional sarcoplasmic reticulum (jSR) to form a functional unit called a “couplon” [3]. The Ca^{2+} influx activates the nearby cluster of SR Ca^{2+} release channels in the couplon, the ryanodine receptors (RyRs) to produce Ca^{2+} sparks [4]. Synchronization of the Ca^{2+} sparks during the AP produces the cell-wide $[Ca^{2+}]_i$ transient that activates contraction. Voltage and Ca^{2+}-dependent inactivation rapidly terminate I_{Ca}. Because of the exquisite nano-anatomy of the couplon, the opening of single or small cluster of L-type Ca^{2+} channels could activate a Ca^{2+} spark [5-7]. This central component of EC coupling may thus link changes in AP morphology to EC coupling and the $[Ca^{2+}]_i$ transient.

Recent studies suggest that modification of the ventricular action potential is a critical early step in the chain of events linked to the development hypertrophy and heart failure [8-12]. Indeed, changes in the AP waveform can be observed as early as 48 hours after myocardial insult, even in the absence of measurable cardiac hypertrophy. These changes include reductions in the rate of depolarization and peak depolarization during phase 0, a decrease in the repolarization during phase 1 (or even complete loss of the notch), and an increase in the duration of the action potential.

Decreased $[Ca^{2+}]_i$ transients and contractility are also a hallmark of pathological hypertrophy and heart failure. The hypothesized mechanisms underlying these changes in function can be subdivided in two general categories. The first involves a decrease in the coupling strength between L-type Ca^{2+} channels and ryanodine receptors [13]. The second mechanism involves decreased Ca^{2+} storage in the sarcoplasmic reticulum. The later mechanism is likely due to decreased SERCA function and enhanced Na^+/Ca^{2+} exchanger expression.

In the current issue of the *Journal of Molecular and Cellular Cardiology*, an article from Mark Cannell’s laboratory [1] revisited the following important question: what is the relationship between changes in action potential waveform and EC coupling during heart failure? In this study, they focused on the importance of the loss of the notch during phase 1 of the human cardiac AP during heart failure (HF) with respect to the magnitude and the kinetics of local and global $[Ca^{2+}]_i$ transients. Although the functional importance of phase 1 of the action potential on EC coupling has been examined before under physiological [14] and pathological [15] conditions, the elegant study by Cooper et al. [1] provides novel mechanistic insights into the mechanisms underlying changes in EC coupling during HF.

By voltage clamping single cardiac myocytes from rat and rabbit using both normal (notched AP) and HF human AP (without notches), the authors show that EC coupling is significantly impaired during a failing AP. I_{Ca} during a failing AP shows a reduced peak amplitude and slower inactivation and the cell-wide (global) $[Ca^{2+}]_i$ transient also shows reduced peak amplitude and slower rise-time. The authors show that the observed change in I_{Ca} and $[Ca^{2+}]_i$ transient amplitude is not caused by action potential prolongation during a failing AP. By varying an artificially simulated notch voltage while fixing the action potential duration, the authors show that both, peak and integrated I_{Ca}, as well as $[Ca^{2+}]_i$ transient amplitude decrease with decreased notch repolarization.

Using confocal microscopy, the authors further show how EC coupling is impaired when a failing AP causes spatial heterogeneity in Ca^{2+} transient and Ca^{2+} "spike" activation [16]. Due to impaired E-C coupling, the SR load is increased, which the authors demonstrate when a Ca^{2+} transient invoked by a normal AP is larger in amplitude when it is immediately preceded by an AP with HF morphology. Increased SR Ca^{2+} can lead to increased risk of arrhythmogenesis [17].

The recent work by Dong et al [18] is at odds with the studies by Cooper *et al.* [1], Sah *et al.* [14], and Harris *et al.* [15]. Dong et al. used the dynamic clamp to quantitatively examine the influence of I_{to} on $[Ca^{2+}]_i$ and contraction of canine left ventricular endocardial and epicardial myocytes. In endocardial myocytes, where the native I_{to} is small, simulation of a large I_{to} increased phase 1 repolarization, but significantly decreased $[Ca^{2+}]_i$ transient and cell shortening. Accordingly, subtraction of the native I_{to} via the dynamic clamp enhanced contractility in epicardial cells. On the basis of these data, Dong et al [18] concluded that there is an inverse correlation between I_{to} levels and myocyte contractility and $[Ca^{2+}]_i$ transient amplitude in canine epicardial and endocardial myocytes. Further experiments may be needed to resolve the conflicts.

Is the decrease in I_{Ca} density associated with the absence of the repolarizing notch seen by Cooper *et al.* sufficient to explain the significant impairment in EC coupling observed during HF? The authors use a modified Monte Carlo stimulation of L-type Ca^{2+} channel and RyR gating, which takes into account Ca^{2+} dependent inactivation of L-type Ca^{2+} channels, to show that the simulated Ca^{2+} sparks agree well with the experimental data. The peak rate of Ca^{2+} spark production is halved, and the rate of initial spark triggering is slowed, while the appearance of late Ca^{2+} sparks due to stochastic L-type Ca^{2+} channel openings is increased. The authors report that the simulation shows that the change in EC coupling caused by the failing AP, with its absence of the repolarizing notch, can be entirely explained by the change in I_{Ca} flux.

In combination with the work by Harris *et al.* [15], the study by Cooper *et al.* [1] is likely to change all current models of EC coupling since there are so many influences in AP morphology. This is particularly relevant for our understanding of the development of EC coupling dysfunction during the onset of HF. Decreased I_{to} is a seemingly general response to myocardial insults, which has been detected as early as 48 hours following myocardial infarction [10]. Down-regulation of I_{to} precedes changes in SERCA, Na^+/Ca^{2+} exchanger, and RyR proteins. The findings by Cooper *et al.* [1] would suggest that this, by itself, would directly translate into a change in EC coupling, which would later be exacerbated by functional decoupling of I_{Ca} and RyRs, down-regulation of SERCA, and/or up-regulation of Na^+/Ca^{2+} exchanger.

Could up-regulation of I_{to} restore EC coupling during HF? A recent study by Jin *et al.* [19] suggest an answer to this important question. They showed that increasing I_{to} density by expressing the accessory subunit KChIP2 in failing ventricular myocytes attenuated HF and improved EC coupling. Thus, I_{to} could be a key, bi-directional regulator of EC coupling and a potential target for restoration of cardiac function during HF.

Although Cooper *et al.* [1]focused on HF, their findings also provide insights into the mechanisms underlying regional variations in EC coupling in the heart. I_{to} and hence phase 1 of the action potential varies throughout the heart. I_{to} is larger in epicardial than in endocardial myocytes of the left ventricular free wall. Yet, $[Ca^{2+}]_i$ transients are larger in endocardial than in pericardial myocytes. In combination with the findings by Cooper *et al.* [1], this suggests that while the changes in the magnitude of phase 1 of the action potential could alter EC coupling, they are not sufficient to explain regional variations in $[Ca^{2+}]_i$

transients in the heart. Under physiological conditions, regional and developmental variations in action potential waveform and the expression and function of Ca^{2+} handling proteins (e.g. Na^{+}/Ca^{2+} exchanger, RyR2) are the likely culprits for heterogeneous EC coupling under physiological conditions [20].

The study by Cooper *et al.* [1] has broad implications on EC coupling. In ventricular myocytes, the probability of Ca^{2+} spark occurrence (P_{Spark}) in a couplon during the action potential is proportional to the open probability (P_o) of Cav 1.2 channels and the square of the Ca^{2+} current and local $[Ca^{2+}]_i$ [7]. A recent study suggested that P_{Spark} approaches unity in specific sites within a cell at the relatively positive membrane potentials (≈ +50 mV) attained during the plateau of the ventricular action potential [5]. This is surprising because, at +50 mV, P_o is less than 1 (≈0.7) and the current through a single L-type Ca^{2+} channel is predicted to be very small (i.e. ≈ 10 fA) at physiological levels of extracellular Ca^{2+}. However, as suggested by Cooper *et al.* [1], by depolarizing to +50 mV the myocyte promotes a high P_o for L-type Ca^{2+} channels, which if followed by a pronounced notch to more negative potentials could enhance the driving force for Ca^{2+} influx and hence the unitary current of L-type Ca^{2+} channels. Indeed, according to the GHK equation repolarization from +50 to +30 during phase 1 would triple the Ca^{2+} influx (≈ 3.09-fold), which would increase P_{Spark} by nine-fold. Although the opening of a single L-type Ca^{2+} channel can activate a couplon at less positive potentials, a larger number of L-type Ca^{2+} channels are likely to be needed at more positive potentials [6-Santana reference][5]. Indeed, experiments and modeling support the larger number of L-type Ca^{2+} channels activated to trigger a couplon at more positive potentials [5, 21][add Ramay & Sobie ?2009]. Phase 1 activation of Ca^{2+} sparks can thus be attributed to increased P_o of the L-type Ca^{2+} channels and also the larger single channel current that would be observed without the notch. Coupled gating of L-type Ca^{2+} channels may also be a mechanisms for the concerted activation of multiple L-type Ca^{2+} channels and thereby contribute to SR Ca^{2+} release and a high P_{Spark} [22]. A note of caution is in order, however: There are distinct cellular and molecular structural difference in human and rat ventricular myocytes (e.g. T-tubule organization, RyR2 number, relationship between L-type Ca^{2+} channels and RyR2 clusters, and more). To some extent excitation-contraction coupling in rat ventricular myocytes may not faithfully reproduce the local and cellular $[Ca^{2+}]_i$ signals of human ventricular myocytes even when the AP is identical. Nevertheless, Cooper *et al.* [1] have provided important new evidence suggesting that the notch of the ventricular AP could be an important determinant of P_{Spark} under diverse and varied physiological and pathological conditions.

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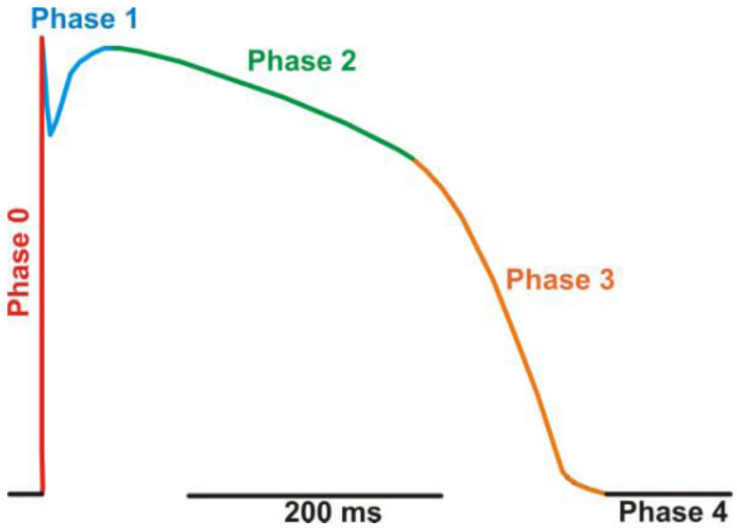

Figure 1.
A typical action potential (AP) waveform of a healthy adult human ventricular myocyte (modified from Cooper et al. [1]).