Rational strategy to stop arrhythmias: Early afterdepolarizations and L-type Ca^{2+} current

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Unlike the brief action potentials (APs) in skeletal myocytes or neurons, the human cardiac AP takes 100s of milliseconds to repolarize the cell. This slow repolarization is essential for proper excitation–contraction coupling in cardiac muscle, and precise control of AP duration contributes to electrical stability. Under various pathological conditions, often when the AP duration is prolonged, repolarization can transiently fail with a sudden transient depolarization of membrane potential (Fig. 1). If such an early afterdepolarization (EAD) reaches threshold, it can trigger a premature AP and thereby initiate potentially fatal ventricular arrhythmias such as torsades de pointes (TdP) and ventricular fibrillation (Cranefield and Aronson, 1991). Thus, understanding the causes of EADs and how one might block them is of significant clinical importance.

Underlying ionic mechanisms responsible for EADs

The physiology underlying EADs is complex, involving multiple inward and outward ionic currents, changes in intracellular ion concentrations, and rapid regulation of ion channels. An EAD occurs when there is a reversal of the normal repolarization during phase 2 or 3 of the cardiac AP and is associated with a reduction in what has been referred to as "repolarization reserve" (Roden, 1998). Repolarization reserve is determined by the dynamic balance of outward currents and inward currents present during repolarization of the AP and implies redundancy of ionic currents in the normal heart to ensure appropriate repolarization. If there is a decrease in normal repolarization reserve, then a regenerative increase in an inward current can overcome and potentially reverse repolarization, leading to an EAD.

The first hint of a diminution of repolarization reserve is frequently an increase in AP duration. Conditions associated with prolongation of the AP are collectively referred to as long QT syndrome (LQTS), reflecting the longer than normal QT interval observed on the surface electrocardiogram. Both acquired and congenital forms of LQTS have been identified. Acquired LQTS occurs in the presence of certain electrolyte abnormalities, most commonly hypokalemia, as well as in response to ischemia, oxidative stress, and certain drugs. In the case of hypokalemia and QT-prolonging drugs, the reduction in repolarization reserve is primarily caused by a reduction in I_{Kr} carried by the hERG K channel. Alternatively, oxidative stress, such as that experimentally induced by H_2O_2 exposure, increases inward currents, including I_{NaL} (late sodium current) and $I_{Ca,L}$, to reduce repolarization reserve (Xie et al., 2009). Congenital LQTS is caused by mutations and dysfunction in a range of ion channels and associated regulatory proteins that either reduce outward repolarizing currents or increase inward depolarizing currents, with at least 13 such genetic defects having been identified (Ackerman et al., 2011). For example, LQTS type I is caused by loss of function mutations in KvLQT1 that reduce the I_{Ks} during AP repolarization. Thus, there are many ways to affect repolarization reserve that can contribute to the generation of EADs and triggered arrhythmias. Although the acquired forms of LQTS are generally reversible by rectifying the insult, e.g., potassium supplementation, revascularization for ischemia, or removing the offending drug, addressing the congenital forms presents more of a challenge.

The upstroke or depolarization of an EAD must be the result of a regenerative inward current, which is also necessary for the EAD to propagate at the tissue level (Zeng and Rudy, 1995). Inward currents that have been suggested to contribute to the upstroke of the EAD include $I_{Ca,L}$ (January et al., 1988), I_{NCX} (Volders et al., 1997), and I_{NaL} (Maltsev et al., 1998); of these, $I_{Ca,L}$ has received the greatest attention. January and Riddle (1989) first convincingly demonstrated in Purkinje fibers that there is a window current for $I_{Ca,L}$ during which steady-state activation and inactivation curves overlap in the membrane potential range where EADs occur. In other words, as the AP repolarizes, $I_{Ca,L}$ can reactivate and contribute to an increasing inward current. Furthermore, interventions that increase $I_{Ca,L}$ currents, such as exposure to BayK8644, a pharmacological channel activator, lead to EADs, as can an increase in sympathetic tone, which acts, in part, by increasing $I_{Ca,L}$ (Tanskanen et al., 2005). Likewise, activation of CaM Kinase II (CaMKII),

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which increased $I_{Ca,L}$ in a mouse model by increasing mode 2 gating of the channels, also stimulated EADs (Dzhura et al., 2000). Thus, strategies to inhibit $I_{Ca,L}$ from generating EADs comprise a logical approach to treatment and prevention of arrhythmias related to LQTS. Unfortunately, doses of classic Ca^{2+} channel blockers sufficient to inhibit EADs also inhibit the influx of Ca^{2+} necessary for excitation–contraction coupling, leading to impaired contraction. Nevertheless, Madhvani et al. (2015), in the previous issue of this journal, reasoned that if they could rationally alter gating parameters of L-type Ca^{2+} channels (LTCCs), then they may be able to identify a modified channel behavior that inhibits the ability of $I_{Ca,L}$ to generate EADs while preserving their essential contribution to excitation–contraction coupling. The long-term goal of such a strategy is to identify small molecule or biological interventions that will produce this ideal channel gating to prevent EADs and thus prevent life-threatening ventricular arrhythmias.

Dynamic clamp to identify gating properties of LTCCs to eliminate EADs

The dynamic clamp technique provided the essential tool that Madhvani et al. (2015) used to systematically test the effect of changes in specific gating properties of LTCCs. In brief, these experiments used isolated rabbit ventricular myocytes that were treated with H_2O_2 or hypokalemic conditions to reproducibly prolong AP duration and induce EADs. After blocking all of the native $I_{Ca,L}$ with a high concentration of nifedipine, which results in dramatic shortening of the AP duration and loss of EADs, the authors introduced a computer-generated virtual $I_{Ca,L}$. This virtual $I_{Ca,L}$ was based on a mathematical model of the current, which in real-time was fed back to the cells in response to the measured voltage (Fig. 1). In a proof-of-principle study of this strategy, this group previously demonstrated that computer-simulated $I_{Ca,L}$ successfully reconstituted the AP and the return of EADs in H_2O_2- or hypokalemia-treated myocytes (Madhvani et al., 2011). They also demonstrated that slight shifts in the voltage dependence of activation or inactivation of the channels could blunt EADs by reducing the window current. In the present study, however, they systematically tested a range of different channel gating properties, examining the slope of voltage-dependent activation and inactivation, the magnitude of the late current, and the time constant of activation, as well as the time constant of inactivation for $I_{Ca,L}$. The winning strategy was to reduce the magnitude of the late or pedestal $I_{Ca,L}$.

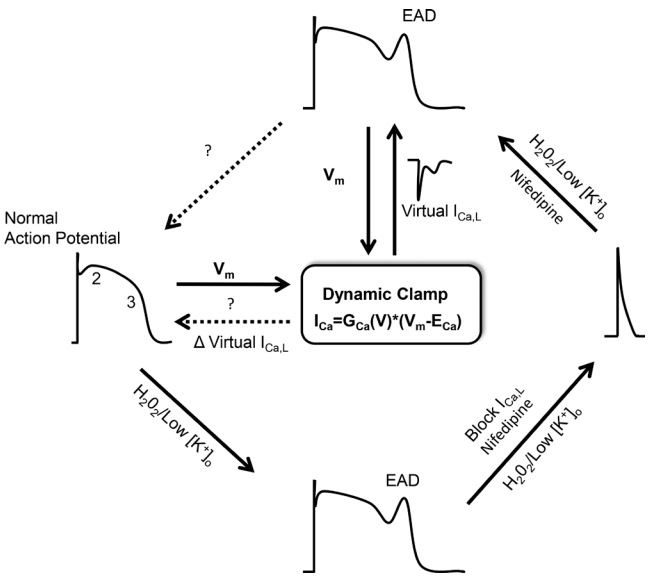

Figure 1. Schematic of cardiac APs studied using dynamic clamp to test properties of virtual $I_{Ca,L}$ to eliminate EADs by Madhvani et al. (2015). A normal ventricular AP (left) with the major phases of AP repolarization indicated (2 and 3). The AP is prolonged in response to oxidative stress (H_2O_2) or hypokalemia (Low$[K^+]_o$), leading to an EAD (bottom). Complete block of $I_{Ca,L}$ with a calcium channel blocker, nifedipine, dramatically shortens the AP and eliminates EAD (right). Using a computer model to generate virtual $I_{Ca,L}$, a dynamic clamp reconstitutes the missing $I_{Ca,L}$ and recapitulates the long AP and EAD (top). By systematically changing the gating properties of virtual $I_{Ca,L}$, Madhvani et al. (2015) test for properties of the current that will preserve peak current and contraction but eliminate EADs.

What is the late component of the L-type Ca^{2+} current?

Madhvani et al. (2015) have found an appealing feature of $I_{Ca,L}$ to target, but what exactly is the late $I_{Ca,L}$? A maintained component of $I_{Ca,L}$ has long been recognized in ventricular myocytes, and single channel experiments suggest it is caused by multiple channel reopenings (Rose et al., 1992). LTCCs exhibit both voltage-dependent inactivation (VDI) and Ca^{2+}-dependent inactivation (CDI; Lee et al., 1985; Peterson et al., 1999). The pedestal current reflects contributions involving both VDI and CDI mechanisms, otherwise the channel would completely inactivate. However, the relationship between VDI and CDI is incompletely defined. Do VDI and CDI share a final common pathway, or are they mediated independently (Findlay, 2004; Kim et al., 2004; Barrett and Tsien, 2008)? For example, in LQT8 or Timothy's syndrome, mutations in $Ca_v1.2$ specifically impair VDI, leading to AP duration prolongation and EADs (Splawski et al., 2004). The study by Madhvani et al. (2015) does not distinguish the respective roles of VDI and CDI in the late $I_{Ca,L}$, which is modeled as a constant. Thus, it remains unclear whether interventions to reduce the pedestal current should ideally target CDI, VDI, or either of the two.

Moreover, LTCCs are not a homogeneous population of channel proteins in cardiomyocytes, making the situation even more complex. Differences in subunit composition, posttranslational modifications, and subcellular localization of channels will all contribute to the heterogeneity of channel behavior observed within a single cell. This raises the question as to whether one specific population of channels is primarily responsible

for the late $I_{Ca,L}$ and may represent the appropriate target. Although the major pore-forming LTCC subunit in ventricular cardiomyocytes is $Ca_v1.2$, different splice variants are expressed and can contribute to heterogeneity of channel gating (Liao et al., 2005). Furthermore, auxiliary subunits modulate the gating behavior of the channel (Singer et al., 1991). The auxiliary β subunit ($Ca_v\beta$) is encoded by four different genes, all of which are expressed in human heart, along with multiple splice variants (Foell et al., 2004). Different $Ca_v\beta$ isoforms differentially regulate inactivation of $I_{Ca,L}$ (Colecraft et al., 2002; Kobrinsky et al., 2004), so it is possible that a subpopulation of LTCCs with a distinct subunit combination may disproportionately or solely contribute to late $I_{Ca,L}$. Posttranslational modifications of the channel, such as phosphorylation by PKA or CaMKII, have been linked with changes in gating that can promote proarrhythmic behavior (De Ferrari et al., 1995; Dzhura et al., 2000). In fact, combining posttranslational modification with unique subunit composition may be critical to susceptibility to EAD, as suggested by a prior study demonstrating that the $Ca_v\beta2a$ subunit was uniquely sensitive to CaMKII modulation in response to oxidative stress, which lead to EADs (Koval et al., 2010). Finally, the distinct subcellular localization of channels in the myocytes may expose the channels to different environments and thereby influence their behavior (Balijepalli et al., 2006; Bhargava et al., 2013). For example, could a subpopulation of channels in caveolae be the source of late $I_{Ca,L}$?

Strategies to block the late component of $I_{Ca,L}$

Defining the optimal way to block late $I_{Ca,L}$ may depend on advancing our understanding of the molecular basis of this current as indicated above; nevertheless, one can speculate that the approach could use small molecules or biological therapies. A precedent for specific late current blockers has been set by the identification of compounds that block the late current conducted by voltage-gated sodium channels in the heart, I_{NaL}, without blocking the peak current. Ranolazine is the prototypic I_{NaL} blocker (Antzelevitch et al., 2004), and new more specific I_{NaL} blockers have been described that have antiarrhythmic properties (Sicouri et al., 2013). So, with this precedent, it seems possible to identify a late $I_{Ca,L}$ blocker. Conceivably, such compounds are already available but were missed in earlier screens of compound libraries for traditional LTCC blockers that focused exclusively on the ability to block peak $I_{Ca,L}$. Alternatively, roscovitine, a purine-based compound that was developed as an anticancer drug (cyclin-dependent kinase inhibitor) has been demonstrated to accelerate $I_{Ca,L}$ inactivation, although it also slows activation gating (Yarotskyy and Elmslie, 2007). Roscovitine has shown promise in the iPS cardiomyocyte model for Timothy syndrome, where it blunted a defect in VDI (Yazawa et al., 2011). Using gene therapy to express regulatory proteins or auxiliary subunits could be considered as an alternative approach. For example, overexpression of a desired $Ca_v\beta$ subunit in cardiomyocytes could modify the gating behavior of endogenous channels (Colecraft et al., 2002). Exactly which $Ca_v\beta$ isoform, or perhaps even a modified $Ca_v\beta$ isoform, would be optimal requires further study.

Cautiously moving forward

The study by Madhvani et al. (2015) illustrates an intriguing strategy to design new therapies to treat arrhythmia syndromes, i.e., using the dynamic clamp in a hybrid computational-experimental approach to identify modifications of $I_{Ca,L}$ gating properties that block a trigger for arrhythmias. However, for such a strategy to succeed, the model must accurately reflect the ionic currents present and the change in $I_{Ca,L}$ gating must achieve the goal of preventing EADs without blunting intracellular Ca^{2+} transients and consequently contraction. Did Madhvani et al. (2015) succeed in selectively eliminating $I_{Ca,L}$ from the native AP to accurately test virtual $I_{Ca,L}$? Although nifedipine is a long-established LTCC blocker, at the high concentration necessary for complete block of $I_{Ca,L}$, it is not certain that off-target effects on other ion channels are not present. Testing another drug to block $I_{Ca,L}$ could provide reassurance that the results are not biased by the particular blocker chosen. A second concern is that virtual $I_{Ca,L}$, unlike native $I_{Ca,L}$, does not lead to influx of Ca^{2+} nor trigger intracellular Ca^{2+} release and hence excitation–contraction coupling. Thus, the authors model intracellular Ca^{2+} transients into $I_{Ca,L}$ gating, but it is difficult to fully recapitulate the effect of the Ca^{2+} transient on multiple ion channels, transporters, and regulatory pathways. In some experiments, the authors included a small fraction of virtual I_{Ks}, a current known to be modulated by intracellular $[Ca^{2+}]$. However, there are certainly other currents, perhaps most importantly I_{NCX}, that could influence the results. Even more difficult to model is the regulation of the LTCCs by CaMKII, which can also be dynamically affected by the intracellular Ca^{2+} transients. Will the reduction in late $I_{Ca,L}$ proposed by the investigators interfere with intracellular Ca^{2+} cycling? The authors argue that maintaining peak $I_{Ca,L}$ will maintain appropriate excitation–contraction coupling, but a reduction in the late component of $I_{Ca,L}$ will reduce overall Ca^{2+} influx during an AP and at steady-state likely reduce intracellular Ca^{2+} stores, leading to a reduction in the Ca^{2+} transient. Whether this will have a significant impact requires further study.

Even if the cell model functions accurately, some questions will remain. Will this intervention focused on reducing late $I_{Ca,L}$ be effective when cardiomyocytes are coupled into a functional tissue or will new concerns/heterogeneities arise? Advancing to multiscale modeling is one approach to address this concern in future studies. How broadly applicable will a reduction in late

$I_{Ca,L}$ be to treat EADs resulting from other causes not studied here? For example, some EADs rely more heavily on I_{NCX}, and these may be more refractory to changes in late $I_{Ca,L}$. However, at the end of the day, existing strategies for developing antiarrhythmic drugs have largely failed, and so new, innovative approaches as described by Madhvani et al. (2015) need to be aggressively pursued and tested.

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