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Deciphering Arrhythmia Mechanisms – Tools of the Trade

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Abstract

Pathophysiological remodeling of cardiac function occurs at multiple levels, spanning the spectrum from molecular and sub-cellular changes to those occurring at the organ-system levels. Of key importance to arrhythmias are changes in electrophysiological and calcium handling properties at the tissue level. In this review, we discuss how high-resolution optical action potential and calcium transient imaging has advanced our understanding of basic arrhythmia mechanisms associated with multiple cardiovascular disorders, including the long QT syndrome, heart failure, and ischemia-reperfusion injury. We focus on the role of repolarization gradients (section 1) and calcium mediated triggers (section 2) in the initiation and maintenance of complex arrhythmias in these settings.

Keywords

Arrhythmias; Repolarization; Calcium; Mapping; Long QT Syndrome; Heart Failure; Alternans

Introduction

Pathophysiological remodeling of cardiac function occurs at multiple levels, spanning the spectrum from molecular and sub-cellular changes to those occurring at the organ-system levels^{46,49,101,102}. With the advent of gene expression profiling techniques, major advances have been made with regards to the characterization of the molecular and genetic fingerprints of the heart at various stages during disease development^{18,41,50}. The combination of these high-throughput strategies with standard electrophysiological and molecular studies have led to a more comprehensive appreciation of the molecular basis of congenital and acquired cardiovascular disorders. Despite these advances, however, mechanisms by which molecular remodeling translates into altered electrophysiological properties at the tissue organ level remain unclear. In fact, defining the exact nature of the electrophysiological substrate of the heart during disease progression and the mechanisms that promote arrhythmic triggers is currently an active subject of intense investigation in many laboratories across the world^{1–4,62,81}. Understanding mechanisms by which molecular and cell signaling pathways promote ion channel dysfunction and alter key electrophysiological properties at the tissue-network level will ultimately facilitate the

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design of new pharmacological as well as cell- and gene-based approaches to combat arrhythmias in patients.

In this article, we illustrate how optical imaging techniques have been effectively exploited by various investigators to advance our basic understanding of arrhythmia mechanisms. Specifically, we focus on a few studies in which optical mapping of ventricular repolarization gradients and intracellular calcium (Ca^{2+}) dynamics have allowed us to decipher arrhythmia mechanisms associated with a broad range of cardiovascular disorders, including the Long QT Syndrome, Heart Failure, and ischemia-reperfusion injury.

Altered Repolarization & Heart Failure

Action potential (AP) prolongation is an electrophysiological hallmark of cells and tissues isolated from failing and hypertrophied hearts^{3,46,48,51,72}. This fundamental change in myocyte biology is caused by the impaired repolarizing capacity of the cardiac cell^{51,55}. Abnormal AP prolongation at the cellular level readily translates to the level of the intact organ resulting in a long and variable QT interval on the surface electrocardiogram (ECG)^{16,79,100}.

Key changes in the early and late phases of AP repolarization have been documented in numerous studies using the patch clamp technique in isolated cardiomyocytes from various small and large animal models of HF (for review see references^{5,51,71}). These cellular electrophysiological changes were mechanistically linked to overall down-regulation of repolarizing potassium currents^{22,51,61}, an increase in late Na current density, as well as major changes in intracellular Ca^{2+} handling proteins^{21,23,45,75}. Notably, HF results in reduced Ca^{2+} load within the sarcoplasmic reticulum (SR). This is caused by defective sequestration of Ca^{2+} by the SR Ca^{2+} ATPase (SERCA2a) coupled with increased diastolic SR Ca^{2+} leak via the ryanodine receptor (RYR2)^{60,68,110}. Abnormal intracellular Ca^{2+} cycling is exacerbated by an up-regulation in the expression and function of the electrogenic Na^{+} - Ca^{2+} exchanger (NCX), which generates a net inward depolarizing current during the plateau phase of the AP further delaying repolarization^{20,21}. At the opposite end of the spectrum, studies in humans and animal models showed delayed global repolarization and enhanced temporal repolarization instability using clinical non-invasive metrics, such as the QT-interval variability index and T wave alternans on the surface ECG. Rosenbaum, Berger, and others have successfully developed and utilized sophisticated algorithms that detect various ECG metrics of global cardiac repolarization to identify patients at high risk of developing sudden cardiac death (SCD)^{8,9,32,33,35,36,58,87,88,111}. Of note are recent findings of a multi-center clinical trial, in which non-invasive T wave alternans testing was shown to significantly enhance the predictability of impending SCD in patients with HF when combined with standard electrophysiological testing in these high risk patients³³.

These cellular and clinical studies highlight the importance of repolarization changes occurring at the tissue level for arrhythmia genesis in HF. Until relatively recently, efforts to investigate the mechanistic link between repolarization changes and reentrant arrhythmias were hampered by technical difficulties in assessing spatio-temporal repolarization gradients across the heart. With the advent of optical imaging techniques using voltage sensitive dyes, a high-resolution measurement of cardiac repolarization at a cellular level within the intact syncytium has become possible^{3,7,17,42,90}. Importantly, a quantitative relationship between altered spatio-temporal repolarization gradients and the incidence of arrhythmias in various animal models of HF have recently emerged^{3,48}. In what follows, we focus on the role of spatial heterogeneities of repolarization in the incidence of reentrant arrhythmias in animal models of HF and the long QT syndrome (LQTS) that are prone to arrhythmias. Specifically, we discuss changes in transmural and transepical repolarization gradients as mechanisms for sustained ventricular arrhythmias.

Transmural repolarization gradients in HF

Antzelevitch and colleagues^{11,13} pioneered the notion that heterogeneities of cellular repolarization in different cell types (epicardial, mid-myocardial, and endocardial) may represent a unifying mechanism underlying a host of arrhythmias in congenital and/or acquired cardiac diseases, such as the long QT, short QT¹⁰, Brugada^{12,24}, Andersen-Tawil¹⁰³, and Timothy syndromes^{95,96}. Of particular importance was the role of mid-myocardial (M) cells in the establishment of transmural repolarization heterogeneity under conditions of prolonged QT interval in various *ex vivo* models of the LQTS^{13,14,113}. Indeed, the functional expression of transmural heterogeneity under conditions of prolonged QT interval has been confirmed in most mammalian species, with the possible exception of porcine myocardium³¹. In non-failing human hearts, a marked transmural APD heterogeneity was elegantly documented at slow pacing rates (0.5 Hz) as mid-myocardial islands of cells with distinctly long APDs and steep local APD gradients were observed⁴². In contrast, human failing hearts exhibited surprisingly reduced transmural APD heterogeneity and lacked prominent local APD gradients⁴². Of note, non-failing human wedges were extracted from donor hearts that were rejected for age, hypertrophy, atrial fibrillation, or coronary artery disease. As such, by necessity, both failing and non-failing preparations were most likely remodeled, which is reflected by their relatively long APD values⁴².

Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall are well recognized, electrotonic flow of current between cells is expected to reduce the functional expression of electrical heterogeneities across the heart¹⁰⁵. This has called into question the functional significance of transmural heterogeneity, in general and M cells, in particular¹⁵. In fact, some elegant studies in which extracellular plunge electrode recordings were used have failed to detect these heterogeneities *in vivo*^{78,107}. It is important to note, however, that by enlarge these studies did not establish conditions favorable for the emergence of M cell behavior (marked QT interval prolongation by bradycardia and/or pharmacological agents). To investigate the functional expression and significance of transmural heterogeneities in intact myocardium, the approach of transmural optical AP mapping was used^{3,7}. This allowed a simultaneous high-resolution measurement of repolarization properties across all muscle layers of the ventricular wall in an intact preparation where the influence of cell-to-cell coupling was present. Furthermore, because QT interval prolongation represents an electrophysiological hallmark of the failing heart, it was hypothesized that LQTS and HF may share important phenotypic properties at the multi-cellular tissue level that predispose them to arrhythmias via similar mechanisms³. By assessing the functional expression of transmural repolarization heterogeneities in arterially perfused canine wedge preparations from normal and failing hearts, we confirmed the role of transmural repolarization gradients in the initiation and maintenance of arrhythmias³. As expected, HF was associated with a marked AP prolongation across all myocardial layers, consistent with findings in isolated myocytes and whole animals. Interestingly, however, AP prolongation was heterogeneous across the left ventricular wall, affecting mid-myocardial and endocardial muscle layers more selectively; thereby, increasing the effective transmural repolarization gradient by ~2-fold³. In support of transmural dispersion of repolarization as a mechanism for arrhythmias associated with various disease etiologies, Yan et al demonstrated that left ventricular hypertrophy in a rabbit model of renovascular hypertension was also associated with significant enhancement of transmural dispersion of repolarization because of selective prolongation of subendocardial APs in this model¹¹².

Mechanisms underlying increased transmural repolarization heterogeneity in HF remain unresolved. These changes, however, likely involve multiple factors, including heterogeneous remodeling of cell-to-cell coupling, ionic currents/exchangers, and Ca²⁺

handling proteins. In an elegant study, Li et al⁶¹ investigated the ionic basis of transmural AP remodeling in HF by measuring the density of key repolarizing potassium (K^+) currents, including the transient outward (I_{to}), the inward rectifier (I_{K1}), and both components of the delayed rectifier (I_K) currents. By enlarge, K current changes were uniform in epicardial, mid-myocardial, and endocardial myocytes of failing hearts, indicating that transmural repolarization heterogeneity observed at the tissue level could not be readily explained by cell-type specific remodeling of repolarizing K^+ currents. In a subsequent study, we measured the expression levels of key alpha and beta subunits encoding these K^+ currents in the three principal myocardial layers of normal and failing hearts⁶. In support of the findings of Li et al⁶¹, we also did not find a K^+ channel molecular basis (neither at the mRNA or protein levels) for the enhanced transmural repolarization heterogeneity observed in the failing heart⁶.

Poelzing et al⁸¹ attributed the location of the maximum transmural repolarization gradient to increased electrical resistivity at that location. Furthermore, they were able to convert basal transmural APD gradients measured across normal preparations into ones that mimicked those in HF simply by perfusing normal preparations with the gap junction inhibitor, Carbenoxolone⁸¹. These findings highlight the potential importance of gap junction uncoupling in the mechanism of increased transmural dispersion of repolarization in HF. We and others have investigated the molecular basis for gap junction uncoupling in HF and have found major changes in the expression, distribution, and phosphorylation state of the main ventricular gap junction protein, Cx43 that develop with varying time-courses during disease progression^{2,4,48,85}. Specifically, end-stage HF was associated with over-all Cx43 downregulation, dephosphorylation, and lateralization. In addition, we recently reported the loss of interaction between Cx43 and the cytoskeletal protein, ZO-1 as a potentially critical event underlying severe conduction slowing and therefore gap junction uncoupling at late stages of remodeling in a model of pressure overload hypertrophy⁴⁸. Interestingly, hyperphosphorylation of Cx43 also occurred at earlier stages of remodeling that were associated with a milder form of conduction slowing⁴⁸. As such disrupted phosphorylation (either increased or decreased) at critical residues within the carboxyl domain of Cx43 may lead to loss of gap junction function via distinct mechanisms. The individual contribution of these complex molecular changes to the establishment of transmural repolarization heterogeneity across the failing heart remains unclear and will require direct investigation.

Altered repolarization & the acquired Long QT Syndrome

The Long QT Syndrome (LQTS) is characterized by QT-interval prolongation often resulting in sudden death caused by Torsade de Pointes (TdP) mediated arrhythmias⁷. To date, several distinct mutations in ion channel and cytoskeletal proteins that directly or indirectly modulate ventricular repolarization have been identified in patients with LQTS. Both triggered activity caused by early afterdepolarization-mediated beats and reentrant excitation have been implicated in the mechanism of TdP. Because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP remained unclear for many years. Using transmural optical AP mapping in *ex-vivo* perfused canine wedge preparations, we investigated the functional topography of M cells and their role in TdP⁷. These studies provided direct evidence that the topographical distribution of M cells promotes unidirectional block and reentrant excitation underlying TdP in this canine wedge model of LQTS⁷. Conduction slowing was not a requirement for reentry, because the path length dictated by the M-cell refractory zone was sufficiently long to allow partial recovery of excitability at former sites of block. Because of the exquisite sensitivity of M cells to rate,

the broad zones of block delineated by M cells collapsed into functional lines of block that shifted dynamically on successive beats⁷. It is noteworthy that similar topographical distributions of M-cells have recently been uncovered in ventricular wedge preparations isolated from human myocardium⁴².

Apex-Base Gradients of Repolarization

In addition to the transmural gradients of repolarization discussed above, optical AP mapping revealed significant apex-base differences in repolarization properties across the heart. Despite species differences in the expression levels and subtypes of cardiac K^+ channels, a qualitatively consistent apico-basal APD gradient of repolarization (shorter APDs at the apex compared to the base) were observed in guinea pig^{30,40,93}, rabbit²⁷, mouse^{17,65}, canine and human⁹⁹ hearts. While the myocardial activation sequence is a determinant of force generation, the apico-basal sequence of repolarization determines the direction of relaxation. This mechanical property appears to be common to all species tested, so far and may be fundamental to cardiac mechanics. Moreover, in a novel rabbit heart preparation isolated with intact and functional autonomic innervation and combined with optical mapping, the repolarization sequence was shown to be reversed during bilateral sympathetic or parasympathetic nerve stimulation. The reversal of repolarization sequence was in large part due to a highly heterogeneous nerve distribution being considerably more dominant at the base than the apex of the heart⁶⁷.

In acquired long QT type 2 elicited by drugs that inhibit the rapid component of the delayed rectifying K^+ current, I_{Kr} , AP prolongation across the heart was not uniform, as it was more pronounced at the apex compared with the base, resulting in large repolarization gradients and a reversal of the repolarization sequence. The heterogeneous effect of I_{Kr} blockers in terms of APD prolongation occurs because of the heterogeneous expression of ERG channels which exhibit a higher density at the apex than the base²⁷.

Repolarization & the congenital Long QT Syndrome

As mentioned above, congenital LQTS is caused by distinct mutations in ion channel related genes. To date, multiple gain and loss of function mutations affecting ion channel pore forming, accessory, or cytoskeletal proteins have been identified. To investigate the arrhythmia phenotype of congenital LQTS and gain a more comprehensive understanding of the role of individual ion channel genes in modulating the electrophysiological substrate, transgenic LQTS mouse models have been created⁹⁴. The technique of optical AP imaging has provided a remarkable tool for deciphering arrhythmia mechanisms in these models¹⁷. Indeed, Salama and coworkers found that epicardial dispersion of repolarization and refractoriness are critical determinants of the arrhythmia phenotype of various congenital forms of LQTS. Importantly, they found that not all loss of function mutations affecting K^+ channel subunits are “created equal”. For example, genetic deletion of the K^+ channel accessory subunit minK, which participates in the formation of I_{Ks} , did not cause major changes in the electrophysiological substrate or the incidence of arrhythmias, despite presence of marked neurological abnormalities (deafness, loss of balance, spinning behavior) in these mice. The lack of cardiac phenotype in minK knockout mice is consistent with the minor role that I_{Ks} is thought to play in murine ventricular repolarization. Also, these data highlight the importance of investigating mutations that affect K^+ channel pore forming and not only auxiliary subunits.

In support of the notion that loss of function mutations affecting K^+ channel subunits can produce divergent outcomes, these authors also found that genetic deletion of Kv4.2, which encodes a pore forming subunit of I_{to} , a major murine repolarizing current resulted in a paradoxical suppression of the apico-basal repolarization gradient across the heart. This

decrease in epicardial dispersion of repolarization, which occurred under a condition of prolonged QT (and APD) interval was indeed protective against arrhythmias. In sharp contrast, deletion of Kv1.4, another I_{to} pore forming subunit led to a marked increase in epicardial dispersion of repolarization and a heightened susceptibility to programmed stimulation-induced arrhythmias. As such, these authors elegantly provided compelling proof in favor of dispersion of repolarization and not APD (or QT interval) prolongation as the mechanism of torsade de pointes in the intact heart. These findings further highlight the importance of investigating arrhythmia mechanisms in multi-cellular and intact preparations, and not only in isolated myocytes, because of the clear importance of regional gradients in repolarization properties that develop under conditions of LQTS, even within a single muscle layer (i.e. epicardium).

Using optical AP imaging, Liu et al.⁶² also uncovered the electrophysiological basis for sex differences in susceptibility to Torsade de pointes. Specifically they found that the greater predisposition of adult female rabbits to E4031 mediated arrhythmias was reversed in pre-pubertal animals, which exhibited a male predominance in terms of drug induced APD prolongation, EAD formation, and arrhythmia induction. These elegant findings highlight the dynamic nature of the electrophysiological substrate, revealing how a given agent (E4031) can produce highly divergent electrophysiological outcomes that are both age and sex dependent⁶².

Excitation-contraction coupling (ECC) is the process by which Ca^{2+} cycling and force generation are tightly controlled by the AP. The reverse is also true, as the shape and duration of the AP depend on Ca_i . A host of ion channels give rise to this “mechano-electrical coupling” including $I_{Ca,L}$, I_{NCX} , Ca^{2+} -sensitive Cl^- channels (I_{Cl}), and I_{Ks} . At steady state, Ca^{2+} influx must equal efflux across the plasma membrane and the SR; any imbalance can only be transiently tolerated. In fact, modifications that alter the balance of Ca^{2+} influx and efflux typically lead to rhythm and/or pump dysfunction.

There is now a growing appreciation that the balance of Ca^{2+} influx to efflux is compromised in a wide range of pathological conditions that lead to lethal arrhythmias due to the inter-dependence of the AP and Ca_i cycling. Hence aberrations in Ca_i cycling can be the result of: a) a metabolic deficit that compromises Ca^{2+} pump function which may occur in ischemia/reperfusion, b) either a gain or a loss of function of a cardiac ion channel which may occur in congenital and acquired long QT syndrome and c) ion channel remodeling that directly or indirectly compromises the balance of Ca_i fluxes which may occur in heart failure (HF)⁸⁹. The development of techniques to simultaneously map APs and Ca_i transients at high spatial (hundreds to thousands of site) and temporal (1–5kHz) resolution has provided compelling evidence that aberrations of Ca_i transients can initiate arrhythmias and may influence the dynamics of ventricular fibrillation (VF).

Simultaneous optical mapping of voltage (V_m) and intracellular free Ca^{2+} (Ca_i)

Although many excellent voltage-sensitive⁶³ and Ca^{2+} indicator³⁷ dyes were available for dual mapping, several problems had to be overcome. A) The two probes needed to have the same excitation wavelength to avoid mechanical components for wavelength switching which would reduce acquisition rates. B) The V_m and Ca_i probes needed to have different Stokes's shifts to avoid cross-talk between V_m and Ca_i signals. C) The probes had to yield large fluorescence changes during a cardiac beat with a high signal to noise ratio to eliminate the need to time average the signals; fluorescent probes were chosen because they pick up less motion artifacts compared to absorption dyes⁷⁰. The first apparatus was based on the combination of RH 237 and Rhod-2AM to measure V_m and Ca_i and a set of two photodiode arrays with 16×16 pixels (Hamamatsu America Inc)³⁰. A significant improvement in signal to noise ratio was achieved by using a new voltage sensitive dye with

a longer emission wavelength Pittsburgh 1 (PGHI)^{80,91} which can be excited at 540 nm (like Rhod-2) but fluoresces above 640 nm with a peak emission at 790 nm. As a result, a wider band pass interference filter (560–620 nm) can be used to pick up the Cai signals from Rhod-2's fluorescence and to pick up the Vm signals from PGHI with a cut-off filter at 630 nm with no cross talk between the two parameter. Another improvement in spatial resolution was achieved with CMOS cameras (SciMedia, Ultima One) with 100×100 pixels that can be scanned at up to 10,000 frames/s²⁸. The CMOS cameras have a limited interval of uninterrupted recordings of tens of seconds such that in a number of applications the photodiode arrays with lower spatial resolution have the disadvantage of continuous recordings through a long lasting experimental protocol. For example, with photodiode arrays it is possible to record V_m and Ca_i during an ischemia (12–15 min) followed by a reperfusion (30–45 min)²⁹. Another application more suitable for photodiode arrays are mapping studies during a period of sympathetic nerve stimulation followed by its recovery^{67,74}. A recent review of methods used to simultaneously map Vm and Cai offers extensive technical details⁹². The need to better understand the role of Ca_i aberrations on cardiac arrhythmias was the driving force to develop a high speed imaging system for V_m and Ca_i and the method is now routinely used by numerous investigators. Future developments to simultaneously map V_m and extracellular K⁺ accumulation are not far behind and are expected to be equally promising⁵⁴.

Ca²⁺ and the genesis of Early Afterdepolarizations

Early afterdepolarizations (EADs) are thought to trigger TdP under conditions of congenital and acquired LQTS. There is appreciable variability in the kinetics of EADs but those that exhibit rapid upstrokes are considered to trigger TdP because they can capture, propagate and trigger propagating APs³⁴. EADs in the setting of impaired repolarization have been classically attributed to the spontaneous reactivation of the L-type Ca²⁺ current, I_{CaL}, during the abnormally long plateau and repolarization phases of the AP^{47,104}. Alternatively, EADs may be triggered by SR Ca²⁺ overload, leading to spontaneous SR Ca²⁺ release during phases 2 or 3 of the AP, and the activation of forward mode I_{NCX}^{98,106}.

Simultaneous optical APs and Ca_i transient mapping in rabbit hearts demonstrated that perfusion with E4031 (0.5 μM) caused marked prolongation of APD, reversal of repolarization gradient, and a 3–5 fold increase in dispersion of refractoriness^{17,27}. EADs developed rapidly (within minutes) and high frequency Ca_i-mediated EAD firing evolved into TdP. Normally, AP upstrokes are followed (within approx 10 ms) by a rise of Ca_i. During an EAD, V_m followed the rise of Ca_i by only 4–6 ms. To determine whether EADs were generated primarily by Purkinje fibers, liquid N₂ was used to cryoablate the conduction system and the endocardium to produce hearts with a thin layer of surviving epicardium (~1mm). After blocking I_{Kr}, EADs were elicited at the same frequency even after the ablation of Purkinje fibers. The dynamic relationship between Ca_i and V_m during an EAD supports the notion that SR Ca²⁺ overload and spontaneous SR Ca²⁺ release activates I_{NCX} which triggers I_{CaL} to produce EADs. The role of spontaneous SR Ca²⁺ release as a trigger of EADs was further emphasized in adult female hearts paced at a bradycardic rate treated with the I_{Kr} blocker, dofetilide, as a model of acquired LQT2. In these conditions, Ca_i oscillations appeared during long APDs but before the onset of EADs and TdP. Such Cai oscillations and the subsequent TdP were abolished by perfusion with Nifedipine, with low external Ca²⁺ or Ryanodine plus Thapsigargin⁷³.

Ca²⁺ and sex differences in arrhythmia risk

Numerous studies have shown an increase of TdP in women versus men following an exposure to agents that block K⁺ channel HERG and inhibit I_{Kr}^{56,59,66,84}. The increase in vulnerability to sudden death in women has been reported for cardiac drugs^{66,59} as well as

numerous non-cardiac drugs³⁸ and are the result of regulation of ionic channel by sex steroids³⁹. In congenital LQT2, the underlying genetic defects of K^+ channels may be asymptomatic in some conditions but in the presence of a mild block of I_{K_r} may precipitate TdP in women more frequently than in men due to their reduced cardiac 'repolarization reserve'⁸⁶. Human registry data for congenital LQT2 showed that sex and age had differential effects on arrhythmia risk^{43,114}. Adult females had significantly higher risk; whereas, in children (<14 years old), boys had higher risk of cardiac events⁴³.

Rabbit hearts exhibit the same sex and age differences of LQT2-related arrhythmias as man. In LQT2 induced with E4031 (0.5 μ M), adult female hearts fired EADs within 5 min that evolved to TdP (n=24/24). In contrast, adult male hearts rarely had an EAD or TdP (n=16/18)⁹⁷. In pre-pubertal rabbits, males were more prone to TdP than females, even though females had longer APDs.⁶² In adult rabbit hearts, the arrhythmia risk was reversed and females were more prone to TdP than males^{62,97}. Optical mapping of EADs showed that the first site to exhibit EADs were sites with greater Ca^{2+} overload and were located at the base of the ventricles in both adult female and pre-pubertal male hearts⁹⁷. The reason for the greater Ca^{2+} overload at the base of the epicardium was investigated by measuring the current densities of $I_{Ca,L}$ and I_{NCX} in voltage-clamp experiments. Myocytes isolated from the base of adult female hearts were found to have a 25–30% greater peak $I_{Ca,L}$ compared to myocytes from the apex and from adult male myocytes (apex and base)⁹⁷. The higher $I_{Ca,L}$ density at the base of adult female myocytes was due to higher expression levels of the main subunit of L-type Ca^{2+} channels, Cav1.2 α ⁹⁷. On-going studies show that the higher expression levels of Cav1.2 α are up-regulated by estrogen by a regional genomic mechanism. This new link between arrhythmia risk and the higher expression of Cav1.2 α provides compelling evidence for Ca_i overload as the mechanism for EADs and TdP.

In transgenic rabbits with congenital LQT2 through the over-expression of a nonfunctional human HERG channel, females had more lethal arrhythmias than males²⁵. Closer investigations of transgenic rabbit hearts revealed a greater dispersion of repolarization, slower AV conduction and a propensity to discordant alternans rather than the typical EADs and LQT2-related TdP^{76,115}.

SR Ca^{2+} release and Arrhythmias

Besides LQTS, a number of other pathologies have been linked to anomalies in Ca_i handling. In myocytes, Ca^{2+} overload and abnormal SR Ca^{2+} release can be induced by rapid pacing, inhibition of the Na^+/K^+ pump or β -adrenergic agonists⁸². In human and animal models of heart failure (HF), reduced amplitude and slower decay of Ca_i transients caused by reduced expression/function of SERCA2a are often accompanied by increased I_{NCX} and decreased I_{K1} amplitudes. Under these conditions, residual adrenergic activity may cause SR Ca^{2+} overload leading to spontaneous Ca^{2+} release events and triggered arrhythmias^{44,64,83}. Ischemia, through an inhibition of oxidative metabolism, causes important changes in ionic concentrations: intra and extra-cellular acidosis, elevations of Ca_i , Na_i , Mg_i and of external K^+ ^{19,26}. During ischemia, excessive production of free-radicals leads to oxidation of sulfhydryl groups on proteins and channels, including RyRs resulting in increased channel activity^{26,69}. Reperfusion also has dire consequences. The prompt recovery of pH_o creates an outward H^+ gradient, a rise of Na_i via Na/H exchange and Ca^{2+} overload by Ca^{2+} entry via Na/Ca exchange. Substantial cellular evidence exists in support of this mechanistic scenario that causes Ca^{2+} overload and reperfusion-arrhythmias⁵².

Indeed, optical mapping of V_m and Ca_i can provide evidence that spontaneous Ca_i elevation leads to triggered activity of sufficient magnitude to capture, propagate and initiate arrhythmias. In a guinea pig model of ischemia/reperfusion, ~ 17% of electrical instabilities

were preceded by spontaneous Ca_i oscillations implicating aberrant Ca_i cycling as a potentially important trigger of premature beats, bigeminal or trigeminal rhythms⁵⁷. In a canine wedge model of drug-induced LQT1, Ca^{2+} -induced triggered activity was typically initiated on the endocardium⁵³. In pig hearts, the inter-dependence of V_m and Ca_i during VF induced by burst pacing was analyzed using Mutual Information (MI), as a statistical measure of the extent to which one variable, V_m predicts another, Ca_i ⁷⁷. MI was relatively high during pacing and VT but fell dramatically during VF, suggesting that spontaneous SR Ca_i release may sustain VF by promoting wavebreak formation⁷⁷. Although the latter findings were not reproduced in non-ischemic hearts in VF^{108,109}, there is less controversy regarding the role of Ca_i overload in ischemia and hypoxia⁸⁹.

SUMMARY

Pathophysiological remodeling of cardiac function occurs at multiple levels, spanning the spectrum from molecular and sub-cellular changes to those occurring at the organ-system levels. Of key importance to arrhythmias are changes in the electrophysiological substrate and the incidence of Ca^{2+} mediated triggers. The role of Ca_i aberrations is central because any deviation between Ca^{2+} influx and efflux is poorly tolerated and can come about by a number of routes. In LQT2, APD prolongation lengthens the interval during which Ca^{2+} influx occurs because $I_{\text{Ca,L}}$ inactivates rapidly due to Ca^{2+} -dependent inactivation but a component of voltage-dependent inactivation maintains a small persistent level of Ca^{2+} influx. Thus the higher the expression levels of $I_{\text{Ca,L}}$ (as in the female heart) the greater the propensity to Ca_i overload and Ca^{2+} -triggered EADs and arrhythmias. In ischemia and hypoxia, the metabolic deficit compromises ATP-dependent Ca^{2+} uptake by the SR pumps resulting in an accumulation of cytosolic Ca^{2+} . A compensatory response arising from lower ATP levels activates ATP-dependent K^+ channels, increases I_{KAPT} , shorten APDs thereby reducing Ca^{2+} influx. In reperfusion, oxidative stress causes SR Ca^{2+} leaks by activating ryanodine receptors and/or enhancing $I_{\text{Ca,L}}$. In HF, the weaker cardiac muscle arises from slower and attenuated Ca_i transients which has been attributed to SR Ca^{2+} leaks via RyR2. In VF induced by burst pacing, the high firing frequencies reduce the diastolic intervals during which Ca^{2+} efflux would normally reset cellular Ca^{2+} levels. Hence, high-resolution optical imaging has been effectively used by various laboratories to decipher arrhythmia mechanisms across broad cardiovascular disorders.

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