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Electrophysiological Remodeling in Heart Failure

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Abstract

Ion channel remodeling in heart failure modulates key cellular electrophysiological properties, predisposing to arrhythmias and sudden death. Heart failure induced ion channel dysfunction prolongs the action potential, increases spatio-temporal gradients of repolarization, promotes arrhythmogenic triggers and results in conduction abnormalities. Understanding fundamental ionic mechanisms of normal and abnormal electrogenesis is a key requirement for the development of effective and safe therapies. Elucidating the underlying molecular mechanisms and functional consequences of ion channel remodeling at the cellular and organ levels presents a unique opportunity for the development of novel pharmacological, device, gene, and cell based approaches for the treatment of arrhythmias in heart failure.

Keywords

Heart failure • Cardiac resynchronization therapy • Arrhythmias • Ion channels • Conduction • Repolarization

Introduction

Heart failure (HF) claims over 200,000 lives annually in the US alone [1]. Approximately 50 % of these deaths are sudden and unexpected, and presumably the consequence of lethal ventricular arrhythmias [2]. Our limited understanding of fundamental arrhythmia mechanisms at the ionic and molecular levels has hampered the development of effective pharmacological treatments for these patients [3]. In fact, the proarrhythmic tendency of ion channel targeting agents has resulted in the premature termination of the Cardiac Arrhythmia Suppression Trial (CAST) [4]. These proarrhythmic effects are due to the heterogeneity of ion channel expression and function [5, 6], the nonspecific nature of most pharmacological

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agents that target ion channels, the cross-talk between individual ion channels, and the complex remodeling that occurs dynamically in the context of left ventricular dysfunction. Indeed, elucidation of arrhythmia mechanisms in HF at the basic ionic level is expected to improve existing pharmacological therapies and facilitate the design of novel approaches, including cell- and gene-transfer strategies designed to target ion channels and transporters. In this chapter, we focus on key HF-induced ion channel remodeling at the cellular level and its pro-arrhythmic manifestations at the tissue level, with the intent of identifying molecular targets for anti-arrhythmic therapy in HF.

Ionic Basis of the Ventricular Action Potential

A unique signature of any excitable tissue is its action potential (AP) profile, which reflects a delicate balance in the activity of several depolarizing and repolarizing ion channels, transporters and exchangers. Myocytes are characterized by a long AP, which is initiated by the influx of sodium ions (Na^+) when voltage-gated Na^+ channels (encoded by $Na_v1.5$) open. The transient opening of these channels gives rise to a rapid phase of depolarization (phase 0) which is terminated by the inactivation of the fast inward Na^+ current (I_{Na}) within a few milliseconds. The AP upstroke is followed by a brief interval of early repolarization (phase 1), caused by the activation of the voltage-gated transient outward potassium (K^+) current (I_{to}). Influx of calcium ions via L-type Ca^{2+} channels carries a small depolarizing current ($I_{Ca\text{-}L}$) which stimulates the release of calcium from the sarcoplasmic reticulum (SR) though ryanodine receptors (RyR2). This regenerative process known as calcium-induced calcium-release results in tropomyosin translocation and myofilament contraction. Cytosolic Ca^{2+} levels are restored to diastolic levels by the coordinated activities of the Na^+–Ca^{2+} exchanger (NCX) and the SR calcium ATPase (SERCA2a). A delicate balance between inward and outward currents results in the plateau phase of the AP. Finally, AP repolarization is sculpted by the orchestrated activities of the delayed rectifier K^+ currents (I_{Kr} and I_{Ks}), the inward rectifier K^+ current (I_{K1}) along with a gradual decrease in net depolarizing currents. The membrane potential of fast response myocytes is effectively locked at rest (phase 4) by the background I_{K1} until the next wave of depolarization activates I_{Na} resulting in a new propagating AP.

Since most ion channels are time- and voltage-dependent, even a subtle change in one ionic transport mechanism can modulate the activity of many other channels and transporters, often profoundly altering the AP duration and profile. As in the long QT syndrome, HF results in a major prolongation of the cellular AP, which translates clinically into QT interval prolongation on the surface electrocardiogram (ECG). Numerous studies using cellular electrophysiology, molecular biology, protein chemistry, high-throughout gene expression profiling and proteomics have considerably advanced our understanding of ion channel remodeling in HF [5, 7]. Complementary studies in multicellular tissue preparations are bridging major gaps in our knowledge of the functional consequences of ion channel remodeling and dysfunction in generating arrhythmias [8–10]. In this Chapter, we summarize some of the key developments in this rapidly evolving field of investigation, with a view towards identifying appropriate ion channel targets for antiarrhythmic therapy in HF.

Potassium Channel Remodeling in Heart Failure

Transient Outward K^+ Current

I_{to} inscribes the notch of the AP during early repolarization and sets the membrane potential at which activator calcium is initiated. As such I_{to} indirectly controls excitation–contraction coupling and AP duration by modulating the take-off potential at which subsequent plateau currents are activated [11]. I_{to} varies regionally (right ventricle > left ventricle) and transmurally (epicardium > midmyocardium > endocardium). Functional down-regulation of I_{to} is consistently observed in most models of HF [7]. The effect of HF-induced I_{to} down-regulation on

AP duration varies across species. Since I_{to} is the major repolarizing current in rodents (with the exception of guinea pigs), its reduction is directly responsible for AP prolongation in these species [12]. On the other hand, in humans and large animal models reduction of I_{to} influences the early part of the AP (phase 1), reducing Ca^{2+} entry through $I_{Ca\text{-}L}$ and shortening the AP duration [13]. The discrepancy in how I_{to} modulates AP duration highlights the importance of exercising caution when applying knowledge gained from rodent studies of repolarizing currents to humans before carefully investigating large animal models.

Kv4.3 (Shal-related subfamily, member 3) encodes the pore-forming alpha subunit of cardiac I_{to} in large mammals [14]. An additional component of I_{to} (encoded by *Kv1.4*) with slower inactivation kinetics also exists preferentially in the endocardial layer of rodents [15]. The role of *Kv1.4* channels in large animals and humans remains unclear. Despite abundant expression of *Kv1.4* in the myocardium, we recently argued against its functional role in the formation of native I_{to} in human and canine ventricular myocytes [16]. Our findings highlight important differences in the molecular composition of I_{to} and other repolarizing currents across species. Such species differences may profoundly impact the response to pharmacological agents targeting ion channel components. I_{to} down-regulation in HF is transcriptionally regulated as *Kv4* mRNA levels are reduced in both humans [14] and dogs with HF [6]. Interestingly, gene transfer of *Kv4.3* shortened AP duration and limited the hypertrophic response in rat hearts that underwent transaortic constriction [17]. The effects of *Kv4.3* over-expression in large animal models whose terminal repolarization is sculpted by delayed rectifier K^+ currents, however, remain unknown.

Other accessory subunits participate in the formation of native I_{to}, including Kv-channel interacting proteins (KChIPs) [18] and the CD26-related dipeptidyl aminopeptidase-like protein 6 (DPPX) [19]. Interestingly, both of these accessory subunits likely contribute to the formation of the transmural I_{to} gradient [20, 21]. Recently, another beta subunit (MiRP-1; potassium voltage-gated channel subfamily E member 2) originally known to modulate I_{Kr} has also been shown to physically associate with, and functionally regulate Kv4- and Kv1-encoded channels in mammalian expression systems [22]. Elucidation of the macromolecular complex that forms native I_{to} is expected to offer new opportunities for regulating this current, which is strongly remodeled by HF, and whose dysregulation has been linked to various arrhythmic syndromes [23, 24]. The strong influence of KChIP2 and MiRP-1 on multiple ion channels suggests that macromolecular complexes involving the same accessory subunits could indeed regulate diverse electrophysiological properties [18, 22]. As such, selective targeting of these proteins is expected to produce complex physiological outcomes that are difficult to predict, especially in the setting of HF, in which these subunits are differentially remodeled.

Inward Rectifying K^+ Current

I_{K1}, encoded by the *Kir2.x* family of genes, maintains the resting membrane potential and contributes to terminal repolarization. Reduced I_{K1} density in HF promotes AP prolongation and enhanced susceptibility to spontaneous membrane depolarizations [25, 26]. Indeed, the genetic suppression of I_{K1} converts fast-response myocytes to cells with automatic activity (i.e. biological pacemakers) [27]. Disease-induced downregulation of I_{K1} could, therefore, enhance automaticity in the failing heart. Interventricular differences in I_{K1} density (left ventricle > right ventricle) underlie the localization of high-frequency sources of activation during ventricular fibrillation [28]. Moreover, pharmacological suppression of I_{K1} reduces the dominant frequency of the so-called 'mother rotor' underlying arrhythmias in structurally normal hearts [28]. Conversely, overexpression of I_{K1} leads to the acceleration and maintenance of fibrillatory rotors [29]. While I_{K1} down-regulation promotes triggers and enhanced automaticity, I_{K1} overexpression can shorten the AP and the cardiac wavelength, thereby stabilizing reentrant activity. This delicate balance supports the notion that ion channels are regulated to operate within a narrow physiological range, such that excessive modulation (reduction or enhancement) of ion channel activity or expression can have equally

deleterious effects. This property adds another challenge to pharmacological and gene transfer approaches targeting ion channels, whose expression is differentially altered across myocardial layers and regions of the failing heart.

The underlying basis for I_{K1} downregulation in HF remains unknown, as no consistent changes in the steady-state levels of *Kir2.1* (inward rectifier potassium channel 2) mRNA [14] or protein [6] have been found. Surprisingly, *Kir2.1* mRNA and protein levels are up-regulated in terminally failing human hearts [30]. Clearly, further investigation into the molecular identity and regulation of I_{K1} by HF is warranted.

Delayed Rectifier K^+ Currents

The delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) play a prominent role in the control of terminal cardiac repolarization in large animals and humans. In fact, a relatively small reduction of either component leads to significant AP prolongation [31]. The reduction in the outward current, over the plateau range of voltages in various models of HF, predisposes to the development of arrhythmogenic early afterdepolarizations (EADs) [8, 32]. Indeed, K^+ current downregulation in HF underlies key phenotypic similarities between HF and the long QT syndrome, including AP and QT-interval prolongation, reduced repolarization reserve and arrhythmia propensity [33, 34].

Despite knowledge of the major alpha and beta subunits that form I_{Kr} and I_{Ks}, the molecular mechanisms underlying I_{Kr} and I_{Ks} downregulation in HF remain controversial. We measured the expression of *HERG* (encoding I_{Kr}) and *KvLQT1* (encoding I_{Ks}) in normal and failing canine ventricles. Surprisingly, we found a paradoxical increase in *HERG* expression with no change in *KvLQT1* protein levels [6]. Other groups reported decreased *HERG* expression in human HF [35]. These discrepant findings highlight the fact that ion channel function is dependent on complex factors that are well beyond the expression levels of the alpha and beta subunits that form these channels. For example, changes in protein assembly, folding, trafficking, membrane insertion, internalization, and degradation can significantly modulate channel behavior.

Evidence is emerging for the existence of macromolecular complexes that include diverse proteins in the proper assembly, trafficking and function of cardiac ion channels, such as I_{Kr} and I_{Ks}. Interestingly, a strong physical and functional interaction between *KvLQT1* and *HERG* was recently demonstrated [36]. Specifically, *KvLQT1* was shown to modulate both the distribution and biophysical properties of *HERG*. Indeed, this elegant demonstration of an interaction between two K^+ channel alphasubunits underscores the complexity of selectively targeting individual ion channels without affecting others [36].

Gene transfer of various alpha and beta subunits that encode I_{Kr} and I_{Ks} has been used to modulate AP duration in small animal models [26, 37]. Whether or not these strategies can reverse remodel the failing heart, normalize AP duration, reduce repolarization gradients, and prevent arrhythmias remain to be seen. Indeed, the complex regulation of ion channel function by post-translational modifications may strongly limit the ability of gene transfer of individual alpha and beta subunits to completely and safely restore ion channel function in the failing heart.

ATP-Sensitive K^+ Current

Sarcolemmal (Sarc) K_{ATP} channels link membrane excitability to metabolism [38]. They are regulated by intracellular nucleotides, membrane phospholipids, protein kinases and phosphatases [38]. The ATP-sensitive K^+ current ($I_{K\text{-}ATP}$) is the principal mediator of AP shortening under conditions of increased metabolic demand and/or energy deficit. Due to their abundance in the plasma membrane, the opening of sarcK_{ATP} channels causes rapid AP shortening, loss of intracellular K^+, and reduction in myocyte excitability. Interestingly, AP shortening in ischemia is exaggerated in cells from hypertrophied compared to normal ventricles [39]. We described a novel mechanism by which oscillations in the mitochondrial membrane potential ($\Delta(\text{delta})\Psi(\text{psi})_m$) under conditions of oxidative stress, can produce oscillations in the AP duration via cyclical activation of $I_{K\text{-}ATP}$, setting the stage for conduction block (which was termed 'metabolic sink') and arrhythmias [40, 41]. While hypertrophied and

failing hearts are associated with oxidative stress and $\Delta(\text{delta})\Psi(\text{psi})_m$ instability [42, 43], the exact role of sarcK_{ATP} channels in HF-mediated arrhythmias remains unclear.

Pacemaker Current

The hyperpolarization-activated pacemaker or 'funny' current (I_f) is a nonselective cation current originally described in automatic tissues such as the sinoatrial node [44]. Although I_f has been observed in human ventricular myocytes [45, 46], it appears to activate at negative voltages outside the physiological range of membrane potential. Nonetheless, increased I_f density in hypertrophy suggests its possible role in either promoting disease progression or arrhythmic triggers [47]. The *HCN* (hyperpolarization-activated mammalian cation channels) family of genes encoding I_f has been cloned [48, 49]. In a canine model of HF, *HCN* expression is significantly decreased in the sinoatrial node and increased in the right atrium, likely contributing to sinus node dysfunction and atrial ectopy [50].

Adenoviral-mediated gene transfer of an engineered *HCN* construct, which exhibits a shift in its inactivation kinetics to more depolarized potentials, unleashes a biological pacemaking source in guinea pig ventricles and pig atria [51]. Increased I_f in the setting of reduced I_{K1} is likely to predispose the failing heart to enhanced automaticity. A strategy for suppressing ventricular I_f could, therefore, be appealing in HF. Indeed, Ivabradine, a potent I_f inhibitor that suppresses the spontaneous depolarization rate in sinoatrial nodal cells, improved cardiac structure and function in HF [52]. The **S**ystolic **H**eart Failure Treatment with the $\boldsymbol{I_f}$ Inhibitor **T**rial (SHIFT) has shown significant efficacy in reversing left ventricular remodeling in a large cohort of patients with HF [53].

Calcium Channel Remodeling in Heart Failure

Excitation–contraction coupling refers to the fundamental principle by which a myocyte's ionic (excitation) properties tightly coordinate its mechanical function. Defective Ca^{2+} handling in HF has a profound electrophysiological impact because the intracellular calcium transient and the AP are intricately linked by a variety of Ca^{2+}-mediated channels and transporters.

L-Type and T-Type Ca^{2+} Channels

Ca^{2+} entry through $I_{Ca\text{-}L}$ triggers SR Ca^{2+} release. The density of $I_{Ca\text{-}L}$, dictated in part by the stage of HF [54, 55], is increased in mild-to-moderate hypertrophy and decreased in more advanced stages of hypertrophy and failure [56, 57]. Interestingly, myocytes from failing hearts also exhibit attenuated augmentation of $I_{Ca\text{-}L}$ in response to beta-adrenergic stimulation [58]. Finally, slowing of $I_{Ca\text{-}L}$ inactivation in HF alters Ca^{2+} handling and prolongs the AP [59]. The molecular mechanisms underlying these changes are unknown, but could involve a dephosphorylation defect that alters open channel probability [60]. Sipido et al. demonstrated that the negative force–frequency relationship in dilated cardiomyopathy is due to impaired recovery of $I_{Ca\text{-}L}$ inactivation at fast stimulation frequencies. These findings provided a potential ionic target for improving myocardial contractile reserve [61].

The pore-forming subunit of $I_{Ca\text{-}L}$ is encoded by *CACNA1C* (calcium channel, voltage-dependent, L type, alpha 1C subunit) and is highly regulated by a variety of accessory subunits that affect channel trafficking, current density and kinetics. *In vitro* and *in vivo* knockdown of the L-type Ca^{2+} channel accessory beta subunit, using a short hairpin RNA template sequence, reduced $I_{Ca\text{-}L}$ and attenuated the hypertrophic response, without compromising systolic performance [62].

Another class of sarcolemmal calcium channels is the low-voltage-activated transient Ca^{2+} channel ($I_{Ca\text{-}T}$), encoded by the *Cav3.x* family of genes [63]. Since these channels activate at hyperpolarized potentials, they might contribute to enhanced automaticity [64], especially in the failing heart, in which $I_{Ca\text{-}T}$ density is considerably enhanced [65]. Interestingly, $I_{Ca\text{-}T}$ was observed in myocytes from endocardial, but not epicardial, layers of the normal canine ventricle [66]. As such, $I_{Ca\text{-}T}$ could also contribute to the electrical and contractile heterogeneity seen

across the transmural wall. Whether or not this intrinsic heterogeneity is increased or decreased in HF remains unknown.

Since abnormal calcium cycling is a central feature of mechano-electrical dysfunction of the failing heart, pharmacological and genetic modulation of individual Ca^{2+} channel subunits could be useful strategies for restoring defective excitation-contraction coupling and electrophysiological properties. However, these strategies have the strong potential to exacerbate intracellular calcium overload and associated dysfunction (increased calcium entry) or result in atrioventricular conduction delays and block (decreased calcium entry).

Sarcoplasmic Reticulum (SR) Ca^{2+} Pump

The amplitude and rate of decay of the intracellular calcium transient are blunted in cells and tissues from failing hearts [67]. These changes are caused by defective sequestration of Ca^{2+} by the SR due, in large part, to reduced SERCA2a expression and function [68]. Since compromised SR Ca^{2+} re-uptake causes abnormal contractile and electrical function in HF, increasing the expression and activity of SERCA2a could be clinically beneficial. Pharmacological stimulation of the pump enhances mechanical function [69]. Furthermore, numerous studies using adenoviral-mediated gene transfer approaches to increase myocardial SERCA2a expression, have demonstrated the efficacy of restoring impaired intracellular Ca^{2+} handling and normalizing contractile dysfunction [70]. The safety of this approach has been confirmed by experiments that demonstrated improved contractility at no metabolic cost (i.e. cost of oxygen) in normal [71] and failing hearts [72]. Targeted gene-transfer techniques to increase the expression levels of SERCA2a using adeno-associated vectors have also been developed [72], and a First-in-man multicenter trial (CUPID, **C**alcium **U**pregulation by **P**ercutaneous Administration of Gene Therapy **I**n Cardiac **D**isease) has been completed [73, 74]. Whether or not enhancing SERCA2a expression and/or function in the failing heart will protect against or exacerbate arrhythmias remains to be fully determined, although no adverse electrical outcomes were reported in the Phase 1 and Phase 2a CUPID trials [73, 74]. Finally, SERCA2a over-expression using gene transfer has been recently shown to suppress arrhythmogenic repolarization alternans in structurally normal hearts [75].

Phospholamban

Phospholamban (PLB), a key regulator of SERCA2a, blunts the rate of SR Ca^{2+} re-uptake [76]. The inhibitory influence of PLB on SERCA2a is reduced when the protein is phosphorylated [77]. Therefore, restoration of intracellular calcium cycling in HF could be achieved by targeting PLB. To that end, PLB silencing using an anti-sense strategy successfully enhanced SERCA2a activity and improved contractile function [78]. In contrast, PLB gene ablation in a transgenic mouse model failed to improve global cardiac function, possibly because of compromised metabolic properties in that animal model [79]. Pharmacological manipulation aimed at dissociating PLB from SERCA2a has also been successfully tested in the laboratory [80]. Potential differences in the electrophysiological consequences of enhancing SR calcium uptake by targeting SERCA2a versus PLB requires direct investigation. Indeed, it is expected that these complementary approaches may be associated with unique benefits and potential pitfalls depending on the disease etiology and the sympatho-adrenergic state, as these calcium regulatory proteins are modulated by local kinase and phosphatase signaling mechanisms.

Na^+/Ca^+ Exchanger

In its 'forward' mode of operation, NCX extrudes intracellular Ca^{2+} via an electrogenic exchange for extracellular Na^+ (one Ca^{2+} for three Na^+ ions), thereby generating a net inward current [81]. Indeed, forward-mode NCX function compensates for defective SR Ca^{2+} uptake at the expense of depleting the releasable pool of Ca^{2+} with repetitive stimulation. In contrast, reverse-mode exchange (Na^+ out and Ca^{2+} in) could provide inotropic support to the failing ventricle while shortening AP duration [82].

NCX expression and function are increased in HF [83, 84], contributing to AP prolongation and

repolarization instability. Importantly, partial inhibition of NCX enhances SR Ca^{2+} load by shifting the balance of Ca^{2+} flux away from transsarcolemmal efflux [85]. As such, NCX blockade could represent an effective therapeutic strategy for improving contractility in HF [85]. Unfortunately, the efficacy of abrogating arrhythmias by targeting NCX will have to await the development of more selective pharmacological agents with improved lipophilicity and bioavailability properties.

Ryanodine Receptors

RyR2 channels are downregulated in HF, both at the mRNA [86] and protein [87] levels. Hyperphosphorylation of RyR2 causes FKBP12.6 (FK506-binding protein 1B) dissociation resulting in diastolic Ca^{2+} leak and the generation of Ca^{2+} waves underlying triggered activity [88]. Whether protein kinase A [88], CaMKII [89], or both mediate RyR2 hyperphosporylation and diastolic calcium leak in HF has been the subject of intense debate. Identification of the specific molecular sites of RyR2 phosphorylation might lead to pharmacological strategies designed to suppress diastolic Ca^{2+} leak and associated arrhythmias. Marks and colleagues identified a class of small molecules that enhance the binding affinity of FKBP12.6 for RyR2 [90]. While these compounds are effective in suppressing catecholaminergic polymorphic ventricular tachycardia in mouse models [91, 92], their utility in preventing HF-related arrhythmias remains to be tested in clinically relevant large animal models of acquired structural heart diseases.

Calmodulin and Calcium/Calmodulin-Dependent Protein Kinase II

Calmodulin kinase II (CaMKII), the main target of Calmodulin binding, is a multifunctional protein capable of phosphorylating several Ca^{2+}-handling proteins, including RyR2, PLB, and SERCA2a. In human HF, CaMKII activity is markedly increased [93, 94], possibly as a compensatory mechanism for altered Ca^{2+} homeostasis. Since phosphatase activity is also enhanced in human HF, the net effect with respect to the phosphorylation state of any given target protein is locally controlled within the subcellular milieu [95]. Over-expression of CaMKII has been shown to increase SR Ca^{2+} leak through RYR2, without altering myocyte contractility [96]. Anderson and colleagues demonstrated that CaMKII inhibition could represent an effective strategy for improving myocardial function and preventing atrial and ventricular arrhythmias in various animal models [97].

Sodium Channel Remodeling in Heart Failure

In light of the important interplay between Na^+ and Ca^{2+} in the cardiac cell, changes in intracellular Na^+ levels are expected to alter both mechanical and electrophysiological properties [98]. Moreover, as Na^+ channels are critical to normal impulse propagation in the heart, perturbations in I_{Na} could lead to conduction slowing, block, and arrhythmias. In what follows, we summarize some of the major changes in Na^+ transport mechanisms that occur in HF.

Na^+ Channels

Normal impulse formation and conduction depend on the fast inward I_{Na}. An increase in the late component of this current can markedly prolong AP duration and promote polymorphic ventricular tachycardia [99]. Changes in I_{Na} density and kinetics could, therefore, promote arrhythmias, either by disrupting conduction or prolonging repolarization. In a canine model of myocardial infarction, major downregulation of I_{Na}, acceleration of its inactivation kinetics and slowing of its recovery from inactivation were observed in myocytes isolated from the infarct border zone [100]. In another canine model of repeated microembolization-induced HF, a considerable increase in the late component of I_{Na} was demonstrated [101]. As such, changes in I_{Na} are likely to depend on the specific disease etiology, and could have profound implications for arrhythmogenesis given the relative abundance and importance of this current to myocyte excitability.

Over-expression of $Na_V1.5$ (also known as *SCN5A*), which forms the alpha subunit of I_{Na} exerted an antifibrillatory effect by enhancing excitability in monolayers of neonatal rat ventricular myocytes [102]. In addition to *Nav1.5*, I_{Na} activity is modulated by a variety of auxiliary beta subunits, kinases, phosphatases and cytoskeletal proteins. HF-induced disruption of the macromolecular complex that regulates I_{Na} results in marked changes in current density and kinetics, which alter conduction (by affecting the amplitude of I_{Na}) and repolarization (by affecting the late component of I_{Na}). Finally, pharmacological inhibition of the late I_{Na} with Ranolazine has shown significant promise in improving mechano-electrical function and suppressing atrial and ventricular arrhythmias [103–106]. Indeed, a prospective, proof-of-concept study (RAnolazine for the Treatment of Diastolic Heart Failure, RALI-DHF) was recently initiated to determine the efficiacy of late I_{Na} blockade in improving diastolic function in HF patients with preserved ejection fraction [107].

Na^+/K^+ ATPase

Intracellular Na^+ is increased in ventricular hypertrophy and HF by approximately two to three fold [108]. This causes a secondary rise in Ca^{2+} influx via reverse-mode NCX [108], which in turn increases SR Ca^{2+} load and enhances contractility. This seemingly beneficial, positive inotropic effect comes at a price — enhanced susceptibility to spontaneous SR Ca^{2+} release through RyR2, activation of the transient inward current and the development of delayed afterdepolarizations, especially in the setting of beta-adrenergic stimulation [81]. Hence, tight regulation of intracellular Na^+ is required for normal electrogenesis.

The Na^+/K^+ ATPase exchanges extracellular K^+ for intracellular Na^+, with a stoichiometry of 2:3, thereby generating a net outward repolarizing current. Reduced Na^+/K^+ ATPase activity in some models of HF contributes to intracellular Na^+ and Ca^{2+} overload, AP prolongation and arrhythmias. Molecular mechanisms underlying altered Na^+/K^+ ATPase activity may include changes in the expression of its three alpha isoforms. In addition, major changes in the expression and phosphorylation of Phospholemman, a key regulatory subunit of the Na^+/K^+ ATPase pump have been identified in a rabbit model and in human HF samples. Indeed, recent advances in our understanding of the regulation and physiological properties of Phospholemman under normal and stress conditions are likely to yield new opportunities for controlling the Na^+/K^+ ATPase pump, intarcellular Na^+ homeostasis and electromechanical function in HF.

Na^+–H^+ Exchanger

The Na^+–H^+ exchanger (NHE) regulates intracellular pH via proton extrusion, driven by the transmembrane Na^+ gradient. NHE inhibition is cardioprotective against myocardial ischemia–reperfusion injury [109]. Prevention of intracellular Na^+ accumulation and excessive Ca^{2+} influx via reverse-mode NCX, have been proposed as the mechanism of cardioprotection by NHE inhibition. Since HF is associated with elevated intracellular Na^+ levels, NHE could represent a promising target for antiarrhythmic therapy. The **GUARD** During **I**schemia **A**gainst **N**ecrosis (GUARDIAN) trial was designed to determine the efficacy of Cariporide, a selective NHE inhibitor, in reducing mortality. Although GUARDIAN failed to demonstrate an overall clinical benefit in patients at risk of myocardial necrosis [110], it is clear that NHE inhibition preserves mitochondrial function and therefore the metabolic status of the myocyte. As such, NHE inhibition is likely to play a critical role in preventing cellular apoptosis and related dysfunction under conditions of oxidative stress, such ischemia-reperfusion injury. The role of NHE inhibition in modulating the electrophysiological substrate and preventing arrhythmias in HF requires direct exploration. Given the central role of mitochondria in regulating excitability and arrhythmias, it is conceivable that NHE inhibition may play a prominent role in restoring normal electrogenesis to the failing heart.

In what follows, we illustrate how ion channel remodeling at the cellular level alters the electrophysiological substrate at the organ level.

Particularly, we focus on mechanisms by which transmural and intra-ventricular repolarization gradients predispose the failing heart to lethal arrhythmias.

Electrophysiological Remodeling at the Tissue Level

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 ECG [2, 111, 112]. 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. 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 developed sophisticated algorithms that detect various ECG metrics of global cardiac repolarization to identify patients at high risk of sudden cardiac death (SCD) [113–122]. Of key importance are recent findings of a multi-center clinical trial (**A**lternans **B**efore **C**ardioverter **D**efibrillator, ABCD), 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 [116].

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 [8, 9, 87, 123, 124]. Importantly, a quantitative relationship between altered spatio-temporal repolarization gradients and the incidence of arrhythmias in various animal models of HF have recently emerged [8, 125]. In what follows, we focus on the role of spatial heterogeneities of repolarization on the incidence of reentrant arrhythmias in clinically relevant large animal models of HF that are prone to arrhythmias. Specifically, we discuss changes in transmural and intra-ventricular repolarization gradients as mechanisms for ventricular arrhythmias in non-ischemic dilated cardiomyopathy and dyssynchronous heart failure, respectively.

Transmural Repolarization Heterogeneity in Dilated Cardiomyopathy

Antzelevitch and colleagues [126, 127] advanced the notion that heterogeneities of cellular repolarization in different cell types (epicardial, mid-myocardial, and endocardial) represent a unifying mechanism underlying a host of arrhythmias in congenital and/or acquired cardiac diseases, such as the long QT, short QT [128], Brugada [129], Andersen-Tawil [130], and Timothy syndromes [131, 132]. 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 long QT syndrome [127, 133, 134]. Because QT interval prolongation represents an electrophysiological hallmark of the failing heart, we hypothesized that both disease states (long QT syndrome and HF) may share important phenotypic properties at the multi-cellular tissue level that predispose them to arrhythmias via similar mechanisms [8]. This hypothesis was directly investigated by determining the functional expression of repolarization gradients across myocardial layers and their potential role in the mechanism(s) of arrhythmias in a model of non-ischemic dilated cardiomyopathy produced by chronic rapid pacing in the dog [8]. As expected, HF was associated with a marked AP prolongation across all myocardial layers, consistent with findings in isolated myocytes and

whole animals. Interestingly, AP prolongation was heterogeneous across the left ventricular (LV) wall, affecting mid-myocardial and endocardial muscle layers more selectively; thereby, increasing the effective transmural repolarization gradient by ~2-fold [8]. In support of transmural dispersion of repolarization as a unifying mechanism for arrhythmias associated with various disease etiologies, Yan et al. elegantly demonstrated that LV 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 [135]. Transmural repolarization heterogeneity in HF produced an arrhythmic substrate, as premature stimuli introduced at a critical window during AP repolarization resulted in intramural decremental conduction and block. Importantly, conduction block which was localized at the interface of the mid-myocardial layer was followed by the initiation of ventricular tachycardia.

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 calcium handling proteins. Li et al. [32] investigated the ionic basis of transmural AP remodeling in HF by measuring the density of key repolarizing K^+ currents, including I_{to}, I_{K1}, I_{Kr} and I_{Ks}. In general, 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 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 hearts [136]. In support of the findings of Li et al. [32] 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 heart.

Poelzing and Rosenbaum [137] attributed the location of the maximum transmural repolarization gradient to increased electrical resistivity at that location. Furthermore, they converted transmural repolarization gradients measured across normal preparations into gradients that mimicked those in HF simply by perfusing normal preparations with the gap junction inhibitor, Carbenoxolone [137]. 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 [9, 125, 138, 139]. 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 hypertrophy [125]. Interestingly, hyperphosphorylation of Cx43 also occurred at earlier stages of remodeling that were associated with a milder form of conduction slowing [125]. 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 requires direct investigation.

Intra-ventricular Repolarization Heterogeneity in Dyssynchronous Heart Failure

Left ventricular (LV) dyssynchrony caused by delayed activation of the lateral free wall results in heterogeneous mechanical stress across the ventricle [140]. We hypothesized that regional differences in local mechanical function across the dyssynchronously contracting failing ventricle produce important changes in electrophysiological, metabolic, and molecular properties, which ultimately worsen outcome in

these patients. We further hypothesized that re-establishing LV synchrony using bi-ventricular pacing in cardiac resynchronization therapy (CRT) could potentially reverse-remodel the electrophysiological substrate. Indeed, the late activating lateral LV endocardium of dyssynchronously failing hearts is distinguished by selective remodeling of calcium handling, gap junction, and stress related molecules [141]. These regionally heterogeneous molecular changes were linked to key electrophysiological abnormalities. Specifically, Aiba et al. [142] found that myocytes isolated from the lateral LV of dyssynchonrously failing hearts were characterized by excessive AP prolongation and enhanced propensity for early after depolarization mediated triggered beats. The ion channel basis of these changes was also investigated. Specifically, dyssynchronous mechanical contraction of the failing heart reduced K^+ current densities (I_{to}, I_K, and I_{K1}) in both anterior and lateral regions. In contrast, $I_{Ca\text{-}L}$ was differentially remodeled across the dyssynchronously failing heart, potentially underpinning differences in repolarization properties and susceptibility to early afterdepolarizations. In particular, a significantly greater reduction in $I_{Ca\text{-}L}$ amplitude and decay rate in myocytes isolated from the high-stress lateral compared to the low-stress anterior LV wall was described. Interestingly, CRT partially restored abnormal repolarization of lateral LV myocytes, reducing the regional disparity in electrophysiological function across the failing ventricle.

In a subsequent study, the effect of mechanical dyssynchrony on changes in regional gene expression was also investigated [143]. Electromechanical dyssynchrony resulted in complex cardiac transcriptome remodeling, causing major gene expression changes in the anterior wall. Once again, CRT corrected the region specific alterations in gene expression, highlighting the importance of mechanical synchrony/dyssynchrony on global and regional gene expression remodeling, which is likely to impact diverse cellular processes. As such, the electrophysiological and molecular changes induced by CRT may indeed suppress ventricular arrhythmias and potentially promote mechanical and metabolic function.

Finally, the influence of mechanical dyssynchrony per se on key electrophysiological properties was investigated independently of LV dysfunction [144]. Normal hearts were subjected to chronic left bundle branch block, inducing mechanical dyssynchrony with preserved LV function. We found that mechanical dyssynchrony in non-failing canine hearts was sufficient to cause regional changes in conduction and repolarization properties [144]. In addition, the distribution of the main ventricular gap junction protein, Cx43 was significantly more lateralized in the late-activating lateral LV, which was associated with slower conduction and faster repolarization [144]. Taken together, these findings suggest an important mechanosensitive component to chronic remodeling associated with dyssynchronous LV contraction which causes regional changes in protein expression and electrophysiological properties that enhance intra-ventricular repolarization gradients and potentially predispose to reentrant arrhythmias. Understanding mechanisms by which local mechanical stresses and strains regulate electrophysiological properties offers the potential for designing novel device-based strategies for the control of arrhythmias in heart failure.

Conclusions

Complex remodeling of a host of ion channels and Ca^{2+}-cycling proteins modulates key cellular electrophysiological properties, predisposing to arrhythmias and sudden death. HF-induced ion channel dysfunction prolongs the AP, increases spatiotemporal gradients of repolarization, promotes arrhythmogenic triggers and results in conduction abnormalities. Understanding fundamental ionic mechanisms of normal and abnormal electrogenesis is a key requirement for the development of modern therapies. Elucidating the role of individual current components and their underlying molecular identities presents a unique opportunity for the development of novel pharmacological, device, gene, and cell based approaches for the treatment of arrhythmias in HF.

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