Biophysics of Membrane Currents in Heart Failure

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Introduction

The cardiac action potential (AP) is made possible by active and passive processes that maintain highly regulated electrochemical gradients for sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) ions through cell membrane ion channels, transporters, and energy-dependent pumps. The delicate balance between depolarizing and repolarizing ionic currents is subject to disruption in heart failure (HF), leading to arrhythmias and mechanical pump dysfunction. Altered membrane currents are implicated in sudden cardiac death (SCD), which claims up to 450,000 lives annually in the United States [1, 2]. While ischemic heart disease is responsible for three quarters of these deaths, the rest are largely attributable to HF, and a 2.6–6.2-fold increased risk of SCD exists in the HF population compared to those without left ventricular (LV) dysfunction [3]. Moreover, worsening LV dysfunction and higher New York Heart Association HF classes correlate with an increased risk of SCD [4]. Cell surface membrane proteins are altered in numerous ways in HF, including regulation of gene expression, posttranslational modifications, protein assembly and trafficking, membrane insertion, functional control and degradation.

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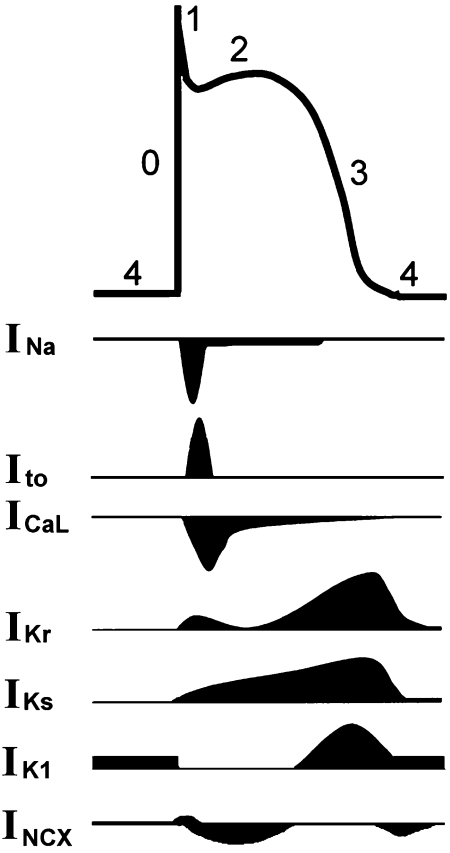

Fig. 1 A schematic representation of the cardiac action potential (AP) with the major contributing cardiac ion channel currents after [5, 6]

New research continues to offer potential therapeutic targets by elucidating the pathways for these observed changes.

While not every contribution to membrane potential during the cardiac AP in myocytes is fully understood, consensus does exist with respect to the main contributing ion fluxes resulting in the five phases of the cardiac AP cycle. As shown in Fig. 1, in a representative cardiac AP of ventricular myocytes [5, 6], Phase 0 is characterized by a rapid depolarizing inward Na^+ current (I_{Na}) that depends upon the voltage-gated Na^+ channel ($Na_v1.5$) encoded by SCN5A. These channels are inactivated within milliseconds of opening. This upstroke is followed by Phase 1, which is characterized by a brief repolarization phase as a result of activation of voltage-gated K^+ channels ($K_v4.x$) that allow for a transient outward K^+ current (I_{to}). Phase 2 is a plateau phase in which an inward Ca^{2+} current (I_{CaL}) through voltage-gated L-type Ca^{2+} channels ($Ca_v1.2$) is balanced by at least two outward K^+ currents: the rapid and slow outward delayed rectifier K^+ currents (I_{Kr} and I_{Ks}), which also contribute to Phase 3, a rapid repolarization phase. I_{Kr} is conducted by HERG and MiRP-1, while I_{Ks} by K_vLQT1 and minK. Additionally,

an inward rectifier K^+ current (I_{K1}) conducted by the Kir2.x family contributes to Phase 3 and maintains the resting membrane potential at baseline or Phase 4 by shifting the membrane potential towards the equilibrium potential of K^+. Other contributing membrane proteins include the Na^+-Ca^{2+} exchanger (NCX), the voltage-dependent T-type Ca^{2+} channel, and the Na^+-K^+ ATPase. Nerbonne and Kass provide an excellent review of the cardiac AP [7]. In HF, changes to cardiac $Na_v1.5$ channel I_{Na} contribute to clinical disease including arrhythmias. While the focus of this chapter will be on $Na_v1.5$, we will briefly review known changes in the other ion channels occurring during HF.

HF affects K^+ currents, and while these changes may be adaptive initially, they have the potential to lead to arrhythmias. For example, downregulation of K^+ currents allows for increased depolarization and time for excitation-contraction (EC) coupling that improves mechanical function in HF. Nevertheless, these changes also lead to afterdepolarizations, heterogeneous repolarization patterns, and ventricular arrhythmias [8–10]. Prolongation of the AP duration (APD) is observed in human HF, and animal models reveal that reductions in I_{to}, I_{K1}, and I_{Ks} are responsible [11–13]. The reduction of these currents has been linked to reduced transcription, translation, and expression of the corresponding channel subunits, such as $K_v4.3$, K_vLQT1, Kir2.1, and accessory proteins including minK and K^+ channel interacting protein 2 [14–17]. Reduced K^+ currents increase membrane resistance, leading to greater depolarization and delayed afterdepolarizations (DADs) [18, 19]. In HF, most studies find that I_{to} is downregulated, although there are conflicting data between human and other large animal studies [14, 18, 20, 21]. In humans, downregulation of I_{to} has been observed at the transcriptional and translational level as $K_v4.3$ mRNA levels are decreased and channel protein processing is altered [16, 22]. One mechanism is that the trafficking of $K_v4.3$ channel is downregulated by forming complexes with angiotensin receptors and increasing $K_v4.3$ internalization [23], which may explain the decreased channel density in HF [20, 21]. I_{K1} is found to be downregulated in HF at the mRNA level leading to increased susceptibility to spontaneous membrane depolarization and increased automaticity [13, 16, 20, 24]. Changes of I_{Kr} and I_{Ks} in HF are not yet well described in humans. The ATP-sensitive K^+ current and the pacemaker current (I_f), are activated at negative membrane potentials. Both are associated with increased automaticity in HF [25–27]. Of note, a repolarization reserve (redundant existence of several K^+ channels with different properties [28]), shields the heart from some pathologic changes seen in HF. Extreme reductions in one current or more may impair this reserve, leading to arrhythmogenic consequences [29]. Abnormalities of repolarization reserve also lead to exaggerated repolarization disturbances under circumstances that further exacerbate repolarization, such as with channel mutations causing decreased K^+ current [30]. Delayed ventricular repolarization can prompt early afterdepolarizations (EADs) generation and lead to ventricular tachycardia [11].

Ca^{2+} currents are dysregulated in HF as well. I_{CaL} has been shown to increase or decrease during HF depending on which channel alterations are dominant. Membrane density of the channel decreases because of reduced channel expression,

however, this is opposed by an increase in channel phosphorylation and a slowing of inactivation, which both serve to prolong the AP thereby increasing EC coupling time but also increasing arrhythmic risk [31–34]. Cardiomyocytes from patients with end-stage HF show decreased amplitude of the peak Ca^{2+} current at higher stimulation frequencies [35], which is because of incomplete recovery and accumulated inactivation of the $Ca_v1.2$ channel accompanied by slow Ca^{2+} removal [36]. This may cause repolarization failure, EADs and DADs. T-tubule loss observed in HF [37, 38] can also result in a loss of $Ca_v1.2$ channel availability and a decrease in I_{CaL}. HF has also been found to alter $Ca_v1.2$ channel modulators, such as the sarcoplasmic reticulum (SR) Ca^{2+} release channels—ryanodine receptors (RyR) and a membrane scaffolding protein, bridging integrator 1. Bridging integrator 1 is found to be reduced in HF, causing impaired $Ca_v1.2$ trafficking to T tubules [39]. A defective coupling of Ca^{2+} influx via the $Ca_v1.2$ channel has been noticed in reduced SR Ca^{2+} release in HF [40]. The NCX is upregulated in HF, which also causes AP prolongation and repolarization instability [41–44]. These examples highlight that there are multiple changes that take place during HF, and Nass et al. provide a detailed review of calcium handling in HF [45].

Changes in the Na^+ Channel with Heart Failure

The voltage-gated cardiac $Na_v1.5$ channel is responsible for the generation of the upstroke of the myocyte AP (Phase 0), which is the main current for excitation propagation [46–48]. The AP upstroke velocity determines AP impulse propagation and conduction velocity in heart tissue, along with the extent of intercellular communication via gap junctions. The cardiac $Na_v1.5$ is involved in determining the APD, since some channels may reopen during the plateau phase, generating a persistent or "late" inward current. The importance of cardiac $Na_v1.5$ channels for functional cardiac electrical activity is highlighted by the emergence of potentially lethal arrhythmias in inherited and acquired sodium channel diseases. Changes of the cardiac $Na_v1.5$ have been implicated in the increased risk of sudden death in HF [49–51]. The macroscopic $\boldsymbol{I}_{Na}$ is reduced in HF [52, 53] and $Na_v1.5$ inactivation is also impaired, causing repolarization failure and EADs and DADs [54–56]. Under pathological conditions such as myocardial ischemia and HF, altered Na^+ channel function causes conduction disturbances and ventricular arrhythmias.

The cardiac $Na_v1.5$ is a transmembrane protein containing a principal pore-forming α-subunit composed of four homologous domains, each containing six transmembrane segments (S1–S6). The four domains are attached to one another by cytoplasmic linker sequences. The positively charged S4 segment of each domain forms the voltage sensor, responsible for increased channel permeability during membrane depolarization [57]. In physiological situations, activation and inactivation properties of $Na_v1.5$ channels are tightly regulated, while during channel dysfunction the gating properties and current kinetics may be altered. The α-subunit interacts with smaller accessory proteins known as the β-subunits that

modulate the channel expression levels and function. The cardiac K^+ and Ca^{2+} channels share similar structures although with different components that result in different regulation and functions.

Since SCN5A, encoding the α-subunit of the cardiac $Na_v1.5$, was cloned and mapped to the chromosomal region 3p21 [58, 59], more than 100 mutations have been found in the gene [60], which cause inherited sudden death syndromes such as Brugada syndrome (BrS) [61], the third variant of long QT syndrome (LQT3) [62], and sudden infant death syndrome (SIDS) [63]. Mutations in the Na^+ channel have been associated with inherited diseases of the conducting system and more commonplace arrhythmias such as atrial fibrillation [64–71]. Loss of function mutations that show decreased or no I_{Na} are thought to confer arrhythmic risk by virtue of slowing conduction or altering the AP that both favor reentry. This can induce BrS, progressive cardiac conduction disease, sick sinus syndrome, or combinations thereof. In cardiomyopathic conditions, treatment with Na^+ channel-directed antiarrhythmic drugs increases sudden death risk, consistent with a downregulation of the channel [72–76]. On the other hand, gain of function mutations that mainly exhibit an increased late Na^+ current lead to AP prolongation, cellular Ca^{2+} overload, and afterdepolarizations. Alterations of I_{Na}, either up- or downregulation, lead to arrhythmias.

Na^+ Channel Current During HF

Various abnormalities of cardiac $Na_v1.5$ have been shown in HF, mainly emerged as reduced macroscopic I_{Na}, which contributes to slower conduction in HF facilitating reentry and ineffective cardiac contraction [18, 77, 78]. The underlying mechanisms for decreased I_{Na} density have been studied in animal models, including posttranscriptional [79] and posttranslational [52, 80, 81] deficiency of $Na_v1.5$. In a canine model of HF, a ~34 % decrease of I_{Na} and a ~30 % reduction of $Na_v1.5$ protein expression are observed without changes in channel mRNA level and gating properties in ventricular cardiomyocytes, indicating a downregulation of $Na_v1.5$ at posttranscriptional level [79]. I_{Na} reduction because of posttranslational alterations in protein kinases dysregulation, channel deglycosylation, channel trafficking disruption, metabolic and oxidative stress-induced channel dysfunction has been reported [52, 80, 81]. In a mouse HF model, a decreased I_{Na} is observed with altered gating properties as a result of deficient $Na_v1.5$ glycosylation, contributing to longer AP and a higher probability of EADs [52]. The cardiac $Na_v1.5$ channel changes in HF on gating, trafficking, and regulation by protein kinases and oxidative stress will be discussed separately. Here, we will focus on the metabolic regulation of cardiac $Na_v1.5$ in HF.

Cardiac injury from many causes is associated with altered metabolism and downregulation of $Na_v1.5$ [52, 53, 82, 83]. HF has been associated with less β-nicotinamide adenine dinucleotide (NAD^+) and increased reduced nicotinamide

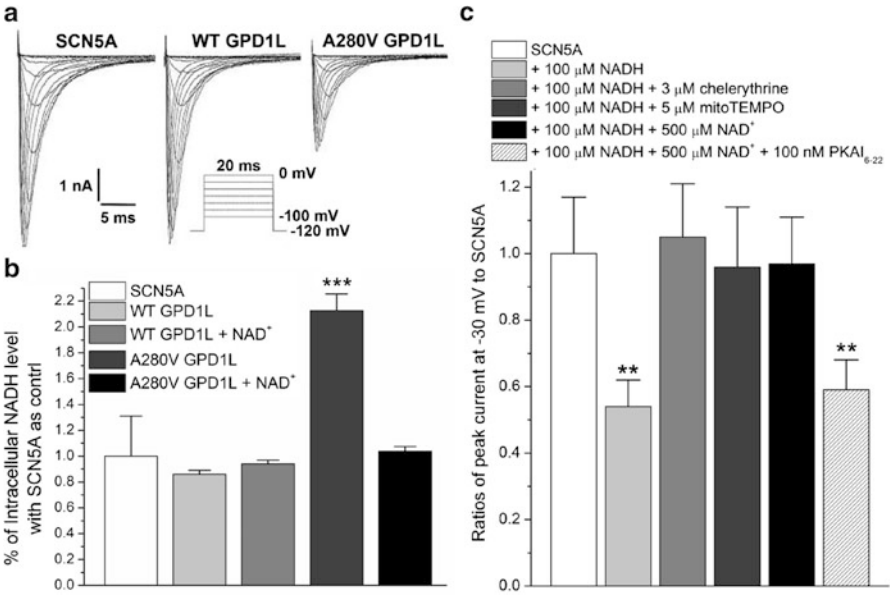

Fig. 2 The A280V GPD1L mutation decreases I_{Na} and increases intracellular NADH level. (**a**) Representative Na^+ current traces from HEK293 cells transfected with SCN5A (*left*), or cotransfected of SCN5A and WT (*middle*) or A280V GPD1L (*right*). (**b**) The intracellular NADH level is increased by A280V GPD1L and is reversed by incubation of NAD^+. ***$P < 0.001$ vs. all other groups. (**c**) The peak current of cardiac $Na_v1.5$ channel is downregulated by NADH and restored by NAD^+, a PKC inhibitor chelerythrine, or mitoTEMPO. The NAD^+ effect can be blocked by a PKA inhibitor, $PKAI_{6–22}$. **$P < 0.01$ vs. all other groups (modified from refs. [81, 89, 91] with permission)

adenine dinucleotide (NADH) [84–86], which can cause a reduction of I_{Na} [53, 87, 88]. For example, mutations of glycerol-3-phosphate dehydrogenase 1 like (GPD1L) protein have been found to induce BrS and SIDS with a decrease of I_{Na} (Fig. 2a) [89, 90]. An elevated NADH level has been associated with the reduction of I_{Na} observed in these cases (Fig. 2b) and the signaling events involve activating protein kinase C (PKC) and inducing oxidative stress, as shown in Fig. 2c [81]. Reduced I_{Na} may contribute to conduction block and arrhythmic risk known to exist with reduced cardiac contractility. Application of NADH results in reduction of I_{Na} and the maximum upstroke velocity of the AP on rat neonatal cardiomyocytes [81, 91]. The NADH-induced decrease of cardiac I_{Na} has also been observed in isolated cardiomyocytes from a mouse model of nonischemic systolic dysfunction HF [92]. This model shows a significantly increased NADH level, decreased I_{Na}, and the conduction velocity. The NADH-induced decrease of I_{Na} in HF can further exacerbate the changes in conduction velocity and contraction that lead to arrhythmic risk. These studies may help explain the link between metabolism and arrhythmic risk [87, 93].

Being in a redox couple with NADH, NAD^+ has been found to antagonize the $Na_v1.5$ channel downregulation by NADH (Fig. 2c) and may represent a new type of antiarrhythmic therapy. In our study of a mouse HF model, we have found that treating either isolated cardiomyocytes or HF animals with NAD^+ is able to increase the cardiomyocyte I_{Na} back to control levels [92]. Nevertheless, the NAD^+ effect does not seem to occur by the same signaling mechanism as does NADH and can be recapitulated by a protein kinase A (PKA) activator or prevented by a PKA inhibitor (Fig. 2c).

While it appears that HF can cause reduced I_{Na}, it is also possible that reduced I_{Na} can cause HF. HF has been reported in patients with SCN5A missense and truncation mutants [70, 94, 95]. For example, the mutation D1275N is associated with a variably expressed phenotype of conduction delay, dilated cardiomyopathy, atrial fibrillation, and impaired automaticity [70]. Two SCN5A mutants R814W and D1595H are reported to be associated with atrial and ventricular arrhythmia [96]. The mechanism whereby loss of Na^+ channel current contributes to HF is unknown.

Abnormal Na^+ Channel Gating in HF

In physiological situations, the biophysical gating properties of the cardiac $Na_v1.5$ are tightly regulated to maintain normal cardiac excitability. Altered gating properties or pathologic gating defects comprises delayed activation, earlier inactivation, faster inactivation, and enhanced slow inactivation [95]. In a mouse model of HF, altered $Na_v1.5$ channel gating properties with decreased I_{Na} has been found to contribute to prolonged APD and a higher probability of EADs [52]. For WT cardiac $Na_v1.5$ channels, there is a well-described component, called late current (I_{NaL}). I_{NaL} was first described in isolated Purkinje fibers in animal models and was found to prolong the AP [55, 97–99]. In disease states including HF and ischemia, more $Na_v1.5$ channels remain in the active state or reopen during Phase 1 and Phase 2 of the AP, causing increased I_{NaL} and further depolarizing the cell membrane. Silencing the $Na_v1.5$ gene expression leads to a 75 % decrease of I_{NaL} and results in reduction of APD and beat to beat AP variability in a HF model, implicating SCN5A as the underlying cause of I_{NaL} [100]. Augmented I_{NaL} and the concomitant increased intracellular Na^+ load in HF lead to dispersion of repolarization and EADs that predispose to ventricular arrhythmias [101–103].

There are likely many contributing factors to allow I_{NaL} to occur and persist in HF and ischemia. $Na_v1.5$ is complex, consisting of a core α subunit and β subunits, and the channel is affected directly by kinases, phosphatases, and trafficking proteins. While $Na_v1.5$ is downregulated in HF, the β subunits remain unchanged and the relatively higher membrane content of these subunits could be involved in generating I_{NaL} [79]. Ca^{2+}-mediated changes may also be responsible for I_{NaL}. There is evidence that Ca^{2+} can directly augment I_{NaL} by binding to the Na^+ channels [104]. Also, the Ca^{2+} binding protein calmodulin may also directly

augment I_{NaL} [105]. Calmodulin may regulate $Na_v1.5$ channel gating via Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). A study that used adenovirus-mediated overexpression of CaMKII found a Ca^{2+}-dependent increase in I_{NaL} [106]. While there are many questions remaining about the underlying mechanisms responsible for I_{NaL}, these different possibilities also offer potential targets for future therapies to block I_{NaL}.

Na^+ Channel Trafficking in HF

Some cases of decreased I_{Na} in HF are because of altered trafficking of cardiac $Na_v1.5$, which results in decreased membrane expression of the channel. Channel trafficking is complex and includes the translocation from the endoplasmic reticulum (ER) to the Golgi apparatus, subsequent trafficking to the sarcolemma, association with accessory subunits, and anchoring to the cytoskeleton, and regulation of endocytosis. Trafficking plays a pivotal role in posttranslational regulation and functional expression of the cardiac $Na_v1.5$ channels under both physiological and pathophysiological conditions. Intracellular trafficking may prove to be a useful tool in manipulating the expression of the cardiac Na^+ channel, and has been shown to be a possible therapeutic target in arrhythmia, epilepsy, anesthesiology, and cancer applications [107–110].

Trafficking changes in human HF are not well studied to date. Downregulation of $Na_v1.5$ channel trafficking has been hypothesized in a chronic canine HF model, which revealed a 30 % decrease of I_{Na} without changes of channel gating properties and channel RNA levels in the failing hearts compared to normal hearts [111]. In spite of lack of knowledge of $Na_v1.5$ trafficking changes in HF, mutations in cardiac $Na_v1.5$ channel and Na^+ channel interacting proteins that cause significant decreases of channel trafficking have been investigated intensively and shown to underlie cardiac arrhythmias and SCD [69, 112–118]. For instance, A G1743R mutation of $Na_v1.5$ was identified in a patient with typical ECG pattern for BrS and ventricular fibrillation [117]. Immunostaining experiments confirmed the retention of G1743R in the ER, which lead to the barely expressed mutant channels on the cell membrane surface and hardly detectable I_{Na}. Baroudi et al. [119] found in BrS patients that a R1432G mutant of human cardiac $Na_v1.5$ colocalized with calnexin in the ER instead of the cell surface. The trafficking deficiency results in significant reduction of $Na_v1.5$ membrane expression and I_{Na}, which can cause slower conduction and increased risks of arrhythmias.

Causes of Changes in Sodium Channel Biophysical Properties in Heart Failure

Studies have revealed that the expression level, channel localization, and biophysical properties of the cardiac Na^+ channel are finely regulated by mediators and modulators in many processes such as gene transcription, RNA processing, protein

synthesis and assembly, and posttranslational processes. Some of the mediators and modulators have been mentioned above, including the cellular metabolic state and $Na_v1.5$ interacting proteins. These mediators are modulated in HF, which in turn affects cardiac $Na_v1.5$ and the susceptibility to arrhythmias. In this section, we will focus on the posttranslational regulation of the cardiac $Na_v1.5$ by protein kinases and the reactive oxygen species (ROS), which have been investigated the most. We will also discuss the genetic control of the $Na_v1.5$ by transcription factors and the roles of neurohumoral systems, G proteins, and coupled receptors on regulation of the $Na_v1.5$ in HF.

Protein Kinases

HF usually develops progressively, during which time the reduced output leads to an increase in neuroendocrine factors like catecholamine, which activate PKC. Activated PKC evokes many signaling molecules that further affect the heart function [118]. PKCs are a family of serine/threonine kinases, comprising three subgroups with at least ten isoforms: the conventional PKCs (α, βI, βII, and γ), the novel PKCs (δ, ε, θ, and η), and the atypical PKCs (ζ, ι/λ). They have been found to play various roles in HF and cardiac ion channels functions. For example, human HF is associated with elevated activation of some conventional PKC isoforms [120, 121]. In HF, the translocation and activation of PKCα are increased [122, 123]. An upregulation of PKCα is observed in spontaneously hypertensive rat model of HF [124] and in a rat model of cardiac hypertrophy [123]. Further activation of PKCα leads to a lethal cardiomyopathy in a hypertrophy heart model, whereas inhibition of PKCα activity improves both systolic and diastolic function of the sick heart [125]. Activation of PKCα has been reported to downregulate the cardiac $Na_v1.5$ channel and decreases the macroscopic I_{Na} through channel phosphorylation, decreasing channel distribution on the plasma membrane, and increasing ROS levels [80, 81, 126]. In our recent study, PKCα seems to play a role in downregulating the $Na_v1.5$ channel in response to NADH [127].

PKCα also regulates other cardiac ion channels. Its activation plays a role in a T-type Ca^{2+} current increase, which seems to contribute to triggering arrhythmias [128]. Activation of PKCα leads to a decrease of the $K_v4.3$ channel current I_{to}, causing shortening of the APD that can cause diastolic dysfunction and arrhythmias [129]. PKCα also shows inhibition on acetylcholine-regulated K^+ current in canine atrial cardiomyocytes of atrial tachycardia-induced remodeling [129].

A significant elevation of PKCβ level and activation has been observed in human HF and in adult cardiac myocytes under hypertrophic stimuli [120, 121, 130–132], although the regulation of PKCβ on cardiac ion channels in HF is not known yet. Nevertheless, a specific study on PKCβII shows that overexpression causes cardiomyopathy exhibiting LV hypertrophy, cardiomyocytes necrosis,

multifocal fibrosis, and decreased LV performance in mice [131]. Ablation of PKCβ is detrimental to pressure-overload-induced HF [133]. Similarly, mice with overexpressed PKCβ show better recovery following ischemia [134], indicating a protective role of PKCβ against HF. Further studies are necessary to understand the roles of PKCβ subtypes on cardiac ion channels in HF.

Studies on PKCε and PKCδ have shown their levels and translocation towards the myocyte membrane are increased, unchanged, or decreased in different types of HF models [135, 136]. The absolute mRNA and protein expression level of PKCδ are enhanced in a rat model of cardiac hypertrophy, while those of PKCε are unaltered [123]. Similar results were obtained in rat aorto-caval fistulas [137]. An increase of PKCε content and activation in myocyte membrane is observed in aortic banding with rats, guinea pigs, and humans [120, 122, 138] and a rat model of hypertrophy [130], but a decrease of PKCε levels have been reported in human and rabbit HF [121, 139]. In the case of cardiac ion channels, activation of PKCδ shows downregulation of the cardiac $Na_v1.5$ channel by inducing an overproduction of mitochondrial ROS level [127], resulting in a significant reduction of I_{Na} that can contribute to slower conduction speed in HF and facilitate reentry and ineffective cardiac contraction. PKCε increases the acetylcholine-regulated K^+ current in canine atrial cardiomyocytes of atrial tachycardia-induced remodeling [129].

PKA is a tetrameric serine/threonine kinase activated by cAMP binding. General effects of PKA on its target present in two ways: protein phosphorylation and protein synthesis. In protein phosphorylation, PKA directly changes the target protein activity, which is a fast process in seconds; while in protein synthesis, PKA first activates CREB, which binds the cAMP response element, altering the transcript and protein synthesis, which may take hours to days. Some of these functions have been found to be modified in HF. For example, β-adrenergic overstimulation-induced PKA activation in HF decreases in $I_{Ca,L}$ through modifications of $Ca_v1.2$ channel together with CaMKII and phosphatases [140], which is different from observations with normal cardiac myocytes where activation of β-adrenergic receptor (β-AR) and consequent PKA increase $I_{Ca,L}$ [141, 142]. A decreased response of the NCX to PKA phosphorylation is observed in a pig model of HF, indicating an increased level of baseline phosphorylation [143]. PKA hyperphosphorylation of the cardiac type RyR2 has been observed in human HF [144] and various animal models of HF [145, 146], causing RyR2 dysfunction with an increased sensitivity to Ca^{2+}-induced activation that can alter $I_{Ca,L}$ and cause prolonged APD and increase arrhythmic risks.

PKA activation upregulates cardiac Na^+ channels and increases I_{Na} through at least three different ways: channel phosphorylation, increasing channel trafficking, and decreasing cellular ROS level that downregulate I_{Na} [81, 92, 147, 148]. In our recent study of a mouse model of nonischemic systolic dysfunction cardiomyopathy, activation of PKA shows salutary effects by decreasing oxidative stress and increasing cardiac I_{Na} [92, 149]. Therefore, PKA may be a therapy to mitigate decreased I_{Na} in HF. PKA also modulates cardiac Ca^{2+} and K^+ currents [150, 151], although the implications in HF are unknown.

The specificity of PKA action is achieved by subcellular targeting of the holoenzyme A-kinase anchoring proteins (AKAPs) [152], which guarantee the correct spatial and temporal action of PKA through generation of multimolecular complexes with PKA and other signaling molecules that participate in the signaling cascades. AKAP binding to PKA is regulated by regulatory subunit II (RII) autophosphorylation. A decrease of PKA RII is observed in HF that may affects PKA relocation and its phosphorylation of other proteins involved in cardiac function [153].

In summary, PKC and PKA are altered in HF. These alterations may explain some of the ion channel changes in HF, such as decreased I_{Na} and $I_{Ca,L}$, and increased I_K. These changes can alter the APD and increase arrhythmic risks in HF.

Reactive Oxygen Species

Oxidative stress is common in HF and cardiovascular disease [84, 86, 154]. Large clinical trials have shown that ROS scavenging by antioxidant vitamins is ineffective or even harmful. On the other hand, prevention of ROS overproduction by targeting various sources of ROS may achieve intriguing benefits. The major sources of ROS overproduction in HF include mitochondrial electron transport chain, uncoupled nitric oxide synthase (NOS), the NADPH oxidase (Nox), and the xanthine oxidase (XO). Elevated ROS levels from these sources are accompanied by an elevation of corresponding enzyme expression and/or activity, or a decrease of antioxidants. For example, the mitochondrial ROS level increases and myocardial antioxidant reserve decreases in HF [155, 156]. Mitochondrial ROS overproduction is observed in mutated GPD1L-induced BrS [91]. Elevated mitochondria ROS decrease cardiac I_{Na} in a mouse model of systolic dysfunction [92]. Elevated mitochondrial ROS in rat neonatal cardiomyocytes [91] and mouse adult cardiomyocytes [127, 149] downregulate cardiac I_{Na} in vitro. The reduction of I_{Na} induced by mitochondrial ROS in HF can increase arrhythmic risk and aggravate cardiomyopathy [53, 87, 88].

Uncoupling of endothelial NOS (eNOS) has been found in diastolic HF, atherosclerosis, diabetes mellitus, ischemia reperfusion injury, and cardiac hypertrophy [157]. Patients and experimental animals with congestive HF have been found to express increased levels of the inducible isoform of NOS in cardiomyocytes [158]. In animal hypertensive HF model studies, uncoupled eNOS is shown to be the major source of superoxide production in the aorta [159]. In the Nox family, the subtypes Nox2 and Nox4 are major sources of superoxide in vascular cells and myocytes and play important roles in atherosclerosis and hypertension [160]. In human HF, higher expression, activity and translocation are observed with Nox4, the regulatory subunit of Nox, $p47^{phox}$ and $p67^{phox}$ [161–163]. The Nox is determined to be a major source of superoxide in a pressure-overload-induced guinea pig model of cardiac hypertrophy [164], with observations of an increased

expression and activity of $p22^{phox}$, $gp91^{phox}$, $p67^{phox}$, and $p47^{phox}$ in cardiomyocytes. An increased XO activity is reported to contribute to abnormal energy metabolism in human dilated HF [165] and in patients with chronic HF [166]. In vitro studies of isolated rat hearts, progressive development of HF is associated with oxidative stress induced by increased myocardial XO levels [167].

Cardiac ion channels have been found to be affected by oxidative stress stemmed from all the sources discussed above. The cardiac $Na_v1.5$ is downregulated by direct application of H_2O_2 and by mitochondrial ROS overproduction [91, 168]. ROS-dependent CaMKII activation enhances I_{NaL}, leading to cellular Na^+ and Ca^{2+} overload and afterdepolarizations, which can contribute to arrhythmias [169] that can be suppressed by the antioxidant N-acetylcarnosine and CaMKII inhibitors [170, 171]. The oxidative stress has been shown to reduce repolarizing K^+ currents by affecting K^+ channel mRNA and protein levels, potentially causing abnormal QT prolongation and arrhythmias in HF [172, 173]. Direct treatment with H_2O_2 triggers an initial K^+ channel HERG activation and subsequent acceleration of channel deactivation, increasing the risks of ectopy [174]. In a chronic rat HF model, the gene expression reduction is observed with the $K_v4.3$ [173]. NO increases the amplitude of the inward rectifying I_{K1} current [175] by increasing the Kir2.1 channel opening probability. Since this channel adjusts the resting membrane potential and influences the APD, redox-dependent changes would be expected to influence arrhythmic risk.

Cardiac Ca^{2+} channels and calcium-handling proteins contain sulfhydryl groups or disulfide linkages that are susceptible to the changes of redox states. A ROS-induced reduction of $I_{Ca,L}$ is observed in the ventricular myocytes of guinea pigs, rats, and rabbits, which contributes to shortening of the APD and an increased potential of reentrant arrhythmias [176–180]. In human cardiomyocytes, ROS can induce a decrease expression of $Ca_v1.2$ [181], but the total I_{CaL} remains unchanged [182, 183]. Single channel recordings of this Ca^{2+} channel from failing human hearts have revealed an increased activity, probably resulting from an increased phosphorylation state [34], which may compensate in part for the decrease in the number of channels and explain the constant Ca^{2+} current. In ferret ventricular myocytes, redox-sensitive thiols in $Ca_v1.2$ diminish $I_{Ca,L}$ under oxidizing conditions, and this mechanism is regulated by NO and *S*-nitrosothiols [184]. The effect of NO on $Ca_v1.2$ has also been studied extensively in different experimental models with variable results, depending on the animal species and the concentration of NO (for review see [84]). Oxidative stress in HF also affects the sarco/endoplastic reticulum Ca^{2+}-ATPase (SERCA), the SR RyR, and the NCX.

In summary, oxidative stress is not only elevated in HF, it also affects cardiac ion homeostasis and structure/function of cardiac ion channels and ion handling proteins, contributing to contractile dysfunction, myocyte apoptosis and necrosis, and aggravating the development of HF. Targeting the specific oxidative stress sources may represent a novel strategy to prevent arrhythmias, which could be safer than the conventional ion channel blockers.

Others Ion Channel Modulators Changed in Heart Failure

Besides protein kinases and ROS, there are many other enzymes and molecules functioning as modulators of cardiac ion channel gene expression, protein folding, trafficking, distribution, and gating properties in HF. In this section, we will discuss some representative modulators and how their dysfunction is linked to HF.

HF may result in changes in ion channel gene transcription. Nuclear factor (NF)-κB, a ubiquitous transcription factor that activates genes expression, has shown effects on many cardiovascular diseases, such as myocardial ischemia/reperfusion injury, cardiac hypertrophy, and HF [185]. NF-κB activation is enhanced in human HF and is required for the development of cardiac hypertrophy in mice and rats and for the transition from hypertrophy to HF in humans [186–190]. Increased NF-κB binding to the cardiac Na^+ channel gene SCN5A promoter can lead to reduction in $Na_v1.5$ transcriptional activity and eventually in I_{Na}. Interestingly, Grabellus et al. show that the elevated activation of NF-κB in human end-stage HF is reversed after installing left ventricular assist devices, indicating the involvement of NF-κB in the process of reverse remodeling [191].

SCN5A gene expression is also regulated by alternative mRNA splicing. In human HF, three C-terminal truncated splice variants have been discovered [87, 168]. Not only are these variants unable to form functional channels, but also they affect the expression of wild-type channel, both of which result in a reduction in I_{Na}. Stimuli for this alternative splicing include hypoxia and angiotensin II, factors associated with ROS and arrhythmic risk. Hypoxia inducing factor-1α (HIFα), a key transcriptional regulator in hypoxia and inflammation, is highly associated to NF-κB regulation of SCN5A [192]. This mechanism is mediated by SCN5A splicing factors RBM 25 and hLuc7A [193, 194]. Targeting these splicing factors may be a possible therapy to increase the membrane expression of wild-type cardiac $Na_v1.5$ and I_{Na} in failing hearts.

Potential Therapies

Despite the fact that a quarter of cardiovascular deaths are to the result of arrhythmias, the therapies available for clinical use are largely limited to ion channel blockers that have proarrhythmic properties and device therapy that does not specifically target the underlying mechanism of each unique arrhythmia [195]. The largest trials of antiarrhythmic medications include the CAST and SWORD studies that both found channel-blocking agents having a negative effect on mortality [196, 197]. Later trials focused on device therapy vs. medical therapy with the consistent finding that devices improve mortality despite treating arrhythmias on a level without focus on specific mechanisms [198–200]. New agents and approaches are needed to manage arrhythmias at the clinical level and the above biophysical considerations suggest possibilities (Table 1).

Table 1 A summary of new therapeutic approaches mentioned in this chapter

Therapy	Targets	Potential roles	Limitations
Gene therapy			
Upregulation of expression	Kir2.1	Stabilize fibrillatory rotors in mice [201] and abbreviate APD without suppressing contractility in HF [203]	Regulation of gene delivery and expression
Adenoviral gene transfer	Drosophila Shaker B K^+channel	Improve AP prolongation and cell mechanical function of failing cardiac myocytes [202]	
New drugs			
Ranolazine	$Na_v1.5$	Suppress I_{NaL} to improve arrhythmia and contractile dysfunction in HF [204]	Unknown efficacy, potential proarrhythmia, and possible off target effects
PD-118057	HERG K^+ channel	Increase I_{Kr} and shorten APD and suppress EADs development [212]	
SEA-0400	NCX	Suppress EADs and DADs, and abolish triggered arrhythmias [213]	
Endothelin antagonists	L-type Ca^{2+} channel and several K^+ channels	Prevent AP prolongation in HF [214, 215]	
Verapamil	L-type Ca^{2+} channel	Convert VF into stable VT [216]	
NAD^+	$Na_v1.5$	Upregulate $Na_v1.5$ [79]	

AP action potential, *APD* action potential duration, *DADs* delayed afterdepolarizations, *EADs* early afterdepolarizations, *HF* heart failure, I_{Kr} rapid delayed rectifier K^+ currents, I_{NaL} late Na^+ current, NAD^+ β-nicotinamide adenine dinucleotide, $Na_v1.5$ cardiac Na^+ channel, *NCX* Na^+-Ca^{2+} exchanger, *VF* ventricular fibrillation, *VT* ventricular tachycardia

Few new antiarrhythmic drugs have been introduced clinically in the last decade, and research has focused on gene therapy to improve perturbations to membrane currents seen in HF. I_{K1} upregulation has been found to accelerate yet also stabilize fibrillatory rotors in mice [201]. Adenoviral gene transfer of an inactivation-defective Drosophila Shaker B K^+ channel in failing cardiac myocytes has been found to improve AP prolongation and cell mechanical function [202]. Similarly, gene therapy with Kir2.1 was found to abbreviate excitation without suppressing contractility in a HF model [203]. Genetic approaches to normalizing membrane currents in disease states will likely prove to be an important area of future clinical research. Nevertheless, there are quite a few difficulties that need to be addressed. Gene delivery systems are not perfected for in vivo models, and furthermore, transgene expression needs to be effectively regulated before this approach can become relevant clinically.

New drug-based approaches to normalizing membrane currents in addition to traditional ion channel-blocking attempts have the potential to yield useful clinical

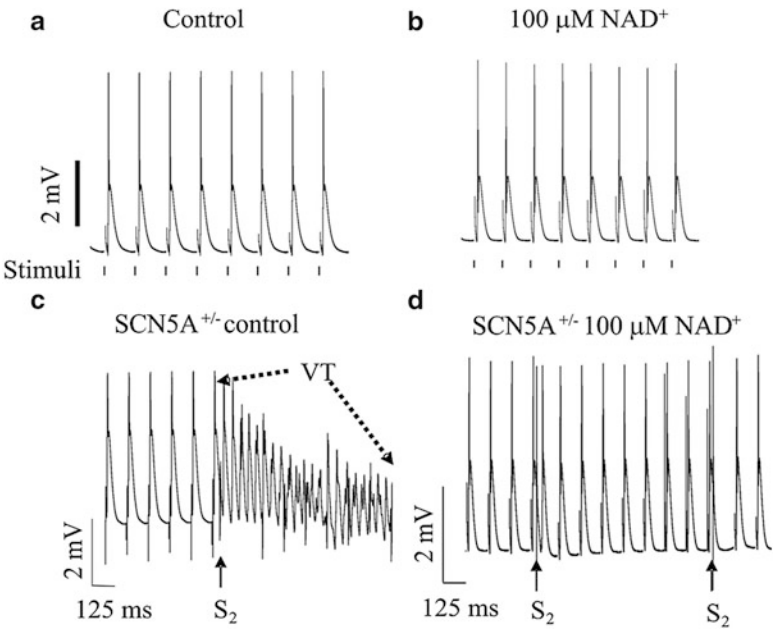

Fig. 3 Representative traces of monophasic action potentials (MAPs) from left ventricular epicardium of Langendorff-perfused SCN5A$^{+/-}$ heart during standard pacing at basic circle length of 125 ms in (**a**) the control condition and (**b**) after 20 min of perfusion with 100 mM NAD$^+$. *Vertical lines* below the MAPs represent the times when electrical stimulations were delivered. (**c**) Representative MAPs recorded during programmed electrical stimulation (PES) showing PES-induced ventricular tachycardia (VT) in SCN5A$^{+/-}$ hearts under control condition. The final six paced beats at 125 BCL (S1) were followed by an extra stimulus (S2) delivered at a S1–S2 interval of 42 ms. PES induced a ventricular tachycardia with cycle length of 20–40 Hz that was sustained for ~19 s. (**d**) Representative trace of PES-induced MAPs recording in same SCN5A$^{+/-}$ heart after 20 min perfusion with 100 mM NAD$^+$. S2 stimuli delivered at a 35 ms S1–S2 interval produced a single MAP but failed to induce VT (modified from ref. [81] with permission)

therapies. For example, ranolazine, a new drug with a 38-fold higher potency for I_{NaL} than for peak I_{Na}, has reported antiarrhythmic effects for both supraventricular and ventricular arrhythmias by suppressing I_{NaL} [204], but there are no large randomized controlled trials to support these findings to date [205–208]. The exact mechanism by which ranolazine alters I_{NaL} is a matter of debate. Two earlier studies identified two binding sites on the $Na_v1.5$ [209, 210]. A recent study suggests that ranolazine blocks I_{NaL} by inhibiting the mechanosensitivity of $Na_v1.5$ independent of previous established binding sites [211]. Evidence suggests that I_{NaL} blockade could be useful to normalize Na^+ and Ca^{2+} handling to treat both arrhythmias and contractile dysfunction in HF. Nevertheless, interpreting the body of literature related to *r* is difficult since off target effects cannot be excluded and a positive control for the effects of the drug is not available.

New compounds have been identified that enhance HERG K^+ channels and result in increased I_{Kr} that can suppress AP prolongation and the development of

EADs [212]. New selective NCX inhibitors have also been found to suppress EADs and DADs in Purkinje fibers [213]. Endothelin antagonists have been developed to prevent AP prolongation in HF, and more importantly, they have been found to counteract the downregulation observed in I_{K1} and I_{to} [214, 215]. Current work on older agents is also promising. A study of verapamil found that it can convert ventricular fibrillation into stable VT by reducing the frequency of rotors and by decreasing wavefront fragmentation, which in turn decreases fibrillatory propagation from the rotor [216].

NAD^+ has emerged as a putative metabolic regulator of transcription, longevity, and several age-associated diseases. Our recent studies show that NAD^+ may represent a new type of antiarrhythmic therapy [81, 91]. NAD^+ modulates the cardiac $Na_v1.5$ channel and mitochondrial ROS formation through PKA (Fig. 2c). This implies that NAD^+ can potentially be given to humans to raise I_{Na} in HF and reduce sudden death risk. NAD^+ has shown antiarrhythmic properties in heterozygous SCN5A knockout mouse hearts that show VT and BrS (Fig. 3) [81]. This opens the possibility of an entirely new paradigm for treatment of arrhythmias by raising rather than blocking $Na_v1.5$.

Further understanding of the changes in currents during HF is likely to lead to new, safer, more effective therapies for arrhythmia. In many senses, we have just begun to reap the benefits of a half century of ion channel biophysics.

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