Journal of the American Heart Association

CONTEMPORARY REVIEW

Neurohormonal Regulation of I_{Ks} in Heart Failure: Implications for Ventricular Arrhythmogenesis and Sudden Cardiac Death

Tyler Shugg, PharmD, PhD; Andy Hudmon, PhD; Brian R. Overholser, PharmD

ABSTRACT: Heart failure (HF) results in sustained alterations in neurohormonal signaling, including enhanced signaling through the sympathetic nervous system and renin-angiotensin-aldosterone system pathways. While enhanced sympathetic nervous system and renin-angiotensin-aldosterone system activity initially help compensate for the failing myocardium, sustained signaling through these pathways ultimately contributes to HF pathophysiology. HF remains a leading cause of mortality, with arrhythmogenic sudden cardiac death comprising a common mechanism of HF-related death. The propensity for arrhythmia development in HF occurs secondary to cardiac electrical remodeling that involves pathological regulation of ventricular ion channels, including the slow component of the delayed rectifier potassium current, that contribute to action potential duration prolongation. To elucidate a mechanistic explanation for how HF-mediated electrical remodeling predisposes to arrhythmia development, a multitude of investigations have investigated the specific regulatory effects of HF-associated stimuli, including enhanced sympathetic nervous system and renin-angiotensin-aldosterone system signaling, on the slow component of the delayed rectifier potassium current. The objective of this review is to summarize the current knowledge related to the regulation of the slow component of the delayed rectifier potassium current in response to HF-associated stimuli, including the intracellular pathways involved and the specific regulatory mechanisms.

Key Words: arrhythmias ■ heart failure ■ I_{Ks} ■ KCNQ1 ■ renin-angiotensin-aldosterone system ■ sympathetic nervous system

Heart failure (HF) refers to a clinically heterogeneous group of pathologic conditions that result in reduced cardiac output and a subsequent reduction in the systemic circulation of oxygenated blood.[1] HF affects nearly 6 million people in the United States and constitutes a leading cause of morbidity, mortality, hospitalization, and healthcare expenditure.[2] HF imparts a 6- to 9-fold increase in the risk for sudden cardiac death (SCD) that occurs secondary to the development of ventricular arrhythmias, such as ventricular tachycardia (including torsades de pointes) and ventricular fibrillation.[3,4] Animal models of HF as well as cardiomyocytes isolated from failing hearts consistently demonstrate prolongation of cardiac action potential duration (APD), which is a risk factor for the development of ventricular arrhythmias.[5,6] Though HF mortality occurs through a variety of mechanisms, arrhythmogenic SCD accounts for up to 40% of mortality in patients with severe HF.[4]

HF is associated with pathological structural (ie, changes in the expression of contractile and extracellular matrix proteins) and electrical (ie, changes in the expression and function of calcium handling proteins, gap junctions, and ion channels) cardiac remodeling that predispose the failing heart to arrhythmogenesis.[7] These remodeling processes largely stem from HF-associated changes in neurohormonal signaling and dysregulated calcium homeostasis.[8] In particular, sustained elevations in the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system

Correspondence to: Brian R. Overholser, PharmD, Purdue University College of Pharmacy, Research Institute 2: Room 402, 950 W. Walnut St., Indianapolis, IN 46202.E-mail: boverhol@purdue.edu

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

Nonstandard Abbreviations and Acronyms

APD	action potential duration
ARBs	angiotensin II receptor blockers
AT_1	angiotensin II type 1 receptor
CaM	calcium-bound calmodulin
CaMKII	calcium/calmodulin-dependent protein kinase II
Epac	exchange protein activated by cyclic adenosine monophosphate
I_{Kr}	rapid component of the delayed rectifier potassium current
I_{Ks}	slow component of the delayed rectifier potassium current
I_{Na}	voltage-gated sodium current
MR	mineralocorticoid receptor
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKA	protein kinase A
PKC	protein kinase C
PKC-ε	epsilon isoform of protein kinase C
RAAS	renin-angiotensin-aldosterone system
SCD	sudden cardiac death
SNS	sympathetic nervous system
SUMO	small ubiquitin-like modifier
β-AR	beta-adrenergic receptor

(RAAS) pathways, as occur in HF, are intimately involved in HF pathophysiology.[9] The roles of the SNS and RAAS in mediating HF disease progression are evidenced by the fact that medications targeting increased catecholaminergic (β-AR [beta-adrenergic receptor] blockers) and angiotensin II (ACE [angiotensin-converting enzyme] inhibitors or angiotensin II receptor blockers [ARBs]) signaling confer mortality benefit and remain standards of care in HF therapy.[10–12] While the mechanistic basis for the pathophysiologic role of alterations in neurohormonal signaling in HF is not completely understood, various investigations have demonstrated that sustained SNS and RAAS activation inhibit a variety of species of ion channels that are important regulators of the cardiac action potential.[13] In particular, the potential for HF-associated changes in neurohormonal signaling to inhibit L-type calcium channels and delayed rectifier potassium channels, including those responsible for the slow component of the delayed rectifier potassium current (I_{Ks}), have been well established.[13]

Cardiac ion channels mediate propagation of autonomous electrical signals in the heart, thereby providing coordinated electrical stimuli that trigger synchronized contraction of cardiac muscle. In this way, ion channels are critical physiologic regulators of cardiac rhythm and contractility. The human ventricle contains many species of ion channels that contribute to cardiomyocyte membrane potential during specific phases of the ventricular action potential via conductance of sodium, calcium, and potassium ions, as illustrated in Figure 1A in the healthy heart.[14] Currents conducted by delayed rectifier potassium channels, including I_{Ks} (Figure 1B), are the primary contributors to the rapid repolarization phase of the ventricular action potential (phase 3) that is responsible for returning cardiomyocytes to resting membrane potential in preparation for subsequent depolarizing electrical stimuli (ie, heartbeats). Accordingly, alterations in ventricular ionic currents in heart failure (summarized in Figure 1C), including reductions in delayed rectifier potassium currents, prolong ventricular APD and thereby prolong the QT interval on the surface ECG.[14]

Given the essential role of ion channels in regulating cardiac rhythm and contractility, dysregulation of ion channel function by HF-associated stimuli underlies increased arrhythmogenesis in HF, including increased development of ventricular arrhythmias.[5,8] Though conflicting data exist, research investigations have largely found reductions in both species of ventricular delayed rectifier potassium currents in limited human studies and in animal and cellular HF models. Specifically, both increased angiotensin II signaling and sustained β-AR stimulation have been demonstrated to inhibit both the rapid component of the delayed rectifier potassium current (I_{Kr}) and I_{Ks}.[15–19] These findings are supported by studies in various models, including isolated ventricular cardiomyocytes from patients with HF, that demonstrate reductions in I_{Ks} and prolongation of APD in HF.[6] HF-associated reductions in I_{Ks} may be of particular clinical importance since I_{Kr} is also reduced in HF, making the contributions of I_{Ks} to phase 3 repolarization in the ventricle more critical.[20,21]

SIGNIFICANCE OF I_{KS} IN HEALTH AND DISEASE

I_{Ks} is conducted through a voltage-gated potassium channel that is formed via protein-protein interaction of the pore-forming subunit, KCNQ1 (gene product of *KCNQ1*; located on chromosome 11p), with the beta subunit, KCNE1 (product of *KCNE1*; 21q).[22] KCNQ1 and KCNE1 are expressed in both human atria and ventricles, where I_{Ks} plays an essential role in phase 3 cardiac repolarization.[23] In the ventricular action potential, I_{Ks} is a major contributor to the ventricular "repolarization reserve" by providing redundant current to maintain rapid repolarization during increases in cardiac rate or during conditions in which other ventricular potassium currents are reduced (eg, pharmacological

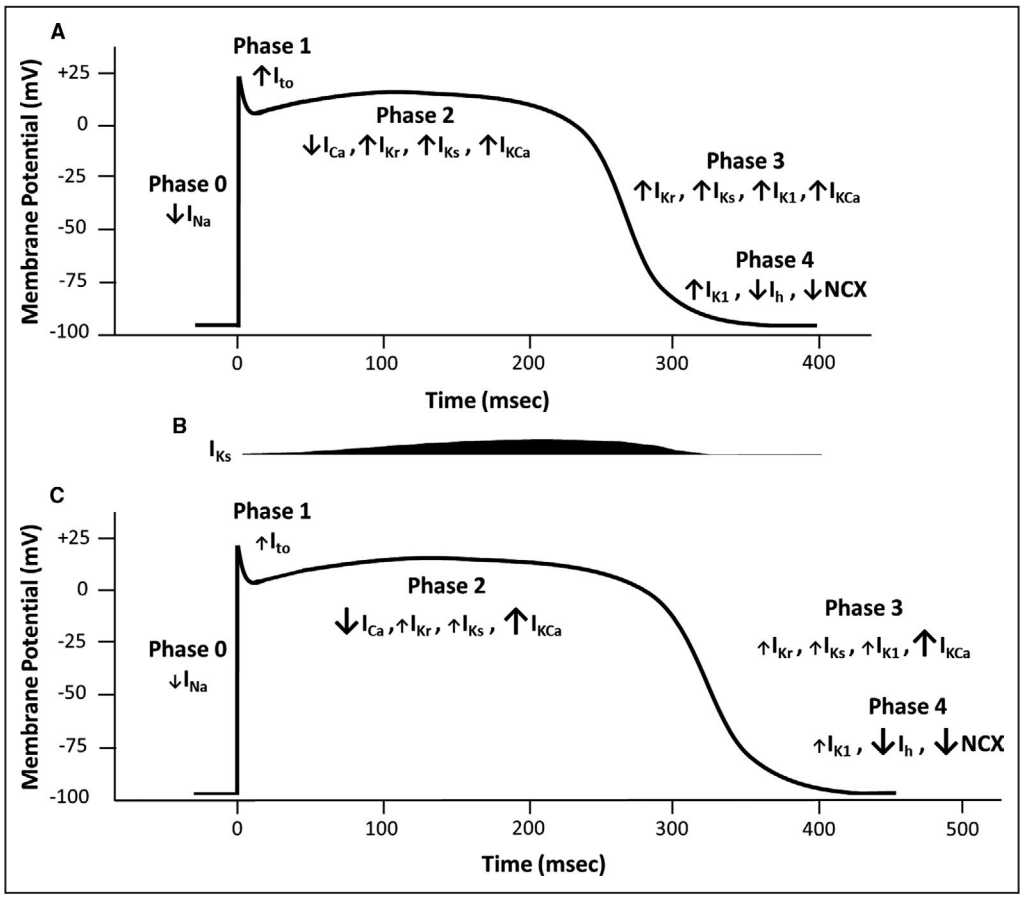

Figure 1. Depiction of the ventricular action potential with the major ionic currents that contribute to each phase in the healthy heart and in heart failure.
A, Diagram of the ventricular action potential in the healthy heart, demonstrating the contributions of ion channels species to each phase of the ventricular action potential, including the upstroke phase (phase 0), the early repolarization phase (phase 1), the plateau phase (phase 2), the late repolarization phase (phase 3), and the resting phase (phase 4). Inward currents are indicated by arrows pointing downward (representing ions moving from the extracellular to intracellular environment), and outward currents are indicated by arrows pointing upward (representing ions moving from the intracellular to extracellular environment). **B**, A simulated trajectory of outward I_{Ks} current density during the ventricular action potential in the healthy heart. **C**, Diagram of the ventricular action potential in the failing heart, with HF-mediated alterations in ionic currents indicated by enlarged (enhanced current) or decreased (reduced current) arrows depicted current direction. I_{Ca} indicates voltage-gated calcium currents; I_h, hyperpolarization-activated cyclic nucleotide–gated current; I_{K1}, inward rectifier potassium current; I_{KCa}, calcium-activated repolarizing potassium current; I_{Kr}, rapid component of the delayed rectifier potassium current; I_{Ks}, slow component of the delayed rectifier potassium current; I_{Na}, voltage-gated sodium current; I_{to}, transient outward potassium current; and NCX, sodium-calcium exchanger.

inhibition of I_{Kr}).[24] In accordance with its contributions to the repolarization reserve, I_{Ks} contributes only modestly to repolarization during normal resting heart rates (during which time I_{Kr} is the predominant repolarizing current). However, I_{Ks} has the potential to be acutely activated by the SNS to increase the rate of ventricular repolarization to compensate for increases in cardiac rate.[25–27] This role is evidenced by the fact that reductions in I_{Ks} may result in APD prolongation and the development of potentially fatal ventricular arrhythmias particularly during sustained adrenergic tone.[26] For example, loss-of-function mutations in *KCNQ1*, referred to as long QT syndrome phenotype 1, constitute the most common causes of congenital long QT syndrome and predispose patients to the development of ventricular arrhythmias and SCD that typically occur during periods of sustained adrenergic stimulation (eg, swimming).[28]

Channel activation to increase I_{Ks} in response to acute release of norepinephrine and other catecholamines comprises a key physiologic mechanism to enhance repolarization during rapid heart rates.[20] Increases in I_{Ks} during rapid heart rates involves multiple mechanisms, including enhancement of I_{Ks} current

amplitude during individual ventricular depolarizations and accumulation of I_{Ks} during rapid pacing attributable to incomplete current deactivation.[29] I_{Ks} activation during acute or intermittent SNS activation is mediated by catecholaminergic activation of β-ARs (primarily cardiac β_1-ARs) and involves a PKA (protein kinase A)-dependent signaling complex that has been well characterized.[30]

In contrast to acute and intermittent β-AR signaling, sustained β-AR stimulation, as occurs as part of the pathophysiology of HF, has consistently been demonstrated to reduce I_{Ks}.[15,19] A number of studies have also shown that sustained increases in angiotensin II signaling, a major signaling molecule of the RAAS pathway that is also upregulated in HF, also result in reduced I_{Ks}.[16–18] Regardless of mechanism, reductions in I_{Ks} have been demonstrated to prolong APD and increase arrhythmia development in animal HF models.[31] In addition, cardiomyocytes isolated from explanted hearts from patients with HF have also demonstrated reduced I_{Ks}.[6,32] Disturbances in I_{Ks} may contribute to known mechanisms of arrhythmia development, including transmural dispersion of repolarization,[33,34] since KCNQ1 and KCNE1 have both demonstrated transmural differences in expression in human ventricular myocardium.[35,36] Experiments in explanted human hearts have demonstrated that the contribution of I_{Ks} to ventricular repolarization is enhanced both during β-AR stimulation and in conditions where I_{Kr} is reduced.[37] Given its increased contribution to repolarization in HF, reductions in I_{Ks} have been shown to potentiate transmural dispersion of repolarization, though conflicting reports exist.[37–39] Therefore, reductions in I_{Ks} in response to various neurohormonal triggers involved in HF pathogenesis may account for increases in the risk of ventricular arrhythmogenesis and SCD seen in HF.

CELLULAR SIGNALING PATHWAYS INVOLVED IN I_{KS} REGULATION IN HF

HF results in a multitude of alterations in neurohormonal signaling that, while initially are adaptive to maintain cardiac output in the failing heart, ultimately lead to cardiac remodeling and disease progression.[7] In particular, chronic activation of the SNS and RAAS signaling pathways are key mediators of HF pathophysiology, and pharmacological targeting of these pathways remains the standard of care for preventing disease progression and HF-related morbidity and mortality.[10] Sustained activation of the SNS and RAAS pathways have also been demonstrated to regulate I_{Ks}, potentially contributing to enhanced arrhythmogenesis in patients with HF. Table 1 summarizes changes in I_{Ks} in response to HF-associated neurohormonal signaling.

Multiple investigations have identified regulation of I_{Ks} in response to SNS signaling, mediated through cardiac α-ARs and β-ARs. Alterations in I_{Ks} by enhanced catecholaminergic signaling through α-ARs, mediated primarily through cardiac α_1-ARs, are less well established relative to β-AR signaling. Acute activation of α_1-ARs has been demonstrated to reduce I_{Ks} by a mechanism that is mediated through PKC (protein kinase C)– and AMP kinase–dependent signaling and involves (1) downregulation of KCNQ1 and possibly KCNE1[46,50] and (2) impaired I_{Ks} via depletion of phosphatidylinositol-4,5-bisphosphate (PIP_2).[47,51] The implications of sustained α_1-AR stimulation on I_{Ks} remain largely unknown, however; while sustained α_1-AR

Table 1. HF-Associated Neurohormonal Signaling Molecules Demonstrated to Regulate I_{Ks}

Signaling Molecule	Receptors Involved	Duration	Effect	Reference
Angiotensin II	Angiotensin II type 1 receptor	Acute	↑ I_{Ks}	Zankov et al[40]
				Matavel et al[41]
			↓ I_{Ks}	Guo et al[17]
				Si et al[42]
				Daleau et al[43]
		Chronic	↓ I_{Ks}	Matavel et al[41]
Aldosterone	Angiotensin II type 1 receptor	Chronic	↓ I_{Ks}	Lv et al[44]
	Mineralcorticoid receptor	Chronic	↓ I_{Ks}	Lv et al[45]
Catecholamines	α_1-Adrenergic receptor	Acute	↓ I_{Ks}	Kurakami et al[46]
				Kienitz et al[47]
	β-Adrenergic receptor	Acute	↑ I_{Ks}	Marx et al[30]
	β-Adrenergic receptor	Chronic	↓ I_{Ks}	Aflaki et al[15]
				Shugg et al[19]
Endothelin	Endothelin receptor	Acute	↑ I_{Ks}	Habuchi et al[48]
			↓ I_{Ks}	Washizuka et al[49]

stimulation might be expected to reduce I_{Ks} via both subunit downregulation of KCNQ1 and KCNE1 and depletion of PIP_2, evidence has shown (1) the potential for desensitization of PIP_2 hydrolysis downstream of chronic α_1-AR signaling[47] and (2) the ability of CaM (calcium-bound calmodulin) to interact with the PIP_2 binding site on the KCNQ1 carboxyl terminus to stabilize I_{Ks}.[51] Acute β-AR stimulation has been consistently shown to enhance I_{Ks} through the aforementioned PKA pathway.[30] In contrast, sustained β-AR stimulation has been demonstrated to reduce I_{Ks} through 2 distinct pathways: the first involving activation of the Epac (exchange protein activated by cAMP) and the second involving activation of CaMKII (calcium/calmodulin-dependent protein kinase II) .[15,19]

Sustained elevations in RAAS signaling, mediated largely through activation of the AT_1 (angiotensin II type 1 receptor) by angiotensin II, have also been demonstrated to affect I_{Ks}. Conflicting data exist regarding the role of acute angiotensin II treatment on regulating I_{Ks}, with investigations demonstrating both enhancements, mediated by phosphorylation of KCNE1 S102 by classical PKC isoforms,[40,41,52] and reductions, mediated by PKC-ε-dependent phosphorylation of KCNQ1 S95, T96, or both and KCNE1 S102.[17,42,43] Sustained treatment with angiotensin II, though less widely studied, has been demonstrated to reduce I_{Ks} through a mechanism involving cellular depletion of PIP_2 in response to G_q-coupled AT_1 signaling.[41] Chronic aldosterone exposure has also been found to reduce I_{Ks} via decreased expression of both KCNQ1 and KCNE1.[45] Interestingly, aldosterone's inhibitory effects on I_{Ks} have been shown to be mediated by signaling through both the MR (mineralocorticoid receptor) and AT_1.[44,45] This finding supports previously described crosstalk between MR activation and subsequent activation of angiotensin II/AT1 signaling.[53–55]

HF also results in chronic elevation of other signaling hormones, including atrial and brain natriuretic peptides, arginine vasopressin, and endothelin. Results from 2 studies assessing the effects of acute endothelin exposure on I_{Ks} within isolated guinea pig cardiomyocytes are conflicting, with one study demonstrating I_{Ks} enhancement and the other showing I_{Ks} reduction.[48,49] The effect of chronic endothelin signaling on I_{Ks} has not been investigated. Similarly, the potential for acute or sustained exposure of atrial natriuretic peptide, brain natriuretic peptide, and vasopressin to regulate I_{Ks} have not been studied.

POTENTIAL FOR CROSSTALK AMONG I_{KS} REGULATORY PATHWAYS

Given that HF-associated changes in neurohormonal signaling have been demonstrated to regulate I_{Ks} through a variety of pathways, there exists the potential for crosstalk among signaling pathways that regulate $I_{Ks.}$ Most notably, the potential for crosstalk between cardiac α_1-ARs and AT_1 receptors, as detailed in Figure 2A, has been consistently demonstrated in response to HF stimuli.[56,57] Specific to I_{Ks}, acute activation of both α_1-ARs and AT_1 receptors have been demonstrated to produce reductions in I_{Ks} through pathways that involve PKC activation and phosphorylation of KCNE1 S102.[17,50,52] Accordingly, simultaneous signaling through acute activation of both pathways may bypass homeostatic mechanisms (eg, downregulation of receptors) involved in either pathway and thus further reduce I_{Ks}.

Evidence also supports crosstalk between AT_1 receptor and cardiac β-AR signaling, including the potential for heterodimerization of the G-protein alpha subunits G_q and G_s downstream of AT_1 signaling.[58–60] As previously mentioned, sustained activation of β-AR and AT_1 receptors have been demonstrated to reduce I_{Ks} through distinct mechanisms.[15,17,19] However, these signaling pathways have been demonstrated to share a common downstream signaling molecule, CaMKII, that is known to be upregulated in HF and has been implicated in HF pathophysiology.[61,62] The delta isoform of CaMKII (δCAMKII) is known to be activated downstream of AT_1 receptor signaling in cardiomyocytes via a mechanism that involves oxidation of methionine 281 or methionine 282 on the enzyme.[63–65] Conversely, sustained β-AR signaling activates the delta isoform of CaMKII through a pathway that involves autophosphorylation at T287 downstream of Epac and PKC-ε signaling.[64,66–68] Therefore, as described in Figure 2B, I_{Ks} reductions may also result from δCAMKII activation downstream of both AT_1 and β-AR signaling.

Finally, results from Lv et al[44] suggest the potential for AT_1 activation downstream of sustained aldosterone activation of the MR. Mechanistically, these results are supported by previous findings that prolonged activation of the MR by aldosterone resulted in increased AT_1 receptor density[53] and increased gene expression of ACE.[54,55] Both of these effects are predicted to increase signaling through AT_1, as shown in Figure 2C, which has been consistently shown to contribute to pathologic cardiac remodeling processes in HF.[69,70] In addition, sustained MR activation has been independently linked to cardiac fibrosis[71] and adverse HF outcomes.[72,73]

MECHANISMS OF I_{KS} ELECTRICAL REMODELING IN HF

A multitude of investigations have explored potential mechanisms for how HF-associated stimuli, including

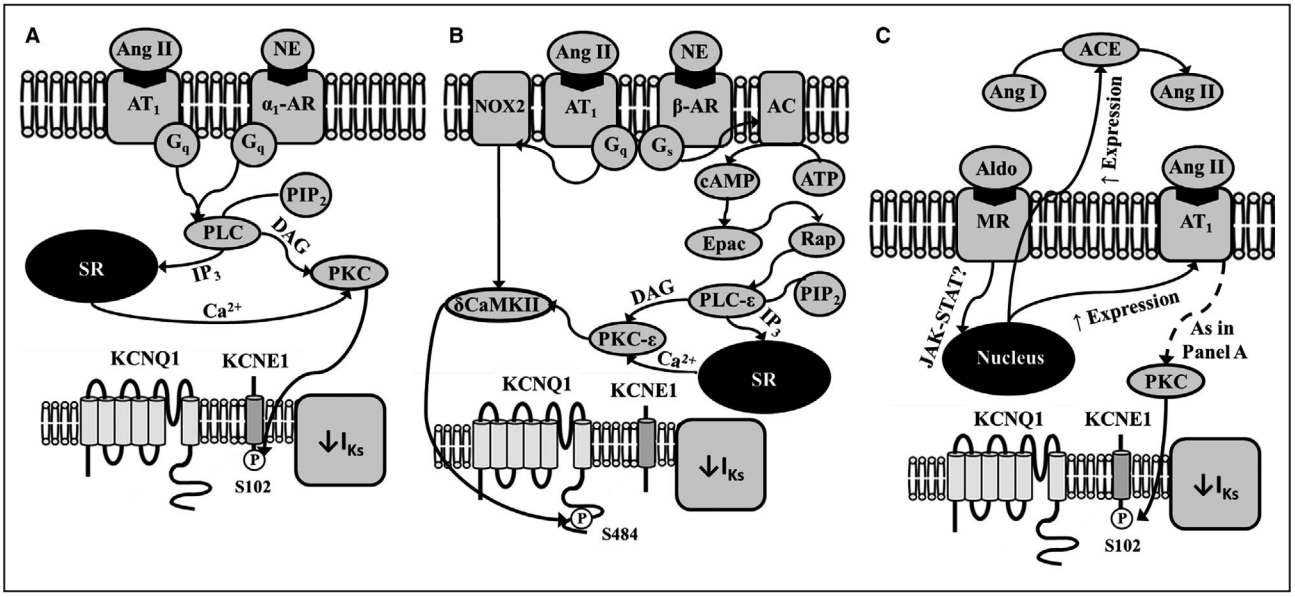

Figure 2. Potential for cardiac-specific crosstalk between signaling pathways demonstrated to regulate I_{Ks}.
Schematic diagrams show putative pathways involved in (left) activation of PKC downstream of acute activation of both AT_1 and α_1-AR receptors, (center) activation of δCaMKII downstream of sustained activation of both AT_1 and β-AR activation, and (right) activation of AT_1 receptors downstream of sustained MR receptor activation. AC indicates adenylyl cyclase; ACE, angiotensin-converting enzyme; Aldo, aldosterone; Ang I, angiotensin I; Ang II, angiotensin II; AT_1, angiotensin II type 1 receptor; ATP, adenosine triphosphate; Ca^{2+}, calcium; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; Epac, exchange protein directly activated by cAMP; G_q, G_q protein alpha subunit; G_s, G_s protein alpha subunit; IP_3, inositol triphosphate; JAK-STAT, Janus kinase-signal transducer and activator of transcription proteins pathway; MR, mineralcorticoid receptor; NE, norepinephrine; NOX2, NADPH oxidase 2; P, phosphorylation; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PKC-ε, epsilon isoform of protein kinase C; PLC, phospholipase C; PLC-ε, epsilon isoform of phospholipase C; S, serine; SR, sarcoplasmic reticulum; α_1-AR, alpha-1 adrenergic receptor; and β-AR, beta-adrenergic receptor; and δCaMKII, delta isoform of calcium/calmodulin-dependent protein kinase II.

alterations in neurohormonal signaling, may pathologically regulate I_{Ks}. These mechanistic analyses have largely focused on (1) changes in the expression of KCNQ1, KCNE1, and other KCNE isoforms; (2) posttranslational regulation of KCNQ1 and KCNE1 subunits; and (3) effects on the interactions among I_{Ks}-related proteins, including interaction of KCNQ1 and KCNE1 with each other and with various accessory proteins. Regardless of mechanism, the arrhythmogenic potential of reductions in I_{Ks} has been corroborated within investigations that have shown the development of ventricular arrhythmias during pharmacological inhibition of I_{Ks} and in canine and rabbit HF models wherein I_{Ks} is reduced.[31] Below, we discuss the existing literature for each regulatory mechanism.

CHANGES IN KCNQ1 AND KCNE EXPRESSION IN HF

Although I_{Ks} reductions and APD prolongation have been consistently observed clinically and in cellular and animal HF models, the mechanism supporting I_{Ks} reduction remains elusive. In particular, changes in the expression of KCNQ1 and KCNE1 within animal HF models and samples from patients with HF have shown conflicting results, as summarized in Table 2. The results from animal studies, in which HF has been simulated by atrioventricular blockade, tachycardiac pacing, coronary artery occlusion, coronary artery microembolization, or treatment with the compounds isoproterenol (β-AR agonist) or aldosterone (MR agonist), have generally been mixed. Multiple studies have found reductions in mRNA and protein expression of KCNQ1 and KCNE1, while others have demonstrated no change in expression.[15,21,34,45,74–78] In contrast, human studies that have analyzed ventricular tissue explanted from patients with HF have demonstrated either no change or increases in the mRNA expression of KCNQ1 and KCNE1 relative to healthy controls.[32,79]

Additionally, multiple KCNE isoforms, including KCNE2, KCNE3, KCNE4, and KCNE5, are expressed in human ventricular cardiomyocytes and may form heteromeric channels with KCNQ1 and KCNE1 to alter I_{Ks}.[80–82] Coexpression of KCNE2, KCNE4, and KCNE5 along with KCNQ1 and KCNE1 reduce I_{Ks} amplitude, whereas coexpression of KCNE3 with KCNQ1/KCNE1 has been shown to enhance I_{Ks} amplitude.[81–83] While data describing HF-associated changes in these KCNE isoforms are sparse, one investigation found that mRNA expression of KCNE2 and KCNE5 were reduced approximately 3- and 4-fold, respectively, in explanted left ventricular tissue from patients with

Table 2. Studies Assessing Changes in the Expression of KCNQ1 and KCNE1 in Animal or Human Models of HF

HF Simulation Method	Experimental Model	Percent Change in mRNA Expression		Percent Change in Protein Expression		Authors
		KCNQ1	KCNE1	KCNQ1	KCNE1	
Chronic aldosterone treatment	Guinea pig left ventricle	↓60%*	↓60%*	↓40%*	↓40%*	Lv et al[45]
Chronic atrioventricular block	Canine ventricle	↓80%	↓70%	↓50%	↓60%	Ramakers et al[34]
Chronic atrioventricular block + bradycardiac pacing	Rabbit left ventricle	↓53%	↓43%	↓53%	↓33%	Tsuji et al[21]
Chronic atrioventricular block + bradycardiac pacing	Rabbit right ventricle	↓76%	↓58%	↓72%	↓37%	Tsuji et al[21]
Chronic atrioventricular block + tachycardiac pacing	Rabbit left ventricle	↓51%	↓43%	↓53%	↓37%	Tsuji et al[21]
Chronic atrioventricular block + tachycardiac pacing	Rabbit right ventricle	↓64%	↓74%	↓78%	↓45%	Tsuji et al[21]
Chronic ischemia (coronary artery occlusion)	Rabbit myocardium	↔	↔	↓80%	NS†	Guo et al[77]
Chronic ischemia (coronary microembolization)	Canine myocardium	NS	NS	↔‡	↓37%	Liu et al[76]
Chronic isoproterenol treatment	Guinea pig ventricle	↔	↓45%	↔	↓51%	Aflaki et al[15]
Chronic isoproterenol treatment	Guinea pig ventricle	↔	NS	↔	NS	Soltysinska et al[74]
Tachycardiac pacing	Canine left ventricle	↔	↔	↔	↔	Akar et al[78]
Tachycardiac pacing	Rabbit ventricle	↔	↔	↔	↔	Rose et al[75]
None (clinical heart failure)	Human left ventricle	↑160%	NS	NS	NS	Borlak et al[79]
None (clinical heart failure)	Human left ventricle	↑400%*	↑400%*	NS	NS	Lundquist et al[81]
None (clinical heart failure)	Human right ventricle	↑400%	NS	NS	NS	Borlak et al[79]
None (clinical heart failure)	Human right ventricle	↔	↑25%	NS	NS	Watanabe et al[32]

↑ indicates increased expression; ↓, decreased expression; ↔, no significant change in expression; HF, heart failure; and NS, not studied.

*Numerical results for the change in expression of KCNQ1 and KCNE1 were not presented in the Lv et al and Lundquist et al articles. The presented percentages were estimated based on the presented graphical results.

† Because of limitations in detecting KCNE1 within protein immunoblot experiments, Guo et al[77] did not report results for changes in KCNE1 protein expression.

‡Liu et al found no change in expression of the predominant KCNQ1 isoform (KCNQ1.1), but did observe an increase in expression of a truncated KCNQ1 isoform (KCNQ1.2), which they predicted to inhibit I_{Ks} in a dominant-negative fashion.

dilated or ischemic cardiomyopathy relative to healthy tissue.[81] In contrast, mRNA expression of KCNE3 and KCNE4 were enhanced approximately 5- and 2-fold, respectively, in the cardiomyopathic left ventricle.[81]

In summary, reductions in mRNA and protein expression of KCNQ1, KCNE1, and other KCNE isoforms in patients with HF and in animal HF models have not been consistently reproduced. These inconsistent findings are likely attributable to differences related to the HF model and the method of HF simulation. Regardless, these inconsistencies, along with the fact that the limited clinical data do not support reduced expression of KCNQ1 and KCNE1, suggest that alternative or additional molecular mechanism may mediate reduced I_{Ks} in HF.

POSTTRANSLATIONAL REGULATION OF I_{KS} IN HF

A number of investigations have assessed the potential for aberrant posttranslational regulation of KCNQ1 and KCNE1 subunits to mediate reductions in I_{Ks} in response to HF-associated changes in neurohormonal signaling. These studies have identified regulatory sites on the intracellular amino and carboxyl termini of KCNQ1 and the intracellular carboxyl terminus of KCNE1 with functional implications for I_{Ks}. Figure 3 diagrams the KCNQ1 and KCNE1 subunits, showing demonstrated sites of posttranslational regulation in response to HF-related stimuli.

Biology of KCNQ1

The KCNQ1 subunit is 676 amino acids in length and contains a 120-residue intracellular amino terminus and a 325-residue intracellular carboxyl terminus.[84] These termini are separated by a transmembrane region with 6 membrane-spanning domains, with the region connecting the fifth and sixth segments serving as the pore and multiple positively charged residues in the fourth segment serving as the voltage sensor.[85] The canonical I_{Ks} channel is formed when 4 KCNQ1 subunits coassemble via protein-protein interactions with 2 KCNE1 auxiliary

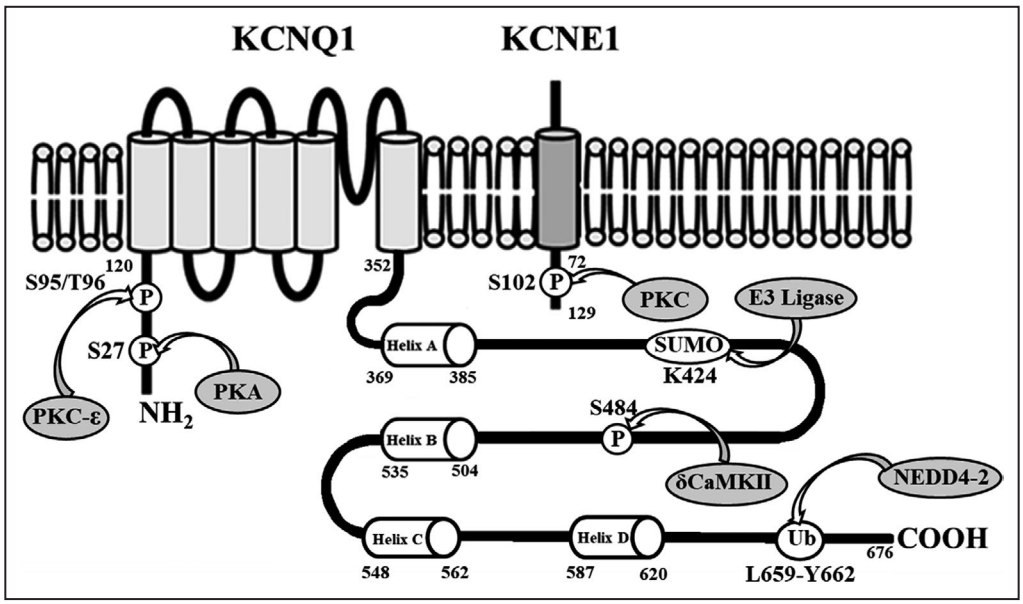

Figure 3. Demonstrated sites of posttranslational regulation on KCNQ1 and KCNE1.
Subunit diagram of KCNQ1 and KCNE1 showing sites of post-translational regulation, the type of posttranslational regulation, and the signaling molecules involved in response to HF-related stimuli. E3 ligase indicates E3 ubiquitin ligase (the specific ligase responsible for SUMO linkage to KCNQ1 K424 has not been elucidated); L, leucine; NEDD4-2, neural precursor cell expressed developmentally down-regulated 4-like; P, phosphorylation; PKA, protein kinase A; PKC, protein kinase C; PKC-ε, epsilon isoform of protein kinase C; S, serine; SUMO, small ubiquitin-like modifier; T, threonine; Ub, ubiquitination; Y, tyrosine; and δCaMKII, delta isoform of calcium/calmodulin-dependent protein kinase II.

subunits.[86] The intracellular amino and carboxyl termini of KCNQ1 contain residues and domains that are critical for channel function, including domains that affect (1) the biophysical properties of I_{Ks} current, (2) coassembly of KCNQ1 tetramers, (3) trafficking of KCNQ1 channels, and (4) interaction of KCNQ1 subunits with KCNE1 and other interacting proteins and molecules (eg, PIP_2 and CaM).[51,87–90] Given the established functional importance of the intracellular domains of KCNQ1, multiple investigations have assessed the potential for experimental models of HF to regulate the intracellular regions of KCNQ1 and thereby affect I_{Ks}.

Posttranslational Regulation of KCNQ1 in HF

Posttranslational regulation of the intracellular amino and carboxyl termini of KCNQ1 has been demonstrated to regulate I_{Ks} in cellular and animal models in response to HF-related signaling cascades. Specifically, the KCNQ1 amino terminus contains residues, including S27 and possibly S92, that mediate activation of I_{Ks} during acute β-AR stimulation via a well-characterized signaling complex that includes PKA, alpha-kinase anchoring protein 9 (also called yotiao), protein phosphatase 1, phosphodiesterase 4D3, and adenylate cyclase 9.[30,91–94] However, it is has been consistently demonstrated that responsiveness to β-AR signaling becomes reduced or "uncoupled" during sustained β-AR stimulation by a mechanism that involves downregulation of G_s;[74,95,96] these findings suggest that PKA-mediated enhancement of I_{Ks} during acute β-AR stimulation, which plays a critical physiologic role in shortening ventricular repolarization during rapid heart rates, is diminished during HF and may contribute to pathological electrical remodeling.[25] Site-specific phosphorylation of the KCNQ1 amino terminus has also been shown to regulate I_{Ks} in response to enhanced angiotensin II signaling. Acute increases in angiotensin II signaling alter I_{Ks} via a signaling cascade that involves PKC,[42] with a recent investigation by Gou et al[17] implicating the PKC-ε (epsilon isoform of protein kinase C) as the regulator via a pathway that involves phosphorylation of S95, T96, or both on the amino terminus of KCNQ1 and a carboxyl residue (S102) on KCNE1. It should be restated that the implications of acute angiotensin II exposure on I_{Ks} have demonstrated conflicting results, with 2 studies demonstrating I_{Ks} enhancement[40,41] and 3 others finding I_{Ks} reductions.[17,42,43]

Posttranslational regulation of the KCNQ1 carboxyl terminus in response to HF-related signaling has also been described. Sustained β-AR stimulation was recently shown to reduce I_{Ks} via a mechanism that involves δCAMKII-mediated phosphorylation of S484 on the KCNQ1 carboxyl terminus.[19] Ubiquitination of

a PY motif by the E3 ubiquitin-protein ligase NEDD4-2 (neural precursor cell expressed developmentally downregulated 4-like) in the distal KCNQ1 carboxyl terminus (L659-Y662) has also been demonstrated to reduce I_{Ks} via downregulation of KCNQ1 plasma membrane expression.[46,97] More recently, findings by Kurakami et al[46] elucidated the signaling pathway involved in KCNQ1 ubiquitination, which involves AMP kinase and PKC and occurs secondary to acute α_1-AR activation. The effects of sustained α_1-AR activation on KCNQ1 ubiquitination and plasma membrane expression have not been studied. Finally, an investigation by Xiong et al[98] has identified the SUMO (small ubiquitin-related modifier) protein as a key regulator of activation gating. "SUMOylation," the process by which an interacting protein is covalently bound by a SUMO protein, has been demonstrated at K424 on each KCNQ1 subunit (incorporating up to four SUMO proteins in a functional I_{Ks} channel) in a process that is KCNE1-dependent.[98] The regulatory role of SUMOylation on I_{Ks} may be of significance in HF since past investigations have found that SUMO proteins, and thereby the process of SUMOylation, are downregulated in HF; decreased SUMOylation in HF has been shown to result in pathological changes in processes involved in cardiac contractility and stress adaptation, including specific pathological regulation of the sarco/endoplasmic reticulum $Ca2^+$-ATPase.[99,100]

Biology of KCNE1

The KCNE1 subunit is 129 amino acids in length and contains a 44 residue extracellular amino terminus and a 58 residue intracellular carboxyl terminus.[101] These termini are separated by a single membrane-spanning transmembrane domain.[101] Functional interaction with KCNQ1 involves multiple locations within the transmembrane domain and carboxyl terminus of KCNE1 and produces the characteristic properties of physiologic I_{Ks}, including slowing of channel activation, increasing conductance, and reducing or eliminating channel inactivation relative to currents produced by KCNQ1 alone.[22,101,102] Based on the necessity of KCNQ1-KCNE1 interaction for proper channel function, investigations have assessed the functional implications of posttranslational modifications of KCNE1 in response to HF-related signaling pathways.

Posttranslational Regulation of KCNE1 in HF

Multiple investigations have elucidated a role for α_1-AR signaling in mediating reductions in I_{Ks} via a mechanism that involves PKC-dependent phosphorylation of KCNE1 S102.[50,52] While the exact isoform of PKC that phosphorylates S102 has not been fully elucidated, it has been demonstrated to be a calcium-dependent PKC isoform, such as alpha (PKC-α), which is the predominant isoform expressed in the heart, or beta (PKC-β), which is upregulated in HF.[103–105] Regardless of the specific PKC isoform involved, I_{Ks} reduction in response to α_1-AR stimulation mechanistically involves enhanced clathrin- and dynamin-dependent endocytosis of plasma membrane-bound KCNQ1-KCNE1 channel complexes.[50,52]

HF EFFECTS ON INTERACTION AMONG I_{KS}-RELATED PROTEINS

In addition to changes in KCNQ1 and KCNE1 expression or posttranslational subunit regulation, HF-associated changes in neurohormonal signaling have also been demonstrated to modulate the interaction of KCNQ1 and KCNE1 with each other and other interacting proteins. These alterations may have important implications for I_{Ks} in HF.

HF-Associated Modulation of the KCNQ1-KCNE1 Subunit Interaction

HF-associated signaling changes may modulate the protein-protein interaction between KCNQ1 and KCNE1 subunits and thereby reduce I_{Ks}. Work by Dvir et al,[90] which investigated the mechanisms for how long QT syndrome mutations in the KCNQ1 and KCNE1 carboxyl termini affect I_{Ks}, demonstrated an important role for PIP_2 in facilitating interaction of KCNQ1 and KCNE1. The importance of PIP_2 in regulating I_{Ks} was also demonstrated by Kienitz et al.[47] Their work found I_{Ks} reductions following sustained α_1-AR stimulation via enzymatic depletion of cellular PIP_2 through a pathway involving chronic PKC activation, which is a known pathologic regulator in HF.[106,107] Accordingly, HF-associated increases in SNS signaling are predicted to disrupt the KCNQ1-KCNE1 subunit interaction by depleting cellular stores of PIP_2, thereby reducing I_{Ks}.

HF-Associated Modulation of KCNQ1-KCNE1 Interaction With Other Interacting Proteins

HF-associated changes in neurohormonal signaling have also been demonstrated to impact the functional interaction between KCNQ1-KCNE1 complexes and other interacting proteins necessary for channel function. For instance, an investigation by Aye et al[108] demonstrated that, relative to tissue from control hearts, explanted heart tissue in patients with HF showed impaired interaction of PKA with alpha-kinase anchoring protein 9, thus potentially blunting I_{Ks} via PKA-mediated (and alpha-kinase anchoring

protein 9–dependent) phosphorylation of KCNQ1 S27 during acute or intermittent rises in adrenergic signaling. Reductions in alpha-kinase anchoring protein 9–dependent I_{Ks} responsiveness to acute β-AR stimulation are predicted to exacerbate an already decreased responsiveness to β-AR stimulation in HF (ie, because of PKA uncoupling secondary to reduced G_s expression). Together, these HF-mediated signaling adaptations are predicted to act in concert to impart an increased risk of QT prolongation and ventricular arrhythmogenesis.[74]

HF-associated changes may also influence interaction of KCNQ1 with CaM, a calcium-binding second messenger that is a regulator of I_{Ks} inactivation gating and channel assembly.[89] Specifically, CaM has been shown to interact with KCNQ1 via 2 CaM-binding domains within helices A and B of the KCNQ1 carboxyl terminus in a process that is required for channel tetramerization.[89,109] Given the essential role of CaM in regulating I_{Ks}, reduced expression of CaM, as has been consistently demonstrated in HF, may be involved in the pathologic regulation of I_{Ks} in HF.[79,110]

CONCLUSION

HF imparts an enormous health burden, and cardiac electrical remodeling underlies a major mechanism of HF-related pathophysiology.[2,4] HF is associated with alterations in neurohormonal signaling, including sustained elevations in SNS and RAAS signaling, that prolong APD and predispose to arrhythmogenic SCD via pathologic regulation of ion channels, including I_{Ks}.[7,13] Herein, we present the current experimental evidence related to the regulation of I_{Ks} during normal physiology and in response to HF-associated stimuli. Specifically, we focus on (1) the cellular receptors and intracellular pathways involved in HF pathophysiology and (2) the specific mechanisms through which HF-associated neurohormonal signaling impact I_{Ks}. By aggregating findings from the basic science and clinical literature, we provide a comprehensive review of both the pathologic regulation of I_{Ks} in HF and the resulting clinical implications on electrical instability and arrhythmogenesis.

Although this review summarizes the literature related to pathological remodeling to alter I_{Ks}, HF is also known to pathologically regulate a wide variety of ion channels and calcium-handling proteins, which contribute to HF-associated ventricular arrhythmogenesis. Past investigations have convincingly demonstrated pathological regulation of ventricular sodium (eg, late I_{Na}), calcium (eg, ICa,L), and potassium currents (eg, the transient outward potassium current, IKr, the inward rectifier potassium current) along with calcium-handling proteins, including the ryanodine receptor, the sodium-calcium exchanger, and sarco/endoplasmic reticulum Ca2+-ATPase, in HF.[6,21,111–115] HF-associated changes in affected ionic currents are summarized in Figure 1C. Given that HF results in deficits in other repolarizing ionic currents, including I_{Kr} and the inward rectifier potassium current, the proarrhythmogenic potential of reductions in I_{Ks} is enhanced in HF.[20,21,25] Indeed, it has been demonstrated that HF-associated reductions in I_{Ks} contribute to the proarrhythmogenic effects of other calcium-related ion carriers that are activated in response to β-AR stimulation (ie, $I_{Ca,L}$, the sodium-calcium exchanger).[116,117] Therefore, the enhanced ventricular electrical instability and arrhythmogenesis characteristic of HF stem from complex interaction of multiple ionic currents that undergo pathological remodeling in HF.

While the therapeutic success of targeting SNS and RAAS signaling in HF is well documented,[11,12] progress toward the understanding of HF pathophysiology continues to elucidate novel potential therapeutic targets. In particular, advances in basic science research have revealed putative therapeutic targets downstream of membrane-bound receptors that have the potential to overcome challenges associated with signaling pathway crosstalk, such as Epac and CaMKII.[118,119] As detailed in this review, the potential benefits of these promising therapeutic targets may be, at least in part, attributable to their ability to prevent or ameliorate pathological I_{Ks} remodeling. Further research is needed to explore the therapeutic potential of these novel targets in preventing and improving clinical outcomes in HF, including arrhythmogenic SCD.

ARTICLE INFORMATION

Affiliations

From the Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN (T.S., B.R.O.); Department of Medicinal Chemistry and Molecular Pharmacology (A.H.) and Department of Pharmacy Practice, Purdue University College of Pharmacy, Indianapolis, IN (B.R.O.).

Sources of Funding

This work was supported in part by a grant from the National Institutes of Health, National Heart, Lung, and Blood Institute; R03 HL141619.

Disclosures

None.

REFERENCES

1. Francis GS, Cogswell R, Thenappan T. The heterogeneity of heart failure: will enhanced phenotyping be necessary for future clinical trial success? *J Am Coll Cardiol*. 2014;64:1775–1776.
2. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, Deo R, et al. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. *Circulation*. 2018;137:e67–e492.

3. Saltzman HE. Arrhythmias and heart failure. *Cardiol Clin*. 2014;32:125–133.
4. Tomaselli GF, Zipes DP. What causes sudden death in heart failure? *Circ Res*. 2004;95:754–763.
5. Coronel R, Wilders R, Verkerk AO, Wiegerinck RF, Benoist D, Bernus O. Electrophysiological changes in heart failure and their implications for arrhythmogenesis. *Biochem Biophys Acta*. 2013;1832:2432–2441.
6. Li GR, Lau CP, Leung TK, Nattel S. Ionic current abnormalities associated with prolonged action potentials in cardiomyocytes from diseased human right ventricles. *Heart Rhythm*. 2004;1:460–468.
7. Cutler MJ, Rosenbaum DS, Dunlap ME. Structural and electrical remodeling as therapeutic targets in heart failure. *J Electrocardiol*. 2007;40:S1–S7.
8. Nattel S, Maguy A, Le Bouter S, Yeh YH. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. *Physiol Rev*. 2007;87:425–456.
9. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. *J Am Coll Cardiol*. 1992;20:248–254.
10. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines and the heart failure society of America. *Circulation*. 2017;136:e137–e161.
11. Shibata MC, Flather MD, Wang D. Systematic review of the impact of beta blockers on mortality and hospital admissions in heart failure. *Eur J Heart Fail*. 2001;3:351–357.
12. Garg R, Yusuf S. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. *JAMA*. 1995;273:1450–1456.
13. Kurokawa J, Abriel H. Neurohormonal regulation of cardiac ion channels in chronic heart failure. *J Cardiovasc Pharmacol*. 2009;54:98–105.
14. Katz AM. *Physiology of the heart*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2011.
15. Aflaki M, Qi XY, Xiao L, Ordog B, Tadevosyan A, Luo X, Maguy A, Shi Y, Tardif JC, Nattel S. Exchange protein directly activated by cAMP mediates slow delayed-rectifier current remodeling by sustained beta-adrenergic activation in guinea pig hearts. *Circ Res*. 2014;114:993–1003.
16. Gonzalez-Juanatey JR, Garcia-Acuna JM, Pose A, Varela A, Calvo C, Cabezas-Cerrato J, de la Pena MG. Reduction of QT and QTc dispersion during long-term treatment of systemic hypertension with enalapril. *Am J Cardiol*. 1998;81:170–174.
17. Gou X, Wang W, Zou S, Qi Y, Xu Y. Protein kinase C epsilon mediates the inhibition of angiotensin II on the slowly activating delayed-rectifier potassium current through channel phosphorylation. *J Mol Cell Cardiol*. 2018;116:165–174.
18. Rivard K, Paradis P, Nemer M, Fiset C. Cardiac-specific overexpression of the human type 1 angiotensin II receptor causes delayed repolarization. *Cardiovasc Res*. 2008;78:53–62.
19. Shugg T, Johnson DE, Shao M, Lai X, Witzmann F, Cummins TR, Rubart-Von-der Lohe M, Hudmon A, Overholser BR. Calcium/calmodulin-dependent protein kinase II regulation of IKs during sustained beta-adrenergic receptor stimulation. *Heart Rhythm*. 2018;15:895–904.
20. Jost N, Virag L, Bitay M, Takacs J, Lengyel C, Biliczki P, Nagy Z, Bogats G, Lathrop DA, Papp JG, et al. Restricting excessive cardiac action potential and QT prolongation: a vital role for IKs in human ventricular muscle. *Circulation*. 2005;112:1392–1399.
21. Tsuji Y, Zicha S, Qi XY, Kodama I, Nattel S. Potassium channel subunit remodeling in rabbits exposed to long-term bradycardia or tachycardia: discrete arrhythmogenic consequences related to differential delayed-rectifier changes. *Circulation*. 2006;113:345–355.
22. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature*. 1996;384:80–83.
23. Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. *Physiol Rev*. 2005;85:1205–1253.
24. Roden DM. Long QT syndrome: reduced repolarization reserve and the genetic link. *J Intern Med*. 2006;259:59–69.
25. Jost N, Papp JG, Varro A. Slow delayed rectifier potassium current (IKs) and the repolarization reserve. *Ann Noninvas Electro*. 2007;12:64–78.
26. Banyasz T, Jian Z, Horvath B, Khabbaz S, Izu LT, Chen-Izu Y. Beta-adrenergic stimulation reverses the I Kr-I Ks dominant pattern during cardiac action potential. *Pflugers Arch*. 2014;466:2067–2076.
27. Volders PG, Stengl M, van Opstal JM, Gerlach U, Spätjens RL, Beekman JD, Sipido KR, Vos MA. Probing the contribution of IKs to canine ventricular repolarization: key role for beta-adrenergic receptor stimulation. *Circulation*. 2003;107:2753–2760.
28. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation*. 2000;102:1178–1185.
29. Stengl M, Volders PG, Thomsen MB, Spatjens RL, Sipido KR, Vos MA. Accumulation of slowly activating delayed rectifier potassium current (IKs) in canine ventricular myocytes. *J Physiol*. 2003;551:777–786.
30. Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496–499.
31. Cheng HC, Incardona J. Models of torsades de pointes: effects of FPL64176, DPI201106, dofetilide, and chromanol 293B in isolated rabbit and guinea pig hearts. *J Pharmacol Toxicol Methods*. 2009;60:174–184.
32. Watanabe E, Yasui K, Kamiya K, Yamaguchi T, Sakuma I, Honjo H, Ozaki Y, Morimoto S, Hishida H, Kodama I. Upregulation of KCNE1 induces QT interval prolongation in patients with chronic heart failure. *Circulation*. 2007;71:471–478.
33. Lee HC, Rudy Y, Po-Yuan P, Sheu SH, Chang JG, Cui J. Modulation of KCNQ1 alternative splicing regulates cardiac IKs and action potential repolarization. *Heart Rhythm*. 2013;10:1220–1228.
34. Ramakers C, Vos MA, Doevendans PA, Schoenmakers M, Wu YS, Scicchitano S, Iodice A, Thomas GP, Antzelevitch C, Dumaine R. Coordinated down-regulation of KCNQ1 and KCNE1 expression contributes to reduction of I(Ks) in canine hypertrophied hearts. *Cardiovasc Res*. 2003;57:486–496.
35. Liu DW, Antzelevitch C. Characteristics of the delayed rectifier current (IKr and IKs) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. A weaker IKs contributes to the longer action potential of the M cell. *Circ Res*. 1995;76:351–365.
36. Szabó G, Szentandrássy N, Bíró T, Tóth BI, Czifra G, Magyar J, Bányász T, Kovács L,Nánási PP. Asymmetrical distribution of ion channels in canine and human left-ventricular wall: epicardium versus midmyocardium. *Pflugers Archiv: Eur J Physiol*. 2005;450:307–316.
37. Kang C, Badiceanu A, Brennan JA, Gloschat C, Qiao Y, Trayanova NA, Efimov IR. Beta-adrenergic stimulation augments transmural dispersion of repolarization via modulation of delayed rectifier currents IKs and IKr in the human ventricle. *Sci Rep*. 2017;7:15922.
38. Burashnikov A, Antzelevitch C. Prominent I(Ks) in epicardium and endocardium contributes to development of transmural dispersion of repolarization but protects against development of early afterdepolarizations. *J Cardiovasc Electrophysiol*. 2002;13:172–177.
39. Pajouh M, Wilson LD, Poelzing S, Johnson NJ, Rosenbaum DS. IKs blockade reduces dispersion of repolarization in heart failure. *Heart Rhythm*. 2005;2:731–738.
40. Zankov DP, Omatsu-Kanbe M, Isono T, Toyoda F, Ding WG, Matsuura H, Horie M. Angiotensin II potentiates the slow component of delayed rectifier K+ current via the AT1 receptor in guinea pig atrial myocytes. *Circulation*. 2006;113:1278–1286.
41. Matavel A, Lopes CM. PKC activation and PIP(2) depletion underlie biphasic regulation of IKs by Gq-coupled receptors. *J Mol Cell Cardiol*. 2009;46:704–712.
42. Si M, Xu J, Zhang F, Wang C, Du X, Zhang H. Involvement of protein kinase A and C in norepinephrine- and angiotensin II-induced modulation of cardiac IKs. *Pharmacology*. 2013;92:217–226.
43. Daleau P, Turgeon J. Angiotensin II modulates the delayed rectifier potassium current of guinea pig ventricular myocytes. *Pflugers Arch*. 1994;427:553–555.
44. Lv Y, Wang Y, Zhu X, Zhang H. Aldosterone downregulates delayed rectifier potassium currents through an angiotensin type 1 receptor-dependent mechanism. *Am J Transl Res*. 2018;10:1413–1421.
45. Lv Y, Bai S, Zhang H, Zhang H, Meng J, Li L, Xu Y. Aldosterone down-regulates the slowly activated delayed rectifier potassium current in adult guinea pig cardiomyocytes. *Br J Pharmacol*. 2015;172:5596–5608.

46. Kurakami K, Norota I, Nasu F, Ohshima S, Nagasawa Y, Konno Y, Obara Y, Ishii K. KCNQ1 is internalized by activation of alpha1 adrenergic receptors. *Biochem Pharmacol*. 2019;169:113628.
47. Kienitz MC, Vladimirova D, Muller C, Pott L, Rinne A. Receptor species-dependent desensitization controls KCNQ1/KCNE1 K+ channels as downstream effectors of Gq protein-coupled receptors. *J Biol Chem*. 2016;291:26410–26426.
48. Habuchi Y, Tanaka H, Furukawa T, Tsujimura Y, Takahashi H, Yoshimura M. Endothelin enhances delayed potassium current via phospholipase C in guinea pig ventricular myocytes. *Am J Physiol*. 1992;262:H345–H354.
49. Washizuka T, Horie M, Watanuki M, Sasayama S. Endothelin-1 inhibits the slow component of cardiac delayed rectifier K+ currents via a pertussis toxin-sensitive mechanism. *Circ Res*. 1997;81:211–218.
50. Kanda VA, Purtell K, Abbott GW. Protein kinase C downregulates I(Ks) by stimulating KCNQ1-KCNE1 potassium channel endocytosis. *Heart Rhythm*. 2011;8:1641–1647.
51. Tobelaim WS, Dvir M, Lebel G, Cui M, Buki T, Peretz A, Marom M, Haitin Y, Logothetis DE, Hirsch JA, et al. Ca(2+)-Calmodulin and PIP2 interactions at the proximal C-terminus of Kv7 channels. *Channels*. 2017;11:686–695.
52. Ronzier E, Parks XX, Lopes CM. KCNE1 subunit phosphorylation leads to IKs internalization in response to chronic calcium-dependent PKC activation. *Biophys J*. 2019;116:398A.
53. Ullian ME, Schelling JR, Linas SL. Aldosterone enhances angiotensin II receptor binding and inositol phosphate responses. *Hypertension*. 1992;20:67–73.
54. Sugiyama T, Yoshimoto T, Tsuchiya K, Gochou N, Hirono Y, Tateno T, Fukai N, Shichiri M, Hirata Y. Aldosterone induces angiotensin converting enzyme gene expression via a JAK2-dependent pathway in rat endothelial cells. *Endocrinology*. 2005;146:3900–3906.
55. Harada E, Yoshimura M, Yasue H, Nakagawa O, Nakagawa M, Harada M, Mizuno Y, Nakayama M, Shimasaki Y, Ito T, et al. Aldosterone induces angiotensin-converting-enzyme gene expression in cultured neonatal rat cardiocytes. *Circulation*. 2001;104:137–139.
56. Vittorio TJ, Fudim M, Wagman G, Kosmas CE. Alpha-1 adrenoceptor-angiotensin II type 1 receptor cross-talk and its relevance in clinical medicine. *Cardiol Rev*. 2014;22:51–55.
57. Henegar JR, Brower GL, Kabour A, Janicki JS. Catecholamine response to chronic ANG II infusion and its role in myocyte and coronary vascular damage. *Am J Physiol*. 1995;269:H1564–H1569.
58. Schutzer WE, Xue H, Reed JF, Roullet JB, Anderson S, Mader SL. Angiotensin II enhances beta-adrenergic receptor-mediated vasorelaxation in aortas from young but not old rats. *Am J Physiol Heart Circ Physiol*. 2000;279:H2807–H2814.
59. Ostrom RS, Naugle JE, Hase M, Gregorian C, Swaney JS, Insel PA, Brunton LL, Meszaros JG. Angiotensin II enhances adenylyl cyclase signaling via Ca2+/calmodulin. Gq-Gs cross-talk regulates collagen production in cardiac fibroblasts. *J Biol Chem*. 2003;278:24461–24468.
60. Barki-Harrington L, Luttrell LM, Rockman HA. Dual inhibition of beta-adrenergic and angiotensin II receptors by a single antagonist: a functional role for receptor-receptor interaction in vivo. *Circulation*. 2003;108:1611–1618.
61. Mishra S, Ling H, Grimm M, Zhang T, Bers DM, Brown JH. Cardiac hypertrophy and heart failure development through Gq and CaM kinase II signaling. *J Cardiovasc Pharmacol*. 2010;56:598–603.
62. Hoch B, Meyer R, Hetzer R, Krause EG, Karczewski P. Identification and expression of delta-isoforms of the multifunctional Ca2+/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. *Circ Res*. 1999;84:713–721.
63. Wagner S, Dantz C, Flebbe H, Azizian A, Sag CM, Engels S, Mollencamp J, Dybkova N, Islam T, Shah AM, et al. NADPH oxidase 2 mediates angiotensin II-dependent cellular arrhythmias via PKA and CaMKII. *J Mol Cell Cardiol*. 2014;75:206–215.
64. Erickson JR, Patel R, Ferguson A, Bossuyt J, Bers DM. Fluorescence resonance energy transfer-based sensor Camui provides new insight into mechanisms of calcium/calmodulin-dependent protein kinase II activation in intact cardiomyocytes. *Circ Res*. 2011;109:729–738.
65. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, et al. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell*. 2008;133:462–474.
66. Baltas LG, Karczewski P, Bartel S, Krause EG. The endogenous cardiac sarcoplasmic reticulum Ca2+/calmodulin-dependent kinase is activated in response to beta-adrenergic stimulation and becomes Ca2+-independent in intact beating hearts. *FEBS Lett*. 1997;409:131–136.
67. Curran J, Hinton MJ, Rios E, Bers DM, Shannon TR. Beta-adrenergic enhancement of sarcoplasmic reticulum calcium leak in cardiac myocytes is mediated by calcium/calmodulin-dependent protein kinase. *Circ Res*. 2007;100:391–398.
68. Pereira L, Metrich M, Fernandez-Velasco M, Lucas A, Leroy J, Perrier R, Morel E, Fischmeister R, Richard S, Benitah JP, et al. The cAMP binding protein Epac modulates Ca2+ sparks by a Ca2+/calmodulin kinase signalling pathway in rat cardiac myocytes. *J Physiol*. 2007;583:685–694.
69. Wollert KC, Drexler H. The renin-angiotensin system and experimental heart failure. *Cardiovasc Res*. 1999;43:838–849.
70. Ohta K, Kim S, Wanibuchi H, Ganten D, Iwao H. Contribution of local renin-angiotensin system to cardiac hypertrophy, phenotypic modulation, and remodeling in TGR (mRen2)27 transgenic rats. *Circulation*. 1996;94:785–791.
71. Tesch GH, Young MJ. Mineralocorticoid receptor signaling as a therapeutic target for renal and cardiac fibrosis. *Front Pharmacol*. 2017;8:313.
72. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med*. 2003;348:1309–1321.
73. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med*. 1999;341:709–717.
74. Soltysinska E, Thiele S, Olesen SP, Osadchii OE. Chronic sympathetic activation promotes downregulation of beta-adrenoceptor-mediated effects in the guinea pig heart independently of structural remodeling and systolic dysfunction. *Pflugers Arch*. 2011;462:529–543.
75. Rose J, Armoundas AA, Tian Y, DiSilvestre D, Burysek M, Halperin V, O'Rourke B, Kass DA, Marban E, Tomaselli GF. Molecular correlates of altered expression of potassium currents in failing rabbit myocardium. *Am J Physiol Heart Circ Physiol*. 2005;288:H2077–H2087.
76. Liu XS, Jiang M, Zhang M, Tang D, Clemo HF, Higgins RS, Tseng GN. Electrical remodeling in a canine model of ischemic cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2007;292:H560–H571.
77. Guo X, Gao X, Wang Y, Peng L, Zhu Y, Wang S. IKs protects from ventricular arrhythmia during cardiac ischemia and reperfusion in rabbits by preserving the repolarization reserve. *PLoS One*. 2012;7:e31545.
78. Akar FG, Wu RC, Juang GJ, Tian Y, Burysek M, Disilvestre D, Xiong W, Armoundas AA, Tomaselli GF. Molecular mechanisms underlying K+ current downregulation in canine tachycardia-induced heart failure. *Am J Physiol Heart Circ Physiol*. 2005;288:H2887–H2896.
79. Borlak J, Thum T. Hallmarks of ion channel gene expression in end-stage heart failure. *FASEB J*. 2003;17:1592–1608.
80. Jiang M, Xu X, Wang Y, Toyoda F, Liu XS, Zhang M, Robinson RB, Tseng GN. Dynamic partnership between KCNQ1 and KCNE1 and influence on cardiac IKs current amplitude by KCNE2. *J Biol Chem*. 2009;284:16452–16462.
81. Lundquist AL, Manderfield LJ, Vanoye CG, Rogers CS, Donahue BS, Chang PA, Drinkwater DC, Murray KT, George AL Jr. Expression of multiple KCNE genes in human heart may enable variable modulation of I(Ks). *J Mol Cell Cardiol*. 2005;38:277–287.
82. Wu DM, Jiang M, Zhang M, Liu XS, Korolkova YV, Tseng GN. KCNE2 is colocalized with KCNQ1 and KCNE1 in cardiac myocytes and may function as a negative modulator of I(Ks) current amplitude in the heart. *Heart Rhythm*. 2006;3:1469–1480.
83. Mazhari R, Nuss HB, Armoundas AA, Winslow RL, Marbán E. Ectopic expression of KCNE3 accelerates cardiac repolarization and abbreviates the QT interval. *J Clin Investig*. 2002;109:1083–1090.
84. Aromolaran AS, Subramanyam P, Chang DD, Kobertz WR, Colecraft HM. LQT1 mutations in KCNQ1 C-terminus assembly domain suppress IKs using different mechanisms. *Cardiovasc Res*. 2014;104:501–511.
85. Wiener R, Haitin Y, Shamgar L, Fernandez-Alonso MC, Martos A, Chomsky-Hecht O, Rivas G, Attali B, Hirsch JA. The KCNQ1 (Kv7.1) COOH terminus, a multitiered scaffold for subunit assembly and protein interaction. *J Biol Chem*. 2008;283:5815–5830.

86. Yu H, Lin Z, Mattmann ME, Zou B, Terrenoire C, Zhang H, Wu M, McManus OB, Kass RS, Lindsley CW, et al. Dynamic subunit stoichiometry confers a progressive continuum of pharmacological sensitivity by KCNQ potassium channels. *Proc Natl Acad Sci U S A*. 2013;110:8732–8737.
87. Schwake M, Athanasiadu D, Beimgraben C, Blanz J, Beck C, Jentsch TJ, Saftig P, Friedrich T. Structural determinants of M-type KCNQ (Kv7) K+ channel assembly. *J Neurosci*. 2006;26:3757–3766.
88. Howard RJ, Clark KA, Holton JM, Minor DL Jr. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. *Neuron*. 2007;53:663–675.
89. Ghosh S, Nunziato DA, Pitt GS. KCNQ1 assembly and function is blocked by long-QT syndrome mutations that disrupt interaction with calmodulin. *Circ Res*. 2006;98:1048–1054.
90. Dvir M, Strulovich R, Sachyani D, Ben-Tal Cohen I, Haitin Y, Dessauer C, Pongs O, Kass R, Hirsch JA, Attali B. Long QT mutations at the interface between KCNQ1 helix C and KCNE1 disrupt I(KS) regulation by PKA and PIP(2). *J Cell Sci*. 2014;127:3943–3955.
91. Lundby A, Andersen MN, Steffensen AB, Horn H, Kelstrup CD, Francavilla C, Jensen LJ, Schmitt N, Thomsen MB, Olsen JV. In vivo phosphoproteomics analysis reveals the cardiac targets of beta-adrenergic receptor signaling. *Sci Signal*. 2013;6:rs11.
92. Lopes CM, Remon JI, Matavel A, Sui JL, Keselman I, Medei E, Shen Y, Rosenhouse-Dantsker A, Rohacs T, Logothetis DE. Protein kinase A modulates PLC-dependent regulation and PIP2-sensitivity of K+ channels. *Channels*. 2007;1:124–134.
93. Li Y, Hof T, Baldwin TA, Chen L, Kass RS, Dessauer CW. Regulation of IKs potassium current by isoproterenol in adult cardiomyocytes requires type 9 adenylyl cyclase. *Cells*. 2019;8:981.
94. Kurokawa J, Motoike HK, Rao J, Kass RS. Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation. *Proc Natl Acad Sci U S A*. 2004;101:16374–16378.
95. Vatner DE, Vatner SF, Nejima J, Uemura N, Susanni EE, Hintze TH, Homcy CJ. Chronic norepinephrine elicits desensitization by uncoupling the beta-receptor. *J Clin Investig*. 1989;84:1741–1748.
96. Fowler MB, Laser JA, Hopkins GL, Minobe W, Bristow MR. Assessment of the beta-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation*. 1986;74:1290–1302.
97. Jespersen T, Membrez M, Nicolas CS, Pitard B, Staub O, Olesen SP, Baro I, Abriel H. The KCNQ1 potassium channel is down-regulated by ubiquitylating enzymes of the Nedd4/Nedd4-like family. *Cardiovasc Res*. 2007;74:64–74.
98. Xiong D, Li T, Dai H, Arena AF, Plant LD, Goldstein SAN. SUMOylation determines the voltage required to activate cardiac IKs channels. *Proc Natl Acad Sci U S A*. 2017;114:E6686–E6694.
99. Mendler L, Braun T, Muller S. The ubiquitin-like SUMO system and heart function: from development to disease. *Circ Res*. 2016;118:132–144.
100. Kho C, Lee A, Jeong D, Oh JG, Chaanine AH, Kizana E, Park WJ, Hajjar RJ. SUMO1-dependent modulation of SERCA2a in heart failure. *Nature*. 2011;477:601–605.
101. Kang C, Tian C, Sonnichsen FD, Smith JA, Meiler J, George AL Jr, Vanoye CG, Kim HJ, Sanders CR. Structure of KCNE1 and implications for how it modulates the KCNQ1 potassium channel. *Biochemistry*. 2008;47:7999–8006.
102. McCrossan ZA, Abbott GW. The MinK-related peptides. *Neuropharmacology*. 2004;47:787–821.
103. Ou J, Rice JJ, Ruwald MH, Parks XX, Ronzier E, Moss AJ, Zareba W, Lopes CM. Impaired IKs channel activation by Ca(2+)-dependent PKC shows correlation with emotion/arousal-triggered events in LQT1. *J Mol Cell Cardiol*. 2015;79:203–211.
104. Braz JC, Gregory K, Pathak A, Zhao W, Sahin B, Klevitsky R, Kimball TF, Lorenz JN, Nairn AC, Liggett SB, et al. PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nat Med*. 2004;10:248–254.
105. Bowling N, Walsh RA, Song G, Estridge T, Sandusky GE, Fouts RL, Mintze K, Pickard T, Roden R, Bristow MR, et al. Increased protein kinase C activity and expression of Ca2+-sensitive isoforms in the failing human heart. *Circulation*. 1999;99:384–391.
106. Singh RM, Cummings E, Pantos C, Singh J. Protein kinase C and cardiac dysfunction: a review. *Heart Fail Rev*. 2017;22:843–859.
107. Rohde S, Sabri A, Kamasamudran R, Steinberg SF. The alpha(1)-adrenoceptor subtype- and protein kinase C isoform-dependence of Norepinephrine's actions in cardiomyocytes. *J Mol Cell Cardiol*. 2000;32:1193–1209.
108. Aye TT, Soni S, van Veen TA, van der Heyden MA, Cappadona S, Varro A, de Weger RA, de Jonge N, Vos MA, Heck AJ, et al. Reorganized PKA-AKAP associations in the failing human heart. *J Mol Cell Cardiol*. 2012;52:511–518.
109. Sun J, MacKinnon R. Cryo-EM structure of a KCNQ1/CaM complex reveals insights into congenital long QT syndrome. *Cell*. 2017;169:1042–1050.e9.
110. Jeck CD, Zimmermann R, Schaper J, Schaper W. Decreased expression of calmodulin mRNA in human end-stage heart failure. *J Mol Cell Cardiol*. 1994;26:99–107.
111. Kääb S, Dixon J, Duc J, Ashen D, Näbauer M, Beuckelmann DJ, Steinbeck G, McKinnon D, Tomaselli GF. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. *Circulation*. 1998;98:1383–1393.
112. Li D, Melnyk P, Feng J, Wang Z, Petrecca K, Shrier A, Nattel S. Effects of experimental heart failure on atrial cellular and ionic electrophysiology. *Circulation*. 2000;101:2631–2638.
113. Maltsev VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure slows late sodium current in human and canine ventricular myocytes: implications for repolarization variability. *Eur J Heart Fail*. 2007;9:219–227.
114. Pogwizd SM, Bers DM. Calcium cycling in heart failure: the arrhythmia connection. *J Cardiovasc Electrophysiol*. 2002;13:88–91.
115. Schillinger W, Schneider H, Minami K, Ferrari R, Hasenfuss G. Importance of sympathetic activation for the expression of Na+-Ca2+ exchanger in end-stage failing human myocardium. *Eur Heart J*. 2002;23:1118–1124.
116. Kamp TJ, Hell JW. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. *Circ Res*. 2000;87:1095–1102.
117. Stengl M, Ramakers C, Donker DW, Nabar A, Rybin AV, Spätjens RL, van der Nagel T, Wodzig WK, Sipido KR, Antoons G, et al. Temporal patterns of electrical remodeling in canine ventricular hypertrophy: focus on IKs downregulation and blunted beta-adrenergic activation. *Cardiovasc Res*. 2006;72:90–100.
118. Pinilla-Vera M, Hahn VS, Kass DA. Leveraging signaling pathways to treat heart failure with reduced ejection fraction. *Circ Res*. 2019;124:1618–1632.
119. Swaminathan PD, Purohit A, Hund TJ, Anderson ME. Calmodulin-dependent protein kinase II: linking heart failure and arrhythmias. *Circ Res*. 2012;110:1661–1677.