Leucine/isoleucine zipper coordination of ion channel macromolecular signaling complexes in the heart: roles in inherited arrhythmias

R.S. Kass*, J. Kurokawa, S.O. Marx, and A.R. Marks

Departments of Pharmacology and Medicine

Columbia University

New York, NY 10032

*Address for correspondence:

Robert S. Kass, Ph.D.
Department of Pharmacology
College of Physicians & Surgeons of Columbia University
630 W. 168th St.
New York, NY 10032
Phone: 212-305-7444
Fax: 212-342-2703
E-mail: rsk20@columbia.edu

The sympathetic nervous system controls the force and rate of contraction of the heart. The rapid response to stress and exercise mediated by increased sympathetic nervous system (SNS) activity requires the coordinated regulation of several ion channels in response to activation of β adrenergic receptors (β-ARs). The microenvironment of target channels is mediated by the assembly of macromolecular signaling complexes in which targeting proteins recruit phosphatases and kinases and in turn bind directly to the channel protein via highly conserved leucine/isoleucine zippers (LIZs). Disruption of local signaling by disease associated LIZ mutations unbalances the physiologic response to SNS stimulation and increases the risk of arrhythmia in mutation carriers.

Control of the rate and force of contraction of the heart by the autonomic nervous system, which consists of the sympathetic and parasympathetic divisions, is a fundamental property of the cardiovascular system. Stimulation of the sympathetic nervous system (SNS) in response to exercise or emotional stress results in a rapid and dramatic increase in heart rate which, in order to ensure adequate diastolic filling time between beats, is accompanied by a concomitant reduction of the ventricular action potential duration (APD) at the cellular level and the corresponding QT interval of the electrocardiogram (ECG). Defective regulation of cardiac electrical activity in the face of SNS activity, can lead to arrhythmias (Wit et al. 1975).

SNS control of cardiac electrical activity is mediated by the activation of β-adrenergic receptors (β-ARs) that regulate the function of select ion channel proteins via phosphorylation by cAMP-dependent protein kinase A (PKA). Targets of PKA include key channels that are involved in the regulation of cellular calcium ion concentration via modulation of the amplitude and duration of the surface electrical signal (membrane action potential) as well as release of calcium ions from the internal calcium store, the sarcoplasmic reticulum (SR). PKA-dependent phosphorylation modulates the activity of L-type calcium channels so that calcium entry is augmented on a beat-by-beat basis. This enhanced calcium entry contributes to action potential prolongation as well as an increase in intracellular calcium available for subsequent uptake by the cardiac SR. PKA

phosphorylation also activates the major intracellular calcium release channel on the SR, the type 2 ryanodine receptor (RyR2) (Marx et al. 2000b) which is responsible for releasing calcium that triggers muscle contraction.

SNS stimulation also leads to cAMP-dependent increase of a slowly activating potassium channel current, I_{Ks}. This modulation increases repolarization current, which is essential to counter the stimulatory effects of PKA on L-type calcium channels (Kass and Wiegers 1982). The result is a balance of inward and outward membrane currents to regulate the duration of the ventricular action potential, and consequently the QT interval, in response to SNS stimulation. Because action potential duration indirectly controls cell calcium, this balance of modulated currents can be thought of as a necessary mechanism to regulate calcium homeostasis in the face of SNS activity.

Inherited mutations in ion channel proteins have been associated with pathological changes that are exacerbated by SNS activity. In one case, the role of I_{Ks} in this process is of interest, because the genes that encode the subunit components of the I_{Ks} channel, *KCNQ1* and *KCNE1* (Sanguinetti et al. 1996, Barhanin et al. 1996), have been linked to the congenital long QT syndrome (LQTS). LQTS, a rare disease in which the QT interval of the ECG is prolonged owing to dysfunctional ventricular repolarization, is associated with syncope, seizures, and sudden death (Keating and Sanguinetti 2001). Mutations in *KCNQ1*, which codes for the α subunit of the I_{KS} channel, cause LQT-1, and mutations in *KCNE1*, the gene coding for the auxiliary β subunit of the I_{Ks} channel, cause LQT-5 (Splawski et al. 2000). In mutation carriers, triggers of arrhythmias are

gene-specific and carriers of mutations in either KCNQ1 or KCNE1 are at greatest risk of experiencing a fatal cardiac arrhythmia in the face of elevated SNS activity (Schwartz et al. 2001). Thus, unraveling the molecular links between the SNS and regulation of the KCNQ1/KCNE1 channel has direct implications for the mechanistic basis of triggers of arrhythmias in this disorder.

In a second case, RyR2 has recently been shown to be involved in at least two forms of sudden cardiac death (SCD): (1) catecholaminergic polymorphic ventricular tachycardia (CPVT) or familial polymorphic   VT (FPVT) (Priori et al. 2001, Laitinen et al. 2001); and (2) arrhythmogenic right ventricular dysplasia type 2 (ARVD2) (Tiso et al. 2001). In both cases fatal cardiac arrhythmias are triggered by stress-induced activation of the SNS either by exercise or startling. However, there is no evidence of APD prolongation or long QT in these individuals, and it has been proposed (but not yet proved) that aberrant diastolic SR calcium release via the mutant RyR2 may trigger the arrhythmias (Marks et al. 2002b).

β-AR signaling: coordination of localized regulation of channel proteins

This complex regulation of key ion channel proteins by β-AR stimulation is mediated by compartmentalization of cAMP-dependent protein kinase (PKA) and protein phosphatases (Bauman and Scott 2002, Smith and Scott 2002) which is achieved through association with targeting proteins, the A-kinase anchoring proteins (AKAPs) (Michel and Scott 2002). AKAPs are a group of structurally diverse proteins with the common

function of binding to PKA, PP1, and protein kinase C (PKC) and other signaling

proteins determine their localization and substrate specificity, enabling these regulatory

enzymes to be present at high concentrations at the site of their substrates within the cell.

Discrete local signaling complexes are formed that are required for the proper processing

of signaling events. Disruption of the local complexes can imbalance the response

leaving some, but not all, pathways intact. Uncoupling of local complexes by disruption

of targeting protein mediated protein-protein interactions is an example of such a

mechanism.

Leucine isoleucine zippers coordinate protein-protein interactions

Marks and colleagues (Marx et al. 2000b, Marks et al. 2002a) were the first to show that

the cardiac calcium release channel/ryanodine receptor (RyR2) is regulated via a

macromolecular signaling complex in which kinases and phosphatases are targeted to the

channel via leucine/isoleucine zipper (LIZ)-mediated protein-protein interactions and to

suggest that this may be a common motif for coordination of ion channel signaling

complexes(Marx et al. 2001b). Subsequent investigations have shown that this is, in fact,

the case for at least two other ion channels that are regulated by PKA: L-type calcium

channels (Hulme et al. 2002) and I_{Ks} (KCNQ1/KCNE1) potassium channels(Marx *et al.*,

2002).

The LIZ domain is an α helical structure that forms coiled coils and was originally

identified as highly conserved motifs mediating the binding of transcription factors to

DNA(Landschulz *et al.*, 1988). Coiled coils are comprised of heptad repeats (*abcdefg*)$_n$ in

which hydrophobic residues occur at positions "*a*" and "*d*" and form the hydrophobic face

of the helix, while "*b,c,e,f,g*" are hydrophilic residues and form the solvent exposed part of

the coiled coil (Lupas, 1996). LZs classically contain a leucine in position "*d*" because of its

flexible side chain, although the canonical leucine residue can be replaced by an isoleucine

or valine (as is the case in mixed lineage kinase-3) (Leung and Lassam 1998). Electrostatic

interactions between side-chains in the "*e*" and "*g*" sites from neighboring helices are

believed to help specify binding partners (Walshaw and Woolfson 2001) and charged

residues at these positions may be destabilizing when not involved in ion pairs (Kohn et al.

1998). In addition, residues occupying the "*e*" and "*g*" positions exhibit a restricted range of

substitutions based upon the volume occluded by adjacent structures (Simmerman et al.

1996).

Mutagenesis has been successfully utilized to study the sequence specificity of interacting

helices in proteins such as GCN4 DNA binding domain (Harbury et al. 1993),

phospholamban (Simmerman *et al.*, 1996), myosin binding subunit/cGKIα (Surks et al.

1999), ryanodine receptor type 1 and 2 (Marx et al. 2001b), and the KCNQ1/KCNE1

channel (Marx et al. 2002). Substitution of an alanine for one or more of the "*d*" position

leucines or isoleucines in the motif diminished the ability of the LZ to mediate protein-

protein interaction without disrupting the native α helical structure (Moitra et al. 1997,

Simmerman et al. 1996).

LZ-mediated local signaling complexes in the heart

Ryanodine receptors

In the case of RyR2 in cardiac muscle and RyR1 in skeletal muscle, both channels are comprised of macromolecular signaling complexes in which the enormous cytoplasmic domain of the RyRs serves as a scaffold for proteins that regulate the channel's function (Jayaraman et al. 1992, Brillantes et al. 1994, Marx et al. 2000a, 2001a). The RyR2 macromolecular complex includes the stabilizing protein FKBP12.6, PKA (both regulatory and catalytic subunits and its targeting protein mAKAP), PP1 and its targeting protein spinophilin, PP2A, and its targeting protein PR130. In each case highly conserved LIZ motifs in RyR2 bind in a completely specific manner to LIZ motifs on the targeting proteins for PKA, PP1 and PP2A (reviewed in Marks et al. 2002a). This macromolecular complex regulates the PKA phosphorylation of a single serine residue (Ser^{2809}) on RyR2. PKA phosphorylation of Ser^{2809} induces the dissociation of FKBP12.6 from the channel, thereby regulating the function of the channel by adjusting its sensitivity to calcium-dependent activation (Marx et al. 2000a). In this manner activation of the SNS can increase the release of SR calcium via PKA phosphorylation of RyR2 resulting in enhanced contractility of the heart and increased cardiac output. This signaling pathway is part of one of the most primitive responses in biology – the "fight or flight" response which is essentially a stress pathway that is required for survival.

KCNQ1/KCNE1 channels

Similarly, the KCNQ1/KCNE1 channel forms a macromolecular signaling complex that is coordinated by the binding of the targeting protein yotiao (Lin et al. 1998) via a LZ

motif in the KCNQ1 carboxy (C-) terminal domain(Marx et al. 2002) (Fig. 1). PKA and

protein phosphatase 1 (PP1), which bind to yotiao, are thus recruited directly to the

channel microdomain and regulate it by phosphorylation of a single amino terminal

KCNQ1 residue, Ser[27] (Marx et al. 2002). Reconstitution of PKA and PP1 mediated

regulation of the KCNQ1/KCNE1 current in Chinese hamster ovary (CHO) cells requires

co-expression of KCNQ1/KCNE1 and yotiao, and is ablated by mutation of the KCNQ1

LZ, which prevents yotiao binding to the channel, resulting in ablation of PKA

phosphorylation of Ser[27].

The functional consequences of PKA phosphorylation of the KCNQ1/KCNE1 channel

complex is a profound increase in the activity if these channels, a hyperpolarizing shift in

the voltage-dependence of channel activation, and a slowing of the return of activated

(open) to resting (closed) channels during diastole. These effects together ensure that

during membrane depolarization, there is more KCNQ1/KCNE1 channel activity in the

presence of SNS-stimulation than in its absence. Consequently, the substantial

repolarization reserve is activated in the face of SNS-mediated activity.

Just as artificial mutations such as substitution of alanine residues for the leucines in the

second and third "*d*" positions within the hKCNQ1 LZ motif abrogate its interaction with

yotiao, without disturbing the α helical structure of the motif, so too inherited mutations

can disrupt LZs and uncouple signaling molecules from their substrates. As noted above,

residues occupying the "*e*" and "*g*" positions in the LZ motif exhibit a restricted range of

substitutions based upon the volume occluded by adjacent structures (Simmerman et al.

1996). Thus, the naturally occurring G589D mutation at an "*e*" position in the LZ motif of

hKCNQ1 disrupts targeting of yotiao to hKCNQ1. The inherited G589D mutation has been

linked to the Long QT Syndrome variant 1 (LQT-1) in Finnish families (Piippo et al.

2001). Moreover, the KCNQ1-G589D mutation, by virtue of the fact that it disrupts the

LZ motif in the carboxy terminus of KCNQ1, results in abrogation of β-adrenergic-

mediated regulation of the channel. The G589D mutation causes a defect in regulation of

the channel by preventing assembly of the macromolecular complex that targets PKA and

PP1 to the C-terminus of the channel. Carriers of this mutation suffer from dysfunctional

regulation of QT duration during mental and physical stress (Paavonen et al. 2001) and

are at risk of arrhythmia and SCD during exercise (Piippo et al. 2001). Thus an inherited

mutation of a single residue on the KCNQ1 channel disrupts the KCNQ1/KCNE1

signaling complex and raises the risk of arrhythmia in mutation carriers.

Disruption of LZ-meditated complexes: a novel mechanism of arrhythmia risk

Because it is now clear that PKA-dependent regulation of at least three key ion channels

in the heart (RyR2, L-type channels, and KCNQ1/KCNE1 channels) requires assembly

LZ-mediated macromolecular signaling complexes, it is also clear that disruption of a

subset of these complexes can lead to an imbalanced response to SNS stimulation. The

LQT-1 mutation G589D is the first example of disease-associated disruption of a

microdomain-signaling complex.

How might the disruption of LZ-mediated regulation of I_{KS} (KCNQ1/KCNE1 channels)

contribute to this arrhythmia risk? Phosphorylation of the KCNQ1/KCNE1 channel,

mediated by the SNS, causes at least two important functional changes in channel activity: an increase in current density during and following activating depolarizing pulses and, importantly, a slowing of the deactivation of channels after the termination of activating pulses (Walsh and Kass 1988). Because KCNQ1/KCNE1 channels activate with a slow time course during depolarization (*in vivo* the depolarizing event is the ventricular action potential), during each action potential the accumulation of open KCNQ1/KCNE1 channels will ultimately be one of the key determinants of ventricular action potential, and hence QT interval, duration. SNS-mediated increase in channel activity provides more channel activity per action potential and hence tips the balance of currents in favor of repolarization earlier than in the absence of SNS stimulation, leading to APD shortening. Additionally, because β-AR stimulation slows the deactivation of this channel activity between beats, the accumulation of open KCNQ1/KCNE1 channels will grow faster on a beat-by-beat basis with SNS stimulation (Viswanathan et al. 1999). Thus β-AR-mediated stimulation of the activity of KCNQ1/KCNE1 channels provides a reserve of outward current that acts to speed the repolarization process in the face of increased activity of L-type calcium channels (Kass and Wiegers 1982).

The G589D mutation, which falls within the KCNQ1 carboxy terminus LZ, uncouples the KCNQ1/KCNE1 channel from SNS-mediated regulation without affecting RyR2 nor the L-type calcium channel, both of which retain the ability to be regulated by SNS activity. Thus L-calcium channel and RyR2 channels activities are enhanced but without the normal SNS-stimulated increase in reserve repolarizing potassium channel current. This imbalance in calcium regulatory mechanisms predisposes cells to two types of

calcium-mediated rhythm disturbances: early after depolarizations (EAD's) (January and Moscucci 1992, January and Riddle 1989) and delayed after depolarizations (DAD's) (January and Fozzard 1988, Wit and Rosen 1983, Kass et al. 1978), both of which can lead to triggered arrhythmias in affected cells (Fig. 2). In addition, the ion channel imbalance may lead to amplification of electrical heterogeneities of repolarization that are intrinsic to the ventricular myocardium. SNS stimulation of L-type calcium channel activity in a setting of reduced I_{Ks} has indeed been shown to increase transmural dispersion of repolarization (Shimizu and Antzelevitch, 1998, Shimizu and Antzelevitch, 2000, Anztzelevitch, 2002) thus creating a condition that can lead to the development of Torsade de Points and the development of triggered activity via midmyocardial M cells Antzelevitch and Shimizu, 2002). Thus an inherited mutation of a single residue in the KCNQ1 LZ can disrupt local communication between the brain and the heart and lead to unstable electrical activity driven by calcium-mediated pro-arrhythmic events.

Local signaling domains, coordinated by the assembly of LZ-mediated macromolecular signaling complexes thus become important to our understanding of the genesis of cardiac arrhythmias at the molecular level.

Figure legends.

Figure 1. Disruption of the KCNQ1 macromolecular complex by an inherited mutation.
A. The targeting protein yotiao binds to the carboxy terminal domain of KCNQ1 via a
LIZ (saw tooth in figure) and recruits PKA and PP1 directly to the channel microdomain
to regulate the channel via phosphorylation of Ser^{27} in the amino terminus. B. The LQT-
2 mutation G589D occurs in the KCNQ1 LIZ motif and disrupts the signaling complex.
Regulation of the channel via Ser^{27} phosphorylation is ablated.

Figure 2. Disruption of a local signaling complex can result in electrical instability in the
face of SNS activity. Ventricular muscle action potential simulation (Clancy and Rudy
1999, Luo and Rudy 1994) shows the effects of disrupting enhancement of
KCNQ1/KCNE1 channel activity in the face of SNS stimulation. The black trace
represents the cellular response to SNS when local signaling complexes are intact. The
dashed trace represents the response under conditions in which the KCNQ1 complex is
disrupted, resulting in a failure of reserve potassium channel activity in response to SNS
stimulation. The imbalanced response to SNS stimulation leads to calcium channel-
dependent EADs (arrow, see text), seen as oscillatory depolarizing events in this
example, which can trigger arrhythmias in the ventricle.

Acknowledgments

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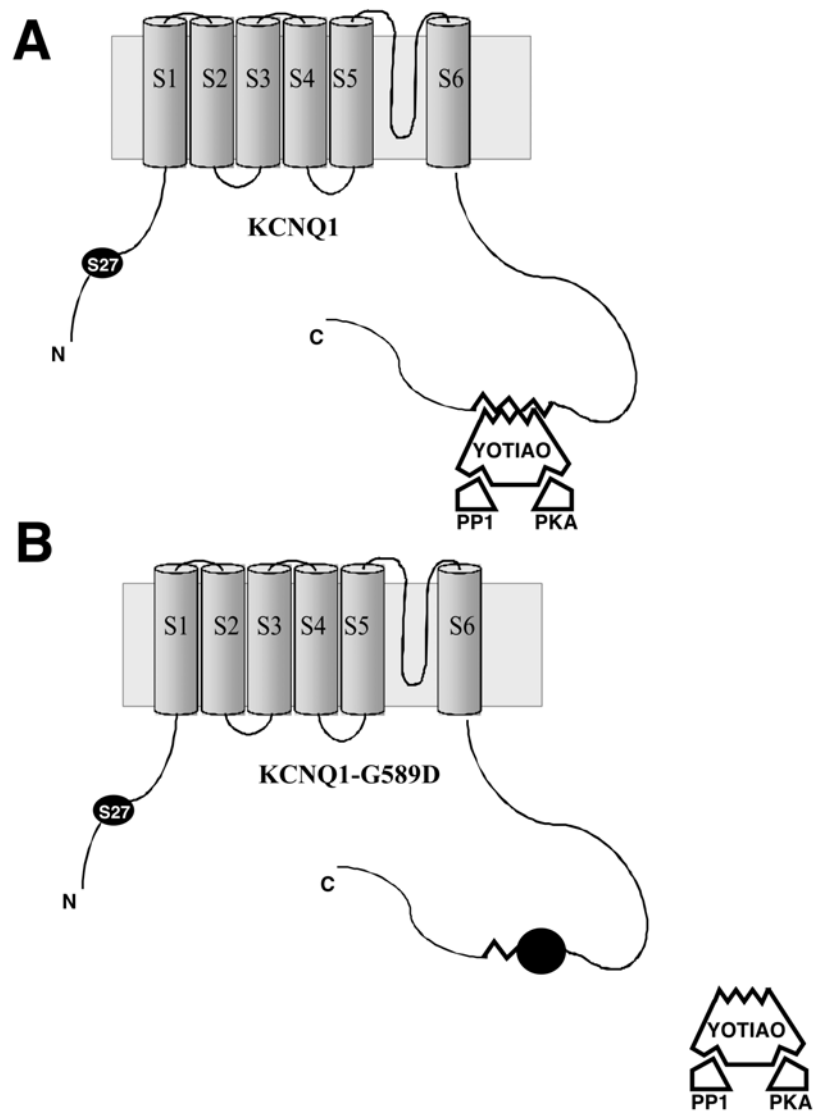Figure 1.

Figure 2.