Control of Cardiac Repolarization by Phosphoinositide 3-Kinase Signaling to Ion Channels

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Abstract: Upregulation of phosphoinositide 3-kinase (PI3K) signaling is a common alteration in human cancer, and numerous drugs that target this pathway have been developed for cancer treatment. However, recent studies have implicated inhibition of the PI3K signaling pathway as the cause of a drug-induced long-QT syndrome in which alterations in several ion currents contribute to arrhythmogenic drug activity. Surprisingly, some drugs that were thought to induce long-QT syndrome by direct block of the rapid delayed rectifier (Ikr) also seem to inhibit PI3K signaling, an effect that may contribute to their arrhythmogenicity. The importance of PI3K in regulating cardiac repolarization is underscored by evidence that QT interval prolongation in diabetes mellitus also may result from changes in multiple currents because of decreased insulin activation of PI3K in the heart. How PI3K signaling regulates ion channels to control the cardiac action potential is poorly understood. Hence, this review summarizes what is known about the effect of PI3K and its downstream effectors, including Akt, on sodium, potassium, and calcium currents in cardiac myocytes. We also refer to some studies in noncardiac cells that provide insight into potential mechanisms of ion channel regulation by this signaling pathway in the heart. Drug development and safety could be improved with a better understanding of the mechanisms by which PI3K regulates cardiac ion channels and the extent to which PI3K inhibition contributes to arrhythmogenic susceptibility. (Circ Res. 2015;116:127-137. DOI: 10.1161/CIRCRESAHA.116.303975.)

Key Words: Ca_{1.2} calcium channel ■ K_{,11.1} voltage-gated channel, human ■ K_{,7.1} potassium channel ■ long QT syndrome ■ Na_{,1.5} voltage-gated sodium channel

Review

Signaling by Class I Phosphoinositide 3-Kinases

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that phosphorylate the 3-hydroxyl position of the inositol head group of phosphoinositides. PI3Ks are grouped into 3 classes according to their primary structures, subunit composition, and substrate specificity. Class 1 PI3Ks phosphorylate phosphatidylinositol 4,5-bisphosphate to produce the second messenger phosphatidylinositol 4,5-trisphosphate (Pl(4,5)P3; Figure 1). Class 1A PI3Ks are heterodimers consisting of a catalytic subunit (p110α, p110β, or p110δ) tightly bound to a regulatory subunit (p85α, p55α, p55δ, or p55γ). The class 1B catalytic subunit p110γ binds to distinct p101 or p97 regulatory subunits. The mammalian heart expresses the p110α, p110β, and p110γ catalytic isoforms along with several different regulatory subunits. Class 2 PI3Ks are monomers (PI3K-C2α, PI3K-C2β, and PI3K-C2γ) that preferentially phosphorylate phosphatidylinositol or phosphatidylinositol 4-phosphate [Pl(4)P]. The class 3 PI3K catalytic subunit Vps34 binds to a Vps15 regulatory subunit and preferentially phosphorylates phosphatidylinositol. PI3K-C2α and Vps34 are also expressed in the heart, but most of the studies that have sought to define a role for PI3Ks in cardiac electrophysiology have centered on the class 1 PI3Ks. Our discussion will focus on these enzymes and will refer to the heterodimers as PI3Kαβ, PI3Kαδ, etc.

Class 1 PI3Ks are tightly regulated by extracellular stimuli that alter the enzyme activity and reposition the PI3Ks to membranes where their lipid substrate is located. A common feature of the class 1A regulatory subunits is the presence of 2 Src homology 2 domains that mediate binding to specific phosphotyrosyl residues in proteins. Exposure of cells to insulin or other growth factors that activate receptor tyrosine kinases leads to phosphorylation of substrate proteins on tyrosyl residues. Class 1A PI3Ks are then recruited to the receptor complexes at the plasma membrane, where the activated enzymes produce Pl(3,4,5)P3 to initiate signaling cascades (Figure 1). Experiments using mouse ventricular myocytes that express a dominant-negative mutant of p110α or that lack p110α, p110β, or p110γ indicated that PI3Kα is the major PI3K isoform that couples to insulin or insulin-like growth factor-1 (IGF-1) receptors in cardiomyocytes. By contrast, PI3Kγ is activated by binding to Gβγ subunits that are released on hormone stimulation of G-protein–coupled receptors (Figure 1). PI3Kβ is also activated by Gβγ subunits, but this mode of regulation has not been demonstrated...
to occur in cardiomyocytes.\textsuperscript{7,8} On the contrary, PI3K\textsubscript{\alpha} is inhibited by G\textsubscript{\alpha}\textsubscript{q} subunits that are released on hormone stimulation of some G-protein–coupled receptors (Figure 1).\textsuperscript{9,10} PI3K\textsubscript{\gamma} has a second function in the heart that is independent of its kinase activity: it binds and activates phosphodiesterases to decrease cAMP (Figure 1).\textsuperscript{11} Dephosphorylation of the 3-hydroxyl group of PI(3,4,5)P\textsubscript{3} by phosphatase and tensin homolog (PTEN) regenerates phosphatidylinositol 4,5-bisphosphate and terminates PI3K signaling (Figure 1). PTEN is a tumor suppressor whose loss or inactivation leads to the upregulation of PI3K signaling in a wide variety of tumors. PI(3,4,5)P\textsubscript{3} can also be sequentially dephosphorylated to yield phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3-phosphate.\textsuperscript{1,2} These 3-phosphoinositides bind to specific domains on PI3K effector proteins to modulate their localization and activity. There are a wide variety of PI3K effectors, including kinases, adaptor proteins, and regulators of small GTPases.\textsuperscript{1,2} We will limit our discussion to protein kinases that have been shown to regulate cardiac ion channels (Figure 1). 3-Phosphoinositide–dependent protein kinase 1 (PDK1) and the protein kinase Akt (also known as PKB) are key PI3K effectors. Binding of PI(3,4,5)P\textsubscript{3} to the pleckstrin homology domain of PDK1 causes the enzyme to translocate to the plasma membrane. Binding of PI(3,4,5)P\textsubscript{3} or phosphatidylinositol 3,4-bisphosphate to the pleckstrin homology domain of Akt causes it to colocalize with PDK1, enabling PDK1 to phosphorylate Akt at a site that partially activates the enzyme. Maximal activation of Akt occurs after phosphorylation of a second site by mechanistic target of rapamycin complex 2, which itself is controlled by PI3K in an as yet undetermined way.\textsuperscript{12} Serum- and glucocorticoid-regulated kinases (SGK) and atypical PKC isoforms are also activated by phosphorylation by PDK1 and mechanistic target of rapamycin complex 2, and some isoforms also possess 3-phosphoinositide binding domains that contribute to their regulation.\textsuperscript{13}

![Diagram of Phosphoinositide 3-kinase (PI3K) signaling pathways regulating cardiac ion channels](http://circres.ahajournals.org/)

**Figure 1. Phosphoinositide 3-kinase (PI3K) signaling pathways regulating cardiac ion channels.** Receptor tyrosine kinases (RTKs) such as the insulin receptor activate PI3K\textsubscript{\alpha} to produce phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P\textsubscript{3}), which recruits Akt and 3-phosphoinositide-dependent protein kinase 1 (PDK1) to the plasma membrane, resulting in Akt activation. RTKs can also activate atypical PKCs (aPKC) and serum- and glucocorticoid-regulated kinase (SGK) via PDK1. G\textsubscript{\beta\gamma} subunits released from G-protein–coupled receptors (GPCRs) activate PI3K\textsubscript{\gamma} to increase PI(3,4,5)P\textsubscript{3} production and activate Akt, but the G\textsubscript{\alpha} subunits inhibit PI3K\textsubscript{\alpha}. Akt, PDK1, aPKC, SGK, and possibly other downstream effectors of PI3K regulate ion channels that conduct potassium, sodium, and calcium currents. Phosphatase and tensin homolog (PTEN) dephosphorylates PI(3,4,5)P\textsubscript{3} to antagonize PI3K signaling. PI3K\textsubscript{\gamma} also binds to and activates phosphodiesterases (PDE) to decrease cAMP, a second messenger that regulates many cardiac ion channels. This function of PI3K\textsubscript{\gamma} is independent of its kinase activity. I\textsubscript{Ca,L} indicates L-type calcium current; I\textsubscript{Kr}, rapid delayed rectifier current; I\textsubscript{Kr}, slow delayed rectifier current; and I\textsubscript{Na}, sodium current.
Long-QT Syndromes

Long-QT syndromes are a family of diseases of multiple causes whose common outcome is prolongation of the QT interval on the ECG. Activation of the ventricular myocardium is reflected on the ECG by the onset of the Q wave, and final repolarization is defined by the end of the T wave. Although the QT interval normally varies with cardiac rate, a pathological increase in the QT interval corrected for rate using various algorithms (ie, QTc) indicates heightened risk for torsades de pointes, an arrhythmia that can cause sudden death.\textsuperscript{14} Primary prolongation of the QT interval (ie, which is independent of an altered QRS complex on the ECG) results from lengthening of the action potential duration (APD) in ventricular myocytes. An increase in depolarizing currents (sodium and calcium) or a decrease in repolarizing currents (potassium) that are major determinants of the action potential waveform can cause an increase in the myocyte APD that is manifested clinically as QT interval prolongation.

Most congenital long-QT syndromes arise from mutations that cause a reduction in the rapid delayed rectifier current (\(I_{Kr}\)) or slow delayed rectifier current (\(I_{Ks}\)).\textsuperscript{15-18} Gain-of-function mutations that cause an increase in the persistent (late) sodium current (\(I_{Na}\)) or L-type calcium current (\(I_{Ca,L}\)) are found in a smaller number of patients.\textsuperscript{19-22} The mutations can affect trafficking, gating, binding to other proteins, or other channel functions.\textsuperscript{23} Acquired long-QT syndromes are far more prevalent than the congenital forms and can arise from drug exposure,\textsuperscript{24} diabetes mellitus,\textsuperscript{25-27} or other conditions. The prevailing view on drug-induced long-QT syndrome is that it is mainly an \(I_{Kr}\) disease resulting from direct blockade of the channel pore or disruption of channel trafficking to the cell surface.\textsuperscript{28-32} We now know that some forms of drug-induced long-QT syndrome, in particular those caused by tyrosine kinase inhibitors, are because of inhibition of PI3K signaling.\textsuperscript{33}

PI3K and Drug-Induced Long-QT Syndrome

Small molecule inhibitors of tyrosine kinases and PI3Ks have entered clinical use or are in clinical trials as anticancer drugs. The package inserts for the tyrosine kinase inhibitors Tasigna (nilotinib) and Sprycel (dasatinib) contain warnings about the risks of QT prolongation, cardiac arrhythmia, and sudden death (www.FDA.gov/drugs/). We showed that nilotinib and PI-103 also caused an increase in QTc in the perfused mouse heart.\textsuperscript{34} In searching for a PI3K whose inhibition might mediate these effects, we found that p110α-null hearts exhibited QTc prolongation. In addition, myocytes lacking p110α exhibited prolonged APD\textsubscript{90}, that was reversed by intracellular application of PI(3,4,5)P\textsubscript{3}. Deletion of PI3K\(\beta\) had little or no effect on APD\textsubscript{90} \textsuperscript{35} Deletion of p110γ did not affect APD in the absence of calcium transients, but APD was prolonged when calcium transients were present.\textsuperscript{34} Gain-of-function mutations in p110α are often found in human cancers, and development of inhibitors to target this enzyme has been a major priority of the pharmaceutical industry.\textsuperscript{35} If PI3K\(\alpha\) plays a major role in regulating the human cardiac action potential as it does in the mouse, then we predict that PI3K inhibitors such as GDC-0941 (Genentech) and BEZ235 (Novartis) that have entered clinical trials will prolong the QT interval in patients.

To our surprise, we found that terfenadine, the nonsedating antihistamine on which the \(I_{Kr}\) hypothesis was based,\textsuperscript{6,37} also seemed to inhibit PI3K signaling because most of its effects on APD\textsubscript{90} were reversed by PI(3,4,5)P\textsubscript{3}. It will be important to learn how prevalent PI3K inhibition is among proarrhythmic drugs, including those that have been classified as \(I_{Kr}\) blockers. In answer to this question, a recent study showed that chronic exposure of adult mouse myocytes, which lack \(I_{Kr}\), to the prototypical \(I_{Kr}\) blocker dofetilide caused APD prolongation that was reversed by PI(3,4,5)P\textsubscript{3}. In tests using \(I_{Kr}\) blockers from multiple therapeutic classes, 6 (including dofetilide) had effects consistent with PI3K inhibition, whereas 2 others (including moxifloxacin) did not. Dofetilide also caused a reduction in Akt phosphorylation that was not seen with moxifloxacin.\textsuperscript{38} Thus, some \(I_{Kr}\) blockers also inhibit PI3K/Akt signaling, and a drug with both activities might have increased potential to cause QT prolongation. Additional studies are needed to characterize how \(I_{Kr}\) blockers, such as terfenadine and dofetilide, inhibit PI3K signaling and to determine the extent to which PI3K inhibition contributes to their arrhythmogenic activity.

Long-QT Syndrome in Diabetes Mellitus

The association of diabetes mellitus with prolonged QTc and cardiovascular death is strongly supported by multiple epidemiological studies.\textsuperscript{25-27} Experiments in animal models showed that streptozotocin-induced diabetic rats,\textsuperscript{39} type 2 diabetic \(db/db\) mice\textsuperscript{40} and alloxan-induced diabetic dogs\textsuperscript{41} and rabbits\textsuperscript{42} exhibited QTc prolongation and APD lengthening in ventricular myocytes. Hyperglycemia per se might not be the cause of these repolarization defects: mice lacking the insulin receptor only in cardiac myocytes also exhibited APD prolongation, even though the animals were euglycemic.\textsuperscript{35} Because reduced production of or sensitivity to insulin in diabetes mellitus results in decreased activation of PI3K, we hypothesized that downregulation of cardiac insulin/PI3K signaling plays a role in QT interval prolongation in diabetes mellitus.\textsuperscript{44} In support of this hypothesis, we found that APD\textsubscript{90} prolongation incubation but not after acute application of 1 to 5 minutes, indicating that the drugs were probably not acting as direct channel blockers.
in ventricular myocytes of diabetic db/db mice and insulin-deficient Ins2Akita mice was reversed by intracellular delivery of PI(3,4,5)P3, but not control phospholipids. Adenoviral expression of constitutively active p110α also corrected APD90 in cultured myocytes from both types of animals. In addition, perfused hearts from db/db and Ins2Akita mice exhibited QTc prolongation. Circulation of insulin through the Ins2Akita heart corrected the abnormal QTc, and this effect was blocked by PI-103.34

Thus, acquired long-QT syndromes caused by diabetes mellitus and some drugs can arise from a common mechanism of suppressed PI3K signaling. It is not surprising that these 2 syndromes are caused by changes in multiple ion channels, some of which have been shown to be PI3K dependent. At least 5 cardiac currents—INa, peak and persistent, IKr, ICaL, and IBK—are affected by PI3K signaling, and the following sections will discuss these currents in more detail.

**Sodium Channel**

In considering the cardiac sodium current INa, it is important to separate effects on peak INa that generates the action potential upstroke and conduction speed from effects on persistent INa that sustains the plateau. Because the predominant evidence suggests that both are generated by the same Na,1.5 channel protein,39,45 effects on channel number or trafficking are likely to alter both currents in the same direction, whereas effects on gating could differentially increase one while decreasing the other. Indeed, treatment of canine ventricular myocytes for 2 hours with nilotinib or PI-103 caused a decrease in peak INa and an increase in persistent INa.33 Effects of nilotinib on both of the sodium currents were reversed by intracellular delivery of PI(3,4,5)P3 through the patch pipette or by extended washout of the drug. These results suggested that INa is regulated by PI(3,4,5)P3, which is slowly depleted during incubation of myocytes with nilotinib or PI-103 and gradually replenished after drug washout. Two lines of evidence demonstrated that elevated persistent INa contributes to the drug effects on repolarization. First, treatment of myocytes with the sodium channel blocker mexiletine at a concentration selective for persistent INa prevented the PI3K inhibitor-induced prolongation of APD90 and early after-depolarization generation.33 Second, computer simulations of the canine ventricular action potential indicated a role of elevated persistent INa in the lengthening of APD90.33 Terfenadine, dofetilide, and several other INa blockers also caused a time-dependent increase in persistent INa that was reversed by PI(3,4,5)P3.33,38 These results suggest that screening drug candidates for chronic effects on persistent INa and for acute INa block might improve drug safety.

To identify the PI3K isoform that regulates INa, the current was studied in mouse myocytes lacking p110α or p110β.33 INa was not altered in p110β-null myocytes, but the p110α knockout recapitulated the effects of drugs on INa in canine ventricular myocytes. The changes in peak and persistent INa in p110α knockout myocytes were eliminated by intracellular perfusion of PI(3,4,5)P3, and QTc prolongation was reversed after mexiletine treatment of the p110α knockout hearts.33 This study suggests that the tyrosine kinase inhibitors and PI3K inhibitors exert their effects on INa through the inhibition of PI3Kα. Whether INa blockers also target PI3Kα remains to be determined using biochemical assays.

PI3K signaling has been reported to upregulate gene expression of sodium channel subunits. Induction of constitutively active p110α in the heart of adult mice increased Akt phosphorylation and mRNA levels of both the α (Scn5a) and β (Scn1b) subunits of the cardiac sodium channel.46 Treatment of the mice with an Akt inhibitor did not block increases in channel expression, suggesting that p110α regulates sodium channel mRNA levels independently of Akt.46 By contrast, increased transcription of Scn5a in rat ventricular myocytes exposed to transforming growth factor β-1 was attributed to activation of PI3K and subsequent phosphorylation of the transcription factor Fox1 by Akt at a site that eliminates its ability to suppress Scn5a expression (Figure 2).57

SGK1 and SGK3 (the major SGK isoforms in the heart), like Akt, can be activated by insulin, IGF-1 or constitutively active p110α in some cell types.58,49 Because Akt and SGKs have similar substrate specificities and phosphorylate some proteins on the same physiologically important sites,50 it seems possible that some effects of PI3K on persistent INa may be mediated by SGKs. Interest in the regulation of the cardiac sodium channel by SGKs was prompted by experiments with the amiloride-sensitive epithelial sodium channel that mediates sodium transport in the kidney and lung.51 Epithelial sodium channel binds to the ubiquitin-protein ligase neural precursor cell-expressed, developmentally downregulated 4-2 (NEDD4-2), leading to ubiquitination and internalization of the sodium channel.52 SGK1 increases channel activity at least, in part, by phosphorylating NEDD4-2 and blocking its interaction with epithelial sodium channel, resulting in an increased number of channels on the plasma membrane.53

Similar studies in Xenopus oocytes expressing the cardiac sodium channel showed that NEDD4-2 decreased the peak sodium current with no alteration in voltage dependence of activation or inactivation, consistent with a loss of Na1.5 because of ubiquitination.54 Regulation of NEDD4-2 and Na1.5 currents by SGK was studied in Xenopus oocytes55 and in mice with cardiac-specific expression of constitutively active SGK1.56 Ventricular myocytes from these mice exhibited increases in peak INa density and cell surface localization of Na1.5, with no change in the total amount of channel protein.56 There was a marked decrease in NEDD4-2 bound to Na1.5 in the transgenic hearts, suggesting that active SGK1 increased peak INa by blocking ubiquitination of Na1.5 and increasing its abundance on the cell surface (Figure 2). Peak INa in myocytes from the transgenic mice also showed a －10 mV shift in voltage dependence of activation and a －5 mV shift in steady-state inactivation. These gating changes should increase and shift the window current to more hyperpolarized potentials, lengthening the action potential at more negative potentials and allowing for greater recovery from inactivation of the calcium channel. Persistent INa was also increased 3.6-fold in transgenic versus wild-type myocytes.56 Not surprisingly, the transgenic SGK1 myocytes exhibited APD90 prolongation, early after-depolarizations, and delayed after-depolarizations,
all of which were reversed by the treatment with the sodium channel blocker ranolazine at a concentration selective for persistent \( I_{\text{Kr}} \). QTc prolongation and lethal ventricular arrhythmias were also improved by treating the mice with ranolazine. Five candidate SGK1 phosphorylation sites were identified in Na,1.5, one of which (T1590) is located in a region important for channel inactivation, suggesting that phosphorylation of this site by SGK1 might be involved in upregulating persistent \( I_{\text{Na}} \) (Figure 2).\(^{56}\) No peptide containing T1590 was detected in a study that identified 11 basal phosphorylated sites in Na,1.5 from mouse ventricular tissue, but the other putative SGK1 sites were found to be phosphorylated.\(^{57}\) It is interesting that inhibition of PI3K/\( \alpha \)/Akt signaling and constitutive activation of SGK1 seem to have the same effects on persistent \( I_{\text{Na}} \). This apparent discrepancy might be because of differential regulation of the current by different effectors downstream of PI3K (Figure 2).

Few studies have examined \( I_{\text{Na}} \) in diabetes mellitus. One noted that peak \( I_{\text{Na}} \) was not altered in alloxan-induced diabetic rabbits.\(^{42}\) We found that mexiletine treatment reversed APD\(_{90}\) prolongation in ventricular myocytes from diabetic \( \text{Ins}2\text{Akita} \) and \( \text{db/db} \) mice, suggesting that persistent \( I_{\text{Na}} \) was increased.\(^{44}\) Measurement of persistent \( I_{\text{Na}} \) confirmed that this was the case. No difference in the amount of Na,1.5 protein was detected in diabetic versus wild-type hearts.\(^{44}\) Intracellular delivery of PI(3,4,5)\(_3\)P, or adenoviral expression of constitutively active p110\( \alpha\) reduced persistent \( I_{\text{Na}} \) in \( \text{Ins}2\text{Akita} \) or \( \text{db/db} \) myocytes to wild-type levels. Conversely, treatment of wild-type myocytes with an Akt inhibitor increased persistent \( I_{\text{Na}} \), but not to the levels seen in diabetic or p110\( \alpha\)-null myocytes. These results suggested that the repolarization defect in diabetes mellitus is due in large part to an increase in persistent \( I_{\text{Na}} \), which is caused by the suppression of PI3K\( \alpha \) signaling to Akt and perhaps other effectors.\(^{44}\)

In summary, current evidence suggests that PI3K\( \alpha \)/Akt signaling leads to phosphorylation of Na,1.5 on a site that regulates its gating properties, thus suppressing persistent \( I_{\text{Na}} \) (Figure 2). Therefore, suppression of PI3K\( \alpha \) signaling because of either diabetes mellitus or drug inhibition contributes to the QT prolongation by decreasing the phosphorylation of Na,1.5 and increasing persistent \( I_{\text{Na}} \). On the contrary, the decrease in peak \( I_{\text{Na}} \) after the inhibition of PI3K\( \alpha \) is likely because of decreased abundance of Na,1.5 on the cell surface and could, if large enough, slow action potential conduction (Figure 2).

**Voltage-Dependent Potassium Channels**

\( I_{\text{Ks}} \) and \( I_{\text{Kr}} \) are major regulators of cardiac repolarization in humans and other large mammals but contribute little to the action potential in adult mice. For that reason, the native currents are not usually studied in genetically modified mice with altered PI3K signaling. Another complication in studying PI3K as a regulator of potassium currents is that some PI3K inhibitors can block \( I_{\text{Ks}} \) or other channels in a PI3K-independent manner.

For example, direct block of 2 slowly inactivating potassium currents \( I_{\text{Ks,slow,1}} \) (K,1.5) and \( I_{\text{Ks,slow,2}} \) (K,2.1) and \( I_{\text{Kr}} \) (K,1.5) by LY294002, a commonly used nonselective PI3K inhibitor, can result in APD prolongation.\(^{58,59}\) We measured reductions in \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) in canine ventricular myocytes treated for 2 hours with nilotinib or PI-103.\(^{33}\) Inhibition of \( I_{\text{Kr}} \) by nilotinib was reversed by adding PI(3,4,5)\(_3\)P to the patch pipette solution, suggesting that it was PI3K dependent. \( I_{\text{Kr}} \) required an extended washout period to recover from nilotinib inhibition, indicating that the drug was not acting as a channel blocker. Computer simulations showed that alterations in both \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) contributed to nilotinib-induced APD\(_{90}\) prolongation. The 60% decrease in \( I_{\text{Kr}} \) alone accounted for less than half of the change in APD\(_{90}\) induced by nilotinib or PI-103, whereas the combined alterations in \( I_{\text{Kr}} \) and persistent \( I_{\text{Ks}} \) accounted for \( \approx 80\% \) of APD\(_{90}\) prolongation.\(^{33}\)

Many of the studies on PI3K regulation of the channel that mediates \( I_{\text{Kr}} \) (K,11.1, also known as hERG) were performed...
using heterologous expression systems in which signaling, channel-interacting proteins, and trafficking may differ from the heart. Constitutively active p110α or Akt enhanced the function of Kv11.1 stably overexpressed in human embryonic kidney 293 cells, whereas dominant-negative mutants of p110α or Akt or treatment with wortmannin (a nonselective PI3K inhibitor) reduced the current.60 Potentiation of Kv11.1 current by SGK3 or constitutively active Akt was also demonstrated in the *Xenopus* oocyte expression system.61 Mutation of 2 putative SGK/Akt phosphorylation sites in Kv11.1 to alanine decreased basal channel function but did not abolish current activation by SGK3, suggesting that SGK3 does not regulate Kv11.1 by direct phosphorylation. A subsequent study in human embryonic kidney 293 cells found that SGK1 and SGK3 increased the level of Kv11.1 on the cell surface by phosphorylating and inhibiting NEDD4-2 and by promoting Rab11-mediated channel recycling (Figure 3).62 The Rab11-dependent process is likely regulated by phosphoinositide kinase, FYVE finger-containing, a lipid kinase that converts phosphatidylinositol 3-phosphate to PI(3,5)P2. Phosphoinositide kinase, FYVE finger-containing is phosphorylated and activated by isoforms of Akt and SGK and was shown to increase the number of Kv11.1 channels on the surface of *Xenopus* oocytes.63 Treatment of neonatal rat cardiomyocytes with dexamethasone increased the expression levels of SGK1 and Kv11.1 and increased *I* _kr_, but whether this is a PI3K-dependent process remains an open question.62

Thyroid hormone increases Kv11.1 currents in rat pituitary cells by inducing the dephosphorylation of T895 in Kv11.1 by protein phosphatase PP5, which is activated by the small GTPase Rac1 downstream of PI3K.64–67 A polymorphism in human Kv11.1 (T897, as opposed to the most common K897) was found to disrupt the kinase recognition site surrounding T895 and create a new Akt phosphorylation site at T897.68 The Kv11.1 T897 variant is associated with a shorter QT interval at baseline.69 However, because thyroid hormone inhibited the Kv11.1 T897 current in a PI3K/Akt-dependent manner, Gentile et al60 predicted that increased PI3K/Akt signaling in people with Kv11.1 T897 would cause QT prolongation. Indeed, a recent study found that 9 of 13 patients who presented with QT prolongation and torsades de pointes during the subacute phase of myocardial infarction (during which Akt is activated) carried the Kv11.1 T897 polymorphism.69 A limitation of this study is that the number of patients who developed torsades de pointes was small, and the enrichment for Kv11.1 T897 could be coincidental.

The slow delayed rectifier current *I* _ks_ is conducted by the Kv7.1 channel (encoded by *KCNQ1*) and its accessory subunit KCNE1 (also called minK). All 3 SGK isoforms and Akt were shown to increase the Kv7.1/KCNE1 current expressed in *Xenopus* oocytes.70,71 Later studies showed that, similar to Na1.5 and Kv11.1, PI3K/SGK1 signaling promoted cell surface localization of Kv7.1/KCNE1 by inhibiting NEDD4-2–mediated internalization (Figure 3).72 NEDD4-2 was also shown to regulate native *I* _ks_ in guinea pig ventricular myocytes.73 An SGK1/phosphoinositide kinase, FYVE finger-containing/PI(3,5)P2/Rab11-mediated pathway was also demonstrated to enhance insertion of the channel into the

![Figure 3. Hypothetical regulation of cardiac delayed rectifier currents by phosphoinositide 3-kinase-α (PI3Kα).](http://circres.ahajournals.org/)

Phosphorylation of neural precursor cell-expressed, developmentally downregulated 4-2 (NEDD4-2) by kinases downstream of PI3Kα increases the currents by preventing the ubiquitination and internalization of the 2 channels. In the presence of PI3K inhibitors or diabetes mellitus, NEDD4-2 is dephosphorylated, it binds to and ubiquitinates K7.1 and K11.1, and the channels are internalized. PI3Kα may also increase cell surface expression of the channels by a second mechanism that involves phosphorylation of phosphoinositide kinase, FYVE finger-containing (PIKfyve) and activation of Rab11-mediated trafficking of channel subunits located in intracellular vesicles (circle) to the cell surface. PI3Kα also upregulates transcription of the potassium channel genes KCNH2 and KCNJ1 by unknown mechanisms.
plasma membrane (Figure 3). Some mutant K, 7.1 channels that cause long-QT syndrome were found to respond in the opposite manner to SGK1, with a decrease in current because of altered trafficking. Conversely, a study of monoyzotic and dizygotic twins found that polymorphisms in the SGK1 gene that are associated with increased blood pressure (presumably because of an increase in SGK1 activity) were associated with a shortened QT interval.

Treatments that activate PI3K signaling have also been reported to suppress some potassium currents in cardiac myocytes. Acute treatment of rat ventricular myocytes with IGF-1, induction of volume-overload cardiac hypertrophy (which is associated with increased IGF-1 signaling and Akt activation), or adenoviral expression of Akt or constitutively active PI3K in rat neonatal cardiomyocytes were all reported to decrease the delayed rectifier current \( I_{Kr} \) and the inward rectifier \( I_K1 \). On the contrary, repolarizing potassium currents in ventricular myocytes were upregulated in 2 models of physiological hypertrophy, one produced by cardiac-specific expression of constitutively active p110α and the other by swim training. Protein and mRNA expression of numerous potassium channel subunits was also increased (Figure 3). Action potential waveform and QT interval were normal in hearts expressing \( I_{Kr} \) and \( I_K1 \). In rat db/db and \( \alpha \)2β1 transgenic mice, \( I_{Kr} \) was increased in proportion to cell size to maintain normal current densities. Inhibition of Akt did not reverse the effects of enhanced cardiac PI3Kα signaling on the potassium channels. The downstream effector of PI3Kα that mediates this response has not been identified.

Before our discovery that an increase in persistent \( I_{Ks} \) contributes to long-QT syndrome in diabetic mice, other investigators described alterations in cardiac potassium currents in animal models of diabetes mellitus. Reductions in the transient outward current \( I_{to} \) were seen in streptozocin-induced diabetic rats and db/db mice, \( I_{K1} \), \( I_{Ks} \), and \( I_{to} \) were decreased in alloxan-induced diabetic rabbits, and \( I_{Ks} \) and \( I_{to} \) were attenuated in alloxan-induced diabetic dogs. Lower abundance of the affected ion channel protein was observed in some cases. Computer simulations suggested that the decrease in \( I_{Ks} \) was the major driver of QT prolongation in the diabetic rabbit. Chronic treatment of alloxan-treated rabbits with insulin completely restored \( I_{Ks} \) function and increased the expression of \( K \), 11.1 to above control levels, even though the animals were still hyperglycemic. Chronic insulin treatment also prevented QTc prolongation, spontaneous ventricular tachycardias, and APD prolongation in ventricular myocytes.

Taken together, the current evidence suggests that PI3Kα signaling upregulates the cell surface expression of \( K \), 11.1 and K, 7.1. Therefore, decreased PI3Kα signaling because of drug inhibition or diabetes mellitus leads to decreased \( I_{Ks} \) and \( I_{Kr} \), contributing to QT prolongation (Figure 3).

**L-Type Calcium Channel**

Early work in neurons and smooth muscle myocytes established PI3Kα as mediators of \( I_{Ca,L} \) potentiation by hormones acting through tyrosine kinase or G-protein–coupled receptors. In rat cerebellar granule neurons, IGF-1 signaling through a class 1A PI3K and Akt increased \( I_{Ca,L} \) and shifted the voltage dependence of activation, with a 4-fold larger current at more hyperpolarized potentials. In rat portal vein myocytes, angiotensin II acting through the G-protein–coupled receptor AT1, upregulated \( I_{Ca,L} \) through \( G \beta y \) activation of PI3Kγ and production of PI(3,4,5)P3.

PI3K/Akt signaling also positively regulates \( I_{Ca,L} \) in ventricular myocytes, and PI3Kα plays a central role in this process. Sun et al determined that IGF-1 signals through PI3Kα and Akt to increase \( I_{Ca,L} \) in mouse myocytes. Transgenic expression of constitutively active p110α also upregulated \( I_{Ca,L} \) and increased the expression of the calcium channel pore subunit (Cav 1,2) and accessory subunits (Caδ2 and Caα2δ1). Transcriptional upregulation of Cav 1,2 and Caα2δ1 was not blocked by treatment with an Akt inhibitor (Figure 4). Akt density was also significantly larger, and inactivation kinetics were faster in myocytes expressing constitutively active Akt. Transgenic expression of nuclear-targeted Akt did not alter \( I_{Ca,L} \), indicating that Akt signaling at the sarcolemma is important for calcium channel regulation. PTEN-null cardiomyocytes, in which Akt is highly active because of the accumulation of PI(3,4,5)P3, exhibited an increase in \( I_{Ca,L} \) and a negative shift in the voltage dependence of activation, but no change in Cav 1,2 protein levels was observed. Treatment of PTEN-null myocytes with a PI3K inhibitor or expression of dominant-negative p110α reversed the increase in \( I_{Ca,L} \) density and the shift in voltage dependence of activation, whereas an Akt inhibitor reversed the increase in \( I_{Ca,L} \) density only. These results established PI3Kα as the mediator of changes in \( I_{Ca,L} \) seen in PTEN-null myocytes. Perhaps PTEN constrains PI(3,4,5)P3 production by PI3Kα in a microdomain that also contains the calcium channel.

PI3Kα-mediated activation of \( I_{Ca,L} \) by receptor tyrosine kinases may be counterbalanced by PI3Kα-mediated inhibition of \( I_{Ca,L} \) by receptors that couple to \( G \alpha q \). Active \( G \alpha q \) binds to PI3K and inhibits its activity. Expression of a \( G \alpha q \) mutant that inhibits insulin/PI3Kα/Akt signaling but that does not activate phospholipase Cβ caused a marked suppression of \( I_{Ca,L} \) in mouse ventricular myocytes that was reversed by PI(3,4,5)P3.

In contrast to the finding that IGF-1 increases \( I_{Ca,L} \) in mouse ventricular myocytes, we found that insulin treatment or intracellular PI(3,4,5)P3 infusion of wild-type myocytes did not increase the current. There was also a disparity in the requirement for basal PI3K/Akt signaling to maintain \( I_{Ca,L} \) in different myocyte preparations. In one case, reducing basal PI3K/Akt activity through the application of nonselective inhibitors of PI3K or Akt by expression of dominant-negative p110α did not cause a significant decrease in \( I_{Ca,L} \) density. By contrast, we found that deletion of p110α caused a 23% reduction in \( I_{Ca,L} \) density, and intracellular delivery of PTEN to dephosphorylate PI(3,4,5)P3, or the pleckstrin homology domain of Grp1 to sequester PI(3,4,5)P3 caused a ≈30% decrease in \( I_{Ca,L} \) density in wild-type mouse cardiomyocytes. In addition, treatment of canine ventricular myocytes with PI-103, nilotinib, or an Akt inhibitor caused a reduction in \( I_{Ca,L} \) and, in the case of PI-103, a shift in the steady-state inactivation curve to the right.
by PI-103, and intracellular delivery of PI(3,4,5)P3, PI3Kα, or activated Akt1 caused a rapid increase in I$_{Ca,L}$ density in p110α-null cardiomyocytes. We found that the reduction in current in p110α-null myocytes was because of a marked decrease in the fraction of Ca$_{1,2}$ on the cell surface, consistent with an earlier study that used transfected COS-7 cells to demonstrate that PI3K/Akt-dependent phosphorylation of a Ca$_{1,2}$ accessory subunit promotes trafficking of calcium channels to the plasma membrane (Figure 4). It is possible that variations in basal PI3K/Akt signaling among myocyte preparations affect Ca$_{1,2}$ surface localization and, therefore, the response of I$_{Ca,L}$ to PI3K inhibition or activation.

Akt-dependent phosphorylation of Ca$_{1,2}$ also regulates Ca$_{1,2}$ protein stability, as revealed in experiments using PDK1-null myocytes. Deletion of PDK1 in the heart of adult mice caused a progressive decrease in Akt phosphorylation, loss of Ca$_{1,2}$ protein, and death by heart failure within 5 to 10 days. Expression of constitutively active Akt increased the amount of Ca$_{1,2}$ in PDK1 knockout myocytes, and experiments in transfected COS-7 cells showed that Ca$_{1,2}$ was susceptible to degradation under conditions of low Akt signaling (Figure 4). Several PEST (rich in proline, glutamic acid, serine, and threonine) sequences that signal rapid protein degradation were identified in Ca$_{1,2}$, as was an Akt phosphorylation site in Ca$_{1,2}$. It was proposed that Akt-mediated phosphorylation of Ca$_{1,2}$ protects Ca$_{1,2}$ from PEST-dependent degradation, thus increasing I$_{Ca,L}$.

Unlike PI3Kα, PI3Kβ is not required to maintain basal I$_{Ca,L}$. Deletion of p110β in cardiac myocytes of adult mice or incubation of canine ventricular myocytes with a PI3Kβ-selective inhibitor (TGX-221) did not change current density. On the contrary, intracellular delivery of phosphatidylinositol 4,5-bisphosphate plus PI3Kβ increased the current in mouse ventricular myocytes whose I$_{Ca,L}$ was suppressed because of the expression of a G$_{o_s}$ protein. It is possible that injection of PI3Kβ into myocytes allows it to enter a subcellular compartment in which it is not normally present to regulate I$_{Ca,L}$.

PI3Kγ suppresses cardiac cAMP levels by activating phosphodiesterases in a manner that is independent of its lipid kinase activity. Two p110γ-null mouse lines have been studied, both of which exhibit elevated cAMP levels in the heart. In ventricular myocytes from the first p110γ-null mouse line, basal I$_{Ca,L}$ density and the response to isoproterenol were abnormally high because of increased cAMP. These effects were attributed to a decrease in phosphodiesterase 3 activity (Figure 4). In the second p110γ-null mouse line, basal I$_{Ca,L}$ density was normal but the rate of calcium-induced current inactivation was increased because of increased sarcoplasmic reticulum calcium release and load. Additional studies suggested that PI3Kγ, phosphodiesterase 3, and phosphodiesterase 4 regulate basal cAMP in microdomains in the vicinity of the sarcoplasmic reticulum that do not contain the L-type calcium channel, and that PI3Kγ is required for phosphodiesterase 4 activity. Interestingly, I$_{Ca,L}$ density in pacemaker cells from the sinoatrial node of these p110γ knockout mice was increased, and the voltage dependence of activation was shifted negative when compared with controls. These changes were reversed by the treatment with a cAMP antagonist, suggesting that PI3Kγ regulates I$_{Ca,L}$ in the sinoatrial node by suppressing cAMP (Figure 4).

Some studies reported that I$_{Ca,L}$ density is diminished in ventricular myocytes of rats with type 1 diabetes mellitus. In rabbits, the inactivation kinetics of Ca$_{1,2}$ in the sinoatrial node by suppressing cAMP were slowed, and the protein expression of Ca$_{1,2}$ was reduced. We found that I$_{Ca,L}$ density was reduced in ventricular myocytes of diabetic Ins2Akita mice, with shifts in the voltage dependence of activation and inactivation to more positive potentials. Intracellular delivery of PI(3,4,5)P3 increased I$_{Ca,L}$ density to control levels and normalized the defect in inactivation. Similar to PI3Kα-null myocytes, the amount of Ca$_{1,2}$ on the surface of Ins2Akita myocytes was reduced, but the total amount of protein was the same as in nondiabetic cells. Incubation of Ins2Akita myocytes with taxol to block microtubule-dependent trafficking did not affect basal I$_{Ca,L}$ density, but it almost completely inhibited current activation provoked by PI(3,4,5)P3 infusion or perfusion with insulin. These results suggest that a major factor underlying the suppression of I$_{Ca,L}$ in Ins2Akita hearts is reduced trafficking of the channel to the cell surface because of reduced insulin/PI3Kα/Akt signaling (Figure 4).

Defects in I$_{Ca,L}$ have also been seen in ventricular myocytes from type 2 diabetic db/db mice. I$_{Ca,L}$ density was decreased, and steady-state activation was shifted toward more depolarized potentials when compared with nondiabetic controls.
The activity of single channels was unchanged, but the expression of the Cα.1.2 protein was reduced in db/db hearts.97 Infusion of db/db myocytes with PI(3,4,5)P3, Akt1, or Akt2 increased current density almost to the wild-type level.98 Infusion of atypical PKC-ι increased current density, but to a smaller extent than Akt.96 The positive shift in steady-state activation in db/db myocytes was completely reversed by infusion of PI(3,4,5)P3 or PKC-ι, whereas Akt1 or Akt2 was without effect (Figure 4).98

In summary, decreased insulin/PI3Kα signaling to Akt and atypical PKCs in myocytes from diabetic mice causes several alterations in I\textsubscript{Ca,L} that can be partially or completely reversed by supplementing the cells with the affected signaling molecules (Figure 4). The current information does not permit a clear definition of the role that I\textsubscript{Ca,L} plays in QT prolongation after downregulation of PI3Kα signaling. In contrast to PI3Kα, PI3Kγ regulates I\textsubscript{Ca,L} by modulating cAMP levels (Figure 4).

Conclusions

Accumulating evidence indicates that PI3K signaling is a key regulator of multiple cardiac ion channels and as a result defines the duration of the cardiac action potential. PI3K signaling affects ≥4 ion channels in cardiac myocytes—Na\textsubscript{L}, 1.5, K\textsubscript{11.1}, K\textsubscript{7.1}, and Cα.1.2—and regulates many aspects of channel function, including protein expression levels, trafficking, and gating. The mechanistic details of these regulatory processes remain unclear, and many questions remain unanswered. For example, chronic changes in PI3K signaling seem to affect transcript levels of many ion channel subunits. Is transcription of ion channel genes in the heart is a relatively transient mechanism by which drugs that inhibit PI3K signaling affects cardiac L-type Ca\textsuperscript{2+} currents via increased PI3Kα/PKB signaling. Circ Res 2006;98:1390–1397. doi: 10.1161/01.RES.0000223321.34482.8c.


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Control of Cardiac Repolarization by Phosphoinositide 3-Kinase Signaling to Ion Channels

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