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

Lisa M. Ballou, Richard Z. Lin, Ira S. Cohen

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 (I\textsubscript{K\text{r}}) 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

Signaling by Class 1 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 subtype specificity.1,2 Class 1 PI3Ks phosphorylate phosphatidylinositol 4,5-bisphosphate to produce the second messenger phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P\textsubscript{3}; Figure 1). Class 1A PI3Ks are heterodimers consisting of a catalytic subunit (p110\textsubscript{α}, p110\textsubscript{β}, or p110\textsubscript{δ}) tightly bound to a regulatory subunit (p85\textsubscript{α}, p55\textsubscript{α}, or p55\textsubscript{γ}). The class 1B catalytic subunit p110\textsubscript{γ} binds to distinct p101 or p97 regulatory subunits. The mammalian heart expresses the γ catalytic isoforms along with sever-

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Nonstandard Abbreviations and Acronyms

<table>
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<tr>
<th>Acronym</th>
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<tbody>
<tr>
<td>APD</td>
<td>action potential duration</td>
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<tr>
<td>APD90</td>
<td>action potential duration at 90% repolarization</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor-1</td>
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<tr>
<td>L-type calcium current</td>
<td></td>
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<tr>
<td>I_r</td>
<td>rapid delayed rectifier current</td>
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<tr>
<td>I_s</td>
<td>slow delayed rectifier current</td>
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<tr>
<td>I_Na</td>
<td>sodium current</td>
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<tr>
<td>NEDD4-2</td>
<td>neural precursor cell-expressed, developmentally downregulated</td>
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<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase</td>
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<tr>
<td>PI(3,4,5)P3</td>
<td>phosphatidylinositol 3,4,5-trisphosphate</td>
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<tr>
<td>PTEN</td>
<td>phospatase and tensin homolog</td>
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<td>SGK</td>
<td>serum- and glucocorticoid-regulated kinase</td>
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Dephosphorylation of the 3-hydroxyl group of PI(3,4,5)P3 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)P3 can also be sequentially dephosphorylated to yield phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3-phosphate.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.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 (PKB) and the protein kinase Akt (also known as PKB) are key PI3K effectors. Binding of PI(3,4,5)P3 to the pleckstrin homology domain of PKB causes the enzyme to translocate to the plasma membrane. Binding of PI(3,4,5)P3 or phosphatidylinositol 3,4-bisphosphate to the pleckstrin homology domain of Akt causes it to colocalize with PKB, enabling PKB 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.1,2 Serum- and glucocorticoid-regulated kinases (SGK) and atypical PKC isoforms are also activated by phosphorylation by PKB and mechanistic target of rapamycin complex 2, and some isoforms also possess 3-phosphoinositide binding domains that contribute to their regulation.13

Figure 1. Phosphoinositide 3-kinase (PI3K) signaling pathways regulating cardiac ion channels. Receptor tyrosine kinases (RTKs) such as the insulin receptor activate PI3Kα to produce phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3), which recruits Akt and 3-phosphoinositide-dependent protein kinase 1 (PKB) to the plasma membrane, resulting in Akt activation. RTKs can also activate atypical PKCs (aPKC) and serum- and glucocorticoid-regulated kinase (SGK) via PKB. Gβγ subunits released from G-protein–coupled receptors (GPCRs) activate PI3Kγ to increase PI(3,4,5)P3 production and activate Akt, but the Gαq subunits inhibit PI3Kα. Akt, PKB1, 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)P3 to antagonize PI3K signaling. PI3Kγ also binds to and activates phosphodiesterases (PDE) to decrease cAMP, a second messenger that regulates many cardiac ion channels. This function of PI3Kγ is independent of its kinase activity. I_Ca,L indicates L-type calcium current; I_Kr, rapid delayed rectifier current; I_Ks, slow delayed rectifier current; and I_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 (i.e., QTc) indicates heightened risk for torsades de pointes, an arrhythmia that can cause sudden death. Primary prolongation of the QT interval (i.e., 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). Gain-of-function mutations that cause an increase in the persistent (late) sodium current (I_Na,L) or L-type calcium current (I_Ca,L) are found in a smaller number of patients. The mutations can affect trafficking, gating, binding to other proteins, or other channel functions. Acquired long-QT syndromes are far more prevalent than the congenital forms and can arise from drug exposure, diabetes mellitus, 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. 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.

**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. 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_90 that was reversed by intracellular application of PI(3,4,5)P_3. Deletion of PI3Kβ had little or no effect on APD_90. Deletion of p110γ did not affect APD in the absence of calcium transients, but APD was prolonged when calcium transients were present. 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. If PI3Kα 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 nonselective antihistamine on which the I_Kr hypothesis was based, also seemed to inhibit PI3K signaling because most of its effects on APD_90 were reversed by PI(3,4,5)P_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_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. 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. Experiments in animal models showed that streptozotocin-induced diabetic rats, type 2 diabetic db/db mice and alloxan-induced diabetic dogs and rabbits 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. 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. In support of this hypothesis, we found that APD_90 prolongation
in ventricular myocytes of diabetic db/db mice and insulin-deficient Ins2A(10) 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 Ins2A(10) mice exhibited QTc prolongation. Circulation of insulin through the Ins2A(10) 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—IKr (peak and persistent), IKs, and ICa-L—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,35,36 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 IKr 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 isofor 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 IKr 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.66 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.66 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 FoxO1 by Akt at a site that eliminates its ability to suppress Scn5a expression (Figure 2).77

SGK1 and SGK3 (the major SGK isofoms in the heart), like Akt, can be activated by insulin, IGF-1 or constitutively active p110α in some cell types.68,69 Because Akt and SGKs have similar substrate specificities and phosphorylate some proteins on the same physiologically important sites,66 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.51

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 Na,1.5 because of ubiquitination.54 Regulation of NEDD4-2 and Na,1.5 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 Na,1.5, with no change in the total amount of channel protein.56 There was a marked decrease in NEDD4-2 bound to Na,1.5 in the transgenic hearts, suggesting that active SGK1 increased peak INa by blocking ubiquitination of Na,1.5 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, delayed after-depolarizations,
all of which were reversed by the treatment with the sodium channel blocker ranolazine at a concentration selective for persistent $I_{Na}$. 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 $I_{Na}$ (Figure 2). 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. It is interesting that inhibition of PI3K/ Akt signaling and constitutive activation of SGK1 seem to have the same effects on persistent $I_{Na}$. This apparent discrepancy might be because of differential regulation of the current by different effectors downstream of PI3K (Figure 2).

Few studies have examined $I_{Na}$ in diabetes mellitus. One noted that peak $I_{Na}$ was not altered in alloxan-induced diabetic rabbits. We found that mexiletine treatment reversed APD$_{90}$ prolongation in ventricular myocytes from diabetic $Ins_{2Akita}$ and $db/db$ mice, suggesting that persistent $I_{Na}$ was increased. Measurement of persistent $I_{Na}$ confirmed that this was the case. No difference in the amount of $Na_{1.5}$ protein was detected in diabetic versus wild-type hearts. Intracellular delivery of PI(3,4,5)P$_3$ or adenoviral expression of constitutively active p110$\alpha$ reduced $I_{Na}$ after the inhibition of PI3K$\alpha$. This decrease in peak $I_{Na}$ 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

$I_{Ks}$ and $I_{Kr}$ 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 $I_{Ks}$ or other channels in a PI3K-independent manner. For example, direct block of 2 slowly inactivating potassium currents [$I_{Ks\text{-slow1}}$ ($K_{1.5}$) and $I_{Ks\text{-slow2}}$ ($K_{2.1}$)] and $I_{Kr}$ ($K_{1.5}$) by LY294002, a commonly used nonselective PI3K inhibitor, can result in APD prolongation. We measured reductions in $I_{Kr}$ and $I_{Ks}$ in canine ventricular myocytes treated for 2 hours with nilotinib or PI-103. Inhibition of $I_{Kr}$ by nilotinib was reversed by adding PI(3,4,5)P$_3$ to the patch pipette solution, suggesting that it was PI3K dependent. $I_{Ks}$ 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 $I_{Kr}$ and $I_{Ks}$ contributed to nilotinib-induced APD$_{90}$ prolongation. The 60% decrease in $I_{Kr}$ alone accounted for less than half of the change in APD$_{90}$ induced by nilotinib or PI-103, whereas the combined alterations in $I_{Ks}$ and persistent $I_{Kr}$ accounted for $\approx$80% of APD$_{90}$ prolongation.

Many of the studies on PI3K regulation of the channel that mediates $I_{Kr}$ ($K_{11.1}$, 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 K_{11.1} 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. Potentiation of K_{11.1} current by SGK3 or constitutively active Akt was also demonstrated in the Xenopus oocyte expression system. Mutation of 2 putative SGK/Akt phosphorylation sites in K_{11.1} to alanine decreased basal channel function but did not abolish current activation by SGK3, suggesting that SGK3 does not regulate K_{11.1} by direct phosphorylation. A subsequent study in human embryonic kidney 293 cells found that SGK1 and SGK3 increased the level of K_{11.1} on the cell surface by phosphorylating and inhibiting NEDD4-2 and by promoting Rab11-mediated channel recycling (Figure 3). 

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 K_{11.1} channels on the surface of Xenopus oocytes. 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.

Thyroid hormone increases K_{11.1} currents in rat pituitary cells by inducing the dephosphorylation of T895 in K_{11.1} by protein phosphatase PP5, which is activated by the small GTPase Rac1 downstream of PI3K. A polymorphism in human K_{11.1} (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. The K_{11.1} T897 variant is associated with a shorter QT interval at baseline. However, because thyroid hormone inhibited the K_{11.1} T897 current in a PI3K/Akt-dependent manner, Gentile et al predicted that increased PI3K/Akt signaling in people with K_{11.1} 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 K_{11.1} T897 polymorphism. A limitation of this study is that the number of patients who developed torsades de pointes was small, and the enrichment for K_{11.1} 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 K_{7.1}/KCNE1 current expressed in Xenopus oocytes. Later studies showed that, similar to Na_{1.5} and K_{11.1}, PI3K/SGK1 signaling promoted cell surface localization of K_{7.1}/KCNE1 by inhibiting NEDD4-2–mediated internalization (Figure 3). NEDD4-2 was also shown to regulate native I_{Ks} in guinea pig ventricular myocytes. 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 cell surface.

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**Figure 3. Hypothetical regulation of cardiac delayed rectifier currents by phosphoinositide 3-kinase-α (PI3Kα).** 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 K_{7.1} and K_{11.1}, 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 KCNQ1 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 monozygotic 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 $I_{K}$ 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 constitutively active p110α because ion channel expression was increased in proportion to cell size to maintain normal current densities. Inhibition of Akt did not reverse the effects of enhanced cardiac PI3K on signaling on the potassium channels.

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 $I_{h}$ were seen in streptozocin-induced diabetic rats and db/db mice, and $I_{K}$ and $I_{m}$ were decreased in alloxan-induced diabetic rabbits, and $I_{K}$ and $I_{m}$ 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 $I_{K}$ was the major driver of QT prolongation in the diabetic rabbit. Chronic treatment of alloxan-treated rabbits with insulin completely restored $I_{K}$ 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 $I_{Km}$ and $I_{Kr}$ contributing to QT prolongation (Figure 3).

L-Type Calcium Channel

Early work in neurons and smooth muscle myocytes established PI3Ks 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 Gβγ 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 (Ca, 1.2) and accessory subunits (Caβ2 and Ca α2δ1). Transcriptional upregulation of Ca, 1.2 and Ca, α2δ1 was not blocked by treatment with an Akt inhibitor (Figure 4). $I_{Ca,L}$ 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 Ca, 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 Gαq. Active Gαq binds to PI3Kα and inhibits its activity. Expression of a Gαq 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 or 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 ≈50% 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 IₖCa,L density in p110α-null cardiomyocytes.³ We found that the reduction in current in p110α-null myocytes was because of a marked decrease in the fraction of Caᵥ1.2 on the cell surface,⁷ consistent with an earlier study that used transfected COS-7 cells to demonstrate that PI3K/Akt-dependent phosphorylation of a Caᵥ2,3 accessory subunit promotes trafficking of calcium channels to the plasma membrane (Figure 4).⁶ It is possible that variations in basal PI3K/Akt signaling among myocyte preparations affect Caᵥ1.2 surface localization and, therefore, the response of IₖCa,L to PI3K inhibition or activation. Akt-dependent phosphorylation of Caᵥβ₂ also regulates Caᵥ1.2 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 Caᵥ1.2 protein, and death by heart failure within 5 to 10 days.⁹¹ Expression of constitutively active Akt increased the amount of Caᵥ1.2 in PDK1 knockout myocytes, and experiments in transfected COS-7 cells showed that Caᵥβ₂ caused Caᵥ1.2 to become 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 Caᵥ1.2, as was an Akt phosphorylation site in Caᵥβ₂.⁹² It was proposed that Akt-mediated phosphorylation of Caᵥβ₂ protects Caᵥ1.2 from PEST-dependent degradation, thus increasing IₖCa,L.

Unlike PI3Kα, PI3Kβ is not required to maintain basal IₖCa,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.¹ On the contrary, intracellular delivery of phosphatidylinositol 4,5-bisphosphate plus PI3Kβ increased the current in mouse ventricular myocytes whose IₖCa,L was suppressed because of the expression of a Gαq protein.⁹³ 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 IₖCa,L.

PI3Kγ suppresses cardiac cAMP levels by activating phosphodiesterases in a manner that is independent of its lipid kinase activity.¹¹ Two p110γ-null mouse lines have been studied, both of which exhibit elevated cAMP levels in the heart.¹¹,88 In ventricular myocytes from the first p110γ-null mouse line, basal IₖCa,L density and the response to isoproterenol were abnormally high because of increased cAMP.⁹² These effects were attributed to a decrease in phosphodiesterase 3 activity (Figure 4).³⁷ In the second p110γ-null mouse line, basal IₖCa,L density was normal but the rate of calcium-induced current inactivation was increased because of increased sarcoplasmic reticulum calcium release and load.⁴,³⁴ 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.³⁴ Interestingly, IₖCa,L density in pacemaker cells from the sinoatrial node of these p110γ knockout mice was increased, and the voltage dependence of activation was shifted negatively when compared with controls.⁹³ These changes were reversed by the treatment with a cAMP antagonist, suggesting that PI3Kγ regulates IₖCa,L in the sinoatrial node by suppressing cAMP (Figure 4).

Some studies reported that IₖCa,L density is diminished in ventricular myocytes of rats⁴⁶ or rabbits with type 1 diabetes mellitus. In rabbits, the inactivation kinetics of IₖCa,L were slowed, and the protein expression of Caᵥ1.2 was reduced.⁴² We found that IₖCa,L density was reduced in ventricular myocytes of diabetic Ins2²Akita mice, with shifts in the voltage dependence of activation and inactivation to more positive potentials.⁹⁵ Intracellular delivery of PI(3,4,5)P₃ increased IₖCa,L density to control levels and normalized the defect in inactivation. Similar to PI3Kα-null myocytes,¹ the amount of Caᵥ1.2 on the surface of Ins2²Akita myocytes was reduced, but the total amount of protein was the same as in nondiabetic cells.⁹⁵ Incubation of Ins2²Akita myocytes with taxol to block microtubule-dependent trafficking did not affect basal IₖCa,L density, but it almost completely inhibited current activation provoked by PI(3,4,5)P₃ infusion or perfusion with insulin.⁹⁸ These results suggest that a major factor underlying the suppression of IₖCa,L in Ins2²Akita hearts is reduced trafficking of the channel to the cell surface because of reduced insulin/PI3Kα/Akt signaling (Figure 4).

Defects in IₖCa,L have also been seen in ventricular myocytes from type 2 diabetic db/db mice. IₖCa,L density was decreased, and steady-state activation was shifted toward more depolarized potentials when compared with nondiabetic controls.⁹⁷
The activity of single channels was unchanged, but the expression of the Ca$_{1.2}$ protein was reduced in db/db hearts.\textsuperscript{97} Infusion of db/db myocytes with PI(3,4,5)P$_3$, Akt1, or Akt2 increased current density almost to the wild-type level.\textsuperscript{98} Infusion of atypical PKC-\textit{t} also increased current density, but to a smaller extent than Akt.\textsuperscript{99} The positive shift in steady-state activation in db/db myocytes was completely reversed by infusion of PI(3,4,5)P$_3$ or PKC-\textit{t}, whereas Akt1 or Akt2 was without effect (Figure 4).\textsuperscript{98}

In summary, decreased insulin/PI3K$\alpha$ signaling to Akt and atypical PKCs in myocytes from diabetic mice causes several alterations in $I_{\text{Ca,L}}$ 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 $I_{\text{Ca,L}}$ plays in QT prolongation after downregulation of PI3K$\alpha$ signaling. In contrast to PI3K$\alpha$, PI3K$\gamma$ regulates $I_{\text{Ca,L}}$ 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 \$\geq 4$ ion channels in cardiac myocytes—Na$_{\text{1.5}}, K_{\text{11.1}}, K_{\text{7.1}},$ and Ca$_{\text{1.2}}$—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 transcriptional downregulation of these genes part of the long-term mechanism by which drugs that inhibit PI3K signaling cause QT prolongation? The role of PI3K in regulating the transcription of ion channel genes in the heart is a relatively unexplored area. Another question relates to how suppression of PI3K$\alpha$ signaling modulates Na$_{\text{1.5}}$ such that an increase in persistent $I_{\text{Na,p}}$ occurs concurrently with a reduction in peak $I_{\text{Na}}$. Additional studies will be needed to identify the specific downstream effectors that modulate these ion channel functions. This information may lead to the development of clinically useful drugs that specifically block persistent $I_{\text{Na,p}}$ without affecting peak $I_{\text{Na}}$. Going forward, it will be important to identify physiological and pathological conditions that alter cardiac PI3K signaling and to identify the affected PI3K isoforms. Even though all 4 class 1 PI3K catalytic isoforms produce the lipid second messenger PI(3,4,5)P$_3$, it is unclear why PI3K$\alpha$ seems to be the predominant isoform involved in ion channel regulation. It is possible that PI3K signaling modules may be confined to microdomains that target certain extracellular inputs to particular channels. Finally, it is already clear that the high-throughput assays currently used to test candidate drugs for the ability to block $I_{\text{Kr}}$ are not sufficient to identify all drugs that might cause long-QT syndrome. Strategies that incorporate screening of drug candidates for effects on PI3K signaling or persistent $I_{\text{Na}}$ will be helpful for pharmaceutical development.

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