Phosphoinositide 3-kinase γ Regulates Cardiac Contractility by Locally Controlling Cyclic Adenosine Monophosphate Levels

Benoit-Gilles Kerfant¹, Robert A. Rose¹, Hui Sun, and Peter H. Backx*

Phosphoinositide 3-kinases (PI3Ks) are enzymes with both protein and lipid kinase activities that regulate important cellular functions in many tissues. In the heart, subclass IA PI3Ks (mainly PI3Kγ) regulate cell growth, apoptosis, cell division and cell size, whereas PI3Kβ, the only member of subclass IB, has been shown to regulate cardiac contractility. We have shown that the loss of PI3Kγ (PI3Kγ−/− mice) enhances cardiac excitation–contraction coupling by modulating cyclic adenosine monophosphate (cAMP) levels in subcellular domains containing the sarcoplasmic reticulum. Specifically, PI3Kγ−/− mice show enhanced sarcoplasmic reticulum Ca2+ cycling in association with increased cAMP. Surprisingly, L-type Ca2+ current, a prototypic target of cAMP-dependent protein kinase A phosphorylation, is largely unchanged in PI3Kγ−/− mice. In this article, we discuss the consequences and implications of cAMP compartmentation in cardiomyocytes. We also review the different roles of PI3Kγ in the heart, particularly as they relate to cardiac contractility, intracellular cAMP levels, and the regulation of β-adrenergic receptor signaling in physiologic and pathologic states. (Trends Cardiovasc Med 2006;16:250–256) © 2006, Elsevier Inc.

¹ Both authors contributed equally to this work.

* Address correspondence to: Peter H. Backx, DVM, PhD, Fitzgerald Building, Heart and Stroke/Richard Lewar Centre, 150 College St., Toronto, Ontario, Canada M5S 3E2. Tel.: (+1) 416-946-8112; fax: (+1) 416-340-8380; e-mail: p.backx@utoronto.ca.

PII S1050-1738(06)00072-7

Class I phosphoinositide 3-kinases (PI3Ks) are enzymes with both protein and lipid kinase activities that regulate important cellular functions in many tissues. In the heart, subclass IA PI3Ks (mainly PI3Kγ) regulate cell growth, apoptosis, cell division and cell size, whereas PI3Kβ, the only member of subclass IB, has been shown to regulate cardiac contractility. We have shown that the loss of PI3Kγ (PI3Kγ−/− mice) enhances cardiac excitation–contraction coupling by modulating cyclic adenosine monophosphate (cAMP) levels in subcellular domains containing the sarcoplasmic reticulum. Specifically, PI3Kγ−/− mice show enhanced sarcoplasmic reticulum Ca2+ cycling in association with increased cAMP. Surprisingly, L-type Ca2+ current, a prototypic target of cAMP-dependent protein kinase A phosphorylation, is largely unchanged in PI3Kγ−/− mice. In this article, we discuss the consequences and implications of cAMP compartmentation in cardiomyocytes. We also review the different roles of PI3Kγ in the heart, particularly as they relate to cardiac contractility, intracellular cAMP levels, and the regulation of β-adrenergic receptor signaling in physiologic and pathologic states. (Trends Cardiovasc Med 2006;16:250–256) © 2006, Elsevier Inc.
(PKB/Akt). PI3Kζ and PI3Kγ, which are members of PI3K subclass IA and IB, respectively, are the two main isoforms expressed in cardiomyocytes (Prasad et al. 2003). While PI3Kζ regulates heart growth (Luo et al. 2005), PI3Kγ has emerged as an important regulator of cardiac contractility (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Aliotti et al. 2005, Kerfant et al. 2005) because of its ability to modulate cyclic adenosine monophosphate (cAMP) metabolism (Kerfant et al. 2005). The available data indicate that PI3Kγ regulates cAMP in compartmentalized microdomains within ventricular cardiomyocytes (Kerfant et al. 2005). This review will focus on the compartmentalized regulation of cAMP metabolism and cardiac ECC by PI3Kγ. We begin with a general description of ECC and cAMP compartmentation in the heart.

Cardiomyocyte contraction is controlled by a series of events in which electrical signals generated at the plasma membrane of the cardiomyocyte lead to contraction of the myofilaments (Bers 2002), a process called cardiac excitation-contraction coupling (ECC). Specifically, during the cardiac action potential, sarcoplasmal Na+ and L-type Ca2+ channels open in response to changes in membrane voltage. Ca2+ influx through L-type Ca2+ channels induces local elevations of intracellular Ca2+ ([Ca2+]i), leading to the opening Ca2+ release channels (called “ryanodine receptors”) in the sarcoplasmic reticulum (SR), by the process of Ca2+-induced Ca2+ release. The Ca2+ released from the SR, in combination with the influx of Ca2+ from L-type Ca2+ channels, raises [Ca2+]i to a level (~600 nM) sufficient to activate myofilament contraction. To maintain Ca2+ homeostasis, cytosolic Ca2+ is returned to the SR via the sarcoendoplasmic reticulum Ca2+-ATPase pump type 2a (SERCA-2a) and extruded from the cell primarily by sarcoplasmal Na+-Ca2+ exchangers as well as secondarily by plasmalemmal Ca2+ pumps (reviewed in Bers 2002).

Several of the proteins involved in cardiac ECC are sensitive to phosphorylation and dephosphorylation events by kinases and phosphatases. The best-characterized example of this type of regulation is the positive inotropic and lusitropic response of cardiomyocytes to ß-adrenergic receptor (ß-AR) activation. Agonist stimulation of ß-ARs (mainly ß1-ARs) activates the heterotrimeric guanosine triphosphate-binding protein, Goα, causing the activation of adenyl cyclase (AC) enzymes. Adenyl cyclase stimulates cAMP production, which activates the cAMP-dependent protein kinase A (PKA), leading to the phosphorylation of proteins such as L-type Ca2+ channels, phospholamban (PLN), ryanodine receptor type 2 (RyR2), myosin-binding protein C, and troponin I (Bers 2002). Thus, the regulation of intracellular cAMP levels is a critical determinant of the heart’s contractile function.

- **cAMP Compartmentation in the Heart**

In addition to the ß-ARs, there are several other hormones and compounds that bind to G-protein-coupled receptors and increase cAMP; however, not all of these hormones produce the inotropic and lusitropic responses seen with ß-AR stimulation in the heart. For example, both glucagon-like peptide-1 and prostaglandin E1 increase cAMP to a level comparable with that elicited by the ß-AR agonist isoproterenol (ISO), but neither of these hormones elicit the contractile responses observed with ISO (Keely 1979, Vila Petroff et al. 2001). These observations suggest that cAMP signaling may be compartmentalized in cardiomyocytes and that different hormones activate distinct pools of downstream signaling molecules (reviewed in Steinberg and Brunton 2001, Bers and Ziolo 2001).

Phosphodiesterases (PDEs), the enzymes responsible for the hydrolysis of cAMP, appear to be critical for cAMP compartmentation (Jurievius and Fischmeister 1996, reviewed in Baillie et al. 2005). For example, in studies measuring L-type Ca2+ current (ICa,L) with two electrodes, the application of forskolin (a direct activator of AC) to a selected region of the cell membrane increased ICa,L throughout the cell, whereas local application of ISO only increased ICa,L locally. However, the same local application of ISO increased ICa,L throughout the entire cell when PDEs were inhibited. These results establish that PDEs restrict cAMP signaling and PKA-dependent phosphorylation in cardiomyocytes to local cellular compartments surrounding ß-ARs. In a separate study, mice with cardiac-specific overexpression of human AC type 8 showed increased cardiac contractility in association with elevated Ca2+ transients and accelerated relaxation but without alterations of the ICa,L amplitude (Georget et al. 2003). These compartmentation effects were linked to the reorganization of specific PDEs within cardiomyocytes (Georget et al. 2003). A more recent study has shown that PDE4D3 binds to RyR2 in adult cardiomyocytes and locally regulates cAMP levels at the Z-line region of the sarcomere (Lehnart et al. 2005), suggesting the PDE4 family may be important in cAMP compartmentation in SR regions containing the RyR2 proteins in cardiomyocytes.

Additional molecules that contribute to the compartmentation of cAMP signaling are anchoring proteins for PKA and phosphatases (Bers and Ziolo 2001, Baillie and Scott 2002, Wong and Scott 2004). For example, A-kinase anchoring proteins (AKAPs) play a critical role in the organization and localization of macromolecular complexes of signaling molecules in microdomains of cells. A-kinase anchoring proteins often bind directly to proteins that are targets for PKA-dependent phosphorylation. One such molecule is muscle-specific A-kinase anchoring protein (mAKAP), which dynamically associates with both PKA and PDE4D3 and functions to locally control PKA activity by controlling cAMP levels in microdomains (Baillie and Scott 2002, Wong and Scott 2004). In summary, protein–protein interactions between AKAPs, PKA, and PDEs (as well as other proteins) result in the formation of dynamic microdomains for spatial and temporal control of cAMP signaling in cardiomyocytes.

- **PI3Kγ and cAMP Compartmentation in Ventricular Cardiomyocytes**

Two separate mouse strains with targeted deletion of the PI3Kγ gene (PI3Kγ−/−) have been created (Hirsch et al. 2000, Sasaki et al. 2000). These transgenic mice are viable and fertile, with normal heart rates and normal mean arterial pressures (Crackower et al. 2002). PI3Kγ−/− mice show significantly enhanced cardiac function and increased cardiomyocyte contractility in association with elevated basal intracellular cAMP levels (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004,
Alloatti et al. 2005), but without alterations in PKB/Akt activity. Consistent with increased levels of cAMP, PI3K<sup>-/-</sup> mice display elevated phosphorylation of PLN, which correlates closely with the increases in cardiac contractility and lusitropy (Crackower et al. 2002, Patrucco et al. 2004). These data suggest that PI3K<sub>y</sub> negatively regulates cAMP levels. Remarkably, the cAMP changes observed in PI3K<sup>-/-</sup> mice were eliminated by overexpression of a kinase-dead PI3K<sub>y</sub> gene in the hearts of PI3K<sup>-/-</sup> mice, supporting the conclusion (although see below) that the inhibition of cAMP by PI3K<sub>y</sub> is not dependent on alterations in PIP<sub>2</sub>/PIP<sub>3</sub> levels (Patrucco et al. 2004).

We have demonstrated that cAMP/PKA activity is enhanced in subcellular compartments of PI3K<sup>-/-</sup> ventricular myocytes containing the SR, but not in the vicinity of the sarcolemma (Kerfant et al. 2005). Specifically, we found that cardiomyocytes lacking PI3K<sub>y</sub> have enhanced contractility due to increased SR Ca<sup>2+</sup> release (Figure 1) and SR Ca<sup>2+</sup> load, which were eliminated after PKA inhibition (Kerfant et al. 2005). These findings suggest that the effects of PI3K<sub>y</sub> deletion were related to PKA-dependent phosphorylation of PLN. Indeed, PLN phosphorylation is increased in PI3K<sup>-/-</sup> mice without obvious changes in the level of SERCA-2a or PLN expression (Crackower et al. 2002, Patrucco et al. 2004). Despite enhanced cAMP levels in PI3K<sup>-/-</sup> cardiomyocytes (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Alloatti et al. 2005), and although it has been shown that I<sub>Ca,L</sub> was modestly elevated (~20%) in another PI3K<sup>-/-</sup> mice model (Alloatti et al. 2005), we did not detect differences in I<sub>Ca,L</sub> amplitude between PI3K<sup>-/-</sup> and control cardiomyocytes under conditions where Ca<sup>2+</sup> transients were present (Figure 1). This was somewhat surprising because I<sub>Ca,L</sub> is profoundly regulated by cAMP-dependent PKA, which enhances the open channel probability resulting in larger I<sub>Ca,L</sub> density (Bers 2002). Although I<sub>Ca,L</sub> amplitude was unaffected by PI3K<sub>y</sub> ablation, the Ca<sup>2+</sup>-dependent phase of I<sub>Ca,L</sub> inactivation was accelerated in PI3K<sup>-/-</sup> cardiomyocytes, consistent with increased SR Ca<sup>2+</sup> release. Protein kinase A inhibition did not affect I<sub>Ca,L</sub> amplitude in PI3K<sup>-/-</sup> or control mice (Kerfant et al. 2005, Sun et al. 2006), but it did normalize the Ca<sup>2+</sup>-dependent inactivation phase of I<sub>Ca,L</sub> between PI3K<sup>-/-</sup> and wild-type mice.

- **Implications and Possible Mechanism(s) of PI3K<sub>y</sub> Regulation of cAMP**

Intracellular cAMP levels are highly regulated in heart and other tissues by the β-AR signaling pathways (Bers 2002).

**Figure 1.** Sarcoplasmic reticulum Ca<sup>2+</sup> release is elevated in a cAMP-dependent manner, whereas I<sub>Ca,L</sub> is unchanged in PI3K<sup>-/-</sup> cardiomyocytes. (A) Mean fluorescence-voltage relationship of Ca<sup>2+</sup> transients recorded in PI3K<sup>+/+</sup> (circles) and PI3K<sup>-/-</sup> (squares) cardiomyocytes before (open symbols) and after (closed symbols) cell dialysis with the cAMP antagonist Rp-cAMP (100 μM). After subtraction of the background fluorescence, the fluorescence signal (F) was normalized to the fluorescence signal before depolarization (F<sub>0</sub>) (adapted with permission from Circ Res. 2005;96:1079-1086). (B) Current-voltage relationship of mean I<sub>Ca,L</sub> densities recorded simultaneously with Ca<sup>2+</sup> transients (adapted with permission from Circ Res. 2005;96:1079-1086). Asterisk indicates a significant difference (P < 0.01) between genotypes; dagger, a significant difference between control and drug treatment within the same genotype. I<sub>Ca,L</sub> was elicited, simultaneously to Ca<sup>2+</sup> transient, by applying 100-millisecond voltage clamp steps between -50 and +60 mV (0.1 Hz) from a holding potential of -80 mV. A 500-millisecond voltage ramp to -50 mV was applied before the voltage steps to inactivate the Na<sup>+</sup> current (I<sub>Na</sub>). The superfusate contained the following (in mM): 140 NaCl, 0.5 MgCl<sub>2</sub>, 5 CsCl, 5.5 glucose, 5 HEPES, and 1.8 CaCl<sub>2</sub> (pH adjusted to 7.4 with NaOH); the pipette solution contained the following (in mM): 130 CsCl, 1 MgCl<sub>2</sub>, 1 Na<sub>2</sub>H<sub>2</sub>P<sub>4</sub>O<sub>7</sub>, 3.6 Na<sub>2</sub> phocreatine, 2 KCl, 5 MgATP, 0.05 fluo-3 pentapotassium salt, and 10 HEPES (pH adjusted to 7.2 with CsOH).
Previous studies have indicated that PI3K activity is enhanced by β2-ARs (Leblais et al. 2004) or β2-ARs (Chesley et al. 2000, Zhu et al. 2001, Jo et al. 2002) (both) as well as by AC activation (Leblais et al. 2004). Although it appears that PI3Kγ is not able to directly hydrolyze cAMP (Patrucco et al. 2004), genetic ablation of PI3Kγ clearly increases cAMP levels in cardiomyocytes (Crackower et al. 2002, Nienaber et al. 2003, Patrucco et al. 2004, Alloatti et al. 2005). Despite elevated cAMP levels in PI3Kγ-deficient cardiomyocytes, combined β1-AR and β2-AR stimulation with ISO increased I_{Ca,L}, and Ca^{2+} transients as well as SR Ca^{2+} release equally (~2-fold) in PI3Kγ+/− and control mice (Kerfant et al. 2005), although baseline Ca^{2+} transients were larger in PI3Kγ−/− mice. These findings demonstrate that, although cAMP levels and signaling are enhanced in the region of the SR (but not in sarcolemmal regions containing L-type Ca^{2+} channels) under baseline conditions in PI3Kγ−/− mice, β-AR stimulation can induce further increases in SR Ca^{2+} release and Ca^{2+} transients, possibly because of increases in I_{Ca,L}. Although a cAMP-dependent acceleration of Ca^{2+} transient relaxation was observed in PI3Kγ−/− compared with control myocytes under baseline conditions, the Ca^{2+} transient relaxation was further accelerated in PI3Kγ−/− myocytes after ISO application. This observation suggests that cAMP levels and cAMP-dependent signaling in the SR region are not saturated by the loss of PI3Kγ. Our results do, however, contrast somewhat with another study that used a different mouse strain reporting that ISO increases contractile force in papillary muscles to a greater extent in PI3Kγ−/− mice than in wild-type mice (Alloatti et al. 2005). The basis for the strain differences in PI3Kγ-deficient mice will require further investigation.

Although the findings discussed above establish direct modulation of cAMP levels by PI3Kγ under baseline conditions, inhibition of PI3Kγ can also influence cAMP-dependent signaling by β-AR and may contribute to the (poorly understood) differences in signaling between β1-ARs and β2-ARs. For example, activation of β2-ARs with zinterol increases cAMP levels to a greater extent in PI3Kγ−/− hearts compared with control hearts (Kerfant et al. 2005). Altered Gi-mediated by changes in the SR and the L-type Ca^{2+} channels is still unclear, as indicated by question marks. Genetic ablation of PI3Kγ results in increased intracellular local cAMP levels, enhanced phosphorylation of PLN, enhanced SERCA-2a activity (indicated by larger arrows), increased SR Ca^{2+} load (darker color), and decreased PDE and Gi (dashed circle) activities. In our PI3Kγ− knockout model, Ca^{2+} release from the SR is significantly enhanced without change in I_{Ca,L} levels (note arrow sizes in figure).
mice (PI3K<sub>KD/KD</sub>) to eliminate elevated contractility in mice lacking endogenous PI3K<sub>y</sub>, supported the conclusion that PI3K<sub>y</sub> activates PDE3B by direct protein–protein interactions (Marcantoni et al. 2006, Patrucco et al. 2004), independent of PIP<sub>2</sub>/PIP<sub>3</sub> levels. However, the regulation of cAMP and myocyte contractility by PI3K<sub>y</sub> may be more complex for several reasons. First, PDE3A is the most abundant PDE isoform in cardiomyocytes, whereas PDE3B is expressed almost exclusively in vascular smooth muscle in the heart (Beavo 1995, Movsesian 2002, Maurice et al. 2003). Second, in HEK293 cells transfected with PDE3B, coexpression of PI3K<sub>y</sub> subunits does not inhibit PDE3B activity (Voigt et al. 2006), implying that additional factors or proteins are required. Several candidate proteins that interact with PI3K<sub>y</sub> and/or PDEs, such as the PI3K<sub>y</sub> regulatory subunit P101 (Oudit et al. 2004) or AKAPs (Bauman and Scott 2002, Wong and Scott 2004), may be involved in mediating the inhibition of PDE3B activity by PI3K<sub>y</sub> in heart. One protein of particular interest is the novel regulatory subunit of PI3K<sub>y</sub> called “P87Pikap,” which physically interacts with both PDE3B and PI3K<sub>y</sub> and which is highly expressed in heart, although coexpression of PI3K<sub>y</sub> and P87Pikap with PDE3B did not highly inhibit PDE3 activity (Voigt et al. 2006). Third, elimination of the activity of phosphatase and tensin homologue deleted on chromosome 10 (a lipid phosphatase which counteracts PI3K<sub>y</sub>) causes a reduction in myocardial contractility that was reversed by simultaneous loss of PI3K<sub>y</sub>, supporting a role for kinase-dependent PIP<sub>3</sub> changes in the regulation of cAMP by PI3K<sub>y</sub> (Crackower et al. 2002). PIP<sub>2</sub>/PIP<sub>3</sub> regulation of cAMP levels and contraction in myocytes is also supported by the ability of PI3K inhibition, with wortmannin or LY 294002, to alter β<sub>1</sub>-AR and β<sub>2</sub>-AR signaling, as mentioned above. In addition, the activation of PI3K, as opposed to the mere physical presence of PI3K<sub>y</sub>, has been shown to stimulate PDE3B activity, thereby reducing cAMP in other tissues such as the hypothalamus (Sahu and Metlakunta 2005).

Overall, the mechanism for the regulation of cAMP by PI3K<sub>y</sub> seems complex (Figure 2). Further investigations are clearly needed to discriminate between kinase-dependent and kinase-independent actions of PI3K<sub>y</sub> on cAMP metabolism. It also remains unclear whether PI3K<sub>y</sub> regulates cAMP activity by activating the enzymatic activity of specific PDE isoforms (in both kinase-dependent and kinase-independent fashions) or whether PI3K<sub>y</sub> acts as a scaffold protein in a macromolecular complex to physically localize PDE(s) to appropriate specific microdomains in ventricular myocytes, possibly in conjunction with other proteins such as AKAPs. Some of these issues could conceivably be addressed by making use of the recently described selective PI3K<sub>y</sub> inhibitor, AS-604850 (Camps et al. 2005).

### Perspectives and Significance

A prominent feature of diseased myocardium is reduced Ca<sup>2+</sup> transient amplitude resulting from decreased SR Ca<sup>2+</sup> uptake without changes in I<sub>Ca,L</sub> density (Gwathmey et al. 1987, Beuckelmann et al. 1992, Gomez et al. 1997, Benitah et al. 2002, Bers et al. 2003). These observations suggest that alterations in the regulation of cAMP by PI3K<sub>y</sub> may contribute to the functional changes observed in heart disease. Consistent with this idea, PI3K<sub>y</sub> activity and expression is increased in cardiac disease (Naga Prasad et al. 2000, Patrucco et al. 2004), along with elevated activity of selected PDEs (Takahashi et al. 2002) and G<sub>i</sub> (Bohm et al. 1997). Thus, enhanced PI3K<sub>y</sub> effects could work in conjunction with reduced SERCA-2a expression (Neticadan et al. 2000) to decrease SR Ca<sup>2+</sup> uptake, thereby impairing Ca<sup>2+</sup> handling and contractility, as observed in heart disease. These observations suggest that reducing PI3K<sub>y</sub> levels or function may be beneficial in heart disease. Consistent with this, PI3K<sub>y</sub>−/− mice are protected from ISO-induced heart failure (Oudit et al. 2003). However, chronic pressure overload induced by transverse aortic constriction in PI3K<sub>y</sub>−/− mice leads to more extensive dysfunction and myocardial damage than in control mice (Patrucco et al. 2004), suggesting that the role of PI3K<sub>y</sub> may vary depending on the nature of disease and that elevated cAMP levels may be detrimental in the response of the heart to certain challenges. Future studies will clearly be required to more fully assess the role of PI3K<sub>y</sub> and local cAMP signaling in normal and diseased hearts.
• Acknowledgments

This study was supported by funding from the Canadian Institutes of Health Research (CIHR) to PHB, who is a Career Investigator with the Heart and Stroke Foundation (HSF) of Ontario. B-GK holds a postdoctoral fellowship from the HSF of Canada and the TACTICS-CIHR program at the University of Toronto. RAR holds postdoctoral fellowships from the HSF of Canada and the Alberta Heritage Foundation for Medical Research.

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PII S1050-1738(06)00073-9 TCM