Phosphoinositide 3-Kinase γ-Deficient Mice Are Protected From Isoproterenol-Induced Heart Failure

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Background—We have recently shown that genetic inactivation of phosphoinositide 3-kinase γ (PI3Kγ), the isoform linked to G-protein–coupled receptors, results in increased cardiac contractility with no effect on basal cell size. Signaling via the G-protein–coupled β-adrenergic receptors has been implicated in cardiac hypertrophy and heart failure, suggesting that PI3Kγ might play a role in the pathogenesis of heart disease.

Methods and Results—To determine the role for PI3Kγ in hypertrophy induced by G-protein–coupled receptors and cardiomyopathy, we infused isoproterenol, a β-adrenergic receptor agonist, into PI3Kγ-deficient mice. Compared with controls, isoproterenol infusion in PI3Kγ-deficient mice resulted in an attenuated cardiac hypertrophic response and markedly reduced interstitial fibrosis. Intriguingly, chronic β-adrenergic receptor stimulation triggered impaired heart functions in wild-type mice, whereas PI3Kγ-deficient mice retained their increased heart function and did not develop heart failure. The lack of PI3Kγ attenuated the activation of Akt/protein kinase B and extracellular signal-regulated kinase 1/2 signaling pathways in cardiac myocytes in response to isoproterenol. β1- and β2-adrenergic receptor densities were decreased by similar amounts in PI3Kγ-deficient and control mice, suggesting that PI3Kγ isoform plays no role in the downregulation of β-adrenergic receptors after chronic β-adrenergic stimulation.

Conclusions—Our data show that PI3Kγ is critical for the induction of hypertrophy, fibrosis, and cardiac dysfunction function in response to β-adrenergic receptor stimulation in vivo. Thus, PI3Kγ may represent a novel therapeutic target for the treatment of decreased cardiac function in heart failure. (Circulation. 2003;108:2147-2152.)

Key Words: heart failure ■ signal transduction ■ hypertrophy

Heart disease is frequently associated with elevated wall stress as a result of pressure overload, such as in hypertension or volume overload seen in valvular disorders. Increased wall stress in the heart triggers a hypertrophic response.1 What initially reflects a compensatory response turns into pathological hypertrophy, which eventually leads to cardiac decompensation and heart failure. The signaling molecules that regulate the hypertrophic response and cardiac function under pathologic conditions are not well understood.1

In flies and mammals, hypertrophic responses can be initiated via phosphoinositide 3-kinase (PI3K) signaling pathways.2,3 PI3Ks constitute a family of evolutionarily conserved lipid kinases that regulate a vast array of fundamental cellular responses, including proliferation, adhesion, cell size, and protection from apoptosis.4 Activated PI3K phosphorylates the 3’-position of phosphatidylinositol (PtdIns)(4,5)P2, leading to the generation of PtdIns(3,4,5)P3, which activates multiple downstream targets, including Akt/protein kinase B (PKB).4-6 Four different type I PI3Ks have been described, 3 of which (PI3Kα, β, and δ) (class IA) are activated by receptor tyrosine kinase pathways. In contrast, PI3Kγ is activated by the βγ subunit of G-proteins and acts downstream of G-protein–coupled receptors (GPCRs) (class 1B).3,4,7 The tumor suppressor PTEN (phosphatase and tensin homologue on chromosome ten) is a lipid phosphatase that dephosphorylates the D3 position of PtdIns(3,4,5)P3 and therefore antagonizes the activity of the PI3K isoforms in a wide range of cell types, including cardiomyocytes.3,8

Recent genetic evidence indicates that PI3Kγ is the sole PI3K that couples to GPCRs and its function can be uncoupled from the class IA PI3K isoforms.3,5,6 We have recently...
reported that mice deficient for p110γ, the catalytic subunit of PI3Kγ, display increased cardiac contractility but normal heart size via increased cAMP signaling. Moreover, we showed that cardiac hypertrophy and myocardial contractility can be genetically uncoupled. However, sustained adrenergic stimulation has been implicated in the development of pathological hypertrophy and progression of heart failure in animal models and humans, suggesting that PI3Kγ may play a role in agonist-induced cardiac hypertrophy and disease. Increased cardiac contractility seen in p110γ−/− hearts also suggests that loss of PI3Kγ function may protect from GPCR-induced heart failure. In the present study, we investigated the cardiac responses of the PI3Kγ-deficient mice to chronic β-adrenergic stimulation.

Methods

Mice
p110γ−/− mice have previously been described. Hypertrophy and heart failure were induced by chronic infusion of isoproterenol (Sigma) for 7 days at a dose of 15 mg/kg per d using Alzet osmotic mini-pumps. Pumps were removed 24 hours before echocardiography and hemodynamic measurements. All experiments were performed in accordance with institutional guidelines. Only littermate male mice of 10 to 12 weeks of age were used.

Heart Morphometry, TUNEL Assay, Echocardiography, and Hemodynamic Measurements
For heart morphometry, hearts were arrested with KCl, fixed with 10% buffered formalin, and embedded in paraffin. Myocardial interstitial fibrosis was determined as collagen volume fraction (PSR-stained sections) using color-subtractive computer-assisted image analysis (Image Processing Tool Kit version 2.5). In situ DNA fragmentation was labeled using the TUNEL assay (ApopTag Plus kit) (Oncor). After removal of the osmotic pumps for 24 hours, echocardiographic assessments and invasive hemodynamic measurements were carried out as previously described.

Signaling, mRNA, and Western Blot Analyses
Total RNA was prepared from hearts using Trizol, resolved on a 0.8% formamide gel, blotted to nylon membranes (Amersham), and

Figure 1. Signaling and cardiac hypertrophy responses in p110γ−/+ and p110γ−/− mice. A, Heart sections from p110γ−/+ and p110γ−/− mice treated with vehicle or isoproterenol for 7 days. B, Quantitation of heart to body weight ratio and heart weight to tibial length ratio in vehicle and isoproterenol-treated mice; n=9 per group. *P<0.05; **P<0.01 compared with vehicle. C, Northern blot analysis and quantitation of cardiac hypertrophy markers in p110γ−/+ and p110γ−/− mice treated with vehicle (1) or isoproterenol (2) for 7 days. GAPDH expression was used as a loading control. Baseline levels of the indicated markers were comparable between p110γ−/+ and p110γ−/− hearts. D, ERK1/2 phosphorylation in isolated p110γ−/+ and p110γ−/− neonatal cardiomyocytes. Cells were (1) left untreated or stimulated with (2) isoproterenol (10 μmol/L) and (3) bFGF (250 ng/mL) for 10 minutes. Western blot data are shown. E, Impaired Akt/PKB activation in total heart extracts from p110γ−/+ and p110γ−/− mice treated with vehicle or isoproterenol for 7 days; n=5 per group. *P<0.01 between all groups. Values are mean±SEM. MyHC indicates myosin heavy chain; ANF, atrial natriuretic factor; and BNP, brain natriuretic peptide.
probed with cDNA probes for atrial natriuretic factor, brain natriuretic peptide, α-myosin heavy chain, and GAPDH. Akt/PKB kinase activity was measured using a commercial in vitro kinase assay kit according to the manufacturer’s protocol (Upstate Biotechnologies). We therefore stimulated purified ventricular cardiomyocytes from p110γ−/− mice (Figure 2D). Cardiac hypertrophy is confirmed impaired cardiomyocyte hypertrophy in p110γ−/− mice (Figure 2D). Cardiac hypertrophy is associated with prototypical alterations in gene expression. We therefore assessed the mRNA levels of hypertrophy markers in hearts of p110γ−/− and p110γ−/− mice chronically stimulated with isoproterenol. Intriguingly, despite the attenuation of hypertrophy in p110γ−/− hearts, we observed a similar alteration in expression profiles in atrial natriuretic factor, brain natriuretic peptide, and α-myosin heavy chain compared with p110γ−/− hearts (Figure 1C).

It is unclear whether PI3Kγ is essential for GPCR signaling in cardiomyocytes. We therefore stimulated purified ventricular cardiomyocytes from p110γ−/− and p110γ−/− littersmates with the GPCR-agonist isoproterenol or the tyrosine kinase–based receptor agonist basic fibroblast growth factor (bFGF) (Figure 1D). Whereas bFGF-induced ERK1/2 activation occurred normally, isoproterenol-induced ERK1/2 phosphorylation was completely abolished in p110γ−/− cardiomyocytes (Figure 1D). Similarly, in p110γ−/− hearts, chronic isoproterenol infusion did not increase endogenous Akt/PKB activity (Figure 1E).
Isoproterenol Stimulation Leads to Reduced Interstitial Fibrosis in p110γ-Deficient Mice

Decompensation of the hypertrophic myocardium and the progression to cardiomyopathy is accompanied by increased fibrosis and disorganization of myocytes. We therefore carried out histological analysis to study the cardiac remodeling response. In control hearts (p110γ+/−), treatment with isoproterenol resulted in disorganization of the myocytes and a marked increase in cardiac fibrosis (Figure 2A). Quantification of interstitial fibrosis showed a significant increase in collagen deposition in the hearts of isoproterenol-treated control mice (Figure 2B). By contrast, p110γ−/− mice displayed no significant increase in cardiac fibrosis or alteration in myocyte organization (Figures 2A and 2B). Cardiomyocyte cell death was comparable between isoproterenol-treated p110γ−/− and p110γ+/− mice (Figure 2C).

Isoproterenol-Induced Cardiovascular Dysfunction Is Reduced in p110γ-Deficient Mice

Given the marked differences in signaling and hypertrophic and fibrotic responses, we studied the functional changes in heart function in mice infused with isoproterenol after withdrawal of the drug. Chronic infusion of isoproterenol into p110γ−/− mice led to a significant reduction in heart function with decreased fractional shortening, velocity of circumferential fiber shortening, and peak aortic velocity (Table). Invasive hemodynamic measurements showed increased left ventricular end-diastolic pressure and decreases in ±dP/dtmax and blood pressure in p110γ−/− animals (Table), again indicating severe impairment of heart function. By contrast, chronic isoproterenol stimulation of p110γ−/− mice caused a markedly smaller reduction in cardiac function and blood pressures. Moreover, these hearts retained an increased pump function relative to untreated control hearts (Table).
Lack of Differential Downregulation of β-Adrenergic Receptors in p110γ-Deficient Mice

PI3K signaling has been proposed to mediate β-adrenergic downregulation after agonist stimulation. β1- and β2-adrenergic receptors were downregulated by ~75% and ~50%, respectively, in membrane fractions from p110γ+/− hearts (ventricular tissue) from mice infused with isoproterenol compared with vehicle-treated mice (Figures 3A and 3B). Importantly, there were no differential changes in β1- or β2-adrenergic receptor expression in membrane fractions from p110γ+/− and p110γ+/− hearts (ventricular tissue) at baseline and in response to chronic in vivo isoproterenol stimulation (Figures 3A and 3B). Expression levels of the major cardiac adenylate cyclase isoforms V and VI were also comparable between p110γ+/− and p110γ+/− hearts and were not affected by chronic β-adrenergic activation (Figures 3A and 3B).

Discussion

Excessive catecholamines and adrenergic stimulation have been clearly linked to cardiac hypertrophy and disease. Indeed, altered signaling via the G-protein–coupled β-adrenergic receptors has been implicated in heart failure in animal models and humans. Although the increased protein expression of PTEN in response to chronic isoproterenol stimulation may serve to negatively regulate the activity of PI3K, the precise role of PI3Kγ isoform in heart failure remains unresolved. We have shown previously that loss of p110γ leads to an increase in cardiomyocyte contractility. Our present data show that PI3Kγ, the PI3K-isoform linked to GPCRs, is critical for the induction of myocardial hypertrophy, interstitial fibrosis, and cardiac dysfunction in response to β-adrenergic receptor stimulation in vivo. Importantly, p110γ+/− mice display a resistance to the effects of isoproterenol on cardiac structure and function. Because it has been suggested that an increase in cardiac function may serve to improve myocardial performance in models of dilated cardiomyopathy, we confirmed that p110γ+/− hearts maintained their hypercontractility after chronic adrenergic stimulation. However, the induction of several hypertrophy markers in response to isoproterenol was not affected by the loss of p110γ, which suggests that p110γ is not required for alteration in fetal gene expression seen in myocardial hypertrophy and that these genetic changes can be uncoupled from the hypertrophic process.

Our data show that chronic activation of β-adrenergic signaling results in markedly decreased heart function and the onset of cardiomyopathy in p110γ+/− mice. In contrast, loss of p110γ renders animals resistant to increased interstitial fibrosis and cardiac dysfunction. Akt/protein kinase B (PKB), a major molecular target of the PI3K signaling pathway, is involved in cell hypertrophy in multiple systems, and the
mitogen-activated protein kinase ERK1/2 also plays a central role in cardiac hypertrophy. Genetic evidence in hematopoietic cells shows that PI3Kγ is the sole PI3K that couples to GPCRs. Importantly, infusion of isoproterenol into p110γ−/− mice showed that β-adrenergic receptor stimulation induced in vivo activation of Akt/PKB and treatment of isolated cardiomyocytes with isoproterenol leads to activation of ERK1/2. These genetic data demonstrate for the first time that after GPCR stimulation, PI3Kγ is the essential PI3K isoform required for Akt/PKB and ERK1/2 activation in cardiomyocytes, which is consistent with our data in hematopoietic cells. In other models of heart disease (pressure-overload hypertrophy), there is also activation of Akt/PKB via the PI3Kγ isoform. As such, whereas the PI3Kγ isoform plays no role in the control of basal (physiological growth) Akt/PKB levels, under GPCR stimulation, there is clearly activation of Akt/PKB via PI3Kγ, possibly via the interaction with Gβγ subunits. Consistent with our recent observations, the intact stimulation of ERK1/2 by the tyrosine receptor agonist bFGF in p110γ−/− cardiomyocytes confirms that the activity of PI3K class Iγ isoforms (PI3Kα, β, and δ) can be genetically uncoupled from the class Iγ isoform, PI3Kγ. Despite the lack of activation of Akt/PKB and ERK1/2 pathways, which are clearly antiapoptotic pathways, apoptosis was not increased in the p110γ−/− mice, which is likely attributable to the antiapoptotic effects mediated by enhanced β-adrenergic receptor signaling in p110γ−/− cardiomyocytes.

Because PI3Kγ also has a role in GPCR signaling in isolated cardiac fibroblasts, it is possible that the reduced interstitial fibrosis in the heart is the direct consequence of attenuated β-adrenergic receptor–mediated stimulation of cardiac fibroblasts in the p110γ−/− hearts. Recently published reports suggest that PI3Kγ might be involved in β-adrenergic receptor downregulation (internalization). However, the loss of PI3Kγ has no apparent effect on the downregulation of β-adrenergic receptors in response to chronic adrenergic stimulation. However, we cannot rule out the role of other PI3K isoforms in the downregulation of β-adrenergic receptors, and there exists the distinct possibility that receptor desensitization could be differentially affected, ie, functional responses are different. In summary, we have shown that the PI3Kγ has an important role to maintain heart function under the pathologic condition of chronic adrenergic stimulation, and our results represent the first genetic evidence for a potential therapeutic role of the modulation of PI3Kγ signaling in heart failure. The role of PI3Kγ inhibition in other genetic and acquired models of heart failure and the mechanisms of cardiac protection such as the role of PI3Kγ in intracellular Ca2+ regulation (eg, phosphorylation state of phospholamban) and the reduction in interstitial fibrosis need be further evaluated.

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