Phosphatidylinositol 3-Kinase Offsets cAMP-Mediated Positive Inotropic Effect via Inhibiting Ca\(^{2+}\) Influx in Cardiomyocytes


Abstract—Phosphoinositide 3-kinase (PI3K) has been implicated in β\(_2\)-adrenergic receptor (β\(_2\)-AR)/G\(_i\)-mediated compartmentation of the concurrent G\(_s\)-cAMP signaling, negating β\(_2\)-AR-induced phospholamban phosphorylation and the positive inotropic and lusitropic responses in cardiomyocytes. However, it is unclear whether PI3K crosstalks with the β\(_1\)-AR signal transduction, and even more generally, with the cAMP/PKA pathway. In this study, we show that selective β\(_1\)-AR stimulation markedly increases PI3K activity in adult rat cardiomyocytes. Inhibition of PI3K by LY294002 significantly enhances β\(_1\)-AR-induced increases in L-type Ca\(^{2+}\) currents, intracellular Ca\(^{2+}\) transients, and myocyte contractility, without altering the receptor-mediated phosphorylation of phospholamban. The LY294002 potentiating effects are completely prevented by βARK-ct, a peptide inhibitor of β-adrenergic receptor kinase-1 (βARK1) as well as G\(_{βγ}\) signaling, but not by disrupting G\(_i\) function with pertussis toxin. Moreover, forskolin, an adenyl cyclase activator, also elevates PI3K activity and inhibition of PI3K enhances forskolin-induced contractile response in a βARK-ct sensitive manner. In contrast, PI3K inhibition affects neither the basal contractility nor high extracellular Ca\(^{2+}\)-induced increase in myocyte contraction. These results suggest that β\(_1\)-AR stimulation activates PI3K via a PKA-dependent mechanism, and that G\(_{βγ}\) and the subsequent activation of βARK1 are critically involved in the PKA-induced PI3K signaling which, in turn, negates cAMP-induced positive inotropic effect via inhibiting sarcolemmal Ca\(^{2+}\) influx and the subsequent increase in intracellular Ca\(^{2+}\) transients, without altering the receptor-mediated phospholamban phosphorylation, in intact cardiomyocytes. (Circ Res. 2004;95:1183-1190.)

Key Words: PI3K ■ PKA ■ cardiac contractility ■ L-type calcium current ■ β\(_1\)-adrenergic receptor

PI3K family has been implicated in multiple vital cellular functions, including cell survival, cell proliferation, cytoskeletal remodeling, and vesicle trafficking. Based on their structure and substrate specificity, PI3Ks are divided into three classes (I, II, and III). The most well characterized class I PI3Ks can be further categorized into two subgroups, IA and IB. Activation of class IA PI3Ks is controlled by stimulation of various receptors with intrinsic or associated tyrosine kinase activity, and class IB (also known as PI3Kγ) by activation of G protein-coupled receptors (GPCRs) via G\(_{βγ}\) subunits, although some exceptions have been reported.4,4

Increasing evidence suggests that PI3Ks are involved in cardiac β\(_2\)-adrenergic receptor (β\(_2\)-AR) signaling. In rodent cardiac myocytes, β\(_2\)-AR stimulation delivers an antiapoptotic

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Materials and Methods

Cell Contraction, Intracellular Ca\(^{2+}\) Transient, and I\(_C\) Measurements

Ventricular cardiac myocytes were isolated from 2- to 4-month-old Sprague-Dawley rats, using a standard enzymatic technique.\(^9\) Cell contraction was indexed by the percent shortening of cell length after electrical stimulation at 0.5 Hz at 23°C, as previously described.\(^8\) In some experiments, cells were treated with 1.5 μg/ml PTX (Sigma Chemical Co) for 3 hours at 37°C.\(^9\) In another set of experiments, myocytes were loaded with a fluorescent Ca\(^{2+}\) probe, indol3-acetoxymethyl ester (Molecular Probes), to measure intracellular Ca\(^{2+}\) (Ca\(_t\)) transient, as described previously.\(^8\)

I\(_C\) was measured via the whole-cell patch clamp technique using an Axopatch 1D amplifier (Axon Instruments Inc) in freshly isolated rat ventricular myocytes, as previously described.\(^9\) Briefly, cells were voltage-clamped at −40 mV to inactivate the sodium and T-type calcium channels. Potassium currents were eliminated by using K\(^+\) free solutions with tetraethylammonium (TEA). The patch pipette (1.4–1.8 MΩ) was filled with pipette solution containing (in mmol/L) NaCl 140, CaCl\(_2\) 1.8, CsCl 1.8, CsCl 5.4, MgCl\(_2\) 2, and HEPES 5 (pH 7.3, adjusted with CsOH), whereas myocytes were perfused with bath solution containing (in mmol/L) NaCl 140, CaCl\(_2\) 1.8, CsCl 1.8, MgCl\(_2\) 5, and HEPES 5 (pH 7.3, adjusted with NaOH).

Culture and Adenoviral Infection of Adult Rat Ventricular Myocytes

Myocytes were cultured and infected with adenovirus-βARK-ct (an adenovirus vector carrying a gene encoding β-AR kinase1 carboxyl-terminal fragment) or adenovirus-β-gal (an adenovirus vector with a reporter gene lacZ, as a negative control), both at multiplicity of infection of 100. All experiments were performed after 24 hours of adenoviral infection.

PI3K Activity Assay

Suspensions of cardiac myocytes were first incubated with appropriate pharmacological agents. Measurement of PI3K activity was then performed, as previously described.\(^5,10\)

PKA-Dependent Phosphorylation of Phospholamban at Ser\(^{16}\)

PKA-dependent phosphorylation of PLB at Ser\(^{16}\) was assayed as described previously.\(^11,12\)

Materials

Isoproterenol (ISO), norepinephrine (NE), prazosin, forskolin (FSK), insulin-like growth factor-1 (IGF-1), and ICI 118,551 (ICI) were purchased from Sigma Chemical Co and LY294002 from Calbiochem.

Statistical Evaluations

All data are presented as mean±SE of n number of experiments. Unpaired or paired Student t test, or ANOVA were used for statistical comparisons when appropriate, and differences were considered significant at P<0.05.

Results

\(β_1\)-AR Stimulation Increases PI3K Activity in Freshly Isolated Adult Rat Ventricular Myocytes

We first examined the effect of \(β_1\)-AR stimulation on PI3K activity in adult rat ventricular myocytes, using a lipid kinase assay. As expected, IGF-1 stimulation, a known PI3K activator,\(^13,14\) elevated PI3K activity in a LY294002 (a PI3K inhibitor)-sensitive manner (Figure 1). Selective \(β_1\)-AR stimulation by NE (5×10\(^{-7}\) mol/L), in the presence of an \(α_1\)-AR antagonist prazosin (10\(^{-5}\) mol/L) and a \(β_1\)-AR antagonist ICI 118,551 (10\(^{-7}\) mol/L), also markedly increased PI3K activity, similar to nonselective \(β_1\)-AR stimulation with ISO (10\(^{-6}\) mol/L) (Figure 1). Interestingly, FSK (10\(^{-8}\) mol/L), an AC activator, also potently augmented PI3K activity to an extent similar to that induced by \(β_1\)-AR activation (Figure 1). These results indicate that activation of PKA or \(β_1\)-AR stimulation augments PI3K activity in intact cardiomyocytes.

PI3K Signaling Negatively Regulates \(β_1\)-AR-Mediated Positive Contractile Response

We next determined whether activation of PI3Ks modulates \(β_1\)-AR-mediated contractile response. Although inhibition of PI3K by LY294002 (5.10\(^{-7}\) mol/L) had only a minor effect on baseline contraction (107.8±1.4% of control, n=50 cells from 10 hearts; also see Figures 2 and 3), it clearly enhanced the contractile response (Figure 2A and 2B). The potentiating effect of LY294002 occurred regardless of the experimental protocol. When LY294002 was applied after ISO-mediated contractile response was stabilized, it further increased contraction amplitude from 164.9±12.42% to 208.0±16.63% of baseline (n=8, P<0.001; Figure 2C). The effect of LY294002 appeared rapidly and was completely reversible on washout. Likewise, in cells pretreated with LY294002 for 10 minutes, ISO increased the contraction amplitude to 188.87±18.77% of baseline (n=8, P<0.001; Figure 2D), which was comparable to the response induced by ISO plus LY294002 (Figure 2C). Another PI3K inhibitor, wortmannin also similarly enhanced ISO-mediated contractile response in adult rat cardiomyocytes (data not shown).
Because stimulation of $\beta_1$-AR serves as the most predominant mechanism in regulating cardiac contractility in response to stress or exercise, we next measured the contractile response to selective $\beta_1$-AR stimulation by NE ($5 \times 10^{-8}$ mol/L, a concentration close to the EC$_{50}$ plus prazosin at $10^{-5}$ mol/L and ICI 118,551 at $10^{-7}$ mol/L), in the presence or absence of the PI3K inhibitor. LY294002 administrated after $\beta_1$-AR stimulation further enhanced NE-induced contractile response (Figure 3A). Similar to the situation of mixed $\beta$-AR stimulation with ISO, pretreatment of cells with LY294002 also significantly augmented NE-induced positive inotropic effect (Figure 3B). In both cases, the potentiating effects of LY294002 were completely reversible on washout (Figure 3A and 3B). As illustrated in Figure 3C, the whole concentration-response course of NE to increase cell contraction amplitude was markedly shifted leftward by LY294002 treatment. These results indicate that $\beta_1$-AR–mediated positive contractile response is negatively regulated by the receptor-activated PI3K signaling.

Surprisingly, the PI3K inhibitor augmented $\beta_1$-AR–positive inotropic effect without enhancing $\beta_1$-AR–mediated relaxant effect. In the absence and presence of LY294002,
NE-induced abbreviation of 50% and 90% relaxation times (T_{50} and T_{90}, respectively) were not significantly different (T_{50}: 82.8±3.3% versus 84.7±6.1% of baseline; T_{90}: 75.7±4.2% versus 78.8±4.7%). The inability of LY294002 to enhance NE-induced lusitropic effect is not attributable to the β_{1}-AR lusitropic effect already reaching the maximum, because NE at 10^{-6} mol/L caused a significantly greater abbreviation in T_{50} (74.1±6.5% versus 84.7±6.1%; n=5 to 8 cells; P<0.05).

**Negative Inotropic Effect of PI3K Requires Activation of the cAMP/PKA Signaling**

To define the potential target of PI3K in the β_{1}-AR signaling cascade, we evaluated the effect of PI3K inhibition on the contractile response to postreceptor manipulations: activation of AC by FSK (5×10^{-7} mol/L) or increasing intracellular Ca^{2+} by elevating extracellular Ca^{2+} concentration from 1.0 to 1.5 mmol/L (high extracellular [Ca^{2+}]). Although both treatments increased the contraction amplitude to a comparable extent, LY294002 selectively enhanced the FSK-induced response, without affecting the high extracellular [Ca^{2+}]-induced increase in myocyte contractility (Figure 4A and 4B). LY294002 also exhibited a moderate, but significant, potentiation effect on the contractile response to CPT-cAMP, an active cAMP analogue (data not shown). These results indicate that modulation of cardiomyocyte contractility by PI3K signaling requires the activation of the cAMP/PKA pathway. In addition, as is the case for β_{1}-AR stimulation, inhibition of PI3K augmented FSK-induced positive inotropic effect, but not its lusitropic effect (data not shown).

**PI3K Inhibition Does Not Affect β_{1}-AR Induced Phosphorylation of Phospholamban at Ser^{16}**

PLB, a primary regulator of the sarcoplasmic reticulum Ca^{2+}-pump, can be phosphorylated at two adjacent sites, Ser^{16} and Thr^{17}, by PKA and Ca^{2+}/calmodulin-dependent kinase II, respectively, resulting in positive inotropic and lusitropic effects. We found no detectable effect of the PI3K inhibitor, LY294002, on β_{1}-AR–induced phosphorylation of PLB at Ser^{16} (Figure 5A and 5B). The concentration-response curves of NE-induced increase in PLB phosphorylation in the absence and presence of the PI3K inhibitor virtually overlapped with each other. This result is consistent with the inability of LY294002 to augment β_{1}-AR–mediated relaxant effect.

**Enhanced Contractile Response By PI3K Inhibition Is Associated With Increased Responses of I_{Ca} and Intracellular Ca^{2+} Transient to β_{1}-AR stimulation**

Because L-type Ca^{2+} current (I_{Ca}) is the key factor of cardiac excitation-contraction coupling, we next determined whether it is involved in the inhibitory effect of PI3K on β_{1}-AR contractile response. Similar to the contractile response, I_{Ca} response to β_{1}-AR stimulation by NE (5×10^{-3} mol/L, in the absence and presence of the PI3K inhibitor virtually overlapped with each other. This result is consistent with the inability of LY294002 to augment β_{1}-AR–mediated relaxant effect.
presence of prazosin and ICI 118,551) was clearly augmented by the PI3K inhibitor, LY294002 (Figure 6A and 6B). On average, LY294002 enhanced the β1-AR–stimulated current by 22.4±3.3% (n=9 cells; P<0.01). These results suggest that PI3K signaling offsets β1-AR positive inotropic effect likely via inhibiting β1-AR–induced Ca2+ influx through L-type Ca2+ channels. Notably, inhibition of PI3K exhibits a very minor effect on β1-AR–induced increase in ICa (data not shown).

To further delineate the mechanism underlying the potentiating effect of LY294002, myocyte contraction and Ca2+ transient were simultaneously measured in indo1-loaded myocytes. Both parameters were examined in response to positive inotropic agents at a concentration close to EC50, including ISO (10-9 mol/L), NE (5×10^{-8} mol/L, plus prazosin and ICI 118,551), or FSK (5×10^{-7} mol/L), in the presence or absence of LY294002 (5×10^{-6} mol/L). As shown in Figure 7, the LY294002-induced increase in contraction amplitude in response to the aforementioned agonists was accompanied by a proportional increase in Ca2+ transient, without altering its baseline (data not shown). These results indicate that PI3K antiadrenergic effect is mainly mediated by blunting β1-AR/PKA–dependent increase in ICa, and the consequent augmentation in the Ca2+ transient, instead of altering myofilament response to intracellular Ca2+. This conclusion is further supported by the fact that LY294002 could not enhance the high extracellular [Ca2+]–induced positive inotropic effect (Figure 4).

**ARK1 As Well As Gβγ Signaling Is Involved in β1-AR–Mediated PI3K Activation**

Disruption of Gβγ signaling with PTX cannot block the potentiating effect of LY294002 on β1-AR–stimulated contraction response (Figure 8A), indicating that Gβγ is not involved in β1-AR–mediated PI3K activation. In contrast, infection of cells with an adenovirus expressing βARK-ct (a peptide inhibitor of βARK1)17 as well as Gβγ, signaling20) completely abolished the potentiating effect of the PI3K inhibitor (Figure 8B). In this subset of experiments, a lower concentration (10^{-7} mol/L) of NE was chosen to avoid possible saturation of cell contractile response. These data suggest that either Gβγ, dissociated from Gβ, or βARK1 plays an essential role in β1-AR–induced PI3K signaling. To distinguish these two possibilities, we next examined the possible effects of LY294002 on FSK-induced contractile response in the presence or absence of βARK-ct, and found that the potentiating effect of LY294002 on FSK response was also abolished by βARK-ct (Figure 8B), indicating that βARK1 is required for the function of PI3K in inhibiting cAMP/PKA-mediated contractile response in cardiomyocytes.

**Figure 6. Potentiating effect of LY294002 on β1-AR–induced increase in ICa.** A, Representative example of an additional significant increase of ICa amplitude (circles, time course) produced by LY294002 (LY, 5×10^{-6} mol/L) on the top of β1-AR stimulation by NE (5×10^{-8} mol/L, in the presence of prazosin at 10^{-6} mol/L and ICI 118,551 at 10^{-7} mol/L). B, Selected original ICa traces from the same experiment shown in A (indicated by arrows for Control, NE+ICI, and NE+ICI+LY, respectively).

**Figure 7. Potentiating effect of LY294002 on contraction is associated with an increase in intracellular Ca2+ (Ca) transient.** Average effect of LY (5×10^{-6} mol/L) on the contractile (A) and the Ca transient (B) responses to mixed β1-AR stimulation by ISO (10^{-7} mol/L), selective β1-AR stimulation by NE (5×10^{-6} mol/L plus prazosin 10^{-5} mol/L and ICI 118,551 10^{-7} mol/L), or direct AC activation by FSK (5×10^{-7} mol/L). Data, expressed in percentage of baseline value, are mean±SE (n=7 to 11 cells for each group). *P<0.05, †P<0.01, ‡P<0.001 vs no LY. Baseline contraction amplitude is 5.75±0.46% (n=33 cells), whereas the basal Ca transient is 0.161±0.008 (n=33 cells).
Discussion

Both cAMP- and Gβγ-BARK1-Dependent Signaling Pathways Are Likely Involved in β1-AR–Induced PI3K Activation

The present study provides biochemical and physiological evidence that β1-AR is able to increase PI3K activity, which in turn, evokes a negative regulation of the receptor-mediated increases in Ica and Ca, transients, thus offsetting the receptor-induced positive inotropic effect. Disruption of Gs signaling by PTX cannot block the potentiating effect of LY294002 on β1-AR contractile response. In contrast, BARK-ct blocks the PI3K inhibitor–induced potentiating effect, indicating that either BARK1 or the βγ heterodimer dissociated from Gs plays an essential role in PI3K-mediated negative regulation of β1-AR contractile response. Interestingly, we have found that BARK-ct fully abolishes the potentiating effect of LY294002 on FSK-mediated contractile response. These results indicate that BARK1 is important for the normal functionality of PI3K, and plays an essential role in PI3K-mediated inhibition of cAMP-, as well as β1-AR-, mediated positive contractile response. This conclusion is based on following lines of evidence. BARK-ct, a well-characterized inhibitor of BARK1 in addition to blocking Gβγ signaling, fully blocks the ability of LY294002 to enhance the contractile response to FSK. Because FSK activates the cAMP-PKA pathway via stimulation of AC in the absence of increased free Gβγ, the basal βARK1 activation might be required for the function of PI3K in inhibiting cAMP/PKA-mediated contractile response in cardiomyocytes. This possibility is corroborated by the fact that PI3K and BARK1 can form a tight physical complex in cardiac myocytes.

Because Gβγ signaling is necessary for BARK1 activation, the present results cannot rule out the possible involvement of Gβγ in β1-AR–induced activation of PI3K. In this regard, previous studies have shown that the class IB PI3K (PI3Kγ isoform) is a downstream target of Gβγ signaling. Recent studies, however, suggest that stimulation of β-AR activates PI3Kα and β in cultured neonatal rat cardiac myocytes. Regardless of the specific isoform of PI3Ks responding to β1-AR stimulation, the present results indicate that β1-AR stimulation markedly increases PI3K activation via a signaling mechanism involving Gβγ, and Gβγ-activated BARK1 in cardiomyocytes.

In addition to increased free Gβγ subunits and the subsequent activation of BARK1, the Gαρ-AC-cAMP-PKA signaling cascade may also contribute to β1-AR–induced PI3K activation, because direct activation of AC by FSK induces a robust increase in PI3K activity and because inhibition of PI3K selectively enhances the positive inotropic effect induced by FSK but not by high extracellular [Ca2+]. Thus, it is likely that the Gαρ-AC-cAMP-PKA, the free Gβγ, and BARK1 participate in the receptor-evoked PI3K signaling, which leads to negative regulation of β1-AR contractile response in cardiomyocytes.

PI3Ks Activated by β1-AR Stimulation Differ From That Activated by β2-AR Stimulation in Regulating Cardiac Excitation-Contraction Machinery and Myocyte Survival

Accumulating evidence indicates that coexisting cardiac β-AR subtypes, mainly β1-AR and β2-AR, activate different signaling cascades with β1-AR coupling to the classic Gs-PKA pathway and β2-AR to concomitant Gq and G12,13 cascades. The coupling of β2-AR to Gq proteins compartmentalizes the Gq-mediated cAMP signaling, resulting in specific modulation of L-type Ca2+ channels without affecting other intracellular regulatory proteins. Inhibition of PI3K, similar to FSK treatment, enables β2-AR stimulation to induce a marked increase in phosphorylation of PLB at the PKA site, Ser16, thus enabling β2-AR stimulation to cause a de novo relaxant response and a markedly enhanced positive inotropic response in adult rat cardiomyocytes. Thus, PI3K is a key downstream event of acute β2-AR/Gq, signaling that negates the Gq-mediated cAMP signaling.

Notably, PI3Ks activated by β1-AR stimulation distinctly differ from that activated by β2-AR stimulation in many important ways. β1-AR–evoked PI3K signaling is unable to suppress the receptor-induced PLB phosphorylation. To the contrary, β1-AR– but not β2-AR–activated PI3Ks offset the receptor-mediated increase in Ica. These results underscore that PI3Ks following different β-AR subtype stimulation exhibit different selectivity for target proteins, although both β-AR subtypes increase total PI3K activity to a similar extent and their positive inotropic effects are negatively regulated by PI3K signaling.

In addition to regulating cardiac excitation-contraction coupling, β1-AR and β2-AR cause opposing effects on cardiomyocyte survival and death. Whereas β1-AR protects myocytes against apoptosis, β2-AR promotes myocyte apoptotic death. These contrasting effects of β-AR subtypes were initially explained by the β1-AR–coupled Gq/11-activated PI3K signaling pathway. However, the present study has documented that β2-AR stimulation is equally efficient in activating PI3Ks in these cardiomyocytes. It awaits further investigation to determine why β1-AR–activated PI3K signaling, unlike that of β2-AR, does not protect cardiomyocytes.

The exact mechanism underlying the differences between β1-AR– and β2-AR–activated PI3Ks in their target protein selectivity and in regulating myocyte survival and death remains largely elusive. Several candidate mechanisms might be involved. First, β1-AR and β2-AR might stimulate different PI3K isoforms. In this regard, it has been shown that different PI3K isoforms exhibit distinct functional roles. Second, the differential interaction of β-AR subtypes with PI3Ks might be, in part, attributed to the distinct subcellular distribution of these β-AR subtypes, therefore accessing distinct downstream signaling pathways. In addition, stimulation of β2-AR, but not β1-AR, activates PI3Ks in a PTX-sensitive manner. Thus, distinct G protein coupling and subcellular localization of β1-AR and β2-AR may render the subtype-specific β-AR/PI3K interaction in regulating cardiac excitation-contraction coupling and cell survival.
Physiological and Pathophysiological Relevance

In many biological systems, stimulation of a GPCR by agonists rapidly blunt the receptor signaling efficiency (receptor desensitization). It has been well established that βARK1 plays an essential role in agonist-initiated β-AR desensitization by phosphorylation of activated β-ARs both in vitro and in vivo.17,32 Because PI3K is able to physically associate with βARK1 and promotes agonist-dependent β-AR internalization,19 activation of PI3K may be involved in agonist-dependent β-AR desensitization. The inhibitory effect of PI3K on the β-AR-mediated contractile response may explain, at least in part, agonist-dependent desensitization of the receptor. Indeed, the present results indicate that PI3K is a key downstream event of acute β-AR–Gₛ-PLCβ₂ signaling and subsequent βARK1 activation that negates the receptor-mediated cAMP signaling in terms of changes in cardiac \( I_{Ca} \) and contractility.

β-AR signaling can be modulated by other GPCRs expressed in cardiac sarcolemmal membranes, including PTX-sensitive Gₛ-coupled adenosine receptors,33 acetylcholine receptors,34 or opioid receptors.35,36 For example, activation of M₂ acetylcholine receptors increases PI3K activity in rat neonatal myocytes.5 The counteraction of PI3K signaling on β-AR–mediated contractile response raises an important question whether PI3K signaling plays a role in the antiadrenergic effect of the aforementioned PTX-sensitive GPCRs. Our data have further revealed that βARK1 activation plays an essential role in PKA-evoked PI3K signaling. These unexpected findings suggest that PKA might elicit βARK1 activation, or alternatively, the basal activation of βARK1 is necessary for the function of PI3K in inhibiting PKA-mediated contractile response. Altogether, these observations imply that PI3K activation may be generally involved in regulating multiple signal transduction cascades in cardiomyocytes, perhaps in many other cell types as well, and that the present study may only demonstrate some important facets of PI3K-mediated regulation of cardiac performance.

The interaction between PI3K and β-AR-Gₛ-PKA in regulating cardiomyocyte \( I_{Ca} \) and contractility may also have important pathophysiological relevance. In this regard, it has been shown that enhanced PI3K activity plays essential roles in pressure overload-induced cardiac hypertrophy,37 β-AR–mediated hypertrophy, and hypertrophy marker gene expression.23,38 In light of its role in pathological cardiac hypertrophy and its negative inotropic effect, exaggerated PI3K activation might be implicated in the overall process of cardiac remodeling and chronic heart failure. This perception has been corroborated by the recent notion that disruption of the association of PI3K to β-AR prevents catecholamine-induced β-AR downregulation and ameliorates the development of heart failure in response to pressure overload.39 Thus, the inhibitory effect of PI3K on β-AR–mediated positive inotropic response contributes to the pathogenesis of heart failure, and inhibition of the interaction of the receptor and PI3K might open a novel therapeutic avenue to restore β-AR signaling and improves the function of the failing heart. Additionally, recent studies have suggested a heart failure-associated upregulation of β-ARs and alteration of its negative inotropic effect.40,41 It merits future investigation to determine the potential role of PI3K signaling in β-AR–mediated negative inotropic effect, particularly in the failing heart.

In summary, β-AR stimulation elevates PI3K activity likely via both the \( G_{a_{cr}} \) and the \( G_{a_{cr}} \)-βARK1-directed signaling pathways in adult rat cardiomyocytes. Enhanced PI3K signaling negatively regulates β-AR–induced contractile response, mainly via interfering with the receptor-mediated increases in \( I_{Ca} \) and intracellular \( Ca^{2+} \) transients, without altering myofilament \( Ca^{2+} \) response or the receptor-mediated phosphorylation of PLB. The PI3K-induced inhibition of β-AR contractile response might be involved in agonist-induced desensitization of the receptor, and when exaggerated, contributes to the pathogenesis of heart failure.

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References
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