Norepinephrine- and Epinephrine-induced Distinct β2-Adrenoceptor Signaling Is Dictated by GRK2 Phosphorylation in Cardiomyocytes*\(^3\)

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Agonist-dependent activation of G protein-coupled receptors induces diversified receptor cellular and signaling properties. Norepinephrine (NE) and epinephrine (Epi) are two endogenous ligands that activate adrenoceptor (AR) signals in a variety of physiological stress responses in animals. Here we use cardiomyocyte contraction rate response to analyze the endogenous β2,AR signaling induced by Epi or NE in cardiac tissue. The Epi-activated β2,AR induced a rapid contraction rate increase that peaked at 4 min after stimulation. In contrast, the NE-activated β2,AR induced a much slower contraction rate increase that peaked at 10 min after stimulation. Whereas both drugs activated β2,AR coupling to G\(_i\) proteins, only Epi-activated receptors were capable of coupling to G\(_i\) proteins. Subsequent studies showed that the Epi-activated β2,AR underwent a rapid phosphorylation by G protein-coupled receptor kinase 2 (GRK2) and subsequent dephosphorylation on serine residues 355 and 356, which was critical for sufficient receptor recycling and G\(_i\) coupling. In contrast, the NE-activated β2, ARs underwent slow GRK2 phosphorylation, receptor internalization and recycling, and failed to couple to G\(_i\). Moreover, inhibiting β2,AR phosphorylation by βARK C terminus or dephosphorylation by okadaic acid prevented sufficient recycling and G\(_i\) coupling. Together, our data revealed that distinct temporal phosphorylation of β2,AR on serine 355 and 356 by GRK2 plays a critical role for dictating receptor cellular events and signaling properties induced by Epi or NE in cardiomyocytes. This study not only helps us understand the endogenous agonist-dependent β2,AR signaling in animal heart but also offers an example of how G protein-coupled receptor signaling may be finely regulated by GRK in physiological settings.

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\(^{3}\) The abbreviations used are: GPCR, G protein-coupled receptor; NE, norepinephrine; Epi, epinephrine; GRK, G protein-coupled receptor kinase; PTX, pertussis toxin; AR, adrenoceptor; OA, okadaic acid; PKA, cAMP-dependent protein kinase; Iso, isoproterenol; GFP, green fluorescent protein; βARKct, βARK C terminus; FLISA, fluorescence-linked immunosorbent assay; ANOVA, analysis of variance.
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FIGURE 1. Epi- and NE-activated β₂-ARs undergo different trafficking in neonatal cardiomyocytes. A, FLAG-tagged mouse β₂-ARs were expressed in the β₂,AR-KO myocytes and visualized by immunocytochemistry. β₂-ARs were mainly localized on the cell surface at steady state. Epi induced rapid receptor internalization whereas NE-activated receptor underwent much slower internalization. Punctate intracellular staining of FLAG-β₂-ARs was observed after 5 min of Epi stimulation and after 30 min of both Epi and NE stimulation. B, β₂-ARs were stimulated with Epi or NE for 10 min before recovery by washing out the drugs. The Epi-activated FLAG-β₂-AR efficiently recycled back to the cell surface after removal of the drug, although the NE-activated β₂-AR remained inside the cell. C, the cell-surface receptor level was measured by FLISAs after agonist-induced internalization and recycling. The quantitative data in C represent the mean ± S.E. of N different experiments. Con, control. * p < 0.05 in Student’s t test.

group of drugs that either activate or inhibit βARs in a variety of clinical conditions, including heart failure, hypertension, coronary artery disease, and asthma. In fact, specific drug-dependent signaling properties are proposed to explain the clinical observations under treatment with different β-blockers (11). Whereas carvedilol has been used as an effective long term therapy for heart failure, other drugs in the same class have failed the clinical trials (12).

Studies in cardiac tissue are of prime interest because adrenergic signaling properties are not only manipulated with β-blockers in managing heart failure but are also linked to the progression of this disease (13). Here we analyzed the NE- or Epi-activated β₂-AR signaling for physiological contraction response on primary cardiomyocytes. We have for the first time uncovered that agonist-dependent phosphorylation of β₂-AR receptor on Ser-355 and -356 by GRK2 plays a critical role to differentiate the receptor signaling activated by Epi or NE to regulate cardiomyocyte contraction rate response.

EXPERIMENTAL PROCEDURES

Cell Culture and Recombinant Adenoviruses—Spontaneous beating neonatal cardiac myocytes were prepared from hearts of 1-day-old wild type, β₁AR-knock-out (β₁,AR-KO), or β₁,β₂AR-knock-out (β₁β₂,AR-KO) mouse pups as published previously (14). Neonatal myocytes were infected with viruses at a multiplicity of infection as indicated in the text after being cultured for 24 h. Recombinant adenoviruses expressing FLAG-tagged human or murine β₂,ARs have been described previously (15). The receptor expression levels were equivalent in myocytes determined by ligand binding assays and Western blots as described before (16). Adenoviruses expressing βARKct were a gift from Walter Koch (Thomas Jefferson University, Philadelphia).

Immunofluorescence Microscopy and Spectroscopy—Mycocyte images were obtained using a Zeiss Axioplan 2 microscope with Metamorph software (Universal Imaging). Epitope-tagged receptors were detected using M1 anti-FLAG antibody (Sigma) followed by Alexa-488- or Alexa-594-conjugated secondary antibodies (Molecular Probes). Surface receptor levels were determined with FLISA as described before (10) in the myocytes expressing the indicated FLAG-β₂-ARs. Cells were serum-starved for 2 h before stimulation with 10 µM epinephrine, norepinephrine, or isoproterenol (Sigma). The recycling of β₂,AR was done by washing out agonists after 10 min of drug stimulation to allow receptor recovery for an additional 30 or 60 min.

Cardiomyocyte Contraction Rate Assay—Measurement of spontaneous contraction rates from cardiomyocytes expressing either the endogenous or the indicated FLAG-β₂-ARs was carried out with or without the use of pertussis toxin (PTX) as described previously (14). In some assay, okadaic acid (OA, 1 µM) was applied 30 min before addition of Epi.

Determination of β₂-AR Phosphorylation or Dephosphorylation with Phosphoserine-specific Antibodies—Antibodies to the C terminus of the β₂,AR and to the phosphorylated serine (355, 356) of β₂-AR were from Santa Cruz Biotechnology (Santa Cruz, CA). Neonatal cardiomyocytes were serum-starved for 2 h prior to addition of epinephrine or norepinephrine for different times. Alternatively, myocytes were pretreated with 1 µM OA for 30 min before adding drugs. The cardiomyocytes were chilled, washed, and harvested in lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 20 mM Na₂HPO₄, 10H₂O, 50 mM NaF, 1 mM Na₃VO₄, 0.1 µM OA, and Complete Mini protease inhibitor (Roche Applied Science)). The lysates were clarified at 13,200 × g for 20 min. The supernatants were resolved on SDS-polyacrylamide gels and blotted with the polyclonal anti-phosphoserine (355, 356)-specific
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RESULTS

In neonatal cardiomyocytes, the Epi-activated β₂ARs displayed similar characteristics of trafficking to those activated by isoproterenol (Iso) (data not shown), showing fast internalization (Fig. 1A) (16) and sufficient recycling (Fig. 1, B and C). In contrast, NE-activated β₂ARs displayed much slower internalization (Fig. 1A) and recycling (Fig. 1, B and C), which led to an intracellular accumulation of the receptor (Fig. 1B, lower right panel). We have previously established that agonist-dependent β₂AR internalization and recycling is necessary for the receptor to switch coupling from Gs to Gi proteins to modulate the myocyte contraction rate (16). We then examined the potential difference in β₂AR signaling-mediated myocyte contraction rate response after the endogenous receptors were stimulated with Epi or NE in β₁AR-KO myocytes. When the β₁ARs were activated by a saturating concentration (10 μM) of Iso, the myocyte contraction rate response displayed an initial increase followed by a sustained decrease dropping the rate below the basal level (Fig. 2A). The response is because of sequential coupling of the receptor to Gα and Gβγ (16). Both Epi and NE induced a dose-dependent maximum contraction rate increase, and the maximum contraction rate increase was attenuated by membrane-permeable peptide PKI, a selective PKA inhibitor (Fig. 2D and supplemental Fig. S1). Moreover, Epi-induced contraction rate increases (maximized at 4 min) were much faster than NE-induced ones (maximized at 10 min, Fig. 2A and supplemental Fig. S1). These data suggest that both NE- and Epi-activated β₂ARs couple to the Gα/Gi pathway to regulate myocyte contraction rate.

At saturating concentrations of 10 μM, both Epi- and NE-induced myocyte contraction rate responses lacked the secondary decrease induced by Iso during the late phase stimulation (Fig. 2A). The time courses of contraction rate increases induced by 10 μM NE or Epi were significantly different (Fig. 2A). This was not because of the activation of α₁ARs by NE or Epi because β₁β₂AR-KO myocytes treated with these two drugs did not display significant change on myocyte contraction rates (data not shown). The lack of the secondary decrease of contraction rate in NE- or Epi-treated myocytes implies a minimum role of the receptor/Gi coupling in the contraction responses (16). Interestingly, only the Epi- but not the NE-induced contraction rate response was further enhanced by inhibiting the Gβγ protein with PTX, a Gβγ inhibitor (Fig. 2B and C). Therefore, only Epi-activated β₂AR had sufficient coupling
is necessary for the efficient receptor coupling to Gi in cardiomyocytes. This notion is further supported by an experiment utilizing a mutant β2AR that cannot recycle. Previously, the C-terminal PDZ motif of β2AR has been shown necessary for receptor recycling after agonist-induced internalization (16). When the mutant β2AR lacking this motif was expressed in β2AR-KO cardiomyocytes and activated by Epi or NE, the receptor signaling-mediated myocyte contraction rate responses were not sensitive to PTX treatment (Fig. 2, E and F).

The agonist-dependent GPCR internalization and recycling is regulated by receptor phosphorylation by the GRK family and dephosphorylation by phosphatase 2A (17). We examined agonist-dependent phosphorylation on β2AR in cardiomyocytes. Both Epi and NE induced a dose-dependent phosphorylation of serine residues 355 and 356 of β2AR in cardiomyocytes (S2). At 10 μM, β2AR stimulated with Epi displayed a rapid increase of phosphorylation on these serine residues that peaked at 5 min followed by a decrease over 60 min after drug administration (Fig. 3, A and B). In contrast, β2AR stimulated with 10 μM NE displayed a much slower increase in phosphorylation of the serine residues that peaked at 15 min and remained at peak level until 60 min after drug treatment (Fig. 3, A and B). To rule out the possibility that lower agonist occupancy by NE accounts for the slower phosphorylation of the receptor, we titrated Epi to a concentration equivalent to 10 μM NE in terms of potency to activate G protein for cAMP accumulation. The cellular cAMP accumulation induced by Epi displayed a dose-dependent increase (supplemental Fig. S3). At a concentration of 500 nM, which was equivalent to 10 μM NE in inducing cAMP, Epi induced a rapid receptor phosphorylation at Ser-355 and Ser-356 (supplemental Fig. S3). The time course of the receptor phosphorylation induced by 500 nM of Epi resembled that induced by 10 μM of Epi (Fig. 3A and supplemental Fig. S3). In addition, inhibiting phosphatase 2A with okadaic acid (OA) attenuated dephosphorylation of Ser-355 and -356 on the activated β2ARs at 30 min of Epi treatment but did not alter the phosphorylation level of these residues on the NE-activated receptors (supplemental Fig. S4). To examine whether the NE-activated β2ARs undergo dephosphorylation, myocytes were treated with agonists for 5 min before being washed. Both Epi- and NE-activated receptors displayed time-dependent dephosphorylation on Ser-355 and -356 after removal of drugs, which was partially but significantly blocked by pretreatment with OA (Fig. 3, C and D). The failure of OA treatment to fully restore the receptor phosphorylation level indicates that other phosphatases may be involved in the β2AR dephosphorylation in cardiac myocytes. Together, Epi and NE induced distinct temporal phosphorylation of Ser-355 and -356 on β2AR in cardiomyocytes. These data are consistent with the β2AR signaling-mediated myocyte contraction rate responses under stimulation of NE and Epi (compare Fig. 3B and 2A).

We then examined the effect of these agonist-induced Ser-355 and -356 phosphorylations on receptor trafficking and signaling in cardiomyocytes by inhibiting receptor dephosphorylation with OA. As expected, OA treatment reduced the recycling of the Epi-activated β2ARs after internalization, which resulted in an intracellular accumulation of the receptors (Fig. 4, A and B). In the β2AR-KO cardiomyocytes stimulated by Epi, OA treatment alone neither significantly changed basal myocyte contraction rate nor significantly altered the Epi-induced contraction rate response (Fig. 4C). However, whereas additional PTX treatment enhanced the contraction rate increase induced by Epi, OA treatment diminished the effects of PTX on inhibiting G signaling (Fig. 4, C and E). Without OA treatment, inhibition of G protein with PTX significantly enhanced both initial maximum contraction rate increases (Fig. 4D) and the late stage contraction rate increases (Fig. 4E) during 30 min.
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A, FLAG-tagged mouse β2ARs were expressed in the β2AR-KO myocytes and stimulated with Epi before removal of the drugs for recycling. Epi-activated β2AR underwent internalization and recycling in cardiomyocytes; pretreatment with OA blocked sufficient receptor recycling. B, cell-surface receptor densities were determined with FLISA. Con, control. C–E, cardiomyocytes from the β2AR-KO mice were pretreated with OA and/or PTX before stimulation with Epi or NE. C, OA did not affect Epi-induced contraction rate in β2AR-KO neonatal cardiac myocytes but blocked the additional inhibitory effects of PTX on Gi signaling. The effects of OA and PTX on initial maximum contraction rate increase (Δ) and the late stage (30 min after drug stimulation) contraction rate increase (Δ) were analyzed after Epi or NE stimulation on β2AR-KO cardiomyocytes. The contraction response curves in C represent the mean ± S.E. of N beating dishes from M different myocyte preparations. *p < 0.05 in Student’s t test. **p < 0.05; time course curves were significantly different between the PTX + Epi and the others (C) by two-way ANOVA.

FIGURE 4. Okadaic acid inhibits Epi-mediated β2AR recycling and coupling to G1 in cardiomyocytes. A, FLAG-tagged mouse β2ARs were expressed in the β2AR-KO myocytes and stimulated with Epi before removal of the drugs for recycling. Epi-activated β2AR underwent internalization and recycling in cardiomyocytes; pretreatment with OA blocked sufficient receptor recycling. B, cell-surface receptor densities were determined with FLISA. Con, control. C–E, cardiomyocytes from the β2AR-KO mice were pretreated with OA and/or PTX before stimulation with Epi or NE. C, OA did not affect Epi-induced contraction rate in β2AR-KO neonatal cardiac myocytes but blocked the additional inhibitory effects of PTX on Gi signaling. The effects of OA and PTX on initial maximum contraction rate increase (Δ) and the late stage (30 min after drug stimulation) contraction rate increase (Δ) were analyzed after Epi or NE stimulation on β2AR-KO cardiomyocytes. The contraction response curves in C represent the mean ± S.E. of N beating dishes from M different myocyte preparations. *p < 0.05 in Student’s t test. **p < 0.05; time course curves were significantly different between the PTX + Epi and the others (C) by two-way ANOVA.

of stimulation with Epi. After pretreatment with OA, the PTX-dependent effect on the initial maximum contraction rate increases were blunted (Fig. 4D), and the PTX effect on contraction rate increase during the late stage of stimulation was completely absent (Fig. 4E), suggesting a diminished Gi signaling. Meanwhile, additional OA treatment only slightly reduced both the initial maximum contraction rate increase and the late contraction rate increase induced by NE (Fig. 4, D and E). These data together with the data from Fig. 3 suggest that OA treatment blocks the Epi-activated β2AR dephosphorylation and subsequent recycling after internalization. The internalized receptors are accumulated at intracellular compartments, which results in limited coupling to Gi protein in cardiomyocytes. These observations are consistent with the notion that the agonist-dependent receptor trafficking is necessary for the efficient β2AR coupling to Gi in cardiomyocytes.

DISCUSSION

In this study, we have shown that distinct β2AR phosphorylation by GRK2 plays a critical role in differentiating NE- and Epi-induced receptor signaling to regulate physiological cardiomyocyte contraction rate responses. Ligand-dependent pharmacological and cellular efficacies have been documented on a growing list of GPCRs, including opioid receptor (19), angiotensin receptor (20), and adrenoreceptor (21). These efficacies include divergent signaling pathways activated by the same GPCR under different drug stimulations (22) as well as divergent cellular sorting pathways after agonist-induced receptor internalization (23). In the case of β2AR, antagonist
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FIGURE 5. βARKct, a GRK2-specific inhibitor, affects β2-AR trafficking and the receptor signaling mediated contraction rate response in neonatal cardiomyocytes. A, FLAG-β2-AR and βARKct were co-expressed in β2,AR-KO myocytes. Epi- or NE-induced GRK2 phosphorylation at serine 355 and 356 was blocked by βARKct in an expression level-dependent manner. B, quantitative analysis of the levels of phospho-β2-AR in A by normalizing as percentage of no infected control (Con). C, Epi- or NE-induced GRK2 phosphorylation at serine 355 and 356 was selectively blocked by βARKct expression but not the GFP control. D, quantitative analysis of the levels of phospho-β2-AR in C by normalizing as percentage of no infected control. E, βARKct reduced β2-AR internalization and recycling after Epi stimulation and caused intracellular accumulation of receptor in β2-AR-KO myocytes. F, βARKct almost completely blocked β2-AR internalization after NE stimulation. G–I, βARKct enhanced the contraction rate increase mediated by Epi-activated β2-AR signaling in β2,AR-KO myocytes (G), but blocked the additional effect of PTX on contraction rate (H). In contrast, βARKct did not alter the contraction rate increase mediated by NE-activated β2-AR signaling in β2,AR-KO myocytes (I). The contraction response curves represent the mean ± S.E. of N beating dishes from M different myocyte preparations. *, p < 0.05; time course curves were significantly different between Epi and βARKct + Epi (G) by two-way ANOVA. **, p < 0.05 in Student’s t test.

alprenolol and inverse agonist ICI118551 failed to activate the β2-AR coupling to Gs protein but are capable of activating the mitogen-activated protein kinase (MAPK) signaling cascade in HEK293 fibroblasts (21). These studies suggest that diversified signaling pathways can be potentially activated by the same GPCR in physiological contexts. Meanwhile, different clinical outcomes from long term therapy of heart failure support the idea that different β-blockers can induce distinct cellular effects in patients (24). Epi and NE are two endogenous ligands that activate the adrenoceptor family in vivo. Recent studies suggest that NE- and Epi-activated human β2-AR can preferentially couple to distinct G protein signaling pathways when overexpressed in mouse heart (25). Despite these in vivo observations, cellular and molecular mechanisms underlying the physiological implication of βAR signaling activated by NE and Epi remain unclear. Here we analyzed the signaling induced by Epi and NE in cardiac tissue by examining the effects on cardiomyocyte contraction rate response. We have shown for the first time that NE- and Epi-activated β2,ARs induce distinct cellular signals in regulating physiological cardiomyocyte contraction responses. At saturating concentrations, whereas both drugs induced β2-AR coupling to Gs protein, only Epi-activated receptors were capable of coupling to Gi proteins because of its sufficient recycling in cardiomyocytes. Subsequent studies showed that a rapid GRK2 phosphorylation and subsequent dephosphorylation of the Epi-activated β2-AR were critical for sufficient receptor recycling and Gi coupling. These data suggest that NE and Epi induce different signaling and functional properties of β2-AR in animal heart.

Myocytes stimulated by Epi at different concentrations displayed a rapid Gs/PKA pathway-dependent contraction rate increase (Fig. 2A). After reaching peak level, the contraction rate underwent an immediate decrease representing the combination of receptor desensitization and receptor/Gi coupling. In contrast, when activated by NE at different concentrations, the receptor displayed a much slower Gi/PKA-dependent contraction rate increase that peaked around 10 min after drug administration (Fig. 2A). This delayed increase is likely due in part because of a slow desensitization of the NE-activated receptor by GRK2 phosphorylation (Fig. 3A), which results in a prolonged coupling to Gi protein. The difference in receptor signaling and
GRK2 phosphorylation was not because of the difference in these two agonist binding affinities. In fact, stimulation with 500 nM Epi, a concentration equivalent to 10 μM NE in terms of stimulation of Gs to increase cAMP, also induced a rapid receptor phosphorylation (supplemental Fig. S3), an observation that is consistent with the receptor phosphorylation at Ser-355 and Ser-356 reported in HEK293 cells (26). In our study, the GRK2-mediated phosphorylation was detected on human β2AR expressed in mouse myocytes. Despite different signaling and biochemical properties reported between human and mouse β2ARs (15, 27), these two receptors resemble each other. Our recent studies on β2ARs expressed in mouse cardiomyocytes show that these two receptors are remarkably similar in activating Gs and Gι for myocyte contraction and undergoing agonist-dependent internalization and recycling (15). Here the tight correlation between the endogenous mouse β2AR-mediated myocyte contraction change and the exogenously expressed human β2AR phosphorylation under agonist stimulation confirms the striking similarity between these two species. We have previously reported that Epi and NE can induce distinct conformational changes on β2AR (10). Thus the difference in the GRK2 phosphorylation of β2AR may be due to the Epi-activated receptors having a higher GRK2 binding affinity or serving as better GRK2 substrates than the NE-activated ones. Alternatively, it may be due to the recruitment of additional cellular factors to the agonist-activated β2ARs that regulate the GRK2-mediated phosphorylation of the receptor.

GRK-mediated phosphorylation of a GPCR has been implicated in receptor desensitization and subsequent internalization (29). The phosphorylated receptors possess increased binding affinities to a scaffold protein β-arrestin for internalization. The internalized GPCRs dissociate from arrestin complexes and undergo dephosphorylation for recycling (17). Our data indicate the GRK2-mediated phosphorylation plays a key role for the agonist-dependent trafficking (internalization and recycling). The dephosphorylation of the β2ARs activated by NE and Epi appeared to be equivalent (Fig. 3, C and D). Thus, in a simple model, when the β2ARs are activated by Epi, the rapid GRK2 phosphorylation leads to transient Gs coupling and faster internalization, which is followed by sufficient dephosphorylation allowing recycling and Gι coupling in myocytes. Blocking β2AR dephosphorylation by okadaic acid inhibits the receptor coupling to Gι. Disrupting the GRK2-mediated phosphorylation of β2AR with βARKct inhibits the receptor internalization and subsequent recycling, which enhances the Epi-induced maximum contraction rate increases, and inhibits the receptor/Gι coupling for contraction rate responses. These data are consistent with recent reports showing that GRK2 and GRK3 preferentially regulate receptor/G protein coupling (30, 31), but do not rule out the possible additional role of GRK5 and GRK6 in β2AR cellular signaling and...
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 Trafficking in cardiomyocytes. In fact, the receptor phosphorylation by other kinases may contribute to the remaining internalization of Epi-activated β₂ARs after GRK2 is inhibited by βARKct (Fig. 5E).

 In contrast, the NE-activated β₂ARs undergo a slow but persistent GRK2 phosphorylation, which may contribute to a prolonged Gᵢ₅ coupling, slow receptor trafficking, and minimum Gₛ coupling. It is therefore not surprising that disruption of GRK2 phosphorylation by βARKct has a minimal effect on the receptor-mediated contraction rate response by NE (Fig. 5H). These data also indicate that the NE-mediated acute myocyte contraction response is not dependent on the slower receptor phosphorylation by GRK2 and subsequent trafficking. Because the NE-activated β₂ARs are accumulated inside of cells, and fail to display sufficient cell surface recovery and Gₛ coupling, our data suggest that the NE-induced β₂AR phosphorylation by GRK2 may play a role in inducing prolonged β₂AR desensitization. These data, however, do not exclude the possibility that other cellular kinases are involved in modulating the GRK phosphorylation of β₂AR under NE stimulation, which may regulate receptor signaling for contraction rate responses. Based on the recycling rates, GPCRs can be classified into two classes: rapid (including β₂AR) and slow recycling receptors (32). The slow recycling receptors usually form stable complexes with arrestin in endosomes, which are essential for initiating additional signaling pathways during a prolonged period of stimulation (32). Therefore, it will be interesting to check whether the NE-activated β₂ARs form stable complexes with arrestin to regulate additional signaling pathways in cardiomyocytes. Meanwhile, it remains to be examined whether the intracellularly accumulated β₂ARs, after stimulation by NE, are targeted to lysosome for degradation. The slow recycling of the NE-activated β₂AR also implies a prolonged desensitization of receptor at post-synaptic regions of sympathetic synapses in animal heart.

 Diseases such as heart failure and asthma are characterized by dysfunction of βAR signaling, including down-regulation and desensitization of receptors (8, 33–35), and much evidence points to GRK as a culprit (35–39). Thus, there is great interest in elucidating the cellular mechanisms by which GRK-medi- ated receptor phosphorylation and function are regulated. Our studies linked GRK2 phosphorylation of adrenoreceptors to agonist-dependent, physiologically significant receptor signaling in cardiomyocytes. Although our data indicate that βARKct can block the β₂AR/Gᵢ₅ coupling, it is interesting to point out that the coupling of β₂AR to Gᵢ₅ protein plays a protective role against insults on cardiac myocytes (28). Thus, our data appear to be at odds with the beneficial effects of the peptide when overexpressed in mouse heart (36, 38). The differences could be due to the different time course of the results observed in these two model systems. Although the effect of βARKct in mice is mainly attributed to the recovery of βAR density and response in animal heart under chronic conditions (36, 38), our results address the acute effect of βARKct on βAR signaling in neonatal cardiomyocytes. Moreover, the observed difference may imply the different roles of GRK2 phosphorylation of βARs in physiological versus pathological settings. Further studies along this direction as well as studies with adult myocytes will provide more mechanistic details on the effects of GRK regulation of βAR signaling in physiological and pathophysiological conditions.

 In summary, we hereby revealed the critical role of GRK2 phosphorylation underlying the distinct cellular events and signaling properties of β₂AR induced by Epi or NE in cardiomyocytes. This finding opens the door to further explore the differential physiological relevance of these two endogenous ligands upon binding to adrenoreceptors. These data not only help us understand the physiological and pathophysiological significance of βAR activation by Epi and NE in vivo but also support the utility of the combinatorial manipulation of βAR and GRK activities in the treatment of a wide range of chronic conditions in both cardiovascular and pulmonary systems.

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