Abstract—Intermolecular interactions between members of both similar and divergent G protein–coupled receptor subfamilies have been shown in various experimental systems. Here, we demonstrate heterodimerization of predominant β-adrenergic receptor (βAR) subtypes expressed in the heart, β1AR, and β2AR, and its physiological relevance. In intact adult-mouse cardiac myocytes lacking native β1AR and β2AR, coexpression of both βAR subtypes led to receptor heterodimerization, as evidenced by their coimmunoprecipitation, colocalization at optical resolution, and markedly increased binding affinity for subtype-selective ligands. As a result, the dose-response curve of myocyte contraction to βAR agonist stimulation with isoproterenol (ISO) was shifted leftward by ≈1.5 orders of magnitude, and the response of cellular cAMP formation to ISO was enhanced concomitantly, indicating that intermolecular interactions of βAR subtypes resulted in sensitization of these receptors in response to agonist stimulation. In contrast, the presence of β2AR greatly suppressed ligand-independent spontaneous activity of coexisting β2ARs. Thus, heterodimerization of β1AR and β2AR in intact cardiac myocytes creates a novel population of βARs with distinct functional and pharmacological properties, resulting in enhanced signaling efficiency in response to agonist stimulation while silencing ligand-independent receptor activation, thereby optimizing β-adrenergic modulation of cardiac contractility. (Circ Res. 2005;97:244-251.)

Key Words: receptor dimerization  ■ β-adrenergic receptor  ■ G protein–coupled receptors  ■ cardiac contractility  ■ ligand binding

G protein–coupled receptors (GPCRs) represent the largest family of transmembrane molecules involved in cell signal transduction. GPCRs have traditionally been thought to function as monomers, but increasing evidence suggests that GPCRs may exist as both homodimers or heterodimers.1–7 The idea that GPCRs might undergo dimerization was first proposed in 1982.8 The physical interaction of GPCRs within or among different families leads to a multitude of changes in ligand binding and signaling properties of these receptors.1–7,9–11 However, a close inspection of previous studies reveals that most have been conducted in naive cell lines or in vitro experimental settings. An important question is, however, whether GPCR dimerization occurs in a physiological context such as the intact cardiomyocyte and, if so, whether this has physiological or pathophysiological relevance.

As prototypical members of the GPCR superfamily, β-adrenergic receptors (βAR) consist of 3 pharmacologically and genetically distinct subtypes, β1AR, β2AR, and β3AR, which are often coexpressed in many types of cells and tissues. In cardiomyocytes, mainly β1AR and β2AR subtypes are coexpressed and fulfill distinct functional roles via activation of subtype-specific signaling pathways.12 Our previous studies have shown that heterodimerization between β1AR and β2AR subtypes inhibits β2AR internalization and its ability to activate ERK1/2 MAPK signaling in HEK293 cells.11 The present study is aimed to characterize potential heterodimerization of these βAR subtypes in the heart and its impacts on the functional and signaling properties of these receptors. To create “pure” β1AR, β2AR, or β1AR/β2AR coexistent systems with a matched total receptor density, we expressed either the mouse β1AR or β2AR, or both subtypes in cardiomyocytes from the adult β1AR and β2AR double knockout (β1AR/β2AR DKO) mice,13 in conjunction with adenoviral gene transfer techniques.14

Our results indicate that β1AR and β2AR are able to form heterodimers in intact cardiomyocytes, and that the heterodimeric receptors exhibit altered ligand binding profiles, enhanced signaling efficiency in regulating myocyte cAMP
production and contractility, and suppressed βAR spontaneous activity in the absence of agonist stimulation. Thus, we conclude that β1AR and β2AR heterodimerization is required for optimal β-adrenergic regulation of cardiac contractility.

Materials and Methods

Cardiac Myocyte Adenoviral Infection and Cell Contraction Measurement

Single cardiomyocytes were isolated from the hearts of ~2- to 3-month-old male β1/β2AR DKO or β1AR KO or wild-type (WT) mice with an enzymatic technique, then cultured and infected with adenoviral vectors for 24 hours, as described previously.4,9 Cultured cells were then perfused with a HEPES-buffered solution (in mmol/L: NaCl 137, KCl 5.4, MgCl2 1.2, NaH2PO4 1, CaCl2 1, Na2HPO4 10, glucose 20, and HEPES 20, pH 7.4), and electrically stimulated at 0.5 Hz at 23°C. Cell contraction was measured by the percent shortening of cell length in response to electrical stimulation.15

cAMP Measurement

Intracellular cAMP levels were assayed by radioimmunobassay, as previously described.16 Briefly, cultured mouse cardiomyocytes were incubated with isoproterenol (ISO) for 10 minutes, and cellular cAMP formation was determined using a radioimmunobassay kit from Amersham with a duplicate in each experiment.

Radioligand-Binding Assay

As described previously,16 binding assays were performed on 25 μg of membrane proteins using saturating amounts of the βAR specific ligand [125I]cyanopindolol ([125I]-CYP). Nonspecific binding was determined in the presence of 20 μmol/L propranolol. Bmax for ICYP were determined by Scatchard analysis of saturation binding isotherms. Data of competition experiments were analyzed using 1- or 2-site competition binding curves with GraphPad PRISM.14,16

Immunocytochemical Staining and Confocal Imaging

Confocal Imaging

Immunostaining and confocal imaging were performed in β1/β2AR DKO cells infected by either Adv-β1AR tagged with hemagglutinin (HA), or Adv-β2AR, or a combination of both, at multiplicity of infection (moi) 100 for 24 hours, as described previously.16 Horse anti-mouse IgG secondary antibodies and goat anti-rabbit IgG secondary antibodies were used for β1AR and β2AR staining, respectively. Immunofluorescence was then detected by a confocal microscope (LSM-510, Zeiss) with an optical section thickness of 1.0 μm.

Coimmunoprecipitation and Western Blotting

Myocytes expressing HA-tagged β1AR, Flag-tagged β2AR, or both receptors were lysed in RIPA buffer (in mmol/L: 50 Tris pH 7.4, 150 NaCl, 20 β-glycerophosphate, 20 NaF, 0.2 Na3VO4, 5 EDTA, 5 EGTA, 10 benzamidine, 0.5 PMSF, 1 PMSF, 25 μg/mL leupeptin, 1% Triton X-100, and 0.5% sodium deoxycholate) for 30 minutes at 4°C. For immunoprecipitation, 100 to 200 μg of protein was incubated with 1 to 2 μg of anti-Flag (1:100) or anti-HA (1:100) overnight at 4°C to pull-down Flag-tagged β1AR or HA-tagged β2AR, respectively. Immunocomplexes were isolated by incubation with 10% vol/vol protein G-Sepharose for 2 to 3 hours. The immunoprecipitate was then treated with 100 mmol/L DTT in the sample buffer and subjected to SDS/PAGE and Western blotting to detect the presence of β1AR or β2AR with the anti-HA monoclonal antibody or the anti-Flag antibody, respectively. In addition, we have quantified the relative percentage of heterodimer of β1/β2AR (pull-down with anti-HA antibody or anti-Flag antibody, normalized by anti-body pull-down efficiency) compared with the total β1/β2AR pool (cell lysate).

Materials

Unless otherwise indicated, all chemicals were purchased from Sigma. [125I]Cyanopindolol was purchased from NEN Life Science Products, Inc. (Boston, Mass). Anti-HA monoclonal antibody, anti-Flag, and β2AR polyclonal antibody were purchased from Berkeley Antibody Co. (Berkeley, Calif) and Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif), respectively. The secondary antibodies were purchased from Vector Laboratories (Burlingame, Calif).

Statistical Analysis

Data were expressed as mean±SE. Statistical comparisons used 1-way ANOVA followed by the Bonferroni procedure for multiple-group comparisons. A P<0.05 was considered statistically significant.

Results

Colocalization of βAR Subtypes in Cardiomyocytes

To investigate possible intermolecular interactions between β1AR and β2AR subtypes, we expressed either or both βAR subtypes in cultured ventricular myocytes from β1β2AR DKO mice using adenosine-mediated gene transfer at moi of 100. After 24 hours infection, the densities of β1AR and β2AR were comparable to that of cells expressing both βAR subtypes (Table 1). Using confocal immunocytochemical imaging, we visualized that the specific immunofluorescence of β1AR or β2AR was largely concentrated on cell surface membranes, including transverse tubules, with enriched staining of the perinuclear area (Figure 1A). An overlay of the images of HA-β1AR and β2AR revealed an excellent pixel-to-pixel correlation (r2=0.78, Figure 1B), an indication of colocalization of β1AR and β2AR at optical resolution.

Coimmunoprecipitation of β1AR and β2AR

To directly demonstrate physical association of βAR subtypes, we expressed HA-tagged β1AR or Flag-tagged β2AR or both in the null background of DKO myocytes and then performed immunoprecipitation and Western blot assays. Total cellular proteins containing either or both βAR subtypes were first immunoprecipitated with a rabbit polyclonal antibody or the anti-Flag antibody, respectively. The pull-down of Flag-tagged β2AR was largely concentrated on cell surface membranes, including transverse tubules, with enriched staining of the perinuclear area (Figure 1A). An overlay of the images of HA-β1AR and β2AR revealed an excellent pixel-to-pixel correlation (r2=0.78, Figure 1B), an indication of colocalization of β1AR and β2AR at optical resolution.

<table>
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<tr>
<th>TABLE 1. Densities and Ratios of the Coexpressed β1AR:β2AR in WT, β1AR KO or Adenovirus-Transfected DKO Mouse Cardiomyocytes</th>
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<td>DKO+ β1β2AR</td>
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Note: ventricular myocytes from WT or β1AR KO mice or those from DKO mice infected by Adv-β1AR (moi of 100) or Adv-β2AR (moi of 100) or both (moi of 50 for each), (n=3 to 4 independent experiments each performed in duplicate).
Three major species (Mr: 52, 70, 150 kDa) were visualized with the Western blot. Three similar immunoreactive species (Mr: 50, 70, 150 kDa) were illustrated by Western blot using anti-HA in the total extracts from cells expressing HA-β2AR (Figure 1D). The 50 kDa species likely represents the monomeric form of HA-β2AR, and the 150 kDa form likely represents SDS-resistant homodimeric receptors (or oligomers). The 70-kDa species might represent the monomeric core glycosylated form of HA-β2-AR. As a negative control, there was no detectable HA-immunoreactivity in myocytes expressing either β1AR alone or β-gal (Figure 1D). Conversely, we performed the coimmunoprecipitation experiments with the anti-HA antibody to pull-down β1ARs and detected coimmunoprecipitated Flag-tagged β2ARs by Western blot using rabbit polyclonal anti-Flag (Figure 1E). The specificity of anti-Flag was confirmed by the immunoreactive signals in the total extracts from cells expressing Flag-β2AR but not in those expressing HA-β1AR alone or β-gal (Figure 1F). In an attempt to quantify the relative proportions of receptor heterodimers, we determined the pull-down efficiency of each antibody (by comparing pull-down with whole cell lysate) and measured the relative amount of the other tagged receptor coimmunoprecipitated. Heterodimers represented 18.9 ± 4.6% (n = 3) and 21.0 ± 5.6% (n = 3) of the total β1ARs and β2AR populations, as indexed by their coimmunoprecipitation. These
results indicate that intermolecular interactions occur between \( \beta_1 \)-AR and \( \beta_2 \)-AR in adult-mouse cardiomyocytes.

**Suppression of Spontaneous \( \beta_2 \)-AR Activation by \( \beta_2 \)-AR Coexpression**

To determine the functional consequences of \( \beta_1 \)-AR-\( \beta_2 \)-AR heterodimerization in adult-mouse ventricular myocytes, we first examined constitutive \( \beta_2 \)-AR activity in the absence of agonist stimulation. The baseline contractility of cells expressing \( \beta_2 \)-AR was enhanced by 1.6-fold relative to myocytes expressing \( \beta_1 \)-AR or those uninfected cells from WT or DKO mice (Figure 2A). In contrast, expression of \( \beta_1 \)-AR at a receptor density that matched the \( \beta_2 \)-AR density did not alter basal ligand-independent myocyte contracture amplitude (Figure 2A). ICI 118 551 (ICI, 5 \( \times \) 10\(^{-7} \) mol/L), a \( \beta_2 \)-AR inverse agonist, completely reversed the enhanced basal contraction (Figure 2B), without altering the baseline contraction in cells expressing \( \beta_1 \)-AR (data not shown). These results are consistent with the previous notion that \( \beta_2 \)-AR, but not \( \beta_1 \)-AR, exhibits spontaneous activity. Surprisingly, the \( \beta_2 \)-AR infection-induced augmentation in the baseline contractility was fully prevented when cells were coinfected with \( \beta_1 \)-AR (50 moi for each) (Figure 2A).

Figure 2B shows representative examples of the inhibitory effect of ICI in a cell expressing either \( \beta_1 \)-AR or both \( \beta \)-AR subtypes (top and bottom, respectively). Clearly, coexpression of \( \beta_1 \)-AR virtually abolished spontaneous \( \beta_2 \)-AR activity, as manifested by the inability of the \( \beta_2 \)-AR inverse agonist to reduce basal myocyte contraction (Figure 2A and 2B).

**Heterodimerization of \( \beta \)-AR Subtypes Enhances Cardiomyocyte Contractile Response to \( \beta \)-AR Agonist Stimulation**

Next, we determined the potential impact of coexpression of these receptors on the myocyte contractile response to agonist-induced \( \beta \)-AR stimulation. In cells expressing either \( \beta_1 \)-AR or \( \beta_2 \)-AR, stimulation of these \( \beta \)-AR subtypes with the same agonist, isoproterenol (ISO), produced comparable maximal contractile responses despite their distinct basal contraction amplitudes (Figure 3A). When the concentration-response curves were normalized by their corresponding basal level or maximal response (Figure 3B and Figure 3C, respectively), it is clear that the concentration-response curves of \( \beta_1 \)-AR- and \( \beta_2 \)-AR-mediated increases in myocyte contractility virtually overlapped with each other with pD2 (\(-\log \text{EC}_{50}\)) of 9.02 \pm 0.01 vs Adv-\( \beta_2 \)-AR infected, or Adv-\( \beta_1 \)-AR and Adv-\( \beta_2 \)-AR coinfected, or uninfected WT or DKO myocytes). The solid bars show effects of ICI 118 551 (0.5 \( \mu \)mol/L) on the baseline contraction amplitude in myocytes infected with Adv-\( \beta_2 \)-AR at moi of 100 or 50, or in those coinfected with Adv-\( \beta_1 \)-AR and Adv-\( \beta_2 \)-AR (moi of 50 for each). B, Typical time course of the ICI 118 551 effect on basal contraction amplitude in DKO myocytes infected by Adv-\( \beta_2 \)-AR (50 moi, top) or those coinfected by Adv-\( \beta_1 \)-AR and Adv-\( \beta_2 \)-AR (50 moi for each, bottom).
induced relative increase in myocyte contractility was shifted leftward by 1.5 orders of magnitude (pD2 10.41±0.49; P<0.01 versus the β1AR or the β2AR group). Thus, βAR subtype heterodimerization sensitizes the contractile response to ligand-induced receptor stimulation in cardiac myocytes.

**Cellular cAMP Responses in β1AR- or β2AR- or Mixed β1β2AR-Expressing Cardiomyocytes**

Because both β1AR- and β2AR-induced positive inotropic effects are mediated by a cAMP-dependent mechanism,21–23 we next investigated the cAMP response to βAR stimulation in DKO cardiomyocytes expressing either or both βAR subtypes. Compared with that of uninfected WT or DKO cells, the baseline cAMP level was unchanged in myocytes expressing β1AR, but augmented by 2.1-fold in cells infected with Adv-β2AR (100 moi) (Figure 4A), caused by spontaneous β2AR activity.16–20 Coexpression of the β1AR with β2AR fully suppressed the β2AR-induced increase in basal cAMP production (Figure 4A), as was the case for the baseline contractility (Figure 2). The absolute increase in cAMP formation in response to ISO in cells expressing β2AR was increased versus that in cells expressing β1AR (Figure 4B). However, the relative response of cAMP formation (% of basal level) to ISO was greater in β1AR-expressing cells compared with that in those expressing β2AR (Figure 4C). Remarkably, when these βAR subtypes were coexpressed in β1β2AR DKO cardiomyocytes at matched levels of total receptor expression, the ISO-induced absolute or relative increase in cAMP formation was almost 2-fold greater than that in cells expressing either β1AR or β2AR alone (Figure 4B and 4C), consistent with the profile of myocyte contractile response to either spontaneous or ligand-induced βAR subtype activation.

**Ligand Binding Profiles of β1AR, β2AR, and Coexpressed β1AR Subtypes**

In addition to the aforementioned immunocytochemical, physiological, and biochemical data, differences in ligand-receptor interactions would provide strong pharmacological evidence for receptor dimerization. In this regard, we examined ligand-binding profiles in WT or in DKO mouse cardiomyocytes when βAR subtypes were individually expressed or coexpressed. There were 2 βAR subpopulations in WT mouse heart with 73.6±2.7% and 26.4±2.9% for β1AR for β2AR, respectively. Most importantly, radioligand binding assays revealed that the binding affinity of separately expressed β1AR or β2AR in DKO cells for their selective ligands, CGP 20712A or ICI 118 551, was reduced by ≈30-fold and ≈10-fold, respectively, compared with that in WT cells or DKO myocytes expressing both βAR subtypes (Table 2). The coexpression-induced decrease in the binding affinity for subtype-selective ligands was not influenced by the absolute densities or the ratio of these βAR subtypes, because it occurred in both WT and coinfected DKO mouse myocytes regardless of their different densities or the ratio of the coexisting βAR subtypes (Tables 1 and 2). That coexpression enhances the affinity of both β1AR and β2AR for their selective ligand binding further supports the notion that these receptors form heterodimers in intact adult-mouse cardiomyocytes.

**Table 2.** Competition of 125I-CYP Binding With β1AR- or β2AR-Specific Antagonists, ICI 118 551 and CGP 20712A, Respectively, in Adult Mouse Cardiomyocytes

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<tr>
<th></th>
<th>ICI 118 551</th>
<th>CGP 20712A</th>
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<tr>
<td></td>
<td>pKi</td>
<td>Hill Coefficient</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>WT</td>
<td>9.12±0.08*</td>
<td>6.48±0.07</td>
</tr>
<tr>
<td>β1AR KO</td>
<td>7.89±0.07</td>
<td>1.125±0.092</td>
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<tr>
<td>DKO + β1AR</td>
<td>6.39±0.08</td>
<td>0.987±0.123</td>
</tr>
<tr>
<td>DKO + β2AR</td>
<td>8.16±0.01</td>
<td>1.104±0.052</td>
</tr>
<tr>
<td>DKO + β1β2AR</td>
<td>8.89±0.13*</td>
<td>6.55±0.31</td>
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Note: ventricular myocytes from WT or β1AR KO mice or those from DKO mice infected by Adv-β1AR (moi of 100) or Adv-β2AR (moi of 100) or both (moi of 50 for each). *P<0.01 vs β1AR KO or DKO expressing either β1AR or β2AR (n=3 to 6 independent experiments each with duplicates).
**Discussion**

**β₁AR and β₂AR Heterodimerization Underlying Altered Signaling Properties of These Receptors**

In the present study, using immunocytochemical, biochemical, physiological, and pharmacological approaches, we have provided the first documentation of intermolecular interactions between β₁AR and β₂AR in intact adult mouse cardiac myocytes. Heterodimerization of these receptors suppresses constitutive ligand-independent β₂AR signaling, but facilitates receptor-ligand interactions, thereby increasing the signaling efficiency of both βAR subtypes and optimizing βAR-mediated modulation of cardiac contractility. This conclusion is based on several independent lines of evidence. First, when coexpressed in adult-mouse cardiac myocytes lacking native βARs, β₁AR and β₂AR are physically associated with each other, as manifested by their coimmunoprecipitation and intracellular colocalization at optical resolution. Second, coexpression of β₁AR and β₂AR creates a novel population of βARs endowed with increased binding affinity for subtype-specific ligands regardless of the total receptor density or the ratio of these receptor subtypes (Tables 1 and 2). Perhaps most importantly, the presence of β₁AR fully quenches the spontaneous activity of coexpressed β₂ARs, as evidenced by a full reversal of agonist-independent β₂AR-mediated augmentation in basal myocyte cAMP production and contractility. This finding provides compelling evidence for constitutive intermolecular interactions between these βAR subtypes, because downstream regulatory events are unlikely to be involved in the absence of agonist stimulation. The enhanced ligand binding might contribute, at least in part, to the sensitization of myocyte contractile and cAMP responses to βAR agonist stimulation (Figures 3 and 4).

Interestingly, there are important differences between the present study in native cardiac myocytes and our previous studies performed in HEK 293 cells.11,24,25 Specifically, high-affinity binding to subtype-selective ligands was reduced, rather than increased, in HEK 293 cells coexpressing both β₁AR and β₂AR relative to those expressing a single subtype.25 Moreover, coexpression of both βAR subtypes is not associated with an increased cAMP formation in response to agonist stimulation in HEK 293 cells.11 These obvious discrepancies between intact cardiomyocytes and naive cells underscore that cell-specific factors certainly influence the properties of receptor signaling, and highlights the importance and necessity to validate these issues in a native cellular context.

**Optimizing βAR-Mediated Modulation of Cardiac Contractility by Subtype Heterodimerization**

Although a large number of other GPCRs have been previously reported to undergo homodimerization or heterodimerization,1-10 the present study provides the first demonstration that β₁AR and β₂AR, prototypical members of the GPCR superfamily, form heterodimers in the physiological context of intact cardiac myocytes. When β₁AR and β₂AR are coexpressed, myocyte contractile or cAMP response to βAR agonist stimulation is profoundly sensitized compared with the “pure” β₁AR or β₂AR system, indicating that β₁AR and β₂AR interact in a synergistic fashion. Similar functional synergy between β₁AR and β₂AR in regulating cAMP production has been previously reported in cultured rat C6 glioma cells.26 These present findings might imply that in cardiac myocytes, the native β₁AR and β₂AR may require mutual support from each other to maintain optimal sympathetic control over heart rate and myocardial contractile performance, allowing the heart to increase its output several times within seconds in response to a “fight-or-flight” situation. In this regard, previous studies have demonstrated that in mice lacking native β₁AR, stimulation of the native cardiac βAR with ISO was unable to elicit a positive inotropic effect in vivo.77 This further supports the perception that β₁AR and β₂AR exhibit a synergistic interaction in their modulation of cardiac contractility.

It is noteworthy that heterodimerization of β₁AR and β₂AR, although enhancing agonist-induced signaling, silences the spontaneous activation of β₁AR, suggesting that heterodimerization might mutually stabilize both receptor subtypes in their respective inactive conformations in the absence of agonist. As a result, it might reduce the signaling background, but optimize the responsiveness of dimeric receptors to agonist stimulation, thus further synchronizing the sympathetic control over cardiac performance in response to exercise or stress. The exact mechanism underlying the inhibitory effect of β₁AR on β₂AR spontaneous activation awaits future investigation. Altogether, our present and previous studies11,24,25 have demonstrated that intermolecular interactions between β₁AR and β₂AR create a new population of receptors in terms of their pharmacology, trafficking, signaling, and functionality.

It has been shown that during the progression to heart failure caused by a variety of etiologies, there is a selective downregulation of β₁AR with little or no change in β₂AR density.28-30 The heart failure–associated decrease in the ratio of β₁AR to β₂AR might alter the heterodimerization of the remaining βARs, thus contributing to the diminution of βAR contractile support or an upregulation of anti-apoptotic β₁AR signaling.31-33 These hypotheses merit further investigation.

**Heterodimerization Between βAR and Members From Other GPCR Families**

In addition to the complicated impacts of heterodimerization of the closely related β₁AR and β₂AR on their trafficking and signaling properties, recent studies have revealed evidence for heterodimerization of β₁AR with other members of adrenergic receptor family, including β₁AR, α₁AAR, and α₁PARK in HEK 293 cells.34-36 Interestingly, whereas either β₁AR or β₂AR alone couples to both G₁ and G₃ proteins, the β₂AR-β₁AR heterodimer is unable to activate G₁ signaling.34 Equally appealing, the heterodimerization of β₁AR with either α₁AAR or α₁PARK leads to cross-internalization of the receptors on agonist stimulation of either β₁AR or the α₁AR subtypes,35,36 and enables α₁PARK to regulate intracellular Ca²⁺ mobilization in response to agonist stimulation.39 Moreover, oligomerization of opioid peptide receptors with β₁AR also alters receptor trafficking and signal transduction.37-39

Additionally, it has been demonstrated that intermolecular interactions between βAR and angiotensin II type 1 receptor
(AT1R) occurring in the heart leads to a cross-inhibition of their downstream signaling and trafficking by either type of receptor antagonist. Thus, oligomerization of GPCRs from the same or different families not only increases the complexity of GPCR signaling and their functional diversity, but also raises important therapeutic considerations.

In summary, the present results indicate that the βAR and βAR are able to form heterodimers in adult-mouse cardiomyocytes, and that the heterodimeric receptors exhibit altered pharmacological and signaling properties, resulting in more potent cAMP and contractile responses to agonist stimulation, while silencing ligand-independent spontaneous βAR activity. The heterodimeric βAR-βAR may represent a pharmacologically and functionally distinct population of βARs. Thus, many well-established paradigms for βAR signaling and function may need to be revisited in the context of the coexistence of multiple receptor subtypes and their homo- or heterodimerization.

Acknowledgments

This work is supported by the National Institutes of Health intramural research grant (W.Z.Z., K.C., S.J.Z., D.Y., H.C., E.G.L., and R.P.X.), and in part by Chinese National Natural Science Foundation (30100215), Peking University 985 Project, Chinese National Key Project 973 (G2000056906), and Chinese Young Investigator Award (30225036). T.E.H. is a MacDonald Scholar of the Heart and Stroke Foundation of Canada (HSFC). The authors would like to thank Dr Brian K. Kobilka at Stanford University School of Medicine for kindly providing mice lacking native βAR or both βAR and βAR. The authors are also grateful to Dr Hal Spurgeon and B. Ziman for their excellent technical support.

References


Heterodimerization of $\beta_1$- and $\beta_2$-Adrenergic Receptor Subtypes Optimizes $\beta$-Adrenergic Modulation of Cardiac Contractility

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_Circ Res._ 2005;97:244-251; originally published online July 7, 2005;
doi: 10.1161/01.RES.0000176764.38934.86

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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