Recent Advances in Cardiac 
\(\beta_2\)-Adrenergic Signal Transduction

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Abstract—Recent studies have added complexities to the conceptual framework of cardiac \(\beta\)-adrenergic receptor (\(\beta\)-AR) signal transduction. Whereas the classical linear Gs-adenyl cyclase–cAMP–protein kinase A (PKA) signaling cascade has been corroborated for \(\beta_1\)-AR stimulation, the \(\beta_2\)-AR signaling pathway bifurcates at the very first postreceptor step, the G protein level. In addition to \(G_s\), \(\beta_2\)-AR couples to pertussis toxin–sensitive \(G_i\) proteins, \(G_i1\) and \(G_i2\). The coupling of \(\beta_2\)-AR to \(G_i\) proteins mediates, to a large extent, the differential actions of the \(\beta\)-AR subtypes on cardiac Ca\(^{2+}\) handling, contractility, cAMP accumulation, and PKA-mediated protein phosphorylation. The extent of \(G_i\) coupling in ventricular myocytes appears to be the basis of the substantial species-to-species diversity in \(\beta_2\)-AR–mediated cardiac responses. There is an apparent dissociation of \(\beta_2\)-AR–induced augmentations of the intracellular Ca\(^{2+}\) (Ca) transient and contractility from cAMP production and PKA-dependent cytoplasmic protein phosphorylation. This can be largely explained by \(G_i\)-dependent functional compartmentalization of the \(\beta_2\)-AR–directed cAMP/PKA signaling to the sarcolemmal microdomain. This compartmentalization allows the common second messenger, cAMP, to perform selective functions during \(\beta_2\)-AR subtype stimulation. Emerging evidence also points to distinctly different roles of these \(\beta\)-AR subtypes in modulating noncontractile cellular processes. These recent findings not only reveal the diversity and specificity of \(\beta\)-AR and G protein interactions but also provide new insights for understanding the differential regulation and functionality of \(\beta\)-AR subtypes in healthy and diseased hearts. (Circ Res. 1999;85:1092-1100.)

Key Words: \(\beta\)-adrenergic receptor subtype ■ G protein ■ cAMP compartmentalization ■ heart failure

Overview: Myocardial \(\beta\)-Adrenergic Receptors

Adrenergic receptor (AR) stimulation by catecholamines provides the most important regulatory mechanism for cardiovascular performance. The adrenergic receptors were classified as \(\alpha\) (for excitatory) and \(\beta\) (for inhibitory) by Ahlquist1 in 1948 on the basis of their functional behavior in blood vessels, ie, vasoconstriction versus vasodilation. Ahlquist’s classification was expanded by Lands et al,2 who recognized that both \(\alpha\) and \(\beta\)-ARs could be further categorized into 2 distinct subtypes based on their relative potencies for the ligands available at that time. The cloning of a cDNA for the human \(\beta_2\)-AR and subsequent studies indicate that the \(\beta\)-ARs are members of the G protein–coupled receptor superfamily, which shares a common feature, the 7-transmembrane-spanning domains.3,4

A dogma of cardiac \(\beta\)-AR signal transduction is that the agonist-bound \(\beta\)-AR selectively interacts with the stimulatory G protein (\(G_s\)), which activates adenyl cyclase, catalyzing cAMP formation. Subsequently, activation of cAMP-dependent protein kinase A (PKA) leads to phosphorylation of regulatory proteins involved in cardiac excitation–contraction (EC) coupling and energy metabolism, including L-type Ca\(^{2+}\) channels, the sarcoplasmic reticulum (SR) membrane protein phospholamban (PLB), myofilament proteins, and glycogen phosphorylase kinase. PKA also phosphorylates and thereby activates an endogenous protein phosphatase inhibitor I, which further ensures the PKA-mediated protein phosphorylation by inhibiting protein phosphatases.5,6 In addition to these acute effects, chronic \(\beta\)-AR stimulation affects multiple cellular functions, including gene transcription, cell growth, and death. After its activation, \(\beta\)-AR signaling is modulated at both the receptor level and downstream of the cascade by the coordinated actions of at least 3 groups of enzymes: G protein–coupled receptor kinases (GRKs), which phosphorylate and thus desensitize the receptor; cyclic nucleotide phosphodiesterases (PDEs), which hydrolyze cAMP; and phosphatases, which dephosphorylate phosphoproteins.

Although several \(\beta\)-AR subtypes have been cloned and pharmacologically characterized, those expressed in cardiomyocytes were initially thought to be exclusively the \(\beta_1\)-AR subtype.2 However, the traditional notion that only \(\beta_1\)-AR modulates cardiac contractile function has been challenged by recent studies that provide compelling evidence that at least both \(\beta_1\)-AR and \(\beta_2\)-AR functionally coexist in cardiomyocytes of many mammalian species, including humans. Many investigators have struggled to understand whether the coexpression of \(\beta\)-AR subtypes represents functional redun-
dancy. An alternative, but equally appealing, concept is that these β-AR subtypes mobilize identical signal transduction pathways but fulfill distinct physiological and pathophysiological roles. Another possibility is that β-AR subtypes elicit distinct cardiac responses through different signaling pathways. Over the past decade, increasing evidence has demonstrated striking qualitative and quantitative differences in the functions and signaling mechanisms of the cardiac β₁-AR and β₂-AR subtypes. In the present review, we will highlight recent advances in cardiac β-AR subtypes (particularly β₂-AR signal transduction) that reveal a new level of diversity and specificity of cardiac β-AR subtype stimulation and provide a conceptual framework for our understanding of the physiological and pathophysiological significance of the coexistence of receptor subtypes.

**Distinct β-AR Subtype Actions in the Heart**

Despite many similarities, β₁-AR and β₂-AR are genetically and pharmacologically distinct entities. The amino acid sequences of human β₁-AR and β₂-AR share only 71% identity in the 7-transmembrane-spanning domains and 54% identity overall. Thus, it is plausible that β-AR subtypes could couple to distinct signal transduction pathways and elicit different cellular responses. In this section, we will examine evidence for qualitative and quantitative differences between β₁-AR and β₂-AR with respect to G protein coupling, cAMP handling, target protein phosphorylation, and modulation of cardiac EC coupling.

**β-AR Subtypes Differentially Regulate Ca²⁺ Handling and Contractility**

Cardiac EC coupling is initiated by a Ca²⁺ influx through voltage-dependent sarcolemmal L-type Ca²⁺ channels during an action potential. This Ca²⁺ influx per se is insufficient to produce a contraction, but it triggers a large Ca²⁺ release from the SR via ryanodine receptors through a Ca²⁺-induced Ca²⁺ release mechanism. The resultant intracellular Ca²⁺ (Caᵢ) transient activates contractile proteins, producing a contraction; Caᵢ is subsequently removed from the cytoplasm by the SR Ca²⁺-ATPase (Ca²⁺-pump) and the sarcolemmal Na⁺-Ca²⁺ exchanger. β-AR stimulation modulates virtually all of these important components of the cardiac EC coupling cascade and therefore plays a prominent role in the regulation of cardiac performance.

There are several striking physiological differences between β₁-AR and β₂-AR subtypes. In rat ventricular myocytes, whereas stimulation of both β-AR subtypes increases L-type Ca²⁺ currents (I₅ᵥ), Caᵢ transients, and contraction amplitude (positive inotropic effect), only β₁-adrenergic stimulation markedly accelerates the Caᵢ transient decay and contractile relaxation (positive lusitropic or relaxant effect). The absence of β₂-AR–mediated cardiac relaxation has been also observed in many other mammalian species (eg, cat and sheep), but human and canine hearts are exceptions. Since β-AR–induced cardiac relaxation is mainly mediated by PKA-dependent phosphorylation of PLB, an SR membrane protein, the different relaxant effects of β-AR subtypes may largely be attributable to their distinct effects on PLB phosphorylation (see below). Additionally, β₁-AR but not β₂-AR stimulation reduces Caᵢ-myofilament interaction. The differential regulation of myofilament sensitivity to Ca²⁺ by β-AR subtypes may be also related to the β₁-AR–induced phosphorylation of myofilament proteins (see below), which inhibits the myofilament response to Ca²⁺. Thus, both an increased SR Ca²⁺ uptake and a decreased myofilament Ca²⁺ sensitivity contribute to the relaxant effect after stimulation of β₁-AR but not β₂-AR. Concomitant with the enhanced SR Ca²⁺ recycling, only β₁-AR stimulation increases the resting cytosolic Ca²⁺ and the likelihood of spontaneous Ca²⁺ oscillations in several mammalian species. This observation suggests that β₁-ARs may be more prone than β₂-ARs to elicit Ca²⁺-dependent arrhythmias. These functional differences between β₁-AR and β₂-AR stimulation suggest that there may be substantial differences in their intracellular signal transduction pathways.

**β₂-AR–Stimulated Caᵢ and Contractile Responses Can Be Dissociated From Global Increases in cAMP and PKA-Mediated Phosphorylation of Cytoplasmic Proteins**

In rat ventricular myocyte preparations, the dose-response curve of total cAMP accumulation induced by β₂-AR stimulation with norepinephrine (NE) overlaps that induced by β₂-AR stimulation with zinterol. However, the maximal increase in the particulate cAMP induced by zinterol is ~50% of that caused by β₁-AR, suggesting differential compartmentalization of cAMP after β₂-AR subtype stimulation. Surprisingly, the β₂-AR–stimulated increases in the particulate and total cAMP levels are apparently dissociated from the positive inotropic effect or the increase in Caᵢ transients in adult rat myocytes; in contrast, β₁-AR–stimulated increase in cAMP in either fraction is closely correlated with the cardiac functional changes. A more striking example of this dissociation has been observed in canine cardiomyocytes, in which the β₂-AR–mediated augmentation of the Caᵢ transient or contraction occurs in the absence of a measurable elevation of cAMP production.

To elucidate β-AR subtype signaling downstream from cAMP, the PKA activity in both soluble and particulate fractions and the phosphorylation state of major regulatory proteins involved in cardiac EC coupling or in energy metabolism have been systematically examined in canine hearts. Stimulation of β₂-AR fails to increase PKA activity in either fraction. In both rat and canine ventricular myocytes, β₁-AR activation has only a negligible effect on PLB phosphorylation, relative to that after β₂-AR activation. These results obtained from single isolated cardiomyocytes have been recently validated in intact animals. Anesthetized dogs exhibited positive chronotropic, inotropic, and lusitropic effects during β₂-AR stimulation, and these effects occur without a detectable increase in PKA activation or PKA-dependent phosphorylation of nonsarcolemmal target proteins, such as glycogen phosphorylase kinase in the cytoplasm, troponin I and C proteins in myofilaments, and PLB in the SR membrane. In contrast, β₁-AR–stimulated cardiac effects are well correlated with an increase in phosphorylation of these key regulatory proteins. Thus, the lack of PKA-mediated protein phosphorylation during β₂-AR...
stimulation in rat and dog hearts clearly indicates that, unlike β₁-AR, β₂-AR does not transmit its signal to cytoplasmic regulatory proteins, at least in these species. The reason is unclear for the PLB and troponin I phosphorylation-independent lusitropic effect of β₂-AR stimulation in canine myocytes.

**Localized cAMP Signaling During Cardiac β₂-AR Stimulation**

The aforementioned observations demonstrate that β₂-AR signaling modulates Iᵥ, but cannot phosphorylate regulatory proteins remote from the cell surface membrane, suggesting that β₂-AR signaling is tightly localized near the subsarcolemmal microdomain, in the vicinity of L-type Ca²⁺ channels (Figure). More direct evidence supporting localized β₂-AR signaling has emerged from on-cell patch-clamp single L-type Ca²⁺ channel recordings. Although the effect of β₁-AR stimulation is rather diffusive, the effect of β₂-AR stimulation is extremely localized: the L-type Ca²⁺ channel responds only to local (agonist included in pipette solution) but not remote (agonist added in bathing solution) β₂-AR stimulation. These results are in general agreement with the observation that in frog cardiomyocytes, in which the β₂-AR subtype predominates, a local β-AR stimulation by isoproterenol applied to one end of the cell has little stimulatory effect on remote L-type Ca²⁺ channels. These results, particularly those in canine hearts, initially evoked doubts as to whether β₂-AR cardiac response is mediated by a cAMP-dependent signaling pathway. It has been proposed that the cardiac effects of β-AR (subtype not specified) might be, in part, mediated by a direct interaction between Gᵢα subunits and L-type Ca²⁺ channels. However, accumulating evidence indicates that the effect of β-AR stimulation on cardiac Iᵥ is mediated exclusively by a cAMP-dependent mechanism. To delineate a role of cAMP-dependent PKA activation in β-AR subtype signaling, specific PKA inhibitors, including Rp-cAMP, H-89, and a peptide PKA inhibitor (PKI), have been used. Most studies, except one in adult rat myocytes, have demonstrated that PKA inhibitors (Rp-cAMP and H-89) not only block the effect of β₂-AR stimulation but also completely reverse the effects of β₂-AR. Similarly, in human and frog cardiac myocytes, the β₂-AR–induced augmentation of Iᵥ is totally prevented by PKI. Taken together, several lines of evidence strongly support the idea that cAMP-dependent PKA activation is obligatory for β₂-AR–mediated cardiac responses, but this β₂-AR–stimulated cAMP/PKA signaling in some species is highly localized to the surface membrane and cannot transmit to nonsarcolemmal proteins (Figure).

**Dual Coupling of β₂-AR to Gᵣ and Gᵢ Proteins**

In many biological systems, Gᵣ and Gᵢ proteins engage in cross talk with each other. This cross talk is usually mediated through different receptor families. For example, activation of muscarinic or adenosine receptors, prototypic Gᵢ-coupled receptors, markedly antagonizes the positive inotropic effect of β-ARs. Interestingly, early work in lipid vesicles had demonstrated a physical potential for a single receptor to couple promiscuously to more than one class of G proteins. β-AR, for instance, couples to both Gᵣ and Gᵢ proteins. Using a photoaffinity labeling technique, recent studies have demonstrated such promiscuous G protein coupling of β₂-AR in intact cardiomyocytes, as manifested by a β₂-AR–stimulated incorporation of a photoreactive GTP analogue, [³²P]GTP-azidoanilide, into α subunits of Gᵣ and Gᵢ, in addition to Gᵣ. The maximal effect of β₂-AR stimulation on Gᵣ proteins is comparable to that of carbachol, a muscarinic receptor agonist. Pertussis toxin (PTX) pretreatment or the β₂-AR antagonist (ICI 118,551) prevents the β₂-AR–mediated Gᵣ activation. Because β₁-AR stimulation is not able to increase Gᵣ activity, the coupling to Gᵣ is specific for β₂-AR subtype. Thus, β₂-AR signaling exemplifies a unique mode of receptor–G protein interaction; ie, a given receptor simultaneously activates more than one class of G proteins routing to functionally opposing pathways. Furthermore, β₁-AR and β₂-AR exhibit subtype-selective coupling to Gᵣ. A more recent in vitro study has revealed that β₂-AR couples to different split variants of Gᵣ and that the β₂-AR coupled to the long splice variant displays ligand-independent constitutive activity. Mechanisms underlying the differential coupling of β-AR subtypes to G proteins are not well understood. At the molecular level, studies on chimeric or mutated G protein–coupled receptors (including the major subtypes of adrenergic receptors) have shown that the third cytoplasmic loop that connects transmembrane domains V and VI of these receptors is an important determinant for G protein coupling. For example, replacement of the cytoplasmic loop of muscarinic receptor with that of β₂-AR can induce Gᵣ activation in response to muscarinic agonists. It has also been shown that a proline-rich region of the third intracellular loop determines the different Gᵣ coupling and sequestration of β₁-AR versus β₂-AR coupling.
β2-AR.33 Thus, the differences between β1-AR and β2-AR in G protein coupling could be eventually ascribed to some critical differences in the sequences of the cytoplasmic loop of the receptors. Recent evidence also suggests a potential role of posttranslation receptor modification in the receptor/G protein coupling. In HEK293 cells, PKA-mediated phosphorylation of β2-AR switches the receptor coupling preference from Gs to Gi.38 At the cellular level, it has been suggested that β-AR subtypes are located in 2 distinct cellular fractions after agonist stimulation: β1-AR in caveolae and β2-AR in coated pits.39,40 The difference in the subcellular distribution of β-AR subtypes may also contribute to their distinct G protein coupling and differences in signaling. In other words, β2-ARs but not β1-ARs might be geometrically colocalized with the G proteins.

Involvement of β2-AR/Gi Coupling in Local Control of β2-AR-Stimulated cAMP Signaling

Functional localization of cAMP/PKA signaling could be ascribed to compartmentalization of cAMP or PKA per se (because of a localized activation of adenylyl cyclase or PDE)23,41 or to specific anchoring proteins of PKA.42,43 A close spatial association of L-type Ca2+ channels with adenylyl cyclase and PKA42,44,45 would provide a structural basis for the β2-AR–mediated cAMP-dependent local regulation of the channel. Nevertheless, in view of the fact that both cAMP and active PKA catalytic subunits are readily diffusible, additional constrictive mechanisms must be involved in the local control of the β2-AR cAMP/PKA signaling. In principle, this local control could also arise from counter–signal transduction pathways that locally negate the cAMP/PKA signaling.

Involvement of β2-AR–coupled Gi signaling in the compartmentalization of the Gi-mediated cAMP/PKA signaling has been investigated by using PTX to inhibit Gi functions. In rat ventricular myocytes, PTX treatment, which has no significant effect on baseline parameters, specifically potentiates the β2-AR–mediated positive inotropic effect.46,47 More remarkably, PTX treatment permits β2-AR stimulation to induce a robust dose-dependent increase in PLB phosphorylation,48 accompanied by a marked relaxant effect.46,48 Thus, inhibition of Gi proteins by PTX causes β2-AR signaling to closely resemble that of β1-AR. In contrast, the β1-AR–mediated effects on cardiac EC coupling in rat ventricular myocytes are insensitive to PTX pretreatment,46,48 consistent with the inability of β1-AR to activate Gi proteins.78 These studies indicate that at least in rat cardiomyocytes, β2-AR/Gi coupling underlies the functional compartmentalization of the β2-AR/Gi–directed cAMP/PKA signaling, which may largely account for the qualitative and quantitative differences between β1-AR– and β2-AR–mediated cardiac actions.

It has been noted that in rat ventricular myocytes, PTX treatment has no significant effect on the β2-AR–mediated global cAMP accumulation27 or PKA activation.48 The simplest explanation for these observations is that the cross talk of β2-AR–coupled Gi and Gs signaling occurs downstream from PKA rather than at the G protein or adenylyl cyclase level (Figure). In this regard, some evidence suggests that typical Gi-coupled receptors, such as the muscarinic receptor M2 or adenosine receptor A1, counteract the effect of PKA, in part, via activation of protein phosphatases.99,50 The role of protein phosphatases in β2-AR/Gi signaling has been recently suggested.48 Alternatively, other evidence indicates that PDE is involved in the compartmentalization of β2-AR–mediated cAMP signaling in canine41 and frog23 cardiac myocytes. Whether β2-AR/Gi coupling activates protein phosphatases, PDE, or other unknown second messengers to functionally compartmentalize the β2-AR/Gi–directed cAMP/PKA signaling awaits further study (Figure).

Possible Role of pH, in Mediating the β2-AR Positive Inotropic Response

Recently, a G protein–independent mechanism underlying a β2-AR–mediated cellular response has been demonstrated.51 Specifically, β-AR agonists can induce a direct physical association of Na+/H+ exchange (NHE) regulatory factor (NHERF), an inhibitor of Na+/H+ exchanger type 3 (NHE3), to the C-terminus of β2-AR, relieving the inhibitory effect of NHERF on NHE3. Thus, β2-AR stimulation can exert opposing effects on NHE3 activity: a stimulatory effect induced by the direct association of β2-AR with NHERF and an inhibitory effect mediated by PKA-dependent phosphorylation of NHERF, thereby activating NHERF. The relevance of this phenomenon to β2-AR signaling in cardiomyocytes, however, has not yet been explored. It has been proposed that β2-AR activation in adult rat ventricular myocytes increases pHi, in a PKA-independent and Na+/H+–independent manner, resulting in an enhanced contractile response.29 In contrast, most studies have found that the β2-AR–mediated positive inotropic effect is closely related to a proportional increase in Ca2+, transient or Ica,8,13,14,19,27,28 and can be completely reversed by specific PKA inhibitors.14,27,28,48 Quantitative studies are required to determine the relative contributions of changes of pH, and Ca, to the β2-AR contractile response.

Diversity of Cardiac β2-AR Signaling Among Mammalian Species

In addition to the differences in the behavior of β-AR subtypes within species, there is also a great diversity in β2-AR–mediated cardiac responses among species. At one extreme, in murine and guinea pig cardiomyocytes, β2-AR stimulation normally elicits no significant contractile response.28,52 In mouse cardiomyocytes, PTX treatment unmask a de novo β2-AR–mediated contractile response.28 At the other extreme, in the chronically failing human atrium and ventricle, β2-AR stimulation induces positive inotropic and lusitropic effects and phosphorylation of regulatory proteins.11,12 Between these extremes, in isolated rat and canine ventricular myocytes, β2-AR–mediated Ica, Ca, transient, and contractile responses are present but can be further enhanced by PTX treatment.46,48 It is possible that this species-dependent diversity of cardiac β2-AR signaling may be largely accounted for by differences in the extent of β2-AR/Gi coupling among species. For example, the β2-AR/Gi coupling would be expected to be extremely robust in mouse heart, but it might be less efficient in human hearts compared with mouse and rat hearts. It should be cautioned that most human studies have been conducted in preparations from chronically
failing hearts, and to date, no data are available on the effect of PTX on β2-AR responsiveness in normal human myocardial preparations. Thus, it is not clear whether these pathophysiological conditions, such as chronic heart failure, influence the β2-AR coupling to G, versus to G1 proteins.

It is noteworthy that avian species appear to use markedly different signal transduction pathways. For example, in embryonic chick ventricular myocytes, β2-AR stimulation by zinterol increases the Ca(2+) transient and contractility by increasing arachidonic acid, which is negatively regulated by cAMP/PKA signaling pathway.53

**Developmental Changes in Cardiac β-AR Subtype Signaling**

In contrast to adult rat myocytes, β2-AR stimulation by zinterol in neonatal rat ventricular myocytes leads to increased intracellular cAMP accumulation and enhanced phosphorylation of PLB and troponin I, associated with an increase in the amplitude and an acceleration of the kinetics of the contraction and Ca transient.54 All of these responses are similar to those elicited by β1-AR stimulation in neonatal rat myocytes. Interestingly, the dose-response curve of contraction in response to β2-AR stimulation by zinterol is shifted ~2 orders of magnitude leftward in neonatal myocytes compared with adult myocytes. Thus, β2-ARs may play a more important role in mediating the response to catecholamines in the noninnervated neonatal (or transplanted) heart than in the innervated adult heart. This developmental change in cardiac β2-AR responsiveness is not due to a higher proportion of β2-ARs in the neonatal heart, because the ratio of β2-ARs to β1-ARs is similar in both preparations.54 It is noteworthy that the contractile dose-response relation to the β2-AR agonist zinterol in neonatal rat myocytes is similar to that in PTX-treated adult rat myocytes.54 Thus, it might be speculated that the β2-AR coupling to G, proteins might be acquired or reinforced during development, and this could explain the greater sensitivity of neonatal β2-AR stimulation in the absence of PTX. In other words, in neonatal rat myocytes, the β2-AR/G coupling is lacking or insufficient to negate the G1-mediated relaxant effect and phosphorylation of regulatory proteins.

**β2-AR Signal Transduction in Genetically Manipulated Murine Models**

Rapid advances in mouse genetics have provide powerful tools to unravel the molecular secrets that govern cardiovascular structure and function in health and disease. Several transgenic mouse models have been developed to manipulate β-AR signal transduction pathways.55–58 In addition, β1-AR or β2-AR and β1-/β2-AR double-knockout mice have recently been generated.59–61 In this section, we will briefly discuss some principles and lessons learned from these transgenic and gene-targeted mice. Discussion involving genetic manipulation and β-AR signaling in the setting of cardiac hypertrophy and heart failure will be deferred to “Implications of β2-AR Signaling in Heart Failure.”

**Spontaneous β2-AR Activation**

In TG4 transgenic mice, cardiac-specific overexpression of β2-AR by ~200-fold leads to an agonist-independent enhancement in both the baseline adenyl cyclase activity and myocardial contractility in vivo55 and in isolated atria57 or single cardiomyocytes.28 This transgenic model opens a new avenue in the study of ligand-free β2-AR signaling. According to the prevailing 2-state model, G protein–coupled receptors, like β-ARs, exist in an equilibrium between 2 functionally and conformationally distinct states: an inactive conformation (R) and an active conformation capable of activating G proteins (R*).55,57,62 In the absence of a receptor ligand, the receptor can undergo a spontaneous transition to the activated state; the equilibrium between R and R* sets the level of basal receptor activation. Thus, an overexpression of a given receptor would be expected to proportionally increase the number of R* state receptors. Receptor agonists have a higher affinity for R*, thereby shifting the equilibrium to the active conformation R*. Neutral antagonists bind with equal affinity to R and R*, and therefore inhibit receptor signaling without altering the equilibrium between R and R*. A third class of receptor ligands known as inverse agonists preferentially binds to R, driving the equilibrium toward the inactive conformation R. As shown in TG4 mice, the enhanced basal cardiac adenylyl cyclase activity and contraction are reversed by a β2-AR inverse agonist, ICI 118,551, both in vivo and in vitro.28,55,57 At the moment, it is unclear whether β1-AR and β2-AR have similar abilities to undergo spontaneous activation.

**Spontaneous and Agonist-Induced Active β2-ARs Exhibit Different Signaling**

Although a 2-state model for the adrenergic receptor is sufficient to explain many aspects of β2-AR activation, multiple active functional states of the receptor have been demonstrated.63–65 This idea is reinforced by several important differences between spontaneously activated β2-ARs and agonist-stimulated β2-ARs in TG4 cardiomyocytes. First, whereas spontaneously active β2-ARs significantly increase the baseline contractility of the TG4 heart, β2-AR agonists, at maximal concentrations, are unable to further increase contraction amplitude, even though the contractility is not yet saturated. Second, whereas β2-AR agonists induce a marked increase in ICa, ligand-independent constitutive β2-AR activation increases cardiac contractility but cannot modulate ICa.66 Finally, spontaneously activated β2-ARs and agonist-stimulated β2-ARs may differentially couple to G, proteins, because PTX robustly enhances the β2-AR agonist–mediated contractile response but only slightly enhances the effect of spontaneous β2-AR signaling on basal contractility in TG4 heart cells.28 These occurrences suggest that spontaneously active β2-ARs and agonist-activated β2-ARs may represent functionally distinct conformational states of the receptor.

**Compensatory Changes in Genetically Manipulated Animal Models**

A peculiar feature of TG4 mice is that β2-AR overexpression causes a marked reduction in PLB expression, which contributes to the accelerated basal cardiac relaxation.67 Additionally, in transgenic mice overexpressing Gαs, there is a significant upregulation of β-adrenergic receptor kinase (βARK).68 In fact, in many transgenic models, a specific or even a global
remodeling process such as hypertrophy is accompanied by
the upregulation or downregulation of a gene or set of
genes60,70 (for a review, see Reference 71). Although these
compensatory changes might be beneficial for the animal’s
survival, they introduce complications in understanding their
phenotypes. Thus, caution should be exercised when drawing
a direct mechanistic link between genetic manipulations and
phenotypes.

Implications of \( \beta_2 \)-AR Signaling in Heart Failure

A large body of evidence has demonstrated that the cardiac
response to \( \beta \)-AR stimulation decreases in chronically failing
hearts in human and animal models, and that there is a
positive correlation between increased plasma catecholamine
levels and the degree of the diminution of the \( \beta \)-AR re-
response.72–75 The demonstration of promiscuous \( \beta_2 \)-AR cou-
pling to Gs and Gi also provides new insights into the
pathogenesis of heart failure.

Distinct Phenotypes Induced by Chronic \( \beta_1 \)-AR
Versus \( \beta_2 \)-AR Stimulation

The \( \beta \)-AR subtypes have markedly different chronic effects
on cardiac hypertrophy, as manifested by the distinct phen-
types of transgenic mice overexpressing cardiac \( \beta_1 \)-AR versus
\( \beta_2 \)-AR. For example, vast overexpression of cardiac
\( \beta_2 \)-AR by \( \approx \)200-fold does not induce detectable cellular
hypertrophy or heart failure, at least in the short term.28,55,57
In contrast, low level (5- to 15-fold) overexpression of \( \beta_2 \)-AR
results in cardiac hypertrophy and heart failure.58 This pheno-
type is similar to that obtained by transgenic overexpres-
sion of cardiac Gs in mice76 or by chronic \( \beta \)-AR stimulation
by agonist infusion.77,78 Furthermore, heart-specific overexpres-
sion of \( \beta_2 \)-AR at \( \approx \)30-fold not only rescues ventricular
function but also reverses cardiac hypertrophy induced by
transgenic overexpression of Gs.79 Emerging evidence also
indicates that these \( \beta \)-AR subtypes even have opposing
effects on apoptosis in cultured rat cardiomyocytes: \( \beta_1 \)-AR
stimulation induces apoptosis,90,91 whereas \( \beta_2 \)-AR stimulation
inhibits apoptosis.81,82 These studies shed new light on
whether long-term \( \beta \)-AR stimulation is beneficial. Answering
this question may require discriminating \( \beta \)-AR subtypes,
because \( \beta_1 \)-AR and \( \beta_2 \)-AR have distinct, even opposite,
chronic effects.

Differential Regulation of \( \beta_1 \)-AR Versus \( \beta_2 \)-AR in
Failing Hearts

Except for mitral valve disorder–induced heart failure,83 most
studies involving end-stage human heart failure73–75,84 and
some heart failure animal models72–85 have shown a selective
decrease in \( \beta_1 \)-AR number with little or no loss of \( \beta_2 \)-AR. The
selective reduction in \( \beta_1 \)-AR density may be attributed to
elevated plasma levels of NE, which has a higher affinity for
\( \beta_1 \)-AR.7 In the line of the distinct phenotypes of transgenic
overexpression of \( \beta_1 \)-AR versus \( \beta_2 \)-AR, the selective down-
regulation of \( \beta_1 \)-AR may reflect a protective mechanism in
the failing heart. However, the \( \beta_2 \)-AR–mediated cardiac
response, similar to that of \( \beta_1 \)-AR, is also significantly
diminished.73–75,84,85 Next, we will discuss possible role of
\( \beta_2 \)-AR/Gi coupling in the loss of \( \beta_2 \)-AR contractile response
in heart failure.

Upregulation of Gi Proteins in Heart Failure

Studies in rat and guinea pig have shown that chronic
infusion of catecholamines increases the expression of Gs,86,87
In transgenic mice, in which the human \( \beta_2 \)-AR is overex-
pressed, the Gi protein abundance is also significantly en-
hanced.28 These results suggest that chronic \( \beta \)-AR stimulation
increases Gi expression. Increasing evidence has demonstrated
that heart failure is associated with elevated plasma catechol-
amine levels, which could result in an increase in Gi protein
abundance or function. Indeed, in chronic heart failure in both
humans88,89 and animal models,72 marked increases in Gi
mRNA levels, PTX-induced ribosylation, and Gi abundance
have been reported. Because the coupling of \( \beta_2 \)-AR to Gi
proteins negatively regulates the Gi–mediated contractile
response in the heart of many mammalian species,27,28,46–48
the enhanced Gi signaling might serve as a mechanism
underlying the diminution of the \( \beta_2 \)-AR–stimulated positive
inotropic effect. In human chronic heart failure, some evi-
dence suggests that Gs proteins may be also involved in the
diminution of \( \beta_2 \)-AR contractile response.90

G Protein–Coupled Receptor Kinases in
Failing Hearts

Agonist-dependent desensitization can be initiated by phos-
phorylation of activated receptors by members of the GRK
family.91,92 \( \beta \)-ARK1 is a prototypic GRK that has been shown to
phosphorylate activated \( \beta_2 \)-AR and \( \beta \)-AR subtypes in vitro.92,93 Phosphorylated receptors become binding sub-
strates for a class of inhibitor proteins, \( \beta \)-arrestins, which
inhibit further G protein coupling.94 Recently, it has been
demonstrated that overexpression of a second myocardial
GRK, \( \beta \)-ARK5, in transgenic mice also leads to significant
heart failure, the levels and enzymatic activity of \( \beta \)-ARK1 are signifi-
cantly elevated.96 In heart failure animal models, \( \beta \)-ARK1 activity is
also correlated with \( \beta \)-AR responsiveness.97 Transgenic mice
with myocardial overexpression of \( \beta \)-ARK1 (3- to 5-fold)
have a blunted contractile response to \( \beta \)-AR stimulation by
isoproterenol96–98 or NE.99 More strikingly, a genetic model of
urine heart failure (MLP\(-/-\)) can be largely reversed by an
overexpression of \( \beta \)-ARK1 inhibitor.100 Taken together,
chronic heart failure is associated with a marked increase in
Gi proteins, a selective downregulation of \( \beta_1 \)-AR (higher
\( \beta_2 \)-\( \beta_1 \)), and an increased expression and activity of GRK2
(\( \beta \)-ARK1). The exaggerated \( \beta_2 \)-AR/Gi signaling and the en-
hanced \( \beta \)-ARK1 activity may contribute to the heart failure–
associated dysfunction of \( \beta \)-AR stimulation and play a
critical role in the pathogenesis of heart failure.

Summary and Perspectives

In summary, recent studies have demonstrated that the
cardiac \( \beta_1 \)-AR and \( \beta_2 \)-AR are markedly different regarding to
G protein coupling, cAMP handling, target protein phosphor-
ylation, and modulation of cardiac EC coupling. Many of
these differences can be explained by the additional coupling
of \( \beta_2 \)-AR to Gi proteins, which, in some species and devel-
opmental stages at least, functionally localizes the β2-AR/Gi-mediated cAMP/PKA signaling to the subsarcolemmal microdomain. This β2-AR/Gi pathay may also contribute to the distinct phenotypes of overexpression of cardiac β2-ARs versus β2-ARs in transgenic mouse models. Thus, it is necessary and important to distinguish β2-ARs from β1-ARs with regard to their physiological and pathophysiological roles.

Many important questions remain to be answered. Further studies are required to determine the molecular, structural, and microarchitectural bases underlying the differential G protein coupling of β2-AR versus β1-AR. Information on downstream signaling of β2-AR–coupled Gi proteins only begins to emerge. In addition, studies of the chronic noncontractile effects of cardiac β-AR subtype stimulation, eg, on cardiac cell growth and death, will broaden and deepen our understanding of the differential regulation and functionality of β-AR subtypes in health and disease. Finally, the possible role of β2-AR/Gi coupling in the pathogenesis of chronic heart failure merits future investigation.

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