β-Adrenergic Signaling in the Heart: Dual Coupling of the β_2-Adrenergic Receptor to G_s and G_i Proteins

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β-adrenergic receptor (AR) subtypes are archetypical members of the G protein-coupled receptor (GPCR) superfamily. Whereas both β_1AR and β_2AR stimulate the classic G_s-adenylyl cyclase-3′,5′-adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling cascade, β_2AR couples to both G_s and G_i proteins, activating bifurcated signaling pathways. In the heart, dual coupling of the β_2AR to G_s and G_i results in compartmentalization of the G_s-stimulated cAMP signal, thus selectively affecting plasma membrane effectors (such as L-type Ca^{2+} channels) and bypassing cytoplasmic target proteins (such as phospholamban and myofilament contractile proteins). More important, the β_2AR-to-G_i branch delivers a powerful cell survival signal that counters apoptosis induced by the concurrent G_s-mediated signal or by a wide range of assaulting factors. This survival pathway sequentially involves G_i, Gβγ, phosphoinositide 3-kinase, and Akt. Furthermore, cardiac-specific transgenic overexpression of βAR subtypes in mice results in distinctly different phenotypes in terms of the likelihood of cardiac hypertrophy and heart failure. These findings indicate that stimulation of the two βAR subtypes activates overlapping, but different, sets of signal transduction mechanisms, and fulfills distinct or even opposing physiological and pathophysiological roles. Because of these differences, selective activation of cardiac β_2AR may provide catecholamine-dependent inotropic support without cardiotoxic consequences, which might have beneficial effects in the failing heart.

Overview

G protein-coupled receptors (GPCRs) constitute the largest class of cell surface signaling molecules in eukaryotes and some prokaryotes. They share a common overall structure feature: seven hydrophobic transmembrane helical domains. In the worm *Caenorhabditis elegans*, GPCR-encoding genes constitute 5% of the genome with ~1100 members (*1*), whereas there are more than 700 GPCRs in the human genome (*2*). By activating their cognate heterotrimeric guanosine triphosphate (GTP) binding proteins (G proteins), GPCRs transduce stimulatory or inhibitory signals for a wide array of endogenous hormones and neurotransmitters, and ambient physical and chemical stimuli, as well as exogenous therapeutic reagents. Occupation of these receptors with agonists promotes guanosine diphosphate—guanosine triphosphate (GDP-GTP) exchange on the Gα subunit and subsequent dissociation of Gα from Gβγ, leading to activation of Gα and release of free Gβγ heterodimers (*3-6*). Both Gα and Gβγ then serve as signaling mediators to directly interact with a variety of effector proteins, including enzymes and ionic channels (*7, 8*).

Intracellular propagation of GPCR signaling is an intricate process orchestrated by a myriad of G proteins. In mammals, there are at least 27 Gα, 5 Gβ, and 13 Gγ subtypes (*9*). On the basis of the primary sequences of the Gα subunits, G proteins can be divided into four families: G_s, G_i, G_q, and G_{12} (*10*). Historically, specificity and selectivity in GPCR signaling was thought to be achieved by coupling of a given GPCR to a single class of G proteins. As the prototypical GPCR, β-adrenergic receptor (AR) was found to interact exclusively with G_s, which in turn activated adenylyl cyclase (AC), catalyzing 3′,5′-adenosine monophosphate (cAMP) formation. Subsequently, activation of cAMP-dependent protein kinase (PKA, also known as A-kinase) would lead to phosphorylation of target proteins. This paradigm, however, has been shifted with the finding that multiple GPCRs can couple to more than one G protein pathway. Whereas the β_1AR subtype appears to stimulate solely the G_s pathway, compelling evidence indicates that β_2AR conducts a duet of signaling that includes both G_s and G_i. The additional G_i pathway not only reshapes the spatiotemporal pattern of the G_s-AC-cAMP signaling, but also delivers G_s-independent signals. These βAR subtypes are currently believed to fulfill distinct, sometimes even opposite, physiological and pathological roles.

This review will highlight recent advances in our understanding of signal transduction by these βAR subtypes, particularly β_2AR, in the heart. It will attempt to unravel the intricacies of how multiple signals are compartmentalized and integrated in space and time to achieve diversity and specificity for GPCR signaling. Furthermore, a conceptual framework for understanding the physiological and pathophysiological relevance of the coupling of GPCRs to multiple G proteins and of the coexistence of receptor subtypes will be provided.

Dichotomous Coupling of Native β_2AR to G_s and G_i in the Heart

Cardiac tissue expresses at least two subtypes of βAR: β_1AR and β_2AR. In the heart, nonselective βAR stimulation activates the G_s-AC-cAMP cascade, leading to PKA-dependent phosphorylation of a set of regulatory proteins involved in cardiac excitation-contraction coupling and energy metabolism, including L-type Ca^{2+} channels, the sarcoplasmic reticulum (SR) membrane protein phospholamban (PLB), myofilament proteins, and glycogen phosphorylase kinase. A hallmark of β_1AR or mixed

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βAR stimulation is to increase cardiac contractility (positive inotropic effect), accelerate cardiac relaxation (positive lusitropic effect), and increase heart rate (positive chronotropic effect). However, in adult rat ventricular myocytes, although stimulation of both βAR subtypes increases the amplitudes of L-type Ca^{2+} currents (I_{Ca}), intracellular Ca^{2+} transient, and contraction strength, β_2AR stimulation fails to accelerate the decay of the intracellular Ca^{2+} transient and the contractile relaxation (*11, 12*). The absence of β_2AR-mediated relaxation occurs in many other mammalian species, including cats and sheep (*13, 14*), but not in humans and dogs (*15-18*). These observations provide the first clue that there can be substantial differences in intracellular signal transduction pathways initiated by β_2AR, compared to those activated by β_1AR.

In the search for the answer to the "anomalous" behavior of cardiac β_2AR stimulation, studies over the past decade have provided evidence for the coupling of native β_2AR to at least two pathways under physiological conditions. Studies using physiological conditions at the single-cell level demonstrated that disrupting G_i signaling by pertussis toxin (PTX)-mediated G_i ribosylation markedly enhances β_2AR-induced contractile response in rat and mouse ventricular myocytes (*19, 20*). These results suggest that PTX-sensitive G_i proteins may partially negate the G_s-mediated contractile response in cardiac myocytes. Moreover, photoaffinity labeling of G proteins with [^{32}P]azidoanilide-GTP in conjunction with immunoprecipitation of endogenous G proteins with antibodies specific for $G\alpha_s$ and $G\alpha_i$ provided direct biochemical evidence that native β_2AR interacts with both G_s and G_i (specifically, G_{i2} and G_{i3}) signaling pathways in freshly isolated adult mouse cardiac myocytes (*20*). The maximal effect of β_2AR stimulation on the G_i proteins in the mouse cardiomyocytes is comparable to that induced by carbachol, a muscarinic acetylcholine M_2 receptor agonist (*20*). PTX treatment or application of a β_2AR antagonist (ICI 118,551) prevents the β_2AR-mediated activation of G_i. Under the same experimental conditions, β_1AR stimulation does not increase G_i activity; thus, the coupling to G_i is specific for β_2AR (*20*). Similarly, in human myocardium, cardiac G_i is activated by stimulation of β_2AR; this property is not shared by β_1AR (*21*). Thus, whereas β_1AR activates only the G_s pathway, β_2AR can activate both G_s and G_i signaling pathways.

Several other GPCRs, including histamine, serotonin, and glucagon receptors, stimulate both G_s and G_i proteins in human heart (*21*). Thus, multiple G protein coupling appears to be rather common, albeit not universal, among GPCRs. These findings raise important questions regarding the consequences of coupling one receptor to multiple G proteins in physiological and pathophysiological contexts.

β_2AR-to-G_i Signals Compartmentalize G_s-Mediated cAMP Signaling

A species-dependent diversity has been documented with respect to β_2AR-stimulated cAMP accumulation and PKA activation. In the human heart, β_2AR stimulation efficiently increases cellular cAMP and PKA-dependent phosphorylation of intracellular regulatory proteins [PLB, troponin-I (TnI), and C protein], similar to β_1AR stimulation (*15, 16, 22*). In freshly isolated canine ventricular myocytes and cultured 18-day embryonic mouse cardiomyocytes, however, β_2AR elevates neither total cellular cAMP nor PKA activity, whereas β_1AR induces a robust increase in cAMP accumulation under the same experimental conditions (*17, 18, 23*). Between these extremes, in freshly isolated rat ventricular myocytes, the dose-response of cAMP to β_2AR overlaps that to β_1AR stimulation (*12, 24, 25*). Nevertheless, both biochemical evidence and biophysical evidence indicate that β_1AR-generated cAMP signaling can broadcast throughout the cell, whereas β_2AR-initiated cAMP signaling is confined to subsarcolemmal microdomains (*26*). Specifically, in adult rat and canine hearts, β_1AR stimulation increases phosphorylation of PLB, which accelerates Ca^{2+} sequestration into SR, resulting in accelerated cardiac relaxation (*11, 17-19, 24, 27, 28*). β_1AR stimulation also promotes phosphorylation of TnI and C protein (*18*), which reduces myofilament sensitivity to Ca^{2+}. In contrast, β_2AR stimulation modulates specifically sarcolemmal L-type Ca^{2+} channels without affecting the aforementioned intracellular regulatory proteins in these species (*11, 17-19, 24, 27, 28*). Furthermore, experiments with patch-clamp single-channel recordings showed that in rat cardiomyocytes, β_2AR stimulation modulated single L-type Ca^{2+} channel activity only in a local mode (agonist included within the patch pipette with tip diameter ~1.0 μm) and not in a remote mode (agonist perfused outside the patch), whereas β_1AR stimulation acted in either mode (*29*). These results are in general agreement with the observation that in frog cardiomyocytes, in which the β_2AR subtype predominates (*30*), local βAR stimulation by isoproterenol applied to one end of the cell has little stimulatory effect on L-type Ca^{2+} channels residing on the other end (*31*).

These studies initially evoked doubts as to whether the β_2AR cardiac response is mediated by a cAMP-dependent signaling pathway. One theory proposed is that the cardiac effects of βAR (subtype not specified) might be, in part, mediated by a direct interaction between $G\alpha_s$ and L-type Ca^{2+} channels (*32, 33*). However, in other studies, except one in rat ventricular myocytes (*34*), specific PKA inhibitors, including a peptide inhibitor (PKI), an inactive cAMP analog (RP-cAMP), and a synthetic compound (H-89), not only blocked the effects of β_1AR stimulation, but also completely inhibited the effects of β_2AR stimulation (*18, 20, 27, 29*). [Although H-89 has been widely used as a PKA inhibitor, recent studies indicate that H-89 is also a potent blocker of both βAR subtypes (*35*)]. The results obtained through PKA inhibition corroborate the notion that the effect of nonselective βAR stimulation by isoproterenol on cardiac I_{Ca} is mediated exclusively by a cAMP-dependent mechanism. Specifically, the I_{Ca} response to isoproterenol is ablated by PKI (*36*). Hence, the modulation of I_{Ca} by β_2AR should require cAMP-dependent PKA activation, but this β_2AR-stimulated cAMP-to-PKA signaling appears to be tightly localized to the surface membrane in the vicinity of L-type Ca^{2+} channels and cannot be transmitted to nonsarcolemmal proteins (Fig. 1).

Several lines of evidence indicate that activation of the β_2AR-to-G_i signaling pathway is essential for the spatial localization and effector selectivity of the G_s-stimulated cAMP-to-PKA signaling. First, disrupting G_i function with PTX permits β_2AR to stimulate remote L-type Ca^{2+} channels (*29*). Second, PTX treatment leads to a robust β_2AR-mediated phospholamban phosphorylation and a positive relaxant effect not normally present in β_2AR cardiac signaling (*19, 28*). Thus, coupling of the cardiac β_2AR to multiple G proteins can paradoxically enhance, rather than compromise, the spatial and temporal specificity of the receptor signaling.

A challenging question is how β_2AR-to-G_i signaling results in the compartmentalization of β_2AR-to-G_s-to-cAMP signaling.

Possible mechanisms for limiting the cAMP signaling pathway include physical restriction of cAMP diffusion, a local imbalance between AC and phosphodiesterase activities, restriction of the diffusion of PKA, or regulation of the pathway downstream of PKA activity. The diffusible second messenger cAMP can traverse a micrometer-scale distance on a millisecond time scale; hence, it seems unlikely that limiting cAMP diffusion is the mechanism. There is evidence that compartmentalization is a consequence of regulation of the pathway downstream of PKA. PTX treatment, which abrogates the functional compartmentalization of β_2AR-to-cAMP signaling in freshly isolated rat ventricular myocytes, has no significant effect on the β_2AR-mediated global cAMP accumulation or PKA activation (*27, 28*). Other G_i-coupled receptors, such as the muscarinic receptor M_2 or adenosine receptor A_1, counteract the effect of PKA, in part, through activation of protein phosphatases (*37, 38*). Emerging evidence suggests that inhibition of protein phosphatases with calyculin A, an inhibitor of phosphatases 1 (PP1) and 2A (PP2A), mimics the effects of PTX treatment and enhances β_2AR-mediated positive contractile response (*28*). Because the effects of PTX treatment and calyculin A are not additive, the mechanism by which β_2AR-coupled G_i signaling compartmentalizes the concurrent G_s signaling may be through activation of protein phosphatase(s) (*28*). Activation of the β_2AR-coupled G_i proteins stimulates a phosphoinositide 3-kinase (PI3K)-Akt (also known as protein kinase B) cell survival signaling pathway in rat and mouse cardiac myocytes (*39, 40*) (see below). A question to be answered is whether PI3K signaling also contributes to the G_i-dependent localization of β_2AR-to-cAMP signaling; if so, it will be necessary to determine the relation of the PI3K signaling to the G_i-activated protein phosphatases.

Another candidate mechanism underlying compartmentalization of cAMP signaling is the structural restriction of PKA diffusion by specific A-kinase anchoring proteins (AKAPs) (*41, 42*). For example, a peptide inhibitor of AKAP can inhibit the modulation of L-type Ca^{2+} channels by PKA, which suggests that AKAPs are necessary for targeting PKA to this substrate (*43*). Interestingly, AKAPs not only traffic the bound PKA and other enzymes (such as protein phosphatases) to specific compartments, but also functionally modulate the activity of the bound enzymes. This is clearly demonstrated by the inhibition of PKA and stimulation of PP1 by certain AKAPs (*44-46*). Increasing evidence indicates that direct interaction of β_2AR with some AKAPs (such as gravin and AKAP79/150) is essential for agonist-induced β_2AR trafficking and desensitization (*47-51*). A potentially interesting question to be examined is whether AKAPs participate in the G_i-dependent compartmentalization of the β_2AR to G_s-mediated cAMP signaling.

β_2AR-to-G_i Coupling Delivers Cell Survival Signals

In addition to the modulation of cardiac excitation-contraction coupling by acute βAR stimulation, as discussed above, both in vivo and in vitro studies have shown that prolonged βAR signaling stimulates cardiac myocyte apoptosis (*52-54*). Apoptosis has been implicated in cardiac ischemic and reperfusion injury and is involved in the transition from cardiac hypertrophy to decompensated heart failure (*55-59*). Pharmacological evidence suggests that β_1AR and β_2AR stimulation may exert different effects on cardiac cell survival (*60, 61*). To avoid complicated interactions between βAR subtypes, we created a genetically "pure" β_1AR or β_2AR experimental system by individually expressing either βAR subtype in the null background of β_1AR and β_2AR double-knockout adult mouse cardiac myocytes in culture (*40, 62*). These studies provided evidence that stimulation of β_1AR leads to cardiac apoptosis, whereas stimulation of β_2AR activates concurrent proapoptotic and antiapoptotic signals, with the net effect being cell survival (*40*). The distinct effects of β_1AR and β_2AR on cardiac cell survival and cell death have been further confirmed using gene-targeted mice lacking either βAR subtype or in cultured wild-type adult mouse ventricular myocytes using βAR subtype-selective agonists and antagonists (*63*).

These differences between the two βAR subtypes might be simply explained by their differential coupling to cAMP. However, this is unlikely because cardiac-specific overexpression of adenylyl cyclase types V or VI in transgenic mouse models markedly increases cAMP and cardiac contractility without apoptotic effects (*64, 65*). In transgenic mouse hearts or cultured adult mouse cardiomyocytes, overexpression of human β_2AR significantly elevates basal cAMP level but is not associated with myocyte apoptosis (*66-68*). In addition, it has been suggested that myocyte apoptosis induced by β_1AR stimulation is independent of cAMP signaling (*69*).

Alternatively, the differential regulation of cardiac cell survival and cell death by these βAR subtypes can be explained by the additional coupling of β_2AR to PTX-sensitive G_i proteins. This conclusion is supported by several independent lines of evidence. First, β_2AR stimulation leads to myocyte apoptosis only under conditions in which G_i is inhibited with PTX (*40*). Second, β_2AR, but not β_1AR, activates a G_i-Gβγ-PI3K-Akt signaling pathway. Inhibition of G_i-to-Gβγ signaling with PTX or βARK-ct (a peptide inhibitor of Gβγ signaling), or inhibition of PI3K activity with LY294002, completely abolishes β_2AR-stimulated Akt activation; more important, it converts β_2AR signaling from survival to apoptotic (*40*). Therefore, PI3K constitutes an intracellular messenger of the β_2AR-to-G_i pathway, which protects myocytes against G_s-mediated apoptosis through activation of the survival factor Akt (Fig. 1). Third, pretreatment of cultured neonatal rat cardiac myocytes with β_2AR agonists (zinterol or isoproterolol plus a β_1AR antagonist, CGP20712A) protects these myocytes from a range of apoptotic assaults, including hypoxia or reactive oxygen species (ROS), through the G_i-dependent, PI3K-mediated mechanism (*39*).

In addition to the PI3K survival pathway, it has been suggested that in cultured adult rat cardiac myocytes, both βAR subtypes activate p38 mitogen-activated protein kinase (MAPK) in a G_i-dependent manner, and that the activated p38 MAPK results in an antiapoptotic effect (*70*). However, this finding contradicts earlier observations from the same laboratory that β_1AR and β_2AR exhibit opposing effects on cardiac myocyte apoptosis because of the specific G_i coupling to β_2AR, but not β_1AR (*60*). In fact, evidence obtained from the mouse β_1AR-β_2AR knockout system argues against the possibility that p38 MAPK is involved in β_2AR-mediated cardiac myocyte survival. This is because both β_1AR and β_2AR increase p38 MAPK activation through a cAMP-to-PKA signaling pathway, but not by a G_i-dependent mechanism (*40, 71*), and because pharmacological inhibition of p38 by SB 203580 (10 μM) cannot block the β_2AR survival effect. These studies indicate that p38 MAPK activation is not related to the β_2AR-stimulated, G_i-mediated antiapoptotic effect in adult mouse cardiac myocytes (*40*). Further-

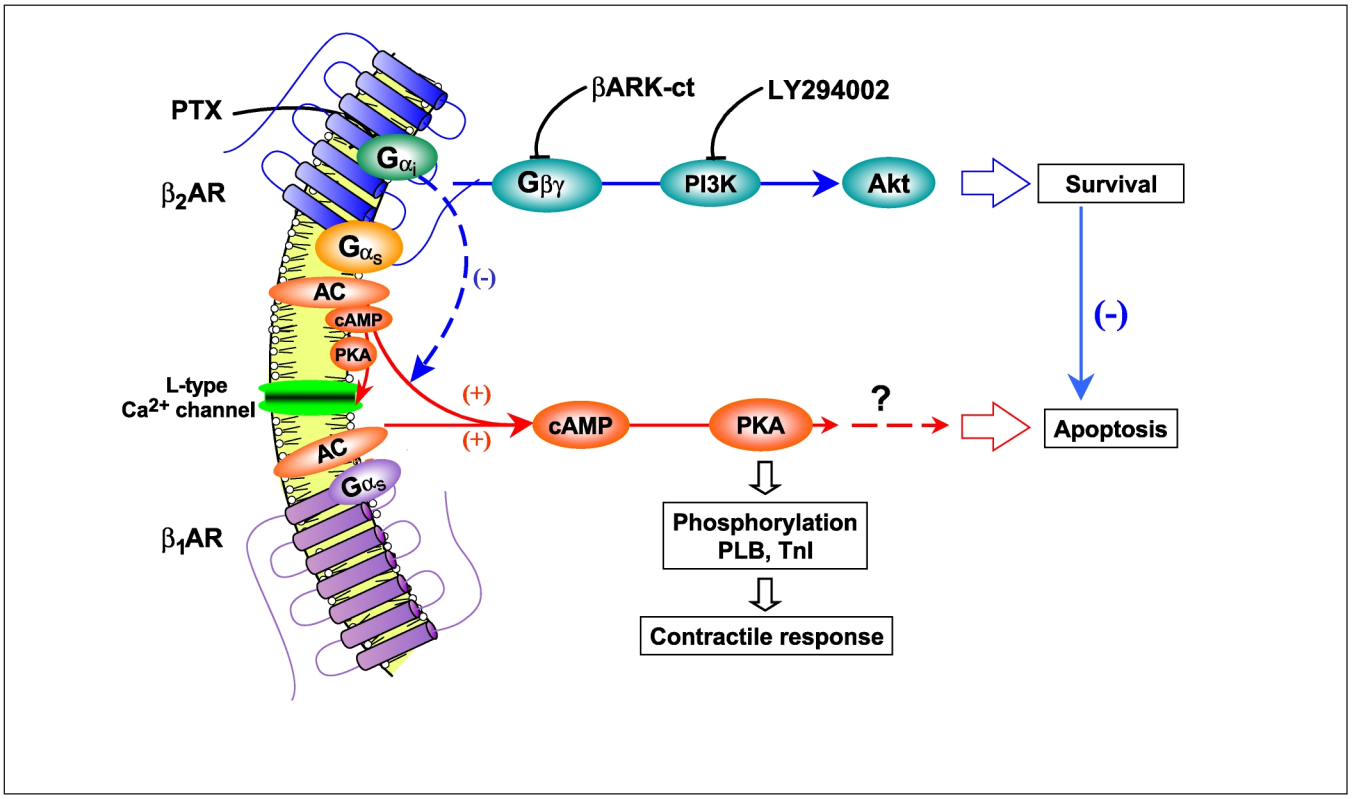

Fig. 1. Dual coupling of β_2AR to G_s and G_i proteins in cardiac myocytes. The activation of β_2AR-coupled G_i proteins functionally localizes the concurrent G_s-mediated cAMP-to-PKA signaling to the subsarcolemmal microdomain. The G_i coupling also delivers cell survival signals through a G_i-Gβγ-PI3K-Akt pathway (PTX, pertussis toxin; βARK-ct, a peptide inhibitor of Gβγ signaling; LY, a PI3K inhibitor; Akt, protein kinase B). The arrow from $G_{i\alpha}$ to global cAMP (but not to the local cAMP) indicates that the G_i coupling functionally localizes the G_s-stimulated cAMP signaling. The local modulation of the sarcolemmal L-type Ca^{2+} channel by β_2AR constitutes the major mechanism for the receptor-mediated positive contractile response. In contrast, β_1AR couples exclusively to G_s, which induces a global cAMP signal. AC, adenylyl cyclase.

more, in vivo activation of p38 MAPKs using transgenic overexpression of activated mutants of upstream kinases MKK3bE and MKK6bE neither induces nor suppresses cardiomyocyte apoptosis or hypertrophy in mice (*72*). Thus, it appears unlikely that p38 MAPK plays an essential role in β_2AR-induced antiapoptotic effect.

Other members of the MAPK family, particularly the extracellular signal-regulated protein kinases (ERK1 and ERK2), can also protect cells from apoptosis (*73, 74*). Stimulation of β_1AR or β_2AR is able to activate ERK1 and ERK2 in multiple cell types, including cardiac myocytes (*39, 75, 76*). Interestingly, the effect of β_2AR, but not β_1AR, on ERK is markedly attenuated by PTX treatment, which suggests that ERK is a downstream target of β_2AR-coupled G_i signaling (*39*). However, inhibition of ERK activation with the inhibitor PD98059, which inhibits the upstream kinase MEK1, cannot prevent a β_2AR-mediated antiapoptotic effect (*39*).

Molecular and Cellular Mechanisms Underlying β_2AR-to-G_i Coupling

The mechanisms underlying the differential coupling of βAR subtypes to G proteins are not well understood. Multiple hierarchical mechanisms may act in concert to render the subtype-specific βAR-to-G protein interaction. At the molecular level, β_1AR and β_2AR are genetically distinct entities. The human β_1AR gene is located at chromosome 10 and encodes a protein of 477 amino acids (*77*), whereas the β_2AR gene is located on chromosome 5 and encodes a protein of 413 amino acids (*78*). The sequences of β_1AR and β_2AR share 71% and 54% amino acid identity in the seven transmembrane spanning domains and in overall sequence, respectively (*77-79*). Studies on chimeric or mutated G protein-coupled receptors (including the major subtypes of α- and β-adrenergic receptors) have shown that the third intracellular loop of these receptors is an important structural determinant for G protein coupling (*80-82*). The third intracellular loop of β_1AR is considerably longer than its β_2AR counterpart because of the presence of a proline-rich motif that has been implicated as a negative modulator of βAR-G_s coupling. This may, at least in part, explain the difference in the efficacy of β_1AR and β_2AR coupling to G_s and AC (*83, 84*). Our preliminary results suggest that replacement of the third intracellular loop and the COOH-terminal tail of β_1AR with those of β_2AR allows the chimeric receptor to activate both G_i and G_s signaling pathways (*85*). Thus, the distinct G protein coupling of β_1AR and β_2AR could eventually be ascribed to some critical differences in the sequences of the third intracellular loops and the COOH-terminal tails of the receptors. Furthermore, a potential contribution of receptor posttranslational modifications to

receptor-G protein selectivity has been demonstrated in HEK 293 cells, in which PKA-mediated phosphorylation of β_2AR switches the receptor coupling preference from G_s to G_i (*75*).

The distinct G protein coupling of these βAR subtypes might, to some extent, be attributable to differential subcellular localization of the receptor subtypes and G proteins. In the absence of agonist stimulation, β_1ARs are enriched in noncaveolar cell surface membranes, whereas β_2ARs are located predominantly in the caveolar membrane fraction of cardiac myocytes (*86*). The difference in the subcellular distribution of βAR subtypes suggests that β_2AR might physically colocalize with G_i proteins, so that G_i proteins are preferentially accessible to β_2ARs. This hypothesis is further supported by the fact that $G\alpha_i$ proteins are most abundant in caveolae, whereas $G\alpha_s$ and $G\beta\gamma$ subunits are distributed in both caveolar and noncaveolar cell surface membranes in cardiac myocytes (*86*).

β_2AR-to-G_i Signaling in Developing Hearts

In contrast to the situation in adult cardiac myocytes, the β_2AR-mediated contractile response is insensitive to PTX treatment in neonatal rat cardiac myocytes (*87*). In those cells, β_2AR stimulation, like β_1AR, induces phosphorylation of PLB and TnI, and accelerates contractile relaxation (*12*). The dose-response curve of contraction in response to the β_2AR agonist zinterol is shifted ~2 orders of magnitude leftward in neonatal myocytes, as compared to that of adult myocytes. Thus, β_2AR may play a more important role in mediating the contractile response to catecholamines in the noninnervated neonatal heart than in the innervated adult heart. This developmental change in cardiac β_2AR responsiveness appears not to be caused by a difference in the amount of receptor expression, because there is no postnatal change in β_2AR density (*12*). The contraction dose-response to zinterol in neonatal rat myocytes (*12*) is similar to that in PTX-treated adult rat myocytes (*19*). Thus, β_2AR coupling to G_i proteins might be acquired or reinforced by the onset of innervation during development or by agonist stimulation.

The lack of PTX sensitivity of β_2AR contractile response in neonatal rat cardiac myocytes appears to contradict the fact that simultaneous β_2AR stimulation and β_1AR blockade results in an antiapoptotic effect through a G_i-dependent survival pathway (*39*), similar to the case in adult myocytes (*40*). These studies suggest that in neonatal cardiac myocytes, β_2AR-to-G_i coupling is rather effective in regulating certain vital cellular processes such as cell survival, whereas it is relatively weak in terms of inhibiting the G_s-mediated positive inotropic effect and phosphorylation of intracellular target proteins that control contraction. Thus, it is possible that the aforementioned compartmentalization mechanisms may not yet be in place in the developing heart.

Interaction Between β_2AR and Other G_i-Coupled Receptors

In cardiac myocytes, β_2AR differs from β_1AR regarding their interaction with several cardiac G_i-coupled receptors. In neonatal rat ventricular myocytes, the β_1AR-mediated cAMP accumulation and its inotropic and lusitropic effects are all prevented by M_2-muscarinic acetylcholine receptor stimulation with carbachol. In contrast, the β_2AR-induced cAMP accumulation and the inotropic effect persist in the presence of carbachol, although β_2AR-stimulated phosphorylation of PLB and TnI and the lusitropic response are abolished by carbachol treatment (*87*). Interestingly, in the absence of agonist stimulation, M_2-muscarinic receptors colocalize with β_1ARs, but not β_2ARs, in noncaveolar cell surface membranes (*88*). This may explain, in part, the differential interactions of M_2 receptors with β_1ARs versus β_2ARs.

In adult rat myocardium, there is also a striking difference between these βAR subtypes with respect to their cross-talk with G_i- and G_o-coupled δ-opioid receptors. A δ-opioid receptor agonist, leucine enkephalin, markedly inhibits β_1AR-mediated positive inotropy (*89, 90*). In contrast, leucine enkephalin has no effect on β_2AR-mediated increase in cardiac contractility (*89*), indicating that δ-opioid receptor signaling selectively interacts with cardiac β_1AR, but not β_2AR, signaling. The exact mechanism underlying the differential interaction of the βAR subtypes and G_i-coupled receptors, both in neonatal and adult rat cardiomyocytes, merits further study.

β_2AR, but Not β_1AR, Undergoes Spontaneous Activation

According to the extended ternary complex model (*91, 92*) and the cubic ternary complex model (*93*), GPCRs, including βARs, exist in an equilibrium of states, including two functionally and conformationally distinct states: an inactive conformation (R) and an active conformation capable of activating G proteins (R*) (*66, 94, 95*). 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, the overexpression of a given receptor would be expected to proportionally increase the number of R* state receptors. Indeed, in a transgenic mouse model (TG4), cardiac-specific overexpression of β_2AR by a factor of ~200 leads to an agonist-independent enhancement in both the baseline AC activity and myocardial contractility (*20, 66, 95*). These results from the transgenic animals are corroborated by acute gene manipulation in cultured wild-type or β_1AR-β_2AR double-knockout adult mouse ventricular myocytes, in which adenovirus-directed overexpression of the human β_2AR also results in agonist-independent increases in cellular cAMP production and in contractility (*67, 68*). These studies suggest that cardiac contractility can be enhanced through genetically manipulating the β_2AR system, which might hold therapeutic promise for improving the function of the failing heart.

Studies on constitutively active GPCRs have suggested the concept of inverse agonists, that is, drugs that preferentially bind to R and inhibit basal receptor activity (*66, 94, 95*). In this regard, ICI 118,551 has been identified as an inverse agonist of the β_2AR. Although the two-state ternary complex model for the βAR is sufficient to explain many aspects of β_2AR activation, there are several important differences between spontaneously activated β_2ARs and agonist-stimulated β_2ARs in terms of their effector selectivity. In TG4 ventricular myocytes, β_2AR agonists produce a marked increase in I_{Ca}, whereas ligand-independent constitutive β_2AR activation increases cardiac contractility without affecting I_{Ca} (*96*). Hence, spontaneously activated β_2AR and agonist-activated β_2AR may represent functionally distinct conformational states of the receptor. This is in agreement with recent reports that β_2ARs exhibit multiple active states (*97, 98*).

The property of spontaneous activation is not shared by the β_1AR, the predominant (75 to 85%) cardiac βAR subtype. In the mouse β_1AR-β_2AR null background, overexpression of β_1AR to similar levels, or even greater levels, relative to overexpression of β_2AR has virtually no effect on cAMP accumula-

tion, contraction amplitude, or contractile kinetics (*67*). These observations are consistent with the results from transgenic mice overexpressing β_1AR (by a factor of 5 to 15 relative to the wild type) (*99*). Apparently, β_1AR, unlike β_2AR, does not readily undergo spontaneous activation. Similarly, dopamine receptor subtypes 1A and 1B exhibit strikingly different constitutive activities (*100*). Thus, not all GPCRs appear to undergo spontaneous activation.

Pathophysiological Relevance of β_1AR versus β_2AR Signaling

β_1AR and β_2AR manifest strikingly different or opposing effects on gene expression, cell growth, and cell death. Specifically, stimulation of β_1ARs can produce hypertrophy in cultured neonatal rat cardiac myocytes through activation of a PI3K-Akt-glycogen synthase kinase-3β (GSK-3β)-GATA4 (a member of zinc finger transcription factor family) signaling pathway (*101, 102*). However, this appears to be independent of PTX-sensitive G_i signaling (*103*) or ERK activation (*101*). In addition, β_1AR activation can exhibit robust apoptotic effects in vivo and in cultured adult myocytes (*40, 60, 61, 104*). In sharp contrast, β_2AR stimulation does not cause cardiomyocyte hypertrophy or apoptosis. Instead, β_2AR activation protects myocytes against apoptosis induced by a wide array of assaulting factors, including enhanced β_1AR signaling, hypoxia, and ROS (*39, 40, 60*). Furthermore, chronic stimulation of each βAR subtype in the heart elicits distinctly different phenotypes and results in differences in prognosis in terms of cardiac hypertrophy and heart failure in transgenic mouse models. Overexpression of cardiac β_1AR by a factor of 5 to 40 leads to cardiac hypertrophy, myocyte apoptosis, and fibrosis within a few weeks after birth, and heart failure within several months (*99, 104*). Overexpression of cardiac β_2AR by a factor of 100 to 200 does not produce hypertrophy or heart failure, at least up to the age of 1 year (*66, 105, 106*). However, higher levels of expression of β_2AR (such as 350 to 1000 times the normal levels) result in pathological phenotypes (*105, 106*), perhaps caused by a mechanical and metabolic overload due to spontaneous β_2AR activation. The opposing effects of βAR subtypes on cardiac myocyte growth and cell death may explain, at least in part, the inverse relationship between the plasma concentration of norepinephrine (with higher affinity for β_1AR than for β_2AR) and survival in patients with chronic heart failure (*107*) and the salutary effects of βAR blockade on morbidity and mortality in heart failure patients (*108*). These insights into the cellular responses to βAR subtype stimulation also imply that the selective down-regulation of β_1ARs in the failing heart (*109-112*) may represent a protective mechanism to slow the progression of cardiomyopathy and myocyte apoptosis. This idea is further supported by the fact that the second and third generations of relatively β_1AR-selective blockers used clinically (such as metoprolol, bisoprolol, and carvedilol) effectively reduce mortality and morbidity of heart failure patients (*113*), whereas for the first generation of nonselective βAR antagonists (such as propranolol), the drug intolerability rate is prohibitively high because of myocardial depression and worsening of cardiac contractile dysfunction (*114*).

Although activation of β_2AR-coupled G_i protects cardiac myocytes against apoptosis, an imbalance of β_2AR-initiated G_s and G_i signaling pathways may have pathological consequences. Chronic heart failure in human and animal models is characterized by a diminished contractile response to βAR stimulation (*109-112, 115, 116*) and is accompanied by an increase in the amount or activity of G_i proteins (*112, 115-118*) and a selective down-regulation of β_1AR, leading to a higher β_2/β_1 ratio (*109-112*). In light of the G_s and G_i dichotomy, the up-regulation of G_i may participate in the reduced βAR inotropic effect in the decompensated failing heart. This idea is supported by the fact that PTX treatment restores the diminished βAR inotropic response in a rat myocardial infarction heart failure model (*119*) and in myocytes from failing human hearts (*120*). On the basis of these findings, it is speculated that the selective down-regulation of β_1AR and the up-regulation of β_2AR to G_i signaling in the functionally compensated hypertrophic heart or in the early stages of heart failure may represent a cardiac protective mechanism. This change in the balance of β_1AR and β_2AR signaling may protect against myocyte apoptosis and consequently slow the progression of cardiomyopathy and contractile dysfunction. However, exaggerated β_2AR-to-G_i signaling may blunt G_s-mediated contractile support, contributing to the phenotype of decompensated heart failure.

Therapeutic Implications of Cardiac β_2AR Signaling

Whether enhancing βAR signaling is beneficial or deleterious for the failing heart has been a matter of much controversy. The prevalent view is that chronically increasing nonselective βAR stimulation is toxic to the heart. However, the discovery of (i) the new paradigm of β_2AR signaling (dual G protein coupling), (ii) the opposing effects of stimulation of these βAR subtypes on cardiomyocyte apoptosis, and (iii) the distinct phenotypes of cardiac-specific overexpression of β_1AR versus β_2AR underscore the necessity and importance of distinguishing β_2AR signaling from that of β_1AR in terms of their cardiac functional roles and therapeutic implications.

Selective enhancement of β_2AR signaling may provide a therapeutic strategy for the prevention and treatment of chronic heart failure because of its evident antiapoptotic and positive inotropic effects. Indeed, crossing transgenic mice overexpressing moderate amounts of cardiac β_2AR with transgenic mice overexpressing $G\alpha_q$ not only improves cardiac performance, but also reverses hypertrophy in the $G\alpha_q$ overexpression heart failure model (*105*). Because extremely high levels of β_2AR overexpression fail to rescue the genetic mouse heart failure model and can be detrimental (*105, 106*), caution must be exercised when designing therapies to enhance β_2AR signaling so that the beneficial levels of activity are not exceeded. The beneficial effect of β_2AR stimulation in the context of heart failure is clearly supported by the analysis of β_2AR polymorphisms in chronic heart failure patients. The prognosis of heart failure patients with Ile^{164} polymorphism (a Thr-to-Ile switch at amino acid 164 with reduced β_2AR signaling efficacy) is much worse than the prognosis of patients without the β_2AR variant (*121*). Thus, moderate selective activation of the β_2AR subtype may have beneficial effects in the failing heart. Given that epinephrine is a potent β_2AR agonist, it would be interesting and informative to determine whether the beneficial effects of exercise might be, in part, attributable to increased cardiac β_2AR stimulation by epinephrine.

Other βAR Subtypes in the Heart

The third class of βARs, β_3AR, was previously named an "atypical βAR" and was considered genetically and pharmacologically different from either β_1AR or β_2AR (*122, 123*). Recent studies provide strong evidence that β_3ARs, important regulators of

the physiologic properties of adipose tissue and the gastrointestinal tract (and thus the target for antiobesity and antidiabetic drugs), are also present in human cardiomyocytes. In contrast to β_1AR and β_2AR, they have been implicated as inhibitors of contractile function (*124*), apparently through a PTX-sensitive G protein-dependent activation of a nitric oxide synthase pathway (*125*). β_3AR also plays an important role in regulating smooth muscle relaxation, which could reflexively influence cardiac contractility. It is noteworthy that β_3AR function is up-regulated in the failing heart (*126*), suggesting that enhanced β_3AR signaling may contribute to the phenotype of chronic heart failure. Stimulation of β_3AR activates both G_s and G_i signaling pathways in cultured neonatal cardiomyocytes from β_1AR-β_2AR double-knockout mice. In the absence of PTX, β_3AR stimulation has a small and relatively brief inhibitory effect on the spontaneous cell contraction rate, whereas inhibition of G_i with PTX unmasks a positive chronotropic effect (*127*). In addition, a fourth βAR subtype has been reported to mediate positive chronotropic and inotropic effects in the human heart (*128*). This "receptor" is now described as a low-affinity state of the β_1AR (*129, 130*), although its genetic identity and pharmacological properties await confirmation.

Beyond G Protein Doctrine: G Protein-Independent βAR Signaling

Possible G protein-independent mechanisms underlying βAR-mediated cellular responses have also been demonstrated. For instance, physical binding of Na^+/H^+ exchange regulatory factor (NHERF), an inhibitor of Na^+/H^+ exchanger type 3 (NHE3), to a PDZ domain at the β_2AR COOH-terminus relieves the NHERF inhibitory effect on NHE3 (*131*). The relevance of this phenomenon to β_2AR signaling in cardiomyocytes, however, has not yet been explored. Another observation in HEK 293 cells demonstrates that the COOH-terminal SH3 domain of the endophilin SH3p4 specifically binds to the proline-rich motif of the β_1AR third intracellular loop (*132*). This protein-protein interaction is implicated in promoting agonist-induced internalization and in decreasing the G_s coupling efficacy of β_1ARs (*132*). Thus, βAR signaling is highly diversified not only through coupling to multiple G proteins, but also through G protein-independent protein-protein interactions between βARs and various effector proteins.

Concluding Remarks

The discovery of the dichotomous coupling of β_2AR to G_s and G_i has challenged the linear one receptor-one G protein paradigm of GPCR signaling in physiological systems. The additional G_i coupling of β_2AR creates a functional barrier that localizes the concurrent G_s-mediated cAMP signaling, thus enhancing receptor signaling specificity and effector selectivity. The G_i branch also delivers a G_s-independent cardioprotective signal through the G_i-Gβγ-PI3K-Akt pathway, which not only counteracts the G_s-mediated apoptotic effect but also protects cells from a variety of apoptosis-triggering assaults. Further, the differential G protein coupling, to a large extent, accounts for the distinctly different physiological and pathological roles in the heart for β_2AR versus those of β_1AR. The delicate balance of G_s and G_i signaling in space and time might be crucial to normal cellular functions, whereas an imbalance may have important pathophysiological relevance and clinical implications. Thus, selectively targeting βAR signaling pathways might afford novel therapeutic strategies for improving the function of the failing heart. Furthermore, these advances in understanding the signaling pathways begin to unravel the logic of multiple G protein coupling of GPCRs and the coexistence of GPCR subtypes in a single cell.

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