Cell Logic for Dual Coupling of a Single Class of Receptors to G$_s$ and G$_i$ Proteins

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G-protein–coupled receptors mediate transmembrane signal transduction for a variety of hormones, neurotransmitters, chemokines, and synthetic ligands. It had been thought that a given receptor couples only to a single class of heterotrimeric G proteins to achieve signaling selectivity and specificity. As a model system of G protein–coupled receptors, β-adrenergic receptor (βAR) was believed to activate exclusively a G$_s$-adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade. This doctrine, however, has been challenged by recent findings that, although seeming illogical, βAR dually couples to G$_i$ and G$_s$ proteins in rodent and canine hearts$^2$–$^3$ (for review, see Reference 4). In this issue of Circulation Research, Kilts et al$^5$ present a solid study that provides additional evidence supporting a dual G protein coupling for βAR and extended the previous finding to several other G$_i$-coupled receptors in human heart.

G$_s$ and G$_i$ Dichotomy

Early studies showed that stimulation of βAR, but not βAR, induces a relaxant effect$^6$–$^7$ a hallmark of cAMP-dependent cardiac modulation. Moreover, βAR-induced augmentations in intracellular Ca$_{2+}$ transient and contractility are apparently dissociated from cellular cAMP accumulation and PKA-mediated protein phosphorylation in rat ventricular myocytes, whereas the classic linear G$_s$-adenylyl cyclase-cAMP-PKA signaling cascade is corroborated for βAR stimulation.$^8$ These findings provided the first clue that there are some substantial differences between βAR and βAR signaling pathways.

In search for answers to the anomalous behavior of cardiac βAR stimulation, recent studies have revealed dichotomous G protein coupling for native βAR under physiological conditions. Disrupting G$_s$ signaling by pertussis toxin (PTX)–mediated ribosylation enhances βAR-induced contractile response in rat ventricular myocytes$^1$ and unmasks the βAR positive inotropic effect in mouse cardiac myocytes, in which G$_i$ signaling fully negates βAR/G$_i$-mediated contractile response.$^2$ More recently, photoaffinity labeling of G proteins with $[^32$P]$\text{azidoanilido-GTP}$ in conjunction with immunoprecipitation with antibodies specific for G$_{ia}$ and G$_{ia}$ provided direct biochemical evidence that βAR activates both G$_s$ and G$_i$ (G$_{ia_2}$ and G$_{ia_3}$) signaling pathways, whereas βAR selectively activates G$_i$ in adult mouse cardiac myocytes.$^2$ By photolabeling human atrial membranes with $[^32$P]$\text{azidoanilido-GTP}$, Kilts et al$^2$ further demonstrated that cardiac G$_{ia_3}$ is activated by stimulation of βAR and several other G$_{ia_3}$-coupled receptors, including histamine, serotonin, and glucagon receptors. Thus, promiscuous G protein coupling seems to be a rather common pattern of receptor–G protein interaction in the physiological context, although this property is not shared by βAR. These findings raise important questions regarding physiological and pathophysiological relevance of the additional G$_i$ coupling of G$_i$-coupled receptors.

Cell Logic for Receptor Coupling to More Than One G Protein

Transmembrane receptor signaling is a complex biological process orchestrated by a myriad of receptors and G proteins. The interplay of G$_i$ and G$_s$ signaling has been clearly elucidated in crosstalk between different receptor families. For example, stimulation of G$_i$-coupled muscarinic receptors attenuates the positive inotropic effect of βAR stimulation.$^9$–$^{10}$ However, cardiac βAR and several other G$_i$-coupled receptors present intriguing cases in which crosstalk occurs between concurrent G$_i$ and G$_s$ signaling pathways originating from a single class of receptors. We are only beginning to appreciate the cellular logic behind the “one receptor, two G protein” signaling mechanism.

Although G$_s$ is named for its inhibitory effect on adenylyl cyclase and activation of G$_i$ may counteract the ability of G$_s$ to stimulate adenylyl cyclase, as demonstrated by Kilts et al,$^2$ it is noteworthy that the G$_s$ and G$_i$ interaction is not necessarily confined to the cyclase level.$^2$–$^3$ Counterintuitively, promiscuous G protein coupling may enhance, rather than compromise, the receptor signaling specificity. This point is perhaps best exemplified by βAR subtype stimulation. In rodent and canine hearts, βAR stimulation increases phosphorylation of phospholamban, which accelerates Ca$_{2+}$ sequestration into the sarcoplasmic reticulum, resulting in accelerated cardiac relaxation,$^4$–$^6$–$^8$–$^{12}$ and increases phosphorylation of troponin I and C protein,$^12$ which reduces myofilament sensitivity to Ca$_{2+}$. In contrast, βAR stimulation modulates specifically L-type Ca$_{2+}$ channels, bypassing the aforementioned intracellular regulatory proteins.$^3$–$^6$–$^8$–$^{12}$ In a direct approach involving on-cell patch-clamp recordings, it has been shown that βAR stimulation modulates single L-type Ca$_{2+}$ channel activity only in a local mode (agonist included in pipette solution) but not in a remote mode (agonist added to bathing solution outside the patch), whereas βAR stimulation does so in either mode.$^{13}$ G$_i$ activation is essential to the spatial localization and effector selectivity of β$_1$ AR signal-
Pathophysiological Relevance

Programmed cell death or apoptosis has been recently implicated as a consequence of cardiac ischemic/reperfusion injury, contributing to the transition from cardiac hypertrophy to decompensatory heart failure. In vivo and in vitro studies indicate that β1AR and β2AR exhibit distinctly different, even opposing, effects on cardiac myocyte apoptosis. In cultured adult rat cardiac myocytes, stimulation of β2AR, but not β1AR, induces myocyte apoptosis. Using a β2AR/β1AR double knockout mouse model in conjunction with adenoviral gene transfer, we have created pure β2AR subtype experimental settings and found that β2AR stimulation markedly induces myocyte apoptosis. In contrast, β1AR stimulation activates concurrent apoptotic and survival signals mediated by Gs and Gi, respectively, with the Gi-mediated survival effect predominant (Zhu W-Z, Zheng M, Kolbikla BK, Xiao R-P, unpublished data, 2000). Moreover, activation of β2AR-coupled Gs protects cardiac myocytes from a range of apoptotic assaults, including hypoxia or reactive oxygen species–induced apoptosis (Chesley A, Ohtani S, Asai T, Xiao R-P, Lunberg MS, Lakatta EG, Crow MT, unpublished data, 2000).

Chronic stimulation of these βAR subtypes in the heart also elicits strikingly different phenotypes in murine transgenic models. Overexpression of cardiac β2AR by ≈5- to 46-fold induces cardiac hypertrophy, apoptosis, and fibrosis within a few weeks after birth and heart failure within several months. Ironically, overexpression of cardiac β1AR by ≈100- to 200-fold does not induce hypertrophy or heart failure, at least up to the age of 1 year. However, overwhelming expression of β2AR (eg, ≈350- to 1000-fold) induces pathological phenotypes, perhaps caused by a mechanic and metabolic overload (markedly enhanced baseline adenyl cyclase activity and cardiac contractility) due to spontaneous β2AR activation. Whether the distinct phenotypes of β2AR versus β1AR mouse transgenic models are related to their different G protein coupling merits further investigations.

Although activation of β2AR-coupled Gs protects cardiac myocytes against apoptosis, an imbalance of β2AR-initiated Gs and Gi signaling pathways may induce pathological consequences. Chronic heart failure in human and animal models is characterized by a diminished contractile response to βAR stimulation, accompanied by a selective downregulation of β2AR (higher β2/β1 ratio) and an increase in the amount or activity of Gi proteins. In light of the Gi and Gs dichotomy, the upregulation of Gi may participate in the defect of βAR inotropic effect in the decompensated failing heart. This idea is supported by recent observations that PTX treatment restores the diminished βAR inotropic response in a rat myocardial infarction heart failure model and in myocytes from failing human hearts.

On the basis of these findings, we speculate that the selective downregulation of β2AR and the upregulation of β1AR/Gi, signaling in functionally compensated hypertrophic heart or early stage of heart failure may represent a cardiac protective mechanism (eg, against myocyte apoptosis), which slows the progression of cardiomyopathy and contractile dysfunction. However, exaggerated β1AR/Gi signaling may blunt Gi-mediated contractile support, contributing to the phenotype of decompensated heart failure.

Therapeutic Implications

It has been highly controversial as to whether enhancing βAR signaling is beneficial or deleterious for the failing heart. The prevalent view is that chronically increasing nonselective βAR stimulation is toxic to the heart, because there is an inverse relationship between the plasma level of norepinephrine and the survival of patients with heart failure and because βAR blockade (eg, by β1AR antagonists bisoprolol and metoprolol or a nonselective βAR blocker carvedilol) reduces both the morbidity and mortality in patients with heart failure. However, the discovery of a new paradigm of β1AR signaling (ie, the dual G protein coupling), the opposing effects of stimulation of these βAR subtypes on cardiomyocyte apoptosis, and the distinct phenotypes of cardiac-specific overexpression of β2AR versus β1AR underscore the necessity and importance of distinguishing β1AR signaling from that of β2AR in terms of their cardiac functional roles and therapeutic implications.

In our opinion, selectively enhancing β2AR signaling may provide a novel therapeutic strategy in the prevention and treatment of chronic heart failure. Indeed, crossing transgenic mice overexpressing cardiac β2AR at appropriate levels (eg, 30-fold) with transgenic mice overexpressing Gi not only improves the cardiac performance but also reverses hypertrophy in the Gi overexpression heart failure model, although extremely high levels of β2AR overexpression (eg, 350- to 1000-fold) fail to rescue the genetic mouse heart failure model. Additionally, the beneficial effect of β2AR stimulation in the context of heart failure is clearly supported by the analysis of polymorphisms of β2AR in chronic heart failure patients. The likelihood of earlier aggressive intervention or cardiac transplantation is significantly greater in heart failure patients with Ile164 polymorphism (a Thr to Ile switch at amino acid 164 with reduced β2AR signaling efficacy) relative to patients without the β2AR variant.
In summary, coupling of one receptor to more than one class of G proteins may represent a common property of many Go-coupled receptors, rather than a unique quality of β2AR. The additional Go coupling not only confers spatial and temporal control of Go-stimulated signals, enhancing the receptor signaling specificity, but also enriches signaling diversity by delivering Go-independent signals. The discovery of the Go and Gq ditachomies reshapes our current understanding of receptor–G protein interactions in physiological systems. An imbalance of the concurrent Go and Gq signals may have important pathophysiological relevance and clinic implications.

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References


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