Alternative signaling: cardiomyocyte β_1-adrenergic receptors signal through EGFRs

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Acute stimulation of cardiac β_1-adrenergic receptors (β_1ARs) by norepinephrine represents the strongest endogenous mechanism for increasing cardiac function, but long-term stimulation induces cardiomyocyte apoptosis and contributes to cardiac disease. These effects have been attributed to coupling of the β_1AR to the stimulatory G protein (G_s) and classical cAMP-mediated signaling. In this issue of the *JCI*, Noma and colleagues report that cardiomyocyte β_1ARs may in addition deliver an antiapoptotic signal through transactivation of EGFRs (see the related article beginning on page 2445). Their findings provide a perspective for a novel class of receptor ligands that may direct β_1AR signaling toward alternative signaling pathways.

In cardiac failure, enhanced levels of norepinephrine resulting from activation of the sympathetic nervous system lead to chronic stimulation of cardiac β-adrenergic receptors (βARs) (1). While this acutely serves to adapt cardiac output to the systemic needs, chronic stimulation of the β_1 adrenergic receptor (β_1AR) is clearly detrimental and contributes to cardiomyocyte hypertrophy, cell death, and progression of the disease (2–4). The deleterious consequences of β_1AR stimulation are thought to be mediated by coupling of the β_1AR to the stimulatory G protein (G_s) and subsequent activation of a defined set of downstream targets (Figure 1). The heart adapts to the chronically elevated norepinephrine concentrations by blunting the response to agonist stimulation, a process termed desensitization (3, 5). However, desensitization is not sufficient to compensate entirely for the chronic overstimulation of the system, and over prolonged periods of time the toxic consequences of β_1AR stimulation prevail (6, 7). Mechanistically, desensitization involves a reduction in β_1AR number (downregulation) and function (uncoupling). The latter occurs through phosphorylation of the third intracellular loop of β_1AR and the C terminus by PKA and, more importantly, through G protein–coupled receptor kinases (GRKs) (8), followed by translocation and binding to the receptor by the multifunctional protein β-arrestin (9). Phosphorylation and subsequent desensitization of the β_1AR is appreciated predominantly as a self-protective mechanism that partially decreases G_s-mediated signal transduction.

In this issue of the *JCI,* Noma and coworkers (10) present exciting new evidence that may change the way we think about β_1AR desensitization in the heart. Their data suggest that GRK-mediated phosphorylation of the β_1AR not only serves to reduce G_s/PKA-mediated signal transduction, but in parallel, serves to initiate a powerful antiapoptotic signal by mediating transactivation of the EGFR through a β-arrestin–dependent pathway (Figure 1). This process crucially depends on two cardiac GRK isoforms — GRK5 and GRK6 — that have to date not been intensely studied with respect to their cardiac function. The data from Noma et al. provide additional evidence that β-arrestins serve as multifunctional proteins that may induce G protein–independent intracellular signaling (9).

EGFR transactivation and cardiovascular biology

Transactivation of receptor tyrosine kinases through G protein–coupled receptor (GPCR) activation was first described for G_q-coupled receptors that mediate ERK/MAPK activation in fibroblasts (11). Shedding of an extracellular domain of heparin-binding EGF (HB-EGF) that then acts as an agonist at EGFRs was identified as the underlying mechanism (Figure 1). These early studies linked two signaling paradigms that had previously been regarded as separate entities. Subsequent work has detailed the mechanism of HB-EGF shedding through membrane proteins of the ADAM (a disintegrin and metalloproteinase domain) family of metalloproteinases, which are activated upon GPCR stimulation (12). Thus, in addition to their matrix-remodeling functions, metalloproteinases may directly control EGFR signaling by proteolytically activating EGFR ligands.

Since then, GPCR-mediated transactivation has been demonstrated for a wide variety of G_q- and G_i-coupled receptors (13). Comparably less evidence has been obtained for primarily G_s-coupled receptors. For the β_2AR, a PKA-induced switch from G_s to G_i coupling has been shown to mediate EGFR transactivation and subsequent ERK activation (14, 15). Remarkably, β_2AR-mediated EGFR transactivation was found to be independent of metalloproteinase activation and involves βγ-subunits and c-Src (16).

Several studies have implicated signaling through EGFRs in cardiovascular biology, with marked divergence as to their presumed physiological role. Asakura et al. have identified ADAM12-mediated shedding of HB-EGF and subsequent EGFR activation as a critical step in angiotensin II type 1a receptor– (AT_{1A}R-) and α_1AR-mediated cardiac hypertrophy (17). Similar data have been obtained for α_1AR-mediated vascular smooth muscle hypertrophy (18). Thus, EGFR transactivation may be viewed as being detrimental according to these studies. In contrast, mice with cardiomyocyte-restricted deletion of the EGFR ErbB2 display dilated cardiomyopathy, suggesting a cardioprotective role (19). Also, more indirect evidence based on the cardiac side effects of trastuzumab (an antibody directed against ErbB2) in breast cancer therapy suggests that EGFR

Nonstandard abbreviations used: ADAM, a disintegrin and metalloproteinase domain; β_1AR, β_1-adrenergic receptor; AT_{1A}R, angiotensin II type 1A receptor; GRK, G protein–coupled receptor kinase; G_s, stimulatory G protein; HB-EGF, heparin-binding EGF.

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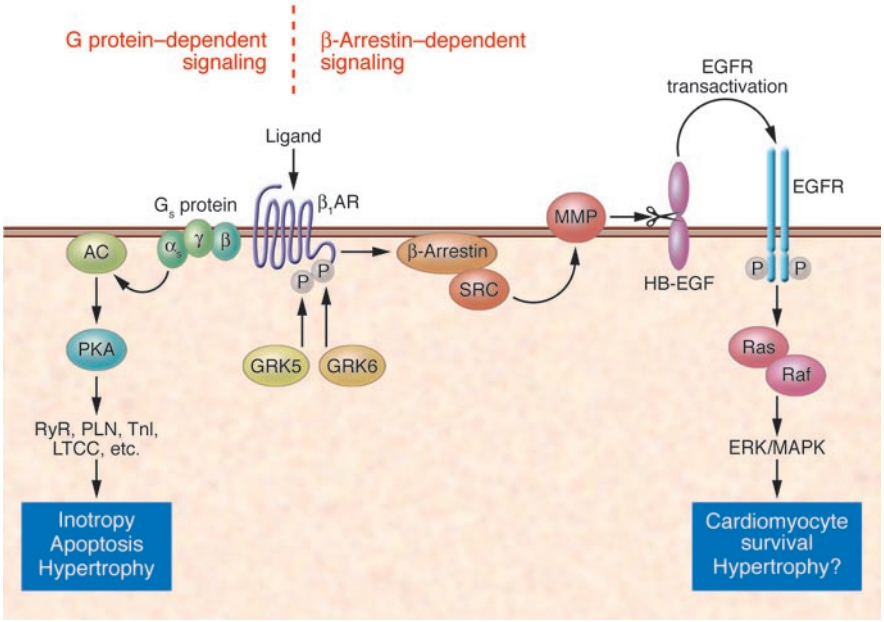

Figure 1

β_1AR-mediated signal transduction in cardiomyocytes. Classical ligand-activated βARs enhance cardiac contractility by coupling to G_s, formation of cAMP by adenylyl cyclase (AC), and PKA-dependent phosphorylation of various target proteins (e.g., ryanodine receptor [RyR]; phospholamban [PLN], troponin I [TnI], and the L-type Ca^{2+} channel [LTCC]). Chronic β_1AR stimulation is detrimental and induces cardiomyocyte hypertrophy and apoptosis. In this issue of the *JCI*, Noma et al. (10) have delineated a novel signaling pathway leading to GRK- and β-arrestin–dependent Src-kinase (SRC) and MMP activation. MMP activation in turn sheds HB-EGF from the cell surface, and this serves as a ligand for cardiomyocyte EGFRs, which mediate ERK/MAPK activation. This pathway protects from β_1AR-induced cardiomyocyte apoptosis but has been associated with cardiac hypertrophy.

activation may be required for maintaining cardiac integrity (20). However, these data have to be interpreted with caution, as the cardiotoxic side effects of trastuzumab may also be related to cellular or complement-dependent cytotoxicity initiated by binding of the antibody to cardiomyocytes. With respect to downstream signaling, it will be interesting to determine whether direct activation of the EGFR or activation via transactivation involve different downstream signaling pathways in cardiomyocytes.

Alternative signaling of the β_1AR to EGFRs

The exciting data by Noma et al. (10) represent the first evidence that the β_1AR may signal through the EGFR to induce a survival signal in cardiomyocytes. This study may change the linear way we think about adrenergic receptor signaling in the heart. Naturally, this raises new questions. What is the molecular chain of events that leads to metalloproteinase activation? The mechanisms by which GPCRs mediate activation of metalloproteinases are generally not well understood and may involve Gα, Gβγ, c-Src, and PKC (21). Noma et al. have convincingly demonstrated the involvement of GRK5, GRK6, Src-kinase (SRC), and β-arrestin. Subsequent work will be needed to elucidate the detailed mechanism integrating these players. It is remarkable that both GRK5 and GRK6 are needed to mediate EGFR transactivation. In this respect it will be interesting to determine whether direct phosphorylation of the β_1AR by GRK5 and GRK6 is needed and, if so, what the sites of receptor phosphorylation are.

Further studies will also be needed to determine the cardiomyocyte metalloproteinase responsible for EGFR ligand shedding and whether EGFR signaling is also beneficial for the heart when chronically activated, which is presumably the case in heart failure. With respect to a role in heart failure, other groups have shown that EGFR signaling is involved in the pro-hypertrophic effects of angiotensin II in cardiomyocytes (17). Thus, the heart may ultimately pay a high price (in the form of hypertrophy) for decreasing cardiomyocyte apoptosis through chronic EGFR signaling. Follow-up studies in chronic models of cardiac disease will be needed to answer these questions.

Alternatively, the discrepancies between ATR- and βAR-mediated EGFR transactivation might involve spatial compartmentation of receptors and their downstream signaling or different kinetics of βAR versus ATR signals.

These studies also prompt the question: What are the downstream signaling mechanisms of β_1AR-mediated EGFR transactivation besides ERK/MAPK activation? Are they different from direct activation of the EGFR? Recent evidence suggests that this might be indeed the case (21), and this could help to explain the uncertainties regarding the role of EGFR signaling in cardiomyocyte biology. In addition, it will be interesting to delineate the molecular determinants that cause different modes of receptor transactivation for the β_2AR and the β_1AR. Further insight into this issue might be gained from the identification of the relevant residues phosphorylated by GRK5 and GRK6 and through studies using receptor chimeras.

Therapeutic perspectives

Most importantly, the findings of Noma et al. (10) may unlock the door to novel therapeutic interventions. This possibility may seem unlikely at first glance, as both the deleterious effects through G_s as well as the protective signal through EGFR transactivation are activated through the β_1AR and current cardiovascular treatment regimes heavily rely on blockade of βAR signaling. However, recent evidence indicates that GPCR ligands may target a receptor signal to specific intracellular effectors (22), and direct analysis of receptor conformational changes during activation by fluorescence resonance energy transfer microscopy has revealed marked differences for different antagonists as to the conformational change of the β_1AR (23). Ultimately, this could lead to the development of ligands that may act as antagonists toward a certain (deleterious) intracellular effector while acting as agonist to another (beneficial) effector. This would allow for the development of GPCR blockers that might prove superior to currently existing substances.

The findings of Noma et al. (10) are likely to gain additional importance in disease

contexts. This is because crucial elements of EGFR transactivation are upregulated during cardiac hypertrophy or failure, such as metalloproteinases, HB-EGF, EGFR, and ERK. Thus, under conditions of cardiac growth and disease, "biased" antagonists that favor signaling through EGFR transactivation may prove particularly effective.

Taken together, the results reported in this issue by Noma et al. (10) support the existence of a novel β_1AR signaling pathway in the heart. Apart from their "classical" signaling properties, β_1ARs are able to signal via activation of EGFRs to the Ras/Raf/MAPK pathway and thereby compensate, at least in part, for the deleterious effects caused by chronic G_s/PKA signaling.

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