β-Adrenergic Receptors Cooperate With Transcription Factors
The “STAT” of Their Union

J.-L. Balligand, MD, PhD

Through their wide tissue/cellular distribution, β-adrenoceptors are key regulators of cardiovascular function and remodeling. Classically, beta1- and beta2-adrenoceptors positively influence all aspects of cardiac contractility through \( \alpha \)-s coupling to adenylyl cyclase and \( \alpha \)/protein kinase A phosphorylation of critical effectors of excitability through \( \beta \)-s coupling to adenylyl cyclase and cAMP/cGMP receptors positively influence all aspects of cardiac contractility and remodeling. Classically, beta1-2Adrenoceptors are also known to initiate signaling that is independent of protein kinase A/cAMP and involves \( \beta \)-arrestin–dependent activation of extracellular signal-regulated kinases. Moreover, like many \( \beta \)-protein–coupled receptors, B1-2ARs can transactivate receptor tyrosine kinases, for example, epidermal growth factor receptor, thereby producing wider effects on cellular growth and survival.

In addition to acute regulation of EC coupling, catecholamines, like many neurohormones, exert profound effects on tissue remodeling, which involves engagement of specific transcription programs, leading to hypertrophy, fibrosis, and angiogenesis, but also profound changes in cell metabolism or survival, all of which participate in the initial adaptation to cardiac stress but eventually culminate in the chronic deterioration of cardiac function. Extensive experimental work has led to the identification of the signaling pathways driving the underlying transcriptional changes, including through epigenetic regulation.

The traditional view has kept these 2 phenomena relatively apart, with little interplay between them. More recently, attention has been focused on the activation of transcription pathways as a consequence of ionic derangements (ie, changes in intracellular \( \mathrm{Ca}^{2+} \) concentrations) in specific subcellular compartments, leading to activation of \( \mathrm{Ca}^{2+} \)-responsive pathways such as calcium/calmodulin-dependent protein kinase or calcium–nuclear factor of activated \( T \) cells. In addition, the stressed myocardium produces and is responsive to an array of paracrine signaling molecules, including growth factors and cytokines. These in turn modulate tissue remodeling and cell survival through activation of another class of receptors, receptor tyrosine kinases.

The work by Zhang et al \(^1\) in this issue of Circulation proposes a paradigm that bridges the 2 systems, that is, EC coupling and transcriptional regulation by a single factor, signal transducer and activator of transcription-3 (STAT3). STAT3 belongs to a family of 7 STAT transcription factors that are widely expressed in cardiovascular tissues, \(^1\) where they classically mediate the pleiotropic effects of cytokines and growth factors on receptor tyrosine kinases such as gp130 in response to the interleukin (IL)-6 family (eg, IL-5, IL-6, IL-11, leukemia inhibitory factor, oncostatin M, and cardiogenic-1). On ligand binding and subsequent homodimerization or heterodimerization of the receptor, Janus kinase and tyrosine kinase within the receptor intracytoplasmic domain phosphorylate STAT3 at the specific Y705, allowing STAT homodimerization or heterodimerization and downstream signaling.\(^2,3\) A number of additional Ser/Thr kinases can phosphorylate STAT3 on S727, an event that critically regulates its transcriptional activity through the recruitment of coactivators such as the histone acetyltransferase p300/CREB binding protein.\(^4\) Activated STAT3 dimers then translocate to the nucleus, where they activate specific interferon-\( \gamma \)-activated sequence transcription sites. Of note, STAT3 can also inhibit gene expression, for example, after acetylation of K685 in the SH2 domain, which allows interaction with DNA methyltransferase-1, repressing transcription.\(^5\) This would result in downregulation of protein-coding mRNA but also potentially noncoding RNA (including microRNA).\(^6\) Moreover, STAT3 was shown to regulate cell respiration, metabolism, and redox stress in the mitochondria,\(^7,8\) illustrating a highly versatile signaling capacity (Figure).

Zhang et al observed that short-term treatment of mice with isoproterenol intraperitoneally or treatment of adult cardiac myocytes with dobutamine in vitro induces nuclear translocation of STAT3 after as little as 30 minutes in vivo and 15 minutes in vitro and a later phosphorylation (P-Y705) signal at 1 hour (in vivo). Previous work had demonstrated delayed STAT3 phosphorylation/activation by G-protein–coupled receptors through different signaling kinases,\(^9,10,11\) including after autocrine production of IL-6.\(^12\) Using β-adrenoceptor antagonists and Src inhibitor 1 in cardiac myocytes, as well as embryonic fibroblasts genetically deficient in B1-2AR or Src/Yes tyrosine kinases, they show this to be mediated by β-adrenoceptor and Src activation but that it is independent of classic G-\( \alpha \)-s coupling (at least in fibroblasts). They also provide evidence against indirect effects on STAT3 through epidermal growth factor receptor transactivation. Zhang et al used a genetic mouse model of cardiac myocyte-specific deletion
extracts from STAT3 CKO mice. Furthermore, they detected and voltage-gated L-type calcium channel subunits) in cardiac cyclase, protein kinase A subunits, ryanodine receptor 2, coupling (including mRNAs coding β1-adrenoceptor, adenyl cyclase, protein kinase A subunits, ryanodine receptor 2, and voltage-gated L-type calcium channel subunits) in cardiac extracts from STAT3 CKO mice. Furthermore, they detected downregulation of transcripts for the major components of EC coupling, eg, Adrb1, Prkca, and RyR2, thereby sustaining contractility, and to inhibit transcription of Cacna1h, coding T-type calcium channels, thereby putatively attenuating adverse remodeling (not illustrated). Others have shown negative transcriptional regulation of microRNAs such as miRNA-7a and miRNA-199a, resulting in preservation of metabolism and energy production under adrenergic stress. Right. This is reinforced by STAT3 association with mitochondrial membrane with protective effects, eg, in ischemia/reperfusion. Top, In addition, cardiac STAT3 controls the production of signaling peptides (eg, erythropoietin), acting paracrinely to modulate angiogenesis and interstitial fibrosis. Note that excessive stimulation of cardiac STAT3 results in opposite effects, with myocyte damage and increased fibrosis (not illustrated), emphasizing the importance of balanced signaling. See text for more details.

Figure. Pleiotropic signaling by signal transducer and activator of transcription-3 (STAT3) in cardiac myocytes. Left, B1-2ARs potentiate excitation-contraction (EC) coupling through cAMP/protein kinase A (PKA) phosphorylation of L-type calcium channel (LTCC), ryanodine receptor-2 (RYR2) (and sarcoplasmic/endoplasmic reticulum calcium ATPase-2a, not illustrated). Center, beta1- and beta2-adrenoceptors phosphorylate and activate cardiac STAT3 through Src tyrosine kinase. Dimerized STAT3 enters the nucleus to activate transcription of genes coding key elements of EC coupling, eg, Adrb1, Prkca, and RyR2, thereby sustaining contractility, and to inhibit transcription of Cacna1h, coding T-type calcium channels, thereby putatively attenuating adverse remodeling (not illustrated). Others have shown negative transcriptional regulation of microRNAs such as miRNA-7a and miRNA-199a, resulting in preservation of metabolism and energy production under adrenergic stress. Right, This is reinforced by STAT3 association with mitochondrial membrane with protective effects, eg, in ischemia/reperfusion. Top, In addition, cardiac STAT3 controls the production of signaling peptides (eg, erythropoietin), acting paracrinely to modulate angiogenesis and interstitial fibrosis. Note that excessive stimulation of cardiac STAT3 results in opposite effects, with myocyte damage and increased fibrosis (not illustrated), emphasizing the importance of balanced signaling. See text for more details.
compensatory changes as a result of lifelong STAT3 deletion). Nonconditional STAT3 CKO mice are well known to develop progressive cardiomyopathy (eg, with repeated pregnancies in females or aging in males) that in itself is accompanied by generic alterations of EC coupling as observed here, including decreased calcium transients and contractility. However, in the present work, young animals (age, 2 months) were used, and phenotypes may differ somewhat between genetic models used in different laboratories. In any case, the demonstration of the regulatory role of STAT3 for the catecholamine-induced inotropic effect (as well as cAMP, calcium transient, and sarcomeric reticulum load increases) was done in isolated adult myocytes (in the tet-on-off inducible mouse model). Together with the observation of dobutamine-induced phosphorylation of STAT3 and its nuclear translocation in adult myocytes, where it may control transcription of β1-adrenoceptor and protein kinase A, this finding highlights an unprecedented role for this transcription factor in the control of key elements of EC coupling.

Whether these transcriptional effects explain all of the remodeling phenotype under stress is less certain. Indeed, previous work has highlighted the pleiotropic effects of STAT3 in myocardial protection against, for example, ischemia/reperfusion, doxorubicin toxicity, or multiple pregnancies. The last was attributable to the transcriptional control by STAT3 of cardiac manganese superoxide dismutase. In the absence of STAT3, increased oxidant stress activates cathepsin D, which cleaves the lactating hormone prolactin into a 16-kDa derived peptide with prominent antiangiogenic properties. STAT3 depletion then results in a microvascular cardiomyopathy underlying the development of postpartum cardiomyopathy (PPCM) when high prolactin and oxidant stress are combined after labor. Such changes (decreased STAT3 and high 16-kDa prolactin) have been validated in human PPCM and justified the launching of a multicentric clinical trial testing the effect of bromocriptine (a dopaminergic agonist that decreases prolactin secretion) for the treatment of PPCM. Because prolactin is a stress hormone, its release under stresses other than labor (ie, sepsis, infarction, or specific antidopaminergic treatments), combined with increased oxidant stress, may well produce a similar ischemic microvascular cardiomyopathy when cardiac STAT3 is reduced. STAT3 also controls the transcription of several microRNAs that functionally affect myocardial integrity. For example, STAT3 downregulates the expression of miR199a that targets the ErbB4 receptor for neuregulin-1, thereby maintaining important protective (eg, nitric oxide synthase-dependent) paracrine signaling. Cardiac STAT3 was also shown to control the expression of erythropoietin, which acts paracrinally to maintain CCL2/CCR2 signaling, which is critical for the differentiation of stem cell antigen-1+ cardiac progenitors into endothelial cells.

In terms of the adrenergic system, Hilfiker-Kleiner and her team recently demonstrated an exquisite cardiotoxicity of dobutamine or isoproterenol in mice with cardiac deletion of STAT3 that does not involve bromocriptine-sensitive production of 16-kDa prolactin but a global energetic deficit of the myocardium leading to cardiomyopathy and terminal failure. They nailed down the mechanism to STAT3 transcriptional control of 2 microRNAs, miR-7a and miR-199a, both of which directly (miR-7a) or indirectly (miR-199a through ErbB4) regulate the expression of glucose transporter type 4, the key transporter of glucose into cardiac myocytes. STAT3 deletion (and glucose transporter type 4 downregulation) resulted in metabolic inflexibility, characterized by the incapacity of cardiac myocytes to upregulate glycolysis in response to β-adrenergic stimulation (as happens physiologically). Combined with decreased fatty acid β-oxidation, the consequence is a state of metabolic starvation with increased production of mitochondrial oxidant radicals, rapidly leading to myocyte loss and ventricular failure. Notably, the authors retrospectively detected a higher incidence of terminal heart failure in patients with PPCM (known to have decreased STAT3 expression) when treated with dobutamine.

Together, these 2 laboratories independently observed an unprecedented role of cardiac STAT3 in sustaining cardiac myocyte contractility in the face of β-adrenergic stimulation through transcriptional control of key elements of EC coupling (eg, β1-adrenoceptor, protein kinase A) and epigenetic control of cardiac metabolism (through microRNA regulation of glucose transporter type 4). Failure of either (or both) may well explain the adverse remodeling leading to cardiac failure in the CKO mouse model and the exquisite toxicity of catecholamine in patients with acquired or inherited depletion of functional STAT3. Clinically, this finding highlights the need for caution concerning excessive adrenergic stimulation in situations such as PPCM and for pharmacological strategies that would preserve or enhance cardiac STAT3 signaling in the face of catecholaminergic stress.

Sources of Funding
This work was supported in part by an Action de Recherche Concertée—Fédération Wallonie-Bruxelles (ARC11-16035), a European Union Horizon2020 grant (634559), and a grant from the Fonds National de la Recherche Scientifique (FRNS-PDR T.0049.14).

Disclosures
None.

References

Song LS, Huang XY, Shou W. Critical roles of STAT3 in development and disease.


β-Adrenergic Receptors Cooperate With Transcription Factors: The "STAT" of Their Union
J.-L. Balligand

Circulation. 2016;133:4-7; originally published online December 1, 2015;
doi: 10.1161/CIRCULATIONAHA.115.019860

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/133/1/4

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2015/12/02/CIRCULATIONAHA.115.019860v1.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
Correction Notice

Beta-Adrenergic Receptors Cooperate With Transcription Factors:

The “STAT” of Their Union

Running title: Balligand; Cardiac STAT3 modulates beta-adrenergic signaling

J-L Balligand, MD, PhD

Université Catholique de Louvain, Brussels, Belgium

CORRECTION:

Reference #30 was incorrectly listed as In Press; however, it is still under review. Please note the citation should read:
