

**Editorial**

**β-Adrenergic Receptors Cooperate With Transcription Factors**

The “STAT” of Their Union

J.-L. Balligand, MD, PhD

Through their wide tissue/cellular distribution, β-adrenergic receptors are key regulators of cardiovascular function and remodeling. Classically, beta1- and beta2-adrenoceptors positively influence all aspects of cardiac contractility through G-α-s coupling to adenylyl cyclase and cAMP/protein kinase A phosphorylation of critical effectors of excitation-contraction (EC) coupling, whereas β3-adrenoceptors exert antipathetic effects, thereby attenuating those of B1-2AR stimulation. B1-2ARs are also known to initiate signaling that is independent of protein kinase A/cAMP and involves β-arrestin–dependent activation of extracellular signal-regulated kinases. Moreover, like many G-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 Ca2+ concentrations) in specific subcellular compartments, leading to activation of Ca2+-responsive pathways such as calcium/calmodulin-dependent protein kinase or calcineurin–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 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, 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 cardiotoxin-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. 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. Activated STAT3 dimers then translocate to the nucleus, where they activate specific interferon-γ–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.

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, including after autocrine production of IL-6. Using β-adrenoceptor antagonists and Src inhibitor 1 in cardiac myocytes, as well as embryonic fibroblasts genetically deficient in B1-2AR or Src/Yes/Fyn tyrosine kinases, they show this to be mediated by β-adrenoceptor and Src activation but that it is independent of classic G-α-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...

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From the Université Catholique de Louvain, Brussels, Belgium.

Correspondence to J.-L. Balligand, MD, PhD, Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique, Department of Medicine, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, FATH B1.53.09, 52 Ave Mounier, 1200 Brussels, Belgium. E-mail jl.balligand@uclouvain.be

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Article see p 48
 extracts from STAT3 CKO mice. Furthermore, they detected and voltage-gated L-type calcium channel subunits) in cardiac coupling (including mRNAs coding 1-adrenoceptor, adenylyl cyclase, protein kinase A subunits, ryanodine receptor 2, calcium sparks, and phosphorylation of troponin-I. This is probably not attributable to a nonspecific, generalized alteration of EC coupling as a consequence of the chronic genetic deletion because the phenotype was reproduced in mice with acute, tetracycline-dependent (tet/on-off) cardiac-specific deletion of STAT3. In addition, STAT3-deficient cardiac myocytes still responded to the positive inotropic effect of ouabain.

STAT3 deletion produced even more dramatic changes in remodeling after minipump infusion of isoproterenol for 7 days, with enhanced hypertrophy, fibrosis, and apoptosis compared with control hearts. What accounted for all these changes? Consistent with the traditional function of STAT3, the authors examined transcriptional changes. They first screened databases available from previous chromatin immunoprecipitation/microarray experiments in embryonic stem cells looking at STAT3 genome-wide promoter occupancy and found target genes with functional relevance to cardiac biology (Table I in the online-only Data Supplement10). These included, for example, Adrb2 (coding B2AR) and RyR2 (coding the cardiac ryanodine receptor). They confirmed the downregulation of transcripts for the major components of EC coupling in cardiac extracts from STAT3 CKO mice. Furthermore, they detected STAT3 binding to conserved binding sites in the promoters of Adrb1 (coding β1-adrenoceptor) and Prkaca (coding a catalytic unit of protein kinase A) by chromatin immunoprecipitation, but many other transcripts may be dysregulated as an indirect consequence of broader biological effects of cardiac STAT3 deletion a fortiori because these transcript measurements have not been repeated in the conditional knockout (thereby allowing

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 para-

of STAT3 (CKO mice) to examine its functional role on catecholamine-induced contraction. Surprisingly, they found a depressed contractile response to dobutamine in Langendorff-perfused hearts, paralleled by downregulation of all elements of EC coupling, that is, cAMP, calcium transients, calcium sparks, and phosphorylation of troponin-I. This is probably not attributable to a nonspecific, generalized alteration of EC coupling as a consequence of the chronic genetic deletion because the phenotype was reproduced in mice with acute, tetracycline-dependent (tet/on-off) cardiac-specific deletion of STAT3. In addition, STAT3-deficient cardiac myocytes still responded to the positive inotropic effect of ouabain.

Although efonidipine has some preferential affinity for T-type calcium channels, it is not absolutely specific,31 and its protective effect through systemic administration is no definitive proof of the causality of the cardiac T-type channel in the phenotype of STAT3 CKO mice. In all probability, additional players are involved, as also suggested by the protection of the drug against some (but not all) aspects of remodeling. Besides Cacna1h, Zhang et al have validated the direct binding of STAT3 to only a few additional gene promoters regulating EC coupling by chromatin immunoprecipitation, but many other transcripts may be dysregulated as an indirect consequence of broader biological effects of cardiac STAT3 deletion a fortiori because these transcript measurements have not been repeated in the conditional knockout (thereby allowing
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 myocardies (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 oxidative 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 (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 for caution concerning excessive adrenergic stimulation in patients with acquired or inherited depletion of functional STAT3. Clinically, this finding highlights the need for attention 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.

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Disclosures

None.

References

7. Gao H, Wang F, Wang W, Makarewicz CA, Zhang H, Kubo H, Berretta RM, Barr LA, Molkentin JD, Houser SR. Ca(2+) influx through L-type 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 attention 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.


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Correction Notice

Beta-Adrenergic Receptors Cooperate With Transcription Factors:

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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:
