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EDITORIAL COMMENT

Alterations in β3-Adrenergic Cardiac Innervation and Nitric Oxide Signaling in Heart Failure*

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Sympathetic activation of cardiac beta-adrenergic signaling pathways modulates inotropic, chronotropic, and lusitropic responses, providing fundamental control over cardiac reserve responses (1). Classically these effects are mediated through the 7-transmembrane spanning, G-protein coupled receptors, β1- and β2-adrenoceptors (ARs), which couple to adenylyl cyclase production of cyclic adenosine monophosphate (cAMP) via G_s (Fig. 1). Over the past 2 decades, it has become appreciated that the adrenergic nervous system within the heart also contains a negative regulator of inotropy and cardiac reserve, acting through a third β-AR (β3) (2). Activation of the β3-AR exerts its effects through G_i coupling to cyclic guanine monophosphate-nitric oxide (cGMP-NO) (3). Thus, adrenergic activation of the heart comprises an internal breaking mechanism that offsets maximal activation of cardiac cAMP responses (4).

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β3-ARs have classically been considered mediators of metabolic effects (i.e., promoting lipolysis and energy expenditures) in adipose tissue (5), but the discovery of their expression in human ventricular myocardium (2) has added a crucial insight into the complexities of adrenergic cardiac regulation in heart failure. As β1 and β2 receptors are classically appreciated to be down-regulated in heart failure (6), it has become important to examine the fate of the β3 receptor and its downstream signaling under similar states of stress. β3-ARs are up-regulated in heart failure (6) but whether this up-regulation is a protective response to adrenergic overactivity (a hallmark of heart failure) or a contributor to heart failure remains to be resolved. Furthermore, it is well established that the negative inotropic effects of β3-AR result from downstream production of nitric oxide (NO) by nitric oxide synthase (NOS) signaling (3,4), and could thus have favorable effects. In this context, NOSs are also fundamentally altered in both abundance and spatial localization in the failing heart (1,7), and the impact of this shift on β3 cardiac innervation in heart failure has heretofore remained incompletely elucidated.

The contribution of Niu et al. (8) in this issue of the *Journal* addresses these important issues. Using a mouse model of pressure overload induced by transverse aortic constriction (TAC), they show that β3-AR stimulation with a β3-agonist (BRL 37344) reduced cardiac remodeling and improved cardiac function after 3 weeks of treatment. They propose that this reduction occurs via restoration in nitroso-redox balance (9), arising from a neuronal NOS (nNOS)–dependent increase in NO generation accompanied by reactive oxygen species reduction. Using specific nNOS inhibitors and nNOS knockout (KO) mice, they further demonstrate that nNOS as opposed to endothelial NOS (eNOS) was the primary downstream isoform maintaining NO and reactive oxygen species balance in the failing heart.

These findings fit well within the existing knowledge base regarding nNOS translocation in the failing heart, in which nNOS increases in abundance and translocates from the sarcoplasmic reticulum (SR) to the sarcolemma under conditions of cardiac stress (10–12). Although the current investigation did not examine nNOS subcellular localization, this finding is highly consistent within animal models of myocardial injury and in humans with cardiomyopathy (10–12). It has previously been well established that under physiological conditions, eNOS is the primary NOS isoform participating in β3-induced negative inotropy (3,4). Yet, in this current study, Niu et al. (8) show that eNOS may actually be deactivated by β3-AR stimulation in that Ser1177 phosphorylation was decreased, whereas Ser114 phosphorylation was increased following BRL treatment. Furthermore, eNOS uncoupling induced by TAC was not reversed by BRL treatment (8).

In an important additional twist on the interaction between β3-AR and nNOS, Niu and colleagues show that β3-AR stimulation further up-regulated nNOS protein, consistent with a feed-forward mechanism. Moreover, BRL stimulation of β3-AR worsened cardiac hypertrophy and left ventricular (LV) dysfunction induced by TAC in nNOS KO mice (8). This finding shows very clearly that nNOS up-regulation (likely accompanied by translocation to the sarcolemma) is an adaptive mechanism in the stressed heart, and is consistent with studies showing that nNOS KO mice developed more severe LV remodeling and impaired β-adrenergic reserve following myocardial infarction (MI) compared with control mice (13,14).

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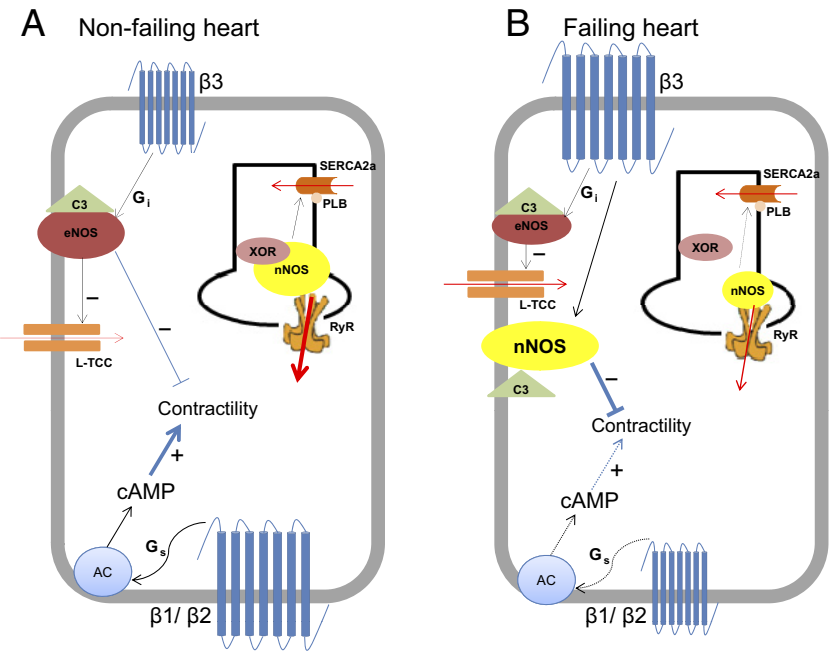

Figure 1 *β*-AR and NOS Signaling in Healthy and Failing Hearts

(A) In the healthy heart, β1- and β2-adrenoceptors (ARs) mediate a positive inotropic effect via the Gs-cAMP pathway, whereas stimulation of the β3-AR exerts a counter-vailing, negative inotropic effect via the Gi-nitric oxide (NO) pathway. Endothelial NO synthase (eNOS) bound to caveolin-3 (C3) is normally found at the sarcolemma, whereas neuronal NO synthase (nNOS) bound to the ryanodine receptor (RyR) and xanthine oxidoreductase (XOR) is found in sarcoplasmic reticulum (SR). **(B)** In heart failure, β1- and β2-ARs are down-regulated or desensitized, and eNOS levels are down-regulated, whereas β3-ARs and nNOS levels are up-regulated. Furthermore, nNOS translocates from SR to sarcolemma. This translocation results in nitroso-redox imbalance contributing to depressed contractility and impaired calcium handling. As shown by Niu et al. (8), nNOS at the sarcolemma is adaptative as transverse aortic constriction in nNOS knockout mice showed detrimental effects. AC = adenylyl cyclase; L-TCC = L-type Ca^{2+} channel; PLB = phospholamban; SERCA2a = sarcoplasmic reticulum Ca^{2+} ATPase.

The mechanism by which the nNOS pathway is involved in regulating cardiac contractility was not examined in the current study. As previously mentioned, in normal hearts (12,15), nNOS is localized to cardiac SR, where it co-localizes with RyR2 and xanthine oxidoreductase and mediates SR Ca^{2+} release (16). Furthermore, our lab has shown that RyR2 receptors in nNOS KO mice are hyponitrosylated, which, in turn, leads to increased diastolic Ca^{2+} leak and reduced intra-SR Ca^{2+} content (17). This leakage, in turn, decreases contractility indicating that in normal hearts, nNOS facilitates myocardial contractile reserve (7,15,17). Following MI and in failing myocardium due to dilated cardiomyopathy, nNOS translocates to the sarcolemma where it binds to caveolin-3 and PMCA4b (10–12). Translocation of nNOS from SR to sarcolemma may have dual effects on myocardial contractility. Increased NO production in caveolae may augment feedback inhibition of L-type Ca^{2+} channels, whereas decreased local NO production at the SR may disrupt normal S-nitrosylation-mediated regulation of SR Ca^{2+}-ATPase (SERCA2A) and RyR2 (18). Furthermore, loss of nNOS-derived NO from SR may remove the inhibition of xanthine oxidoreductase, leading to substantial increases in O_2^- production (16), which is up-regulated in failing hearts (19). The resulting nitroso-redox imbalance may enhance the risk of unregulated and irreversible RyR2 activation by O_2^-, diastolic Ca^{2+} leak, and depletion of SR Ca^{2+} stores contributing to depressed cardiac contractility (19).

What are the clinical relevance and future perspectives of the Niu et al. (8) findings? Their findings suggest that β3-ARs, due to their protective and negative inotropic effects on the myocardium, represent an attractive candidate target for a novel pharmacological strategy in heart failure. Previous studies using β3-AR agonists in the treatment of obesity and type 2 diabetes resulted in unfavorable outcomes in animal and human studies due to lack of potency or deleterious side effects such as tachycardia and muscle tremor arising from lack of β3-AR selectivity (5). However, the use of these agonists in heart failure may be more promising. First, β3-ARs are expressed at levels that can mediate physiological responses in healthy myocardium (2). Second, unlike β1-AR, β3-AR is elevated in the myocardium in patients with heart failure from ischemic or dilated cardiomyopathies (6). Third, β3-AR are more resistant (than β1 and β2) to homologous desensitization (20). Finally, co-treatment with β-blockers further increases the expression of β3-AR (21). Recent studies using nebivolol, which has combined properties of a β1-AR antagonist and β3-AR agonist, resulted in concentration-dependent negative inotropic effects through activation of the NOS path-

way in nonfailing transplanted human hearts (22), and improved LV systolic function in mice subjected to MI (23). While these early observations are exciting and suggest that β3-AR pathway has the potential to become an important therapeutic target in the treatment of heart failure, more longitudinal studies in animal models and patients are needed to examine the effect of long-term stimulation of cardiac β3-ARs.

In summary, we have now unraveled another layer in understanding the role of the β3-AR-NO pathway in heart failure. As shown by Niu and colleagues, the increased abundance of β3-AR interfaces with another important intracellular adaptation in the failing heart, the translocation of nNOS from SR to sarcolemma. Together these adaptations lead to enhanced β3-NOS signaling, which favorably offsets the remodeling process. Disrupting the nNOS downstream signaling is clearly deleterious in TAC, reminiscent of previous work in MI (13,14). Together, this entire body of work supports translational efforts to modulate β3-AR-nNOS signaling in the stressed heart due to a variety of insults, including pressure overload, ischemic injury, and cardiomyopathy.

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Key Words: β3-adrenergic receptor ■ heart failure ■ hypertrophy ■ nitric oxide synthase ■ oxidative stress.