β-Adrenergic Receptor Signaling: An Acute Compensatory Adjustment—Inappropriate for the Chronic Stress of Heart Failure?

Insights from Gsα Overexpression and Other Genetically Engineered Animal Models

Stephen F. Vatner, Dorothy E. Vatner, Charles J. Homcy

Under normal physiological conditions, the heart must be able to increase its output 5-fold to supply the required blood flow to the coronary circulation and skeletal muscles during severe stress. This is normally met by ≈5-fold increases in myocardial contractility, ≈3-fold increases in heart rate, and additional increases in stroke volume. This increased load requires a commensurate increase in myocardial blood flow, because oxygen extraction across the heart is nearly complete, even under normal conditions. Accordingly, the design of the cardiovascular system evolved to conserve myocardial metabolic demand, and consequently coronary blood flow, at rest, but with considerable reserve that can be called on rapidly in times of stress. There is a host of compensatory adjustments, including changes in metabolic substrates and kinetics, as well as oxygen-carrying capacity, that may be recruited in response to stress. However, none is more important than the autonomic nervous system in general, and the sympathetic arm in particular, in terms of providing large, rapid changes in cardiac function. When this compensatory mechanism is unavailable, eg, after treatment with propranolol, the 3-fold increases in heart rate and 5-fold increases in myocardial contractility in response to exercise cannot be achieved.1

In this connection, it is recognized that heart failure is a state characterized by enhanced sympathetic tone, but when the failing myocardium is challenged by β-adrenergic stimulation in vivo or in vitro, the most frequent result is β-adrenergic downregulation or desensitization.2–5 An impairment of cardiac function leads to autocrine, paracrine, and neurohormonal adjustments, including a strong sympathetic component (Figure 1); under acute conditions, these reflex adjustments are beneficial, as noted above. However, when the sympathetic nervous system is chronically and tonically stimulated, as occurs in the pathogenesis of heart failure, desensitization mechanisms are called into play, such that the effects of sympathetic stimulation are muted.2–5 These mechanisms include decreased β-adrenergic receptor density, decreased adenyl cyclase activity, and uncoupling the β-adrenergic receptor from Gs, in conjunction with an increase in β-adrenergic receptor kinase (βARK) activity, as well as an increase in the content of the inhibitory GTP-binding protein, Gi.2–5 If one of the consequences of chronic stress is the generation or development of desensitization, then one might argue that the desensitization response is appropriate. However, there is no consensus regarding this point, and indeed, this aspect of cardiovascular pathophysiology and therapy has been controversial for the past half century. Diametrically opposing camps have emerged, one supporting a role for β-adrenergic supplementation in heart failure6–8 and the other suggesting that further inhibition of β-adrenergic signaling and enhancing desensitization is palliative.9–12 In fact, a β-adrenergic receptor agonist, dobutamine, is still frequently administered acutely to patients with cardiac failure, because it may provide short-term benefit. However, a recent study suggested that patients receiving intravenous dobutamine have an increased risk of death.13 This is the crucial point that must be kept in mind: the differences between the initial salutary action of sympathomimetic amines and the effects of chronically and tonically stimulating this pathway.

There have been several approaches to resolving this controversy related to whether chronic sympathetic stimulation or inhibition is better in heart failure therapy. Most recently, a variety of genetic approaches have been used, in which key components of the β-adrenergic receptor signaling pathway have either been overexpressed or diminished in mice.6,7,14–17 One goal of this review is to summarize the results from these experiments and, importantly, to point out again, as noted above, the critical differences between the consequences of acute and chronic β-adrenergic receptor stimulation.

One unifying feature for all of these models is that in young animals, enhancement of β-adrenergic receptor signaling,
whether through overexpression of β1- or β2-adrenergic receptors, adenyl cyclase, or Gsα, leads to enhanced cardiac function. Two major approaches have been used to chronically enhance β-adrenergic signaling in genetically engineered animals, as follows: (1) augmenting the stimulating component (overexpression of β1- or β2-adrenergic receptors), overexpression of adenyl cyclase, and overexpression of Gsα or (2) inhibiting β-adrenergic receptor kinase. Some of these approaches have proven useful in “rescuing” pathological phenotypes that develop cardiomyopathy and heart failure. For example, when Gaq-overexpressing mice, which develop cardiac hypertrophy and dysfunction, are mated with mice overexpressing β2-adrenergic receptors at a relatively low level (30-fold), the crossbred mice fared better in terms of cardiac function and development of hypertrophy than did the mice solely overexpressing Gaq. Positive results have been even more impressive with mice that express the β-adrenergic kinase inhibitor. When those mice were mated with MLP-1 mice, which develop dilated cardiomyopathy as a result of ablation of a muscle-restricted gene that encodes the muscle LIM protein, the development of cardiomyopathy was attenuated. Furthermore, heart rate variability, as occurs in heart failure, was restored. Similarly, one wonders whether a similar picture of cardiomyopathy might be observed in other models of enhanced β-adrenergic receptor signaling with age. Only recently have some of these data become available, which also support the concept that chronically and tonically enhanced β-adrenergic receptor signaling is deleterious. One facet of chronically enhanced sympathetic stimulation is chronic tachycardia with reduced heart rate variability, as occurs in heart failure. It also must be appreciated that enhanced β-adrenergic signaling is more complex than simply an augmentation in contractility following an increase in cAMP generation in response to receptor occupancy by agonist. There appear to be cAMP-independent actions, as well, that can augment cardiac contractility. For example, there is evidence that β-adrenergic stimulation through the Gs protein can affect myocyte function, independent from cAMP generation, potentially by a direct action on the L-type calcium channel. In myocytes from the mice with overexpressed cardiac Gsα, a significant increase in myocyte contractility and Ca2+ channel activity still occurs with β-adrenergic stimulation even after the cAMP pathway is blocked with Rp-cAMP, a diastereoisomer of adenosine 3’,5’-phosphorothioate and inhibitor of protein kinase A. It remains to be determined whether the adverse effects of chronic β-adrenergic signaling in the overexpressed Gsα mouse is solely cAMP-dependent or involves a cAMP-independent, albeit Gsα-mediated, activity. The latter pathway could synergistically contribute to the

Figure 1. Proposed scheme demonstrating the role of reflex, autonomic, endocrine, and paracrine pathways that are called into play to compensate for an acute impairment in cardiac function. Under acute conditions, these adjustments are beneficial. However, under chronic conditions, a vicious cycle is established, in which, in the presence of limited coronary reserve, these pathways can be deleterious, and conversely, desensitization pathways may be protective.

First of all, one of the limitations to many cardiovascular studies is the “snapshot” experiment, in which 1 or at least a few rapid measurements are made before the experiment is terminated. In addition, most experiments in animals are carried out in young adults, despite the fact that heart failure is usually a disease of older patients. In larger mammals, it is not generally possible to conduct serial experiments over their lifetime, because that time span may exceed the productive period of the investigator. However, this is possible in murine models, particularly transgenic mice, with lifetimes of ≈2 years. Nonetheless, the overwhelming majority of studies have reported the results from genetically altered mice at 1 or 2 times in their lifetime, most often recording the last measurement in young adulthood.

One exception to that rule is the murine model of cardiomyopathy. These animals exhibit enhanced β-adrenergic receptor signaling and normal myocardial architecture as young adults. However, as they age, they develop a picture resembling cardiomyopathy in humans. They exhibit a dilated heart with reduced ejection fraction, ventricular arrhythmias, sudden death, myocardial hypertrophy, interstitial myocardial fibrosis, and apoptosis in humans (Figure 2). These characteristics of cardiomyopathy were not due to aging, per se, given that age-matched wild-type littermates were entirely normal. However, although aging, per se, is not the cause of the cardiomyopathy, it is conceivable that there are age-related alterations in gene expression or activity of signaling pathways that predispose the overexpressed Gsα mouse, but not normal-aged mice, to develop cardiomyopathy.
adverse effects of enhanced cAMP signaling. This question could be addressed by chronically interrupting the β-adrenergic signaling pathway at the level of adenylyl cyclase or protein kinase A activation in the Gsa mouse model.

The nature of the downstream pathway responsible for the deleterious phenotype in the setting of chronically enhanced β-adrenergic signaling is also likely to be complex, potentially involving alterations in stress-activated kinase pathways as well as aberrant energy production and utilization,²⁷ potentially leading to alterations at the transcriptional level as well. It is important to keep in mind that the physiological responses induced by the altered genotype may well invoke other genetic and/or biochemical changes in the heart, which might play a role in determining the end result of cardiomyopathy. This latter point is not generally appreciated, ie, that the phenotype of many transgenic models is more complex than being simply the consequences of the changed genotype, because other compensatory mechanisms may be invoked. Moreover, dissection of other compensatory mechanisms, eg, identification of altered gene expression, remains an intriguing avenue of research that is now possible with the development of transgenic models of cardiac dysfunction. To reiterate, the mouse overexpressing Gsa recapitulates the pattern of chronic cardiac sympathetic overdrive that occurs in the pathogenesis of human heart failure, but in the absence of primary myocardial overload or dysfunction. Thus, it offers the potential to delineate more precisely the pathways and genes activated or inhibited by chronic sympathetic stimulation of the heart.

How is it possible to reconcile the apparently diametrically opposing conclusions from our studies on the transgenic mouse with overexpressed Gsa and the transgenic mice with overexpressed β-adrenergic receptors? In both models there is enhanced β-adrenergic signaling and cardiac function in young adult animals. However, the Gsa mice were also studied after they had aged. It is our thesis, and one of the major themes of this review, that the effects of chronically enhanced β-adrenergic signaling over the lifetime of the animal are deleterious, particularly in the face of ineffective desensitization mechanisms. Until the other models of enhanced β-adrenergic signaling are studied for comparable periods of time, and the extent of desensitization is analyzed, this hypothesis cannot be tested fully.

As proof of principle, it would be useful to block the enhanced β-adrenergic signaling, and to determine whether the cardiomyopathy that develops in the older Gsa mice is averted. We recently accomplished this in the overexpressed cardiac Gsa model by treating the animals chronically with a β-adrenergic receptor antagonist propranolol.²⁸ In that study, we began administering propranolol in the drinking water to mice.²⁸ Most interestingly, the β-adrenergic receptor blockade arrests myocyte damage and preserves cardiac function in the transgenic Gsa mouse. Kaplan-Meier survival curves of propranolol-treated and untreated TG mice are shown (Figure 2). Propranolol treatment abolished (log-rank test, P<0.05) premature mortality in TG mice. (Reproduced with permission from Asai K, Yang G-P, Geng Y-J, Takagi G, Bishop S, Ishikawa Y, Shannon R, Wagner T, Vatner D, Homcy C, Vatner S. β-Adrenergic receptor blockade arrests myocyte damage and preserves cardiac function in the transgenic Gsa mouse. J Clin Invest. 1999;104[5]:551–558).

Figure 2. Left ventricular ejection fraction (LVEF; top left), myocyte cross-sectional area (top right), and terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL)-positive myocytes (bottom left) were quantified in untreated older transgenic (TG) mice (solid bars), and propranolol-treated older TG mice (shaded bars) and older wild-type (WT) mice (open bars). Chronic propranolol administration reduced the apoptosis and hypertrophy characteristic of older Gsa mice. Kaplan-Meier survival curves of propranolol-treated and untreated TG mice are shown (bottom right). Propranolol treatment abolished (log-rank test, P<0.05) premature mortality in TG mice. (Reproduced with permission from Asai K, Yang G-P, Geng Y-J, Takagi G, Bishop S, Ishikawa Y, Shannon R, Wagner T, Vatner D, Homcy C, Vatner S. β-Adrenergic receptor blockade arrests myocyte damage and preserves cardiac function in the transgenic Gsa mouse. J Clin Invest. 1999;104[5]:551–558).
positive beneficial effects of chronic β-adrenergic receptor blockade in patients with heart failure.9–12 These clinical studies, which have been summarized recently,29 clearly demonstrate the positive beneficial effects of β-adrenergic receptor blockade therapy in heart failure, improving left ventricular function and survival. Some of these drugs relieve β-adrenergic desensitization in heart failure, whereas others do not.30,31

Even more recently, supporting evidence for the adverse effects of chronic β-adrenergic signaling has become apparent from transgenic murine models of overexpressed β1-adrenergic receptors32 as well as β2-adrenergic receptors (Figure 3). Again, the extent of overexpression (clearly high levels of overexpression are deleterious), the duration of the enhanced β-adrenergic receptor signaling, and the extent to which desensitization mechanisms attenuate the adverse effects of chronic β-adrenergic signaling all play a role. In this connection, it is important to keep in mind that the overexpressed Gsa mice do not fully desensitize, ie, enhanced isoproterenol stimulation of adenylyl cyclase is still observed in the older animals. In contrast, in animals with overexpressed β1-adrenergic receptors, isoproterenol-stimulated adenylyl cyclase is downregulated, which most likely tempers the downstream action of the enhanced β-adrenergic receptor signaling (K.-L. He, unpublished observations, 1999). In addition, there may be a differential effect of β1- versus β2-adrenergic overexpression, given that β1-adrenergic stimulation is a more potent stimulus for hypertrophy.33

Although complete elucidation of the role of β-adrenergic receptor signaling in heart failure is important, it is just 1 component in this complex process. If the concept proposed in this review is correct, ie, enhanced β-adrenergic signaling is counterproductive in heart failure, it simply represents 1 step forward in our understanding of the complex process termed heart failure. Nevertheless, resolution of this controversy, which has been at the forefront of cardiovascular pathophysiology and heart failure therapy over the past half century, remains important for both the basic cardiovascular scientist and the clinician. Before the controversy regarding the role of β-adrenergic receptor signaling in heart failure is fully resolved, there will be many other dialectical positions proposed, including short-term adrenergic stimulation, followed by chronic blockade or intermittent gene therapy, or myocyte-specific activation. However, at this time, the majority of evidence supports the position that chronic and tonic β-adrenergic receptor signaling is deleterious in the pathogenesis of heart failure, whereas interruption of this pathway appears salutary. This holds for enhanced β-adrenergic signaling accomplished by overexpressing β1-adrenergic receptors (5- to 15-fold) or Gsa (5-fold). Apparently, β2–adrenergic receptors have to be overexpressed quantitatively more, ie, >100-fold, to result in cardiomyopathy. Why there is this dose-related difference between β1- and β2-adrenergic receptor overexpression needs to be resolved. The key to this problem may reside in differences in distal signaling pathways. Additional important factors include the chronicity of stimulation and the extent to which desensitization mechanisms are effective.

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References


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