Editorial

How Do β-Blockers Improve Ventricular Function in Patients With Congestive Heart Failure?

William H. Barry, MD; E. Michael Gilbert, MD

The binding of β-adrenergic agonists such as norepinephrine and isoproterenol to the β-1 adrenergic receptor (AR) in the sarcolemma of the ventricular myocyte increases intracellular levels of cAMP via G_s protein-induced stimulation of adenyl cyclase. cAMP activates protein kinase A (PKA), which causes phosphorylation of proteins involved in Ca^{2+} homeostasis, such as phospholamban and the L-type Ca^{2+} channel, increasing the intracellular calcium ion concentration ($[Ca^{2+}]_i$) transient, and thus causing a positive inotropic effect. It is now very well established that patients with congestive heart failure due to ischemic or idiopathic dilated cardiomyopathy are in a hyperadrenergic state.[1] Treatment of these patients with β-adrenergic receptor-blocking drugs reduces morbidity and mortality,[2] improves ventricular function, and reverses pathological remodeling.[3] Clinical and experimental animal studies have suggested a number of mechanisms by which chronic exposure to this class of drugs, which have a negative inotropic effect in normal myocardium, could have an apparently paradoxical beneficial effect in failing myocardium.

See p 2459

Seminal work by Bristow and associates[4] showed that β-1 AR density is reduced in the failing myocardium, and receptor density is increased by treatment with some β-AR blockers.[5] An increase in β-receptor density may restore toward normal an available positive inotropic reserve in patients with heart failure. In isolated myocytes, a cytotoxic effect of prolonged adrenergic stimulation can be demonstrated,[6] suggesting that β-blockade may reduce a deleterious effect of the chronic hyperadrenergic state on myocyte survival. Treatment with β-blockers also slows the heart rate. Because failing myocardium displays a decrease in contractility with increasing rate of stimulation,[7] this may improve ventricular function. At slow rates of stimulation, the myocyte action potential is prolonged, allowing for more Ca^{2+} influx via Na/Ca exchange, and permitting more complete relaxation and reloading of the sarcoplasmic reticulum (SR) with Ca^{2+}, despite a reduced expression of SR Ca^{2+} ATPase SERCA2a, which is present in the myocardium of many patients with heart failure.[8] Finally, the reduced expression of SERCA2a and α-myosin heavy chains[9] may be restored toward normal by changes in gene expression induced by β-blocking drugs.[10] This could improve systolic and diastolic function.

In this issue of *Circulation*, Reiken et al[11] describe another possible mechanism by which β-blockers may improve SR function and thus calcium homeostasis and contractility in failing myocardium: A reduction in PKA-mediated hyperphosphorylation of the SR calcium release channel. The cardiac calcium release channel, or ryanodine receptor 2 (RyR2), is a macromolecular complex comprised of homotetramers, with each of the 4 subunits containing a PKA phosphorylation site and capable of binding 1 molecule of FK506-binding protein (FKBP12.6). The channel complex, which is opened by exposure to Ca^{2+} entering the myocyte via the L-type Ca^{2+} channel during excitation-contraction coupling, also includes phosphatases, which can induce dephosphorylation. As Reiken et al[11] discuss, work from their group has shown that FKBP12.6 is dissociated from RyR2 by exposure to FK506, or by hyperphosphorylation, and this causes the RyR2 calcium release channel to display an increased sensitivity to Ca^{2+}, a greater open probability resulting in a "leaky" channel that could cause SR Ca^{2+} depletion, and impaired cooperativity between RyR subunits. Reiken et al[11] studied myocardium obtained from hearts of 9 patients with heart failure not treated with β-blockers, from 10 patients with heart failure treated with β-blockers, and from 5 normal patients. In patients with heart failure, there was a reduced binding of FKBP12.6 to RyR2 associated with hyperphosphorylation of RyR2 detected by a back phosphorylation technique and abnormal RyR2 channel function in planar lipid bilayers characterized by an increased opening probability and greater prevalence of subconductance states. All these abnormalities were reversed toward normal in myocardium from patients with heart failure who had been treated with β-blockers. Reiken et al[11] suggest that RyR2 hyperphosphorylation due to increased activation of PKA (and reduced phosphatase activity) results in dissociation of FKBP12.6 from RyR2, thus inducing a SR Ca^{2+} leak with SR Ca^{2+} depletion and hence a negative inotropic effect. Treatment with β-blockers is proposed to improved myocardial function by decreasing the degree of phosphorylation of RyR2, thus decreasing the degree of dissociation of FKBP12.6 and enhancing SR function.

It is somewhat surprising that hyperphosphorylation of RyR2 is present in heart failure, in which there is downregulation of β-ARs. Indeed, some studies have shown that phosphorylation of phospholamban, another substrate for PKA-mediated phosphorylation, is actually reduced in myo-

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Cardiology Division, University of Utah Health Sciences Center, Salt Lake City.

Correspondence to William H. Barry, MD, Division of Cardiology, University of Utah Health Sciences Center, 50 North Medical Dr, Salt Lake City, UT 84132. E-mail whbarry@med.utah.edu

(*Circulation* 2003;107:2395-2397.)

© 2003 American Heart Association, Inc.

***Circulation* is available at http://www.circulationaha.org**
DOI: 10.1161/01.CIR.0000070984.65122.9C

cardium from patients with heart failure.[12] However, recent work[13] has demonstrated that there are discrete microdomains within cardiac myocytes in which effects of PKA may be regulated by the binding of the enzyme to its anchoring proteins and by co-localization of phosphodiesterases and phosphatases that can regulate regional concentrations of cAMP and of phosphorylated substrates, respectively. Variable alterations in these factors may explain why one target of PKA might be hyperphosphorylated whereas another might be hypophosphorylated in the failing myocyte. There are data from several experimental studies in animal models that are consistent with the hypothesis that hyperphosphorylation of RyR2 does occur with heart failure.[11] That this causes dissociation of FKBP12.6 from RyR2 and results in SR Ca^{2+} depletion is supported by work of Yano et al.[14] These investigators found that defective interaction of FKBP12.6 with RyR2 in a canine model of pacing-induced heart failure is associated with an abnormal SR Ca^{2+} leak, which they suggest may contribute to impaired function of the myocardium. In addition, we recently reported that dissociation of FKBP12.6 from RyR2 by exposure of rabbit ventricular myocytes to FK506 causes depletion of SR Ca^{2+} stores and a resulting decrease in the myocyte $[Ca^{2+}]_i$ transient.[15]

Despite these supportive findings, there are some results that seem to challenge the concept that hyperphosphorylation-induced dissociation of FKBP12.6 is an important factor in abnormal Ca^{2+} homeostasis in heart failure. Jiang et al[16] have reported that RyR2 isolated from failing human myocardium displayed no differences in vitro in open probability or the incidence of subconductance states as compared with controls. In addition, they detected no difference in the degree of RyR2 phosphorylation. Preliminary work by Terentyev et al[17] has shown that in permeabilized myocytes from rats with heart failure induced by chronic isoproterenol administration, exposure to phosphatases PP1 and PP2a induced activation of Ca sparks and depletion of Ca^{2+} stores. They suggest that their results are inconsistent with the idea that heart failure is associated with hyperphosphorylation of RyR, rendering the Ca^{2+} release channel leaky to Ca^{2+}. To some extent, these discrepancies may result from the fact that different experimental techniques have been used in different animal models of heart failure, and that different species were used. For example, dissociation of FKBP12.6 from RyR2 by exposure to FK506 is associated with an increase in the $[Ca^{2+}]_i$ transient in rat[18] and mouse[15] myocytes, but causes a decrease in the $[Ca^{2+}]_i$ transient in rabbit myocytes.[15] Nevertheless, the importance of hyperphosphorylation of RyR2 in influencing myocyte contractility in heart failure needs and is certain to receive further investigation.

Finally, we should consider whether phosphorylation-induced dissociation the FKBP12.6 from RyR2 is important in normal physiology and whether alteration of the FKBP12.6–RyR2 interaction may be significant in other clinical situations. Reiken et al[11] suggest that the acute increase in RyR2 phosphorylation induced by PKA may be a component of the "fight or flight" response by increasing RyR2 sensitivity to Ca^{2+} and thus enhancing SR Ca^{2+} release and the $[Ca^{2+}]_i$ transient. This would cause an acute increase in contractility, along with PKA-induced increases in the SR Ca^{2+} uptake induced by phospholamban phosphorylation, and the increase in the L-type Ca^{2+} current induced by phosphorylation of the Ca^{2+} channel. This is plausible, but one might question why a positive inotropic effect, rather than a negative inotropic effect resulting from depletion of SR Ca^{2+} due to Ca^{2+} release channel leakiness, would predominate in the presence of hyperphosphorylation-induced dissociation of FKBP12.6 from RyR2. The reason may relate to the degree to which SR Ca^{2+} stores can be maintained in the face of an increased SR Ca^{2+} leak. For example, FK506 induces depletion of SR Ca^{2+} in rabbit myocytes and decreases the $[Ca^{2+}]_i$ transient, whereas in mouse SR Ca^{2+} stores are not depleted and FK506 increases the transient.[15] Thus, in normal myocardium, during sympathetic stimulation with activation of β-adrenergic receptors, stimulation of SR Ca^{2+} uptake by phosphorylation of phospholamban might be sufficient to maintain SR Ca^{2+} stores despite a SR Ca^{2+} release channel leak with a resulting positive inotropic effect due to increased sensitivity of RyR2 to Ca^{2+}. In failing myocardium, because of downregulation of SERCA2a and possibly reduced phosphorylation of phospholamban,[12] this compensatory increase in SR Ca^{2+} uptake might be inadequate to maintain SR Ca^{2+} stores.

The immunosuppressive actions of FK506 (tacrolimus) are well known and result from the ability of a complex of FK506 and FKBP to bind to calcineurin and inhibit its phosphatase activity, resulting in the inhibition of T-lymphocyte activation.[19] Use of FK506 in pediatric transplant recipients has been reported to be associated with the development of hypertrophic cardiomyopathy.[20] Long-term exposure to ryanodine, which impairs SR Ca^{2+} release channel function, has been reported to induce hypertrophy in rats.[21] Therefore, FK506 could possibly induce hypertrophy in young patients because of its ability to cause dissociation of FKBP12.6 from RyR2, thus inducing SR dysfunction. No significant myocardial effects of the use of tacrolimus in adult patients have been recognized, but could theoretically occur. Further study of the clinical significance of modulation of RyR2 function by drugs, intracellular signaling pathways, and other RyR2-associated proteins such as sorcin[22] in heart failure and other conditions is clearly warranted.

References

1. Cohn JN, Levine TB, Olivari MT, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med*. 1984;311:819–823.
2. Foody JM, Farrell MH, Krumholz HM. β-blocker therapy in heart failure. *JAMA*. 2002;287:883–889.
3. Olsen SL, Gilbert EM, Renlund DG, et al. Carvedilol improves left ventricular function and symptoms in chronic heart failure: a double-blind randomized study. *J Am Coll Cardiol*. 1995;25:1225–1231.
4. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med*. 1982;307:205–211.
5. Gilbert EM, Abraham WT, Olsen S, et al. Comparative hemodynamic, left ventricular functional, and antiadrenergic effects of chronic treatment with metoprolol versus carvedilol in the failing heart. *Circulation*. 1996; 94:2817–2825.
6. Mann DL, Kent RL, Parsons B, et al. Adrenergic effects on the biology of the adult mammalian cardiocyte. *Circulation*. 1992;85:790–804.

7. Alpert NR, Leavitt BJ, Ittleman FP, et al. A mechanistic analysis of the force-frequency relation in non-failing and progressively failing human myocardium. *Basic Res Cardiol*. 1998;93(suppl 1):23–32.
8. Barry WH. Na^{+}-Ca^{2+} exchange in failing myocardium. *Circ Res*. 2000; 87:529–531.
9. Miyata S, Minobe W, Bristow MR, et al. Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circ Res*. 2000;86: 386–390.
10. Lowes BD, Gilbert EM, Abraham WT, et al. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *N Engl J Med*. 2002;346:1357–1365.
11. Reiken S, Wehrens XHT, Vest JA, et al. *β*-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation*. 2003;107:2459–2466.
12. Frank KF, Bolck B, Brixius K, et al. Modulation of SERCA: implications for the failing human heart. *Basic Res Cardiol*. 2002;97(suppl 1):I72–I78.
13. Zaccolo M, Pozzan T. Discrete microdomains with high concentration of camp in stimulated rat neonatal cardiac myocytes. *Science*. 2002;95: 1711–1715.
14. Yano M, Ono K, Ohkusa T, et al. Altered stoichiometry of FKP12.6 versus ryanodine receptor as a cause of abnormal Ca(2+) leak through ryanodine receptor in heart failure. *Circulation*. 2000;102:2131–2136.
15. Su Z, Sugishita K, Li F, et al. Effects of FK506 on $[Ca^{2+}]_i$ differ in mouse and rabbit ventricular myocytes. *J Pharmacol Exp Ther*. 2003;304:334–341.
16. Jiang MT, Lokuta AJ, Farrell EF, et al. Abnormal Ca^{2+} release, but normal ryanodine receptors, in canine and human heart failure. *Circ Res*. 2002;91:1015–1022.
17. Terentyev D, Viatchenko-Karpinski S, Smirnov A, et al. Activation of SR Ca release by protein phosphatases in myocytes from normal and failing rat hearts. *Biophys J*. 2003;89:200A. Abstract.
18. McCall E, Li L, Satoh H, et al. Effects of FK-506 on contraction and Ca^{2+} transients in rat cardiac myocytes. *Circ Res*. 1996;79:1110–1121.
19. Marks AR. Cellular functions of immunophilins. *Physiol Rev*. 1996;76: 631–649.
20. Atkison P, Joubert G, Barron A, et al. Hypertrophic cardiomyopathy associated with tacrolimus in paediatric transplant patients. *Lancet*. 1995; 345:894–896.
21. Meyer M, Trost SU, Bluhm WF, et al. Impaired sarcoplasmic reticulum function leads to contractile dysfunction and cardiac hypertrophy. *Am J Physiol Heart Circ Physiol*. 2001;280:H2046–H2052.
22. Lokuta AJ, Meyers MB, Sander PR, et al. Modulation of cardiac ryanodine receptors by sorcin. *J Biol Chem*. 1997;272:25333–25338.

KEY WORDS: Editorials ■ heart failure ■ ventricles ■ receptors, adrenergic, beta