Cardiomyocyte Life-Death Decisions in Response to Chronic β-Adrenergic Signaling

Russell S. Whelan, Klitos Konstantinidis, Rui-Ping Xiao, Richard N. Kitsis

β-adrenergic signaling in the heart is a double-edged sword. In the short-term, it enhances cardiac function, whereas chronic high-level activation results in heart failure. In this issue of *Circulation Research*, Zhang et al[1] investigate mechanisms that mediate β-adrenergic–induced cell death and, in doing so, uncover a survival pathway of potential clinical relevance.

Article, see p 498

A basic principle of cardiac pharmacology is that acute activation of β-adrenergic receptors (referred to herein as β-receptors) increases heart rate (chronotropy), contractility (inotropy), and relaxation of heart muscle (lusitropy) to augment cardiac systolic and diastolic performance. These responses couple cardiac function with physiological demands, such as exercise, and provide compensatory mechanisms when the circulation is threatened by insults, such as hemorrhage or sudden deterioration in cardiac function. The major ligand in this context is norepinephrine, a catecholamine derived primarily from the postganglionic sympathetic neurons that innervate the heart and, to a lesser extent, from the adrenal medulla.

Given the beneficial hemodynamic effects of acute β-adrenergic signaling, cardiologists in the 1980s hypothesized that activation of this pathway may benefit patients with advanced heart failure, a condition with an astounding 5-year mortality of ≈50%. Indeed this approach improved cardiac hemodynamics in the short term, but it soon became apparent that chronic treatment results in marked increases in patient mortality.[2] Conversely, β-receptor blockade reduced mortality,[3] a finding presaged by small studies decades earlier.[4] These paradoxical findings were at odds with the accepted notion that improvement of hemodynamics alone should be sufficient to stem the progression of heart failure. The resolution of these data ultimately necessitated a new paradigm. In this model, catecholamines initially help the failing heart to compensate for decreased pump function. However, when present chronically at high levels, they function as toxins that promote adverse cardiac remodeling and progressive deterioration of function.

How does chronic β-adrenergic stimulation damage the heart? This is thought to occur through dysfunction of viable cardiomyocytes (eg, abnormalities in Ca^{2+} handling)[5] and cell death. Cardiomyocytes may die via apoptosis, necrosis, or perhaps autophagy. Multiple genetic and pharmacological studies in cells and intact mice indicate that the β_1-receptor working through $G\alpha_s$ not only mediates acute increases in chronotropy and inotropy but, when activated chronically, also plays the primary role in cell death[6–11] (Figure). In contrast, some data indicate that the β_2-receptor may signal survival when its coupling switches from $G\alpha_s$ to $G\alpha_i$.

Although it is clear that chronic β_1-receptor activation induces cell death, considerable controversy has surrounded the mechanism. The major bone of contention has been whether protein kinase A (PKA) plays a critical role. Inactive PKA is composed of 2 inhibitory subunits bound to 2 catalytic subunits. The binding of 2 cAMP molecules to each of the regulatory subunits releases the catalytic subunits, which then become enzymatically active after the binding of ATP. Although the liganding of agonists to the β-receptor activates PKA, the question is whether PKA activation is needed for killing in cardiomyocytes.

Initial investigations, using H89 or KT5720, small molecules that competitively inhibit the binding of ATP to the catalytic subunits, found that β-adrenergic–induced death of isolated neonatal and adult rat cardiomyocytes is dependent on PKA.[7,8] Unfortunately, H89 and KT5720 are now recognized to have multiple off-target effects, including inhibition of other kinases and even β-adrenergic receptors.[12]

A subsequent study used more specific approaches to inhibit PKA:[13] Rp-8-CPT-cAMPS, which competitively inhibits cAMP binding to PKA regulatory subunits; and PKI, a peptide derived from an endogenous protein inhibitor of PKA that binds the catalytic subunits at the site occupied by the regulatory subunit in the inactive state.[14] These independent approaches demonstrated the PKA independence of β-adrenergic–induced death.

Zhang et al[1] addresses this issue for the first time in vivo, using mice that express an inducible, cardiomyocyte-specific PKI-GFP transgene. Isolated adult feline cardiomyocytes were also studied using adenoviral-mediated gene transfer. In both contexts, β-adrenergic–induced cardiomyocyte death was found to be dependent on PKA over a range of acute and chronic time points. In addition, inhibition of PKA in the transgenic mice abrogated β-adrenergic–induced increases in cardiac hypertrophy and fibrosis and decreases in systolic

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

From the Departments of Medicine (R.S.W., K.K., R.N.K.) and Cell Biology (R.S.W., K.K., R.N.K.), Wilf Family Cardiovascular Research Institute (R.S.W., K.K., R.N.K.), Diabetes Research Center (R.N.K.), and Albert Einstein Cancer Center (R.N.K.), Albert Einstein College of Medicine, Bronx, NY; Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA (K.K.); and Institute of Molecular Medicine, Center for Life Sciences, Peking University, Beijing, China (R.-P.X.).

Correspondence to Richard N. Kitsis, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail richard.kitsis@einstein.yu.edu

(*Circ Res*. 2013;112:408-410.)

Circulation Research **is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.113.300805

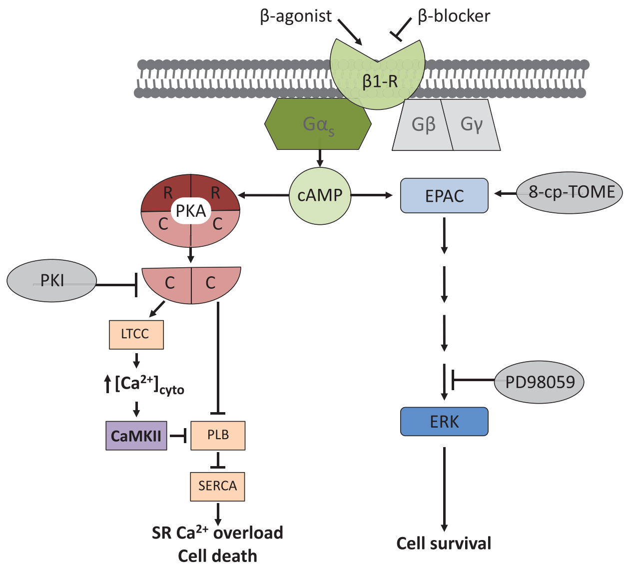

Figure. Chronic β_1-adrenergic receptor activation induces cardiomyocyte death. The binding of a β-adrenergic ligand to the β1-receptor activates adenyl cyclase through $G\alpha_s$ resulting in the production cAMP. The binding of 2 cAMP molecules to each of the protein kinase A (PKA) regulatory subunits (R) releases the catalytic subunits (C), which are enzymatically active after the binding of ATP. PKA phosphorylates many targets including the L-type calcium channel resulting in the entry of Ca^{2+} into the cytoplasm. Increases in intracellular Ca^{2+} activate calmodulin-dependent protein kinase II (CaMKII). PKA and CaMKII phosphorylate phospholamban relieving its inhibition of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), thereby resulting in the entry of Ca^{2+} into the sarcoplasmic reticular (SR) lumen. When signaling is sustained, the SR becomes Ca^{2+}-overloaded resulting in the induction of apoptosis and necrosis through a variety of mechanisms (not shown). cAMP also binds to and activates exchange protein directly activated by cAMP (EPAC), leading to a cascade (not shown) that activates extracellular signal-related kinase (ERK), which signals cell survival. Note that the events depicted are highly simplified. PLB indicates phospholamban; PKI, protein kinase inhibitor of PKA (see text); 8-cp-TOME, small molecule activator of EPAC; and PD98059, small molecule that inhibits ERK activation.

function. Although the conclusions of the in vivo and cell culture experiments in this study are concordant, they are opposite to the results of the cell culture experiments in the PKI study described above, which found that β-adrenergic–induced killing was PKA-independent.[13] A potential explanation for this discrepancy is that the PKI constructs in these studies were not identical. Although both peptides were successful in inhibiting endogenous PKA activity, it is theoretically possible that they differ with respect to other biological effects. A more obvious difference, however, is the species of the cultured cardiomyocytes used—adult cat in the current study versus adult mouse in the previous one. It is possible that the higher levels of cytosolic Ca^{2+} in unpaced isolated murine cardiomyocytes[15] lower the threshold for cell death, thereby bypassing the requirement for PKA. Future experiments could test alternative strategies to inhibit PKA, such as other dominant negative alleles and simultaneous knockdown of the isoforms of the catalytic subunits. However, given that the possible discrepancy in PKA dependence may be attributable to species differences, it might be most informative to conduct studies in a more clinically relevant species, such as the pig, using pharmacological and in vivo viral gene transfer approaches.

The present study goes on to address events downstream of PKA. In agreement with previous work,[13] intracellular Ca^{2+} and Ca^{2+}/calmodulin-dependent protein kinase II play important roles in β-adrenergic–induced cardiomyocyte killing. Mechanisms linking PKA with calmodulin-dependent protein kinase II remain controversial, however. This study presents pharmacological evidence that calmodulin-dependent protein kinase II activation in response to β-agonists requires PKA-stimulated increases in Ca^{2+} influx through L-type calcium channels. In accordance with this model, PKI blocks these increases. The investigators also found that increases in sarcoplasmic reticular [Ca^{2+}] seem to be particularly important in β-adrenergic–induced cell death. Although this sarcoplasmic reticular Ca^{2+}-overload model is consistent with mechanisms of cardiomyocyte apoptosis and necrosis in other settings, it also needs to be considered in the context of data demonstrating that long-term β-adrenergic stimulation depletes the sarcoplasmic reticular of Ca^{2+}.[5] Insights into this conundrum may be provided by a more precise delineation of the time course of sarcoplasmic reticular [Ca^{2+}] during β-stimulation.

Perhaps, the most exciting aspect of Zhang et al[1] is the identification of a β-adrenergic–induced survival pathway. Exchange protein directly activated by cAMP (EPAC)[16] is a guanine nucleotide exchange protein for the small GTPases, Rap1 and Rap2. As its name indicates, it is activated by cAMP, independently of PKA. Although prior work has shown that EPAC can promote or inhibit cell death depending on the cellular context, it seems to promote survival in cardiomyocytes. By using PKI to block the PKA-mediated death arm of the pathway, this study reveals that β-adrenergic stimulation activates EPAC and promotes cardiomyocyte survival in a manner dependent on extracellular signal-related kinases (ERKs). Although the necessity of EPAC for these survival effects still requires testing, its sufficiency was demonstrated using 8-cp-TOME, a cAMP analogue that directly activates EPAC. These cell culture findings were then taken to a mouse model of postmyocardial infarction heart failure. PKI-mediated inhibition of PKA was more effective in preserving cardiac function 4 weeks after infarction than was metoprolol (a β_1-specific antagonist), suggesting that targeted inhibition of the PKA-dependent death pathway is superior to ablation of both death and survival pathways downstream of the β_1-receptor.

What are the potential clinical implications of these data? While one might consider substituting peptide or small molecule inhibitors of PKA for β_1-blockers in the treatment of postmyocardial infarction heart failure, it must be kept in mind that β-blockers provide beneficial effects beyond those considered here. A more attractive option might be to supplement β-blockade with a small molecule activator of the EPAC-ERK pathway.

Zhang et al[1] have provided critical insights into the bigger picture of cardiomyocyte life-death decisions resulting from chronic β-adrenergic stimulation. The hypotheses generated by these data may provide important opportunities for clinical translation.

Acknowledgments

We thank Andrew R. Marks, Evangelia Kranias, Alexander Kushnir, Joan Heller Brown, and W. Jonathan Lederer for helpful discussions.

Sources of Funding

This work was supported by grants to R.N. Kitsis from the National Institutes of Health (5R01HL060665-14, 1R03DA031671-02, 5U01HL099776-04) and The Harrington Project for Discovery and Development. R.N. Kitsis is supported by the Dr Gerald and Myra Dorros Chair in Cardiovascular Disease. We are most grateful to the Wilf Family for their ongoing generosity and support.

Disclosures

None.

References

1. Zhang X, Szeto C, Gao E, et al. Cardiotoxic and cardioprotective features of chronic β-adrenergic signaling. *Circ Res*. 2013;112:498–509.
2. Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, Kukin ML. Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. *N Engl J Med*. 1991;325:1468–1475.
3. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *N Engl J Med*. 1996;334:1349–1355.
4. Swedberg K, Hjalmarson A, Waagstein F, Wallentin I. Prolongation of survival in congestive cardiomyopathy by beta-receptor blockade. *Lancet*. 1979;1:1374–1376.
5. Wehrens XH, Lehnart SE, Huang F, et al. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell*. 2003;113:829–840.
6. Shizukuda Y, Buttrick PM, Geenen DL, Borczuk AC, Kitsis RN, Sonnenblick EH. beta-adrenergic stimulation causes cardiocyte apoptosis: influence of tachycardia and hypertrophy. *Am J Physiol*. 1998;275:H961–H968.
7. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. *Circulation*. 1998;98:1329–1334.
8. Iwai-Kanai E, Hasegawa K, Araki M, Kakita T, Morimoto T, Sasayama S. alpha- and beta-adrenergic pathways differentially regulate cell type-specific apoptosis in rat cardiac myocytes. *Circulation*. 1999; 100:305–311.
9. Bisognano JD, Weinberger HD, Bohlmeyer TJ, Pende A, Raynolds MV, Sastravaha A, Roden R, Asano K, Blaxall BC, Wu SC, Communal C, Singh K, Colucci W, Bristow MR, Port DJ. Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. *J Mol Cell Cardiol*. 2000;32:817–830.
10. Asai K, Yang GP, Geng YJ, Takagi G, Bishop S, Ishikawa Y, Shannon RP, Wagner TE, Vatner DE, Homcy CJ, Vatner SF. Beta-adrenergic receptor blockade arrests myocyte damage and preserves cardiac function in the transgenic G(salpha) mouse. *J Clin Invest*. 1999;104:551–558.
11. Yoo B, Lemaire A, Mangmool S, Wolf MJ, Curcio A, Mao L, Rockman HA. Beta1-adrenergic receptors stimulate cardiac contractility and CaMKII activation in vivo and enhance cardiac dysfunction following myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2009;297:H1377–H1386.
12. Murray AJ. Pharmacological PKA inhibition: all may not be what it seems. *Sci Signal*. 2008;1:re4.
13. Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, Devic E, Kobilka BK, Cheng H, Xiao RP. Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. *J Clin Invest*. 2003;111: 617–625.
14. Ashby CD, Walsh DA. Characterization of the interaction of a protein inhibitor with adenosine 3',5'-monophosphate-dependent protein kinases. I. Interaction with the catalytic subunit of the protein kinase. *J Biol Chem*. 1972;247:6637–6642.
15. Bers DM. Cardiac Na/Ca exchange function in rabbit, mouse and man: what's the difference? *J Mol Cell Cardiol*. 2002;34:369–373.
16. de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature*. 1998;396:474–477.

Key Words: apoptosis ■ β-adrenergic signaling ■ cell death ■ EPAC ■ heart failure ■ necrosis ■ PKA

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Cardiomyocyte Life-Death Decisions in Response to Chronic β-Adrenergic Signaling

Russell S. Whelan, Klitos Konstantinidis, Rui-Ping Xiao and Richard N. Kitsis

Circ Res. 2013;112:408-410
doi: 10.1161/CIRCRESAHA.113.300805
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

Correction

In the *Circulation Research* article by Whelan et al (Whelan RS, Konstantinidis K, Xiao RP, Kitsis RN. Cardiomyocyte life-death decisions in response to chronic β-adrenergic signaling. *Circ Res.* 2013;112:408–410. DOI: 10.1161/CIRCRESAHA.113.300805), the Sources of Funding section needed to be updated as follows:

This work was supported by grants to R.N. Kitsis from the National Institutes of Health (5R01HL060665-14, 1R03DA031671-02, 5U01HL099776-04) and The Harrington Project for Discovery and Development. R.N. Kitsis is supported by the Dr Gerald and Myra Dorros Chair in Cardiovascular Disease. We are most grateful to the Wilf Family for their ongoing generosity and support.

This update has been corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/112/3/408.full

(*Circ Res*. 2013;112:e181.)

Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/RES.0b013e31829c46a1