Decreased beta-adrenergic responsiveness following hypertrophy occurs only in cardiomyocytes that also re-express beta-myosin heavy chain

Kumar Pandya¹, Kristine Porter², Howard A. Rockman², and Oliver Smithies^{1*}

¹Department of Pathology and Laboratory Medicine, University of North Carolina, 701 Brinkhous Bullitt Building, Chapel Hill, NC 27599-7525, USA; and ²Department of Medicine, Duke University, Durham, NC, USA

Received 25 November 2008; revised 21 March 2009; accepted 2 April 2009

Aims

Cardiac hypertrophy is associated with a reduction in the contractile response to beta-adrenergic stimulation, and with re-expression of foetal genes such as beta-myosin heavy chain (MHC). However, whether these two markers of pathology develop concordantly in the same individual cells or independently in different cells is not known.

Methods and results

To answer this question, we examined the beta-adrenergic response of individual beta-MHC expressing and non-expressing myocytes from hypertrophic hearts, using a previously generated mouse model (YFP/beta-MHC) in which a yellow fluorescent protein (YFP) is fused to the native beta-MHC protein allowing easy identification of beta-MHC expressing cells. Yellow fluorescent protein/beta-MHC mice were submitted to 4 weeks of transverse aortic constriction (TAC), and the contractile parameters of isolated individual myocytes in response to the beta-adrenergic agonist isoproterenol were assessed. Our results demonstrate that the decrease in isoproterenol-induced cell shortening that develops in TAC hearts occurs only in those hypertrophic myocytes that re-express beta-MHC. Hypertrophic myocytes that do not express beta-MHC have contractility indices indistinguishable from non-TAC controls.

Conclusion

These data show that the reduction of beta-adrenergic response occurs only in subsets, rather than in all myocytes, and is coincident with re-expression of beta-MHC.

Keywords

Cardiac hypertrophy • Beta myosin heavy chain • Beta adrenergic receptors

Introduction

Re-expression of beta-myosin heavy chain (MHC) is a well-documented molecular marker of pathological cardiac hypertrophy.¹ Normally, the beta-MHC isoform is expressed in a developmental stage-specific manner in the mouse ventricle. Beta-MHC expression is high during embryonic and foetal stages, but decreases rapidly soon after birth when it is replaced by the expression of alpha isoform.² Beta-MHC is re-expressed in the murine ventricle following cardiac hypertrophy and during cardiac failure.^{1–5} Reversal of cardiac failure is associated with

beta-MHC expression returning to baseline levels.^{3,6} The beta-MHC isoform has a lower ATPase activity, but greater force of contraction than the alpha isoform.⁷ Recent reports have demonstrated that even modest increases in the levels of beta-MHC are accompanied by a significant detriment in pump function of the heart including a decrease in contractility (+dp/dt).^{8,9}

At the cellular level, a functional hallmark of cardiac hypertrophy and heart failure is a depressed contractile response of individual cardiac myocytes to beta-adrenergic stimulation.^{10,11} In myocytes from a healthy adult heart, beta-adrenergic agonists such as

* Corresponding author. Tel: +1 919 966 6912, Fax: +1 919 966 8800, Email: jenny_langenbach@med.unc.edu

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2009. For permissions please email: journals.permissions@oxfordjournals.org.

isoproterenol induce a short-term hypercontractile response by increasing the degree of cell shortening and by increasing the velocities of contraction and relaxation. However, myocytes sampled from the hearts of mice undergoing hypertrophy and heart failure show a markedly decreased response to beta-adrenergic stimulation, manifested as a smaller increase in cell shortening and in the velocities of contraction and relaxation.^{12,13} This effect is a direct consequence of a decrease in the number of beta-adrenergic receptors as well as impaired function of the receptor.^{14–18} The decrease in beta-adrenergic response is directly correlated with the severity of the cardiac phenotype, and genetic and therapeutic interventions that maintain normal beta-adrenergic receptor numbers and signalling have a substantial positive impact on heart function.^{10,12,19–23}

At the level of individual myocytes, the relationship between the re-expression of beta-MHC and reduced beta-adrenergic responsiveness is, however, not known. Thus, it is not known whether these two markers of cardiac pathology always develop together in all the cells that change or arise independently in different responding cells. To answer this question, we evaluated the contractile parameters of individual beta-MHC expressing and non-expressing cardiac myocytes isolated from the same heart exposed to a hypertrophic stimulus.

Methods

The generation of YFP/beta-MHC animals, and procedures for morphometrical analyses, confocal image acquisition, transverse aortic constriction (TAC), and myocyte isolation have been described previously.^{24,25} The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Evaluation of myocyte function

Myocytes were placed in a 0.5 mL chamber with 1.8 mM Ca^{2+} Tyrode's solution at room temperature, and visualized with a Nikon inverted microscope with a solid-state CCD camera attached and displayed on a video monitor. The expression of YFP in individual cells was scored using an epifluorescence apparatus attached to the microscope. Two platinum electrodes connected to a stimulator were used to field stimulate the myocytes with pulse duration of 5 ms and a frequency of 0.5 Hz. Myocyte cell edges are enhanced and processed with a video edge motion detection system (Crescent Electronics) at a sampling rate of 240 Hz. Recordings were performed either under basal conditions or after isoproterenol stimulation ($1 \mu\text{M}$). Calibrated myocyte lengths were converted from analog to digital on-line (MacLab) and stored on computer. All myocytes were studied within 1–2 h after myocyte isolation. Data from three consecutive contractions were averaged. Approximately 15 myocytes from each category were studied. The recordings for the control group were acquired from untreated hearts which contain only non-yellow myocytes. Those for the TAC group were acquired from yellow and non-yellow myocytes isolated from single hearts. The data for basal and isoproterenol stimulation within each of the three groups (control non-yellow, TAC non-yellow, TAC yellow) were acquired from different cells and analysed with ANOVA with Newman–Keuls correction using Graphpad Prism software. Student's *t*-test was used for morphometrical analyses (Figure 1).

Figure 1 (A) Morphometrical analyses. Cross-sectional areas (sq. microns) of myocytes from animals that were untreated (UT) or that underwent TAC for 4 weeks (TAC) grouped according to their yellow fluorescence (yellow cells, Y or non-yellow cells, NY). A total of 300 cells (100 cells/heart) from three hearts/condition were analysed. Error bars denote SE. (B) Yellow fluorescent images (YFP) and Cy5 fluorescence for WGA lectin (pseudo-coloured blue) acquired from the left ventricular free walls of control (UT) or TAC-treated (TAC) hearts with 20x oil objective. (C) Yellow fluorescent confocal images of ventricular sections from untreated (UT) or TAC-treated (TAC). Overlapping images were acquired using a $\times 4$ objective and stitched together using ImageJ. *** $P < 0.0001$.

Results

To determine the association between the state of beta-MHC expression and the contractile parameters of individual cells, we used mice that express a yellow fluorescent protein (YFP) fused to the amino terminal of the beta-MHC (YFP/beta-MHC) as described previously.²⁴ This YFP/beta-MHC allele is appropriately regulated during development, and accurately tracks the expression of the wild-type beta-MHC allele in response to cardiac hypertrophy *in vivo*. In our present experiments, cardiac

Table 1 Echocardiographic parameters

	Untreated	TAC
<i>n</i>	9	10
LVEDD (mm)	3.1 ± 0.2	3.3 ± 0.2
LVESD (mm)	1.8 ± 0.2	2.3 ± 0.2
% FS	43 ± 3	29 ± 3*
IVSD th (mm)	1.1 ± 0.1	1.2 ± 0.1
IVSS th (mm)	1.7 ± 0.1	1.7 ± 0.1
PWD th (mm)	1.2 ± 0.1	1.2 ± 0.1
PWS th (mm)	1.8 ± 0.1	1.5 ± 0.1
HR (b.p.m.)	604 ± 57	506 ± 54

Animals were untreated or TAC treated for 4 weeks.

n, number of animals in each group; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVSD th, inter ventricular septum thickness diastole; FS, fractional shortening; IVSS th, inter-ventricular septum thickness systole; PWD th, posterior wall thickness diastole; PWS th, posterior wall thickness systole; HR, heart rate.

*P < 0.01.

hypertrophy was induced in 4-month-old heterozygous YFP/β-MHC animals by 4 weeks of TAC. Echocardiography was conducted on these animals and the data (Table 1) show that the TAC-treated animals were in a state of heart failure (%FS 29 vs. 43 for control, *P* < 0.01), but without overt LV dilation (no significant differences in the levels of end-systolic and end-diastolic LV diameters). Morphometric analyses (Figure 1) of myocytes from the left ventricular free walls of untreated and TAC-treated hearts show that in the TAC hearts the cross-sectional areas of myocytes are larger than myocytes from untreated hearts regardless of whether the TAC myocytes express YFP/β-MHC or not. Furthermore, the cross-sectional areas of the TAC myocytes that express YFP/β-MHC (column 3) are not significantly different (*P* = 0.6) from TAC myocytes from the same hearts that do not express YFP/β-MHC (column 2), demonstrating that the YFP/β-MHC expressing cells are as hypertrophic as the non-YFP/β-MHC expressing cells. This confirms previous observations by ourselves²⁴ and by others⁹ that cellular hypertrophy is not obligatorily coupled to the re-expression of YFP/β-MHC. Ventricular sections from a TAC or control heart were analysed for YFP fluorescence using confocal microscopy, and Figure 1C illustrates the spatial distribution of YFP expressing cells in the control (UT) or TAC-treated heart.

To test whether the YFP/β-MHC expressing and non-expressing myocytes from TAC hearts have different contractile responses to β-adrenergic stimulation, myocytes from untreated control, and TAC-treated hearts were isolated by collagenase treatment. The contractile parameters were then assessed during basal (unstimulated) conditions or after stimulation with the β-adrenergic agonist isoproterenol (1 μM). Under basal conditions, the percent cell shortening (%CS, Figure 2A), as well as the rates of relaxation (dI/dt, Figure 2B) and shortening (−dI/dt, Figure 2C) of YFP/β-MHC expressing cells from TAC hearts are not significantly different from YFP/β-MHC non-expressing TAC myocytes from the same hearts (*P* > 0.05), or

Figure 2 Cell-shortening parameters of myocytes. Fractional cell shortening (% cell shortening, Y-axis) (A), rate of cell relaxation (dI/dt, B), and rate of cell shortening (−dI/dt, C) of myocytes from YFP/β-MHC animals that were either untreated (columns marked 'UT'), or treated with TAC for 4 weeks (columns marked 'TAC') that either did not express YFP-β-MHC (open bars), or that did express YFP-β-MHC (yellow bars). Myocytes from each group were either unstimulated (−) or stimulated with 1 μM isoproterenol (+). Each experimental group consists of approximately 15 myocytes/heart, and five hearts/condition. Error bars denote SE. *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA with Newman–Keuls correction.

from control hearts (*P* > 0.05). Isoproterenol stimulation of myocytes from control hearts induces a marked increase in the %CS (Figure 2A); as well as in the velocities of relaxation (Figure 2B) and shortening (Figure 2C). Isoproterenol stimulation of YFP/β-MHC non-expressing cells from the TAC hearts likewise induced a marked and significant increase in the %CS, and velocities of relaxation and shortening (Figure 2A–C), and these increases are virtually identical to those of control cells (Figure 2A–C). In marked contrast, isoproterenol stimulation of YFP/β-MHC expressing cells from the TAC hearts changed

the %CS minimally after stimulation (Figure 2A, $P > 0.05$); the %CS in the YFP/beta-MHC expressing TAC myocytes is significantly lower than that in either the YFP/beta-MHC non-expressing TAC myocytes ($P < 0.05$) or the control myocytes (Figure 2A, $P < 0.01$). The changes in rates of relaxation and shortening, of the YFP/beta-MHC expressing TAC myocytes (Figure 2B and C), in response to isoproterenol stimulation, are also significantly lower than those of the YFP/beta-MHC non-expressing TAC myocytes from the same hearts (Figure 2B and C, $P < 0.01$), or from cells of the control heart (Figure 2B and C, $P < 0.01$). These results demonstrate that those cardiac myocytes that re-express YFP/beta-MHC are also those in which the beta-adrenergic response is decreased.

To exclude the possibility that the changes in isoproterenol-induced contractility observed in the YFP/beta-MHC expressing myocytes might be due to the toxic effects of the fluorescent protein expression, we tested the contractile parameters of myocytes from an untreated transgenic animal that expresses GFP under the control of the cardiac alpha MHC promoter at a high level. The data (not shown) show that GFP-expressing myocytes from an untreated transgenic mouse display the same contractile parameters during basal conditions and in response to isoproterenol stimulation as the myocytes from the untreated non-transgenic hearts.

Taken together, our results demonstrate that the decrease in beta-adrenergic responsiveness in individual myocytes isolated from TAC treated hearts is co-incident with YFP/beta-MHC expression. Myocytes that do not express YFP/beta-MHC isolated from the same TAC treated hearts have a normal beta-adrenergic response, even though the YFP/beta-MHC non-expressing cells are as hypertrophic as those that express YFP/beta-MHC.

Discussion

We have previously demonstrated that, during cardiac hypertrophy, beta-MHC re-expression occurs in subsets (approximately 38%) rather than in all hypertrophic myocytes, indicating that the pathways that induce cellular hypertrophy are not obligatorily coupled to the pathways that induce beta-MHC.^{24,26} These YFP-expressing myocytes are mostly found in the left ventricle, generally in the mid-ventricular wall, and surround areas of fibrosis. In our current report, we demonstrate that decreased contractile response to beta-adrenergic stimulation also occurs in subsets of myocytes and is co-incident with beta-MHC re-expression; beta-MHC non-expressing cells from the same TAC hearts show a normal contractile response to beta-adrenergic stimulation even though they are equally hypertrophic.

Although we have demonstrated that the reduction of beta-adrenergic response is coincident with beta-MHC re-expression in individual cells *in vivo*, our data do not show whether the reduction of beta-adrenergic response and beta-MHC re-expression are linked in a cause-and-effect relationship or are the coincidental outcomes of some other change. However, genetic means of rescuing pathological heart failure by decreasing beta-adrenergic stimulation decrease beta-MHC expression.^{3,6} Similarly, beta-blocker therapy of hearts in failure shows a decrease in the levels of beta-MHC mRNA along with the improvement of cardiac function.^{27,28} Furthermore, in

neonatal rat myocytes, isoproterenol induces both a decrease in beta-adrenergic responsiveness, and an increase in beta-MHC expression.²⁹ These observations indicate that agents which increase or decrease beta-adrenergic responsiveness also lead to changes in beta-MHC regulation, and that pathogenic stimulation that promote beta-adrenergic receptor downregulation/desensitization will also cause beta-MHC re-expression. Future studies addressing whether the beta-MHC expressing myocytes also show an altered response to other agonists would be instrumental in dissecting the pathways that link beta-MHC re-expression to other pathological stimuli.

The reduction of beta-adrenergic response in myocytes during TAC and heart failure has been well documented.^{10–13,30,31} Our present results demonstrate that the reduction in beta-adrenergic response in myocytes following hypertrophy induced by TAC occurs in subsets rather than in all myocytes. It has been proposed that the decrease in beta-adrenergic responsiveness of cardiac myocytes during heart failure may be a consequence of increase in circulating catecholamines.³² However, different regions of the same heart have different levels of catecholamines, and myocytes from the right ventricle of animals with increased circulating catecholamines do not show signs of decreased beta-adrenergic response.³³ These results and our current finding that only subsets of myocytes within the heart display decreased beta-adrenergic responsiveness support a hypothesis that local, rather than systemic, increases in catecholamine levels cause the reduction in beta-adrenergic responsiveness and increase in beta-MHC expression that we observe in individual myocytes.

Some comment is required on the apparent discrepancy between our results that show normal basal contractile behaviour in beta-MHC expressing cells and the results of Tardiff *et al.*⁹ that showed a decrease in baseline contractile parameters in beta-MHC expressing cells. Important differences exist between the two studies. The Tardiff study used a mouse model in which beta-MHC was transgenically over-expressed in all cardiac myocytes (12% of total MHC). In contrast, in our studies, beta-MHC was re-expressed in response to pressure overload and occurred in subsets of cells. Furthermore, while the Tardiff study conducted contractility studies (measuring dp/dt) on the whole heart using retrograde perfusion on a Langendorff apparatus, our contractility studies (measuring cell shortening) were carried out at the level of individual isolated cardiac myocytes.

Acknowledgements

We thank D.Tilley, A. Pendse, and J. Arbones-Mainar for critical reading of the manuscript.

Conflict of interest: none declared.

Funding

This work was supported by grants from NIH [HL71266 and HL49277 (O.S.); HL56687 (H.A.R.)]; and the American Heart Association [0325655U and 0525594U (K.P.)]. Authors declare no financial conflict of interest.

References

1. Sadoshima J, Izumo S. The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu Rev Physiol* 1997;**59**:551–571.

2. Weiss A, Leinwand LA. The mammalian myosin heavy chain gene family. *Annu Rev Cell Dev Biol* 1996;**12**:417–439.
3. Abraham WT, Gilbert EM, Lowes BD, Minobe WA, Larrabee P, Roden RL, Dutcher D, Sederberg J, Lindenfeld JA, Wolfel EE, Shakar SF, Ferguson D, Volkman K, Linseman JV, Quaife RA, Robertson AD, Bristow MR. Coordinate changes in Myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. *Mol Med* 2002;**8**:750–760.
4. Schiaffino S, Samuel JL, Sassoon D, Lompre AM, Garner I, Marotte F, Buckingham M, Rappaport L, Schwartz K. Nonsynchronous accumulation of alpha-skeletal actin and beta-myosin heavy chain mRNAs during early stages of pressure-overload-induced cardiac hypertrophy demonstrated by in situ hybridization. *Circ Res* 1989;**64**:937–948.
5. Schwartz K, Carrier L, Chassagne C, Wisniewsky C, Boheler KR. Regulation of myosin heavy chain and actin isogenes during cardiac growth and hypertrophy. *Symp Soc Exp Biol* 1992;**46**:265–272.
6. Blaxall BC, Spang R, Rockman HA, Koch WJ. Differential myocardial gene expression in the development and rescue of murine heart failure. *Physiol Genomics* 2003;**15**:105–114.
7. Krenz M, Sanbe A, Bouyer-Dalloz F, Gulick J, Klevitsky R, Hewett TE, Osinska HE, Lorenz JN, Brosseau C, Federico A, Alpert NR, Warshaw DM, Perryman MB, Helmke SM, Robbins J. Analysis of myosin heavy chain functionality in the heart. *J Biol Chem* 2003;**278**:17466–17474.
8. Krenz M, Robbins J. Impact of beta-myosin heavy chain expression on cardiac function during stress. *J Am Coll Cardiol* 2004;**44**:2390–2397.
9. Tardiff JC, Hewett TE, Factor SM, Vikstrom KL, Robbins J, Leinwand LA. Expression of the beta (slow)-isoform of MHC in the adult mouse heart causes dominant-negative functional effects. *Am J Physiol Heart Circ Physiol* 2000;**278**:H412–H419.
10. Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000;**101**:558–569.
11. Port JD, Bristow MR. Altered beta-adrenergic receptor gene regulation and signaling in chronic heart failure. *J Mol Cell Cardiol* 2001;**33**:887–905.
12. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. *Nature* 2002;**415**:206–212.
13. Tilley DG, Rockman HA. Role of beta-adrenergic receptor signaling and desensitization in heart failure: new concepts and prospects for treatment. *Expert Rev Cardiovasc Ther* 2006;**4**:417–432.
14. Bohm M, Flesch M, Schnabel P. Role of G-proteins in altered beta-adrenergic responsiveness in the failing and hypertrophied myocardium. *Basic Res Cardiol* 1996;**91**(Suppl. 2):47–51.
15. Bohm M, Lohse MJ. Quantification of beta-adrenoceptors and beta-adrenoceptor kinase on protein and mRNA levels in heart failure. *Eur Heart J* 1994;**15**(Suppl. D):30–34.
16. Brodde OE. Beta-adrenoceptor blocker treatment and the cardiac beta-adrenoceptor-G-protein(s)-adenylyl cyclase system in chronic heart failure. *Naunyn Schmiedeberg Arch Pharmacol* 2007;**374**:361–372.
17. Harding SE, Brown LA, Wynne DG, Davies CH, Poole-Wilson PA. Mechanisms of beta adrenoceptor desensitisation in the failing human heart. *Cardiovasc Res* 1994;**28**:1451–1460.
18. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? *Circ Res* 2003;**93**:896–906.
19. Cleland JG, Bristow MR, Erdmann E, Remme WJ, Swedberg K, Waagstein F. Beta-blocking agents in heart failure. Should they be used and how? *Eur Heart J* 1996;**17**:1629–1639.
20. Hjalmarson A, Kneider M, Waagstein F. The role of beta-blockers in left ventricular dysfunction and heart failure. *Drugs* 1997;**54**:501–510.
21. Kaumann AJ, Molenaar P. The low-affinity site of the beta1-adrenoceptor and its relevance to cardiovascular pharmacology. *Pharmacol Ther* 2008;**118**:303–336.
22. Tachibana H, Naga Prasad SV, Lefkowitz RJ, Koch WJ, Rockman HA. Level of beta-adrenergic receptor kinase 1 inhibition determines degree of cardiac dysfunction after chronic pressure overload-induced heart failure. *Circulation* 2005;**111**:591–597.
23. Waagstein F. Efficacy of beta blockers in idiopathic dilated cardiomyopathy and ischemic cardiomyopathy. *Am J Cardiol* 1997;**80**:45J–49J.
24. Pandya K, Kim HS, Smithies O. Fibrosis, not cell size, delineates (beta)-myosin heavy chain reexpression during cardiac hypertrophy and normal aging in vivo. *Proc Natl Acad Sci USA* 2006;**103**:16864–16869.
25. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, Smithies O, Rockman HA. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest* 2006;**116**:1547–1560.
26. Pandya K, Cowhig J, Brackhan J, Kim HS, Hagaman J, Rojas M, Carter CW Jr, Mao L, Rockman HA, Maeda N, Smithies O. Discordant on/off switching of gene expression in myocytes during cardiac hypertrophy in vivo. *Proc Natl Acad Sci USA* 2008;**105**:13063–13068.
27. Lowes BD, Gilbert EM, Abraham WT, Minobe WA, Larrabee P, Ferguson D, Wolfel EE, Lindenfeld J, Tsvetkova T, Robertson AD, Quaife RA, Bristow MR. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *N Engl J Med* 2002;**346**:1357–1365.
28. Yasumura Y, Takemura K, Sakamoto A, Kitakaze M, Miyatake K. Changes in myocardial gene expression associated with beta-blocker therapy in patients with chronic heart failure. *J Card Fail* 2003;**9**:469–474.
29. Sucharov CC, Mariner PD, Nunley KR, Long C, Leinwand L, Bristow MR. A beta1-adrenergic receptor CaM kinase II-dependent pathway mediates cardiac myocyte fetal gene induction. *Am J Physiol Heart Circ Physiol* 2006;**291**:H1299–H1308.
30. Brodde OE. Beta-adrenergic receptors in failing human myocardium. *Basic Res Cardiol* 1996;**91**(Suppl. 2):35–40.
31. Hajjar RJ, Muller FU, Schmitz W, Schnabel P, Bohm M. Molecular aspects of adrenergic signal transduction in cardiac failure. *J Mol Med* 1998;**76**:747–755.
32. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med* 1982;**307**:205–211.
33. Bristow MR, Minobe W, Rasmussen R, Larrabee P, Skerl L, Klein JW, Anderson FL, Murray J, Mestroni L, Karwande SV. Beta-adrenergic neuroeffector abnormalities in the failing human heart are produced by local rather than systemic mechanisms. *J Clin Invest* 1992;**89**:803–815.