Native \(\beta\)-Adrenergic Support for Left Ventricular Dysfunction in Experimental Mitral Regurgitation Normalizes Indexes of Pump and Contractile Function

Masayoshi Nagatsu, MD; Michael R. Zile, MD; Hiroyuki Tsutsui, MD; Phillip G. Schmid, MD; Gilberto DeFreys, BS; George Cooper IV, MD; Blase A. Carabello, MD

**Background** It is generally accepted that the adrenergic nervous system provides inotropic support for the failing heart. However, the magnitude of this support has never been studied extensively. The present study was performed to test the hypothesis that the adrenergic nervous system is capable of maintaining indexes of pump and contractile function in the normal range despite significant innate myocardial depression.

**Methods and Results** We used our model of experimental canine mitral regurgitation, which produces left ventricular dysfunction after 3 months of volume overload. We studied indexes of contractile function on and off \(\beta\)-blockade at baseline and again on and off \(\beta\)-blockade 3 months after chronic mitral regurgitation had induced significant contractile dysfunction. At baseline, acute \(\beta\)-blockade caused insignificant reductions in the mass-corrected slope of the end-ejection stress-volume relation (EESVR), the end-systolic stiffness constant, and the ejection fraction–end-systolic stress and the mean velocity of circumferential fiber shortening (VCF)–end-systolic stress relations. After 3 months of chronic mitral regurgitation, all indexes of contractile function were normal in the unblocked state except for the VCF-stress relation, which was mildly reduced. However, after acute \(\beta\)-blockade after 3 months of chronic mitral regurgitation, the EESVR fell to 303±27 versus 443±24 during acute \(\beta\)-blockade before mitral regurgitation was created (\(P<.05\)), and the end-systolic stiffness constant was reduced to 2.54±0.15 versus 3.27±0.11 (\(P<.05\)). Only after \(\beta\)-blockade was the ejection fraction–stress relation significantly reduced for dogs with chronic mitral regurgitation. The VCF-stress relation became markedly more abnormal. The viscosity-velocity relation of myocytes isolated from the ventricles of the dogs with mitral regurgitation confirmed that substantial innate contractile depression was present.

**Conclusions** After 3 months of chronic mitral regurgitation, the adrenergic nervous system was able to maintain most indexes of contractile function in the normal range despite significant depression in innate contractile function. Thus, in the absence of \(\beta\)-blockade, significant innate contractile depression may be obscured by adrenergic support. *(Circulation. 1994;89:818-826.)*

**Key Words** contractility • mitral valve • regurgitation • receptors, adrenergic, beta

It is generally accepted that the adrenergic nervous system provides inotropic support for the failing heart.\(^1\)\(^6\) Since acute \(\beta\)-blockade may precipitate congestive heart failure in previously compensated patients with ventricular dysfunction, native adrenergic support must be substantial enough to be clinically relevant. Yet, remarkably, the magnitude of native adrenergic support for the failing heart has not been studied extensively. Without this knowledge, it is possible that clinical and experimental studies of contractile function performed in a variety of diseases in the absence of \(\beta\)-blockade detected only subjects with the most severe contractile depression—those in whom adrenergic support failed to normalize indexes of pump and contractile function. Although this group is surely an important one to study, it may not provide an accurate overview of the extent of contractile abnormalities in a given disease.

In this study, we tested the hypothesis that native \(\beta\)-adrenergic stimulation was capable of maintaining indexes of pump and contractile function in or near the normal range despite the presence of a significant reduction in native contractile state. To test this hypothesis, we examined indexes of pump and contractile function off and on \(\beta\)-blockade in normal dogs at baseline and again after 3 months of severe mitral regurgitation had induced myocardial dysfunction. Since all in vivo indexes of contractile function have limitations in application and interpretation, we also examined the contractile function of myocytes isolated from the regurgitant ventricles to corroborate the in vivo estimation of contractility.

**Methods**

**Study Design**

Indexes of pump and contractile function were studied at cardiac catheterization performed under light anesthesia in six normal dogs at baseline. Data from these animals have not
been published previously in any of our other studies. β-blockade was induced by infusing a loading dose of esmolol 1.5 mg/kg followed by a constant esmolol infusion of 0.3 mg·kg⁻¹·min⁻¹, a dose that produces near-total β-blockade in the dog.⁷ Pump and contractile function were studied again. Severe mitral regurgitation was induced by use of our closed-chest chordal rupture technique, and the animals were allowed to recover.⁸ This model consistently produces contractile dysfunction after 3 months of mitral regurgitation.⁹⁻¹² Accordingly, 3 months after mitral regurgitation had been induced, we again studied indexes of pump and contractile function in these same animals in the unblocked and β-blocked state at a time when we suspected that myocardial dysfunction would be present. In a subset of three dogs, contractile function was also studied in myocytes isolated from the ventricles in which contractile function had been studied in vivo.

Assessment of In Vivo Pump and Contractile Function

All studies were performed under light anesthesia provided by infusion of a combination of fentanyl/droperidol by vein and by inhalation of nitrous oxide and oxygen using a ratio of nitrous oxide to oxygen of 3:1. This combination of anesthetic drugs has been shown previously to have little effect on contractile state.¹³ At the initial study, a cutdown was performed over the right cervical vessels, and at the final study, a cutdown was performed over the right femoral vessels. In all other details, the studies were performed identically. All studies met the standards for animal use in experimentation set by the American Physiological Society. A Swan-Ganz catheter was advanced from the jugular or femoral vein to the pulmonary artery to record pulmonary artery and pulmonary capillary wedge pressures and to obtain cardiac output by the thermodilution method. After a 1000 rpm, 2 mL of calcium-free buffer was added to the circumflex artery was isolated, and the artery was cannulated and perfused with collagenase, allowing the disaggregation of viable myocytes. Laser diffraction was then used to analyze the extent and velocity of sarcomere shortening.²⁶²⁷ These studies were performed only on myocytes that suffered no significant damage from the isolation process according to criteria previously described.¹² By variation of the viscosity of perfusate with inert methyl cellulose, the afterload on contracting sarcomeres was varied.²⁸ The viscosity-velocity relation constructed in this manner is analogous to the force-velocity relation of classic cardiac mechanics.

Determination of Catecholamine Concentrations

Ten milliliters of blood was collected and centrifuged at 10,000 rpm at 4°C for 5 minutes, and 2 mL of plasma was saved and stored at −70°C until assayed. Plasma norepinephrine was analyzed by radioenzymatic assay with a commercial kit for determination of catecholamines (CAT-A KIT, Amersham Corp., Arlington Heights, III). Norepinephrine was converted to its meta-[³H]-methoxy derivative, ie, catechol-O-methyltransferase, in the presence of ³H methionine, extracted, and separated by thin-layer chromatography. The derivative [³H]vanillin was further converted to [³H]vanilmandelic acid by periodate oxidation, and β-radioactivity was determined by liquid scintillation counting.²⁸

Calculations

Left ventricular volumes were calculated by the area-length method. This method has previously been validated as providing an accurate measurement of volume and mass in dogs with mitral regurgitation studied in our laboratory.⁹ The regurgitant fraction (RF) was calculated from the equation

\[ RF = \frac{(SV_A - SV_F)}{SV_A} \]
where $SV_e$ is angiographic stroke volume (end-diastolic volume−end-systolic volume) and $SV_f$ is forward stroke volume (thermodilution cardiac output divided by heart rate).

Wall stress was calculated by Mirsky's formula. The EESVR was calculated by linear regression. In this study, as in previous studies, end-ejection volume rather than true end-systolic volume was used because of its clear definition, because little volume is expelled into the left atrium after closure of the aortic valve in this model, and because this method has correlated well with the more rigorous isochronally determined maximum elastance. Although end ejection and end systole may become uncoupled in mitral regurgitation, this problem is largely obviated by matching dicrotic notch pressure with end-ejection volume. The slope of the EESVR was then corrected for the development of hypertrophy by multiplying it by the mass present at the time of study. The VCF was calculated from the equation

$$VCF = \frac{EDD - ESD}{EDD \times ET}$$

where $EDD$ is end-diastolic dimension, $ESD$ is end-systolic dimension, and $ET$ is ejection time. Ejection time was calculated as the total time the left ventricle is ejecting either into the left atrium or aorta, taken from the time of angiographic appearance of contrast into the left atrium to time of aortic valve closure. This definition accounts for the occurrence in mitral regurgitation of substantial ejection before aortic valve opening. The end-systolic stiffness constant ($k$) was determined by fitting end-systolic stress and end-systolic wall thickness data to the curvilinear equation

$$\sigma = C e^{k/L}$$

where $\sigma$ is systolic wall stress, $C$ is a constant, $k$ is the end-systolic stiffness constant, and $\ln(1/H)$ is the natural logarithm of the reciprocal of wall thickness. A detailed discussion of the mathematical derivation of this index is given in the “Appendix.”

Statistics

Dispersion from the mean is noted as ±SEM. Comparisons made regarding given parameters over the course of the study represented multiple repeated comparisons. Therefore, we tested for statistical differences using two-way ANOVA followed by a Newman-Keuls test to locate differences when they were found. Correlation coefficients for EESVR and $k$ relations exceeded .99 in all cases.

Results

Regurgitant fraction was 0.60±0.02 after the creation of mitral regurgitation, and it remained at this value at the 3-month study. Fig 1 demonstrates that serum catecholamines were elevated after 3 months of chronic mitral regurgitation, confirming the potential for increased adrenergic support. At baseline, $\beta$-blockade reduced heart rate from 77±2 to 69±2 beats per minute ($P<.05$). After 3 months of mitral regurgitation, unblocked heart rate was higher than at baseline: 85±3 beats per minute ($P<.05$). $\beta$-Blockade during chronic mitral regurgitation reduced heart rate to 82±2 beats per minute, a nonsignificant difference. Fig 2 demonstrates the effects of acute $\beta$-blockade on pulmonary capillary wedge pressure at baseline and after 3 months of chronic mitral regurgitation. $\beta$-Blockade at baseline caused pulmonary capillary wedge pressure to increase acutely but modestly, still within the normal range for our laboratory. After 3 months of mitral regurgitation, in the absence of $\beta$-blockade, wedge pressure was greater than it had been in the unblocked state at baseline, but it was still
baseline state. Three months later, ejection fraction in the unblocked state was similar to baseline. After β-blockade was instituted after 3 months of chronic mitral regurgitation, ejection fraction fell substantially to a level similar to that at baseline during β-blockade. However, this occurred when the favorable loading conditions of mitral regurgitation should have produced an ejection fraction higher than baseline. The departure from normal was more striking after β-blockade.

Fig 3. Graph showing cardiac output index for body weight (BW) at baseline (Base), during β-blockade at baseline (β-Base), at 3 months of chronic mitral regurgitation without β-blockade (cMR), and after β-blockade was instituted at 3 months of chronic mitral regurgitation (β-cMR). Although somewhat reduced during chronic mitral regurgitation in the absence of β-blockade, cardiac output dropped even more substantially when β-blockade was instituted.

Fig 4. Graph showing ejection fraction at baseline (Base), during β-blockade at baseline (β-Base), at 3 months of chronic mitral regurgitation without β-blockade (cMR), and after β-blockade was instituted at 3 months of chronic mitral regurgitation (β-cMR).

Discussion

An important finding of this study was that native β-adrenergic stimulation could maintain pump function and indexes of contractile function in the normal or nearly normal range despite the presence of significant innate contractile dysfunction. Although it is obvious that β-adrenergic stimulation helps maintain ventricular function, ours is the first study that estimates the magnitude of this support. By assessing innate contractile dysfunction when β-stimulation was blocked, we developed a reference point for comparison of various indexes measured when adrenergic stimulation was not blocked. Wedge pressure, cardiac output, ejection fraction, and indexes of contractile function were normal or nearly normal in the unblocked state 3 months after severe mitral regurgitation had caused contractile dysfunction. Only when β-blockade was instituted were pump and contractile dysfunction revealed. The one exception to this observation was the VCF-stress relation, which was mildly abnormal in the mitral regurgitation dogs in the absence of β-blockade.

It is likely that the overload of mitral regurgitation induces progressively worsening innate contractile function, which can be normalized by progressively using adrenergic reserve. Only when adrenergic reserve is eventually exhausted does contractile dysfunction become obvious in the unblocked state. Thus, it is likely that our previous use of β-blockade in all our studies of mitral regurgitation helped us to detect the dysfunction we found. The implication of this study is that examinations of contractile function that detect a contractile abnormality with the β-adrenergic system intact may select a group of patients with such severe dysfunction that the contractile abnormality is evident despite adrenergic stimulation. Such studies examine a
clinically important group of patients with severe disease but may be relatively insensitive to milder degrees of contractile dysfunction that was masked by adrenergic support. Although the study was not designed to compare the sensitivity of various indexes of contractile function, the study does suggest that the VCF-stress relation is sensitive, since it was the only index that demonstrated contractile dysfunction in the unblocked state.

Reliability of In Vivo Indexes of Contractile Function

Our case regarding the ability of the β-adrenergic nervous system to support contractile function rests largely on our ability to measure contractile function. The ideal index of contractile function would be sensitive to changes in inotropic state, insensitive to changes in loading, and independent of left ventricular size. The slope of the end-systolic afterload-volume relation used...
in this study is relatively load independent and sensitive to inotropic changes but varies indirectly with cardiac size.\textsuperscript{19,21,22} Thus, when a volume overload causes an increase in left ventricular size, there is an expected decrease in the slope of this relation whether or not contractile function decreases. To overcome this problem, we multiplied the slope times the mass present at the time the slope was derived.\textsuperscript{10,11} Thus, as eccentric hypertrophy develops, the expected decrease in slope should be offset by correction with the higher mass. Despite this correction, this index demonstrated contractile dysfunction after 3 months of mitral regurgitation in the \textit{β}-blocked state.

End-systolic stiffness relates strain, which is dimensionless, to stress. Because strain is dimensionless, this index of contractile function is independent of changes in cardiac size or mass that occur as hypertrophy develops. We have previously shown stiffness to be independent of loading and sensitive to changes in inotropic state.\textsuperscript{15} This index also showed depression in contractile function in the dogs with chronic mitral regurgitation when \textit{β}-blockade was instituted.

The intent of the ejection fraction–stress and VCF–stress relation is to correct afterload-dependent indexes for afterload, resulting in a relation that reflects contractility. Both demonstrated reduced contractile function when \textit{β}-blockade was instituted.

To further substantiate our assessment of contractile function, in this study as in the past, we examined an independent indicator of innate contractile function, the contractility of myocytes isolated from the affected ventricles. These studies were obtained by investigators blinded to the \textit{in vivo} results. The reduced viscosity-velocity curves of the myocytes isolated from the mitral regurgitation ventricles are analogous to a reduced force-velocity relation of classic cardiac mechanics.\textsuperscript{24} Importantly, the myocytes are studied in a bath free of catecholamines after thorough washing to remove residual catecholamines that were present in vivo. Thus, cell function was studied in the absence of \textit{β}-receptor stimulation. Both the cell indexes of contractile function in the absence of catecholamines and the \textit{in vivo} indexes of contractile function in the \textit{β}-blocked state were concordant and demonstrated contractile dysfunction. Other support for the presence of contractile dysfunction was the high wedge pressure that occurred in the \textit{β}-blocked state and the reduced ejection fraction in chronic mitral regurgitation during \textit{β}-blockade despite the presence of the favorable loading conditions that mitral regurgitation produces.\textsuperscript{10,32}

The contractile dysfunction we found on institution of \textit{β}-blockade after 3 months of chronic mitral regurgitation was not merely statistically significant but also clinically relevant. \textit{β}-Blockade in chronic mitral regurgitation caused a 75% increase in wedge pressure (10 to 17.5 mm Hg), a 25% decrease in cardiac output, and a substantial fall in all contractility indexes.

**Acute Versus Chronic \textit{β}-Blockade**

This study used acute \textit{β}-blockade to remove the effects of the \textit{β}-adrenergic nervous system, suddenly unmasking its level of support. These effects must not be confused with the gradual institution of chronic \textit{β}-blockade, which may have beneficial effects in congestive heart failure.\textsuperscript{33-38}

In conclusion, we have found that activation of the adrenergic nervous system provides considerable support for the failing heart. Although this was suspected in the past, its impact has been substantiated here. Importantly, many indexes of contractile function appear normal in the natural state. Only after \textit{β}-blockade is instituted is underlying contractile dysfunction revealed. We must caution that these data were obtained in an experimental model of mitral regurgitation, and applicability to other species and diseases is uncertain. However, it is likely that clinical studies and experimental studies that find contractile dysfunction in the natural state probably have detected those subjects whose contractile dysfunction is so severe that contractility cannot be normalized by the \textit{β}-adrenergic nervous system. Such studies may underestimate the prevalence of innate contractile dysfunction in various disease states.

---

**Graphs showing the sarcomere velocity/viscosity relation for normal dogs and for those with mitral regurgitation (left).**

The contractility of cells from the dogs with mitral regurgitation (MR) was significantly depressed (left) and correlated well with an \textit{in vivo} index of contractile function (\textit{k}) obtained during \textit{β}-blockade (right).
Appendix*

Derivation of the Constant of End-Systolic Stiffness

Strain is defined as the deformation of a material caused by application of a force. It is usually expressed in an unstressed dimension, ln(l/l0), or area, ln(A/A0), and is a dimensionless property. We make the assumption that the myocardium is incompressible.29,40 As such, it has a constant volume equal to its area (A) times its thickness (H). Thus, changes in area are reflected by changes in thickness. As noted above, areal strain is defined as ln(A/A0). Assuming that the myocardium is incompressible, ln(A/A0) can be substituted for ln([A(H)/l(H0)]) or ln(H/H0). Myocardial stiffness is described mathematically as the change in stress (dσ) divided by the change in strain (dε) along the minor axis of the equilibrium state. The relation between stress, strain, and curvature of the epicardial surface and passing through a selected point O of the section:

\[ V_m = A \times H \]

Since the myocardium is incompressible, \( V_m \) is constant. If we wish to examine regional work (RW) per unit volume (\( V_m \)) of myocardium (RWM), we divide RW by \( V_m \) (RW/\( V_m \)), which equals RW/AH. Thus, RWM=RW/AH. Recall Equation 1, RW=-\( fT_dA \). Thus,

\[ \text{RWM} = \left( -\int T_dA \right) / AH \]

(9)

Tension (T) divided by thickness (H) is \( T/H = \) wall stress (σ). Thus,

\[ \text{RWM} = -\int \sigma \ln A \]

(11)

This equation describes the area surrounded by the \( \sigma-\ln A \) loop. The formula for calculating stress (σ) is derived by dividing tension (Equation 8) by thickness:

\[ \sigma = \frac{P \cdot b}{2} \left( \frac{3}{2} - \frac{b^2}{2a^2} \right) / H \times 1332 \text{ dynes/cm}^2 \]

(12)

Meaning of \( \ln A \) and Definition of Area Strain

The change in lnA (dlnA), or dA/A, in the \( \sigma-\ln A \) relation expresses a relative change in area ("incremental area strain"). Total area strain (ε) is given by the following equation:

\[ \epsilon = \int_{A_0}^{A} \frac{dA}{A} = \ln A - \ln A_0 = \ln(A/A_0) \]

where \( A_0 \) is the area corresponding to a state of zero stress. Definition of unstressed area (\( A_0 \)) is required to obtain the complete stress/strain relation extrapolated to the x axis. However, \( A_0 \) is not required in the analysis of the stiffness (dσ/dε) strain relation. As noted above, myocardial stiffness is defined as the change in stress (dσ) divided by the change in strain (dε) or dσ/dε. This relation only examines incremental changes in stress and strain to define its slope. Thus, extrapolation to \( A_0 \) is unnecessary. Therefore, stiffness...
(dr/dE) is identical using either definition of strain: lnA or ln(A/Ao). Thus, Ao can be omitted and area strain is defined as

\[ e = \ln A \]

**Use of Reciprocal Wall Thickness (1/H) Instead of A**

Unfortunately, changes in a regional area of interest of the myocardium are difficult to measure. Conversely, wall thickness and changes in wall thickness are easy to measure by conventional echocardiographic or cineangiographic methods. Thus, substitution of thickness (H) for area (A), if possible, would increase the applicability of the method. The following explains how wall thickness (H) can be substituted for area in strain analysis. The myocardium is incompressible.\(^{39,40}\) Thus, the volume of the region of interest (Vw) is constant, even though A and H vary throughout the cardiac cycle. From Equation 9, \( A = V_m/H, \) then

\[ \ln A = \ln(V_w/H) = \ln V_m + \ln(1/H) \]

Since \( V_m \) is constant, \( d(\ln A) = dln(1/H). \) Thus, one can substitute \( \ln(1/H) \) for \( \ln A. \) \( \ln(1/H) \) represents strain in the stress-strain relation.

**Acknowledgments**

This research was supported in part by National Institutes of Health grant HL-38185 (B.A.C.) and Veterans Administration Merit Reviews (B.A.C., M.R.Z.).

**References**


Native beta-adrenergic support for left ventricular dysfunction in experimental mitral regurgitation normalizes indexes of pump and contractile function.
M Nagatsu, M R Zile, H Tsutsui, P G Schmid, G DeFreyte, G Cooper, 4th and B A Carabello

Circulation. 1994;89:818-826
doi: 10.1161/01.CIR.89.2.818
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/2/818

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/