Altered Adrenergic Receptor Density in Myocardial Hibernation in Humans
A Possible Mechanism of Depressed Myocardial Function

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Background—Alterations in adrenergic receptor densities can potentially contribute to myocardial dysfunction. Their relevance to myocardial hibernation in humans is unknown.

Methods and Results—Accordingly, 22 transmural myocardial biopsies were obtained in 11 patients with ischemic ventricular dysfunction during bypass surgery, guided by transesophageal echocardiography. Patients underwent dobutamine echocardiography (DE) and rest scintigraphic studies before revascularization and DE at 3 to 4 months. \(\alpha\)- and \(\beta\)-receptor density (ARD and BRD) and extent of fibrosis were quantified from the myocardial biopsies. Of the 22 segments, 16 had abnormal rest function and 6 were normal. Severely hypokinetic or akinetic segments showed a 2.4-fold increase in ARD with a concomitant 50% decrease in BRD compared with normal segments. An increase in ARD, a decrease in BRD to a lesser extent, and thus an increase in ARD/BRD ratio were seen in dysfunctional segments with contractile reserve compared with normal segments and were most pronounced in those without contractile reserve \((P<0.001)\). Similar findings were observed if recovery of function or scintigraphic uptake was analyzed as a marker for viability. No significant relation between either ARD or BRD and percent myocardial fibrosis was noted \((r=0.37\) and \(-0.39\) respectively).

Conclusions—Thus, graded and reciprocal changes in \(\alpha\)- and \(\beta\)-adrenergic receptor densities occur in viable, hibernating myocardium and may account in part for the observed depression in resting myocardial function and preserved contractile reserve in this entity.\(^{\text{Circulation. 2000;102:2599-2606.}}\)

Key Words: receptors, adrenergic ■ ischemia ■ hibernation ■ dobutamine ■ echocardiography

Depression of myocardial function in myocardial hibernation is thought to be an adaptive response to severe chronic hypoperfusion. However, recent studies have shown that although patients with hibernating myocardium frequently have reduced resting coronary flow, some may have normal flow at rest, invoking a possibility of repetitive myocardial stunning.\(^1,2\) The absence of adequate animal models of chronic myocardial hibernation has necessitated the use of clinical studies to examine the mechanisms of contractile dysfunction.\(^3,4\) Recently, structural derangements have been demonstrated at myocardial biopsy.\(^3,4\) Whether adrenergic receptor density and function are perturbed in hibernating myocardium has not been evaluated. Alterations in the densities of myocardial \(\alpha\)- and \(\beta\)-adrenergic receptors have been described in a variety of pathological states associated with myocardial dysfunction.\(^5,7\) More recently, clinical reports have underscored the potential importance of \(\alpha\)-adrenergic function in blunting the contractile response of viable postischemic myocardium.\(^8,9\) These data may have direct relevance to contractile dysfunction in myocardial hibernation, given that repetitive episodes of postsischemic dysfunction are a possible pathophysiological mechanism. We therefore studied changes in \(\alpha\)- and \(\beta\)-adrenergic receptor density (ARD and BRD) in the myocardium of patients with chronic ischemic left ventricular dysfunction undergoing revascularization with coronary bypass surgery.

Methods
The study population consisted of patients with chronic stable ischemic left ventricular dysfunction in the distribution of \(\geq 1\) coronary artery stenosis \((\geq 70\%\) diameter stenosis) who were already scheduled for bypass surgery. Study results did not change the
management of any of the patients enrolled. Dobutamine echocardiography (DE) and rest scintigraphic studies were performed 1 to 5 days before surgery. During surgery, transmural myocardial biopsies were obtained, guided by transesophageal echocardiography. Three to 4 months later, DE was repeated. The Institutional Review Board of Baylor College of Medicine approved the study protocol, and all patients signed informed consent before enrollment. These patients were not part of previous investigations from our institution.

**Echocardiographic Studies**

Echocardiography was performed in standard parasternal and apical views (Hewlett Packard Sonos 2500, 2.5- or 3.5-MHz transducer). Regional function was assessed in a 16-segment model, visually graded from 1 (normal) to 5 (dyskinesia), and assigned to coronary territories as previously described. Myocardial thickening fraction (TF) was calculated from the parasternal short-axis views as $\frac{\text{end-systolic thickness} - \text{end-diastolic thickness}}{\text{end-diastolic thickness}}$. Measurements were performed in triplicate and averaged (Digisonics Digiview-ERS). The interobserver and intraobserver mean absolute differences in TF were $4\pm6\%$ and $3\pm6\%$, respectively.

Ejection fraction was quantified with the multiple-diameter method. All studies were interpreted without knowledge of scintigraphic or histopathological data. Recovery of regional function was defined as an improvement of $\geq 2$ grades in wall motion.

**Dobutamine Echocardiography**

Dobutamine infusion was started at $2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and increased at 3-minute intervals to 5, 7.5, 10, 20, 30, and $40 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Images at baseline, $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, 7.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and peak dobutamine were digitized online in a quad-screen format. The responses of dysfunctional segments to dobutamine were classified as previously described. Segments with any contractile reserve during DE were considered viable.

**Scintigraphic Perfusion Study**

Rest and 4-hour redistribution $^{201}$TI tomography was performed after administration of 3 mCi of $^{201}$TI, before bypass surgery, as previously described. In patients weighing $>200$ pounds, a resting $^{99}$Tc sestamibi scan was used to evaluate viability. Experienced nuclear cardiologists unaware of all other data analyzed the scintigraphic images. A 16-segment model comparable to that for echocardiography was used. Scintigraphic uptake was determined in each segment with a region of interest $40 \times 40$ pixels in size (matrix $128 \times 128$) and normalized to the segment with highest uptake. A maximal uptake of $>60\%$ was considered indicative of viability.

**Quantitative Coronary Angiography**

Coronary angiography of the right and left coronary arteries in multiple views was performed with the Judkins technique. The angiograms were analyzed and quantified with an automated edge-detection method using the Coronary Angiography Analysis System (CASS; Pie Medical Instruments). The degree of stenosis was expressed as percent reduction of the internal luminal diameter in relation to the normal reference.

**Transmural Left Ventricular Biopsies**

Transmural myocardial biopsies from the anterior, inferior, or lateral walls were obtained with a 20-mm, 14-gauge Tru-cut biopsy needle at the time of bypass surgery, before cardioplegia, guided by transesophageal echocardiography. Two biopsies were acquired per patient: 1 from a dysfunctional segment and another from a normal segment for use as control. When no normal wall was identified, 2 dysfunctional segments were biopsied.

**Analysis at Pathology**

**Visualization, Localization, and Quantification of $\alpha$- and $\beta$-Receptors**

Tissue biopsy specimens were probed by fluorescence deconvolution microscopy with BODIPY 558/568–tagged prazosin for localizing...
Figure 2. Images of a section of cardiac tissue that has been probed to visualize smooth muscle actin and demonstrate vascular elements and vascular nuclei. A, β-Receptors (green/yellow), actin (red), and nuclei (blue). B, We removed actin channel but included smooth muscle actin channel (red, Cy5 high-wavelength-labeled antibody) to visualize a few intramyocyte capillaries and a larger vessel running from top to lower right. C and D are included for comparison, with smooth muscle actin removed in C and receptors removed in D.

α-receptors and BODIPY CGP-12177, an isoproterenol analogue, for visualization of β-receptors (Figure 1). Probes were from Molecular Probes.14–16 Prazosin has a high affinity for α1-adrenergic receptors and has been used successfully in flow cytometry and confocal microscopy.17,18 For β-adrenergic receptors, no subtypes were designated, because the specificity of CGP-12177 for subtypes of β-receptors is less clear.

Fresh biopsy samples of cardiac tissue were embedded in medium containing 10.24% polyvinyl alcohol, 4.2% polyethylene glycol, 85.5% sucrose (O.C.T. Compound, Tissue-Tek) and placed on dry ice. The blocks were sectioned at 4°C, thickness 10±3 μm, with a Reichert HistoSTAT cryotome. Sections were attached to glass coverslips coated with poly-L-lysine (Sigma) and placed in 3.7% paraformaldehyde for 5 minutes at room temperature. Slices were visualized on an Applied Precision DeltaVision scanning fluorescence microscope fitted with an Olympus IX70 microscope and deconvolution capabilities. Sections were stained with the appropriate fluorescent receptor probes (5 nmol/L) for 30 minutes at 37°C and placed on a glass slide on 1 drop of Elvanol (DuPont). To ensure that we had saturation of all β-receptors with CGP-12177, we challenged concentrations of the probe (up to 100 nmol/L) with different concentrations of (−)- and (+)-isoproterenol (up to 100 nmol/L). We found no reduction in fluorescence at the concentration of probe used (5 nmol/L) until the concentration of cold isoproterenol (both forms) had exceeded 50 nmol/L. A Kᵦ value of 0.25 nmol/L was obtained for CGP-12177, and in similar experiments, a Kᵦ of 0.13 nmol/L was obtained for prazosin.

Assessment of Fibrosis
Specimens were fixed in 10% buffered formalin, processed through a series of ethanol solutions, embedded in paraffin, and cut into sections 3 μm thick. Sections were stained with hematoxylin-eosin/Mallory’s trichrome for extent of fibrosis. Fibrosis, which stains purple with the trichrome stain, was distinguished from pink myocardium and quantified with computer image analysis using Optima Bioscan software. Fibrosis was expressed as percent of the total biopsied section.6,11

Statistical Analysis
Results are shown as mean±SEM. ANOVA was used to compare adrenergic receptor densities among the various groups with regard to (1) resting function, (2) contractile reserve, and (3) recovery of function. Unpaired t test was used to compare adrenergic receptor densities between segments with >60% or <60% scintigraphic uptake. Linear regression analysis was used to correlate adrenergic receptor density with (1) TF at baseline, (2) percent scintigraphic uptake, and (3) extent of fibrosis. Significance was set at P<0.05.

Results
Patient Population
The population consisted of 11 patients (5 men) with a mean age of 60 years (range 54 to 70 years) and a mean left ventricular ejection fraction of 33.3% (range 20% to 45%). Eight patients had symptoms of heart failure and 8 stable angina. Three patients were on β-blockers (2 on metoprolol 50 mg/d and 1 on atenolol 25 mg/d), and 3 were on calcium antagonists. Results of ARD and BRD were similar if patients on β-blockers or calcium channel blockers were excluded. Complete revascularization was performed (1 to 3 grafts per patient). All patients underwent DE, and 10 had scintigraphic imaging. Ten of the 11 patients had a DE 3 to 4 months after surgery, and 1 patient died before follow-up. All 10 patients remained stable during follow-up, and none experienced worsening of heart failure or anginal symptoms.

Myocardial Function of Biopsied Segments
A total of 22 segments were biopsied. Six of the 22 segments had normal resting function (TF >30%) and served as control. Ten segments were hypokinetic (TF<30%) at rest, and 6 were akinetic. Ten of the 16 dysfunctional segments had contractile reserve with DE. Of the 14 dysfunctional segments available for follow-up, 5 had recovery of rest function and inotropic reserve and 1 had inotropic reserve without recovery of rest function. In the 3 patients on β-blockers, 2 segments had normal function at rest and 4 had dysfunction, 3 of which had contractile reserve and recovery that were counted individually for content of fluorescence. Figure 1 shows that distinct areas of labeling were apparent, together with larger “clusters” of receptors. These larger areas were subjected to gain reduction until distinct spots within the clusters could be identified. Three independent countings of multiple areas were made to reduce potential errors. Mean values for receptor density on myocytes were determined as the number of receptors in 60×60-μm samples.
of function. Eleven of the 20 segments with scintigraphic data had >60% uptake. Quantitative angiographic parameters were not different between segments with and without recovery of function.

Resting Function Versus Adrenergic Receptor Density
A progressive increase in ARD was observed from normal segments to mildly hypokinetic (TF 20% to 30%) through to severely hypokinetic/akineti c segments (TF 0% to 20%, n=12) (Figure 3). ARD related significantly and inversely to TF at rest (r=−0.62, P<0.002). A reciprocal trend was seen in BRD (Figure 3). Although reductions in BRD did not reach statistical significance, a significant correlation of TF with BRD was noted (r=0.7, P<0.0002). Thus, a graded increase in the ARD/BRD ratio was observed with worsening rest function.

Contractile Reserve With DE
Analysis of the inotropic response to dobutamine revealed a stepwise increase in ARD from normal segments to those with depressed function and preserved inotropic reserve, and was most pronounced in segments without contractile reserve (Figure 4). Conversely, a graded decrease in BRD was seen with worsening inotropic reserve, with a resultant increase in the ratio of ARD/BRD (ANOVA P<0.001; Figure 4).

Recovery of Function
Compared with controls, dysfunctional segments that recovered resting function after revascularization showed an increase in ARD, a decrease in BRD, and thus an increase in ARD/BRD ratio (ANOVA P<0.001; Figure 5). The largest alteration in adrenergic receptor densities was seen in dysfunctional segments that failed to recover function (Figure 5). Figure 1 shows images of fluorescence-labeled α- and β-receptors in normal myocardium and in dysfunctional myocardium with and without recovery of function.

Rest Scintigraphic Uptake
Scintigraphic uptake (%) at rest related significantly to ARD and BRD. An inverse relation was observed between scintigraphic uptake and ARD (r=−0.7, P<0.001), whereas a positive relation was observed with BRD (r=0.61, P<0.008) (Figure 6). A significantly greater ARD was found in segments with <60% uptake compared with those with >60% uptake (P=0.009) (Figure 6, left). A small reduction in BRD, which failed to reach statistical significance, was also noted in the group with <60% scintigraphic uptake (P=0.32) (Figure 6).

Adrenergic Receptor Localization and Relation to Fibrosis
Studies on localization of the adrenergic receptors indicated that there was no preferential localization of adrenergic receptors to nonmyocyte cells (Figure 2). In particular, there was no appreciable change in receptors on the vascular endothelial cells or fibroblasts. The extent of fibrosis in the myocardial biopsies ranged between 12% and 53% (mean 23±3%). Weak and nonsignificant correlations between either ARD or BRD and the extent of fibrosis were observed (r=0.37, P=0.19, and −0.39, P=0.10, respectively).

Discussion
The present study demonstrates for the first time that significant changes in both myocardial ARD and BRD occur in myocardial hibernation. An increase in ARD and decrease in BRD were observed in dysfunctional viable myocardium, irrespective of whether viability was assessed as recovery of myocardial function, presence of contractile reserve, or preserved radionuclide uptake. Levels of ARD and BRD in
hibernating myocardium were intermediate between those of normal segments and nonviable myocardium.

Alterations in Myocardial Adrenergic Receptors in Hibernating Myocardium

Profound reductions in BRD with small changes in ARD have been demonstrated in chronic ventricular dysfunction. In the setting of experimental myocardial ischemia, however, upregulation of myocardial α-adrenergic receptors and increase in ARD are well documented, whereas changes in BRD are less clear. To the best of our knowledge, there are no previous reports on adrenergic receptor density or function in myocardial hibernation in humans. The changes demonstrated in myocardial adrenergic receptor density are concordant with observations in experimental myocardial ischemia. Because hibernating myocardium is present in the

Adrenergic Receptors in Myocardial Hibernation

Figure 4. Mean ARD and BRD and ARD/BRD ratios in segments with normal resting function and contractile reserve and in those with resting dysfunction, with and without contractile reserve.

Figure 5. Adrenergic receptor density and ARD/BRD ratio in normal segments and in those with resting dysfunction, with and without recovery of function 3 to 4 months after revascularization.
setting of severe coronary stenosis, it is exposed to repetitive episodes of resting and/or demand ischemia. Indeed, the presence of contractile reserve and inducible ischemia is the most specific finding of hibernating myocardium. Thus, it is conceivable that the increase in ARD in viable segments may be the result of resting and/or intermittent ischemia, particularly that these alterations were observed regionally, in areas of myocardial hibernation compared with control segments. The significant inverse correlation between scintigraphic uptake and \( \alpha \)-adrenergic receptors also supports this view. Although the changes in BRD may conceivably reflect concomitant ventricular dysfunction, the finding of a regional decrease in BRD in dysfunctional segments, not demonstrated in control regions exposed to similar hemodynamics, supports the thesis that alterations in BRD are also associated with hibernating myocardium.

Our results indicate that the closer a segment approached criteria for nonviability, the greater the increase in ARD and the decrease in BRD. The alterations in adrenergic receptor density in hibernating myocardium, intermediate between normal and less viable myocardium, are reminiscent of the intermediate ultrastructural changes in this entity. The changes observed in ARD and BRD, however, are not a mere reflection of the structural changes in the dysfunctional myocardium. The lack of predilection of adrenergic receptors to the microvasculature or fibroblasts and the care taken to include receptors on myocytes in quantifying adrenergic receptor density support the notion that the measured changes reflect predominantly alterations of adrenergic receptors on myocytes rather than on nonviable areas or vascular tissue. The lack of a significant correlation between adrenergic receptor density and degree of myocardial fibrosis lends further support to these observations.

### Adrenergic Receptors and Contractile Function

Several mechanisms for depression of contractile function in the hibernating myocardium have been postulated: reduction in resting coronary flow, repetitive myocardial stunning, ultrastructural changes, dedifferentiation of cardiac myocytes, and alterations in metabolism. In the present study, worsening myocardial viability was associated with a parallel increase in ARD and decrease in BRD, with a consequent increase in ARD/BRD ratio. Although the current data do not allow us to clearly distinguish whether the changes in receptor density are mechanistically important in contractile impairment or merely a pathological sequela of progressive myocardial dysfunction, several lines of evidence point to the former. The significant regional decline in BRD in dysfunctional segments compared with normal controls in the same patients provides compelling evidence for the impairment of myocardial function at rest. The higher levels of BRD in hibernating tissue compared with nonrecovered segments may also partially explain the preserved inotropic reserve in these segments. The most profound changes in receptor density were seen with ARD. \( \alpha \)-Adrenergic–mediated attenuation of resting myocardial function has been demonstrated in several experimental models of myocardial ischemia. Furthermore, the inhibitory effect of \( \alpha \)-adrenergic activation on \( \beta \)-adrenergic receptor–mediated inotropic effects in dysfunctional myocardium has been consistently reported. Such an interaction between the 2 adrenergic systems may provide an additional explanation for the depressed inotropic reserve as well as the more severe resting dysfunction in segments with high ratios of ARD/BRD. This concept is further supported by reports of improved contractile performance with \( \alpha \)-adrenergic antagonists in animal models of...
nonischemic ventricular dysfunction. Although the increased ARD localized predominantly to the myocytes, an effect of \(\alpha\)-adrenergic tone on the microvasculature cannot be excluded. Indeed, recent data have highlighted the role of \(\alpha\)-adrenergic–mediated microvascular vasoconstriction in the pathogenesis of postischemic left ventricular dysfunction. Moreover, relief of \(\alpha\)-adrenergic constrictor tone has clearly been shown to increase coronary blood flow in experimental models of hypoperfusion. Whether \(\alpha\)-adrenergic receptor blockers would improve resting dysfunction and contractile reserve in patients with myocardial hibernation remains to be determined. Ultimately, whether the alterations observed in adrenergic receptor density in myocardial hibernation are reversible after revascularization and account in part for the recovery of resting function would be difficult to answer clinically and would await confirmation in an experimental model.

**Advantages and Limitations**

Two myocardial biopsies were obtained per patient. Myocardial structure arguably may differ somewhat in different areas, even within the same segment. Because transesophageal echocardiography was used to guide the core biopsies, we believe that the specimens indeed reflect the core tissue in these segments well. The total number of biopsies may be relatively small. However, significant differences were achieved between normal, hibernating, and nonviable myocardium. Although more specimens could have been obtained, this was greatly limited by patient safety.

The binding of prazosin is highly specific for \(\alpha_1\)-adrenergic receptor, and thus, this subtype of adrenergic receptor is very likely being visualized by the BODIPY prazosin tag. A specific subtype for \(\beta\)-adrenergic receptor was not specified in the present study because the binding of the hydrophilic \(\beta\)-adrenergic antagonist CGP-12177 is not clearly specific to subtypes of \(\beta\)-receptors. Comparison of the fluorescence-based method for adrenergic receptors with the more traditional radioligand has shown that estimations of receptor numbers are similar. However, the fluorescence method is, in our view, a more accurate approach than simply use of radioligand binding estimations or overall fluorescence measurements via flow cytometry. Using reconstructed multisecton acquisitions, then applying stepwise intensity reduction, we could locate pinpoints of probe and eventually distinguish individual loci within large fluorescent areas. This methodology, followed by exhaustive stereology, gives a true representation of receptor numbers. Radioligand binding, for example, does not place receptors within a cell or on a cell membrane and is subject to isotope sequestration and non-specific binding. With the current method, superfluous probe can be removed via a challenge with cold agonist/antagonist and image enhancement techniques.

The function of secondary messengers of the \(\beta\)- and \(\alpha\)-adrenergic pathways (eg, cAMP and phosphoinositols as-says) could not be evaluated because of the size limitations of the biopsy samples. Also, the number of probes we used became a limiting factor, ie, our probe for adenylyl cyclase is in the BODIPY wavelength channel, which is required for receptor identification. Analysis of function of secondary messengers will be essential in elucidating the functional significance of the current data and is the subject of further investigations.

**Conclusions**

This study demonstrates for the first time that significant alterations in \(\alpha\)- and \(\beta\)-adrenergic receptor densities exist in patients with myocardial hibernation. Worsening resting function, inotropic reserve, and recovery of function are all associated with a graded increase in ARD and decrease in BRD. Alteration in adrenergic receptors may therefore play a significant role in the observed depression of myocardial function and preserved contractile reserve in myocardial hibernation.

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