Myocardial Force-Frequency Defect in Mitral Regurgitation Heart Failure Is Reversed by Forskolin

Louis A. Mulieri, PhD; Bruce J. Leavitt, MD; Barbara J. Martin, BS; Joe R. Haeberle, PhD; Norman R. Alpert, PhD

Background. Postoperative ejection phase parameters and patient survival rates for mitral valve replacement surgery are considerably lower than for similar aortic valve surgery. While chordal transection probably is the major contributor to the lowered values, there is also evidence for decreased preoperative myocardial contractile reserve in mitral regurgitation patients. This study characterizes abnormalities in the force-frequency relation that may underlie impaired function of myocardium isolated from mitral regurgitation patients with New York Heart Association class II-III heart failure.

Methods and Results. Left ventricular epicardial myocardium was obtained by surgical biopsy during mitral valve replacement surgery in patients with mitral regurgitation heart failure (left ventricular ejection fraction, 0.64±0.05) and during coronary artery bypass surgery in patients with normal ventricular function. The steady-state twitch tension versus frequency relation was measured in myocardial strip preparations (37°C, 12 to 228 min−1) in the absence and presence of forskolin. Relative to normal function, peak isometric twitch tension in mitral regurgitation is depressed by 50% (P<.02) and 74% (P<.003) at contraction frequencies of 60 min−1 and 168 min−1, respectively. The slope of the tension-frequency curve is blunted and its peak is shifted to a lower frequency (mitral regurgitation: 134 min−1; normal function: 173 min−1; P<.02). The myosin heavy chain concentration did not differ between mitral regurgitation and normal function strips (53±4 versus 54±4 nmol/g blotted wt). Forskolin (0.5 μmol/L) completely reversed the tension depression, blunting, and the lowered peak frequency in the mitral regurgitation preparations.

Conclusions. Preoperatively, myocardial tension generation in mitral regurgitation patients is severely depressed, and the force-frequency curve is blunted and has a negative slope in the exercise range of heart rates. The reversal of these defects by forskolin suggests that abnormal excitation-contraction coupling may underlie the decreased contractile reserve in mitral regurgitation patients. (Circulation. 1993;88:2700-2704.)

Key Words • left ventricle • mitral valve

The 10-year survival rate of valvular replacement patients differs considerably for mitral compared with aortic regurgitation (50% versus 80%) despite the similarity of surgical procedures.1 Mitral valve repair/replacement abruptly increases afterload by eliminating the unloading effect of the regurgitant flow to the low-pressure pulmonary circuit. While the frequent outcome of unchanged or decreased postoperative ejection performance was originally thought to be caused by the large increase in afterload, it has now been shown to be associated with removal of the mitral apparatus.2,3 However, even with preservation of the mitral apparatus, low values of ejection fraction can persist,3,4 suggesting that decreased preoperative myocardial contractile reserve (decreased velocity of circumferential fiber shortening with normal wall stress5,6) may also contribute when postoperative ejection fraction is low. While it is likely that chordal preservation during mitral valve repair will by itself ameliorate much of the previous mortality excess, this improvement may be limited if diminished contractile reserve persists. The present study was undertaken to evaluate alterations in the force-frequency relation as potential contributors to diminished contractile reserve in mitral regurgitation myocardium.

Direct evidence for myocardial abnormalities in mitral regurgitation hearts has been obtained from in vitro measurements on left ventricular papillary muscle strips, indicating that the force-frequency relation exhibits a negative slope.6 We have recently reported preliminary results confirming these findings in papillary muscle strips and also showing that twitch tension is depressed, that the peak of the force-frequency relation is shifted to low contraction frequencies,7,9 and that this depressed performance can be improved by the adenylate cyclase activator forskolin.10

The objectives of the present study were to see if comparable depression of myocardial performance exists in the free ventricular wall as observed previously in the papillary muscle and to further test the hypothesis

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that forskolin can reduce or eliminate the depressed contractile performance.

Methods

Myocardial Biopsy and Strip Preparation

Subepicardial tissue (mitral regurgitation) was obtained from four mitral regurgitation patients in New York Heart Association class II-III heart failure (III: n=1; II: n=3; age, 56±3 years). There were three men and one woman. Their ejection fractions ranged from 0.54 to 0.76 (0.64±0.05). Medications included digoxin (n=2), calan (1), isordil (2), capoten (3), lasix (2), coumadin (3), and theechron (1). Control subepicardial tissue (normal function) was obtained from four coronary artery bypass patients aged 67±4 years (P-NS) who had normal left ventricular wall motion and normal ventricular function. Patients gave informed, written consent before participating in the study, which was approved by the Committee on Human Research of The University of Vermont. Surgical biopsies from the anterior segment of the left ventricular wall were obtained shortly after cardioplectic arrest.11 There were no complications resulting from the biopsy procedure in any patient. The excised tissue was immediately submerged in room temperature, preoxyenated BDM-protective solution, and after a 60-minute recovery from surgical trauma, it was dissected into thin strips approximately 0.2 mm in diameter.8,11,12 Experiments were performed on two strip preparations from each of the eight hearts biopsied.

Apparatus and Measurements

Isometric twitch tension was measured at the peak of the tension-length relation (L_max) in each of the two muscle strip preparations simultaneously using the same apparatus, methods, and protocols as described previously.12

The steady-state force-frequency relation of each strip was obtained at 37°C with 5 minutes of stimulation at each frequency investigated starting at 0.2 Hz (12 min⁻¹) and increasing in 0.2-Hz increments. Peak twitch tension and timing parameters of the steady-state myograms were measured by digital readout and averaged at each frequency across all strips in each group.11,12 A 30-minute equilibration was allowed after forskolin addition, and the entire force-frequency relation was again measured. The length at L_max (4.84±0.24 mm) and the blotted weight (0.86±0.13 mg) of the active portion of each muscle strip were measured, and the quotient was used to calculate its cross-sectional area. The strips were frozen by immersion in liquid nitrogen and stored at -71°C for myosin heavy chain content measurements.

Myosin Determination

Myosin content was determined by quantitative SDS-PAGE. The frozen strip preparations were dehydrated in chloroform/methanol (2:1, v/v) at 0°C, dried under vacuum, weighed, placed in 100°C gel dissociation buffer (62.6 mM Tris/base, 3% SDS, 20% glycerol, 6 mg/ml DTT; 1 μL/0.75 μg dry tissue weight) and extracted at 100°C for 5 minutes and at room temperature for 60 minutes. Following overnight storage (4°C), trace bromophenol blue and fresh DTT (2 μL 250 mmol/L DTT/100 μL sample) was added to each sam-


dule. The filtrate (Gelman Z-spin filter, 0.45-μm pore) was incubated at 100°C (2 minutes), vortexed, cooled, and sedimented at 13 000g. The supernatant was loaded (26 μg dry wt per lane) on 5% SDS-PAGE with the buffer system of Porzio and Pearson.13 Gels were stained overnight with 0.1% Coomassie blue in 25% isopropanol and 10% acetic acid and destained in 30% methanol and 10% acetic acid. The myosin heavy chain content in each lane was quantified by densitometric gel scanning (PD/INS with QUANTYTE ONE software) using skeletal muscle myosin as standards. The concentration of myosin in the standards was determined by absorbance (E_{280}^1%=5.0).

Solutions

All measurements were made in Krebs-Ringer solution described previously.8 The BDM-protective solution for dissection consisted of this Krebs-Ringer solution plus 30 mmol/L 2,3-butanedione monoxime.8 Forskolin was dissolved in 95% ethanol and introduced into the 90-mL muscle baths in 2-μL aliquots to give 0.5 μmol/L forskolin and not higher than 20 μmol/L ethanol. The latter did not produce any changes in the twitch response when introduced separately. BDM and forskolin were obtained from Sigma. Glass-distilled water was used for all solutions.

Statistical Analysis

Repeated-measures ANOVA with group, strip, and frequency as factors was used to test for disease and drug-related differences in the tension-frequency relation (SYSTAT, 1988: Systat Inc, Evanston, Ill). Peak twitch tensions were compared at two stimulation frequencies across normal function versus mitral regurgitation and normal function versus mitral regurgitation+forskolin groups using an unpaired t test and a paired t test for comparison of mitral regurgitation+forskolin with mitral regurgitation. Stated P values were Bonferroni corrected for three comparisons. Twitch timing, optimum stimulation frequency, and myosin concentrations were compared using unpaired t test at a single stimulation frequency. All values are expressed as mean±SEM. P≤.05 was considered statistically significant.

Results

Tension-Frequency Relation in Failing Myocardium

The time course of the isometric twitch in the failing myocardium was not different than in the nonfailing myocardium. The average tension-frequency curves obtained from nonfailing and failing myocardial strips are shown in Fig 1. The ANOVA results indicate that a significant group by frequency (P<.001) effect exists. The slopes of the chords between 60 and 168 min⁻¹ are significant (P<.05) for the normal function and the mitral regurgitation+forskolin curves but not for the mitral regurgitation curve. Comparison of tensions at these two frequencies by t test revealed a significant difference exists between normal function and mitral regurgitation and between mitral regurgitation and mitral regurgitation+forskolin at 60 and 168 min⁻¹ but no significant difference exists between normal function and mitral regurgitation+forskolin at either frequency. At a contraction frequency of 60 min⁻¹, the strips from the mitral regurgitation hearts develop only 50% of the tension developed by the normal function preparations.
Fig 1. Plot of average steady-state isometric twitch tension versus stimulation frequency. Each point represents the mean±SEM of eight nonfailing, control preparations (NF, ○) and eight failing, mitral regurgitation preparations (MR, ■). Temperature, 37°C.

(P<.02). At higher stimulation frequencies, the deficit in twitch tension increased. It was 74% (P<.003) at a contraction frequency of 168 min⁻¹ (Table). In addition to this severe blunting of the frequency treppe (“Bowditch treppe”), the position of the peak of the tension-frequency curve (optimum frequency) was shifted from 173 to 134 min⁻¹ (P<.02).

The myosin heavy chain content of the failing myocardial strips did not differ significantly from the nonfailing strips either on a blotted-weight basis (Table) or on a dry weight basis (219±29 nmol/g dry wt versus 220±15 nmol/g dry wt).

Effects of Forskolin on the Tension-Frequency Relation in Failing Myocardium

Forskolin (0.5 μmol/L) caused a threefold potentiation of the twitch response that took 15 to 20 minutes to fully develop and was then stable for many hours. Times to peak twitch tension and to half and full relaxation of the twitch all were decreased by 30% (P<.05) from their control values at 60 min⁻¹. As can be seen in Fig 2 and the Table, the depression, blunting, and shifting of the tension-frequency curve in the failing myocardium are completely reversed in the presence of the drug. Peak twitch tensions at 60 and 168 min⁻¹ as well as optimal stimulation frequency were not significantly different than in the nonfailing preparations.

Discussion

Depression of Tension-Frequency Relation in Mitral Regurgitation

The present finding of 50% depression in peak isometric twitch tension in epicardial muscle strips agrees with our preliminary findings in papillary muscle strips from mitral regurgitation hearts showing that the working myocardium as well is depressed. The depression is not attributable to loss of contractile material since the myosin heavy chain content of the failing epicardial muscle strips is not different than in the nonfailing strips studied. This is consistent with earlier reports of only slight differences in interstitial tissue content and myocyte diameter or in myofibrillar protein content in human mitral regurgitation myocardium.

The blunting of the frequency treppe in the failing myocardium, amounting to a 60% smaller than normal increase in twitch tension with an increase in stimulation frequency from 60 to 120 min⁻¹, constitutes a major loss of contractile reserve in the mitral regurgitation heart. At contraction frequencies above 134 min⁻¹, a further loss of contractile reserve occurs due to premature onset of the descending limb of the tension-frequency curve (40 min⁻¹ lower than in normal function). This additional loss of contractile reserve would further limit exercise capacity in mitral regurgitation.

Isometric Twitch Tension Parameters at 60 and 168 min⁻¹, 37°C

<table>
<thead>
<tr>
<th>Type</th>
<th>Cross-Sectional Area, mm²</th>
<th>Myosin Content, nmol/g blotted wt</th>
<th>Twitch Tension, mN×mm⁻²</th>
<th>Optimum Frequency, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 min⁻¹</td>
<td>168 min⁻¹</td>
</tr>
<tr>
<td>NF</td>
<td>0.23±0.03</td>
<td>54±4.1</td>
<td>15.2±1.5</td>
<td>34.1±4.1</td>
</tr>
<tr>
<td>MR</td>
<td>0.11±0.08 (NS)</td>
<td>53±4.1</td>
<td>7.7±1.5*</td>
<td>8.8±1.9†</td>
</tr>
<tr>
<td>MR+forskolin</td>
<td>...</td>
<td>...</td>
<td>19.5±3.7†</td>
<td>32.3±5.5§</td>
</tr>
<tr>
<td>(MR+forskolin)/NF</td>
<td>...</td>
<td>...</td>
<td>1.28 (NS)</td>
<td>0.95 (NS)</td>
</tr>
</tbody>
</table>

NF indicates nonfailing myocardial strips; MR, mitral regurgitation myocardial strips; forskolin, 0.5 μmol/L g blotted wt, grams of weight of blotted strips; and min⁻¹, contractions per minute.
P values (with Bonferroni’s correction for three comparisons): MR vs NF: * .02; †.0027; (MR+forskolin) vs MR (paired): †.02, §.004; (MR+forskolin) vs NF: NS=1.
patients. Blunting and leftward shifting of the tension-frequency relation similar to these findings in epicardial strips has been observed in previous studies on papillary muscle strips from mitral regurgitation hearts.\(^6\)\(^-\)\(^10\)\(^\text{16}\) In our preliminary studies on mitral regurgitation papillary muscle myocardium, we found\(^10\) the twitch tension to be depressed by 55\% at 60 min \(^{-1}\), similar to the present observation of 51\% in the epicardium, while the tension-frequency curve was flat between 60 and 120 min \(^{-1}\) (Reference 7) with optimum frequency and slope of the frequency treppe reduced by 30\% and 70\%, respectively, compared with normal function.\(^9\) The latter values are comparable to the 23\% and 60\% reductions observed in the present study on mitral regurgitation epicardium. These similarities suggest there is global involvement of myocardial deterioration in mitral regurgitation heart failure. However, our preliminary results with papillary muscle myocardium\(^10\) also suggest regional variations in degree of involvement may exist since addition of forskolin to this preparation only increases twitch tension by 35\% and optimal frequency by 17\% compared with 267\% and 27\%, respectively, in the mitral regurgitation epicardium of the present study. Since nonfailing papillary muscle strips have not been studied, we are uncertain whether these differences are associated with peculiarities in the papillary muscle or are disease related.

**Reversal of Tension-Frequency Defect by Forskolin**

As seen in Fig 2 and the Table, addition of 0.5 \(\mu\)mol/L forskolin to the muscle bath completely reverses both the tension deficit and the blunting and shifting of the tension-frequency relation in the failing myocardium. The present study is the first to demonstrate complete reversal, over the whole range of contraction frequencies, of heart failure–related defects in the force-frequency relation by the drug. Previous studies at lower stimulation frequencies have shown that forskolin can potentiate the twitch in various forms of heart failure\(^15\)\(^-\)\(^19\) and can enhance the treppe in dilated\(^20\) and hypertrophic cardiomyopathy.\(^17\) At 18 min \(^{-1}\) and 30\(^\circ\)C, twitch tension was potentiated by 15\% in a previous study by Feldman et al.\(^14\) Our higher value of 27\% (Fig 2) probably results from the lower degree of activation present at 37\(^\circ\)C before drug application. Bristow et al\(^19\) obtained 60\% of maximal forskolin potentiation with 0.5 \(\mu\)mol/L at 36 min \(^{-1}\) and 37\(^\circ\)C. Our Fig 2 shows peak twitch tension at 36 min \(^{-1}\) with 0.5 \(\mu\)mol/L forskolin to be 52\% of the maximal value reached at the peak of the tension-frequency curve.

**Possible Mechanisms Underlying the Tension-Frequency Defect**

In the absence of any change in the myosin heavy chain content of the mitral regurgitation strips, the tension loss might be attributable to changes in cross-bridge kinetics as suggested by the findings of reduced (50\%) myofibrillar ATPase.\(^15\)\(^,\)\(^21\) However, our previous energetics study in mitral regurgitation myocardium (21\(^\circ\)C, 10 min \(^{-1}\)) indicates that tension loss due to slower kinetics may be compensated by a doubling of the cross-bridge tension-time integral and that tension loss is more likely due to changes in the quantity of calcium cycled (which is reduced by 50\%) in a twitch response in mitral regurgitation.\(^22\) Therefore, tension reduction and blunting of the frequency treppe in mitral regurgitation may result from altered excitation-contraction coupling (ECC). The present observation of reversal of these defects by forskolin could result from reversal of this ECC alteration by elevation of intracellular cAMP levels. We now believe that our previous report\(^10\) that forskolin only partly reverses the depressed tension-frequency relation in papillary muscle mitral regurgitation myocardium does not warrant our previous conclusion ruling out reduced cAMP levels because, contrary to that study, current measurements (unpublished) show that myosin heavy chain content of the papillary muscle myocardium is substantially lower than in nonfailing epicardium. Further experiments to rule out direct action of forskolin on some other intracellular target are needed to support this suggestion of cAMP involvement (see “Study Limitations”). This explanation is attractive because forskolin increases peak intracellular \([\text{Ca}^{2+}]\) without increasing diastolic \([\text{Ca}^{2+}]\) levels\(^17\) and because inhibition of adenylate cyclase converts a positive frequency treppe (Rabbit) into a negative one.\(^23\) The alternative explanation that forskolin drives cAMP and \([\text{Ca}^{2+}]\) levels above normal to compensate for some other defect such as decreased \(\text{Ca}^{2+}\) pump content is not likely since we previously observed (see “Study Limitations”) depression of twitch tension and optimum stimulation frequency by forskolin in preliminary studies on nonfailing myocardium.\(^10\)

**Possible Clinical Relevance of Present Findings**

The depression, blunting, and shifting of the tension-frequency relation in New York Heart Association class II–III failure observed in these mitral regurgitation hearts is almost as severe as in end-stage dilated cardiomyopathic hearts (DCM) from transplant recipients,\(^12\) and it is likely that all of these defects contribute to the decreased contractile reserve of the mitral regurgitation ventricle in vivo. They would limit wall tension generation and blunt the normal potentiating effect of tachycardia. In addition, presence of a negatively sloped force-frequency relation in the exercise range of heart rates may also contribute to development of further dilation of the ventricle both preoperatively and post-operatively.\(^11\)\(^,\)\(^12\) These findings may help to explain the basis of failure to observe large increases in ejection phase ventricular parameters after successful mitral valve repair\(^13\)\(^,\)\(^14\) and suggest that alterations in ECC, possibly related to deficient levels of intracellular cAMP, may underlie this failure. Although the present finding of decreased contractile reserve in mitral regurgitation myocardium suggests this may be a potential contributor (in addition to chordal transection) to the lowered survival rate of mitral regurgitation patients compared with aortic regurgitation patients, similar experiments on myocardium from the latter group with results showing absence of decreased contractile reserve are necessary to support this hypothesis.

**Study Limitations**

Although forskolin has been shown to be a potent activator of human myocardial adenylate cyclase\(^19\) and to raise intracellular cAMP and \(\text{Ca}^{2+}\) transients,\(^24\) in the absence of measurements of intracellular cAMP, the possibility remains that reversal of the force-frequency defect by forskolin is mediated by a direct action of the
drug on some other system. We favor the cAMP mechanism because of the results of a preliminary experiment in which we applied membrane-permeant dibutyryl cAMP (90 μmol/L) or forskolin (0.5 μmol/L) to two ventricular strips prepared from the same papillary muscle biopsy taken from a mitral regurgitation patient. Both drugs increased the slope of the ascending limb of the force-frequency relation to similar values (DB cAMP: 3.7% min⁻¹; forskolin: 3.2% min⁻¹) that compare well with the slope of the normal function preparations in Fig 1 (3.6% min⁻¹). Further experiments of this kind on epicardial strips are needed to confirm the role of cAMP in reversal of the force-frequency defect.

Forskolin was not applied to the nonfailing preparations in the present study because our previous preliminary study showed it to decrease both optimal stimulation frequency (by 26%) and maximal peak twitch tension (by 15%) in control myocardium. This decision is in keeping with the emerging concept that intracellular [cAMP] is not to be maintained within a certain concentration range to manifest a normal force-frequency relation. Too high a concentration may be detrimental because of excessive abbreviation of the twitch myogram and because of possible reduction in myofilament Ca²⁺ sensitivity.

**Note added in proof:** The present results showing a 65% reduction in myocardial twitch tension in mitral regurgitation at 84 min⁻¹ are in good agreement with the recent report of a 68% reduction in ventricular Ewave at 85 min⁻¹ in vivo in mitral regurgitation patients (Starling MK, Kirsh MM, Montgomery DG, Gross MD. Impaired left ventricular contractile function in patients with long-term mitral regurgitation and normal ejection fraction. J Am Coll Cardiol. 1993;22:239-250.).

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