Determinants of contractile reserve in viable, chronically dysfunctional myocardium

Michael D. Banas,1,3,4 Sunil Baldwa,1,2,3,4 Gen Suzuki,3,4 John M. Canty, Jr.,1,3,4,5 and James A. Fallavollita1,3,4

1Veterans Affairs Western New York Health Care System, Buffalo; 2Canandaigua Veterans Affairs Medical Center, Canandaigua, New York; and 3Center for Research in Cardiovascular Medicine, 4Department of Medicine, and 5Department of Physiology and Biophysics, University at Buffalo, Buffalo, New York

Submitted 20 December 2006; accepted in final form 19 January 2007

Banas MD, Baldwa S, Suzuki G, Canty JM Jr, Fallavollita JA. Determinants of contractile reserve in viable, chronically dysfunctional myocardium. Am J Physiol Heart Circ Physiol 292: H2791–H2797, 2007. First published January 19, 2007; doi:10.1152/ajpheart.01384.2006.—There is considerable variability in the sensitivity of inotropic reserve to identify viability in chronically dysfunctional myocardium. This is partially related to the underlying pathophysiology, with more frequent contractile reserve in chronically stunned (with normal resting perfusion) than hibernating myocardium (with reduced flow). This study was undertaken to determine the physiological responses to transient and graded stimulation in chronically stunned and hibernating myocardium to define the relative roles of acute catecholamine desensitization and biphasic responses. Pigs were chronically instrumented with a fixed left anterior descending artery stenosis that resulted in chronically stunned myocardium after 2 mo. One month later, hibernating myocardium was confirmed by regional dysfunction (wall thickening, 3.2 ± 0.3 vs. 5.5 ± 5 mm in remote, P = 0.01) with reduced resting flow (0.70 ± 0.07 vs. 0.92 ± 0.09 ml·min⁻¹·g⁻¹ in remote, P = 0.01) without infarction. Wall thickening in dysfunctional regions significantly increased during both graded and transient epinephrine stimulation in chronically stunned (from 3.6 ± 0.3 to 5.6 ± 0.5 and 4.9 ± 0.5 mm, respectively) and hibernating myocardium (from 3.3 ± 0.3 to 5.4 ± 0.6 and 5.0 ± 0.7 mm, respectively) and returned to baseline within 15 min. Although a biphasic response during graded stimulation was common, the subsequent decrement in function was small and similar in both groups (stunned, 0.7 ± 0.2 mm; hibernating, 1.1 ± 0.3 mm, P = 0.25). We conclude that 1) the extent of contractile reserve during β-adrenergic stimulation is similar in chronically stunned and hibernating myocardium, 2) there are no significant differences between the responses to transient compared with graded catecholamine stimulation, and 3) submaximal catecholamine stimulation does not induce additional stunning in either chronically stunned or hibernating myocardium.

CLINICAL STUDIES HAVE SHOWN that there is considerable variability in the accuracy of contractile reserve during β-adrenergic stimulation to predict myocardial viability. This appears to be at least partially related to the continuum of physiologies associated with viable myocardium, since most studies have shown a greater incidence of contractile reserve in chronically stunned myocardium with normal levels of resting flow compared with hibernating myocardium in which resting perfusion is reduced (4, 5). Among segments with hibernating myocardium, clinical evidence of contractile reserve may be absent in up to 50% (29). In a well-characterized porcine model of hibernating myocardium, our group (14) has previously shown that contractile reserve during β-adrenergic stimulation is significantly blunted compared with the normally perfused remote myocardium. Furthermore, our work documented that this limited responsiveness was not due to the development of acute ischemia, because there was no regional lactate production or change in venous pH (14). Subsequent work by our group (21) has shown that despite normal levels of total β-adrenergic receptors in hibernating myocardium, there is a reduction in high-affinity binding and regional desensitization of receptor signaling.

Our group also has previously demonstrated that pigs chronically instrumented with a proximal coronary stenosis systematically progress from chronically stunned myocardium (at 1 and 2 mo after instrumentation) to develop hibernating myocardium after 3 mo. By using serial studies in the same group of animals, the aims of the present study were to determine 1) whether transient β-adrenergic stimulation elicits greater contractile reserve than a prolonged, graded stimulation protocol (24), 2) whether contractile reserve is different in chronically stunned versus hibernating myocardium, and 3) the relative frequency of a biphasic contractile response during graded catecholamine stimulation in chronically stunned and hibernating myocardium (29).

METHODS

Experimental procedures conformed to institutional guidelines for the care and use of animals in research, and were approved by the University Institutional Animal Care and Use Committee. Pigs (n = 15, weight = 8.0 ± 0.4 kg) were chronically instrumented with a fixed, 1.5-mm (inner diameter) Delrin stenosis on the proximal left anterior descending artery (LAD). At the time of instrumentation this stenosis caused no reduction in resting flow or flow reserve, but the physiological significance of the stenosis increased with animal growth. Previous studies have shown that 1 and 2 mo after instrumentation, there is regional dysfunctional myocardium with normal resting flow, consistent with chronically stunned myocardium (9, 11). However, by 3 mo after instrumentation, there was a critical reduction in flow reserve and resting flow became depressed, consistent with hibernating myocardium (10, 15, 23).

To determine differences in contractile reserve in chronically stunned compared with hibernating myocardium, we studied pigs — 2

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
mo (62 ± 1 days, weight = 44 ± 1 kg) and 3 mo (94 ± 2 days, weight = 68 ± 3 kg) after instrumentation. A previous study in different groups of animals with hibernating myocardium had suggested that the contractile response was greater with transient compared with graded epinephrine stimulation (24), so paired protocols at each time point were performed 4 ± 1 days apart in random order. Animals were initially sedated with a mixture of Telazol (tiletamine and zoletil)-xylazine, and after peripheral vascular access was obtained, anesthesia was maintained with a continuous infusion of propofol (5–10 mg·kg⁻¹·h⁻¹). Blood pressure was noninvasively obtained with an automated cuff (Dinamap; Critikon). Heart rate and oxygen saturation were continuously monitored. Animals were intubated and given supplemental oxygen.

Assessment of regional ventricular function. Regional left ventricular (LV) function was assessed by transhastorial echocardiography through a right parasternal window with the animals on their left side (24), using a 2.5-MHz phased-array transducer (GE Vivid System V; GE Healthcare) (6, 16). Mid-LV short-axis two-dimensional echocardiographic views were obtained, and two to four cycle loops were recorded. Anteroseptal and posterior wall thickness were determined using anatomic M-mode (GE Healthcare) (6). As recommended by the American Society of Echocardiography (30), end diastole was defined as the onset of the QRS complex and end systole was the point of minimum chamber diameter. Regional function was assessed with myocardial wall thickening (end-systolic thickness − end-diastolic thickness) (24). Fractional shortening, an assessment of global function, was defined as [100 × (LV end-diastolic dimension) − (LV end-systolic dimension)]/(LV end-diastolic dimension) (24).

For the transient stimulation protocol, a single stage of stimulation was produced with a dose of epinephrine (0.35 ± 0.01 μg·kg⁻¹·min⁻¹) that would increase heart rate by ~40 beats/min (34). For the graded simulation protocol, epinephrine was incrementally infused in four equal stages to a similar peak epinephrine dose (nominal doses of 0.0875, 0.175, 0.2625, and 0.35 μg·kg⁻¹·min⁻¹) (14). Each stage was maintained for ~10 min to allow heart rate to stabilize before echocardiograms were obtained (24). To assess the recovery of regional function and the possible induction of additional stunning following inotropic stimulation in viable dysfunctional myocardium, we assessed function after transient stimulation every 15 min for 1 h.

Regional perfusion and myocardial sampling. Myocardial perfusion and coronary flow reserve were assessed in 12 animals 95 ± 2 days after instrumentation in the closed-chest, propofol-anesthetized state (6). The remaining three animals died prematurely either as a result of spontaneous sudden death (n = 2) (6) or as a complication of a study (n = 1). Myocardial flow was quantified with fluorescently labeled microspheres injected at rest and during adenosine vasodilation (0.9 mg/kg iv) with phenylephrine coinfused to maintain systolic thickening (14). This was considered statistically significant.

RESULTS

Development of hibernating myocardium in chronically instrumented pigs. Table 1 and Fig. 1 confirm the development of hibernating myocardium in chronically instrumented pigs 3 mo after initial instrumentation (6, 14, 15, 24). Myocardial wall thickening was reduced by 41% in the hibernating LAD region compared with the normally perfused remote region. This was associated with reductions in subendocardial and full-thickness resting perfusion and severe reductions in regional flow reserve in hibernating myocardium (Table 1). There was a critical reduction in subendocardial flow reserve, with flow during adenosine vasodilation (0.61 ± 0.16 ml·min⁻¹·g⁻¹) unable to increase over resting levels (0.75 ± 0.09 ml·min⁻¹·g⁻¹, P = 0.54). These abnormalities in function and perfusion were not the result of myocardial infarction, because fibrosis involved <1% of LV mass by TTC staining in each animal. Although this model of hibernating myocardium is associated with a small regional increase in connective tissue staining (2%) (13), a similar change is present in animals with chronically stunned myocardium (9).

Transient versus graded epinephrine stimulation in chronically stunned myocardium. Myocardial dysfunction under baseline conditions was present in the LAD region of chronically instrumented animals 2 mo after instrumentation (Fig. 1), and function in the LAD region remained significantly reduced compared with the remote region during epinephrine stimulation (ANOVA, P < 0.001 for each protocol). The functional responses to both transient and graded epinephrine stimulation are shown in Fig. 1, top. Both the transient and graded stimulation protocols resulted in significant increases in function in the LAD-perfused region (Fig. 1, top left) as well as remote myocardium (Fig. 1, top right). There were no differences in LAD region function between the two protocols at comparable time points; however, as indicated by ANOVA, function in remote myocardium tended to be higher during the graded stimulation protocol (P = 0.04). At the maximum dose of epinephrine, both protocols resulted in similar improvements in regional function in both regions, and global function was blinded to all study information. Discrepancies in baseline wall thickening of >1 mm were adjudicated with a third observer, and the values in closest agreement were averaged. The changes in wall thickening during each stimulation protocol were assessed with a two-way (myocardial region and protocol stage) analysis of variance (ANOVA; SigmaStat 3.00, SPSS). When significant differences were present, the Holm-Sidak test was used for post hoc comparisons versus baseline. Changes in hemodynamic parameters during a protocol were assessed with one-way ANOVA. Differences in baseline or peak stimulation parameters between myocardial regions or stimulation protocols were compared with paired t-tests. A value of P ≤ 0.05 was considered statistically significant.

| Table 1. Function, perfusion, and flow reserve in hibernating myocardium |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                               | Resting Wall    | Baseline Flow, ml·min⁻¹·g⁻¹ | Adenosine Flow, ml·min⁻¹·g⁻¹ | Flow Reserve (Adenosine/Baseline) |
|                               | Thickening, mm | Endo             | FT               | Endo             | FT               | Endo             | FT               |
| LAD                            | 3.2±0.3*        | 0.75±0.09*       | 0.70±0.07*       | 0.61±0.16*       | 1.31±0.24*†      | 1.3±0.6*         | 2.3±0.8*         |
| Remote                         | 5.5±0.5         | 1.10±0.11        | 0.92±0.09        | 4.72±0.49†       | 5.01±0.48††      | 4.9±0.8          | 6.0±0.8          |

Endo, subendocardial; FT, full thickness; LAD, left anterior descending artery (n = 12); †P < 0.05 vs. remote; ††P < 0.05 vs. baseline.
increased to a similar extent (Table 2). However, the peak improvement in regional function during the graded protocol frequently occurred at a submaximal epinephrine dose (peak LAD function at stage 2.8 ± 0.3). The peak improvement in remote region function during graded stimulation occurred at stage 3.0 ± 0.3 (P = 0.72 vs. LAD). There was no evidence of additional stunning following either stimulation protocol, because wall thickening returned to baseline within 15 min. The hemodynamic parameters associated with these protocols are shown in Fig. 2, left. There were no differences in hemodynamic parameters between the protocols at comparable time points (baseline, maximum epinephrine, and 15 min of recovery). Epinephrine stimulation resulted in a small decrease in LV diastolic dimension and an increase in LAD region diastolic wall thickness (Table 2).

**Transient versus graded epinephrine stimulation in hibernating myocardium.** Myocardial function assessed with wall thickening did not change in the LAD region from 2 to 3 mo after instrumentation, although there was an increase in diastolic wall thickness (Table 2) and function in the remote normally perfused myocardium tended to increase (Fig. 1). The results of both inotropic stimulation protocols in hibernating myocardium were remarkably similar to the data in chronically stunned myocardium (Fig. 1 and Table 2). Regional function (Fig. 1, bottom) and global function (Table 2) improved during each protocol at comparable hemodynamics (Fig. 2, right), but function in the LAD-perfused region remained significantly reduced compared with the normally perfused remote region. There were no differences in regional or global function between the two protocols. In hibernating myocardium (LAD region), the peak functional responses during the two protocols were more similar than the function at the maximum dose of epinephrine (E_{max}), but neither the difference nor change was statistically significant. During the graded stimulation protocol, the peak improvement in LAD function occurred at stage 2.3 ± 0.3 compared with stage 3.1 ± 0.4 in the normally perfused remote region (P = 0.19). Again, there was no evidence of additional stunning following either stimulation protocol.

**Biphasic responses to graded stimulation in viable, chronically dysfunctional myocardium.** As noted above, peak function frequently occurred during a submaximal dose of epinephrine in the graded stimulation protocol at both 2 mo (9 of 12) and 3 mo after instrumentation (8 of 9, P = 0.60 vs. 2 mo). This is consistent with a biphasic response (a fall in regional function from peak function to E_{max}) in the majority of the

---

**Table 2. Diastolic dimensions and left ventricular fractional shortening**

<table>
<thead>
<tr>
<th>LAD</th>
<th></th>
<th>Remote</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diastolic Wall Thickness, mm</td>
<td></td>
<td>LV Diastolic Dimension, mm</td>
</tr>
<tr>
<td></td>
<td>BL Peak E4</td>
<td>BL Peak E4</td>
<td>BL Peak E4</td>
</tr>
<tr>
<td>2 Mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient</td>
<td>10.8 ±0.4 12.3 ±0.8</td>
<td>9.5 ±0.5 10.1 ±0.4</td>
<td>42 ±2 38 ±2*</td>
</tr>
<tr>
<td>Graded</td>
<td>10.5 ±0.4 12.2 ±0.8*</td>
<td>9.9 ±0.4 10.0 ±0.4</td>
<td>42 ±2 39 ±3* 38 ±3*</td>
</tr>
<tr>
<td>3 Mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient</td>
<td>12.5 ±0.5 13.7 ±1.1</td>
<td>10.5 ±0.6 11.1 ±0.6</td>
<td>45 ±3 45 ±2</td>
</tr>
<tr>
<td>Graded</td>
<td>11.9 ±0.6 13.0 ±0.9</td>
<td>12.1 ±0.8 11.4 ±0.5</td>
<td>48 ±2 46 ±2 43 ±2*</td>
</tr>
</tbody>
</table>

BL, baseline; Peak, maximum function during epinephrine; E4, maximum epinephrine dose; LV, left ventricular; *P < 0.05 vs. BL.
animals. However, as illustrated in Fig. 3, the reduction in function from peak response to Emax was usually small, and only rarely greater than one standard deviation of the baseline LAD wall thickening (\( >1.4 \text{ mm} \); 2 mo, 1 of 12; 3 mo, 3 of 9; \( P = 0.27 \)) (24).

**DISCUSSION**

To our knowledge, this is the first systematic comparison of graded versus transient catecholamine stimulation protocols to assess inotropic reserve in both chronically stunned and hibernating myocardium. In contrast to most previous studies of contractile reserve, which have used a semiquantitative wall motion score, M-mode echocardiography was used to quantify regional wall thickening. Our results show that both graded and transient stimulation protocols can elicit significant and comparable improvements in regional function in animals with viable, chronically dysfunctional myocardium. Interestingly, the degree of contractile reserve was similar in chronically stunned and hibernating myocardium, and a biphasic response was common (but small in amplitude) at both time points.

**Transient versus graded catecholamine stimulation in hibernating myocardium.** On the basis of our previous study (24), we had hypothesized that a transient stimulation protocol might produce a greater contractile response than graded epinephrine infusion in pigs with hibernating myocardium (3 mo after instrumentation). In that study, separate groups of animals underwent graded and transient epinephrine stimulation to comparable increases in hemodynamic parameters. Although the difference between the groups was not statistically significant, there was an 80% greater increase wall thickening (peak function - baseline) during the transient protocol (1.8 ± 0.7 mm) compared with the graded epinephrine stimulation (1.0 ± 0.6 mm, \( P = 0.41 \)) (24). This is in contrast to very similar increases in wall thickening in the present study (transient, 2.0 ± 0.6 mm; graded, 1.8 ± 0.3 mm; \( P = 0.67 \)). The disparity between these two studies can be attributed to blunted contractile reserve during the graded epinephrine protocol of the earlier study (24). This finding is likely due to the isoflurane anesthesia that was unique to this group of animals and is consistent with the blunting of myocardial contractility in the presence of volatile anesthetics. Previous investigations have shown that propofol anesthesia maintains functional responses in normal and collateral-dependent myocardium similar to those occurring in conscious animals (25) and only affects \( \beta \)-adrenergic responsiveness at high doses (35). Therefore,
propofol should be the preferred anesthetic for studies of contractile reserve (as used in the present study and the transient stimulation group from the previous study) (25).

**Contractile reserve in viable, chronically dysfunctional myocardium.** Although the presence of contractile reserve is commonly used to identify viable myocardium, previous studies have clearly shown that it is not universally present in viable, dysfunctional myocardium compared with recovery of function after revascularization or metabolic viability assessed with positron emission tomography (PET) (27, 29). For example, Melon et al. (27) have shown that among 142 dysfunctional segments that were viable by PET (normal resting perfusion or preserved $^{18}$F-fluoro-2-deoxyglucose uptake), only 77 (54%) had an improvement in regional wall motion by dobutamine echocardiography. Our assessment of contractile reserve in chronically instrumented pigs supports this clinical observation. Using a semiquantitative wall motion score in pigs with viable myocardium with reduced resting perfusion (hibernating myocardium), our group observed that only 25 of 44 (57%) animals showed a change in regional wall motion (12). However, in animals with a patent coronary artery, wall motion improved in 11 of 12 (92%, P = 0.01 vs. pigs with an occluded artery). This occurred despite the fact that resting perfusion was reduced to a similar extent in both groups of animals (12). We suggested that the greater contractile reserve in hibernating myocardium perfused through a patent artery was the result of greater subendocardial flow during inotropic stimulation and pharmacological vasodilation (12), as has been shown in patients with coronary artery disease (22, 33).

Regional wall motion score is a relatively insensitive assessment of regional function that could underestimate the degree of contractile reserve in viable, dysfunctional myocardium. Consistent with this hypothesis, our group has shown that M-mode echocardiographic quantification of wall thickening (24) and subendocardial segment shortening with sonomicrometry (14) can increase the proportion of animals that demonstrate an improvement in regional function, although even with these approaches, functional improvement could not always be documented in histologically viable myocardium. Similarly, in patients with coronary artery disease, wall thickening measurements with magnetic resonance imaging during dobutamine infusion have shown improved (but not perfect) sensitivity compared with dobutamine echocardiography (28).

Does viable dysfunctional myocardium develop ischemia during inotropic stimulation? An alternate explanation for the absence of contractile reserve in some segments of viable, dysfunctional myocardium is the development of acute ischemia with the exhaustion of subendocardial flow reserve and energy stores (1), as occurs in animal models of prolonged moderate ischemia or “short-term hibernation” (32). This mechanism has also been attributed to the biphasic response to graded inotropic stimulation in which an improvement in regional function is followed by functional deterioration at higher doses (1, 7). Although a biphasic response has been felt to be pathognomonic for hibernating myocardium (1), correlation with regional perfusion is limited to the study of Sawada et al. (29) in which 88% of segments demonstrating a biphasic response had normal levels of resting flow (chronically stunned myocardium). There are no criteria for defining a biphasic response by wall thickening; however, the majority of animals in the present study had small decrements in regional function at higher levels of inotropic stimulation (Fig. 3), with no differences between stunned and hibernating myocardium.

In pigs with hibernating myocardium, our group has shown that although subendocardial flow reserve is exhausted at rest, modest increases in demand and regional function are associated with increases in regional oxygen consumption (14). Furthermore, we have found no metabolic evidence of regional ischemia, with progressive increases in regional lactate consumption and normal levels of venous pH over the same range of epinephrine stimulation that was produced in the present study (14). The absence of acute ischemia is further supported by the lack of additional myocardial stunning following inotropic stimulation. In all four protocols presented in this study, regional wall thickening returned to baseline function within 15 min after the discontinuation of epinephrine.

In the clinical literature, there are conflicting data regarding the extent to which inotropic stimulation can induce acute ischemia in chronically dysfunctional myocardium. Consistent with our porcine data, Lee et al. (22) have shown that even in the absence of contractile reserve (which might suggest the development of ischemia), there is an increase in regional oxygen consumption during dobutamine stimulation. In contrast, Indolfi et al. (20) have shown that intracoronary dobutamine always caused a reduction in arterial-venous lactate differences, associated with regional lactate production in four of nine patients. However, with persistent stimulation, there was stabilization or improvement in arterial-venous lactate differences in all but one patient (20).

These clinical and basic studies support the contention that the development of hibernating myocardium involves intrinsic adaptations that not only downregulate resting flow and oxygen consumption but also blunt the response to inotropic stimulation to limit the development of acute ischemia (4, 14). This hypothesis is supported by the alterations in β-adrenergic signaling identified using tissue from pigs with hibernating myocardium (21). Baseline cAMP production in hibernating myocardium was normal, but the response to isoproterenol stimulation was blunted and associated with an increase in the ratio of inhibitory to stimulatory G proteins (21). Despite normal β-adrenergic receptor density and subtype distribution, there was a reduction in high-affinity binding sites (21), which would be consistent with regional desensitization. Before this study, we hypothesized that transient stimulation might result in greater contractile reserve by limiting any acute desensitization of β-receptors compared with a longer, graded infusion. However, the fact that both protocols resulted in similar improvements in regional function in hibernating myocardium is inconsistent with such a mechanism but, rather, suggests a chronic downregulation.

**Contractile reserve in chronically stunned and hibernating myocardium.** Clinical studies have clearly shown that the presence of contractile reserve is highly specific for myocardial viability (2). However, the results of the present study are in agreement with previous reviews suggesting that responsiveness to catecholamines is not specific for the underlying pathophysiology of dysfunction (17, 18). Nevertheless, contractile reserve as assessed by wall motion score is more common in regions with normal resting perfusion (chronically stunned myocardium) than in regions with reduced resting flow (hibernating myocardium) (22, 29, 31). For example, Sawada et al. (29) detected improvement in wall motion among 61 of 108
(56%) dysfunctional segments with normal resting flow but in only 9 of 28 (32%) dysfunctional segments with reduced resting perfusion but metabolic viability ($P = 0.03$). Nevertheless, despite the differences in the prevalence of contractile reserve, the limited clinical data are conflicting with regard to the extent of functional improvement in chronically stunned and hibernating myocardium. Bountioukos et al. (3) found that baseline pulsed wave tissue Doppler velocity was similar in stunned and hibernating myocardium. However, during dobutamine infusion, the increase in tissue velocity was significantly greater in chronically stunned than in hibernating myocardium (3). In contrast, Mazzadi et al. (26) showed that baseline circumferential shortening by tagged magnetic resonance imaging was similar in dysfunctional segments with matched (chronically stunned myocardium) and mismatched (hibernating myocardium) metabolism and perfusion (26). In agreement with our results in chronically instrumented pigs, they found that dobutamine stimulation increased circumferential shortening to a similar extent in chronically stunned and hibernating myocardium (26).

Methodological limitations. Theses studies in chronically instrumented pigs were performed with epinephrine rather than dobutamine, which is the more commonly used catecholamine for clinical viability testing. Although we cannot confirm that the results would be the same with dobutamine, epinephrine produces a stress that is more physiologically consistent with exercise. Furthermore, we believed it was critical to produce the same level of stress that we have previously shown to be effective in chronic hibernating pigs. Circulation 99: 2798–2805, 1999.

REFERENCES


