Blunted functional responses to pre- and postjunctional sympathetic stimulation in hibernating myocardium

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Ovchinnikov, Vladislav, Gen Suzuki, John M. Canty, Jr., and James A. Fallavollita. Blunted functional responses to pre- and postjunctional sympathetic stimulation in hibernating myocardium. Am J Physiol Heart Circ Physiol 289: H1719–H1728, 2005. First published May 27, 2005; doi:10.1152/ajpheart.00273.2005.—Regional reductions in norepinephrine-tracer uptake are found in pigs with hibernating myocardium. Clinical studies would suggest that this is evidence for denervation; however, the functional responses to sympathetic stimulation have not been evaluated, and our previous studies with β-adrenergic stimulation have not suggested denervation hypersensitivity. Therefore, pigs were chronically instrumented to produce hibernating myocardium characterized by chronic regional dysfunction and histological viability. Open-chest studies were performed to determine changes in regional function in response to both pre- and postjunctional stimulation. Regional segment shortening was reduced at rest in hibernating myocardium compared with controls (13 ± 3% vs. 27 ± 3%, \(P = 0.004\)). During stellate ganglion stimulation, regional function increased in both groups of animals (\(P = 0.008\) vs. baseline), but the increase in hibernating myocardium was blunted compared with controls (\(\Delta\% , 3 \pm 2\% \) vs. \(8 \pm 3\% , \ P = 0.04\)). Similar results occurred with intracoronary tyramine (10 \(\mu g/kg\)). Functional improvement during intravenous epinephrine infusion (0.35 \(\mu g/kg/min\)) was also blunted in hibernating myocardium compared with controls (\(\Delta\% , 7 \pm 1\% \) vs. \(15 \pm 2\% , \ P = 0.04\)). Even when the improvement in function was expressed relative to the reduced baseline, there was no evidence for catecholamine-mediated hypersensitivity in hibernating myocardium. We therefore conclude that functional responses to both pre- and postjunctional sympathetic stimulation are blunted in pigs with hibernating myocardium. In contrast to previous studies of infarcted, denervated, and acutely stunned myocardium, there is no catecholamine-induced hypersensitivity in hibernating myocardium. These data suggest a downregulation in functional responses to stimulation that would protect hibernating myocardium from demand-induced ischemia at the expense of contractile reserve during sympathetic stimulation.

In patients with ischemic heart disease, regional reductions in norepinephrine-tracer uptake occur in areas of infarction (3), as well as in clinically viable myocardium distal to severe coronary stenoses (8, 22). Because sympathetic nerves are thought to be exquisitely sensitive to ischemia, these abnormalities have been interpreted as evidence for sympathetic denervation (3, 6, 22). In a chronic porcine model that recapitulates all of the physiological features of hibernating myocardium (25) in the absence of infarction (19), we have found similar regional reductions in norepinephrine tracer uptake using meta-[\(^{131}\)I]iodobenzylguanidine (MIBG) (30) and [\(^{11}C\)]hydroxyephedrine (HED) (31). However, our previous physiological studies with this model do not support a hypothesis of sympathetic denervation. In contrast to hypersensitivity to catecholamine stimulation (26, 42) associated with an upregulation of β-adrenergic receptor density (42) that is typical of denervated myocardium, we have shown blunted contractile reserve during catecholamine stimulation (18) and normal β-adrenergic receptor density (28). In an effort to reconcile these findings, the present study was performed to determine the functional responses to sympathetic stimulation in pigs with hibernating myocardium and to directly compare the responses to pre- and postjunctional stimuli.

METHODS

All experiments were performed in accordance with institutional guidelines for the care and use of animals in research. Hibernating myocardium was produced in pigs by chronic instrumentation of the left anterior descending coronary artery (LAD), as previously described (19). Briefly, the proximal LAD of juvenile animals (\(n = 17\) pigs) was instrumented with a Delrin stenosis (1.5 mm id). As the animal grows, the fixed diameter stenosis causes progressive reductions in regional flow reserve and regional dysfunction in the absence of infarction (19).

Closed-chest studies. Coronary angiography and contrast ventriculography were performed in all chronically instrumented animals. Animals were sedated with a tiletamine-zolazepam (Telazol)-xylazine mixture (0.022 ml/kg im) followed by propofol (5–10 mg/kg iv). Animals were prophylactically intubated and provided with supplemental oxygen. Frequent blood gas determinations were used to determine the necessity for and adjustment of mechanical ventilation. With the use of sterile technique, a cut down was performed over a brachial artery for placement of a vascular introducer (11). A multipurpose catheter was used to selectively engage the coronary arteries, and iodinated contrast was used to determine LAD stenosis severity and the presence of coronary collaterals (19). Percent diameter stenosis was quantified with caliper measurements of magnified angiograms and referenced to average pre- and poststenosis coronary diameter (19). Anteropapical wall motion was determined with hand injections of contrast in the left ventricle (LV) and quantified by consensus of three reviewers (0, akinesis; 1, mild hypokinesis; 2, severe hypokinesis; and 3, normal) (19). In addition, global and regional myocardial function was assessed with transthoracic echocardiography at the midventricular level. Regional function was quantified by percentage of wall thickening [end-systolic wall thickness – end-diastolic wall thickness]/end-diastolic wall thickness; anatomic M-mode, GE System 5]. Global LV function was determined by fractional shortening based on end-systolic and end-diastolic chamber dimensions [end-diastolic chamber dimension – end-systolic cham-
Open-chest studies of sympathetic function. Regional sympathetic nerve function was assessed in chronically instrumented pigs 112 ± 3 days after initial instrumentation, and results were compared with eight uninstrumented normal control animals. Pigs were initially sedated with diazepam (10 mg/kg po), followed by midazolam (2 mg/kg im) and ketamine (15 mg/kg im). Animals were intubated and mechanically ventilated, and frequent blood gas determinations were used to adjust ventilatory parameters. Anesthesia was maintained with a continuous infusion of propofol (20–75 μg·kg⁻¹·min⁻¹) and ketamine (30–125 μg·kg⁻¹·min⁻¹). This anesthetic regimen was specifically chosen to minimize effects on the sympathetic nervous system. Although at high doses, both ketamine and propofol have been shown to alter autonomic responses, effects at low-to-moderate doses are minimal and opposed (36, 45).

A jugular vein catheter was used for infusion of fluids and anesthetics, and LV pressure was assessed with a transducer-tipped catheter (Millar Instruments) advanced retrogradely from a carotid artery. Normal saline was infused to maintain LV end-diastolic pressure (LVEDP) comparable to the initial baseline obtained in the closed-chest state. The heart was exposed through a midline sternotomy. Pairs of piezoelectric crystals were inserted into the subendocardium in the midanterior wall (~1 cm apart) to quantify regional segment shortening [(end-diastolic segment length – end-systolic segment length)/end-diastolic segment length] (18). The right stellate ganglion was exposed for stimulation without disruption of spinal innervation. Finally, bipolar pacing wires were placed on the right atrial appendage.

Based on review of the literature in dogs (14) and pigs (29), we anticipated using stimulation of the left stellate ganglion to assess the sympathetic innervation of the LV anterior wall. However, electrical stimulation of the left stellate resulted in consistent reduction in anterior segment shortening among normal control animals. In contrast, anterior segment shortening improved during right stellate ganglion stimulation and was therefore used for subsequent analyses. Electrical stimulation was performed by the delivery of 5-ms pulses at 10 Hz for 10 s. Optimal output was initially evaluated in the normal controls, with graded measurements from 1 to 30 V. Anterior wall function plateaued at 10 V; therefore, this maximum level was used in the chronically instrumented animals. Regional function and hemodynamic parameters were averaged over 20 s beginning 10 s after stimulation ended. A representative recording of selected hemodynamic parameters and anterior segment length from a control animal is shown in Fig. 1. Because right stellate ganglion stimulation caused sinus tachycardia, these parameters were also assessed during atrial pacing at 150 beats/min to confirm that the functional improvement was not due to the changes in heart rate.

After hemodynamic parameters returned to baseline, postfunctional stimulation was assessed with intravenous epinephrine (0.35 μg·kg⁻¹·min⁻¹ for 10 min) (32), for comparison with our previous study of metabolism during catecholamine stimulation (18). Regional function and hemodynamic parameters were averaged over 20 s after a steady-state was achieved (~3 min). Finally, tyramine was used to functionally assess sympathetic nerve norepinephrine stores. Tyramine (10 μg/kg over 10 s) was selectively injected into the LAD by an angiographically placed catheter (when the LAD was patent) or via an angiocatheter placed distal to a total occlusion, to limit the reflex changes in hemodynamic parameters during intravenous administration (9). There were no differences in response to tyramine as a function of LAD patency. Regional function and hemodynamic parameters were averaged over 20 s, beginning 30 s after the infusion was completed. After studies were completed, the LV was sectioned into concentric rings, which were stained with triphenyltetrazolium chloride (TTC, 1 g TTC/100 ml phosphate buffer) to exclude infarction. Rings were scanned, digitized, and any area of TTC negativity quantified as a percentage of the LV (Sigma Scan, SPSS).

Data and statistical analyses. Tracings of all hemodynamic parameters and segment length measurements were displayed on a Pentium-based personal computer (sampling rate, 500 Hz) using the HEM analysis program (Notocord Systems). All measurements were calculated from the digitized data and averaged over 20 s. LV change of pressure over time (dP/dt) was determined from the first derivative of the digitized LV pressure. Data are means ± SE with P ≤ 0.05 level considered significant. Differences in closed-chest echocardiographic parameters and baseline open-chest function between groups were assessed with unpaired t-tests. Hemodynamic and functional parameters at each baseline before stimulation within each group were compared with unpaired t-tests. Parameters re-
compared with an ANOVA (SigmaStat 3.0). A two-way repeated measures ANOVA with post hoc Holm-Sidak test was used to assess changes in hemodynamic and functional parameters between the hibernating and control groups at baseline and during stimulation, as well as the absolute and relative changes in function between groups and interventions (SigmaStat 3.0). Linear regression was used to compare changes in regional function between methods of stimulation.

RESULTS

All animals were in good health at the time of study. Of the 17 chronically-instrumented animals, 5 had normal anteroapical wall motion by contrast ventriculography (wall motion score of 3), with an average diameter stenosis of 73 ± 3% and no coronary collaterals. These animals were considered sham studies and were analyzed separately. One animal with regional dysfunction had TTC evidence of myocardial infarction encompassing 3.2% of LV mass. This animal was excluded from the analyses. In the remaining 11 animals, anteroapical wall motion was severely hypokinetic to akinetic (wall motion score of 0.6 ± 0.2), with an average LAD stenosis of 90 ± 4%. Myocardial necrosis in these animals was rare and averaged only 0.4 ± 0.2% of LV mass. Complete LAD occlusion occurred in five animals.

Wall thickening in the LAD territory by transthoracic echocardiography was significantly reduced in the hibernating group compared with shams (25 ± 2% vs. 54 ± 9%, P < 0.001), but there was no difference in function in the normally perfused remote regions (82 ± 6% vs. 86 ± 10%, P = 0.76). Global function assessed by fractional shortening was not significantly reduced in the dysfunctional group (29 ± 2% vs. 35 ± 3% in shams, P = 0.09).

Regional sympathetic stimulation: stellate ganglion stimulation and tyramine infusion. The functional effects of sympathetic stimulation were assessed with regional, subendocardial segment shortening. Consistent with normal wall motion in the closed-chest state, baseline regional segment shortening of sham animals in the open-chest state (26 ± 4%) was the same as normal controls (28 ± 4%). Regional function after stimulation was also similar in these two groups of animals (see below). Thus these two groups were combined into a single control group. Baseline segment shortening was significantly lower in the dysfunctional group (initial values, 13 ± 3% vs. 27 ± 3% in controls, P = 0.004), but global ventricular contractility (maximum dP/dt) was similar (initial values, 1,236 ± 123 vs. 1,203 ± 89 mmHg/s in controls, P = 0.82).

Representative hemodynamic and functional responses to stellate ganglion stimulation in a control animal as well as an animal with hibernating myocardium are shown in Fig. 2. Average hemodynamic parameters for the control and dysfunctional groups at the initial baseline and after stimulation are shown in Table 1. The corresponding changes in segment shortening and maximum dP/dt are illustrated in Figs. 3 and 4, respectively. Figures 5 and 6 show the changes in regional and global LV function as the absolute change from baseline (Figs. 5, top, and 6, top), as well as the improvement in function relative to baseline values (Figs. 5, bottom, and 6, bottom). There were no differences in any resting hemodynamic parameters between groups. With the use of an ANOVA of baseline hemodynamics before each method of stimulation, there was only a change in heart rate over time, decreasing from 89 ± 3 beats/min at initial baseline to 74 ± 4 beats/min before tyramine infusion (P = 0.03). There were no changes in baseline end-diastolic segment length or percent segment shortening (%SS) before stimulation.

Among control animals, stellate ganglion stimulation resulted in consistent improvement in regional and global ventricular function, with segment shortening increasing from 27 ± 3% to 35 ± 4% (P = 0.01, Fig. 3) and a threefold increase in maximum dP/dt (to 3,850 ± 450 mmHg/s, P = 0.001; Fig. 4). There were no differences in functional response to stellate ganglion stimulation between the sham-instrumented and normal control animals. In the hibernating group there were also significant increases in anterior wall segment shortening (15 ± 3% to 18 ± 3%, P = 0.01; Fig. 3) and maximum dP/dt (to 2,914 ± 392 mmHg/s, P = 0.001; Fig. 4), but the absolute improvements in segment shortening (3 ± 2% vs. 8 ± 3%, P = 0.04; Fig. 5, top) and positive dP/dt compared with controls (1,652 ± 356 vs. 2,580 ± 417 mmHg/s, P = 0.04; Fig. 6, top) were significantly blunted. This occurred despite a similar increase in heart rate (Table 1), thus confirming a similar level of stellate ganglion stimulation and suggesting that atrial sympathetic innervation in chronically dysfunctional myocardium was unaffected. As expected, the attenuated response on anterior function in the hibernating group resulted in a blunted increase in blood pressure compared with controls (Table 1). Interestingly, the functional improvement after stellate ganglion stimulation was similar in the two groups of animals when calculated as the percentage change from baseline (Fig. 5, bottom).

The tachycardia associated with stellate ganglion stimulation was not responsible for the improvement in function. As previously described (10, 18), atrial pacing did not improve function. At a pacing rate of 150 beats/min (as compared with an average rate of 151 ± 8 beats/min during stellate stimulation, P = 0.46), there was no significant change in regional function in the control (26 ± 4% to 24 ± 4%, P = 0.11) or hibernating groups (15 ± 3% to 11 ± 2%, P = 0.07). There were also no significant changes in systolic blood pressure (control, 88 ± 10 to 87 ± 10 mmHg, P = 0.94; hibernating, 95 ± 5 to 91 ± 6 mmHg, P = 0.19) or LVEDP (control, 17 ± 2 to 19 ± 2 mmHg, P = 0.41; hibernating, 21 ± 1 to 21 ± 1 mmHg, P = 0.86) during atrial pacing.

The functional effects of electrical stellate ganglion stimulation were confirmed with intracoronary tyramine infusion to release sympathetic nerve norepinephrine stores. As shown in Figs. 3 and 5, the absolute and relative improvement in regional function with tyramine was very similar to that after stellate stimulation in both the control and hibernating groups but with no effect on heart rate (Table 1). The similarity of functional responses to stellate ganglion stimulation and tyramine infusion were also highly correlated among individual animals with very high correlation coefficients for both the control (r = 0.94, P = 0.02) and hibernating groups (r = 0.92, P = 0.01; Fig. 7). The data approach the line of identity [peak tyramine %SS = 1.03 (peak stellate %SS) + 0.03, r = 0.93], supporting the similar magnitude and physiological mechanism for these two methods of stimulation.

Epinephrine stimulation. To assess the responses to postjunctional stimulation, contractile reserve was also assessed with an intravenous infusion of epinephrine. In contrast to the response in control animals (24 ± 2% to 39 ± 3%, P =
Fig. 2. Responses to stellate ganglion stimulation (Stim) and epinephrine infusion. In a representative control animal (top), single beat recordings at baseline (BL) and after stellate ganglion and epinephrine stimulation show significant increases in LVP, LV dP/dt, and systolic segment shortening. Although increases in these parameters also occurred in animals with hibernating myocardium (bottom), degree of improvement was blunted. First vertical line on the segment length tracings denotes end diastole (D), and the second line denotes end systole (S).

0.001, Fig. 3), the improvement in regional function during epinephrine infusion in animals with hibernating myocardium was blunted (14 ± 3% to 21 ± 3%, P = 0.001, Fig. 3), and the absolute increases in segment shortening (7 ± 1% vs. 15 ± 2% in controls, P = 0.04, Fig. 5, top) and positive dP/dt were significantly reduced (3,245 ± 555 vs. 5,241 ± 532 mmHg/s in controls, P = 0.04, Figs. 4, and 6, top). There were no differences in functional response to epinephrine infusion be-

Table 1. Hemodynamic parameters

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<th>Heart Rate, beats/min</th>
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<td>111±6</td>
<td>20.1±1.2</td>
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<td>17.7±1.1</td>
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<td>6,507±557†</td>
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All values are means ± SE. n, number of pigs; LV, left ventricular; dP/dt, change of pressure over time. *P < 0.05 vs. control; †P < 0.05 vs. baseline.
tween the sham-instrumented and normal control animals. The subsequent increase in blood pressure was also blunted among the hibernating group (Table 1). This occurred despite identical doses of epinephrine in the two groups and similar heart rate responses (Table 1). However, as was evident during sympathetic stimulation, the increase in both regional and global LV function during epinephrine infusion relative to the baseline was similar between both groups of animals (Figs. 5, bottom, and 6, bottom). Thus there was no evidence for hypersensitivity to β-adrenergic agonist stimulation in hibernating myocardium as would be expected with sympathetic denervation (26, 42).

Because both pre- and postjunctional stimulation were performed in the same animals, the relative responses may offer insight into the mechanism(s) of adaptation. Figure 7 compares the functional responses between interventions for both the control and hibernating groups. Although function was lower...
in the hibernating group, there was a close correlation of functional responses within each group. The epinephrine responses were shifted upward relative to tyramine responses, consistent with a greater magnitude of stimulation; however, in both groups there was a parallel shift in the correlations. Thus responses to both pre- and postjunctional stimulation were similarly blunted in pigs with hibernating myocardium.

DISCUSSION

This is the first study to assess regional sympathetic innervation by the functional responses to stimulation in chronic ischemic heart disease without infarction. In a chronic pig model that recapitulates all of the physiological features of hibernating myocardium (19), we have documented blunted functional responses to sympathetic stimulation that support our previous reports of regionally reduced sympathetic nerve norepinephrine uptake in this model (30, 31). With evidence for downregulation in the responses to both pre- and postjunctional stimulation, our data support a postjunctional explanation for reduced contractile reserve in chronic ischemic heart disease (7, 16). This is in sharp contrast to the denervation associated with infarction, which is characterized by reduced or absent function responses to direct sympathetic stimulation, and exaggerated responses to β-adrenergic stimulation (hypersensitivity) (26, 42).

Sympathetic denervation and hypersensitivity to β-adrenergic agonists after infarction. We have previously shown that norepinephrine uptake, as assessed by the accumulation of the tracers MIBG (30) and HED (31), is regionally reduced in pigs with hibernating myocardium. The 48% regional reduction in HED uptake that we have documented in hibernating myocardium (31) is similar to the 57% reduction found in patients with infarcted myocardium (3), suggesting that hibernating myocardium may be sympathetically denervated. However, the present findings in pigs with hibernating myocardium clearly show some preservation of sympathetic responses during stellate stimulation as well as when norepinephrine is released from the prejunctional nerve with tyramine. These differ from the responses to pre- and postjunctional sympathetic stimulation associated with infarcted myocardium.

It has been well documented that myocardial infarction results in regional sympathetic denervation, and the interruption of sympathetic nerves at the site of infarction results in heterogeneous denervation of myocardium apical to the coronary occlusion (4). In addition to the loss of both afferent and efferent sympathetic nerve responses (4), denervated myocardium becomes hypersensitive to exogenous catecholamines (26, 42). For example, Barber et al. (4) have shown that a 1–2 wk after transmural infarction in dogs, there was a blunted reduction in the effective refractory period to stellate ganglion stimulation. Nevertheless, in these animals as well as a group subjected to chemical denervation, there was accentuated shortening of effective refractory period after intravenous nor-epinephrine compared with unaffected myocardium (26). Similarly, Vatner et al. (42) showed exaggerated increases in heart rate and LV dP/dt in dogs after cardiac denervation and identified an increase in β-adrenergic density as the mechanism of hypersensitivity.

Although models of infarction have demonstrated heterogeneous responses to sympathetic nerve stimulation ranging from blunted to absent (5, 26), the lack of hypersensitivity to infused catecholamines in pigs with hibernating myocardium clearly distinguishes our results from those of infarcted and denervated myocardium. In our study, the absolute improvement in regional function and global contractility during epinephrine stimulation was reduced compared with controls (Figs. 3 and 4), confirming our previous results using regional wall-thickening (32) and segment shortening (18). Even when the increase in regional function was assessed as a change from the reduced baseline (Fig. 5, bottom), there was no evidence for hypersensitivity in hibernating myocardium, although the magnitude of improvement was similar to controls. Our results were not confounded by averaging of functional responses, because we have previously shown that during graded epinephrine infusion there was no biphasic response (improved function at low levels of stimulation but functional deterioration at higher levels) (18, 32). Thus our data support a generalized downregulation in the responses to both pre- and postjunctional stimulation that would protect hibernating myocardium from demand-induced ischemia at the expense of contractile reserve during sympathetic stimulation.
Sympathetic nerve dysfunction and catecholamine hypersensitivity after acute ischemia. Initial studies of sympathetic nerve function after acute coronary occlusion suggested that sympathetic nerves are especially sensitive to ischemia, with functional responses that were blunted to absent in the setting of stunned myocardium. For example, Ciuffo et al. (14) showed that in stunned myocardium after 25 min of ischemia in dogs, the improvement in regional function in response to stellate stimulation was lost for at least 2 h. However, they noted persistent contractile reserve to intravenous norepinephrine infusion, and the improvement in function as a percentage of the reduced baseline function was much greater than before ischemia, consistent with a hypersensitive response. Gutterman et al. (21) extended these findings to evaluate sympathetic

![Graph showing absolute and relative changes in LV global function during sympathetic stimulation.](http://ajpheart.physiology.org/)

Fig. 6. Absolute and relative changes in LV global function during sympathetic stimulation. Each method of stimulation resulted in a greater absolute increase in peak positive dP/dt (Absolute, top) in the control group (shaded bars) compared with the hibernating group (black bars, \(P = 0.04\)). There were no significant differences between groups when change in positive dP/dt was determined as a percentage of the BL values (Relative, bottom, \(P = 0.18\)). Regardless of the analysis, there was no evidence for hypersensitivity to catecholamine stimulation in hibernating myocardium. All values are means ± SE.

![Graph showing correlation of functional responses in hibernating myocardium.](http://ajpheart.physiology.org/)

Fig. 7. Correlation of functional responses in hibernating myocardium and controls. Peak functional response during stellate ganglion stimulation is plotted against peak response to intravenous epinephrine infusion (shaded squares and dotted lines) and intracoronary tyramine infusion (black diamonds and solid lines). Although function in the hibernating group (right) was reduced compared with controls (left), both groups showed significant correlations between methods of stim (\(P < 0.02\) for all correlations). Parallel relations between the groups of animals support adaptive downregulation to sympathetic stimulation in hibernating myocardium.
responses in coronary resistance vessels. They noted blunted increases in coronary vascular resistance after acute ischemia of 15-min, but not 7-min, duration (21) that was mediated by adenosine (1, 34) and free radicals (33). Similar to the findings of Ciuffo et al. (14), the responses to tyramine or bretylium were preserved (21), suggesting that prejunctional norepinephrine stores were maintained and could be released in the presence of stunned myocardium.

Schulz et al. (39) have concluded that there are no alterations in sympathetic nerve dysfunction in stunned myocardium. After completely reversible ischemia of 15-min duration in dogs, they noted that there was parallel recovery of resting wall thickening and function during stellate stimulation and norepinephrine infusion. During the period of stunned myocardium (up to 4 h postischemia), the absolute change in wall thickening during stellate stimulation was preserved compared with the control group (in contrast to the blunted response illustrated in Fig. 5, top). Nevertheless, stunned myocardium showed a greater improvement in function during catecholamine infusion relative to baseline function compared with control conditions (39) (in contrast to the similar responses in hibernating and control groups in Fig. 5, bottom), suggesting a hypersensitive response. As with denervated myocardium, the mechanism of catecholamine-mediated hypersensitivity in stunned myocardium has been shown to be an increase in \( \beta \)-receptor density (37).

Adaptive alterations in sympathetic responses in chronic ischemic heart disease. Because hibernating myocardium develops in response to progressive reductions in flow reserve (41) and is preceded by a state of chronically stunned myocardium (15), the sympathetic responses in hibernating myocardium would be expected to be similar to those during acute stunning. Similar to the results of Ciuffo et al. (14) but not Schulz et al. (39), the functional responses to sympathetic stimulation in hibernating myocardium were significantly blunted compared with those in the control group. However, in contrast to all of the previous studies in acutely stunned myocardium, hibernating myocardium does not demonstrate hypersensitivity to exogenous catecholamines (Fig. 3, bottom), which is consistent with normal \( \beta \)-receptor density in this model (28). We hypothesize that these differences may be in part related to the relative resistance of hibernating myocardium to develop acute ischemia. Despite a critical reduction in subendocardial flow reserve and progressive reductions in relative flow during submaximal inotropic stimulation, we previously reported no metabolic evidence of acute ischemia in hibernating myocardium (18). In fact, up to a heart rate of \( \sim 150 \) beats/min we previously documented a progressive increase in regional lactate consumption and no lactate production in any animal. In addition, venous pH was identical to that in normal control animals.

The generalized reduction in functional responses to sympathetic nerve stimulation and \( \beta \)-adrenergic agonists in hibernating myocardium is likely explained by postjunctional alterations in the myocyte. We have previously shown that hibernating myocardium is associated with attenuation of \( \beta \)-receptor adenyl cyclase signaling with blunted cAMP production to catecholamines (27) and regional reductions in sarcoplasmic reticulum calcium handling proteins (16). The physiological impact of this latter finding was recently supported by altered calcium transients in isolated myocytes from a similar model (7). The role of regionally reduced sympathetic nerve norepinephrine uptake (30, 31) in effecting blunted sympathetic nerve responses is currently unclear. The simplest interpretation of this finding would be that ischemically mediated damage to sympathetic nerves causes either a partial loss of nerves (i.e., partial denervation) or targeted damage to the norepinephrine uptake mechanism. This could alter junctional norepinephrine concentrations independently of postjunctional alterations. Additional studies will be required to formally test this possibility.

Clinical studies have documented regional reductions in norepinephrine tracer uptake in patients with ischemic heart disease in the absence of infarction (8, 22), analogous to our findings in pigs with hibernating myocardium (30, 31). Thus blunted contractile responses to sympathetic nerve stimulation and \( \beta \)-adrenergic signaling (28) almost certainly occur in patients with chronic coronary disease, and our results may explain the clinical finding of increased mortality associated with the presence of hibernating myocardium (2). As proposed by Zipes and Wells (44), inhomogeneity in sympathetic nerve function would lead to heterogeneity in ventricular repolarization that would be exacerbated during periods of sympathetic activation and predispose to arrhythmic sudden death. Consistent with this hypothesis, our laboratory (11) has recently described that pigs with hibernating myocardium without evidence of infarction have a high rate of arrhythmic sudden death. Furthermore, among patients with chronic coronary artery disease, there is frequent coexistence of hibernating, stunned, and infarcted myocardium. Not only would this produce even more dramatic spatial heterogeneity in both pre- and postjunctional sympathetic nerve function, but infarction has been shown to induce sympathetic nerve sprouting (12), which might further predispose to sudden death (13).

Methodological limitations. Although coronary artery manipulation has been associated with the interruption of sympathetic nerves in some animal models (24), we are confident that this did not occur in our model and did not confound our findings. First, our control group included a series of pigs with identical instrumentation but normal resting wall motion, and sympathetic nerve stimulation resulted in similar improvement in function compared with uninstrumented controls. Second, we have previously documented that both acute and chronic dissection of the LAD have no effect on sympathetic nerve norepinephrine-tracer uptake in pigs (30, 31). We have also excluded a confounding role of myocardial infarction with TTC staining and have previously shown that in this model there is only a 2% regional increase in connective tissue (17, 19). However, it is interesting to note that the functional responses in the animal with subendocardial necrosis were similar to animals with hibernating myocardium.

Some open-chest animal preparations have been associated with an increase in circulating catecholamines that could not only alter baseline cardiac hemodynamics and function but also may impair the ability to detect increases in function associated with sympathetic stimulation. This might also be exacerbated by the medications necessary to provide adequate anesthesia. However, for several reasons we are confident that such effects did not significantly confound our results. First, the baseline hemodynamics in the open-chest anesthetized state in this study were similar to basal parameters in telemetrically monitored animals in our laboratory (heart rate, \( \sim 97 \pm 14 \) beats/
min; systolic pressure, \(\sim 108 \pm 12 \text{ mmHg}\)). Second, the magnitude of improvement in both regional and global function during stellate stimulation is similar to those observed by other investigators (14, 39). Finally, the anesthetic regimen used in this study was specifically chosen to minimize potential effects on sympathetic tone, with continuous infusions of low to moderate doses of propofol and ketamine. At high doses, propofol tends to produce central sympathetic depression, although at low to moderate doses, baroreflexes have been shown to remain intact (43). On the other hand, ketamine has a tendency to produce sympathomimetic effects. Nevertheless, recent studies in autonomically intact animals show that changes in hemodynamic parameters are minimal (only a small increase in heart rate) and that there were no effects on peripheral sympathetic nerve activity, presumably due to a balanced suppression of the central sympathetic nervous system (36). Most importantly, because we observed blunted responses to both pre- and postjunctional stimulation in animals with hibernating myocardium and both groups received the same anesthetic regimen, we are confident that anesthesia did not significantly alter our results. Nevertheless, we cannot exclude the possibility that there may be quantitative differences in functional responses under different anesthesia or in a closed-chest preparation.

In the setting of severe coronary artery disease, increases in oxygen demand, as would occur with catecholamine or sympathetic stimulation, could induce acute myocardial ischemia. This possibility cannot be absolutely excluded as the mechanism for blunted functional responses in hibernating myocardium; however, we have previously shown that a similar level of catecholamine stimulation produces no metabolic evidence for ischemia in this model (18). Although \(\alpha\)-adrenergic constriction during sympathetic nerve stimulation does produce no metabolic evidence in this model (18). Although \(\alpha\)-adrenergic vasoconstriction is minimal in the porcine coronary circulation (20, 40). This is consonant with in vitro studies that have shown minimal constriction of coronary microvessels by \(\alpha\)-agonists (35).

Because stellate ganglion isolation was performed without interruption of the spinal projections, it is possible that reflex responses due to afferent stimulation may have occurred. Nevertheless, this cannot explain the differences between groups because the same isolation protocol was used in all experiments. Finally, a shift in the frequency-response relationship in hibernating myocardium cannot be excluded because only one frequency of stimulation was performed.

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