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¹¹C-meta-hydroxyephedrine defects persist despite functional improvement in hibernating myocardium

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Abstract

Background—Regional cardiac sympathetic nerve dysfunction develops in hibernating myocardium and may play a role in its association with sudden cardiac death. Interventions to improve cardiac function (i.e., revascularization) improve survival, but the potential reversibility of sympathetic nerve dysfunction remains unclear.

Methods and Results—Pigs ($n = 11$) were chronically instrumented with a proximal left anterior descending coronary artery (LAD) stenosis to produce hibernating myocardium. Prior to therapeutic interventions, there was LAD occlusion with collateral-dependent myocardium, reduced regional function (echocardiographic LAD wall-thickening $23\% \pm 4\%$ vs $83\% \pm 6\%$ in Remote, $P < .001$), and large defects in ¹¹C-meta-hydroxyephedrine (HED) PET ($48\% \pm 4\%$ of LV area, $26\% \pm 2\%$ integrated reduction). Successful PCI or pravastatin therapy improved regional (LAD wall-thickening $23\% \pm 4\%$ to $42\% \pm 6\%$, $P < .05$) and global LV function (fractional shortening $24\% \pm 2\%$ to $31\% \pm 2\%$, $P < .01$), but did not alter regional HED uptake, retention, defect size, or defect severity.

Conclusions—Despite significant functional improvement of hibernating myocardium as a result of PCI or pravastatin therapy, there were no changes in HED defect size or severity. Thus, inhomogeneity in myocardial sympathetic innervation persisted, and the lack of plasticity suggests that even in the absence of significant infarction, structural rather than functional defects are responsible for reduced myocardial norepinephrine uptake in chronic ischemic heart disease. (*J Nucl Cardiol* 2009)

Keywords

Hibernating myocardium; isotopes; sympathetic nervous system

Introduction

Repetitive episodes of ischemia as a consequence of chronic coronary artery disease can lead to the development of viable, chronically dysfunctional myocardium (chronically stunned and hibernating myocardium).^{1,2} This physiology is associated with an increased risk of cardiovascular death³ that some data suggest is primarily related to lethal ventricular arrhythmias.^{4,5} Chronically dysfunctional myocardium also develops inhomogeneity in myocardial sympathetic innervation,⁶⁻⁸ which has been considered a potential arrhythmogenic substrate for sudden cardiac death.^{9,10}

Myocardial infarction and cardiac transplantation result in a loss of sympathetic nerves (denervation) that has only limited capability for reinnervation. Since regional norepinephrine uptake is only moderately reduced in hibernating myocardium^{6,7} as compared to infarction^{11,12} or cardiac transplantation,^{13,14} we have hypothesized that the partially dysinnervated myocardium may reflect a functional abnormality of the nerves rather than denervation.⁸ Although previous studies of patients with chronic coronary artery disease have shown very limited potential for improvement in regional dysinnervation,^{11,15,16} this could have been the result of denervation associated with unrecognized infarction. In contrast, we hypothesized that the sympathetic dysinnervation associated with the reversible ischemia in hibernating myocardium^{6,7} may result from a functional, ischemically mediated inhibition of the norepinephrine reuptake mechanism. We further hypothesized that resolution of regional dysfunction in hibernating myocardium would be predicated on improvement in regional ischemia, which would allow recovery of any ischemically mediated sympathetic nerve stunning over a similar time course. This would be consistent with previous studies that have demonstrated sympathetic nerve stunning after brief reversible ischemia due to their exquisite sensitivity to limitations in oxygen delivery.¹⁷

Thus, we undertook the present investigation to examine whether improvements in regional function in hibernating myocardium were accompanied by improvement in sympathetic nerve function *in vivo*.^{6,7} Studies used a clinically relevant animal model of chronic myocardial ischemia where infarction is minimal and could be accurately quantified post-mortem.^{1,5} Furthermore, the extent and severity of defects in ¹¹C-meta-hydroxyephedrine (HED) uptake were quantitatively assessed in conjunction with therapeutic interventions known to improve regional function in this model, namely the administration of pravastatin¹⁸ and revascularization with direct percutaneous coronary intervention (PCI).^{19,20} The results demonstrate dissociation between improved regional function and persistent sympathetic nerve dysfunction. This supports a role of structural loss of sympathetic nerves in response to reversible ischemia.

Materials and Methods

All experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research, and were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

Chronic Instrumentation and Therapeutic Interventions

Studies were conducted in farm-bred pigs that were chronically instrumented to produce hibernating myocardium. The initial instrumentation and experimental protocol have been previously published in detail.^{1,6,7} Briefly, juvenile pigs (7.7 ± 0.5 kg, $n = 11$) were instrumented with a 1.5-mm Delrin or Silastic^{19,20} stenosis on the proximal left anterior descending coronary artery (LAD). Initial studies were performed ~3 months after this instrumentation (94 ± 2 days), a time-point at which our previous studies have documented the development of viable dysfunctional myocardium, consistent with hibernating myocardium.¹

Once hibernating myocardium had developed, baseline functional studies (transthoracic echocardiography and PET) were performed. We then assessed whether therapeutic interventions previously demonstrated to improve regional function could improve myocardial sympathetic innervation assessed using HED (imaging protocol outlined below). Eight pigs received the HMG-CoA reductase inhibitor pravastatin (160 mg/day orally) which we have previously demonstrated to improve regional function in hibernating myocardium.¹⁸ Of these animals, one experienced sudden death prior to repeat echocardiography or PET imaging, and two underwent studies only after therapy. Four animals (including one that was also treated with pravastatin) were studied after percutaneous revascularization of the LAD. In these animals, total LAD occlusion and collateral-dependent myocardium were present at the initial study. The occlusion was crossed with a wire and the Silastic occluder was expanded using a standard balloon angioplasty catheter. Since previous studies demonstrated frequent recoil, patency was maintained with insertion of a bare metal coronary stent and treatment with aspirin (325 mg/day orally) and clopidogrel (300 mg then 75 mg/day orally).^{19,20} Even with this regimen one of the four animals developed reocclusion, and since it was not successfully revascularized the results are presented separately.

After the imaging studies were completed, the animals were euthanized. The heart was sectioned into concentric rings and triphenyltetrazolium chloride staining was used to quantify the extent of fibrosis.^{6,7} Myocardial fibrosis averaged $2.8\% \pm 0.8\%$ of the left ventricle (LV) and was more evident among the animals that underwent PCI ($4.2\% \pm 1.9\%$) than pravastatin therapy ($1.6\% \pm 0.5\%$, $P = .15$).

Positron Emission Tomography

Imaging of cardiac sympathetic nerve function was performed with HED and positron emission tomography (PET) in pigs with hibernating myocardium before ($n = 8$) and 4 weeks (26 ± 2 days, $n = 10$) after therapeutic intervention. We have previously shown that there are no spontaneous changes in regional HED uptake in pigs with hibernating myocardium over this time period.⁷ Pigs were sedated with a mixture of Telazol (tiletamine 50 mg/mL and zolazepam 50 mg/mL) and xylazine (100 mg/mL) (0.022 mL/kg IM), with supplemental doses (0.011 mL/kg IM) administered as needed. Heart rate and blood pressure were noninvasively monitored and supplemental oxygen (2 L/min by nasal cannula) was given to maintain oxygen saturations $>92\%$ while the animal was sedated.

PET imaging was performed on a CTI ECAT EXACT HR+ PET scanner with a 15.5-cm axial field-of-view and a resolution of $\sim 5.4 \text{ mm}^3$ full-width-at-half-maximum at 10 cm.^{7,10} Sympathetic nerve norepinephrine uptake was assessed with 185-370 MBq (5-10 mCi) HED.^{7,21} HED was produced by the direct methylation of 1 mg metaraminol free base in 250 μL dimethylformamide with ^{11}C -methyl iodide, followed by heating at 100°C for 5 minutes and semi-preparative HPLC purification.²² The HED was transported directly from the University at Buffalo 30 MeV Cyclotron Facility to the PET suite via a dedicated pneumatic tube system. In conjunction with intravenous tracer injection, dynamic imaging was performed with the following frame rate: 6×30 seconds, 2×60 seconds, 2×150 seconds, 2×300 seconds, 2×600 seconds for a total of 14 frames over 40 minutes. Attenuation correction of all emission data was performed with a 15-minute transmission scan using a ^{68}Ge rod source.

PET images were reconstructed with a zoom of 2, a Hann filter with cutoff frequency of 0.3 cycles/pixel, and corrected for radionuclide decay. A summed HED image from 15 to 40 minutes post-injection was used to define the three-dimensional shape of the LV with combined cylindrical and hemispherical (bottle-brush) sampling.²³ Dynamic polar maps containing time-activity curves for 496 mid-myocardial sectors were obtained, as previously described.^{7,24} An arterial blood time-activity curve was obtained from an 18 mm^3 cylindrical region of interest

automatically centered in the LV and left atrial cavities.²⁴ One “before” therapy PET scan in a statin-treated animal was uninterpretable due to motion artifact.

Regional tracer activity was assessed using a 17-segment model.²⁵ The mid-antero-septal, mid-anterior, apical-anterior, and apical-septal segments were assigned to the LAD perfusion territory; and the mid-inferior, mid-inferolateral, basal-inferior, and basal-inferolateral segments were assigned to the normally perfused remote myocardium. Regional HED uptake was expressed as a percent of maximal region uptake on a per animal basis, with normal uptake defined as relative HED activity $\geq 75\%$.¹¹ HED was also quantified as retention (segment activity $\div$ integrated arterial activity, in min^{-1}) between 10 and 40 minutes after injection, determined for each polar map sector.^{7,26} Defect size was quantified both as a percentage of the LV surface area with reduced HED uptake and as an integrated volume of myocardium with reduced HED uptake (defect severity, $100 - \text{mean HED uptake as a \% of the maximum region}$).²⁷ In order to account for the increase in heart size between the two studies due to animal growth, defect size was also expressed in grams of myocardium using LV mass estimated by echocardiography.

Transthoracic Echocardiography

Myocardial function was assessed with transthoracic echocardiography in all animals within 1 week of PET imaging (3.6 ± 0.5 days). Images were obtained through a right parasternal window with the sedated animal lying on their left side.⁷ From mid-ventricular short axis images, anatomic M-mode (GE Vivid 7) was used to quantify wall thickness in the LAD-perfused antero-septum and the normally perfused posterolateral wall. Regional function was quantified as regional percent wall-thickening [$100 \times (\text{end-systolic wall thickness} - \text{end-diastolic wall thickness}) / \text{end-diastolic wall thickness}$]. Fractional shortening [$100 \times (\text{end-diastolic LV diameter} - \text{end-systolic LV diameter}) / \text{end-diastolic LV diameter}$] was used to assess global function. LV mass was calculated as recommended by the American Society of Echocardiography²⁸:

$$\text{LV mass} = 0.8 \times \left\{ \left[1.04 (\text{end} - \text{diastolic LV diameter} + \text{anteroseptum diastolic wall thickness} + \text{posterolateral wall diastolic wall thickness})^3 - (\text{end} - \text{diastolic LV diameter})^3 \right] \right\} + 0.6 \text{ g.}$$

Data Analysis

This study was planned as an unpaired analysis of HED imaging before and after therapy in anticipation of scheduling difficulties related to: the 3-month delay between initial instrumentation and therapy, potential problems with animal transportation to the VA, the use of a clinical PET scanner, and the high rate of spontaneous sudden death in this model.⁵ However, since a majority of animals had studies both before and after therapeutic intervention, a paired analysis was also performed.

Data are presented as mean $\pm$ SEM, with the $P < .05$ level considered statistically significant. HED parameters (defect size, defect severity, regional uptake, and regional retention) were compared between groups using a two-way analysis of variance to account for study (before therapy vs after therapy) and group (statin vs PCI). Comparisons between the LAD region and the normal remote region were assessed using paired t -tests, and differences between studies performed before and after therapeutic intervention were compared with both paired and unpaired t -tests (Sigma Stat 3.2).

Results

Effect of Therapeutic Interventions on HED PET

Prior to therapeutic interventions, all animals were in good health with normal hemodynamic parameters: Systolic pressure = 117 ± 6 mmHg, diastolic pressure = 76 ± 5 mmHg, and heart rate = 92 ± 2 bpm. Representative HED PET tomograms are shown in Figure 1. Consistent with our previous studies,^{6,7} there were regional defects in norepinephrine tracer uptake in all animals. Defect size averaged $48\% \pm 4\%$ of the surface area of the LV, with an integrated HED defect severity of $26\% \pm 2\%$ of the LV. With an average LV mass of 134 ± 7 g, HED defect size was 65 ± 8 g with an integrated defect severity of 35 ± 4 g (Figure 2). HED uptake in the LAD region was significantly reduced in comparison to remote myocardium ($63\% \pm 4\%$ vs $94\% \pm 1\%$ of maximum, $P < .001$; Figure 3), with a similar regional reduction in HED retention (0.15 ± 0.01 per minute in the LAD region vs 0.22 ± 0.01 per minute in Remote, $P < .001$; Figure 3).

All animals were in good health at the time of study after therapeutic interventions. There were no significant changes in hemodynamic parameters from the initial study: Systolic pressure = 118 ± 4 mmHg, diastolic pressure = 79 ± 4 mmHg, and heart rate = 99 ± 3 bpm. There was a similar response to therapy between the Statin and PCI-treated animals for each HED parameter (defect size, ANOVA $P = .46$; defect severity, ANOVA $P = .82$; regional uptake, ANOVA $P = .66$; and regional retention, ANOVA $P = .79$). Since both interventions have been shown to improve regional function in pigs with hibernating myocardium,¹⁸⁻²⁰ the two groups of animals were combined for all subsequent analyses.

Despite the significant improvement in regional and global LV function (detailed below), there was no improvement in any HED parameter. There was no change in HED defect size whether it was quantified as a surface area (65 ± 8 g to 78 ± 12 g, $P = .39$) or as an integrated deficit in HED uptake (35 ± 4 g to 42 ± 6 g, $P = .36$; Figure 2). Similar to the initial studies, there was a persistent regional reduction in HED uptake ($66\% \pm 3\%$ in the LAD region vs $93\% \pm 1\%$ of maximum in Remote, $P < .001$) and HED retention (0.15 ± 0.01 per minute in the LAD region vs 0.20 ± 0.01 per minute in Remote, $P < .001$), with no significant changes in either region as compared to the initial study (Figure 3). One animal was excluded and analyzed separately due to reocclusion after coronary PCI. PET imaging in this animal revealed a large increase in both the estimated size (65 - 141 g) and severity (32 - 72 g) of the HED defect (Figure 4).

A paired analysis was also performed for animals that underwent PET imaging both before and after therapeutic intervention. In these six animals, the estimates for both HED defect size (65 ± 10 g to 67 ± 10 g, $P = .89$) and defect severity (35 ± 6 g to 36 ± 3 g, $P = .36$) were nearly identical (Figure 2). HED defect size improved in three animals (two statin-treated and one PCI-treated) and defect severity improved in four (two in each group). There was no significant difference between the statin and PCI groups with regard to the change in defect size ($P = .16$) or defect severity ($P = .49$). Furthermore, these results were not influenced by myocardial fibrosis as there was no significant correlation with either the change in defect size ($r = -0.54$, $P = .21$) or defect severity ($r = -0.51$, $P = .24$).

Effect of Therapeutic Interventions on Left Ventricular Function

Parameters of regional and global LV function are illustrated in Figure 5, and the corresponding measurements are included in Table 1. Consistent with our previous studies,^{1,7} chronically instrumented animals developed regional dysfunction in the LAD region as compared to the remote, normally perfused myocardium (% wall-thickening of $23\% \pm 4\%$ vs $83\% \pm 6\%$, $P < .001$) with very mild impairment in global LV function (fractional shortening of $24\% \pm 2\%$, estimated ejection fraction of $56\% \pm 3\%$). After therapeutic intervention, regional function in

the LAD region was significantly improved as compared to the initial study (% wall-thickening from $23\% \pm 4\%$ to $42\% \pm 6\%$, $P < .05$; Figure 5) but remained abnormal as compared to remote, normally perfused region ($85\% \pm 9\%$ in Remote, $P < .001$ vs LAD). This was accompanied by a significant improvement in global LV function (fractional shortening $24\% \pm 2\%$ to $31\% \pm 2\%$, $P < .01$, Figure 5; estimated ejection fraction $56\% \pm 3\%$ to $67\% \pm 2\%$, $P < .01$). There were no differences in the degree of regional ($P = .80$) or global function ($P = .32$) improvement between the statin and PCI groups, but pathological fibrosis was modestly correlated with the improvement in regional ($r = -0.73$, $P = .07$) but not global function ($r = -0.057$, $P = .90$).

Discussion

The present investigation in pigs with hibernating myocardium provides novel and clinically relevant information on the short-term persistence of HED defects in ischemically mediated sympathetic nerve dysfunction following common therapeutic interventions employed in patients with coronary artery disease. Despite the fact that regional and global LV function improved with therapy, we found no significant change in HED defect size or severity. Thus, these results show that at least in the short term, functional improvement of chronically ischemic myocardium is dissociated from improvement in regional defects in sympathetic nerve norepinephrine uptake.

Sympathetic Nerve Dysfunction Associated with Myocardial Ischemia

In addition to the association of regional sympathetic denervation (loss of sympathetic nerves) with myocardial infarction,^{11,12} sympathetic nerve dysfunction can also develop as a result of reversible myocardial ischemia.^{6,7,29,30} We and others have used the term “dysinnervation” in association with viable myocardium with reduced norepinephrine tracer uptake to reflect the lack of data regarding the status of sympathetic nerves in this setting.^{6,7,31} Although this physiology may reflect partial sympathetic denervation,³² a functional abnormality of the sympathetic nerves (e.g., neural stunning, reduced norepinephrine uptake protein activity, etc.) has not been excluded. In this regard, we have previously shown that the uptake of the guanethidine analog ¹³¹I-meta-iodo-benzylguanidine (MIBG) was regionally reduced in pigs with hibernating myocardium.⁶ Reductions in MIBG were most pronounced in the subendocardium, consistent with an ischemic mechanism of damage.⁶ We have also documented that PET imaging with the more specific norepinephrine tracer HED improves the discrimination between normal and dysinnervated myocardium,⁷ consistent with improved selectivity of HED for the norepinephrine uptake protein.³³ Since the regional abnormality in sympathetic nerve function may underlie the high rate of spontaneous, arrhythmic sudden death in this model,⁵ we and others have hypothesized that HED imaging may become a clinically important tool to assess sudden death risk.¹⁰

Our findings in pigs with hibernating myocardium are consistent with studies of patients with coronary artery disease. For example, MIBG defects have also been demonstrated in small studies of patients with CAD and no clinical history of infarction.^{29,30} Sympathetic dysinnervation was identified in regions with normal resting perfusion supporting the presence of viable myocardium, and the occurrence of defects was correlated with stenosis severity, angina and inducible ischemia. Furthermore, alterations in sympathetic nerve function frequently extend beyond the area of flow reduction in non-Q-wave infarction,¹¹ and more closely correspond to the area at risk of ischemia.^{34,35}

Reversibility of Myocardial Sympathetic Nerve Dysfunction

The majority of studies that have investigated the potential for improvement in the imaging of sympathetic nerve function have evaluated patients after myocardial infarction^{11,12,36} or cardiac transplantation.^{13,14} Within the first year after myocardial infarction, there is little³⁶

or no^{11,12} improvement in sympathetic nerve function. For example, Hartikainen et al showed that between 3 and 12 months after Q-wave infarction, MIBG uptake remained unchanged.¹² Similar results were reported by Allman et al using HED and PET in patients with myocardial infarction.¹¹ They found no change in HED defect size between 7 days and 8 months after infarction ($31\% \pm 11\%$ to $34\% \pm 15\%$ of the LV, $P = \text{ns}$), even though four of the eight patients with follow-up studies did not develop Q-waves.¹¹ In contrast to these studies, Fallen et al detected a modest 15% improvement in ¹⁸F-fluorodopamine uptake between ~2 weeks and 3 months after infarction, with no further improvement after an additional 3 months.³⁶

Cardiac transplants are surgically denervated and provide a clinical approach to study sympathetic reinnervation in humans. Tissue norepinephrine levels in the surgically denervated heart are very low and concordant with nearly absent left ventricular norepinephrine tracer uptake within the first 12-18 months following cardiac transplantation.^{13,14} When evaluated at later time points, partial reinnervation is present in most patients, but this is highly heterogeneous and primarily limited to the basal portions of the anterior wall.^{13,14}

Although both basic and clinical studies have documented abnormal sympathetic nerve function in viable myocardium (as discussed above), there has been little prior investigation on whether these changes are reversible. Clinical studies have produced inconsistent results,^{11,15,16} and may have been confounded by coexistent but unrecognized subendocardial infarction. Furthermore, function was not consistently assessed in order to determine baseline as well as temporal improvement or deterioration in regional function in a setting where recurrent ischemia and myocardial stunning could occur in the absence of symptoms. Nevertheless, in conjunction with their study of HED uptake after myocardial infarction, Allman et al noted that there was also no improvement in HED uptake in the peri-infarct region (defined as the area with initially reduced HED but normal myocardial perfusion).¹¹ Although these patients had all received reperfusion therapy with thrombolysis, revascularization was only performed in two of the eight patients and recurrent ischemia from a residual stenosis could not be excluded as a cause for the persistent HED defects. A similar result was obtained by Fricke et al in 23 patients with coronary artery disease treated with spinal cord stimulation.¹⁵ Thoracic spinal stimulation produced no improvement in segmental HED retention over 1 year and this was true even after the analysis excluded segments with scar ($<70\%$ FDG uptake).¹⁵ Interestingly, in a post-hoc analysis evaluating viable segments with a mild reduction in coronary flow reserve there was a small (4%) but significant increase in HED retention.¹⁵ Additional support for the potential reversibility of defects in HED uptake in viable, dysinnervated myocardium was reported by Guertner et al in a series of patients with MIBG imaging performed before and after percutaneous transluminal coronary angioplasty.¹⁶ MIBG defects were noted to be qualitatively improved in five of eight patients with $\leq 50\%$ stenoses on repeat angiography.¹⁶ In contrast, three of four patients with restenosis showed an increase in MIBG defect size.¹⁶

Our study extends this previous clinical work by demonstrating that reductions in HED uptake are dissociated from improvements in function with or without revascularization. We confirmed this result with four different methods of quantifying HED distribution (defect size, defect severity, segmental uptake, and segmental retention). Furthermore, the use of a well-characterized animal model of chronic hibernating myocardium permitted accurate quantification of associated myocardial fibrosis. Collectively, our results support the contention that there is little if any short-term potential for improvement in sympathetic nerve dysfunction associated with viable myocardium. However, our results very clearly show that functional improvement in viable, chronically dysfunctional myocardium can be dissociated from improvement in regional sympathetic nerve function. Thus, improvement in regional sympathetic nerve dysfunction is not simply due to the effects of neural stunning from ischemia.¹⁸⁻²⁰ This observation is consistent with our previous finding of partial sympathetic

denervation in hibernating myocardium,³² for which new nerve growth would be required for improvement.

It is certainly possible that there were different mechanisms of functional improvement with pravastatin therapy and PCI. We have previously shown that the functional improvement in hibernating myocardium with pravastatin therapy is not accompanied by an increase in resting or vasodilated flow.¹⁸ A critical limitation in subendocardial flow reserve persisted despite therapy, and thus there continued to be a risk of myocardial ischemia with increases in oxygen demand. Although recurrent stunning could be implicated in the residual dysfunction associated with pravastatin therapy, this could not explain the similar degree of persistent dysfunction in the PCI-treated animals in which follow-up angiography confirmed coronary patency. The similar degree of functional improvement in the two groups would suggest a common mechanism for the residual dysfunction exclusive of recurrent ischemia and stunning (discussed in more detail below). Furthermore, a similar argument would suggest a common mechanism for the persistence of the HED defects, and exclude ischemic sympathetic nerve dysfunction as the cause of reduced HED uptake.

Methodological Limitations

In this study, HED imaging was performed within 1 month of therapy; therefore, a longer time-course of improvement in sympathetic nerve function cannot be excluded. Nevertheless, the therapy and time period were sufficient for significant improvement in regional and global LV function (Figure 5). Thus, our results dissociate the time-course of improvement in function from any improvement in sympathetic nerve dysfunction. Although regional LV function in this study did not normalize after the therapeutic interventions, this is consistent with the time-course and incomplete functional recovery of hibernating myocardium in patients with ischemic cardiomyopathy after surgical revascularization.^{37,38} For example, Haas et al have shown that regional function had improved in 43 of 57 segments (75%) with hibernating myocardium within 11 days, but wall motion had normalized in only 2 (3%).³⁷ Even after a mean of 14 months, only 10 of the segments with hibernating myocardium (18%) had normal wall motion.³⁷ Similarly, Bax et al have shown that 3 months after revascularization only 21% of segments (13 of 62) with hibernating myocardium had recovered normal function, although this increased to 58% after 14 months.³⁸ Although unrecognized infarction may play a role in this delayed recovery and residual dysfunction,^{39,40} the limited fibrosis in our porcine model suggests a greater role for cellular dedifferentiation that has been recognized in both patients^{41,42} and pigs with hibernating myocardium.^{43,44} Furthermore, we have shown that there is persistent cellular hypertrophy with down-regulation of proteins involved with oxidative metabolism and electron transport for up to 1 month after coronary revascularization.

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Due to the inherent variability in the size and severity of sympathetic nerve dysfunction, it is impossible to exclude the possibility that the therapeutic interventions resulted in a small improvement in HED uptake. In fact, four of six animals with serial studies showed some degree of improvement. However, the quantitative difference in HED uptake between the two studies was very small and directionally discordant (1.8 g increase in HED defect size, and 0.7 g increase in defect severity), and was very clearly of less magnitude than the improvement in regional LV function. Additional studies will be required to determine the potential for delayed improvement in sympathetic nerve function.

Conclusion

Despite functional improvement of hibernating myocardium as a result of pravastatin therapy or coronary revascularization, the size and severity of defects in regional sympathetic nerve function as assessed with HED and PET remained unchanged. Thus, there was no short-term

improvement in the ischemically mediated sympathetic dyssinnervation associated with viable chronically dysfunctional myocardium, and there was dissociation from the time-course of improvement in regional function. This finding is therefore consistent with partial sympathetic denervation in hibernating myocardium rather than a component of neural stunning that would be ameliorated with the resolution of ischemia. Further studies will be necessary to evaluate the potential for delayed improvement in sympathetic nerve function in viable, chronically dysfunctional myocardium that would likely require new nerve growth to replace nerves lost as a result of reversible myocardial ischemia.

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Figure 1.

Representative tomograms from HED PET imaging before and after therapeutic intervention. Each pair of images is from an animal serially imaged before and after therapy with either pravastatin (**A**) or percutaneous coronary intervention (PCI, **B**). The polar tomograms (*left images*) represent a series of short axis slices from the apex of the left ventricle (*center* of each image) to the base (*outer periphery* of each image). Representative reconstructed short axis (SA), vertical long axis (VLA), and horizontal long axis (HLA) views are also shown. HED uptake is color coded from the maximum segment in each scan (*red*) to no activity (*dark blue*). The size and severity of the HED defects remained remarkably stable between the two studies. S, Interventricular septum; L, lateral wall; I, inferior wall.

Figure 2.

HED defect size and severity before and after therapeutic intervention. These graphs illustrate the quantification of defect size (grams of LV myocardium from the surface area with HED uptake <75% maximum, **A**) and severity (grams of LV myocardium from the integrated volume with reduced HED uptake, **B**) from each image obtained before or after therapeutic intervention. The individual points for animals treated with pravastatin are identified by *black diamonds*, and PCI-treated animals are shown as *white diamonds*. Animals with studies both before and after therapeutic interventions are connected by a *line*. The graphs to the right illustrate the mean results before (*white bars*) and after (*black bars*) therapy for both unpaired and paired comparisons. Despite improvement in regional function after therapeutic

interventions, there were no significant changes in HED defect size (unpaired $P = .39$, paired $P = .89$) or defect severity (unpaired $P = .36$, paired $P = .90$).

Figure 3.

Regional changes in HED uptake and retention. As compared to the remote, normally perfused myocardium, both HED uptake (*left graph*) and retention (*right graph*) were reduced by ~30% in the LAD region. The regional differences prior to therapeutic intervention (*white bars*) persisted when imaging was repeated after interventions (*black bars*), with no significant changes in regional HED uptake or retention between the two studies. Values from the LAD region were the average of the mid-anteroseptal, mid-anterior, apical-anterior, and apical-septal segments, while the remote region consisted of the mid-inferior, mid-inferolateral, basal-inferior, and basal-inferolateral segments.

Figure 4.

HED PET imaging with reocclusion after coronary PCI. The initial defect size and severity of the animal that developed reocclusion after coronary PCI was similar to the other animals [*upper images*, defect size of 47% (65 g) and defect severity of 23% (32 g)]. The polar tomogram is shown on the *left*, with representative short axis (SA), vertical long axis (VLA), and horizontal long axis (HLA) views on the *right*. Reocclusion of the LAD resulted in a large increase in the HED defect [*lower images*, defect size of 77% (141 g) and defect severity of 39% (72 g)]. *S*, Interventricular septum; *L*, lateral wall; *I*, inferior wall.

Figure 5.

Echocardiographic assessment of left ventricular function before and after therapeutic intervention. Prior to any therapeutic intervention (before therapy), pigs chronically instrumented with an LAD stenosis developed regional myocardial dysfunction in comparison to the remote, normally perfused myocardium (% wall-thickening of $23\% \pm 4\%$ vs $83\% \pm 6\%$, $P < .001$; *left graph*). After therapy there was significant improvement in both regional ($23\% \pm 4\%$ to $42\% \pm 6\%$, $P < .05$) and global LV function (fractional shortening $24\% \pm 2\%$ to $31\% \pm 2\%$, $P < .01$; estimated ejection fraction $56\% \pm 3\%$ to $67\% \pm 2\%$, $P < .01$; *right graph*). The mean data are shown as *black circles*. The animals treated with pravastatin are identified by *black diamonds*, and PCI-treated animals are shown as *white diamonds*.

Table 1

Echocardiographic parameters of regional and global LV function

	<i>n</i>	LAD region wall thickness			Remote region wall thickness			Left ventricular dimensions		
		Diastolic (mm)	Systolic (mm)	WT (%)	Diastolic (mm)	Systolic (mm)	WT (%)	Diastolic (mm)	Systolic (mm)	FS (%)
Before therapy	8	8.1 ± 0.3 [*]	10.1 ± 0.6 [*]	23 ± 4 [*]	6.5 ± 0.4	11.8 ± 0.5	83 ± 6	49 ± 2	37 ± 2	24 ± 2
After therapy	9	8.3 ± 0.3	12.3 ± 0.5 ^{*,†}	42 ± 6 ^{*,†}	8.4 ± 0.3 [†]	15.3 ± 0.6 [†]	85 ± 9	46 ± 2	31 ± 2	31 ± 2 [†]

WT, Wall thickening; FS, fractional shortening.

^{*} *P* < .05 vs Remote region;[†] *P* < .05 vs before therapy.