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ORIGINAL CONTRIBUTION

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Inotropic reserve and histological appearance of hibernating myocardium in conscious pigs with ameroid-induced coronary stenosis

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Abstract Inotropic reserve, demonstrated with administration of sympathomimetic amines, is characteristic of hibernating myocardium. The goal of this study was to determine whether inotropic reserve was present following chronic coronary artery constriction in the pig, which is one potential model of hibernating myocardium. The effects of isoproterenol were examined in five conscious pigs 21 ± 2.1 days after ameroid implantation on the left circumflex coronary artery on measurements of left ventricular (LV) pressure, LV dP/dt, and regional wall thickening in the ameroid-dependent zone (posterior wall) and contralateral non-ischemic zone (anterior wall). Isoproterenol, 0.1 μg/kg/min, increased LV dP/dt by 96 ± 11 %, heart rate by 43 ± 13 beats/min, and normalized systolic wall thickening, slightly, but not significantly more in the ameroid-dependent zone (+ 1.57 ± 0.31 mm) than in the contralateral non-ischemic zone (+ 1.04 ± 0.31 mm), although the baseline wall thickening was reduced significantly in the ameroid-dependent zone. This occurred at a time when baseline myocardial blood flow was preserved and myocardial perfusion in the ameroid-dependent zone was derived in part from the native coronary circulation and also through collateral channels. Two weeks later histological evidence of lesions characteristic of hibernating myocardium, i.e., myofibrolysis and increased glycogen deposition, were observed. Thus, these histological changes and the confluence of chronically depressed regional function and residual inotropic reserve in the conscious pig with chronic ameroid-induced coronary constriction support this model for further study of hibernating myocardium.

Key words LV function – coronary stenosis – coronary collaterals – myocardial blood flow – stunned myocardium

Introduction

Although there is debate whether chronic hibernating myocardium involves sustained significant reductions in blood flow (6, 9), it is generally agreed that the concept is important clinically, because the chronically depressed function can be restored following revascularization. One of the clinical tests for hibernating myocardium is the demonstration of inotropic reserve as assessed with low dose dobutamine echocardiography (5). The major limitation to studying this feature, as well as mechanisms involved with hibernating myocardium, is a lack of a suitable animal model (7).

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Recently, our laboratory reported the effects of chronic ameroid-induced coronary constriction in the pig (9), a potential model for hibernating myocardium. That model was characterized by a decrease in function in the zone distal to the ameroid constriction, but did not demonstrate a significant decrease in baseline myocardial blood flow (9). In order to determine whether the model can be used to study hibernating myocardium, it would be important to determine if other features of hibernating myocardium were present, e.g., inotropic reserve demonstrated with low dose sympathomimetic amine stimulation. In five of the animals used in that study (9) isoproterenol was administered after coronary constriction had developed, but these data were not reported. Accordingly, the goal of this study was to focus on whether regional myocardial function would demonstrate inotropic reserve following chronic ameroid-induced coronary constriction in swine, by reporting the effects of isoproterenol. These results are not necessarily predictable, since in that model intense sympathetic stimulation induced by excitement results in a decrease in wall thickening, i.e., paradoxical motion (9). The effects of graded doses of isoproterenol were examined on regional function (wall thickness) in the ameroid dependent region and in the contralateral non-ischemic region. The experiments in this report were conducted at 21 ± 2.1 days after ameroid-induced coronary constriction, a time characterized by significant reduction in regional wall thickening distal to the coronary constriction, but when the constricted coronary artery is still patent, and baseline levels of blood flow remain normal (9). A second goal of the present report is to provide quantitative data on the relative coronary blood flow supply from native vs. collateral vessels during progression of ameroid-induced coronary constriction. This was accomplished by examining blood flow to the entire myocardium perfused by the constricted coronary artery before and after temporary, complete coronary artery occlusion, assuming that the blood flow supply during complete coronary artery occlusion represented collateral blood flow. Finally, the hearts from the animals were examined histologically after 4 – 6 wks of ameroid constriction to determine whether the characteristic findings of hibernating myocardium, i.e., myofibrolysis and enhanced glycogen deposition (1) were present.

Methods

Five minipigs (Charles River Inc., Boston, MA), weighing 26.1 ± 2.5 kg, were sedated with telazol (5 mg/kg i.m.) and atropine (0.05 mg/kg i.m.). General anesthesia was induced with sodium thiamylal (10 to 20 mg/kg i.v.). The animals were intubated and maintained with halothane (0.5 to 1.5 vol%). By use of sterile surgical technique, a left thoracotomy was performed at the fifth intercostal space. Tygon catheters (Norton Plastics) were implanted in the descending aorta and in the left atrium for measurement of pressures and radioactive microsphere injection. A solid-state miniature pressure gauge was implanted in the left ventricular (LV) cavity to obtain LV pressure and dP/dt. An ameroid constrictor and a hydraulic occluder were implanted at the origin of the left circumflex coronary artery. Two pairs of ultrasonic crystals were implanted transmurally across the LV free wall, in the anterior and in the posterior regions, for measurement of regional wall thickness. The subendocardial crystal was introduced obliquely, so that the myocardium between the two crystals would not be impaired by injury or fibrosis. Proper alignment of the epicardial and endocardial crystals was achieved during surgical implantation by positioning the crystals to obtain a received signal on the oscilloscope with the greatest amplitude and shortest transit time. It is also important to note that the crystals distal to the ameroid were implanted in the central ischemic zone, as defined by a brief test coronary artery occlusion at the time of operation. The correct placement of the crystals was also confirmed at autopsy. The wires and catheters were externalized between the scapulae, the incision was closed in layers, and the chest was evacuated. Each pig was treated with 1 g cephalothin (Keflin, Lilly) immediately after surgery and for 1 week after surgery. Animals used in the present study were maintained in accordance with the guideline of the Committee on Animals of the Harvard Medical School and the "Guide for the Care and Use of Laboratory Animals" (Department of Health and Human Services publication No. [NIH] 85-23, revised 1985).

As these five pigs were included in a prior study (9), the effects of isoproterenol challenge and histology will be emphasized in this study, with data which were not included in the prior study. The effects of isoproterenol infusion, 0.05 and 0.1 μg/kg/min × 5 min, i.v., were examined in conscious pigs trained to remain quietly in a sling, while continuous measurements of LV global and regional function were recorded. A higher dose, 0.4 μg/kg/min × 5 min, i.v., was also administered to three of the pigs. Experiments were conducted at 21 ± 2.1 days after implantation of ameroid constriction, a time when the ameroid had not totally occluded the coronary artery, but significant collateralization had occurred (Fig. 1). The extent to which coronary perfusion of the myocardium distal to the ameroid was derived from native or collateral channels was assessed by examining blood flow measured with microspheres before and during temporary (2 min) coronary artery occlusion induced by inflating the hy-

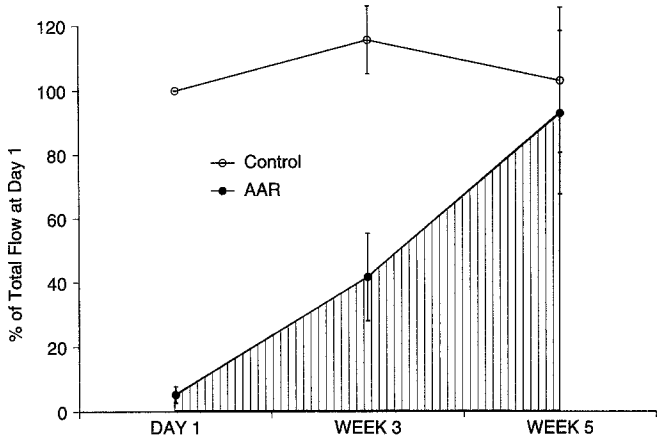

Fig. 1 The distribution of blood flow to the myocardium distal to the ameroid constriction is shown. The top line, connecting open circles, shows the total baseline blood flow at 1 day, 3 weeks, and 5 weeks after ameroid implantation, whereas the line connecting closed circles shows the data with the coronary artery distal to the ameroid temporarily occluded. With the coronary artery occluded all myocardial blood flow must be derived from collateral channels (denoted by the shaded area). At day 1 collateral flow is minimal, whereas at 5 weeks it is entirely responsible for baseline myocardial perfusion in the ameroid-dependent zone. The transmural distribution of blood flow shown at the bottom is in ml/min/g.

draulic occluder at 1 day, 3 weeks, and 5 weeks after ameroid implantation. At the end of the experiment, the heart was perfused *in vitro* to determine the entire myocardial mass perfused by the coronary artery with the ameroid constrictor. The entire area was measured and blood flow in each animal normalized to 100 at baseline on day 1 after the ameroid implantation.

Hemodynamics were recorded continuously on a magnetic tape recorder (Honeywell) and played back on a multiple-channel ink-writting oscillograph (Gould-Brush). Aortic and left atrial pressures were measured with a strain-gauge manometer (Statham Instruments) connected to the respective fluid-filled catheters. The solid-state LV pressure gauge was cross-calibrated against measurements of systolic aortic and left atrial pressures. LV dP/dt was calculated with an operational amplifier connected as a differentiator, which has a frequency response of 700 Hz. Mean arterial pressure was determined by using a resistance-capacitance filter having a 2-s time constant. Regional myocardial function was measured with an ultrasonic transit-time dimension gauge. This instrument measures the transit time of acoustic signals traveling at 1.58×10^6 mm/s between the intramyocardial crystal pairs. The drift of this instrument, although minimal, was effectively compensated for by repeated calibrations. A cardio-tachometer triggered by the LV pressure pulse provided instantaneous and continuous recordings of heart rate.

Regional blood flow was measured by the radioactive microsphere technique. Two to three million microspheres (15 ± 1 μm) labeled with ^{141}Ce, ^{114}In, ^{51}Cr, ^{113}Tin, ^{103}Ru, ^{85}Sr, ^{95}Nb and ^{46}Sc were suspended in a 0.01 % Tween 80 solution (10 % dextran) and placed in an ultrasonic bath for 30 to 60 min. Before the first injection of microspheres, 1 ml of Tween 80 solution was injected to test for potential adverse cardiovascular effects. Microspheres were injected and flushed with saline via the left atrial catheter. Arterial reference blood samples were withdrawn at a rate of 7.75 ml/min for a total of 120 s.

Histological studies were conducted after 4–6 weeks of ameroid constriction. The hearts were removed from the thorax, perfused via the aorta with saline and monastral blue dye to outline the risk region, with the ameroid bed separately perfused with saline at the same pressure. Samples of tissue from both the ameroid supplied bed and the non-ischemic bed were immersion fixed in 10 % phosphate buffered formalin, dehydrated through alcohols, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin, Gomori aldehyde fuchsin trichrome and periodic acid Schiff (PAS). Sections were examined by light microscopy. The remainder of the heart was sectioned for microsphere blood flow analysis.

Data are expressed as means $\pm$ standard error of the mean (SEM). Differences in ameroid and non-ischemic zone changes with time were analyzed with repeated measures ANOVA. Because of the inhomogeneity of the wall thickness data, comparison of two means was performed using the Wilcoxon signed rank test. Statistical significance was accepted at $p < 0.05$.

Results

The experiments were conducted at 21 ± 2.1 days after ameroid implantation. At that time the coronary artery was still patent, but considerable collateral development had occurred, as reflected by the blood flow persisting during temporary complete occlusion of the coronary artery (Fig. 1). Figure 1 compares blood flow to the myocardium perfused by the coronary artery distal to the ameroid before and after temporary, total coronary artery occlusion induced by inflating the hydraulic occluder. Radioactive microspheres were administered under both conditions. At day 1 almost all the blood flow was derived from native vessels, while at 21 ± 2.1 days after ameroid implantation the coronary bed was supplied almost equally by the native vessel and by collateral channels

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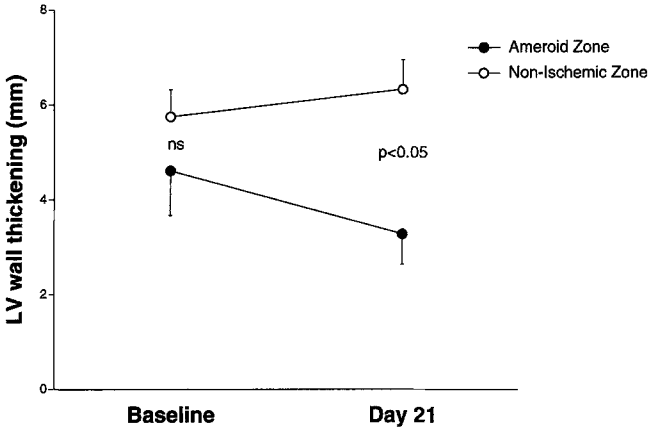

Fig. 2 Left ventricular wall thickening in ameroid-dependent and contralateral non-ischemic zones at baseline (1 day) and 21 days following implantation of the ameroid device. The normalized wall thickening in the posterior, ameroid-dependent zone decreased significantly from 4.61 ± 0.94 to 3.29 ± 0.64 mm from day 1 to 21 following ameroid implantation.

Fig. 3 Phasic waveforms of LV pressure, LV dP/dt, regional wall thickness in the ameroid-dependent zone (posterior wall) and arterial pressure in one conscious pig 21 days after ameroid implantation. Isoproterenol increased wall thickness at the low dose and reduced it at the high dose.

(Fig. 1). Baseline endocardial, epicardial and transmural myocardial blood flows (radioactive microsphere technique) in the ameroid-dependent zone (posterior wall) were assessed at day 1, week 3 and week 5 following ameroid implantation (Fig. 1). These flows did not change during any of these time periods.

Changes in regional left ventricular function with time, as assessed by wall thickening in the anterior (control) and ameroid-dependent regions are shown in Fig. 2. The wall thickening in the posterior, ameroid-dependent zone, normalized to wall thickness in the contralateral control zone, decreased significantly from 4.61 ± 0.94 to 3.29 ± 0.64 mm from day 1 to 21 following ameroid implantation, respectively.

The effects of isoproterenol are shown in Table 1 and Figure 3. Low doses of isoproterenol (0.05 and 0.1 μg/kg/min) increased LV dP/dt and heart rate and wall thickening in both regions. The increase in wall thickening was at least as great in the ameroid-dependent zone. For example, with isoproterenol, 0.1 μg/kg/min, wall thickening in the contralateral non-ischemic zone increased by 1.04 ± 0.31 mm from 5.75 ± 0.57 mm. In the ameroid-dependent zone, wall thickening increased slightly, but not significantly more, by 1.57 ± 0.31 mm from a reduced baseline of 3.29 ± 0.64 mm. With high dose isoproterenol, 0.4 μg/

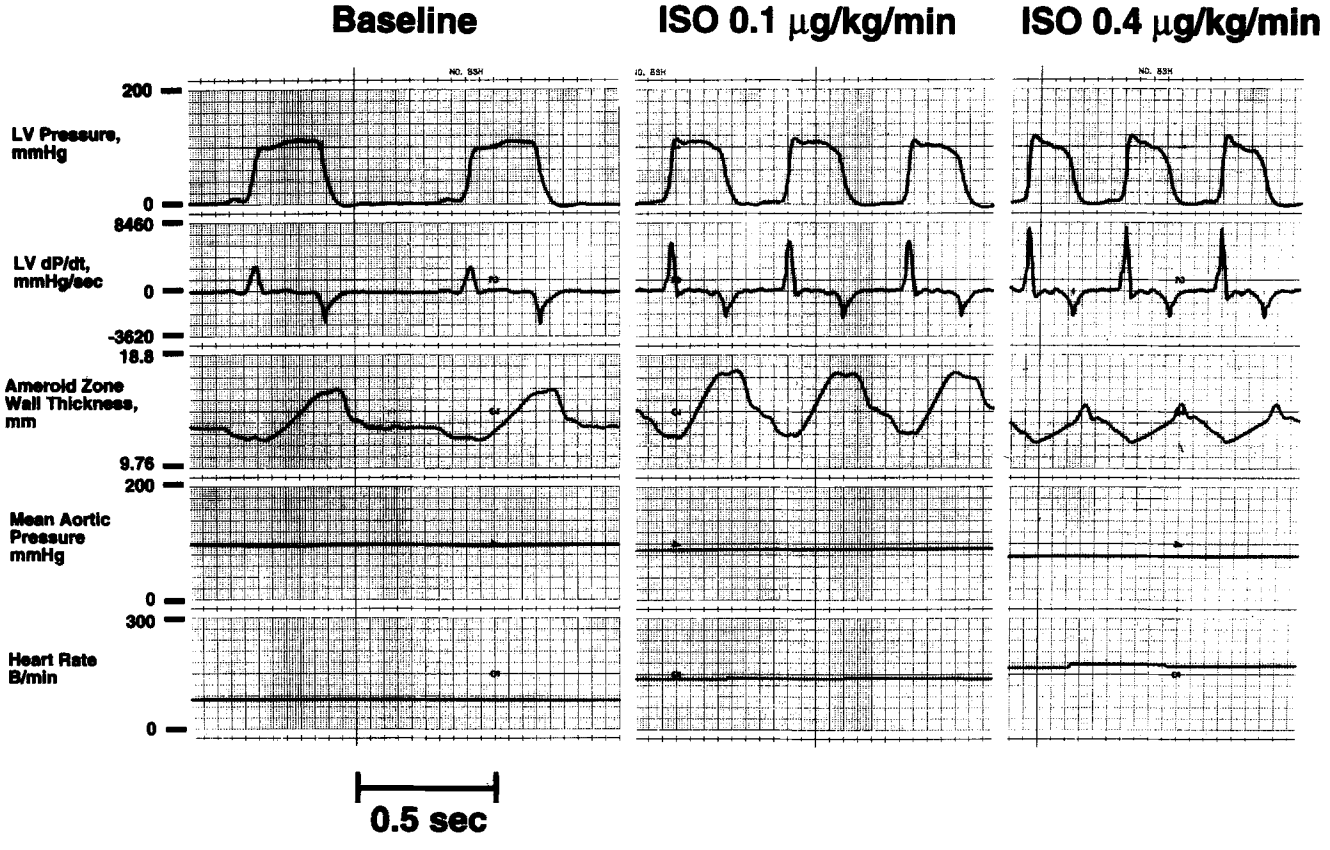

Table 1 Hemodynamic responses to isoproterenol

	Baseline	Change from baseline with isoproterenol 0.05 μg/kg/min	0.1 μg/kg/min
LV pressure (mm Hg)	108±2.6	+8±3.5	+11±3.0*
LV dP/dt (mm Hg)	2385±182	+1216±274*	+2281±269*
Mean arterial pressure (mm Hg)	100±5.8	−3±1.9	−7±2.1*
Heart rate (beats/min)	107±7	+24±9.5	+43±13*
Anterior wall thickening (control) (mm)	5.75±0.57	+0.73±0.17*	+1.04±0.31*
Posterior wall thickening (ameroid) (mm)	3.29±0.64	+0.71±0.14*	+1.57±0.31*

*$p < 0.05$ from baseline

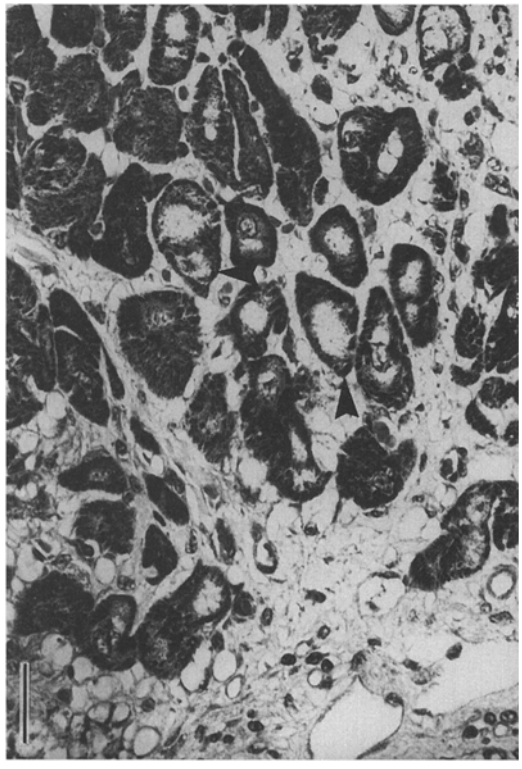

Fig. 4 Light micrograph of subendomyocardial tissue from within the region supplied by the vessel occluded by the ameroid occluder, illustrating several myocytes with central myofibrillar lysis (arrows), surrounding areas of collagenous replacement of myocardium. Hematoxylin and eosin stain. Bar = 25 μm.

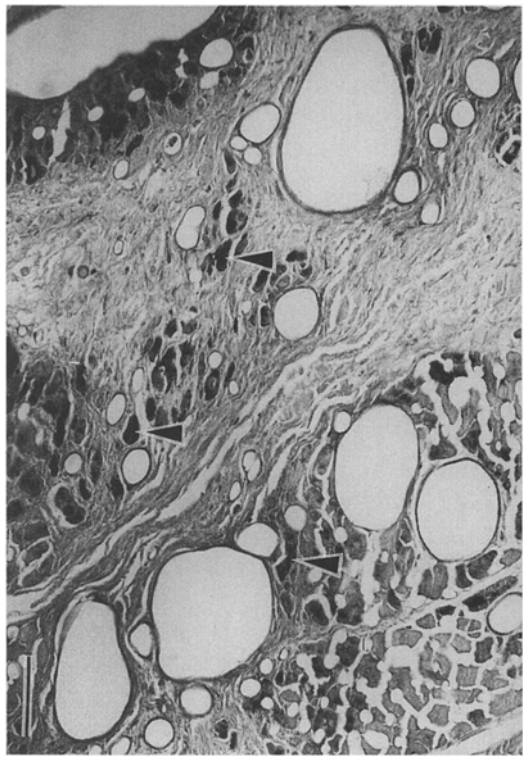

Fig. 5 Light micrograph of subendomyocardial tissue stained with PAS, illustrating several cells in the peri-scar regions with increased glycogen content (arrows). Bar = 50 μm.

kg/min, regional function deteriorated in the ameroid-dependent zone (Fig. 3).

Histologic evaluation of the 5 hearts in this study revealed multifocal areas of fibrosis throughout the inner one-third to one-half of the ameroid-dependent region of the left ventricle. Surrounding these microinfarcts, which generally were less than 500 μm diameter, there were myocytes, often with central myofibrillar lysis (Fig. 4), which contained increased amounts of glycogen, as revealed by PAS stain (Fig. 5). The PAS positive cells were limited to a rim of tissue, three to six cell layers wide, surrounding the microinfarcts. Purkinje fibers were heavily stained by PAS, as expected due to their high glycogen content.

Discussion

The understanding of mechanisms involved in chronic hibernating myocardium is impeded by the absence of a suitable animal model. The ameroid coronary constriction model in the pig shares several features with hibernating

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myocardium. For example, the model demonstrates a significant reduction in regional myocardial function consistent with reports in patients and this occurs over a several week period. Furthermore, as in the clinical setting, the coronary bed in the pig with ameroid-induced coronary constriction is perfused by a combination of native and collateral blood flow. This conclusion is based on the results of Fig. 1 showing the total area perfused by the coronary artery distal to the ameroid. Over time, as the ameroid closes the coronary artery completely, the ameroid-dependent zone becomes supplied entirely by collateral channels. After 4–6 weeks of ameroid constriction, at a time when the coronary artery is essentially totally occluded by the ameroid, patchy areas of necrosis and replacement fibrosis are observed (2, 3, 9). Therefore, measurements of regional distribution of blood flow and function at that time are contaminated by minor areas of scar tissue, which is also a limitation in most studies in patients as well.

The myocardium in the ameroid-dependent zone of these animals was also characterized by multifocal microinfarcts in the subendomyocardium. Surrounding these areas of patchy necrosis, a narrow rim of myocytes with myofibrolysis and increased glycogen in the cytoplasm was observed, similar to hibernating myocytes described by Borgers (1). However, myocardium remote from these microinfarcts did not have these structural abnormalities.

Another feature of hibernating myocardium is its responsiveness to mild inotropic stress, using dobutamine echocardiography (5). The major goal of the current study was to determine if the depressed regional function in the ameroid-dependent zone was responsive to sympathomimetic amines. We observed clear positive inotropic responses to isoproterenol in the ameroid-dependent region at both low doses studied (Table 1). At the highest dose, regional function actually fell below baseline levels (Fig. 3), most likely due to the imbalance between O_2 supply and demand induced by isoproterenol in the presence of limited coronary reserve. This is consistent with what was reported previously in response to excitement, a more potent sympathomimetic stimulant than low dose isoproterenol or dobutamine (9). With excitement the increase in metabolic demand induced by the increase in heart rate and contractility in the face of limited coronary reserve resulted in a clear reduction in regional function (9), even more dramatic than was observed with high dose isoproterenol (Fig. 3). Exercise also induces a deterioration in function in the ameroid-dependent zone (8, 10, 11). Thus, results of sympathomimetic amine stress in studies of hibernating myocardium must be interpreted knowing the severity of the O_2 demand vs. the severity of the limitation in coronary reserve. In support of this Hammond and McKirnan found differential effects of dobutamine and arbutamine on regional function at 5 weeks after ameroid implantation, most likely due to differences in myocardial metabolic demand induced by these two drugs (4).

The measurements of total myocardial blood flow in the ameroid-dependent zone (Fig. 1) support the concept that myocardial blood flow is maintained in this model of hibernating myocardium. The results of the current experiments with isoproterenol provide further indirect evidence that baseline perfusion is normal in the ameroid model of hibernating myocardium. If baseline blood flow were reduced substantially as has been proposed with hibernating myocardium (6), but never verified experimentally, then the amount of isoproterenol delivered to the ameroid-dependent region would be diminished, by definition, and would result in a reduced inotropic response compared with the contralateral zone, simply on the basis of less drug delivery to the ischemic myocardium. This was not observed. If anything, the response to isoproterenol tended to be greater in the ameroid-dependent zone (Table 1), indirectly supporting the contention that baseline perfusion is preserved in this model.

In summary, the histological evidence of lesions characteristic of hibernating myocardium in the setting of chronically depressed regional function and residual inotropic reserve, supports the model of the pig with chronic ameroid-induced coronary constriction for further study of the phenomenon of hibernating myocardium.

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