Mechanisms of Improved Ischemic Regional Dysfunction by Bradycardia

Studies on UL-FS 49 in Swine*

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In anesthetized swine, the left anterior descending coronary artery was cannulated and perfused at constant blood flow levels during two grades of ischemia. In one group (n=10), moderate ischemia reduced percent systolic wall thickening (by sonomicrometry) from 25±7% to 6±2%, whereas in the other group (n=7), severe ischemia reduced percent wall thickening from 24±6% to −0.5±4%. Heart rate was paced in both groups at 91 beats/min. After reperfusion and complete return to control conditions, administration of the bradycardic agent UL-FS 49 (0.37 mg/kg i.v.) decreased the heart rate to 55±5 beats/min. During subsequent ischemia at the same coronary inflow as before bradycardia, percent wall thickening in the ischemic zone during moderate ischemia was increased from 6±2% to 25±6% (p<0.01) (not significantly different from control without ischemia), and during severe ischemia, percent wall thickening increased from −0.5±4% to 13±7% (p<0.01). During moderate ischemia, bradycardia caused an increase in the subendocardial blood flow from 0.24±0.60 to 0.42±0.17 (ml/min)/g (p<0.009) and during severe ischemia, bradycardia caused an increase from 0.14±0.08 to 0.2±0.1 (ml/min)/g (p<0.001). At each level of ischemia, a more marked improvement occurred in subendocardial blood flow per beat ((ml/min)/g/heart rate). The relation between myocardial blood flow and wall function at a heart rate of 55 beats/min (n=14) was plotted and compared with that studied at a heart rate of 122 beats/min in another group of pigs (n=14). The increase in subendocardial blood flow per minute during bradycardia was not sufficient to explain the striking increase in function; thus, an independent relation (p<0.05) between blood flow per minute and contractile function (percent wall thickening) was found for each heart rate. In contrast, when myocardial blood flow was normalized for heart rate and expressed per beat, data from both heart rate groups could be described by a single relation. Thus, the subendocardial blood flow per beat predicted wall function independently of heart rate and accounted for changes in both oxygen supply and demand. (Circulation 1989;80:983–993)

Increased heart rate aggravates myocardial ischemia,1–4 and pharmacologic reduction of heart rate by β-adrenergic blockade has been an important form of therapy in patients with coronary artery disease. Mechanisms involved in the myocardial contractile responses to changes of heart rate in the presence of ischemia are complex because heart rate changes can directly modify coronary blood flow as well as myocardial oxygen demands.5

The effects of bradycardia produced by β-adrenergic blockade and other agents on ischemia have been studied experimentally. In our initial studies, intravenous propranolol was used in conscious, resting dogs with acute coronary stenosis and caused considerable improvement in regional myocardial function in an ischemic region, depression of function in normal zones, and an increase of mean coronary blood flow velocity.6 Later studies showed that propranolol also caused improved myocardial function in an ischemic region during exercise and impairment of the normal increase of function in normal zones.7 Several other investigations have explored mechanisms involved in the

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reduction of regional ischemia by bradycardia, which include increased subendocardial blood flow. However, a number of confounding variables in the studies in resting animals have limited our understanding of the effects of the bradycardia per se on the relations between regional blood flow (as an index of oxygen supply) and regional contraction (as an index of oxygen demand). Thus, changes in coronary stenosis resistance, unknown contributions of collateral blood flow, and possible unopposed α-adrenergic constrictor effects after β-adrenergic receptor blockade can affect total regional blood flow and its distribution. In addition, the negative inotropic actions of β-blocking drugs and some bradycardic agents or the occurrence of sustained “stunning” after periods of ischemia can preclude assessment of the full beneficial effects of bradycardia on regional contraction.

Acute regional myocardial ischemia may be viewed as a condition in which oxygen requirements of the myocardium initially exceed oxygen supply, which then leads to reduced contractile function and development of perfusion-contraction matching during the subsequent steady state. Thus, bradycardia could favorably influence contraction through beneficial changes in both supply and demand. Accordingly, the present study was designed to examine the effects of bradycardia on relations between regional myocardial blood flow distribution and left ventricular regional contractile function in a porcine model, in which collateral blood flow is negligible, and in which coronary stenosis is replaced by reduced coronary inflow that is held constant by pump perfusion. The negative inotropic actions of β-blocking agents were avoided by using a bradycardic drug (UL-FS 49) shown to have no direct cardiodepressant action, and thereby allowing assessment of the role of slowed heart rate on contraction at different levels of ischemia under controlled conditions.

Methods
The animals in this study were handled according to the animal welfare regulations of the American Heart Association and the University of California San Diego, and the experimental protocol was approved by the animal subjects committee of this institution.

Animal Model
Fifteen swine (20–30 kg) were sedated with ketamine hydrochloride (30 mg/kg i.m.) and anesthetized with thiopental sodium (20 mg/kg) administered through an ear vein. A tracheostomy was performed, and an endotracheal tube was positioned and connected to a respirator equipped with an isoflurane vaporizer. Anesthesia was maintained with isoflurane (1.5–2.5%) and oxygen, and ventilation was adjusted to maintain PO₂, PCO₂, and pH within normal ranges: PCO₂, 35±5 mm Hg; PO₂, greater than 150 mm Hg, and pH 7.40±0.10. Both carotid arteries were cannulated, one with a large polyethylene catheter that supplied the blood for an extracorporeal circuit and the other with a small polyethylene catheter that was used for blood sampling. Both internal jugular veins were cannulated, one to infuse saline and the other to return blood from the extracorporeal circuit before the coronary canulation. Rectal temperature was measured periodically, and animals were kept on a circulating hot water pad to prevent hypothermia (body temperature, >36.8°C).

A left lateral thoracotomy was performed in the fourth intercostal space, and the pericardium was opened and sutured to cradle the heart. Electrodes were sutured to the left atrium for electrical pacing (Medtronic 5800, Minneapolis, Minnesota). A micromanometer (Konigsberg P7, Pasadena, California) and a fluid-filled catheter were placed in the left ventricle through the apex for measurement of left ventricular pressure.

Ultrasound crystals were implanted in the anterior wall within the perfusion bed of the left anterior descending coronary artery to measure wall thickness by standard techniques. To verify the stability of the preparation, a set of ultrasonic crystals was also implanted in the lateral wall (control zone) within the perfusion bed of the normally perfused left circumflex coronary artery to measure midwall segmental shortening. Dimensions were measured by the ultrasonic transit time technique (Triton Technologies, San Diego, California).

The proximal left anterior descending coronary artery was dissected free from surrounding tissue for a distance of approximately 1.5 cm. After an infusion of heparin (15,000 IU initial dose, followed by 10,000 IU/hr), the left anterior coronary artery was ligated, rapidly cannulated, and perfused by a pump circuit. Mean perfusion pressure was measured through a distal side arm of the cannula and adjusted to produce a coronary pressure of 115±15 mm Hg. The pressure drop from the cannula tip to the side arm measured in vitro at a flow of 100 ml/min (considerably higher than any level used in these experiments) was 1.2 mm Hg. Because of the minimal error introduced by measuring coronary pressure from the side arm, no correction was made.

Blood was supplied by an extracorporeal circuit having one side port for microsphere injections. The extracorporeal circuit included an occlusive roller pump (Masterflex, Cole-Parmer Instruments, Chicago, Illinois), windkessel, and an electromagnetic flow probe (Micron Medical, Model RC 2000, Los Angeles, California). The pump was calibrated by timed collection so that pump flow could be determined precisely with a calibrated dial and from the calibrated flowmeter. The microsphere injection port was just distal to the pump and windkessel, and the microspheres were mixed with the blood within the perfusion line by a vortex chamber.
Blood Flow Measurements

Regional myocardial blood flow distribution was measured with 12-μm microspheres (Dupont, Boston, Massachusetts) labeled with one of the following radionuclides: 125I, 141Ce, 114In, 51Cr, 113Sn, 103Ru, 98Nb, and 46Sc. The microsphere injection port was just distal to the pump and windkessel, and a chamber with a vortex mixer was positioned just distal to the injection port. Thorough mixing of microspheres with blood by this perfusion system has been documented previously.25 For each measurement, approximately 130,000 microspheres suspended in saline were injected slowly into the extracorporeal circuit perfusing the left anterior descending coronary artery so not to affect perfusion pressure. At least 500 microspheres were required in each tissue specimen for the blood flow data to be considered satisfactory. Microsphere injections were made under each experimental condition after coronary perfusion pressure and hemodynamic variables had reached a steady state. After the study, the perfusion bed of the left anterior descending artery was determined by injecting trypan blue dye through the coronary cannula. The radioactivity within the entire dyed area was counted to determine the total amount of radioactivity for each isotope injected into the perfusion bed. The total counts injected were then related to the total coronary inflow at the time of injection (determined by the calibrated electromagnetic flowmeter) for the calculation of regional blood flow using the equation described previously25: counts per sample/blood flow to sample is equal to total counts injected/coronary inflow. The left ventricular myocardium containing the wall thickness gauge was always well within the dyed perfusion territory. A transmural tissue sample was obtained at this site, divided into transmural thirds, weighed, and then placed into glass tubes for counting gamma radioactivity by a multichannel gamma counter. Calculation of the regional blood flow was corrected for the weight of the tissue. Tissue samples usually contained greater than 1,000 microspheres.

Protocol

Two levels of ischemia were studied, moderate and severe, and separate studies were conducted at each flow level at the higher and lower heart rates. After these relatively brief periods of ischemia, regional function was always allowed to return to the control condition as was regional coronary perfusion pressure. The latter returned more slowly and often required 30 minutes or more for complete recovery, but recovery was complete for each of the four control periods.

Moderate ischemia. In 10 pigs, approximately a 70% decrease in percent wall thickening was produced at a heart rate of 91 ± 2 beats/min by reducing the coronary inflow, and a microsphere injection was made; satisfactory blood flow data were available in seven of these animals. The time required to obtain steady-state ischemia and to perform the microsphere injection was limited to 3–4 minutes to ensure complete recovery of function after reperfusion. After blood flow, wall function, and hemodynamic conditions had returned to the control state, the bradycardic agent UL-FS 49 (Karl Thomae, Biberach, FRG) (range, 0.33–0.65 mg/kg; mean, 0.37 mg/kg i.v.) was administered to reduce the sinus rate below 60 beats/min. After coronary pressure and hemodynamics had reached a steady state, the coronary inflow was reduced to the identical level set during ischemia at 91 beats/min, and another microsphere injection was made.

Regional function (eight pigs), hemodynamics (eight pigs), and regional myocardial blood flow (six pigs) after UL-FS 49 administration during ischemia were also measured when heart rate was returned to the control level (91 beats/min) by atrial pacing.

In six additional animals, the lowered left ventricular systolic pressure existing at a heart rate of 56 beats/min during ischemia with UL-FS 49 administration was matched with the systolic pressure existing at a heart rate of 90 beats/min without ischemia by use of a constrictor on the descending thoracic aorta.

Severe ischemia. In seven pigs, severe ischemia (mild dyskinesia) was induced at a heart rate of 91 beats/min by reducing coronary inflow, and a microsphere injection was made. After obtaining satisfactory blood flow data in six animals, coronary inflow was returned to normal. After hemodynamic values had returned to baseline, the heart rate was reduced by UL-FS 49, and coronary inflow was set at the same flow as that during the previous ischemia at 90 beats/min, and another microsphere injection was made.

Effects of UL-FS 49 under control conditions. The effects of UL-FS 49 on left ventricular systolic and diastolic pressures, peak dP/dt, and coronary vascular resistance were tested in seven animals without ischemia at a constant-paced heart rate of 90 beats/min. Regional myocardial blood flow under control conditions (no ischemia) at a heart rate of 91 beats/min was measured in nine experiments.

Flow-function relations. In two different groups of animals, one group from another study25 and one group from the present series of experiments, the relations between myocardial blood flow and percent wall thickening were compared at average heart rates of 122 beats/min (14 pigs, prior study) and 55 beats/min (14 pigs, eight with moderate and six with severe ischemia from present study). The flow and function points in the former group of animals studied at a higher heart rate were obtained from experiments carried out in the identical swine model with a different protocol25 because much wider ranges of ischemic flow levels and of contractile function were available at more rapidly paced heart rates for comparison with the present data,
TABLE 1. Effects of Heart Rate Reduction by UL-FS 49 on Hemodynamics and Regional Wall Function Under Control Conditions and During Moderate Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Ischemia</th>
<th>Control 2</th>
<th>Ischemia + UL-FS 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>91±2</td>
<td>91±2</td>
<td>91±2</td>
<td>54±6*‡‡</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>92±14</td>
<td>90±15</td>
<td>90±15</td>
<td>73±25*‡‡</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>13±4</td>
<td>17±5</td>
<td>12±2</td>
<td>21±5†</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,149±289</td>
<td>1,019±232†</td>
<td>1,093±288</td>
<td>802±199†‡‡</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>64±41</td>
<td>19±14‡</td>
<td>64±41</td>
<td>19±14†‡</td>
</tr>
<tr>
<td>% Wall thickening</td>
<td>25±7</td>
<td>6±2†</td>
<td>28±8</td>
<td>25±6*</td>
</tr>
<tr>
<td>% Segment shortening</td>
<td>15±7</td>
<td>16±6</td>
<td>16±7</td>
<td>16±7</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=10 pigs.
Coronary blood flow was measured with an electromagnetic flowmeter; % Wall thickening was in the ischemic region; % Segment shortening was in the control region. Control 1, control for ischemia without UL-FS 49; Control 2, control for ischemia with UL-FS 49; LV, left ventricular; +dP/dt, maximal first derivative of LV pressure.
*Different from Ischemia; †different from Control 1; ‡different from Control 2; all p<0.05.

which are restricted to relatively low flow and function levels during ischemia.

Data and Statistical Analysis

Left ventricular pressure, coronary arterial pressure, thickness of the anterior wall, segment length of the lateral wall, and mean coronary blood flow to the left anterior descending coronary artery were recorded on an eight-channel recorder (model 220, Brush, Cleveland, Ohio) and ½-in. magnetic tape (Hewlett-Packard, Palo Alto, California). Magnetic tapes were replayed later for digitization, beat averaging, and analysis (DEC LSI-11 computer, Digital Equipment, Stow, Massachusetts, interfaced with an A/D converter). Fifteen sequential beats were averaged for each measurement. End diastole was defined as the zero crossing point of the first derivative of left ventricular pressure (dP/dt) before its maximum value. End systole was defined as the time of maximum wall thickness occurring within 20 msec before peak negative left ventricular dP/dt.24 Hemodynamic measurements included left ventricular peak and end-diastolic pressures, maximum and minimum left ventricular dP/dt, mean coronary artery pressure, and mean coronary blood flow (electromagnetic flowmeter). To assess regional function, wall thickness and midwall segment length were determined at end diastole and at end ejection as previously described.2,12,13,20 Systolic wall thickening and segment shortening were calculated as percentages of end-diastolic dimensions.24

Results are expressed as mean±SD. Statistical analysis was by analysis of variance (ANOVA) for repeated measures, and when a significant overall effect was detected, Tukey's test was applied to compare single mean values. Significant differences were assumed to be present when p was less than 0.05. A paired t test was used for comparison of myocardial blood flow data; a single comparison was made for each group of swine. We also tested the null hypothesis of whether one regression can be applied to relations between subendocardial flow per minute and percent wall thickening and between subendocardial blood flow per beat and percent wall thickening in two groups of animals.26

Results

Bradycardia During Moderate Ischemia

Regional myocardial function. Hemodynamic and regional function data are summarized in Table 1. Percent systolic wall thickening decreased at the paced heart rate of 91 beats/min by 76% when coronary inflow was reduced from 64 to 19 ml/min. During hypoperfusion at the same coronary inflow and when the heart rate was reduced by UL-FS from 49 to 54 beats/min, percent wall thickening was markedly improved, reaching the control value (Figure 1, upper panel). A typical example of the effects of bradycardia induced by UL-FS 49 under these conditions is shown in Figure 2. During ischemia with bradycardia, the left ventricular systolic pressure and dP/dt decreased and end-diastolic left ventricular pressure increased compared with control ischemia (Table 1).

When the heart rate was paced to 91 beats/min during ischemia after UL-FS 49 administration in a subset of eight animals, the percent wall thickening fell to 6±4% (NS, compared with ischemia at 91 beats/min without UL-FS 49 administration). Table 2 summarizes the hemodynamic values after UL-FS 49 administration during ischemia at matched heart rates. These findings indicate no beneficial or detrimental effects of UL-FS 49 per se in the absence of bradycardia and show that the entire negative effect on global left ventricular function (dP/dt and systolic left ventricular pressure) after UL-FS 49 administration was due to the bradycardia, that is, to a force-frequency effect.

During ischemia after UL-FS 49 administration in a separate group of six pigs, the left ventricular systolic pressure was matched to the pressure previously observed during ischemia. The drop in systolic pressure was considerably less during moderate ischemia (ischemic wall thickening, 6%) with UL-FS 49 administration in this group of animals.
(89–85 mm Hg) compared with those groups in Table 1. Thus, restoring pressure to the prior level at the lower heart rate resulted in a mild reduction of wall thickening (from 27% to 23%, NS, Table 3). These results show a large improvement of regional function with the bradycardic agent at a constant systolic pressure but do not allow an estimate of how much the effect of pressure matching would have inhibited the improvement of function in the first group of animals.

Regional myocardial blood flow. Control blood flows in the subendocardium, midwall, and epicardium were 1.04±0.22, 0.99±0.37, and 1.08±0.46 (ml/min)/g, respectively; subendocardial blood flow per beat was 11±1 ml/g×10⁻³.

The blood flow data during moderate ischemia are summarized in Table 4. When heart rate during ischemia was reduced from 91 to 54 beats/min, significantly higher subendocardial and midmyocardial blood flows were found at the lower heart rate (Figure 3), and the subendocardial to subepicardial ratio increased from 0.55±0.09 to 0.86±0.09% (p<0.001). The increases in subepicardial and transmural flows were not significant. Mean distal coronary perfusion pressure was measured during ischemia and increased from 41±10 mm Hg during control ischemia to 50±25 mm Hg during ischemia after UL-FS 49 administration (NS).

During ischemia after UL-FS 49 administration, the heart rate was paced back to the control level (91 beats/min in eight pigs), and as mentioned above, the hemodynamic variables and regional function during pacing were nearly identical to those obtained in these pigs during moderate ischemia in the absence of UL-FS 49 (Table 2). In a subset of these animals (n=6), regional myocardial blood flow was also measured. The subendocardial and the subepicardial blood flows were 0.23±0.11 and 0.39±0.17 (ml/min)/g, respectively, during pacing after UL-FS 49 administration. These values are not significantly different from control ischemia at a heart rate of 91 beats/min when subendocardial blood flow was 0.23±0.10 and when subepicardial blood flow was 0.38±0.10 (ml/min)/g.

Bradycardia During Severe Ischemia

Regional myocardial function. Hemodynamic and functional data are summarized in Table 5. During severe ischemia, the heart rate was reduced by UL-FS 49 from 90 to 56 beats/min. At a heart rate of 90 beats/min, when coronary inflow was reduced from 71 to 10 ml/min, percent wall thickening decreased from 24% to ~0.5%. After heart rate reduction with UL-FS 49 at the same coronary inflow, percent wall thickening was significantly higher, averaging 13% (Figure 2, lower panel). Also, a significant decrease in left ventricular systolic pressure and an increase in enddiastolic pressure was present after UL-FS 49 administration (Table 5).

Regional myocardial blood flow. Blood flow measurements are summarized in Table 3. Bradycardia during severe ischemia was associated with an increase in subendocardial flow from 0.14 to 0.21 (ml/min)/g (Figure 3) and an increase in the subendocardial to subepicardial flow ratio from 0.46 to 0.75 (Table 4). The increases in subepicardial and transmural flows were not significant. Mean distal coronary perfusion pressure was 43 mm Hg during control ischemia and was also 43 mm Hg with ischemia after UL-FS 49 administration.

Effects of UL-FS 49 Administration Without Ischemia

The hemodynamic effects of UL-FS 49 without ischemia are shown in Table 6. Left ventricular systolic pressure and dP/dt were reduced, and enddiastolic pressure was increased. However, no differences in left ventricular systolic and enddiastolic pressures and dP/dt were found after drug administration when the heart rate was paced back to the control level. Coronary vascular resistance values after UL-FS 49 administration before and after the heart was paced back to a rate of 90 beats/min also were not significantly different. These
findings indicate a lack of direct cardiodepressant and coronary vascular effects of UL-FS 49.

**Flow-Function Relations During Ischemia at Different Heart Rates**

In Figure 4 (upper panel) subendocardial blood flows per minute (in milliliters per minute) per gram during moderate and severe ischemia after UL-FS 49 administration at an average heart rate of 55 beats/min are plotted against corresponding levels of regional percent wall thickening and compared with values from the previously studied group of pigs25 with various degrees of ischemia at an average heart rate of 122 beats/min. It is evident that the flow-function relation is shifted upward by bradycardia. We tested the null hypothesis that one regression could be applied to both groups, and this hypothesis was rejected (p<0.05) (Figure 4, upper panel). Also, when the endocardial flows below 0.5 (ml/min)/g were fitted to linear regressions, the slopes were not different, but the difference in the intercept was highly significant (p<0.005).

In Figure 4 (lower panel) subendocardial blood flow per beat is plotted against regional function in these two groups of animals. When subendocardial blood flow is expressed per beat, a single relation is observed for all animals, and the null hypothesis that one regression applies to both groups was not rejected.

**Discussion**

The major finding of this study is that slowing of the heart rate by about 40 beats/min during ischemia causes a remarkable improvement of contractile function in the ischemic region, which is associated with increased subendocardial blood flow. Thus, during moderate ischemia (75% reduction of systolic wall thickening) after UL-FS 49 administration, systolic wall function was similar to the con-

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**TABLE 2. Hemodynamics and Regional Wall Function During Moderate Ischemia Before and After UL-FS 49 Administration at Constant Paced Heart Rate**

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>Moderate ischemia</th>
<th>+UL-FS 49 and pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>95±15</td>
<td>93±11</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>14±7</td>
<td>12±2</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,100±275</td>
<td>1,013±251</td>
</tr>
<tr>
<td>% Wall thickening</td>
<td>5±2</td>
<td>6±4</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>19±16</td>
<td>19±16</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=8 pigs.
LV, left ventricular; +dP/dt, maximal first derivative of LV pressure.
*Control moderate ischemia.
†Ischemia at the same coronary inflow but after UL-FS 49, with paced heart rate to prevent bradycardia.
Mechanisms and Potential nonischemic level of function. There was less marked, but still significant, improvement of contraction during very severe ischemia (defined as slight dyskinesia) associated with a smaller improvement in the subendocardial blood flow.

**Potential Mechanisms of Improved Contraction**

With moderate ischemia, the improvement of contraction by UL-FS 49 was considerably greater than would be expected from the increase in subendocardial perfusion alone (from approximately 0.24 to 0.41 [ml/min]/g), and during bradycardia (Figure 4, upper panel), there was an upward shift of the entire relation between subendocardial blood flow per minute and function. Thus, there are separate curves relating blood flow per minute to contractile function at different heart rates; that is, for a given level of subendocardial blood flow per minute, systolic wall thickening is inversely related to the heart rate (Figure 4). These data suggest that the modest increase in subendocardial blood flow per minute was not the only factor causing improved function; rather, the more dominant factors were probably the decreased number of active contractions per minute and the increased diastolic perfusion time to the subendocardium that produced increased subendocardial blood flow per beat. Therefore, when perfusion and contraction were related by expressing subendocardial blood flow per beat, a single relation between flow and systolic wall thickening appears (Figure 4, bottom panel) despite different heart rates. These results support the concept of matching subendocardial blood flow per beat and regional contraction during various degrees of ischemia.5 With such an analysis, the present studies show that perfusion-contraction matching (which can exist during any steady state provided there is some residual coronary blood flow5) that accompanies bradycardia in the presence of ischemia can produce a near normal level of contraction despite a sustained flow reduction to the subendocardium (0.41 [ml/min]/g). During exercise-induced ischemia, the heart rate is also a major determinant of the amount of blood flow (and hence oxygen) available per beat, which, in turn, largely determines the level of regional contractile dysfunction in the steady state.5,27

No attempt was made to measure regional MVO2 per minute in these experiments because practical methods for assessing inner and outer wall MVO2 are not available. Although bradycardia is generally stated to reduce MVO2 during ischemia, this assump-

**Table 3. Effects of Heart Rate Reduction by UL-FS 49 on Hemodynamics and Wall Function During Moderate Ischemia With Systolic Pressure Matched During Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>Ischemia + UL-FS 49</th>
<th>Ischemia + UL-FS 49 + pressure match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>90±2</td>
<td>90±2</td>
<td>56±4</td>
<td>56±4</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>93±9</td>
<td>89±7</td>
<td>85±5</td>
<td>89±9</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,406±100</td>
<td>1,245±184</td>
<td>1,066±127†</td>
<td>1,053±189†</td>
</tr>
<tr>
<td>% Wall thickening</td>
<td>33±4</td>
<td>6±1*</td>
<td>27±9</td>
<td>23±7</td>
</tr>
<tr>
<td>% Segment shortening</td>
<td>22±4</td>
<td>23±2</td>
<td>23±4</td>
<td>21±5</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=6 pigs.
LV, left ventricular; +dP/dt, maximal first derivative of LV pressure.
*Different from control; †different from control and ischemia; p<0.05.

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**Table 4. Effect of Heart Rate Reduction by UL-FS 49 on Regional Myocardial Blood Flow During Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Moderate ischemia</th>
<th>Severe ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>91 54</td>
<td>90 56</td>
</tr>
<tr>
<td>Subendocardial blood flow (ml/min/g)</td>
<td>0.24±0.06 0.42±0.17†</td>
<td>0.14±0.08 0.21±0.11*</td>
</tr>
<tr>
<td>Midwall blood flow (ml/min/g)</td>
<td>0.29±0.11 0.38±0.12*</td>
<td>0.19±0.08 0.22±0.11</td>
</tr>
<tr>
<td>Epicardial blood flow (ml/min/g)</td>
<td>0.44±0.07 0.47±0.15</td>
<td>0.28±0.12 0.26±0.11</td>
</tr>
<tr>
<td>Transmural blood flow (ml/min/g)</td>
<td>0.32±0.07 0.42±0.14</td>
<td>0.20±0.09 0.23±0.10</td>
</tr>
<tr>
<td>Endo/epi (ml/g×10^-3)</td>
<td>0.55±0.09 0.86±0.09*</td>
<td>0.46±0.14 0.75±0.20*</td>
</tr>
<tr>
<td>Endo/beat (ml/g×10^-3)</td>
<td>2.6±0.7 7.9±3.2†</td>
<td>1.5±0.8 3.7±1.9*</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=7 pigs (Moderate ischemia) and n=6 pigs (Severe ischemia).
Endo/epi, subendocardial to subepicardial blood flow ratio; Endo/beat, subendocardial blood flow divided by heart rate.
*p<0.05, †p<0.01 vs. same inflow condition at higher heart rate.
tion may not apply to the subendocardium. Thus, during relatively severe steady-state ischemia with near akinesia of the wall, MVO₂ and oxygen use per beat are probably substantially reduced in the subendocardium, whereas development of tension in the epicardial fibers and outer wall\(^2^8\) could cause considerable oxygen expenditure in the outer layers. With bradycardia during ischemia, the decreased number of contractions per minute will cause considerable improvement in contractile function in the subendocardial layers, which therefore must actually increase oxygen use per beat; therefore, the change in MVO₂ per minute of the inner ischemic wall during bradycardia is difficult to predict and actually may not decrease. If this were the case, the mechanism for the observed improvement contraction would relate almost entirely to increased oxygen supply because of the somewhat improved subendocardial flow per minute and the greatly augmented subendocardial flow per beat consequent to the bradycardia. Confirmation of whether this speculation is correct will require reliable measurements of regional MVO₂.

The observed changes in regional function could have been due in part to the reduction of left ventricular systolic pressure associated with heart rate reduction produced by UL-FS 49 because a low impedance to the left ventricular ejection may cause an increase in the extent of shortening.\(^2^9,3^0\) However, a very large improvement in regional function was found after UL-FS 49 at matched left ventricular pressures accomplished with aortic constriction in another set of animals. In conscious, normal dogs, UL-FS 49 has been reported not to decrease systolic or diastolic aortic pressures despite a significant reduction in heart rate\(^2^0-2^3\) perhaps in part because of reflex adjustments.

The beneficial effects of UL-FS 49 on regional myocardial ischemia were completely dependent on the heart rate reduction because with pacing during ischemia to prevent the bradycardia we observed no residual effects of the drug on dP/dt, percent wall thickening, and regional ischemic myocardial blood flow. In contrast, atrial pacing to the heart rate existing before drug administration did not completely reverse the beneficial effects of another bradycardic agent, alinidine, on regional myocardial blood flow\(^1^4\) suggesting that bradycardia-independent factors (i.e., direct negative inotropic effect or vasodilation) are involved in the effects of that agent during ischemia. Similar results have been shown with \(\beta\)-adrenergic antagonists.\(^6,9\) However, in a model of exercise-induced ischemia when the bradycardia produced by atenolol was prevented by pacing, regional myocardial blood flow

### Table 5. Effect of Heart Rate Reduction by UL-FS 49 on Hemodynamics and Regional Wall Function During Severe Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Ischemia</th>
<th>Control 2</th>
<th>Ischemia + UL-FS 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>91±1</td>
<td>90±1</td>
<td>91±0.4</td>
<td>56±2*†‡</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>95±13</td>
<td>85±12</td>
<td>90±20</td>
<td>76±7*†‡</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>10±3</td>
<td>14±6</td>
<td>11±6</td>
<td>18±4*†‡</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,195±278</td>
<td>974±218†</td>
<td>1,094±225</td>
<td>813±190*†‡</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>71±47</td>
<td>10±5†</td>
<td>71±47</td>
<td>10±5§</td>
</tr>
<tr>
<td>% Wall thickening</td>
<td>24±6</td>
<td>-05±4†</td>
<td>25±7</td>
<td>13±7*†</td>
</tr>
<tr>
<td>% Segment shortening</td>
<td>16±6</td>
<td>18±5</td>
<td>16±6</td>
<td>20±4*†‡</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=7 pigs.
Coronary blood flow was measured with an electromagnetic flowmeter; % Wall thickening in the ischemic region;
% Segment shortening was in the control region.
Control 1, control for ischemia without UL-FS 49; Control 2, control for ischemia with UL-FS 49; LV, left ventricular; +dP/dt, maximal first derivative of LV pressure.
*Different from Ischemia; †different from Control 1; ‡different from Control 2; p<0.05.

### Table 6. Hemodynamic Effects of UL-FS 49 Without Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>UL-FS 49</th>
<th>Pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>90±2</td>
<td>54±7*</td>
<td>90±2</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>94±14</td>
<td>81±10*</td>
<td>94±15</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>11±3</td>
<td>20±5*</td>
<td>10±3</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,213±301</td>
<td>917±322*</td>
<td>1,145±286</td>
</tr>
<tr>
<td>-dP/dt (mm Hg/sec)</td>
<td>1,220±307</td>
<td>702±249*</td>
<td>1,102±302</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=8 pigs.
LV, left ventricular; +dP/dt, peak positive first derivative of left ventricular pressure; -dP/dt, peak negative LV dP/dt.
*Different from control by ANOVA, p<0.05.
actually diminished slightly below the value observed during exercise without \( \beta \)-blockade.\(^31\)

**Differences From Prior Studies**

An inverse relation between subendocardial perfusion and heart rate has been shown in exercising dogs with restricted coronary inflow\(^1,32\) and during cardiac pacing in a normally perfused bed with maximal vasodilation,\(^2\) and several other studies have shown beneficial effects of \( \beta \)-blocking agents during different types of ischemia.\(^6,10,12\)

Previous studies on the mechanisms by which bradycardic drugs decrease regional ischemia due to coronary artery stenosis have shown increased subendocardial to subepicardial myocardial blood flow ratios during \( \beta \)-blockade or increased subendocardial blood flow with unchanged subepicardial flow after \( N \)-dimethyl propranolol (which has no \( \beta \)-blocking properties).\(^11\) Other studies with more severe ischemia showed increased subendocardial blood flow without significant change in subepicardial flow after several \( \beta \)-blocking agents were administered and also showed increased distal perfusion pressure and a marked reduction in calculated resistance of a coronary artery stenosis.\(^10\) This redistribution of blood flow was attributed to restoration of autoregulation in the distal bed.

In previous studies, ischemia was often maintained for 15–20 minutes before drug administration,\(^8,14\) which could have produced myocardial "stunning."\(^15\) Also, some bradycardic agents have well-known negative inotropic effects that may independently affect transmural blood flow distribution. These variable effects on coronary stenosis resistance,\(^10\) coronary collateral blood flow,\(^8\) and perhaps unopposed \( \alpha \)-adrenergic tone\(^13\) have not been assessed under controlled conditions and provided the focus for the present experiments.

In a recent report of Dämgen et al,\(^33\) the administration of UL-FS 49 failed to increase regional function, despite a significant increase in subendocardial flow and an increase in the subendocardial to subepicardial ratio from 0.52 to 0.80. In the study by Dämgen et al,\(^33\) the first blood flow measurement was obtained 30 minutes after coronary stenosis at which time UL-FS 49 was infused for 30 minutes (first dose) and subsequently for another 30 minutes. The myocardium might have been stunned, thereby making it unresponsive to the bradycardia and increased perfusion.\(^15,34\) In our experimental model, we used brief (3–4-minute), fully reversible periods of ischemia to avoid stunning.

**Other Mechanisms**

In a previous study from this laboratory, dogs with chronic coronary stenosis treated with UL-FS 49 showed marked attenuation of exercise-induced ischemia and improved regional function, without compromise of contractile function in a non-ischemic area or reduction of global left ventricular contractility.\(^20\) However, left ventricular end-diastolic pressure was elevated during ischemia after heart rate reduction with UL-FS 49. This likely reflected the prolonged diastolic interval with increased ventricular filling.\(^20\) Although elevated left ventricular end-diastolic pressure may impede subendocardial blood flow,\(^35\) the beneficial effects of prolonged diastolic perfusion in that model apparently outweighed any disadvantages due to the elevated left ventricular end-diastolic pressure. A similar conclusion appears to apply in the present experiments because left ventricular end-diastolic pressure was further elevated after UL-FS 49 with ischemia (Table 1). Exercising dogs without coronary stenosis also have a significant increase in subendocardial flow despite an increase in end-diastolic pressure to 20 mm Hg.\(^32\)

**Direct Effects of UL-FS 49 on Left Ventricular Function**

Without ischemia at a constantly paced heart rate, no direct effect of UL-FS 49 on left ventricular contractility was found. Although UL-FS 49 is a
structural analogue of verapamil, it does not have classic calcium channel antagonist activity,20–22 and its action appears to be specific to the sinus node.23 This drug could have advantages over \( \beta \)-adrenergic blocking agents in the clinical setting because the beneficial effect of bradycardia is obtained without direct depression of contractility,20 and without other effects of \( \beta \)-adrenergic blockade.

After bradycardia induced by UL-FS 49, with or without ischemia, a significant reduction in \( \Delta P/\Delta t \) and systolic pressure of the left ventricle was observed. We suggest that these effects can be attributed entirely to the negative inotropic effect of decreased frequency of contraction through the force-frequency relation (negative Bowditch case) acting on the normal myocardium, whereas during ischemia, opposite changes occurred in the ischemic region because of improved oxygen supply-demand relations. Also, in the absence of ischemia, the negative effect was completely eliminated by pacing back to the control heart rate. The elevated left ventricular end-diastolic pressure at the slowed heart rate can be attributed in part to the negative inotropic effect of bradycardia but mainly to the effects of increased cardiac filling after UL-FS 49.20

The positive inotropic effect of increased contraction frequency has been previously reported.36,37

Advantages and Potential Limitations of the Study

Constant flow coronary perfusion may not be truly physiologic. However, mean coronary inflow in the presence of critical coronary artery stenosis does not change significantly during exercise or pacing, thereby representing conditions in which coronary inflow is relatively constant.32 The porcine model obviates effects due to coronary collateral blood flow.16–19 Pump perfusion was used to accurately measure the net effect of heart rate changes on regional function and myocardial blood flow without the need for assumptions concerning the stability of a mechanical, occlusive device or hydraulic occluder or variations in the degree of the coronary stenosis during different interventions.

The left anterior descending coronary artery in swine perfuses both left and right ventricles,36 and restriction of coronary blood flow to this artery affects both chambers. In such a setting, which also occurs in coronary heart disease in humans, a redistribution of myocardial blood flow could occur between the two ventricles, and recently, a right ventricular “steal” has been shown during flow-constant perfusion after intracoronary dobutamine administration in swine.39 Effects involving a “reverse steal” by this mechanism could have occurred in the present experiments because regional transmural blood flow tended to increase (although not statistically significant) despite constant inflow after UL-FS 49, and further studies to explore such a mechanism are planned.

Implications of the Present Study

Reduction of heart rate with UL-FS 49 may have an advantage over \( \beta \)-adrenergic blockade because UL-FS 49 does not cause unmasking of \( \alpha \)-adrenergic vasoconstriction in the large coronary arteries21–23 and because the beneficial effects of reduced heart rate are obtained without direct depression of ventricular contractility or impairment of reflex increases in contractility.20

Reduction of heart rate by a non-\( \beta \)-adrenergic receptor mechanism may prove useful in the clinical setting in ischemic heart disease. Thus, use of a bradycardic drug in the setting of severe ischemia at rest (as in unstable angina pectoris), or non-Q wave myocardial infarction (in which there may be persistent antegrade flow) could markedly improve the oxygen supply-demand balance without direct negative inotropic effects. However, bradycardia may cause lowered arterial pressure through the negative force-frequency effect and require concomitant use of a vasopressor or positive inotropic agent that does not increase heart rate. In this connection, a recent experimental study showed that reduction of heart rate of 50% by a portable stimulator caused a protective effect against catecholamine damage, which was manifested by a smaller extent of necrosis and by the maintenance of normal cardiac pump function at rest and during a short-term administration of norepinephrine.40

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C Indolfi, B D Guth, T Miura, S Miyazaki, R Schulz and J Ross, Jr

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