LABORATORY INVESTIGATION

MYOCARDIAL ISCHEMIA

Effect of the combination of diltiazem and atenolol on exercise-induced regional myocardial ischemia in conscious dogs

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ABSTRACT The effect of combination therapy with diltiazem and atenolol on the regional myocardial blood flow–function relationship was studied in eight conscious dogs with chronic coronary artery stenosis. An aneroid constrictor and hydraulic occluder were placed around the left circumflex coronary artery, sonomicrometers were implanted for measuring wall thickness in control and ischemic regions, and regional myocardial blood flow was measured with the microsphere method. Eighteen days (average) after surgery, resting regional myocardial function and blood flow were normal, but treadmill exercise induced severe regional myocardial dysfunction in the posterior wall (wall thickening during systole reduced from 25.5% to 2.7%, a 90% reduction). Subendocardial blood flow decreased by 68% from the control standing value, while subepicardial flow increased. An identical exercise bout was performed 3 hr after administration of atenolol (1.0 mg/kg orally) and 15 min after administration of diltiazem (0.3 mg/kg iv). Heart rate during running was significantly lower as were left ventricular peak systolic pressure, end-diastolic pressure, and peak dP/dt. Wall thickening in the control region was not augmented during exercise after atenolol and diltiazem. There was less dysfunction in the ischemic region (35% reduction) and the improved performance was accompanied by a substantial increase in subendocardial perfusion (0.31 ± 0.14 vs. 0.61 ± 0.30 ml/min/g, a 36% reduction from rest). Epicardial flow was unchanged, and the endocardial/epicardial ratio increased (0.27 ± 0.13 vs. 0.62 ± 0.29). Recovery time for regional wall thickening also improved. The beneficial effects of the combination of atenolol and diltiazem in a preparation of single-vessel chronic coronary stenosis were shown to be significantly greater than those of either drug alone.


β-ADRENERGIC BLOCKADE is effective in improving exercise tolerance in patients with angina pectoris but sometimes optimum results are not achieved when the drugs are used alone.1-3 A second drug is therefore sometimes added to therapy, such as a long-acting nitrate,4 a calcium blocker,5-7 or occasionally digitalis.8 Calcium channel–blocking drugs, which inhibit the influx of calcium ions during cell depolarization in both smooth and cardiac muscle, are highly effective in the therapy of vasospastic myocardial ischemia and stable angina pectoris.8-15 The efficacy of calcium antagonists in patients receiving propranolol has been studied clinically,5,6 but little is known about how the combination of β-adrenergic blockade with a calcium antagonist acts in man or experimental animals. Also to be determined is whether exercise-induced maldistribution of regional myocardial blood flow and contractile dysfunction are modified by the therapy. Diltiazem is a calcium channel–blocking drug that has been reported in clinical and experimental studies to have smaller negative inotropic and chronotropic properties than verapamil and less inotropic and vasodilator action than nifedipine.16,17 Accordingly, in conscious exercising dogs with chronic coronary artery stenosis we have investigated the effects of diltiazem in combination with the cardioselective β-blocking drug atenolol.

Methods

Animal preparation. Eight adult mongrel dogs weighing 19 to 30 kg (mean 24.7 kg) were anesthetized with sodium pentobarbital (25 mg/kg iv) and ventilated with a Harvard respirator. A left lateral thoracotomy was performed by a sterile technique through the fifth intercostal space. A miniature pressure transducer (Konigsberg P7) and a Tygon fluid-filled catheter (2.2 mm internal diameter) were introduced into the left ventricle.

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through the apex. Left ventricular pressure measured through the fluid-filled catheter (Statham P23Db) was used to calibrate the Konigsberg gauge, zero reference being taken at the estimated level of the right atrium while each dog lay on its right side. Tygon catheters were inserted into the left atrium for injection of microspheres, and into the descending aorta to withdraw reference blood samples.

A 2.5 cm section of the proximal left circumflex coronary artery was dissected free and a single crystal (10 MHz) Doppler flowprobe was placed around the coronary artery. An atherosclerotic constrictor was positioned distal to the flowprobe, and a hydraulic occlusive cuff was placed beyond the constrictor. The atheroconstrictor was encased in a slotted stainless steel ring (5 mm wide, external dimension 8 mm, lumen 2.5 to 3.0 mm). The constrictor was designed to produce gradual constriction of the coronary artery and is known to stimulate development of collateral vessels.19,21

Two pairs of miniature ultrasonic dimension gauges (5 MHz) were implanted in the left ventricular wall for measuring regional myocardial wall thickness as previously described.22 One pair was positioned in the posterior free wall supplied by the circumflex artery to measure posterior (ischemic) wall thickness, and the other was placed in the distribution of the anterior descending artery to measure anterior (control) wall thickness. In brief, one crystal (2 mm diameter) of each pair was inserted obliquely through the myocardium to the subendocardium. The second crystal (8 mm diameter) was sewn on the epicardial surface where the ultrasonic transit time between the crystals was shortest. The pericardium was left open and all wires and catheters were tunneled subcutaneously to the back of the dog and brought out through the skin between the scapulae. The chest was then closed, the pneumothorax evacuated, and an antibiotic (ampicillin, 300 mg/day) was administered intramuscularly for 3 days after surgery. The position of all crystal pairs was carefully determined and recorded at the time of necropsy to verify correct alignment. Of the 16 available pairs of crystals (eight dogs), data from 15 were considered acceptable. Eleven subendocardial crystals (six in the ischemic area and five in the control area) were positioned just inside the endocardial surface spanning nearly all of the wall, while the remaining four inner crystals were found within 2 mm of the endocardial surface, spanning approximately 80% of the full wall thickness. One pair of crystals that was to be used to measure control wall thickness was not usable because of improper alignment. There was no gross indication of infarction, and the crystals were surrounded by fibrous connective tissue (approximately 1 mm), as described previously.22

Regional myocardial blood flow. The reference withdrawal method was used to measure regional myocardial blood flow with 15 μm diameter radionuclide-labeled microspheres (57Co, 114In, 115Sn, 103Ru, 95Nb, or 46Sc; New England Nuclear). The microspheres were diluted in 10% low-molecular weight dextran with Tween 80 added. Details of the methods of regional myocardial blood flow determination in this laboratory have been described previously.23 Left ventricular pressure, wall dimensions, coronary blood flow velocity, and subendocardial electrograms in the ischemic area were monitored continuously during the reference sample withdrawal period and the microsphere injection. If arrhythmias or significant changes in heart rate and left ventricular pressure were observed during the withdrawal period, the measurement in question was discarded. Immediately after the final experiment, each dog was anesthetized with intravenous sodium pentobarbital and killed with a lethal dose of potassium chloride. The heart was removed, the left and right coronary arteries were flushed gently with saline followed by formalin, and the heart was placed in 10% formalin for 3 to 5 days to facilitate sectioning by techniques previously described.23 Histologic examination of the tissue between crystal pairs was performed,23 and the extent of necrosis was determined with a point-counting technique.24

The radioactivity of myocardial and reference blood samples was counted in a Packard Auto-gamma Spectrometer (Model 5912) at window settings corresponding to the primary energy peak of each isotope with a matrix inversion technique for resolving the various isotope windows.25 The myocardial blood flow to each sample was calculated by a standard method.26 Flow was divided by the sample weight and expressed in milliliters per minute per gram (wet weight) of myocardium. Data are reported from only the samples of the tissue containing the crystal pairs, but the entire slice (eight full-thickness samples) was counted and results confirmed that the tissue containing the crystal pair was always representative of the central ischemic zone.

Experimental protocol. Before operation, all dogs were familiarized with the laboratory and trained to run on a motorized treadmill. Starting 7 days after surgery, all eight dogs studied were evaluated every 2 to 3 days. The conscious, unsedated dog rested quietly on its right side, and after basal control recordings of hemodynamics, coronary blood flow velocity, and dimensions were obtained, temporary circumflex coronary artery occlusion was abruptly induced by inflating the hydraulic cuff. Complete coronary occlusion was maintained for 10 sec followed by full release of the cuff; the subsequent reactive hyperemia was recorded for at least 5 min. Seven days after surgery, mean basal blood flow velocity in the circumflex coronary artery was 40.2 ± 10.0 cm/sec (mean ± SD), and mean peak hyperemic flow velocity was 107.4 ± 20.1 cm/sec (268% of resting value). On the first evaluation day (7 days after surgery), brief coronary occlusion caused severe dysfunction in the ischemic area, but as time passed dysfunction of the ischemic wall during coronary occlusion gradually diminished and reactive hyperemia also decreased progressively, indicating collateral development. When peak reactive hyperemic flow velocity was reduced to less than approximately 130% of the preocclusion value treadmill exercise evaluation was performed, and if ischemic dysfunction was produced during this brief evaluation run (for approximately 1 min), the experiment was carried out on the same day. If the exercise-induced dysfunction during control exercise was not severe (systolic wall thickening reduced by less than 50% of standing value), the study was delayed. At an average of 18 ± 4 days after surgery (range 14 to 27 days), the exercise studies were performed. All eight dogs exhibited normal activity, were afebrile, showed normal hematocrits (range 39.0% to 45.5%), and were capable of repeated exercise on the treadmill.

On the study day, in four of eight dogs, circumflex coronary artery blood flow velocity at rest was decreased, averaging 28.2 ± 4.6 cm/sec (p < .01 compared with 7 days after surgery), and peak reactive hyperemic flow velocity was also significantly decreased to 36.2 ± 6.6 cm/sec (128% of rest, p < .01 compared with at 7 days). In the remaining four dogs, blood flow through the circumflex coronary artery was absent because of complete obstruction by the atheroconstrictor. As previously noted,27,28 dogs with occluded arteries were not different from the other dogs with respect to the responses of regional function at rest or during exercise before or after drugs; the average subendocardial blood flow during exercise after drugs tended to be lower in dogs with occluded arteries, but numbers were too small for statistical analysis.

Control recordings and the first microsphere injection were made while the dog stood quietly on the treadmill. Each dog was then run on the treadmill at a speed and grade sufficient to substantially decrease systolic wall thickening in the ischemic area (average 9.6 ± 1.5 km/hr, 5% grade). All treadmill runs
were performed for 3.2 to 4.2 min (average 3.6 ± 0.4 min) with microspheres injected between 2 and 2.5 min into the run when a steady state was apparent.27 Data were recorded and taped during running, for 5 min after the end of the exercise period, and then intermittently for the next ½ hr. At least ½ hr after full recovery from control exercise was confirmed, atenolol (1 mg/kg) was administered orally. Approximately 3 hr after atenolol was given, data were recorded and diltiazem (0.3 mg/kg) was administered via the left atrial catheter over 5 min. Ten to fifteen minutes later, control data for the second exercise period were recorded with the dog standing quietly on the treadmill. The treadmill exercise was then repeated at exactly the same speed and grade, and for the same running time. The third microsphere injection was made when steady-state conditions were achieved (approximately 2 to 2.5 min into the run), as characterized by stable regional function and hemodynamics.

As reported previously, we have found that maximum effect on suppression of exercise-induced tachycardia by atenolol occurs at 3 to 4 hr after oral administration.27 We have previously reported a high degree of reproducibility in sequential runs performed 3 hr apart in this animal preparation without intervention, with very similar responses of regional blood flow, hemodynamics, and regional wall thickening in both ischemic and normal zones.27

**Data analysis.** Recordings during each experiment were made on a Brush forced-ink recorder and on magnetic tape for subsequent analysis. The variables analyzed were wall thickness at end-diastole (identified at the point just before the onset of the positive dP/dt signal) and end-systole (defined as the maximum systolic thickening occurring at or within 20 msec of peak negative dP/dt), extent of systolic wall thickening, left ventricular peak and end-systolic pressures, mean and phasic blood flow velocities in the circumflex coronary artery, and heart rate. The extent of wall thickening was calculated as the difference (in mm) between end-diastolic and end-systolic dimensions and was also expressed as the percentage change from end-diastolic wall thickness. The values for wall thickness were normalized to a 10 mm initial end-diastolic thickness by dividing the end-diastolic and end-systolic wall thickness by the control standing end-diastolic wall thickness and multiplying by 10.24–29 The absolute values of end-diastolic wall thickness in the ischemic and control areas during control standing conditions ranged from 8.21 to 11.67 mm (average 10.35 ± 1.21 mm) and from 8.44 to 11.61 mm (average 9.75 ± 1.28 mm), respectively. Mean ejection phase wall thickening velocity (mWThV) in the ischemic and control walls was calculated by the following equation: mWThV = (ESWTh − EDWTh)/ET, where ESWTh = end-systolic wall thickness; EDWTh = end-diastolic wall thickness; and ET = left ventricular ejection time (defined as the interval between the time of maximum left ventricular positive dP/dt and 20 msec before left ventricular peak negative dP/dt). Hemodynamic, dimensional, and coronary flow velocity data were digitized from magnetic tape with use of a computer system (PDP/11/03). Twenty consecutive cardiac cycles (10 consecutive cardiac cycles at the initial phase of exercise) were sampled and averaged.

Statistical analysis of the difference between control exercise and exercise with the combination of atenolol and diltiazem and comparison of mean values during exercise with those in the resting state were done with a multifactor analysis of variance for repeated measures.30 When an overall difference was detected, Tukey’s least significant difference test was used to determine which means differed significantly. Blood flow data were analyzed with a single-factor (repeated measures) analysis of variance, and multiple analysis of variance was used to analyze differences between blood flow in each layer in the ischemic and control areas.30 An analysis of variance for repeated measures was also used to compare heart rate, left ventricular pressures, and peak left ventricular dP/dt values obtained in the present study with those observed in previous studies in which atenolol27 and diltiazem28 were used alone. In addition, points on the myocardial blood flow-function relationship comparing atenolol and diltiazem combined with values observed in the previous studies using atenolol or diltiazem alone were analyzed by Hotelling’s multivariate t test for two variables.30 Data are reported as mean ± SD; p < .05 was considered indicative of a significant difference.

**Results**

**Hemodynamic responses.** Hemodynamic data are summarized in figure 1. Representative tracings showing hemodynamic variables and dimensions during control exercise and during exercise after administration of atenolol and diltiazem are illustrated in figure 2.

Within 10 sec of the beginning of control exercise, heart rate increased abruptly and remained elevated throughout the run, whereas left ventricular peak systolic pressure and peak (+) dP/dt initially increased, fell somewhat during the first minute, and then stabilized (figures 1 and 2). Left ventricular end-diastolic pressure gradually increased and was significantly elevated by 1 min into the control run. At the time of microsphere injection (approximately 2.5 min into the run), heart rate was increased from 103 ± 14 (standing rest) to 227 ± 31 beats/min (p < .01). Left ventricular peak systolic pressure and left ventricular peak (+) dP/dt were also significantly increased (figure 1). During recovery from control exercise, these values (except for heart rate) returned to near the control standing values within 5 min (figure 1).

Fifteen minutes after diltiazem (3 hr and 15 min after administration of atenolol), left ventricular peak (+) dP/dt in dogs standing at rest was significantly decreased (by 10% from that before control exercise), while resting heart rate, left ventricular peak systolic pressure, and left ventricular end-diastolic pressure were unchanged. During the exercise period after drugs, the initial rapid response of hemodynamic parameters was absent and subsequent increase of those variables was markedly attenuated (figures 1 and 2). Heart rate and left ventricular diastolic pressure increased significantly, but the increases were greatly reduced throughout the entire exercise period (both different, p < .01, compared with control exercise). Left ventricular peak systolic pressure and left ventricular peak (+) dP/dt during exercise with atenolol and diltiazem were not different from resting values before exercise.

In four of the eight dogs, the circumflex coronary artery was still open throughout the studies. Blood flow velocity was 28.2 ± 4.6 cm/sec (at rest) before

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control exercise and it was 33.4 ± 6.9 cm/sec at the time of exercise microsphere injection (NS). After atenolol and diltiazem were given, flow velocity in the circumflex coronary artery in dogs at rest was 25.7 ± 5.7 cm/sec (change NS) and it remained unchanged during exercise.

**Myocardial blood flow.** Average blood flow data at standing control, control exercise, and exercise with atenolol and diltiazem in ischemic and control areas, along with subendocardial to subepicardial flow ratios, are presented in table 1. The transmural flow distributions are summarized graphically in figure 3. At standing rest, mean transmural, subendocardial, midmyocardial, and subepicardial blood flows were not significantly different in the ischemic and the control areas.

**Control area.** During control exercise, the blood flow in the normal area increased uniformly in each layer (figure 3), and subendocardial to subepicardial blood flow ratios in the control area were similar at rest and during exercise (table 1). During exercise after drugs myocardial blood flow was higher in the control area than during control standing (except for epicardial blood flow), but it was markedly reduced in all layers compared with that during control exercise (figure 3).

**Ischemic area.** During control exercise, blood flow distribution in the ischemic area was markedly changed. Subendocardial and midmyocardial blood flows decreased by 68% and 38%, respectively, from the standing values, while subepicardial flow exceeded the standing value by 48% (table 1, figure 3). The subendocardial to subepicardial flow ratio in the ischemic area decreased from 1.17 to 0.27 (table 1).

In the ischemic area during exercise after atenolol and diltiazem subendocardial blood flow decreased by 36% from the control standing level, but this value was significantly higher than that during control exercise (approximately twice the control exercise value) (figure 3). Midmyocardial blood flow was slightly higher and subepicardial blood flow slightly lower, but these values were not significantly different from those at rest or during control exercise (figure 3). After atenolol

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**FIGURE 1.** Hemodynamic responses to exercise and subsequent recovery before (closed circles) and after (open circles) administration of atenolol and diltiazem. All parameters, heart rate (HR), left ventricular end-diastolic pressure (LVEDP), left ventricular peak systolic pressure (LVSP), peak positive left ventricular dP/dt, were reduced during the run after administration of atenolol and diltiazem.

**FIGURE 2.** Tracings obtained during a control run (top) and during a run with atenolol and diltiazem (bottom) from one dog. Early into the run, heart rate, ventricular pressure, dP/dt, and wall thickening in both regions increased. Systolic wall thickening in the posterior wall soon decreased progressively to a steady-state level of severe dysfunction, at which time microspheres were injected (MID). During the run with atenolol and diltiazem, the initial increases in all parameters were absent and the deterioration of wall thickening in the posterior wall was less. Also, wall thickening in the ischemic zone returned to control after 1 min of recovery, whereas dysfunction persisted for a longer period after the control run.
and diltiazem, the subendocardial to subepicardial flow ratio in the ischemic area during exercise increased significantly from that during control exercise (0.27 to 0.62; table 1).

Normalized blood flow data in the ischemic area (expressing flow as a decimal fraction of blood flow in the control area) are graphically presented in figure 4. Normalized subendocardial, midmyocardial, epicardial, and mean transmural blood flows in the ischemic zone during exercise after drugs were greatly improved compared with those during control exercise (all \( p < .01 \)) (figure 4). Subepicardial blood flow in the ischemic area during exercise with atenolol and diltiazem was not significantly different from that in the control area (figure 4). Normalized subendocardial flow during exercise after drug administration was reduced by less than 50% (control decrease 84%), and midmyocardial flow was 33% below the control area value (control decrease 70%).

**Regional myocardial function.** Dimension data during control exercise and exercise with atenolol and diltiazem are summarized in figure 5, and the recordings in figure 2 illustrate changes in these measurements during exercise without and with atenolol and diltiazem in a single dog.

**Control wall.** During control exercise, average end-diastolic wall thickness significantly increased by 4.2% over the control standing value. This was accompanied by significant augmentation of systolic wall thickening at steady state from 27.7 ± 5.6% to 32.9 ± 4.9% (figure 5).

<table>
<thead>
<tr>
<th>Ischemia area (n = 8)</th>
<th>Standing</th>
<th>Control exercise</th>
<th>A + D exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENDO</td>
<td>0.96 ± 0.16</td>
<td>0.31 ± 0.14*</td>
<td>0.61 ± 0.30 B, C</td>
</tr>
<tr>
<td>MID</td>
<td>0.96 ± 0.14</td>
<td>0.60 ± 0.24 B</td>
<td>0.82 ± 0.26</td>
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<tr>
<td>EPI</td>
<td>0.83 ± 0.13</td>
<td>1.23 ± 0.42 A</td>
<td>-0.99 ± 0.27</td>
</tr>
<tr>
<td>Mean</td>
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<td>0.71 ± 0.20</td>
<td>0.81 ± 0.23</td>
</tr>
<tr>
<td>ENDO/EPI</td>
<td>1.17 ± 0.15</td>
<td>0.27 ± 0.13 B</td>
<td>0.62 ± 0.29 B, C</td>
</tr>
</tbody>
</table>

Control area (n = 8)

| ENDO                  | 0.99 ± 0.15 | 2.28 ± 0.35 B | 1.23 ± 0.36 A, C |
| MID                   | 0.94 ± 0.16 | 2.28 ± 0.57 B | 1.31 ± 0.41 B, C |
| EPI                   | 0.79 ± 0.16 | 2.04 ± 0.40 B | 1.04 ± 0.33 C |
| Mean                  | 0.90 ± 0.15 | 2.18 ± 0.43 B | 1.19 ± 0.36 A, C |
| ENDO/EPI              | 1.26 ± 0.20 | 1.13 ± 0.17 | 1.20 ± 0.13 |

ENDO = subendocardium; MID = midmyocardium; EPI = subepicardium.

*\( p < .05 \), *\( p < .01 \) compared with standing; \( p < .01 \) compared with control exercise.

After the administration of atenolol and diltiazem, resting systolic wall thickening was somewhat reduced (24.1 ± 5.1%), but it was not significantly different from the standing value before control exercise. However, increases in systolic wall performance during exercise were abolished after atenolol and diltiazem (figure 5); there was no significant increase in percent change in wall thickness during exercise and it was significantly less than that during control exercise.

**Ischemic wall.** During control exercise, the ischemic wall exhibited severe dysfunction in all dogs (figures 2 and 5). End-diastolic wall thickness increased abruptly during the initial phase of exercise but was not different from the control standing value in later phases of exercise or recovery. Percent change in wall thickness decreased from 25.5% during standing rest to 2.7% at the time of microsphere injection, a 90% reduction (figure 5). Mean systolic wall thickening velocity was initially increased (16.6 ± 3.6 to 25.7 ± 8.1 mm/sec, \( p < .01 \)), but then decreased by 1 min of exercise to 2.3 ± 1.9 mm/sec (\( p < .01 \)). During control exercise, the values for percent change in wall thickness and mean systolic wall thickening velocity were not significantly different at 1 min, the midpoint, and late
During exercise, indicating that a steady state of regional myocardial dysfunction had been achieved. Percent change in wall thickness during systole in the ischemic area remained depressed at 5 min after the end of control exercise (p < .05), but was not significantly different from the control standing value by 30 min (76% of control standing value, NS) (figure 5).

At rest after drug administration, the wall was slightly thinner at end-diastole, but the small decrease in percent systolic wall thickening was not significant (figure 5). During exercise after atenolol and diltiazem there was significant improvement in ischemic wall function (figures 2 and 5). End-diastolic wall thickness was less than that during control exercise, and remained unchanged during exercise. Percent systolic wall thickening decreased compared with the standing value (22.6% to 13.7%), but the systolic wall performance was significantly improved compared with that during control exercise, being reduced by 39% from the resting value (figure 5). Mean wall thickness velocity was improved at steady state from 2.3 ± 1.9 to 9.6 ± 4.3 mm/sec (p < .002).

After exercise with atenolol and diltiazem, recovery of systolic wall function from exercise-induced dysfunction was much faster than after control exercise. One minute after the end of exercise, percent change in wall thickness and mean systolic wall thickening velocity had recovered to 90% and 98%, respectively, of standing values before the second exercise bout (differences from control NS) and were significantly improved from those after control exercise (figure 5).

Comparison of combination of atenolol and diltiazem with either drug alone. The findings in the present study were compared with closely similar exercise runs previously reported in which either atenolol27 or diltiazem28 alone was used.

The hemodynamic conditions during control running were nearly identical, the heart rates at steady state exercise averaging 228 ± 30 and 227 ± 33 beats/min in the two previous studies and 227 ± 31 beats/min in the present study, and the peak left ventricular systolic pressures were not significantly different in the three control runs. After administration of atenolol alone, heart rate during running averaged 181 beats/min vs 204 beats/min after diltiazem alone (p <
.01), values significantly different from those obtained in the present combined drug experiment (155 beats/min) (p < .01). With diltiazem alone, peak systolic pressure was not different from that during control exercise, whereas with atenolol alone it was lower; the difference between exercise with atenolol alone (144 mm Hg) and atenolol plus diltiazem (139 mm Hg) was small but significant (p < .01). Peak (+) dP/dt with exercise after diltiazem alone was not different from that during the control run (5982 mm Hg/sec), but was higher than that after atenolol alone (3787 mm Hg/sec, p < .01); with running after atenolol plus diltiazem, peak (+) dP/dt was 3496 mm Hg/sec, which was not significantly different from that after atenolol alone.

The relationship between normalized regional systolic wall thickening (expressed as a percentage of the resting systolic wall thickening) and the normalized subendocardial blood flow (expressed as a percentage of the control area flow) for all three control runs, the runs with atenolol and diltiazem alone, and the combined atenolol and diltiazem study, are summarized in figure 6. The points in the three control runs are not significantly different. The flow-function points during running after atenolol or diltiazem alone were significantly different from those during control running, but not significantly different from each other (p = .90). Comparison of running after atenolol alone with running after atenolol plus diltiazem revealed a highly significant difference (p < .02). The difference between running with diltiazem alone and running after atenolol and diltiazem was also highly significant (p = .025). Therefore, the improvement of the flow-function relationship observed in the present study after administration of atenolol and diltiazem was significantly greater than with either drug alone.

Histologic examination. At autopsy, there was no gross ischemic damage between crystals. Microscopically, myocardial ischemic damage constituted 2.1 ± 2.9% (range 0 to 7.8%) of the inner one-third layer of the wall, and 1.7 ± 2.2% (0 to 5.8%) of the midmyocardium between the crystals in the ischemic wall. There was no ischemic damage in the outer third of the myocardium in the ischemic area. In the ischemic wall section, scar information around the subendocardial crystals was 7.6 ± 4.6% (range 2.3% to 15.1%) of the inner third of the myocardium.

Discussion

In many patients, severe coronary artery stenosis is associated with normal ventricular function at rest, but exercise produces myocardial ischemia and regional myocardial dysfunction. The canine preparation used in the present study was developed previously by our laboratory to simulate the setting in patients with single-vessel coronary artery disease. Exercise in these dogs induces regional myocardial dysfunction and allows measurements of regional myocardial blood flow with radiolabeled microspheres together with determinations of regional wall thickening dynamics. Additionally, in a prior study we demonstrated that regional blood flow and function during exercise-induced ischemia in this preparation are reproducible in runs performed 3 hr apart without interventions. Thus, our preparation permits study of the effects of running before and after pharmacologic intervention. The combination of β-adrenergic blockade with calcium-channel blockade has been used successfully clinically, yet little was known about the interaction of these agents with respect to how they may affect the regional myocardial blood flow-function relationship during exercise.

In four of the eight dogs used in this study, the circumflex coronary artery was open on the day of the study but exhibited decreased blood flow velocity compared with that measured 1 week after surgery. The other four dogs had no antegrade flow on the study.
day. However, no regional myocardial ischemia at rest was detected either by microspheres or by regional myocardial dysfunction in the eight dogs. Therefore, at rest all animals had adequate myocardial perfusion either through a combination of limited antegrade flow with some collateral flow or solely through collateral channels. The induction of collateral development in this preparation is readily detected with use of serial, transient occlusions of the coronary artery after operation by demonstrating progressively less regional myocardial dysfunction and reduced reactive hyperemia after release of the occluder. These observations are similar to those reported previously from this laboratory and others.20, 21, 28, 31, 32

In this preparation, the control exercise period produced profound dysfunction in the posterior left ventricular wall perfused by the circumflex artery. This hypokinesia, found during steady-state exercise, was preceded by a transient phase of increasing and then decreasing regional function in the collateral-dependent zone over the first minute of exercise. The onset of exercise was associated with an abrupt increase in heart rate, left ventricular peak systolic pressure, and left ventricular (+dP/dt) in the initial 5 to 10 sec. Likewise, mean systolic wall thickening velocity in the ischemic zone initially increased and percent change in wall thickness was not reduced. Soon, however, regional wall performance declined in the ischemic zone such that by 1 min into the exercise bout, wall thickening in the posterior wall was reduced and steady at 2.5% (from a resting value of 25.5%). Mean coronary blood flow velocity in the circumflex coronary artery did not increase in the four dogs with a patent vessel, confirming the existence of critical stenosis.

During this control exercise bout, regional myocardial blood flow to the ischemic zone exhibited a marked perfusion abnormality, with an absolute reduction in regional myocardial perfusion to the subendocardium and midwall, and a mild increase in subepicardial flow. The importance of subendocardial perfusion to maintenance of systolic wall thickening has been reported by Gallagher et al.33 and Roan et al.34 in preparations of acute myocardial ischemia. Roan et al., using conscious dogs, showed a relationship between subendocardial blood flow and systolic wall thickening in zones in and around a region supplied by an occluded vessel.34 Vatner35 has described an exponential relationship between regional subendocardial flow and regional segmental function in conscious dogs with various degrees of ischemia. Gallagher et al.33 found the correlation between subendocardial flow and systolic wall thickening to be high and nearly linear in anesthetized and conscious dogs36 during graded coronary stenosis, and this correlation was also shown during exercise.37, 38 Redistribution of flow away from the subendocardium to the subepicardium (transmural “steal”) has been produced previously in exercising dogs with acute partial coronary stenosis39–41 and it was theorized that this phenomenon relates to the limitation of diastolic perfusion time in the subendocardium with metabolic vasodilation in the subepicardium during exercise.39 Responses during the control run in the present experiments in the presence of chronic coronary stenosis were similar.

After the administration of atenolol and diltiazem, the effect of exercise on regional myocardial blood flow in the ischemic zone was substantially altered, with improvement of the distribution of flow across the ventricular wall. This improvement in ischemic zone blood flow was characterized by an increase in the absolute subendocardial blood flow (compared with control exercise), failure of the subepicardial flow to rise significantly, and a large increase in the endocardial/epicardial ratio. This enhancement of ischemic zone perfusion was associated with significant increases in ischemic zone wall thickening and thickening velocity during exercise after administration of atenolol and diltiazem. Although a regional wall thickening abnormality still occurred during this exercise bout, it should be recognized that resting function in the ischemic zone was somewhat reduced after the drugs and that exercise function in the normal zone was also attenuated. Thus, regional wall function during exercise after drugs decreased by only 39% of the resting value (compared with 90% during the control run) and function in the ischemic zone during exercise after drugs was 55% of that in the normal area, whereas it was only 8% of normal function during control exercise.

Figure 6 illustrates the magnitude of the improvement observed with combined therapy compared with that observed previously when diltiazem and atenolol were used separately. In all three studies, the amount of exercise-induced dysfunction that occurred during control exercise as well as the perfusion to the subendocardium of the ischemic zone were closely similar (ischemia during the control run for the combined study being slightly more severe). Since results of repeat runs 3 hr apart in the same dog without drug intervention are highly reproducible,27 this finding supports the validity of comparing the effects of the combined study with those of the two previous experiments. The magnitude of the effects on the normalized subendocardial flow-function points during running

with atenolol or diltiazem alone were similar (figure 6), although the hemodynamic actions of the two drugs during exercise differed markedly. Thus, exercise after diltiazem alone mildly lowered the heart rate and did not affect peak left ventricular pressure, peak left ventricular dP/dt, or percent change in wall thickness in the normal zone, whereas atenolol further lowered exercise heart rate, markedly reduced peak left ventricular pressure and dP/dt, and diminished percent change in wall thickness in the normal zone during exercise. The hemodynamic responses after the combination of the two drugs resembled those after atenolol alone, although the heart rate was lowered significantly further (155 beats/min), suggesting that the heart rate effect of combined therapy may be the main factor causing the enhanced improvement, although the peak left ventricular pressure was 5 mm Hg lower as well. The combination of these two drugs appears approximately additive (figure 6), and it is interesting to note that the data from all three studies appear to fall close to a linear relationship between subendocardial perfusion and transmural wall thickening. This suggests the existence of a close relationship between subendocardial blood flow and wall thickening during exercise-induced ischemia, as has been found with acute coronary stenosis, even when points on the relationship are modified by different pharmacologic interventions.

Numerous mechanisms may be involved in the beneficial effect of atenolol and diltiazem on the regional blood flow-function relationship during exercise-induced regional myocardial ischemia. Atenolol can be presumed to substantially reduce the myocardial oxygen consumption of the left ventricle, thereby reducing an imbalance between regional oxygen supply and demand. The reduction of myocardial oxygen requirements at steady state by atenolol can be predicted from the substantial decreases in exercise heart rate, left ventricular systolic pressure, and contractility (dP/dt); this lowering of oxygen consumption is reflected in the significant reduction in blood flow in the nonischemic zone during exercise after administration of atenolol and diltiazem (figure 3).

We have hypothesized that "relative ischemia" does not exist in the steady-state condition and that regional function will closely follow the amount of absolute ischemia in the subendocardium. Thus, the concept of "relative ischemia" may be applicable only during the initial transient phase of exercise, when regional function exceeds that predicted on the basis of regional perfusion. Consequently, this is necessarily a transient phase until regional function can stabilize at a lower steady-state level, which can be supported by the available regional subendocardial blood flow.

The decrease in heart rate after atenolol and diltiazem caused improved blood flow per beat because of the increase in diastolic perfusion time. Therefore, while mean transmural blood flow was not affected by atenolol and diltiazem, more flow was available per minute and per beat to the subendocardium to support improved contraction, and the transmural flow distribution was improved with an increase in endocardial/epicardial ratio. This was related not only to increased subendocardial blood flow, but also to reduced subepicardial flow because of the decreased myocardial oxygen requirements after the drugs were given. Thus, transmural steal may have been reduced, further supporting enhanced regional performance. This sequence places emphasis on reduced heart rate in explaining the beneficial effect of atenolol on regional perfusion and, consequently, regional function. However, Tomoike et al., studying the effect of propranolol during acute coronary arterial stenosis, found improved ischemic zone shortening and an improved coronary blood flow pattern that was only partially lost by cardiac pacing at the same rate observed before propranolol. Whether or not such an effect might exist during exercise will require further study.

Of the commonly used calcium antagonists, diltiazem has a relatively minimal negative inotropic effect. When studied alone in a preparation identical to the one used in this study, the reduction in heart rate was smaller than that reported for atenolol, and no negative inotropic effect on the normal region was evident. While the contribution of diltiazem to improved regional perfusion of the ischemic zone may include its modest negative chronotropic effects, other mechanisms may be more important. Specifically, the vasodilative action of diltiazem could have increased perfusion to the ischemic zone, either directly or through an increase in blood flow in the coronary collateral vessels supplying that zone. Diltiazem alone also results in a lower left ventricular end-diastolic pressure during exercise-induced ischemia, which could enhance subendocardial perfusion. In addition, the transmural maldistribution of blood flow could have been reduced by diltiazem. Bache and Tockman have reported that nifedipine limits reactive hyperemia after coronary occlusion and inhibits the redistribution of blood flow away from the subendocardium during coronary arterial stenosis. Therefore, diltiazem could act primarily to augment absolute blood flow to the ischemic zone and improve its distribution within that region.

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The possibility exists that, in addition to increased perfusion, reduced afterload during exercise also could have contributed to improved wall motion in the ischemic zone, since left ventricular peak systolic pressure was 22 mm Hg lower during exercise after drug administration than during control exercise. However, regional function in the normal zone was substantially reduced during running after the drugs compared with during the control run, suggesting lack of a major role of reduced afterload per se in the increase in wall motion.

In conclusion, we have demonstrated a substantial improvement in the regional myocardial blood flow-function relationship during exercise-induced ischemia by combined use of a β-blocker and a calcium channel–blocking drug. The improved regional myocardial performance observed in the ischemic zone appears to relate to a more favorable blood flow distribution within the ischemic zone, with an increase in absolute and relative subendocardial blood flow, and a reduction in subepicardial flow. The additive effect of metoprolol and diltiazem, compared with that of either drug alone, probably relates to differing mechanisms for augmenting flow to the ischemic zone. Our finding of significant improvements in both perfusion and performance in zones subject to ischemia during exercise stress in a conscious canine preparation may provide an objective basis for the use of these agents in combination clinically for the treatment of patients with exercise-induced angina pectoris.

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Effect of the combination of diltiazem and atenolol on exercise-induced regional myocardial ischemia in conscious dogs.
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