Myocardial Hypertrophy in the Ischemic Zone Induced by Exercise in Rats After Coronary Reperfusion

Byung-Hee Oh, MD, PhD; Shiro Ono, MD; Howard A. Rockman, MD; and John Ross Jr., MD

Background. Factors influencing left ventricular (LV) remodeling after coronary artery reperfusion, including adaptive changes in the infarcted region and the role of exercise, have not been well defined. The common application of early reperfusion by thrombolysis after acute myocardial infarction lends potential significance to such remodeling, and a rat model with 45 minutes of regional ischemia followed by reperfusion was developed to study these events. We postulated that the effects of reperfusion in altering LV morphology would be further modified by exercise training, including induction of hypertrophic changes in the outer region of the nontransmural infarction.

Methods and Results. Female Sprague-Dawley rats were subjected either to 15 minutes of left anterior descending coronary artery occlusion followed by reperfusion or to sham operation, and at 5 days after the operation, animals were randomly assigned to sedentary conditions or to 3 weeks of swimming exercise. Animals completing the experiment included a reperfused sedentary group (n=21), a reperfused exercised group (n=20), a sham-operated group (n=10), and a sham-operated group subjected to exercise (sham exercised group, n=9). In addition, in seven rats, myocardial infarction was produced by permanent coronary occlusion, and the animals remained sedentary (permanent occlusion group). In each group, the morphology of the noninfarcted (septal) and the infarcted (anterolateral) regions of the left ventricle was examined 26 days after surgery from midventricular transverse sections 25 μm thick taken after perfusion fixation of the heart at an aortic pressure of 60 mm Hg and an LV cavity pressure of 10 mm Hg. Compared with the permanent occlusion group, LV cavity area in the sedentary reperfused group was smaller (33.5 versus 53.2 mm², p<0.001), the infarcted wall was thicker (1.36 versus 0.53 mm, p<0.001), and the septal wall also was thicker (1.95 versus 1.62 mm, p<0.05), whereas compared with the sham-operated group, the LV cavity area was increased, and infarcted wall thickness was reduced (both p<0.01). Reperfusion resulted in less transmurality of infarction compared with permanent occlusion (38.3% versus 69.5%, p<0.001), with increased subepicardial area in the infarcted zone after reperfusion (7.5 versus 1.9 mm², p<0.001). In the reperfused exercised group, transmurality was further decreased compared with the reperfused sedentary group (31.5% versus 38.3%, p<0.05), and the viable subepicardial area of the infarct zone increased by 32%.

Conclusions. Important remodeling of global and regional LV morphology was evident at 26 days in sedentary rats after 45 minutes of coronary occlusion with reperfusion compared with rats with permanent coronary occlusion, with reduced infarct transmurality and less LV dilation in the reperfused group. Exercise after reperfusion further affected ventricular remodeling by causing hypertrophy with increased wall thickness of the surviving subepicardium of the infarcted zone. (Circulation 1993;87:598–607)

Key Words • left ventricular remodeling • hypertrophy • occlusions • reperfusion

Myocardial infarct expansion, left ventricular (LV) dilation, and compensatory myocardial hypertrophy in noninfarcted regions are involved in late ventricular remodeling after coronary occlusion, and these events are important in determining LV function as well as long-term prognosis in patients after acute myocardial infarction.1-7 LV dilation, which can serve to maintain the stroke volume, and myocardial hypertrophy, which can normalize wall stress, appear to be important compensatory mechanisms that follow the loss of functional myocardium and associated infarct expansion.6,7 With the advent of clinical thrombolysis and early coronary angioplasty for reducing the extent of damage early after acute myoc...
cardial infarction, increasing numbers of patients exhibit nontransmural myocardial infarction, but the effect of coronary artery reperfusion on LV remodeling has not been well defined.

Compensatory hypertrophy in noninfarcted regions away from the infarct site and tissue loss in the infarcted area have been observed to occur over several weeks after permanent coronary occlusion in dogs, and permanent coronary occlusion is associated with infarct expansion in other species, such as rats, that have little or no collateral circulation. After 2 hours of coronary occlusion followed by reperfusion in intact instrumented dogs, however, substantial recovery of contraction in the infarcted region has been observed over a 2-4-week period. Experimentally, a change in remodeling related to reduction in infarct expansion after coronary occlusion followed by reperfusion has been described in rats. However, whether hypertrophy of residual areas of surviving myocardium within the infarcted region and its outer wall can occur and contribute to the functional recovery of the infarcted region after nontransmural myocardial infarction has not been examined. In a recent report, we described marked myocardial cell hypertrophy in the inner and outer walls of the infarcted region in dogs after 2 hours of coronary occlusion followed by reperfusion for 3 weeks, associated with partial recovery of regional function during that period.

Accordingly, in the present study, we developed a model in the rat that has allowed comparison of regional ventricular morphology in the presence of nontransmural infarction several weeks after coronary occlusion with reperfusion compared with transmural infarction. The effects of exercise on infarct expansion and LV dilation after permanent coronary occlusion have been variable, depending on the animal model and type of exercise, but information is not available about the effect of exercise on remodeling after nontransmural myocardial infarction. Therefore, in this model we also studied global and regional LV remodeling after 45 minutes of coronary occlusion with reperfusion in sedentary rats compared with that in animals exercised for 3 weeks.

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 and Surgical Preparation

Female Sprague-Dawley rats weighing 250–300 g were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg body wt i.p.), xylazine (10 mg/kg i.p.), and morphine sulfate (5 mg/kg i.p.). After adequate anesthesia, all animals were placed in the supine position on a table heated by circulating warm water, intubated, and ventilated under positive pressure with a rodent ventilator (model 683, Harvard Apparatus). Under a dissecting microscope, a left thoracotomy was performed in the fourth intercostal space, and the pericardium was opened. The left coronary artery (which is intramural) was encircled within the myocardium between the left atrial appendage and right ventricular outflow tract with a curved needle and 6-0 silk suture. In sham-operated animals, the left coronary artery was not ligated, and in others it was ligated permanently. In other animals, after pilot studies to determine an appropriate period of occlusion to induce nontransmural infarction, the myocardium was reperfused after 45 minutes of occlusion by cutting the ligature around the left coronary artery. For reperfusion, a small piece of plastic foam was tied between the ligature and the myocardium to facilitate cutting of the ligature and to minimize direct injury to the myocardium and artery. The occurrence of distinct color change of the myocardium upon reperfusion, the appearance of reperfusion arrhythmia, or both were considered to indicate reperfusion. The chest was closed in layers and the pneumothorax evacuated.

Experimental Protocol

After surgery, animals were caged in proportion to size, given water and standard rat chow ad libitum, and housed in a climate-controlled environment subjected to 12-hour light/dark cycles. Five days after surgery, reperfused rats and sham-operated rats were randomly assigned to several groups, some of which were exercised by regular swimming: group 1, reperfused sedentary; group 2, reperfused exercised; group 3, sham-operated sedentary; group 4, sham-operated exercised. A separate group of rats with permanent occlusion (group 5) remained sedentary.

The swimming exercise was commenced 5 days after surgery with a single 15-minute swimming period daily. The duration of the exercise period was increased by 5 min/day up to 60 minutes and then continued 7 days a week for 3 weeks until the end of the study. Rats were allowed to swim in groups of five to 10 animals in a 50-cm-deep plastic tub containing water heated to 33–35°C.

Postmortem and Histological Preparations

Twenty-six days after surgery, the rats were killed, and the myocardium was preserved as follows: Under the same anesthesia as used for the previous surgery, rats were mechanically ventilated. The thorax was opened, polyethylene catheters (PE 200) were introduced into the left ventricle (via the left atrial appendage) and into the descending aorta retrogradely, and the heart was arrested with a 2–3-ml injection of saturated KCl solution into the right atrium. After aortic perfusion with heparinized saline (10,000 units/l) for 2–3 minutes to wash out the blood, the myocardium was perfused retrogradely from the aorta with 10% phosphate-buffered formalin at a constant pressure of 60 mm Hg for 20–30 minutes. The right atrium and pulmonary artery were opened to decompress the right ventricle during fixation. To maintain the left ventricle filled throughout fixation and to compare results among groups, the LV intracavitary pressure was maintained at 10 mm Hg by use of the catheter introduced via the left atrial appendage.

After hardening, the heart was excised and immersed into 10% buffered formalin solution for 24 hours. Subsequently, the atria and adhesions were carefully dissected away, and the right and left ventricles were separated and weighed, the interventricular septum being included with the left ventricle. To compare heart weights among groups of animals, especially between the exercised and nonexercised groups, heart weights were normalized by the length of tibia as well as body weight. Tibial length has been reported to be a better
measure of body size, especially under conditions in which the body weight changes, such as exercise.22 Even in weight-matched groups, the variance was smaller when tibial length was used compared with body weight.22 The right tibia was dissected, and its length from the condyles to the tip of the medial malleolus was measured with a micrometer caliper using the method of Yin et al.22 There were close linear relations when heart weights were normalized by tibial length or body weight ($r=0.893–0.975$, $\text{SEE}=0.255–1.311$, $p<0.001$).

**Figure 1.** Color photographs of representative transverse slices of the mid left ventricles in four groups of rats: sham operated (Sham), permanent coronary occlusion (PCO), reperfusion (RP), and reperfusion plus exercise (RP+EX). Milligan's trichrome stain. Original magnification at 1 cm scale shown.
After being kept for more than 2 days in 10% buffered formalin solution, the whole left ventricle was embedded in paraffin. Transverse serial sections 25 μm thick were cut, and every 40th section from the apex to the base (every 1 mm) was mounted and stained with Milligan’s trichrome. Between nine and 12 slides were obtained from each heart.

Morphometric Analyses

On microscopic examination of 25-μm-thick sections, the trichrome stain allowed ready visualization of viable or hypertrophied myocardium (pink) and the scarred region (blue) (Figure 1). The slides were analyzed blindly, and the findings of two independent observers were compared in a large subset. The slides were projected with a microprojector (Jena, Germany) at a magnification of ×17.3. The infarcted area and the endocardial and epicardial borders of the left ventricle were traced on paper, and the following areas were measured by computerized planimetry (Hewlett-Packard digitizer, Sketchpro): 1) areas within the infarction zone, including the infarct and spared epicardial and endocardial areas; 2) the area of the LV cavity and of the entire myocardial ring; and 3) the average thicknesses (measurements taken from radii every 15°) of the noninfarcted (septal) and infarcted (anterolateral) walls, as shown diagrammatically in Figure 2. Measurements were performed on two midventricular slices (5 and 6 mm from the apex) and averaged.

Infarct size was expressed as a percentage of infarcted scar to total LV ring area. Transmurality of the infarct was defined as the ratio of the scar area to the area of the entire wall encompassed by the infarcted region, as subtended by the lateral margins of the infarct (sum of infarcted scar, epicardial, and endocardial areas) (Figure 2). Since in rats the LV free wall is ischemic and infarcted and the interventricular septum is usually spared after left coronary artery occlusion, the thickness of the interventricular septum was considered to represent the noninfarcted wall. The ratio of the LV dimension to the average thickness of either the infarcted or noninfarcted wall was calculated to estimate relative changes of regional LV diastolic wall stress according to the law of Laplace,16,23 since the heart was fixed at a common distending pressure.

Comparison of Measurements Between Midventricular Slices Versus All Slices

to determine whether the average of measurements from two midventricular slices adequately represented those using all slices of the heart, in 13 animals the LV cavity area, absolute infarcted scar area, percent infarct size, and the spared epicardial area within the infarcted zone were measured using all the slides from each heart and from the average of two midventricular slices. The LV cavity area measured from the midventricular slices showed a close linear relation with the sum of the area measurements from all heart slices (r=0.971, SEE=2.602, p<0.001) and also with LV volumes calculated with a modified Simpson’s method (r=0.965, SEE=2.861, p<0.001) (Figure 3A). Percent infarct sizes measured by the two methods were also linearly correlated (r=0.886, slope=0.953, SEE=2.655, p<0.001) (Figure 3B). The subepicardial areas (r=0.805, SEE=1.469, p<0.002) and absolute infarct areas (r=0.866, SEE=1.127, p<0.001) from midventricular slices and the sum of all heart slices also showed good linear correlations.

Interobserver Variation

Interobserver variation was assessed by observing the relations between blinded measurements by two independent observers. There were significant linear relations between these measurements (p<0.001 for each variable), and the ranges of the slopes of linear regressions (0.850–1.014), r values (0.916–0.999), and SEE were low. Interobserver variations for the LV cavity areas and subepicardial areas are shown in Figures 3C and 3D.

Statistics

All values are expressed as mean±SD. Unpaired t tests were used to compare morphological data between infarct-related variables in the animals with permanent coronary occlusion with those in the sedentary reperfused group and to compare data in the reperfused animals with and without exercise. A Bonferroni correction was applied to adjust the values of p for multiple comparisons. A two-way ANOVA was used to examine the effects of reperfusion (sham-operated versus reperfusion) and exercise (exercised versus sedentary) on variables related to global LV remodelling only, such as the LV cavity area and the heart weight/tibial length ratio, since no infarction was present in the sham-operated groups. If there was a significant interaction between the effects of reperfusion and the effects of exercise, further examination of selected pairwise contrasts was undertaken to establish where any differences
existed. The values of p for these multiple post hoc comparisons were corrected by the Bonferroni method. When there was no interaction effect, it can be assumed that any effect of exercise was the same in reperfused and sham-operated hearts or that any difference between the reperfused hearts and sham-operated hearts was the same regardless of exercise. Values of p<0.05 were considered statistically significant.

Results

Operative mortality of the surgical procedure in the reperfused groups was 17%, including early deaths within 24 hours. Operative mortality for the permanent occlusion group was 30%. Three rats in the reperfusion group died during swimming exercise, all of the deaths occurring on the first day of exercise. Of the 70 rats that completed the experimental protocol, 67 hearts were used for the analysis (60 rats in the randomization protocol and seven subjected to permanent occlusion). One heart in the sham-operated group was excluded because of the presence of an unexpected transmural myocardial infarction; two hearts with reperfusion were excluded (one each in the reperfused sedentary and reperfused exercised groups) because of absence of significant infarction; there was only minor scarring of the subepicardium, which appeared to result from the sutures rather than from infarction secondary to permanent coronary occlusion.

Heart Weights

The left and right ventricular weights and their values normalized by tibial length are summarized in Table 1. Differences in values among groups were not statistically significant.

Coronary Reperfusion Compared With Sham and Permanent Occlusion in Sedentary Animals

The LV morphological data at 26 days after surgery are summarized in Table 2, and representative LV slices from each group are shown in Figure 1. Forty-five minutes of left coronary artery occlusion followed by reperfusion produced nontransmural myocardial infarction with sparing of a considerable amount of subepicardium as well as a thin rim of endocardium in the

![Figure 3. Scatterplots showing linear relations between left ventricular (LV) cavity area from midventricular slices (mid) versus calculated LV volume using all slices of the heart (panel A) and between percent infarct sizes calculated from midventricular slices and from all slices (panel B). Close linear correlations existed between LV cavity areas (panel C) and subepicardial areas (panel D) measured by two independent observers. SEE, standard error of estimate.]

<p>| Table 1. Heart Weights and Heart Weight/Tibial Length Ratios in Five Groups of Rats |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>PCO (n=7)</th>
<th>Sedentary (n=21)</th>
<th>Exercise (n=20)</th>
<th>Sedentary (n=10)</th>
<th>Exercise (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV (mg)</td>
<td>779±80</td>
<td>817±88</td>
<td>848±89</td>
<td>782±104</td>
<td>821±87</td>
</tr>
<tr>
<td>RV (mg)</td>
<td>242±113</td>
<td>181±39</td>
<td>182±27</td>
<td>163±22</td>
<td>188±36</td>
</tr>
<tr>
<td>TL (mm)</td>
<td>39.1±0.5</td>
<td>40.1±0.63</td>
<td>39.8±0.9</td>
<td>39.8±1.0</td>
<td>39.7±0.6</td>
</tr>
<tr>
<td>LV/TL (mg/mm)</td>
<td>19.9±2.0</td>
<td>20.4±2.3</td>
<td>21.3±2.4</td>
<td>19.6±2.2</td>
<td>20.7±2.2</td>
</tr>
<tr>
<td>RV/TL (mg/mm)</td>
<td>6.2±2.9</td>
<td>4.5±1.0</td>
<td>4.6±0.7</td>
<td>4.1±0.5</td>
<td>4.7±0.9</td>
</tr>
</tbody>
</table>

PCO, permanent coronary occlusion group; reperfusion, reperfusion group with 45 minutes of left coronary artery occlusion; sham, sham-operated group; reperfusion/sedentary, reperfusion without exercise group; reperfusion/exercise, reperfusion with exercise group; sham/sedentary, sham without exercise group; sham/exercise, sham plus exercise group; LV, left ventricular weight; RV, right ventricular weight; TL, tibial length. All values are mean±SD.
Table 2. Changes of Left Ventricular Topography 26 Days After Surgery in Five Groups of Rats

<table>
<thead>
<tr>
<th></th>
<th>PCO (n=7)</th>
<th>Sedentary (n=20)</th>
<th>Exercise (n=21)</th>
<th>Sedentary (n=10)</th>
<th>Exercise (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV cavity area (mm²)</td>
<td>53.2±5.0*</td>
<td>33.5±8.9§</td>
<td>39.1±6.5‡</td>
<td>28.0±6.7</td>
<td>31.2±6.4</td>
</tr>
<tr>
<td>LV dimension (mm)</td>
<td>8.19±0.36*</td>
<td>6.43±0.87§</td>
<td>7.01±0.63§</td>
<td>5.92±0.82</td>
<td>6.25±0.65</td>
</tr>
<tr>
<td>Total myocardial area (mm²)</td>
<td>37.2±7.0†</td>
<td>48.6±6.1</td>
<td>50.7±5.1</td>
<td>50.0±7.2</td>
<td>53.3±9.9</td>
</tr>
<tr>
<td>Non-inf area (mm²)</td>
<td>26.3±6.6</td>
<td>31.3±6.0</td>
<td>30.5±6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct scar area (mm²)</td>
<td>7.6±1.0</td>
<td>6.6±2.2</td>
<td>6.3±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi-inf area (mm²)</td>
<td>1.9±0.7*</td>
<td>7.5±3.2</td>
<td>9.9±3.9‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endo-inf area (mm²)</td>
<td>1.4±0.6*</td>
<td>3.3±1.2</td>
<td>4.0±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi+endo area (mm²)</td>
<td>3.3±0.7*</td>
<td>10.8±4.0</td>
<td>13.9±3.3#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent infarct (%)</td>
<td>21.0±5.1†</td>
<td>13.8±5.0</td>
<td>12.6±3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmurality (%)</td>
<td>69.5±4.2*</td>
<td>38.3±8.6</td>
<td>31.5±7.1**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninfarcted (septal) wall</td>
<td>1.62±0.29‡</td>
<td>1.95±0.29</td>
<td>1.81±0.23</td>
<td>1.80±0.36</td>
<td>1.82±0.32</td>
</tr>
<tr>
<td>Infarcted (lateral) wall</td>
<td>0.53±0.1*</td>
<td>1.36±0.29</td>
<td></td>
<td>1.51±0.34</td>
<td>2.02±0.33</td>
</tr>
<tr>
<td>LV dim/wall thickness ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninfarcted wall</td>
<td>5.23±1.18†</td>
<td>3.40±0.83</td>
<td>3.95±0.69</td>
<td>3.47±0.98</td>
<td>3.53±0.69</td>
</tr>
<tr>
<td>Infarcted wall</td>
<td>15.98±3.17*</td>
<td>4.99±1.55</td>
<td></td>
<td>4.91±1.35</td>
<td>3.05±0.81</td>
</tr>
</tbody>
</table>

PCO, permanent coronary occlusion group; reperfusion, group on which reperfusion was performed after 45 minutes of left coronary artery occlusion; sham, sham-operated group; reperfusion/sedentary, reperfusion without exercise group; reperfusion/exercise, reperfusion plus exercise group; sham/sedentary, sham without exercise group; sham/exercise, sham plus exercise group; LV, left ventricle; non-inf, noninfarcted zone; epi-inf, subepicardial area in the infarcted zone; endo-inf, endocardial area in the infarcted zone; epi+endo, sum of subepicardial and endocardial areas in the infarcted zone. All values are mean±SD.

* p<0.001, † p<0.01, § p<0.05 in PCO vs. sedentary reperfusion group (t test).
§ p<0.01, † p<0.001 main effect reperfusion sedentary vs. sham sedentary (ANOVA).
¶ p<0.05 main effect reperfusion exercise vs. sham exercise (ANOVA).
** p<0.05, *** p<0.01 exercise vs. sedentary in reperfusion animals (t test).

infarcted zone. Compared with the sham-operated sedentary group, the reperfused sedentary group showed significant increases in LV cavity area (Figure 4A) and in cavity dimension (Table 2), and the infarcted (lateral) wall was thinner (Figure 4B). Compared with the sham group, the ratio of LV cavity dimension to infarcted wall thickness was increased in the reperfused sedentary group (p<0.001), whereas this ratio in the noninfarcted (septal) wall was not different (Table 2).

Compared with the permanent occlusion group, the LV cavity area was much smaller in the reperfused sedentary group (33.5 versus 53.2 mm²) (Figure 4A), and the LV cavity dimension was also smaller (Table 2). Reperfusion was associated with a thicker noninfarcted wall (1.95 versus 1.62 mm), and the thickness of the infarcted wall was much greater (1.36 versus 0.53 mm) (Figure 4B). The percent infarct size was reduced (13.9% versus 21.0% of the total myocardial area), and there was much more sparing of the epicardial and endocardial areas in the reperfused sedentary group than in the permanent occlusion group (Figure 5 and Table 2). Average transmurality was 38.3% in the reperfused sedentary group, much less than in the permanent occlusion group (69.5%); some subendocardial sparing also occurred after reperfusion compared with permanent occlusion (Table 2).

Effects of Exercise on LV Remodeling After Coronary Reperfusion

Swimming exercise increased the LV cavity areas and cavity dimensions significantly (p<0.05, Table 2, Figure 4A). There was no significant interaction, which indicates that the extent of the increases caused by exercise was about the same in both the reperfused exercised and sham-operated exercised groups. Exercise had no significant effect on total myocardial area, wall thickness (Figure 4B), or the LV dimension/wall thickness ratio.

In the reperfused exercised group, the subepicardial area of the infarcted region was significantly increased (by 32%) compared with the reperfused sedentary group (Figure 5); the increase observed in the endocardial area just missed statistical significance, but the total area of spared myocardium in the infarcted region was larger (the sum of the subepicardial and endocardial areas) compared with the reperfused sedentary group (Table 2). Primarily because of increased subepicardial area in the infarcted region, exercise significantly reduced transmurality in the reperfused exercised group compared with the reperfused sedentary group (from 38.3 to 31.5%); the noninfarcted myocardial areas were not significantly different (Table 2). These findings suggest that exercise-induced regional hypertrophy of surviving myocardium occurred in the infarcted region after nontransmural myocardial infarction.

The average absolute areas of the scars were not different between reperfused sedentary and reperfused exercised groups (Figure 5), and the distributions of the infarct areas were closely similar between these two groups (Figure 6).

Discussion

In the rat, the coronary collateral circulation is minimal or absent, and coronary occlusion produces myo-
nontransmural infarction after reperfusion so as to study subsequent LV remodeling and whether or not it can be modified by exercise. We were successful in producing nontransmural infarction with 45 minutes of coronary occlusion with reperfusion.

Effects of Reperfusion in the Rat

Most previous studies in rats have focused on LV remodeling after permanent coronary occlusion. A few studies in rats have used transient left coronary occlusion to evaluate infarct development or to examine the effect of reperfusion on LV topography. However, these studies focused mainly on the beneficial effect of late reperfusion, independent of myocardial salvage, on LV remodeling after 90 minutes or 2 hours of coronary artery occlusion. In some experiments in rats, a 30-minute period of coronary occlusion was used followed by reperfusion, which prevented LV dilation and decreased thinning of the infarcted wall compared with permanent occlusion, although the size of the infarcted region was not assessed.

We chose a 45-minute occlusion period for the nontransmural infarction model because there was significant sparing of subepicardial myocardium in pilot studies, yet the infarction was still substantial. Hale and Kloner reported a midepicardial to subepicardial location of infarction after 20–60 minutes of coronary artery occlusion in rats, but the infarction in the present

nontransmural infarction after reperfusion so as to study subsequent LV remodeling and whether or not it can be modified by exercise. We were successful in producing nontransmural infarction with 45 minutes of coronary occlusion with reperfusion.

Effects of Reperfusion in the Rat

Most previous studies in rats have focused on LV remodeling after permanent coronary occlusion. A few studies in rats have used transient left coronary occlusion to evaluate infarct development or to examine the effect of reperfusion on LV topography. However, these studies focused mainly on the beneficial effect of late reperfusion, independent of myocardial salvage, on LV remodeling after 90 minutes or 2 hours of coronary artery occlusion. In some experiments in rats, a 30-minute period of coronary occlusion was used followed by reperfusion, which prevented LV dilation and decreased thinning of the infarcted wall compared with permanent occlusion, although the size of the infarcted region was not assessed.

We chose a 45-minute occlusion period for the nontransmural infarction model because there was significant sparing of subepicardial myocardium in pilot studies, yet the infarction was still substantial. Hale and Kloner reported a midepicardial to subepicardial location of infarction after 20–60 minutes of coronary artery occlusion in rats, but the infarction in the present
study was typically midendocardial to subendocardial in location with more sparing of subepicardial than subendocardium (Figure 1). The present study cannot be directly compared with that of Hale and Kloner, because in that study measurements were made at 24 hours rather than 26 days after surgery, and tetrazolium staining was used to evaluate the infarction.

We demonstrated significant sparing of subepicardium and a thin rim of subendocardium at 26 days after 45 minutes of coronary artery occlusion followed by reperfusion, and such infarction associated with reperfusion was shown to cause an increase of LV cavity area compared with the sham-operated groups; however, the increase was much less severe than after permanent coronary occlusion. The lack of significant differences in the heart weight/tibial length ratio between the reperfused, the permanently occluded, and the sham-operated groups, despite considerable tissue loss and a thinner infarcted wall, particularly in the permanent occlusion group, provides suggestive evidence of compensatory hypertrophy of surviving myocardium. Extensive loss of tissue in the permanent occlusion group probably also explains the similar area of the infarct scar region compared with the reperfused sedentary groups (Table 2). The occurrence of hypertrophy in noninfarcted regions in the permanent occlusion group is supported by several reports by others of hypertrophy of myocardial fibers in noninfarcted and border zones in rats after permanent coronary occlusion. Our finding of lack of a significant LV weight/tibial length change is similar to that of Pfeffer et al., although that group reported decreased LV/body weight ratio when there was extensive infarction. Different infarct sizes and different timing of measurements may explain some variation in results, since heart weight, especially the LV weight, depends on the balance between tissue loss in the infarcted region and compensatory hypertrophy of surviving myocardium. The trend toward a slightly thicker noninfarcted (septal) region in the reperfused sedentary group, although not statistically significant compared with that in the sham-operated group, supports the possibility of mild myocardial hypertrophy in the noninfarcted zone. The increased wall thickness in the noninfarcted zone in reperfused hearts compared with that in hearts with permanent coronary occlusion is probably related to prevention by reperfusion of the effects of a large infarction to cause marked LV chamber dilation, with inadequate hypertrophy and elevated wall stress, leading to thinning of the noninfarcted wall.

Whether or not progressive compensatory hypertrophy occurred in the infarcted zone in the reperfused sedentary group cannot be determined, since we observed the heart only at one time point 26 days after infarction. However, increased cell size within the infarcted zone and in the subepicardial region of the infarct zone after nontransmural myocardial infarction with coronary reperfusion for 3 weeks in dogs recently has been observed in this laboratory, supporting the possibility that hypertrophy of surviving myocardium occurs in the infarcted region as well as remote regions. Considering that even late reperfusion can decrease the extent of infarct expansion independent of myocardial salvage and contribute to healing of the myocardium, hypertrophy of residual outer wall myocardium or islands of surviving myocytes in the infarcted zone after nontransmural myocardial infarction might be beneficial in preventing progression of an unfavorable remodeling process.

The LV dimension/wall thickness ratio can reflect relative changes of diastolic wall stress, a major determinant of eccentric ventricular hypertrophy. This ratio was elevated in the reperfused sedentary group in the infarcted wall compared with the sham group, which could stimulate hypertrophy in that region, whereas a normal value of this ratio in the noninfarcted wall suggests the occurrence of adequate compensatory hypertrophy. Elevated ratios of LV dimension to wall thickness in both the infarcted and noninfarcted areas within the permanent occlusion group suggest inadequate compensatory hypertrophy in both regions and may promote progressive LV dilatation.

Effect of Swimming Exercise on LV Remodeling

Inconsistent effects of exercise on LV remodeling after permanent coronary occlusion have been observed, although experimental protocols and types of exercise differed. However, the effects of exercise on LV remodeling after coronary reperfusion, especially in the infarcted zone, have not been assessed. We chose swimming because it is convenient to perform in a large number of animals, and it has been reported to increase cardiac mass significantly more than treadmill exercise. The histopathological changes after myocardial infarction occur approximately twice as fast in rats as in humans, with complete healing and scar formation by 3 weeks after infarction in the rat. Therefore, we decided to commence the exercise intervention 5 days after surgery so as to reduce early risk (a longer delay is used in the clinical setting) and to terminate the experiment after 3 weeks of exercise.

Swimming exercise after permanent coronary occlusion in rats can induce thinning of the infarcted wall but in the present study, the infarcted wall was not thinner in the reperfused exercised group than in the reperfused sedentary group. Such a difference seems to be primarily a result of exercise-induced outer wall thickening in this model of nontransmural myocardial infarction (Table 2, Figure 5). Exercise in normal rats is known to increase LV chamber volume, and a trend toward increased cavity area with exercise was noted in the sham-operated animals; LV cavity area was significantly increased by exercise in the reperfused group compared with that in the exercised sham-operated animals. The latter finding is probably related to the fact that LV cavity area was significantly larger in the sedentary reperfused group than in the sedentary sham-operated groups. However, the trend toward a larger cavity area in the reperfused exercised group compared with the reperfused sedentary group was not statistically significant. We would expect LV function at rest to be somewhat reduced by the nontransmural infarction, accompanied by the observed increase in LV cavity area in the sedentary reperfused group, and it may be postulated that exercise-induced hypertrophy in the ischemic zone contributed to limiting any significant further increase in cavity size produced by exercise compared with that caused by exercise in normal animals.

We postulate that the significant increase of 32% in the area of surviving epicardial myocardium with 3 weeks of swimming exercise in the reperfused exercised
group was primarily a result of hypertrophy of surviving myocardium in the outer wall. Such regional hypertrophy caused by exercise has not been demonstrated directly after acute myocardial infarction, although the occurrence of hypertrophy without exercise is shown by increased cell size in the infarcted wall after reperfusion for 3 weeks in dogs, as mentioned.\textsuperscript{18} Myocardial hypertrophy in the infarcted zone may play an important role not only in preventing further infarct expansion but also by explaining, at least in part, the functional recovery after acute myocardial infarction with reperfusion, in addition to the early recovery from reversal of stunning after reperfusion.\textsuperscript{18} Also, after permanent coronary occlusion in rats, Litwin et al.\textsuperscript{19} recently demonstrated reduced LV dilation and improved systolic function when myocardial hypertrophy was induced in noninfarcted regions with an inhibitor of long-chain fatty acid oxidation.

The mechanism for this hypertrophy is unknown. Elevated levels of epinephrine and norepinephrine in plasma and myocardial tissue accompany swimming exercise, and myocyte hypertrophy in the nonischemic region in the absence of exercise may be reduced by α\textsubscript{1}-adrenergic blockade.\textsuperscript{40} findings that suggest that sympathoadrenal activation is associated with exercise-induced myocardial hypertrophy. Both increased regional wall stress and augmented sympathoadrenal activity associated with swimming exercise might be involved in provoking the regional hypertrophic response, an issue addressed further in other studies on the effects of β-adrenergic blockade with exercise in this reperfusion model in the rat.\textsuperscript{41}

Several technical aspects of the methods used in this study should be considered. The long-term course of the global and regional LV morphological changes that we observed after coronary reperfusion are unknown, since measurements were made only at one time point, 26 days after coronary occlusion. However, in contrast to the progressive nature of the LV remodeling after transmural myocardial infarction,\textsuperscript{30} it seems unlikely that progressive LV dilation would occur, because the determinants of diastolic wall stress were largely unchanged in the reperfused exercised group.

We obtained thick serial sections with the entire left ventricle fixed in formalin to analyze gross anatomic changes accurately, an approach not suitable for cell size measurements. Cell size changes may be relatively small after exercise as well as after mild to moderate infarction,\textsuperscript{42} and with large infarctions in sedentary rats, they can involve changes predominantly in cell length.\textsuperscript{26,43} Because the period of exercise in our study was only 3 weeks, such an analysis would probably require large numbers of animals and a different set of experiments to detect regional cell size changes; cell size measurements in multiple regions of the left ventricle after pressure overload hypertrophy have been made with collagenase perfusion to obtain isolated fixed myocytes,\textsuperscript{44} and studies that allow examination of large numbers of cells in our model of future experiments. The evidence for regional hypertrophy is strong, however, judging from the findings of the present study. Edema occurs relatively early after reperfusion (within 48 hours),\textsuperscript{45} and infarct healing in the rat is complete by 3 weeks.\textsuperscript{37} Therefore, in adult rats, the only likely mechanism for the observed significant increase in subepicardial area would be myocyte hypertrophy. In this connection, in rats studied 4 weeks after transmural myocardial infarction, larger myocytes were found adjacent to the lateral border of the infarct than in more distant regions.\textsuperscript{28}

Whether or not exercise training is likely to have favorable effects on LV morphology and function in some patients after thrombolysis for acute myocardial infarction cannot be answered with certainty on the basis of this study, although the morphological changes produced by exercise in our study suggest the potential for a beneficial effect. We did not obtain hemodynamic and functional data in this model of nontransmural myocardial infarction, although baseline hemodynamic findings, especially cardiac output and the LV filling pressure, have previously been reported to be altered in the rat only with extensive transmural myocardial infarction.\textsuperscript{45} A study is under way to evaluate the effects of regional hypertrophy on global and regional ventricular function in this rat model of coronary occlusion with reperfusion.

Acknowledgments

We are indebted to Denice J. Brannigan and Abdul-Wahaab Farid for their expert technical assistance, to Elizabeth Gilpin for her statistical advice, and to Sharon Perry and Noa Katrin for manuscript preparation.

References

9. Braunwald E: The path to myocardial salvage by thrombolytic therapy. \textit{Circulation} 1987;76(suppl II):II-12--II-7
Myocardial hypertrophy in the ischemic zone induced by exercise in rats after coronary reperfusion.
B H Oh, S Ono, H A Rockman and J Ross, Jr

Circulation. 1993;87:598-607
doi: 10.1161/01.CIR.87.2.598
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/87/2/598

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/