Microvascular and myocardial contractile responses to ischemia: influence of exercise training

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Symons, J. David, Stephen V. Rendig, Charles L. Stebbins, and John C. Longhurst. Microvascular and myocardial contractile responses to ischemia: influence of exercise training. J. Appl. Physiol. 88: 433–442, 2000.—We hypothesized that exercise training preserves endothelium-dependent relaxation, lessens receptor-mediated constriction of coronary resistance arteries, and reduces myocardial contractile dysfunction in response to ischemia. After 10 wk of treadmill running or cage confinement, regional and global indexes of left ventricular contractile function were not different between trained and sedentary animals in response to three 15-min periods of ischemia (long-term; n = 17), one 5-min bout of ischemia (short-term; n = 18), or no ischemia (sham-operated; n = 24). Subsequently, coronary resistance vessels (~106 ± 4 µm ID) were isolated and studied using wire myographs. Maximal ACh-evoked relaxation was ~25, 40, and 60% of KCl-induced preconstriction after the long-term, short-term, and sham-operated protocols, respectively, and was similar between groups. Maximal sodium nitroprusside-evoked relaxation was also similar between groups among all protocols, and vasoconstrictor responses to endothelin-1 and U-46619 were not different in trained and sedentary rats after short-term ischemia or sham operation. We did observe that, after long-term ischemia, maximal tension development in response to endothelin-1 and U-46619 was blunted (P < 0.05) in trained animals by ~70 and ~160%, respectively. These results support our hypothesis that exercise training lessens receptor-mediated vasoconstriction of coronary resistance vessels after ischemia and reperfusion. However, training did not preserve endothelial function of coronary resistance vessels, or myocardial contractile function, after ischemia and reperfusion.

Myocardial function; coronary vascular function; coronary resistance arteries; endothelium; vascular smooth muscle; acetylcholine; sodium nitroprusside; endothelin-1; thromboxaneA2; U-46619

MYOCARDIAL ISCHEMIA OCCURS when the metabolic requirement for oxygen exceeds the available supply (5). Episodes of ischemia may arise in individuals with coronary artery disease during a number of situations including increased myocardial oxygen demand (e.g., physical exertion), reductions in blood pressure, spontaneous increases in microvascular tone, and/or surgical procedures (e.g., angioplasty and coronary artery bypass graft surgery) (34). Depending on the intensity and duration of myocardial ischemia, left ventricular contractile dysfunction (e.g., myocardial stunning or hibernation) (7, 18, 29) and/or coronary vascular dysfunction (e.g., reduced endothelium-dependent relaxation and enhanced receptor-mediated constriction) may result (28).

In contrast to ischemia, evidence exists that exercise training enhances left-ventricular contractile function and improves coronary vascular reactivity (1, 13, 19, 20, 27). Although these findings may explain, in part, results of epidemiological studies indicating that chronic exercise training protects against the morbidity and mortality associated with ischemic heart disease (16), it is unclear whether this intervention also might render myocardium and/or coronary vasculature less susceptible to ischemia-induced damage. In this regard, studies examining the effects of exercise training on the myocardial response to ischemia indicate that contractile function may be improved (1, 27), decreased (17), or unaltered (2, 4). To our knowledge, the effects of physical training on the coronary vascular response to myocardial ischemia have not been evaluated.

Accordingly, the present study assessed whether 10 wk of treadmill running would preserve coronary vascular reactivity and myocardial contractile function in response to myocardial ischemia and reperfusion. We hypothesized that ischemia-induced endothelial dysfunction and receptor-mediated vasoconstriction of coronary resistance vessels would be lower, and that regional and global myocardial contractile function would be preserved, in trained compared with sedentary rats.

METHODS

Experimental Animals

All protocols used in this study were approved by the Animal Use and Care Committee at the University of California, Davis, and conformed to the American Physiological Society and Animal Welfare Act guidelines. Female Sprague-Dawley rats (Simonsons Breeders, Gilroy, CA) were housed individually under controlled temperature (23°C) and light conditions (12:12-h light-dark cycle) and were allowed access to food and water ad libitum.

Exercise Training

Rats ran on the treadmill (Quinton, model 42-15) initially for 5–10 min at 13 m/min, 15% grade, 5 days/wk. The...
duration and intensity were increased gradually over 5 wk to 60 min/day and 26.8 m/min, respectively. This workload was maintained for an additional 5–7 wk (13). To verify the ability of this training program to evoke a central cardiovascular adaptation (i.e., a reduction in heart rate at the same submaximal workload), the heart rate response to two submaximal workloads (13.4 and 20.1 m/min; 15% grade) was measured before and after 10 wk of training in six rats. To document peripheral adaptation to chronic exercise, the soleus muscle was excised at the end of the training program from these six rats, frozen immediately in liquid nitrogen, and stored at −70°C until processed for the analysis of citrate synthase activity (30). Results were compared with five age-matched rats that were cage-confined for 10–12 wk.

Surgical Procedures

After 10–12 wk, exercise-trained (n = 26) and sedentary (n = 33) rats were anesthetized using ketamine (30–50 mg/kg im) and xylazine (3–5 mg/kg im). Supplemental doses of this mixture were administered as required. The trachea was intubated, and respiration was maintained artificially (model 661, Harvard Apparatus) by using room air supplemented with 100% oxygen. A catheter was placed in the carotid artery to measure arterial pressure and to obtain samples for blood-gas analysis (Radiometer ABL-3, Westlake, OH). After the heart was exposed through a lateral thoracotomy and the pericardium was opened, a pressure transducer-tipped catheter (2F; Millar Instruments, Houston, TX) was placed through the apex into the left ventricle (LV) to measure LV systolic pressure and the first derivative of LV pressure (LV dP/dt). A 6-0 suture then was passed loosely around the left coronary artery, and both ends were threaded through a vinyl tube. This device served as a snare to occlude the coronary artery and was used to evoke reversible myocardial ischemia. Next, a 20-MHz single-transducer sonomicrometer was sewn on the epicardium in the region perfused by the left coronary artery to measure LV systolic wall thickening (SWth) (26). An illustration of the surgical instrumentation is shown in Fig. 1. Throughout the surgical procedures and experimental protocols, rectal temperature was maintained at 37°C using a heating pad and lamp.

Determination of Ischemia/Reperfusion Protocol

Our primary goal was to evaluate the effects of exercise training on microvascular responses to “short-term” and “long-term” ischemia. In preliminary studies, it was necessary to establish the extent of ischemia required to cause impairment of our primary end point, ACh-evoked relaxation of coronary microvessels. Using a 60- to 65-min in situ protocol, several durations of ischemia were attempted (e.g., one 5-min period, two 10-min periods, three 10-min periods, and three 15-min periods of ischemia). Between each repeated bout of ischemia, 5–10 min of reperfusion were allowed. Because ACh-induced relaxation was similar between three 10-min periods and three 15-min periods of ischemia, the latter was chosen to serve as the “long-term” ischemia paradigm. Similarly, because dysfunction of coronary resistance vessels was similar during one 5-min bout and two 10-min periods of ischemia but was lower (P < 0.05) than that achieved using three 15-min bouts of ischemia, the one 5-min period of ischemia was chosen as the “short-term” ischemia paradigm. ACh-evoked relaxation was lower after three periods of 15-min ischemia and one bout of 5-min ischemia (P < 0.05), compared with sham-operated, time control animals.
periods of ischemia that each lasted 15 min. Five minutes of reperfusion followed the first two bouts of ischemia, whereas 10 min followed the third. The additional 5 min after the third ischemic period allowed the final reperfusion time (i.e., 10 min) to be standardized between protocols 1 and 2. In all protocols, hemodynamic variables were monitored continuously (Hewlett-Packard 78534B) and were processed by a computer through an analog-to-digital interface card (R. C. Electronics, Santa Barbara, CA) that allowed for subsequent off-line quantitative analyses. Arterial blood gases and pH were measured at 0, 30, and 60 min. The volume required for each blood-gas analysis (~0.25 ml) was replaced using 0.6% dextran.

Protocol 2: short-term ischemia. This protocol examined whether exercise training preserves myocardial and coronary vascular function in response to an ischemic challenge of shorter duration relative to protocol 1. Hemodynamic variables were measured in sedentary (n = 8) and trained (n = 10) animals during a 60-min protocol that included one period of ischemia between 45 and 50 min. After 10 min of reperfusion, hearts were excised at 60 min.

Protocol 3: sham-operated, time controls. The purpose of this protocol was to examine the effects of exercise training on myocardial and coronary vascular function in the absence of ischemia (sedentary, n = 15; trained, n = 9). In this 60-min protocol that served as a vehicle control, the snare occluder around the left coronary artery was positioned but was not tightened.

In Vitro Protocols

At termination of the in situ protocols (i.e., 65 min for protocol 1; 60 min for protocols 2 and 3), hearts were excised and placed in ice-cold, oxygenated, normal physiological salt solution (NPSS; pH ~7.40). With use of a dissecting microscope (Leica Stereo Zoom 5), placement of the suture around the left coronary artery was confirmed, and the vessel was traced toward the apex of the heart. Second- and third-order branches of this artery then were isolated, cut from the heart, and prepared for mounting on a microvessel myograph (Jules Osher, Pomona, CA) (23). This apparatus allows direct determination of vessel wall force development while internal diameter is controlled. Two tungsten wires (outer diameter = 20 μm) were inserted in a parallel manner through the lumen of the vessel. One wire was attached to a force transducer (Fort10 transducer, World Precision Instruments, Sarasota, FL) to measure tension development, whereas the other was fixed to a micrometer that was used to stretch the vessel in small increments. Tension data were recorded continuously (Gould Brush 260). Vessels were immersed in a temperature-controlled, 8.5-ml reservoir (i.e., a tissue “bath”) containing oxygenated NPSS (pH ~7.40). Samples from all buffers and each tissue bath were withdrawn and analyzed at ~30-min intervals for PO2, PCO2, and pH.

After the coronary resistance arteries were mounted, the tissue bath was warmed gradually to 37°C, and the vessels were equilibrated at zero tension (~30 min). Ten milligrams of tension then were applied to the artery, and the distance between the wires was measured to calculate the internal diameter of the vessel by using the formula L = (2 + π)W + 2G, where L is internal circumference, W is wire thickness, G is the distance between wires, and vessel internal diameter = L/π (23).

Next, a series of internal circumference-active tension curves was constructed to determine the vessel diameter that evoked the greatest tension development (Lmax) to 100 mM KCl. This involved manually increasing the internal diameter of the vessel using the micrometer, exchanging NPSS in the vessel bath for 100 mM KCl, and measuring maximal developed tension. This procedure was repeated three to six times until the tension evoked by 100 mM KCl differed by <10% in response to successive increments in vessel internal diameter. Lmax was determined for every vessel, and this optimal resting tension was maintained throughout the study. An equilibration period of 30 min preceded the assessment of endothelium-dependent relaxation.

Endothelium-Dependent Relaxation

Vessels were precontracted using 40 mM KCl, and concentration-relaxation curves were constructed through cumulative additions (10 μl; 10–5–10–4 M) of the muscarinic receptor agonist ACh into the vessel bath. Relaxation responses were presented as a percentage of KCl-induced precontraction. After the response to the final dose of ACh was recorded, the NPSS was reintroduced into the tissue bath, and a 30-min equilibration period was initiated during which the bathing medium was reexchanged with NPSS two additional times.

Receptor-Mediated Vasocostriction

After an equilibration period, concentration-contraction curves were constructed in response to cumulative additions of U-46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F2α, 10–9–10–4 M), a prostaglandin H2-thromboxane A2 receptor agonist (9). When the response to the final concentration was stable and recorded, the vessel reservoir was rinsed with NPSS every 15–30 min for ~90 min until the pre-U-46619 tension was attained. Concentration-contractile responses to endothelin-1 (10–11–10–7 M) then were assessed. KCl, U-46619, and endothelin-1-induced contractile responses are presented as milligrams of developed tension.

Endothelium-Independent Relaxation

The contractile response to the last dose of endothelin-1 (i.e., 10–7 M) served as preconstriction for concentration-relaxation curves developed with the endothelium-independent vasodilator sodium nitroprusside (nitroprusside: 10–5–10–3 M). Relaxation responses are presented as a percentage of endothelin-1-induced precontraction.

Drugs and Solutions

NPSS contained (in mM) 125 NaCl, 4.7 KCl, 1.2 KH2PO4, 2.7 MgSO4, 2.5 CaCl2, 18 NaHCO3, 0.026 Na2EDTA, and 11.2 glucose. The KCl concentration was increased in designated solutions (i.e., 40 and 100 mM) by isomolar exchange for NaCl. All solutions were maintained at ~37°C and aerated with 95% O2–5% CO2 at a rate sufficient to maintain pH at ~7.40. NPSS and KCl solutions were prepared daily from concentrated stock. ACh, nitroprusside (Sigma Chemical, St. Louis, MO), U-46619 (Cayman Chemical, Ann Arbor, MI), and endothelin-1 (Peninsula Laboratories, San Carlos, CA) were purchased commercially and prepared daily from stock solutions using distilled deionized water. All doses are expressed as the final concentration of each drug in the vessel bath.

Exclusion Criteria

Vessels that did not develop at least 60 mg of tension in response to 40 mM KCl during the assessment of endothelium-independent function, or that did not relax by ~15% in response to ACh, were excluded from the entire analysis.

Statistical Analyses

The heart rate responses to two submaximal workloads at weeks 0 and 10 were compared in the trained rats using a
repeated-measures ANOVA, followed by a Tukey's post hoc analysis. Citrate synthase activity was compared between trained and sedentary rats using an unpaired Student's t-test.

Hemodynamic variables were calculated at baseline and at 10-min intervals throughout each in situ protocol. Data were analysed using a two-way (time vs. experimental group) repeated-measures ANOVA, followed by a Tukey's post hoc analysis when appropriate. Vascular relaxation and constriction responses also were compared using a two-way (dose vs. experimental group) repeated-measures ANOVA. Planned comparisons were made at each drug dose to determine whether differences existed between groups (i.e., sedentary vs. trained) in each protocol. Before analysis, however, responses from two to four vessels were averaged from each animal; these averages were subsequently counted as one observation. Vessel characteristics were compared among groups using a one-way ANOVA, followed by a Tukey's post hoc test when appropriate. All values are presented as means ± SE. Statistical significance was accepted at P < 0.05 (14).

RESULTS

Exercise Training

In the exercise-trained rats, the heart rate response to 13.4 m/min was lower at 10 wk (458 ± 4 beats/min) compared with week 0 (484 ± 6 beats/min). Similarly, heart rates at 20.1 m/min were lower at week 10 (490 ± 6 beats/min) than week 0 (524 ± 5 beats/min). Soleus citrate synthase activity was greater in these trained rats (38 ± 3 µmol·min⁻¹·g wet wt⁻¹) compared with sedentary animals (24 ± 4 µmol·min⁻¹·g wet wt⁻¹). Body weights at 10 wk in the sedentary and trained animals were not different among protocols and therefore were averaged (sedentary, 287 ± 13 g; trained, 297 ± 15 g).

Arterial Blood Gases During In Situ Protocols

No differences among protocols existed at baseline in arterial blood pH (7.44 ± 0.02 and 7.44 ± 0.01), Pco₂ (34 ± 2 and 34 ± 2 Torr), or Po₂ (197 ± 31 and 214 ± 42 Torr) in comparisons between sedentary and trained rats, respectively. In addition, these variables did not change throughout the duration of the in situ protocols.

Hemodynamic Responses During In Situ Protocols

Protocol 1: long-term ischemia. Heart rates were lower throughout the experimental protocol, and left ventricular developed pressure (LVDP) and SWth were depressed to a greater extent at 20 min during the first reperfusion period in trained compared with sedentary rats (Table 1). Mean arterial pressure, LVDP, LV dp/dt, and SWth were reduced during each bout of ischemia in both groups. In addition, these variables were depressed similarly in trained and sedentary rats during reperfusion at 40 and 60 min compared with their respective baseline values. An original tracing of hemodynamic variables from a sedentary rat at baseline, during ischemia, and at 65 min is shown in Fig. 1 (A, B, and C, respectively).

Protocol 2: short-term ischemia. The only difference between groups was that heart rate was lower during the entire experimental protocol in trained compared with sedentary animals. In both groups of rats, mean arterial pressure, LVDP, LV dp/dt, and SWth were reduced during ischemia, compared with pre-ischemic measures. During reperfusion, these variables were greater relative to measures obtained during ischemia but depressed compared with values recorded before ligation of the coronary artery (Table 2).

Protocol 3: sham-operated, time controls. Although heart rate was lower during the entire experimental protocol in trained compared with sedentary animals, all other hemodynamic variables were similar (Table 3).

Vessel Characteristics

There were no differences among protocols in internal diameter of vessels at baseline (102 ± 4 and 110 ± 4 µm) or Lmax (185 ± 8 and 192 ± 5 µm) or the vessel length (1.2 ± 0.2 and 0.9 ± 0.1 mm), comparing

Table 1. Hemodynamic variables in sedentary and exercise-trained rats: long-term ischemia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 Min (ischemia)</th>
<th>20 Min</th>
<th>30 Min (ischemia)</th>
<th>40 Min</th>
<th>50 Min (ischemia)</th>
<th>65 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td>SEDENTARY</td>
<td>TRAINED</td>
<td>SEDENTARY</td>
<td>TRAINED</td>
<td>SEDENTARY</td>
<td>TRAINED</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>89 ± 6</td>
<td>72 ± 4*</td>
<td>85 ± 5</td>
<td>66 ± 2*</td>
<td>72 ± 4*</td>
<td>65 ± 3*</td>
<td>75 ± 4*</td>
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<tr>
<td></td>
<td></td>
<td>96 ± 8</td>
<td>75 ± 7*</td>
<td>78 ± 7*</td>
<td>73 ± 5*</td>
<td>80 ± 7*</td>
<td>73 ± 3*</td>
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<tr>
<td>LVDP, mmHg</td>
<td></td>
<td>289 ± 24</td>
<td>271 ± 20</td>
<td>272 ± 18</td>
<td>294 ± 15</td>
<td>283 ± 16</td>
<td>295 ± 15</td>
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<tr>
<td></td>
<td></td>
<td>226 ± 6†</td>
<td>210 ± 13</td>
<td>214 ± 16†</td>
<td>230 ± 10</td>
<td>234 ± 10†</td>
<td>232 ± 11†</td>
</tr>
<tr>
<td>LV dP/dt, mmHg/s</td>
<td>94 ± 4</td>
<td>76 ± 5*</td>
<td>92 ± 5</td>
<td>73 ± 4*</td>
<td>79 ± 4*</td>
<td>72 ± 3*</td>
<td>78 ± 4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101 ± 8</td>
<td>79 ± 9*</td>
<td>83 ± 4†</td>
<td>77 ± 4*</td>
<td>83 ± 7*</td>
<td>79 ± 6*</td>
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<tr>
<td>SWth, %</td>
<td></td>
<td>3,570 ± 181</td>
<td>2,744 ± 182*</td>
<td>3,327 ± 160</td>
<td>2,646 ± 124*</td>
<td>2,709 ± 139*</td>
<td>2,509 ± 138*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3,103 ± 152</td>
<td>2,546 ± 204*</td>
<td>3,238 ± 77</td>
<td>2,406 ± 135*</td>
<td>2,526 ± 114*</td>
<td>2,549 ± 116*</td>
</tr>
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<td></td>
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<td>3.9 ± 0.4</td>
<td>0.2 ± 0.6*</td>
<td>3.2 ± 0.4</td>
<td>−0.4 ± 0.6*</td>
<td>2.2 ± 0.6*</td>
<td>−0.5 ± 0.4*</td>
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<td></td>
<td></td>
<td>3.5 ± 0.9</td>
<td>0.2 ± 0.4*</td>
<td>1.9 ± 0.7†</td>
<td>0.7 ± 0.4*</td>
<td>1.4 ± 0.5*</td>
<td>0.3 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; LVDP, left ventricular developed pressure; LV dP/dt, first derivative of left ventricular pressure; SWth, systolic wall thickening. *P < 0.05 vs. respective baseline value; †P < 0.05 vs. sedentary.
sedentary and trained rats, respectively. Maximal developed tension in response to 100 mM KCl was similar between protocols 2 and 3 for both groups and averaged 140 ± 4 mg (sedentary rats) and 177 ± 5 mg (trained rats). In protocol 1, however, 100 mM KCl evoked less tension in trained (87 ± 2 mg) than sedentary (161 ± 5 mg) animals.

Vascular Responses During In Vitro Protocols

Protocol 1: long-term ischemia. Baseline tension at $L_{\text{max}}$ was similar in the sedentary (141 ± 11 mg) and trained animals (132 ± 9 mg). For all protocols, this tension was not altered throughout the experiment. Percent preconstriction evoked by 40 mM KCl (i.e., tension developed by 40 mM KCl divided by tension developed at $L_{\text{max}}$) was not different between sedentary (78 ± 2%) and exercise-trained rats (73 ± 4%). After precontraction, endothelium-dependent relaxation responses to ACh increased in a dose-dependent manner but did not differ between the sedentary and trained groups. In several vessels from sedentary and trained animals in each protocol, precontraction was evoked by using 40 mM KCl, but no ACh was administered. These experiments demonstrated that the developed tension remained stable for the time required (i.e., 30 min) to perform the ACh concentration-relaxation responses (data not shown).

U-46619 and endothelin-1 caused concentration-dependent increases in developed tension. Resistance arteries from sedentary animals evoked greater tension compared with arteries from trained rats in response to the three highest doses of both agents. Percent precontraction evoked by the final dose of endothelin-1 (i.e., tension developed by 10 M endothelin-1 divided by tension developed at $L_{\text{max}}$) was not different between sedentary (107 ± 6%) and exercise-

Table 2. Hemodynamic variables in sedentary and exercise-trained rats: short-term ischemia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 Min</th>
<th>20 Min</th>
<th>30 Min</th>
<th>40 Min</th>
<th>50 Min (Ischemia)</th>
<th>60 Min</th>
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<tbody>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sedentary</td>
<td>98 ± 8</td>
<td>94 ± 6</td>
<td>94 ± 6</td>
<td>91 ± 6</td>
<td>94 ± 6</td>
<td>77 ± 5*</td>
<td>84 ± 5†</td>
</tr>
<tr>
<td>Trained</td>
<td>90 ± 5</td>
<td>84 ± 3</td>
<td>83 ± 3</td>
<td>84 ± 3</td>
<td>82 ± 3</td>
<td>70 ± 4*</td>
<td>75 ± 5†</td>
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<tr>
<td><strong>HR, beats/min</strong></td>
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<tr>
<td>Sedentary</td>
<td>264 ± 6</td>
<td>264 ± 7</td>
<td>267 ± 7</td>
<td>266 ± 7</td>
<td>266 ± 7</td>
<td>268 ± 9</td>
<td>266 ± 8</td>
</tr>
<tr>
<td>Trained</td>
<td>209 ± 6†</td>
<td>211 ± 6†</td>
<td>214 ± 6†</td>
<td>214 ± 6†</td>
<td>213 ± 7†</td>
<td>212 ± 7†</td>
<td>209 ± 8†</td>
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<tr>
<td><strong>LVDP, mmHg</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>104 ± 10</td>
<td>99 ± 7</td>
<td>99 ± 7</td>
<td>96 ± 6</td>
<td>97 ± 6</td>
<td>75 ± 5*</td>
<td>88 ± 5†</td>
</tr>
<tr>
<td>Trained</td>
<td>95 ± 4</td>
<td>90 ± 2</td>
<td>90 ± 2</td>
<td>90 ± 2</td>
<td>89 ± 2</td>
<td>72 ± 3*</td>
<td>82 ± 2‡</td>
</tr>
<tr>
<td><strong>LV dp/dt, mmHg/s</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Sedentary</td>
<td>4,098 ± 189</td>
<td>3,927 ± 125</td>
<td>3,855 ± 118</td>
<td>3,682 ± 106</td>
<td>3,602 ± 146</td>
<td>2,776 ± 97*</td>
<td>2,891 ± 317‡</td>
</tr>
<tr>
<td>Trained</td>
<td>3,414 ± 140</td>
<td>3,382 ± 121</td>
<td>3,396 ± 133</td>
<td>3,409 ± 119</td>
<td>3,214 ± 140</td>
<td>2,671 ± 99*</td>
<td>2,750 ± 226‡</td>
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<tr>
<td><strong>SWth, %</strong></td>
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<tr>
<td>Sedentary</td>
<td>2.6 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>0.4 ± 0.4*</td>
<td>2.0 ± 0.2‡</td>
</tr>
<tr>
<td>Trained</td>
<td>3.1 ± 0.5</td>
<td>3.0 ± 0.4</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>2.8 ± 0.2</td>
<td>0.3 ± 0.2*</td>
<td>2.4 ± 0.3‡</td>
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</table>

Values are means ± SE. *vs. all other time points; †vs. sedentary; ‡vs. all other time points; all P < 0.05.
trained rats (120 ± 11%). Assessment of endothelium-independent relaxation through use of nitroprusside indicated that vascular smooth muscle responsiveness was intact and was similar between groups. In several vessels from sedentary and trained animals in each protocol, endothelin-1-induced precontraction was evoked, but no nitroprusside was administered. These experiments documented stability of the developed tension over the same time period (i.e., 30 min) required to complete the nitroprusside concentration-relaxation curve (data not shown). Vascular responses to ACh, nitroprusside, endothelin-1, and U-46619 for animals in protocol 1 are shown in Fig. 4, A–D, respectively.

Protocol 2: short-term ischemia. Baseline tension at Lmax was similar in the sedentary (119 ± 6 mg) and trained animals (115 ± 9 mg). Percent precontraction evoked by 40 mM KCl was not different between sedentary (70 ± 6%) and exercise-trained rats (74 ± 3%). After precontraction was stable, relaxation responses to ACh increased similarly in a dose-dependent manner between sedentary and trained rats. Unlike the long-term ischemia protocol, both U-46619 and endothelin-1 elicited concentration-dependent increases in tension of resistance vessels that were similar between the two groups.

No differences existed between groups regarding percent precontraction produced by endothelin-1 (104 ± 6%, sedentary rats; 93 ± 6%, trained rats) or the subsequent endothelium-independent responses evoked by cumulative additions of nitroprusside. Vascular responses to ACh, nitroprusside, endothelin-1, and U-46619 for animals in protocol 2 are shown in Fig. 5, A–D, respectively.

Protocol 3: sham-operated time controls. Baseline tension at Lmax was similar in the sedentary (138 ± 6 mg) and trained animals (108 ± 6 mg). Percent precontraction evoked by 40 mM KCl was not different between groups regarding percent precontraction produced by endothelin-1 (104 ± 6%, sedentary rats; 93 ± 6%, trained rats) or the subsequent endothelium-independent responses evoked by cumulative additions of nitroprusside. Vascular responses to ACh, nitroprusside, endothelin-1, and U-46619 for animals in protocol 2 are shown in Fig. 5, A–D, respectively.
between sedentary (65 ± 3%) and exercise-trained rats (71 ± 6%). After the KCl-induced preconstriction was stable, we observed that endothelium-dependent relaxation responses to ACh were concentration dependent but similar between sedentary and trained rats. Although U-46619 and endothelin-1 evoked contractile responses in the resistance vessels, no differences existed between the two groups. The only exception to this was that tension development was lower in trained than sedentary animals at 10⁻² M U-46619.

No differences existed between groups regarding percent precontraction produced by endothelin-1 (120 ± 7%, sedentary rats; 103 ± 8%, trained rats). Nitroprusside evoked concentration-dependent relaxation responses, confirming that endothelium-independent function was intact in both groups of rats. However, we did observe that the extent of relaxation was lower in trained than sedentary animals at several of the highest doses of nitroprusside (10⁻⁷–10⁻⁴ M). Vascular responses to ACh, nitroprusside, endothelin-1, and U-46619 for animals in protocol 3 are shown in Fig. 6, A–D, respectively.

**DISCUSSION**

We tested the hypotheses that exercise training preserves coronary vascular reactivity and myocardial contractile function in response to myocardial ischemia and reperfusion. We observed that ACh-induced relaxation and vascular smooth muscle function of coronary resistance vessels and reductions of regional and global myocardial contractile function were similar between...
trained and sedentary animals after both long- and short-term ischemia. However, our main finding was that tension developed by coronary resistance vessels in response to two structurally dissimilar vasoconstrictors (endothelin-1 and U-46619) was lower after long-term ischemia in exercise-trained compared with sedentary rats. Although these results support our hypothesis that receptor-mediated vasoconstriction of coronary resistance vessels is lower after ischemia and reperfusion in trained than sedentary rats, the hypothesis that exercise training preserves endothelial and myocardial contractile function in response to ischemia and reperfusion was not confirmed.

**Coronary Vascular Function**

The primary focus of the present study was to determine whether exercise training preserves coronary vascular function after ischemia. A strong rationale exists for posing this question. First, coronary resistance vessels from treadmill-trained pigs demonstrate improved endothelium-dependent vasodilation compared with sedentary animals (22), likely through mechanisms associated with increased endothelial cell nitric oxide synthase messenger RNA (35). Because upregulation of endothelial cell nitric oxide synthase gene expression probably contributes to enhanced nitric oxide production, this adaptation could render the endothelium more resistant to damage caused by ischemia and reperfusion. Second, coronary flow is greater during reperfusion after global ischemia in isolated working hearts from treadmill-trained compared with cage-confined rats (2, 4), a finding that suggests that resistance to flow during reperfusion after ischemia is reduced by exercise training. Third, myocardial lipid peroxidation in response to ischemia induced by coronary occlusion is lower in trained compared with sedentary rats (27). Reducing oxidative stress associated with the reintroduction of molecular oxygen during reperfusion would be a beneficial adaptation because reactive oxygen species contribute to vascular dysfunction (12). Together, previous studies provide a strong rationale for our hypothesis that ischemia and reperfusion-induced vascular dysfunction are lessened by exercise training.

We studied the function of coronary resistance arteries because these vessels are responsible for regulating myocardial blood flow (8) and are more susceptible than conductance arteries to endothelial damage caused by ischemia and reperfusion (28). Moreover, although it is well established that ischemia and reperfusion-evoked dysfunction of coronary resistance vessels are dependent on the severity of the ischemic challenge and the duration of reperfusion (10), the potential for exercise training to limit ischemia-induced damage in coronary resistance vessels is unknown.

Our most important findings were that tension development in response to U-46619 and endothelin-1 was lower in trained compared with sedentary rats but only after long-term ischemia (i.e., protocol 1). ACh-induced vasorelaxation and vascular smooth muscle function were similar between the two groups in all protocols. Although extrapolating data from isolated vessels of experimental animals to in vivo conditions in humans is difficult, our findings do suggest that exercise training potentially could reduce reactivity of ischemic coronary resistance vessels to thromboxane A₂ and endothelin-1. This is relevant clinically because these mediators are elevated during myocardial ischemia and other pathophysiological conditions and therefore could contribute to vascular dysfunction including coronary vasospasm (15, 21, 32).

Blunted contractile responses to endothelin-1 and U-46619 of coronary resistance vessels from trained rats cannot be explained by greater opposition from endothelium-derived factors (e.g., nitric oxide) because ACh-evoked vasodilation was similar after ischemia, regardless of training. Moreover, compromised cGMP formation is not responsible for this finding because nitroprusside-induced vasodilation was not different between groups. Instead, it appears that postreceptor-mediated mechanisms may have contributed to the reduced vasoconstrictor responsiveness observed in trained rats. In this regard, maximal tension development in response to 100 mM KCl was lower in trained compared with sedentary animals after long-term ischemia. KCl causes vascular smooth muscle contraction through nonreceptor-mediated mechanisms involving voltage-gated Ca²⁺ channels. Therefore, it is less likely that attenuated contractile responses to U-46619 and endothelin-1 are due to alterations specific to thromboxane A₂ and endothelin-A or endothelin-B₂ receptor subtypes (11) and more probable that postreceptor-mediated mechanism(s) concerning Ca²⁺ regulation and/or sensitivity is involved. This speculation is supported by data from others indicating that coronary contractile responses to agonists that mobilize sarcoplasmic reticulum Ca²⁺ (e.g., endothelin-1) are reduced in trained compared with sedentary animals (3), likely through mechanisms that diminish the increase in myoplasmic free Ca²⁺ (31, 33).

We also were able to assess the influence of exercise training alone (without ischemia) on isolated coronary resistance vessel function (i.e., protocol 3). Our findings indicate that ACh-evoked vasodilation and receptor-mediated constriction are similar in trained and sedentary rats. Interestingly, results from the only other evaluation of exercise training on coronary vessels from rats indicated that ACh-induced relaxation is blunted in proximal but unaltered in distal segments of the same coronary artery (220–226 μm, resting internal diameter) in trained compared with sedentary animals (25). We suggest that our results from coronary resistance vessels are more pertinent to consideration of the actual regulatory element of vascular resistance and blood flow. Coronary vessels of our trained rats in the absence of ischemia also demonstrated reduced responsiveness to lower concentrations of nitroprusside (i.e., 10⁻²–10⁻⁴ M) but similar vasodilation with the highest dose (i.e., 10⁻² M) of this agent. This observation suggests reduced vascular smooth muscle sensitivity in trained compared with sedentary animals. These findings regarding nitroprusside are similar to those ob-
served with coronary conductance (24) but not with resistance (22) vessels from trained pigs. Our results suggest that training may alter cGMP-mediated vasodilation in vascular smooth muscle. However, because nitroprusside-evoked increases in cGMP were not measured, this mechanism remains speculative. Overall, our findings and those from Parker et al. (25) suggest that exercise training exerts little influence on endothelial and vascular smooth muscle function in coronary conductance and resistance arteries of rats in the absence of ischemia.

Myocardial Contractile Function

Our findings do not support the hypothesis that exercise training preserves global and regional myocardial contractile function in response to ischemia. For example, LV dP/dt and SWth reductions during left coronary artery occlusion and reperfusion generally were similar, regardless of training status, after short-term and long-term ischemia. The only hemodynamic variable that was different between groups among all protocols was the expected reduction in heart rate in trained compared with sedentary animals. These data are strengthened by several factors. First, afterload was similar between groups during all protocols. This is important because alterations in this variable can influence both global and regional myocardial contractility (5). Second, because regional and global myocardial function were similar throughout the sham-operated, time control protocol (e.g., protocol 3), reductions in these variables were due to ischemia and reperfusion rather than to a deteriorating experimental preparation.

In general, the present results confirm those obtained previously from rats in response to ischemia after completing a treadmill-training program similar to ours (2, 4) and extend them to include SWth in response to short-term and long-term ischemia. In contrast to our study, Powers et al. (27) reported that systolic pressure was better maintained in situ during myocardial ischemia (one 20-min occlusion of the left coronary artery) and reperfusion (10 min) in treadmill-trained compared with sedentary rats. Although these data suggest that exercise training potentially can lessen ischemia-induced myocardial dysfunction, LV dP/dt, ventricular wall motion, and afterload were not measured.

Our study is the first to assess the effects of physical training on coronary resistance vessel reactivity and myocardial contractile function after ischemia and reperfusion in the same animal. We observed that ischemia-induced alterations in ACh-evoked relaxation and myocardial contractile function were not affected by exercise training. However, tension developed by coronary resistance vessels in response to specific receptor agonists (i.e., endothelin-1 and thromboxane A2) that may be released during ischemia was lower after long-term ischemia in trained compared with sedentary rats. Although precise mechanisms responsible for these observations were not evaluated in the present study, our data suggest that postreceptor-mediated pathways are responsible. These findings are relevant to clinical settings where the coronary circulation is exposed to repeated transient reversible ischemia and when concentrations of endothelin-1 and/or thromboxane A2 are elevated (6, 15). Because coronary resistance vessels primarily regulate regional myocardial blood flow, our results suggest that physical exercise can be beneficial in attenuating receptor-mediated increases in vascular tone.

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