Endurance exercise training attenuates cardiac β₂-adrenoceptor responsiveness and prevents ventricular fibrillation in animals susceptible to sudden death

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Submitted 18 November 2005; accepted in final form 13 December 2005

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Intense exercise training can attenuate cardiac β₂-adrenoceptor responsiveness and prevent ventricular fibrillation in animals susceptible to sudden death. Am J Physiol Heart Circ Physiol 290: H2590–H2599, 2006. First published January 27, 2006; doi:10.1152/ajpheart.01220.2005.—Enhanced cardiac β₂-adrenoceptor (β₂-AR) responsiveness can increase susceptibility to ventricular fibrillation (VF). Exercise training can decrease cardiac sympathetic activity and could, thereby, reduce β₂-AR responsiveness and decrease the risk for VF. Therefore, dogs with healed myocardial infarctions were subjected to 2 min of coronary occlusion during the last minute of a submaximal exercise test; VF was observed in 20 susceptible, but not in 13 resistant, dogs. The dogs were then subjected to a 10-wk exercise-training program (n = 9 susceptible and 8 resistant) or an equivalent sedentary period (n = 11 susceptible and 5 resistant). Before training, the β₂-AR antagonist ICI-118551 (0.2 mg/kg) significantly reduced the peak contractile (by echocardiography) response to isoproterenol more in the susceptible than in the resistant dogs: −45.5 ± 6.5% vs. −19.2 ± 6.3%, respectively. After training, the susceptible and resistant dogs exhibited similar responses to the β₂-AR antagonist: −12.1 ± 5.7% and −16.2 ± 6.4%, respectively. In contrast, ICI-118551 provoked even greater reductions in the isoproterenol response in the sedentary susceptible dogs: −62.3 ± 4.6%. The β₂-AR agonist zinterol (1 μM) elicited significantly smaller increases in isotonic shortening in ventricular myocytes from susceptible dogs after training (n = 8, +7.2 ± 4.8%) than in those from sedentary dogs (n = 7, +42.8 ± 5.8%), a response similar to that of the resistant dogs: +3.0 ± 1.4% (n = 6) and +3.2 ± 1.8% (n = 5) for trained and sedentary, respectively. After training, VF could no longer be induced in the susceptible dogs, whereas four sedentary susceptible dogs died during the 10-wk control period and VF could still be induced in the remaining seven animals. Thus exercise training can restore cardiac β-AR balance (by reducing β₂-AR responsiveness) and could, thereby, prevent VF.

β₂-adrenergic receptor; myocardial infarction; myocardial ischemia

The mammalian myocardium contains β₁- and β₂-adrenoceptors (1). In the normal heart, the dominant β₁-adrenoceptor mediates the inotropic response to sympathetic nerve activation. Under certain pathological conditions, however, activation of β₂-adrenoceptors may become particularly important (1, 11). During heart failure, β₁-adrenoceptor sensitivity decreases substantially, whereas β₂-adrenoceptor number remains relatively constant (1, 11). As a consequence, the failing heart becomes more dependent on activation of β₂-adrenoceptors for inotropic support. Activation of these receptors may help maintain cardiac function in diseased hearts, but not without potentially adverse consequences. β₂-Adrenoceptor activation promotes an increase in the Ca²⁺ current without altering Ca²⁺ reuptake by the sarcoplasmatic reticulum (2). The resulting elevation in intracellular Ca²⁺ could provoke oscillations in membrane potential, which, in turn, could trigger arrhythmias (5). Thus, in the diseased heart, β₂-adrenoceptor activation would tend to reduce the cardiac electrical stability and increase the propensity for the formation of malignant arrhythmias.

Recently, we demonstrated in dogs with healed myocardial infarctions that the nonselective β-adrenoceptor agonist isoproterenol provoked significantly larger increases in heart rate and velocity of circumferential fiber shortening (VF, an index of contractility) in animals that were susceptible to ventricular fibrillation (VF) induced by myocardial ischemia than in animals that were resistant to these malignant arrhythmias (22). The selective β₂-adrenoceptor antagonist ICI-118551 reduced the isoproterenol response to a much greater extent in the susceptible animals, eliminating any differences between the groups (22). In a similar manner, the Ca²⁺ transient amplitude and the single-cell isotonic shortening responses to isoproterenol were larger in myocytes obtained from the hearts of susceptible than resistant dogs, differences that were also eliminated by β₂- but not by β₁-adrenoceptor blockade (9, 22). In the intact dog, β₂-adrenoceptor blockade almost completely suppressed VF induced by acute myocardial ischemia, protecting 10 of 11 susceptible animals (9). When considered together, these data demonstrate that an enhanced β₂-adrenoceptor responsiveness is associated with an increased propensity for VF. One would predict that interventions that restore a more normal β₁-to-β₂-adrenoceptor balance should also protect against VF.

Regular exercise can improve β-adrenoceptor responsiveness in normal animals (3, 38), aged animals (29), and animals with hypertension (27), despite a reduction (3) or no change in the β₁-adrenoceptor density (17, 29). MacDonnell et al. (27) demonstrated that exercise training could correct the defective inotropic response to β-adrenoceptor stimulation in spontaneously hypertensive rats. The effects of exercise training on β₂-adrenoceptor responsiveness, particularly in animals with damaged hearts, have not been examined and remain to be determined.

Exercise training is associated with a reduced incidence of sudden death and arrhythmias in human and animal models (6). For example, from meta-analysis of 22 randomized trials of...
rehabilitation with exercise after myocardial infarction, O’Connor et al. (31) found that exercise training elicited significant reductions in reinfarction and incidence of sudden death. Exercise training also improved cardiac function and reduced arrhythmia frequency in patients with congestive heart failure (16, 19), a patient population with abnormal cardiac β-adrenoceptor function and elevated risk for sudden death (40). In animals, regular exercise reduced the electrical current necessary to induce VF (30, 33) or the susceptibility to VF induced by myocardial ischemia (8, 24). Contributions of changes in cardiac autonomic balance to the protection afforded by exercise training were not extensively examined in these studies and remain largely to be determined. Noakes et al. (30) and Posel and co-workers (33) found that exercise training elicited reductions in myocardial cAMP levels that may reflect corresponding alterations in β-adrenoceptor activity in trained animals.

The purpose of this study was to investigate the effects of exercise training on β2-adrenoceptor responsiveness and susceptibility to VF in a conscious canine model of sudden death. Specifically, we tested the hypothesis that exercise training would attenuate β2-adrenoceptor responsiveness and, thereby, prevent VF induced by myocardial ischemia. The contractile response to increasing doses of isoproterenol, a nonspecific β-adrenoceptor agonist, in the presence or absence of selective β1- or β2-adrenoceptor antagonists was evaluated by echocardiography before and after a 10-wk training program or a 10-wk sedentary period. In addition, the effects of the selective β2-adrenoceptor agonist zinterol were evaluated in ventricular myocytes isolated from hearts of exercise-trained or sedentary animals.

METHODS

The principles governing the care and use of animals as expressed by the Declaration of Helsinki and as adopted by the American Physiological Society were followed at all times during the study. The Ohio State University Institutional Animal Care and Use Committee approved all the procedures used in this study.

Surgical preparation. Sixty heartworm-free mongrel dogs [19.1 ± 0.4 (range 15.4–24.5) kg body wt] were anesthetized and instrumented as previously described (9, 22). Briefly, 24 h before surgery, a transdermal fentanyl patch that delivers 100 μg/h (Duragesic, Jansen Pharmaceutical, Titusville, NJ) was placed on the left side of the animal’s neck and secured with tape. On the day of surgery, anesthesia was induced with 15 mg (1 ml im) of morphine sulfate (Elkins-Sinn, Cherry Hill, NJ) and thiopental sodium (Baxter Healthcare, Glendale, CA; 20 mg/kg iv). The dogs were intubated, and a surgical plane of anesthesia was maintained by inhalation of 1–1.5% isoflurane (Baxter Healthcare). Strict aseptic procedures were used to make a left thoracotomy in the fourth intercostal space. The heart was exposed and supported by a pericardial cradle. The left circumflex coronary artery was dissected free of the surrounding tissue. A 20-MHz pulsed Doppler flow transducer and a hydraulic occluder were then placed around this vessel. Two pairs of silver-coated copper wires were also sutured on the epicardial surface of the heart and used to obtain a ventricular electrogram. One pair of electrodes was placed in the potentially ischemic area (lateral left ventricular wall, an area perfused by the left circumflex artery), and the other pair was placed in a nonischemic region (right ventricular outflow tract). A two-stage occlusion of the left anterior descending artery was then performed approximately one-third of the distance from its origin to produce an anterior wall myocardial infarction. This vessel was partially occluded for 20 min and then tied off. The leads to the cardiovascular instru-

mentation were tunneled under the skin to exit on the back of the animal’s neck.

In addition to the fentanyl patch, morphine sulfate (1.0 mg/kg sc) was given as needed to control any postoperative pain. The long-lasting local anesthetic bupivacaine HCl (0.25%; Abbott Laboratories, North Chicago, IL) was injected in each of three sites (0.5 ml) to block the intercostal nerves in the area of the incision to minimize discomfort to the animals. Each dog was treated with amoxicillin (Teva Pharmaceuticals, Sellersville, PA; 500 mg po) twice daily for 7 days. The animals were placed in a quiet recovery area and returned to their home kennel after the effects of the anesthesia had dissipated. To minimize the incidence of arrhythmias, the dogs were treated with 100 mg of lidocaine HCl (im; Elkins-Sinn, Cherry Hill, NJ) before surgery and an additional 60 mg iv before each of the two stages of the coronary occlusion. The dogs also received procainamide HCl (500 mg im; Abbott Laboratories) before the surgery.

Exercise + ischemia test: classification of the dogs. The studies began 3–4 wk after production of the myocardial infarction (Fig. 1). The susceptibility to VF was tested as previously described (8, 9, 22). Briefly, the animals ran on a motor-driven treadmill while workload was progressively increased until a heart rate of 70% of maximum (~210 beats/min) was achieved. During the last minute (on average, during minute 18) of exercise, the left circumflex coronary artery was occluded, the treadmill was stopped, and the occlusion was maintained for an additional 1 min (total occlusion time = 2 min). The exercise + ischemia test reliably induced ventricular flutter, which rapidly deteriorated into VF. Therefore, large metal plates (11 cm diameter) were placed across the animal’s chest, so that electrical defibrillation (M series defibrillator, Zoll Medical, Burlington, MA) could be achieved with a minimal delay, but only after the animal was unconscious (10–20 s after onset of VF). Sixty dogs underwent surgery; 21 animals could not be tested because of death within 72 h of the myocardial infarction (n = 14, 23.3%) or occluder failure (n = 7). Thus the exercise + ischemia test was performed on 39 of the original 60 animals. The occlusion was immediately released if VF occurred. Twenty-six dogs developed VF (susceptible), and the remaining 13 animals did not (resistant). Three susceptible animals were not successfully defibrillated and, thus, were not available for additional studies. Using the same exercise intensity, we repeated this exercise + ischemia test after a 10-wk exercise-training or a 10-wk sedentary period (see below).

**Fig. 1.** Sequence of events. At 3–4 wk after myocardial infarction, dogs were classified as susceptible or resistant to ventricular fibrillation (VF) with an exercise + ischemia test. Dogs were then randomly assigned to a 10-wk exercise (Ex)-training program or a 10-wk sedentary period. Exercise + ischemia test was repeated at the end of the 10-wk period. Defib, Defibrillation.

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**AJP-Heart Circ Physiol** • VOL. 290 • JUNE 2006 • www.ajpheart.org
**Exercise-training protocol.** The susceptible (n = 23) and resistant (n = 13) dogs were randomly assigned to a 10-wk exercise-training group (n = 9 susceptible and 8 resistant) or 10-wk sedentary group (n = 14 susceptible and 5 resistant; Fig 1) period. The dogs in the exercise-training group ran on a motor-driven treadmill for 10 wk, 5 days/wk, at ~70–80% of maximum heart rate. The exercise intensity and duration progressively increased as follows: 20 min at 4.8 kph and 0% grade in week 1, 40 min at 5.6 kph and 10% grade in week 2, 40 min at 6.4 kph and 10% grade in week 3, 60 min at 6.4 kph and 10% grade in week 4, 60 min at 6.4 kph and 12% grade in week 5, 75 min at 6.4 kph and 12% grade in week 6, 90 min at 6.4 kph and 12% grade in week 7, and 90 min at 6.4 kph and 14% grade in weeks 8–10. Each exercise session included 5-min warm-up and 5-min cool-down periods, i.e., running at a low intensity (4.8 kph and 0% grade). The dogs in the sedentary group were placed in transport cages for equivalent time periods but without exercise. All 17 animals (susceptible and resistant dogs) in the exercise group successfully completed the training program. Four dogs in the susceptible sedentary group died spontaneously between weeks 6 and 10 of the sedentary period and were eliminated from the study. The exercise + ischemia test could not be repeated in three of the sedentary susceptible animals because of failure of the coronary occluder, and these dogs were eliminated from the study (Fig. 1).

**Echocardiography studies.** The echocardiography studies were performed 3–4 wk after surgery (myocardial infarction) and at the end of the 10-wk exercise training or 10-wk sedentary period as previously described (22). These studies were performed before the animals were classified by the exercise + ischemia test. Briefly, the dogs were lightly sedated with acepromazine (0.5 mg/kg iv; Ft. Dodge Animal Health, Ft. Dodge, IA) before the studies. A Sonos 1000 system (Hewlett Packard, Palo Alto, CA) with a 5.5-MHz transducer was used to obtain a conventional M-mode echocardiogram. Fractional shortening and Vcf of the left ventricle were determined. The latter parameter provides a load-independent measure of contractility incorporating the extent and velocity of fiber shortening (10, 22). Vcf was calculated according to the following formula: \((LVIDd - LVIDs)/LVIDd \times ET\), where LVIDd is the short axis of the left ventricle in diastole (in cm), LVIDs is the short axis of the left ventricle in systole (in cm), and ET is the ejection time (in s). Dividing by the square root of the R-R interval from a simultaneously collected ECG corrects for changes in heart rate (10). Fractional shortening was calculated as follows: \((LVIDd - LVIDs)/LVIDd \times 100\).

The total \(\beta\)-adrenoceptor response was quantified by infusion of increasing doses of isoproterenol (Sigma Chemical, St. Louis, MO): 0.005, 0.015, 0.05, 0.15, and 0.5 \(\mu\)g·min\(^{-1}\)·kg\(^{-1}\). The echocardiogram was obtained when a steady-state heart rate response was achieved at each dose. After the mixed \(\beta_1\) and \(\beta_2\)-adrenoceptor in vivo response, a bolus injection of the \(\beta_1\)-adrenoceptor antagonist bisoprolol (0.6 mg/kg; Merck, Darmstadt, Germany) (22) or the \(\beta_2\)-adrenoceptor antagonist ICI-118551 (0.2 mg/kg; RBI, Natick, MA) (9, 22) was administered. The isoproterenol dogs received bisoprolol first; the other half received ICI-118551 first. We previously demonstrated that repeated isoproterenol infusion did not result in downregulation and desensitization of the \(\beta\)-adrenoceptors (22).

**Isolation of ventricular myocytes.** At the end of the study, the exercise-trained (n = 8 susceptible and 6 resistant) and sedentary (n = 7 susceptible and 5 resistant) dogs were anesthetized with pentobarbital sodium (10 mg/kg i.v.; Nembutal, Abbott Laboratories) and the heart was rapidly removed for the preparation of cardiac myocytes. At the same time, a small piece of the diaphragm was placed in liquid nitrogen and stored in a -80°C freezer. The citrate synthase activity of this skeletal muscle was assayed using the modified technique described by Seres (39). The skeletal muscle was harvested ≥48 h after the last exercise session.

**Data analysis.** Values are means ± SE. The in vivo data were digitized (1 kHz) and recorded using a data acquisition system (model MP-100, Biopac Systems, Goleta, CA). The echocardiographic data were averaged over the last 30 s of each exercise level. The coronary occlusion data were averaged over the last 5 s before and 60 s after occlusion onset (i.e., onset of VF). Heart rate variability (0.24- to 1.04-Hz component of R-R interval variability, an index of cardiac vagal tone index) was obtained using a vagal tone monitor triggered by the ECG R-R interval (Delta-Biometrics, Urbana-Champaign, IL). This device employs the time-series signal-processing techniques developed by Porges (32) to estimate the amplitude of respiratory sinus arrhythmia. Details of this analysis have been described previously (7).

The in vivo and in vitro data were analyzed using ANOVA for repeated measures (NCSS, Kaysville, UT). For example, the echocardiographic data (Vcf, fractional shortening, and heart rate) were analyzed using a three-way ANOVA [group (2 levels) × pre-post (2 levels) × drug (4 levels) or drug dose (5 levels)] with repeated measures on two factors (pre-post and drug or drug dose). The effects of exercise training on the heart rate response to exercise or the exercise + ischemia test were analyzed using a two-factor [group × exercise level or time] ANOVA with repeated measures on one factor (exercise level or time). A similar two-factor (group × pre-post) ANOVA with repeated measures on one factor (pre-post) was used to evaluate the effects of the interventions on left ventricular systolic wall thickness. Because repeated-measures ANOVA depends on the homogeneity of covariance, this spurious assumption (i.e., that the variance of the...
difference scores in a within-subject design are equal across the groups) was tested using Mauchley’s test (23). If the sphericity assumption was violated, then the F-ratio was corrected using the Huynh-Feldt correction (23). If the F-ratio exceeded a critical value (P < 0.05), then the difference between the means was determined using Scheffe’s test. For the single-cell isotonic shortening data, cells from a given animal were averaged, and the mean values were compared with a one-way ANOVA. Finally, citrate synthase activity data (exercise trained vs. sedentary) were evaluated using Student’s t-test.

RESULTS

Confirmation of exercise training. Body weight was similar in trained and sedentary animals for the resistant (20.4 ± 1.1 and 19.6 ± 1.8 kg, respectively) or the susceptible (20.0 ± 0.6 and 19.8 ± 0.8 kg, respectively) dogs. However, left ventricular systolic wall thickness was significantly larger (P < 0.025) in the susceptible and resistant exercise-trained than in the sedentary dogs (Fig. 2), indicating that the training provoked a small (10.1 ± 4 and 8.0 ± 2.6% in susceptible and resistant, respectively) ventricular hypertrophy. The heart rate and vagal tone index response to submaximal exercise before and after the 10-wk exercise or 10-wk sedentary period are displayed in Fig. 3 for the susceptible dogs. Training provoked significant (P < 0.0025) reductions in heart rate that were accompanied by significant increases in R-R interval variability (vagal tone index, i.e., 0.24- to 1.04-Hz component of the R-R interval variability, P < 0.04), whereas these variables did not change in the sedentary animals. Similar, but smaller, changes were noted for the resistant dogs (data not shown). Finally, citrate synthase activity was significantly (P < 0.02) higher in skeletal muscle from exercise-trained than sedentary dogs (n = 10 each): 11.6 ± 1.0 vs. 7.5 ± 1.4 μmol·ml⁻¹·min⁻¹. Inasmuch as there were no differences between resistant and susceptible dogs, these data were pooled for the analysis. These data confirm that the exercise-training program was effective; i.e., a significant skeletal muscle and cardiac adaptation was induced by the training program.

Effect of training on the β2-adrenoceptor contractile responses in vivo: echocardiography studies. Isoproterenol elicited significant (P < 0.0001) dose-dependent increases in Vcf, fractional shortening, and heart rate that were reduced by the β1-adrenoceptor antagonist bisoprolol or the β2-adrenoceptor antagonist ICI-118551 in susceptible and resistant dogs. The effects of the antagonist were most obvious at the highest dose of isoproterenol. The peak responses to isoproterenol before and after treatment with the selective β2-adrenoceptor antagonists for the susceptible dogs are displayed in Fig. 4, and data for the resistant animals are shown in Fig. 5. The data are shown before (control) and after completion of the 10-wk training or 10-wk sedentary time period. Inasmuch as there were no differences in the data before the start of the 10-wk period for the sedentary or exercise-trained animals, the data were combined for the sake of clarity.
In the susceptible animals, isoproterenol elicited similar increases in $V_{cf}$ (Fig. 4A), fractional shortening (Fig. 4B), and heart rate (Fig. 4C) before and at the end of the sedentary or training period. $\beta_1$-Adrenoceptor blockade significantly (all $P < 0.01$) reduced $V_{cf}$, fractional shortening, and heart rate responses to isoproterenol to a similar extent before and after completion of the training or sedentary period. These data are consistent with a similar $\beta_1$-adrenoceptor responsiveness before and after training or an equivalent sedentary time period. In marked contrast, $\beta_2$-adrenoceptor blockade elicited significantly (all $P < 0.01$) smaller reductions in $V_{cf}$ (Fig. 4A), fractional shortening (Fig. 4B), and heart rate (Fig. 4C) responses to isoproterenol in the exercise-trained animals. Conversely, this intervention provoked even larger (all $P < 0.01$) reductions in the isoproterenol response of all three variables in the sedentary dogs. These data suggest that training reduced $\beta_2$-adrenoceptor responsiveness, whereas $\beta_2$-adrenoceptor responsiveness increased over time in the sedentary group.

In the resistant animals, isoproterenol elicited similar increases in $V_{cf}$ (Fig. 5A), fractional shortening (Fig. 5B), and heart rate (Fig. 5C) before and at the end of the sedentary or the training period. In agreement with our previous studies (9, 22), the $\beta_2$-adrenoceptor antagonist ICI-118551 provoked smaller reductions in the isoproterenol response in the resistant than in the susceptible dogs. In contrast to the susceptible dogs, $\beta_1$- and $\beta_2$-adrenoceptor blockade significantly (all $P < 0.01$) reduced $V_{cf}$, fractional shortening, and heart rate responses to isoproterenol to a similar extent before and after completion of the training or sedentary period. Thus, in contrast to the susceptible animals, $\beta_2$-adrenoceptor responsiveness was not affected by training, nor did it increase over time in the sedentary animals.

**Effect of training on the $\beta$-adrenoceptor contractile responses in vitro: single-cell shortening studies.** The isotonic single-cell shortening responses to the $\beta_2$-adrenoceptor antagonist zinterol (after $\beta_1$-adrenoceptor blockade with CGP-01217A) are displayed in Fig. 6. Zinterol elicited a significantly ($P < 0.0004$) greater increase in isotonic shortening in cells from sedentary than exercise-trained susceptible dogs: $+42.8 \pm 5.8$ vs. $+7.2 \pm 4.8\%$. The response of the cells from the hearts of the exercise-trained susceptible dogs was similar to that of exercise-trained and sedentary resistant dogs: $+3.0 \pm 1.4$ and $+3.2 \pm 1.8\%$, respectively.

**Effect of training on susceptibility to VF.** The exercise + ischemia test was repeated after completion of the 10-wk exercise-training or 10-wk sedentary period. The heart rate response to coronary artery occlusion is displayed in Fig. 7. Coronary occlusion elicited significant ($P < 0.0002$) increases in heart rate in the sedentary and exercise-trained susceptible
atropine sulfate (50 μg/kg iv injected while the dogs were running 3 min before the coronary occlusion). Atropine significantly (P < 0.01) increased heart rate (36.8 ± 3.2 beats/min) and reduced heart rate variability (−2.3 ± 0.3 ln ms²) before and during the coronary occlusion. This heart rate increase exceeded the maximum heart rate induced by exercise or the coronary occlusion before training. Yet this intervention reintroduced VF, or any other arrhythmia, in only one of eight dogs tested. Thus neither reductions in heart rate nor increases in cardiac vagal regulation are solely responsible for the protection induced by training.

DISCUSSION

The major findings of this study are as follows. 1) β2-adrenoceptor blockade elicited larger reductions in the isoproterenol-mediated increases in ventricular contractility in dogs susceptible to VF than in dogs resistant to these malignant arrhythmias. 2) Endurance exercise training eliminated this enhanced β2-adrenoceptor response, restoring a more normal β-adrenoceptor balance in the dogs previously shown to be susceptible to VF. 3) The β2-adrenoceptor responsiveness increased in the sedentary dogs, suggesting that, over time, these dogs became even more dependent on β2-adrenoceptor activation to maintain cardiac function. 4) Similar results were noted in the isolated ventricular myocytes. The β2-adrenoceptor agonist zinterol elicited a greater single-cell isotonic shortening in myocytes from susceptible sedentary dogs than in cells from susceptible exercise-trained or resistant dogs. After training, the response was no longer different between the susceptible and the resistant (sedentary or trained) animals.
Training completely suppressed VF induced by acute myocardial ischemia, protecting all nine susceptible dogs that completed the 10-wk exercise program. In marked contrast, an equivalent sedentary period failed to protect the susceptible dogs that completed the 10-wk period, and four dogs died spontaneously during this period. These data suggest that exercise training restores a more normal $\beta_2$-adrenoceptor balance by reducing $\beta_2$-adrenoceptor responsiveness and by preventing a further deterioration in the cardiac $\beta_2$-adrenoceptor regulation. As a consequence, exercise training also reduced the incidence of malignant arrhythmias. To the best of our knowledge, these findings represent the first demonstration that exercise training can reduce $\beta_2$-adrenoceptor responsiveness in diseased hearts and protect against ischemically induced VF.

$\beta_2$-Adrenoceptors and susceptibility to VF. An enhanced sympathetic activation can reduce cardiac electrical stability and induce VF (36). Presumably, the activation of myocardial adrenergic receptors mediates the arrhythmogenic effects of catecholamines released from the sympathetic nerve terminals. Until recently, it has been thought that myocardial $\beta_2$-adrenoceptors were primarily the $\beta_1$-subtype. However, it is now

Fig. 8. Representative ECG recordings from 2 different susceptible dogs: before and after completion of a 10-wk endurance exercise program and before and after an equivalent 10-wk sedentary period. Arrhythmias were no longer induced by exercise + ischemia test in exercise-trained dog. Arrow indicates time at which the treadmill was stopped.

Fig. 9. Effect of endurance (10-wk) exercise training on incidence of VF. Exercise training prevented VF in all 9 animals susceptible to this malignant arrhythmia. Exercise + ischemia test induced VF in all 9 susceptible animals that completed a 10-wk sedentary period; 4 animals died spontaneously during this 10-wk time period, and 3 additional animals could not be retested because of failure of the coronary artery occluder. *$P < 0.01$ vs. Pre (after 10 wk).
apparent that ventricular myocytes also contain functional β2-adrenoceptors, which may become particularly important under certain pathological conditions (1, 11). β1-Adrenoceptor sensitivity decreases substantially as the result of heart failure, whereas the number of β2-adrenoceptors remains relatively constant (1, 11). As a consequence, the failing heart becomes more dependent on β2-adrenoceptors for inotropic support. However, the recruitment and activation of these “latent” β2-adrenoceptors may also alter cardiac electrical stability, increasing the propensity for the formation of malignant arrhythmias.

We previously demonstrated that the activation of β2-adrenoceptors in dogs with diseased hearts produced a large increase in L-type Ca2+ current without altering Ca2+ reuptake by the sarcoplasmic reticulum, whole cell cAMP, or phospholamban phosphorylation (2). In contrast, cells from normal control animals exhibited little response to β2-adrenoceptor stimulation. The resulting elevations in intracellular Ca2+ concentration could trigger oscillations in membrane potential that could culminate in the induction of VF (5). The activation of β2-adrenoceptors could, therefore, provoke malignant arrhythmias, whereas the inhibition of these adrenergic receptors could prevent these arrhythmias. Indeed, β2-adrenoceptor activation provoked an increased Ca2+ transient amplitude, aftercontractions, and arrhythmias in rabbits in which heart failure was induced by the combination of aortic insufficiency and aortic constriction (14). Furthermore, the selective β2-adrenoceptor antagonist ICI-118551 prevented VF induced by acute ischemia in animals with healed myocardial infarctions (9). In agreement with the present study, the susceptible dogs also exhibited a β2-adrenoceptor-mediated contractile response in the intact animal and in ventricular myocytes isolated from the hearts of susceptible dogs that was enhanced compared with that in cells obtained from resistant dogs (22). Interestingly, the overexpression of β2-adrenergic receptors elicited a much higher mortality in transgenic than in wild-type mice (81 vs. 4% by 15 mo of age). These transgenic mice also exhibited abnormal ECG waveforms and episodes of polymorphic ventricular tachycardia (15), further evidence that excessive β2-adrenoceptor activation can provoke profound cardiac electrical derangements.

No clinical studies have specifically evaluated the contribution of β2-adrenoceptors to cardiac mortality. However, a number of studies indirectly support this hypothesis. There is overwhelming evidence that β-adrenoceptor antagonists can protect against arrhythmia formation induced by myocardial ischemia and infarction (18, 26). Indeed, this marked reduction in cardiac mortality has been verified in 32 trials involving ∼29,000 patients (18, 26). However, if one carefully examines the clinical studies cited above, it becomes apparent that not all β-adrenoceptor antagonists offer the same level of protection, particularly during acute myocardial infarction. The majority of the studies using the β1-adrenoceptor antagonist metoprolol failed to report significant reductions in the incidence of VF during acute myocardial infarction (20, 21). Furthermore, although atenolol reduced overall mortality by 15%, the number of patients who died as the result of malignant arrhythmias was not altered (25). In contrast, propranolol therapy elicited large reductions in overall mortality (65% decrease) and sudden cardiac death (41% decrease) in post-myocardial infarction patients with persistent S-T segment depression (37), a group of patients known to be at a particularly high risk for subsequent cardiac events (41). These data suggest that a better antiarrhythmic protection can be achieved with complete (i.e., β1- and β2-adrenoceptor), rather than selective (i.e., β1-), β-adrenoceptor blockade (1).

Exercise training and susceptibility to VF. Although exercise conditioning can reduce cardiac sympathetic activity (34), the effects of this intervention on β-adrenoceptors are less clear. Regular exercise can improve β-adrenoceptor responsiveness in normal animals (3, 38), aged animals (29), and animals with cardiovascular disease (27), despite a reduction (3) or no change in the β1-adrenoceptor density (27, 29). In normal Wistar rats, daily exercise provoked significant reductions in β1-adrenoceptor density without altering β2- or β3-adrenoceptor density (3). Although in agreement with the present study, MacDonnell et al. (27) demonstrated that exercise training reversed the defective contractile response to β-adrenoceptor stimulation in spontaneously hypertensive rats, despite the presence of ventricular hypertrophy. These investigators did not determine whether training altered β2-adrenoceptor responsiveness. In the present study, exercise training significantly reduced the in vivo and in vitro contractile response to β2-adrenoceptor stimulation in animals known to be susceptible to VF. After training, the β2-adrenoceptor response of the susceptible dogs was indistinguishable from that of the dogs that were resistant to malignant arrhythmias. In marked contrast, the β2-adrenoceptor responsiveness increased even further in susceptible animals after completion of a comparable sedentary period. Thus exercise training provoked a “remodeling” of the cardiac β-adrenoceptor regulation, restoring a more normal β1-to-β2-adrenoceptor balance in the dogs that were previously susceptible to VF. The resulting attenuation of the β2-adrenoceptor responsiveness would increase cardiac electrical stability, thereby reducing the risk for sudden death.

Regular exercise is associated with a lower risk for arrhythmias and sudden death in humans and animals (6). Bartels et al. (4) found that the incidence of sudden cardiac death was inversely related to the level of regular physical activity; i.e., sedentary individuals had the highest rate of sudden death (4.7 deaths per 105 person-yr), whereas the most active individuals had the lowest incidence of sudden death (0.9 deaths per 105 person-yr). Furthermore, by meta-analysis of 22 randomized trials of rehabilitation with exercise after myocardial infarction, O’Connor et al. (31) found that exercise training elicited significant reductions in the reinfarction rate and the incidence of sudden death. There was an overall reduction in cardiac mortality of 20% (due largely to the reduction in sudden death), a reduction that is comparable to the mortality reductions noted for β-adrenoceptor antagonists (18, 26). Exercise training can also improve cardiac function and reduce arrhythmia frequency in congestive heart failure patients (16, 19). For example, Hertzeneau et al. (19) found that a 6-mo exercise program reduced the frequency and severity of the arrhythmias in postmyocardial infarction patients with poor ejection fractions (<30%).

Experimental studies have reported that exercise training decreases the risk for arrhythmias (6, 8, 24) or the electrical current necessary to induce VF (30, 33). In agreement with the present study, Billman et al. (8) and Hull et al. (24) reported that daily exercise prevented VF induced by acute ischemia in dogs with healed anterior wall myocardial infarctions. How-
EXERCISE TRAINING REDUCES β2-ADRENOCEPTOR RESPONSES

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However, although the mammalian heart contains a large number of β2-adrenoceptors, β2-adrenoceptor agonists have surprisingly small inotropic effects (1). For example, the β1- to β2-adrenoceptor ratio was found to be 59:41 in baboon cardiac myocytes, yet the contractile response to isoproterenol was almost completely abolished by the selective β1-adrenoceptor antagonist metoprolol in vivo (13). It, therefore, seems more likely that exercise-induced changes in β2-adrenoceptor responsiveness would result from alterations in β2-adrenoceptor signaling than from changes in β2-adrenoceptor density. The mechanisms by which exercise training restored cardiac β-adrenoceptor balance remain to be determined.

GRANTS

These studies were supported by National Heart, Lung, and Blood Institute Grant HL-68609.

REFERENCES


Limitations of the study. Extensive coronary collateral vessels in dogs (28) may (35) or may not (12) be increased by exercise training. Therefore, exercise training-induced increases in coronary collateral circulation could contribute to the protection noted in the susceptible dogs. However, acute myocardial ischemia provoked similar increases in heart rate in the susceptible sedentary and susceptible trained dogs before (31.0 ± 8.5 and 32.4 ± 1.1 beats/min, respectively) and after (32.5 ± 7.4 and 29.3 ± 1.9 beats/min, respectively) the 10-wk period. In addition, the exercise + ischemia test provoked a similar S-T segment depression in the sedentary and exercise-trained susceptible dogs before (−4.8 ± 1.2 and −4.7 ± 0.4 mm, respectively) and at the end of the 10-wk period (−4.8 ± 1.7 and −4.8 ± 0.4 mm, respectively). When considered together, these data suggest that the coronary occlusion elicited a similar ischemic response before and at the end of the 10-wk sedentary or 10-wk exercise-training period.

In a similar manner, exercise training reduced the peak heart rate achieved during the exercise + ischemia test. By decreasing metabolic demand, a lower heart rate per se could reduce the risk for arrhythmias. However, atropine pretreatment elicited large increases in heart rate that exceeded the maximum heart rate values induced by the coronary occlusion before training yet only modestly increased the arrhythmia frequency. Thus heart rate reductions alone cannot be responsible for the training-induced protection from VF.

Myocardial infarction size could also contribute to the differences between susceptible and resistant dogs; animals with larger infarctions would be expected to have poorer ventricular function and a higher risk for VF. Myocardial infarction size was not measured in the present study, inasmuch as the hearts were removed for in vitro studies. Therefore, we performed a retrospective analysis of animals in which infarction size was determined and found larger infarctions in the susceptible dogs: 17.7 ± 0.9% for susceptible (n = 93) and 12.6 ± 1.4% for resistant (n = 50) dogs. Inasmuch as the exercise program did not begin until after the myocardial infarction had healed (≥4 wk after induction of the infarction), it seems unlikely that training would reduce the infarction size in these animals.

Finally, β-adrenoceptor density was not measured in the present study. It, therefore, was not possible to determine whether the exercise-training-induced reductions in β2-adrenoceptor responsiveness resulted from reductions in β2-adrenoceptor density (or distribution) or alterations in β2-adrenoceptor signaling (i.e., events downstream from the receptors). However, neither study examined the effects of this exercise program on cardiac sympathetic regulation. The present study confirms and extends these previous studies. Training attenuated β2-adrenoceptor responsiveness, whereas an equivalent sedentary time period resulted in a further enhancement of cardiac β2-adrenoceptor regulation. The restoration of a more normal β-adrenoceptor balance was accompanied by complete suppression of VF. All nine susceptible dogs that completed the 10-wk exercise program were protected from VF induced by acute myocardial ischemia. In marked contrast, an equivalent sedentary period failed to protect any of the susceptible dogs that completed the 10-wk period, and dogs also died spontaneously during this period. These data suggest that exercise training could prevent VF by restoring more normal β-adrenoceptor balance (i.e., reducing β2-adrenoceptor responsiveness).
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