Exercise training normalizes β-adrenoceptor expression in dogs susceptible to ventricular fibrillation

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Holycross BJ, Kukielski M, Nishijima Y, Altschuld RA, Carnes CA, Billman GE. Exercise training normalizes β-adrenoceptor expression in dogs susceptible to ventricular fibrillation. Am J Physiol Heart Circ Physiol 293: H2702–H2709, 2007. First published August 24, 2007; doi:10.1152/ajpheart.00763.2007.—Previous studies demonstrated an enhanced β-AR responsiveness in animals susceptible to ventricular fibrillation (VF) that was eliminated by exercise training. The present study investigated the effects of endurance exercise training on β1-AR and β2-AR expression in dogs susceptible to VF. Myocardial ischemia was induced by a 2-min occlusion of the left circumflex artery during the last minute of exercise in dogs with healed infarctions: 20 had VF [susceptible (S)] and 13 did not [resistant (R)]. These dogs were randomly assigned to either 10-wk exercise training [treadmill running; n = 9 (S) or 8 (R)] or an equivalent sedentary period [n = 11 (S) or 5 (R)]. Left ventricular tissue β-AR protein and mRNA were quantified by Western blot analysis and RT-PCR, respectively. Because β2-ARs were located in caveolae, caveolin-3 was also quantified. β1-AR gene expression decreased (~5-fold), β2-AR gene expression was not changed, and the ratio of β2-AR to β1-AR gene expression was significantly increased in susceptible compared with resistant dogs. β1-AR protein decreased (~50%) and β2-AR protein increased (400%) in noncaveolar fractions of the cell membrane in susceptible dogs. Exercise training returned β1-AR gene expression to levels seen in resistant animals but did not alter β2-AR protein levels in susceptible dogs. These data suggest that β1-AR gene expression was decreased in susceptible dogs compared with resistant dogs and, further, that exercise training improves β1-AR gene expression, thereby restoring a more normal β-AR balance.

β-adrenergic receptors; myocardial infarction; sympathetic nervous system

IT IS WELL ESTABLISHED THAT cardiac β1-adrenoceptor (AR) sensitivity decreases substantially during heart failure, whereas the β2-AR response remains relatively constant (1, 12). As a consequence the failing heart becomes more dependent on the activation of β2-AR for inotropic support. The activation of β2-ARs promotes increased calcium currents without altering calcium reuptake by the sarcoplasmatic reticulum (2). The resulting elevations in intracellular calcium could provoke oscillations in membrane potential that could, in turn, trigger extrasystoles. Thus, in the diseased heart, β2-AR activation would tend to reduce cardiac electrical stability and increase the propensity for malignant arrhythmias, particularly when calcium regulation is further altered by sympathtic activation and/or myocardial ischemia.

We recently demonstrated (10, 18), in dogs with healed myocardial infarctions (MIs), that the nonselective β-AR agonist isoproterenol provoked significantly larger heart rate and inotropic responses in those animals that were susceptible to ventricular fibrillation (VF) induced by myocardial ischemia than in those animals that were resistant to these malignant arrhythmias. The selective β2-AR antagonist ICI-118551 reduced the isoproterenol response to a much greater extent in the susceptible animals, eliminating any differences noted between the groups (10, 18). In a similar manner, both the calcium transient amplitude and the single-cell isotonic shortening responses to isoproterenol were larger in myocytes obtained from the hearts of susceptible compared with resistant dogs, differences that were also eliminated by β2-AR blockade but not by β1-AR blockade (10). In the intact dog, β2-AR blockade also prevented VF induced by acute myocardial ischemia (10). When considered together, these data demonstrate that an enhanced β2-AR responsiveness is associated with an increased propensity for VF. One would predict that interventions that restore a more normal β1-AR to β2-AR balance should also protect against VF.

Regular exercise can both improve β-AR responsiveness and decrease the incidence of malignant arrhythmias (4, 5, 7, 8, 11, 23, 25, 38, 42). We recently demonstrated (11) that a 10-wk endurance exercise program reduced β2-AR responsiveness and prevented VF induced by acute myocardial ischemia. In contrast, β2-AR responsiveness was further enhanced in sedentary (control) dogs that also remained prone to VF (11). These data suggest that exercise training could restore a more normal β-AR balance after MI and could thereby prevent VF. However, it remains to be determined whether the improved β2-AR responsiveness resulted from altered β-AR gene expression, β-AR protein abundance in the cell membrane, or changes in the receptor signal transduction process.

Exercise training-induced changes in the distribution of β-ARs within the sarcolemma could also affect the response of these receptors to stimulation. β1-ARs are widely distributed throughout the cardiac myocyte cell membrane, where agonist stimulation results in elevated intracellular cAMP and calcium flux. (33). β2-ARs are located in caveolae, lipid-enriched invaginations of the cell membrane, both on the outer surface of the cell and throughout the t tubules (29, 37). In adult rat ventricular cardiomyocytes, it appears that β2-ARs must be located in the caveolae to obtain close approximation to sar-
coplasmic reticulum and signaling pathway proteins such as G<sub>i</sub> and the sodium/calcium exchanger (13). Disruption of caveolae results in redistribution of β<sub>2</sub>-ARs and a marked increase in β<sub>2</sub>-AR agonist-induced cardiomyocyte shortening and calcium transients (13). Thus the distribution of β<sub>2</sub>-ARs may be altered in animals susceptible to VF, and, further, exercise training may redistribute the β<sub>2</sub>-ARs to restore a more normal β-AR responsiveness, decreasing the risk for malignant arrhythmias.

It was therefore the purpose of this study to determine the effects of exercise training on β<sub>2</sub>-AR and β<sub>1</sub>-AR gene expression and protein abundance in dogs susceptible or resistant to VF. In particular, the hypothesis that endurance exercise training would restore a more normal β<sub>2</sub>-AR to β<sub>1</sub>-AR balance was tested. Left ventricular (LV) β-AR protein and mRNA were quantified by Western blot and RT-PCR in tissue harvested from either sedentary (10 wk) or exercise-trained (10 wk progressively increasing treadmill running) dogs. This training program was previously shown to prevent VF in these animals (7, 11). Since β<sub>2</sub>-ARs are located in caveolae, caveolin-3 was also quantified.

MATERIALS AND METHODS

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

All studies were performed on archived tissue harvested from dogs that had been randomly assigned to either exercise training (10 wk, progressively increasing treadmill running) or an equivalent sedentary time period. LV tissue from three to five dogs per treatment group was selected at random for RT-PCR and Western blot analysis. In addition, the assays were performed on LV tissue obtained from four control (i.e., no MI) dogs.

The surgical procedures, the exercise training program, and the test (exercise + ischemia) used to classify the dogs as to susceptibility to VF have been described previously (6-11, 39). A flowchart of the animals used in the present study is displayed in Fig. 1.

### Myocardial Infarction

(n = 60, 14 died within 72 hr)

#### Exercise + Ischemia Test

(n = 46, occluder failure n = 7)

- Susceptible
  - (had VF, n = 26, unable to defibr = 3)
  - Ex Training (n = 9)
  - Sedentary (n = 14*)

- Resistant
  - (no VF, n = 13)
  - Ex Training (n = 8)
  - Sedentary (n = 5)

* 4 animals died, unable to re-test 3 dogs due to occluder failure

Fig. 1. Flowchart illustrating the sequences of events in this study. Three to four weeks after myocardial infarction, the dogs were classified as susceptible or resistant to ventricular fibrillation (VF) with an exercise + ischemia test. The animals were then randomly assigned to either a 10-wk exercise (Ex) training program or a 10-wk sedentary period. The exercise + ischemia test was repeated at the end of the 10-wk period. n = No. of animals. defibr, Defibrillate.

Real-Time PCR

A section of left ventricle (posterior lateral free wall, noninfarcted tissue) was snap frozen in liquid nitrogen and then stored at −80°C. Total RNA was isolated from frozen LV tissue with a ToTALLY RNA kit (Ambion, Austin TX) followed by DNase I digestion. Total RNA was quantified via spectrophotometry, and 250 ng of total RNA was used in 25-μl reverse transcription reactions utilizing TaqMan RT reagents as outlined by the manufacturer (Applied Biosystems, Foster City, CA). Real-time polymerase chain reaction (PCR) experiments were conducted in the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) with the SYBR Green PCR Master Mix (24, 45). Each 25-μl reaction was performed in triplicate on a 96-well plate with 12.5 μl of SYBR Green Master Mix, 1 μl of 5 μM sense primer, 1 μl of 5 μM antisense primer, and 1.5 μl of cDNA. Thermocycler conditions were set at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 45 s, and 72°C for 45 s. Before real-time PCR analysis, each primer pair was confirmed to yield a single band at the appropriate size by conventional PCR using the above listed cycling conditions.

Primers were designed with TaqMan Probe and Primer design software and were designed to be 19–21 bp long, with a melting temperature of 59–60°C, and yield a PCR product between 52 and 63 bp long. Primers for PCR were 18S forward: 5′-GGTTGATCTTGCC- CAGTAGCATAT-3′; 18S reverse: 5′-GTACCGCCCGTGCAGAC- TT-3′ (GenBank accession no. AY262732); β<sub>1</sub> forward: 5′-TGCTA- CAACGGACCCCAATG-3′; β<sub>1</sub> reverse: 5′-AGTGACACGAGGC- CATGATG-3′ (GenBank accession no. U73207); and β<sub>2</sub> forward: 5′-CCAGAAGGCAGCTCCAGAGA-3′; β<sub>2</sub> reverse: 5′-CCACTTG- GCTGAAGTTTTGG-3′ (GenBank accession no. AY011309). The primers were subjected to a BLAST search and were found to have no cross-homologies with other canine genes.

Analysis of all genes for a given dog was performed on the same 96-well plate, and dogs from the different treatment groups were randomly assigned to different 96-well plates. For a given gene, analysis parameters were consistent between plates. For each dog, a threshold cycle (C<sub>t</sub>) for each gene was determined. A ΔC<sub>t</sub> was then calculated by subtracting the C<sub>t</sub> for 18S from the C<sub>t</sub> obtained for the β<sub>2</sub>-AR or β<sub>1</sub>-AR. The average ΔC<sub>t</sub> for each gene was calculated for the resistant sedentary group of dogs, and the difference between the gene expression in this group and all other treatment groups was calculated and expressed as the ΔΔC<sub>t</sub>. The actual fold change in expression between treatment groups is calculated as 2<sup>ΔΔC<sub>t</sub></sup>. Values for β<sub>2</sub>- to β<sub>1</sub>-AR expression were calculated for each dog as ΔC<sub>t</sub> β<sub>1</sub> - ΔC<sub>t</sub> β<sub>2</sub> to obtain a ΔΔC<sub>t</sub>. Fold differences in expression were calculated as 2<sup>ΔΔC<sub>t</sub></sup>, and the ratios β<sub>2</sub>/β<sub>1</sub> were calculated as 1/ΔΔC<sub>t</sub> fold change. Because there were no differences in the gene expression between the resistant sedentary and the resistant exercise groups, these data were combined in all subsequent analyses.

Western Blot Analysis

Frozen canine tissue was extracted by two different methods to evaluate receptor sequestered in caveolae compared with total receptor complement in the cell membrane.

SDS extraction. Approximately 100 mg of tissue was rapidly pulverized in liquid nitrogen with a mortar and pestle and placed in 500 μl of SDS buffer consisting of 1% SDS, 10 mmol/l Tris·HCl pH 7.4, 1 mmol/l orthovanadate, 0.1 mmol/l leupeptin, 0.001 mmol/l aprotinin, and 10 mg/ml PMSF as described previously (34). Lysates were boiled in a microwave oven for 15 s (1,100 W) and then centrifuged at 13,000 g for 15 min at room temperature.

Triton X-100 extraction. Approximately 100 mg of tissue was rapidly pulverized in liquid nitrogen with a mortar and pestle and then placed in 500 μl of ice-cold TE buffer containing 20 mmol/l Tris, 1 mmol/l EDTA, 0.1 mg/ml benzamidine, 10 mg/ml PMSF, 5 μg/ml leupeptin, 5 μg/ml pepstatin A, 5 μg/ml aprotinin, and 100 μmol/l sodium orthovanadate as described previously (14). The suspension
was vortexed, and Triton X-100 was added to a final concentration of 2%. Lysates were put on ice for 2 h and then centrifuged at 14,000 g for 10 min at 4°C. For each extraction, the soluble fraction was retained and stored at −80°C. Protein concentration was determined with a microBCA protein assay kit (Pierce), and 30 μg (SDS extracts) or 50 μg (Triton X-100 extracts) was heated to 95°C for 5 min in 3× SDS sample buffer (New England) and then subjected to electrophoresis (1 h, 150 V) on 10% Tris-HCl Criterion Precast Gels (Bio-Rad). Proteins were transferred for 45 min (15 V) to Immobilon-P polyvinylidene difluoride membranes with a semidy bladder (Bio-Rad). Membranes were blocked (1 h, room temperature) with 5% nonfat milk in 0.1% Tween 20 in Tris-buffered saline (TBS) and incubated overnight at 4°C in 5% BSA and 0.1% Tween 20 in TBS with rabbit polyclonal antibodies to β1-AR or β2-AR (Santa Cruz Biotechnology) at 1:500 dilution or mouse monoclonal anti-caveolin-3 antibodies (BD Transduction Laboratories) at 1:2,500 dilution. After three washes (5 min, room temperature) with 0.1% Tween 20 in TBS, the membranes were incubated with 1:1,000 dilution of goat anti-rabbit IgG-horseradish peroxidase (HRP) or goat anti-mouse IgM-HRP in 5% nonfat milk. 0.1% Tween 20 in TBS for 1 h at room temperature. Membranes were then washed six times with 0.1% Tween 20 in TBS (15 min, room temperature). Antibody binding was detected with LumiGlo chemiluminescent substrate (Upstate) and CL-X Posure clear blue X-ray film (Pierce). Films were scanned with ScanSuite software and quantified with ImageJ (National Institutes of Health). Densitometric comparisons were performed in samples run on the same membrane. For Western blots, three to five dog samples were analyzed per treatment group, and each analysis was performed on two or three gels. Normalization for protein loading was calculated after Ponceau staining of the membrane as described by Ping et al. (32). After normalization for loading, the data were normalized to the value of the resistant sedentary group, which was arbitrarily assigned the value of 100%. Because there were no differences in Western blot values between the resistant sedentary and the resistant exercise groups for any of the proteins examined, these data were combined in all subsequent analyses.

Echocardiography Studies

To evaluate the effects of MI on ventricular function, two-dimensional B- and M-mode echocardiograms were obtained from a subset of dogs (7 susceptible and 4 resistant) under butorphanol sedation (0.5 mg/kg im) at baseline (before surgery and infarction) and 8 wk after surgery (GE Vivid 7 echocardiograph).

Statistical Analysis

All data are reported as means ± SD. Statistical analysis was performed with a one-factor ANOVA (NCSS, Kaysville, UT). If the F-ratio exceeded a critical value (P < 0.05), the means were compared by post hoc analysis performed with the Tukey-Kramer test. The echocardiographic variables were compared with a paired t-test.

RESULTS

Effect of Myocardial Infarction on Ventricular Function

In a subset of animals, echocardiograms were obtained to assess LV function and structure. In agreement with our previous studies (9, 18), MI did not induce any significant alterations in contractile function measured as LV fractional shortening in either the susceptible [n = 7; 41.3 ± 4.7% baseline vs. 40.5 ± 2.9% post-MI; P = not significant (NS)] or the resistant [n = 4; 37.5 ± 5.1% baseline vs. 37.0 ± 2.7 post-MI; P = NS] group. Furthermore, MI did not produce any significant LV dilation measured as end-diastolic diameter in either the susceptible [n = 7; 4.2 ± 0.3 cm baseline vs. 4.3 ± 0.3 cm post-MI; P = NS] or the resistant [n = 4; 4.0 ± 0.3 cm baseline vs. 4.3 ± 0.4 cm post-MI; P = NS] group. Similarly, there were no significant changes in LV end-systolic diameter in either the susceptible [n = 7; 2.5 ± 0.2 cm baseline vs. 2.6 ± 0.3 cm post-MI; P = NS] or the resistant [n = 4; 2.5 ± 0.2 cm baseline vs. 2.7 ± 0.2 cm post-MI; P = NS] group.

Effect of Exercise Training on β-AR mRNA and β-AR Protein Abundance

The effects of MI on β-AR expression were evaluated by comparing β-AR mRNA (real-time PCR) and protein abundance (Western blot) in LV tissue from control (no infarction) and postinfarction (sedentary resistant) dogs. The number of cycles to reach threshold (ΔCt) for detection for both β1-ARs [control (n = 4) 15.7 ± 0.8 vs. resistant (n = 4) 15.2 ± 1.6; F = 0.69, P = NS] and β2-ARs [control (n = 4) 18.9 ± 0.6 vs. resistant (n = 4) 19.5 ± 0.6; F = 3.55, P = NS] was similar in both groups. Protein abundance (Western blot, Triton extraction) was also similar in both the infarcted and noninfarcted animals. β1-AR [control (n = 4) 82.3 ± 37 vs. resistant (n = 4) 95.2 ± 28.6; F = 0.31, P = NS], β2-AR [control (n = 4) 166.4 ± 78.4 vs. resistant (n = 4) 98.5 ± 28; F = 3.96, P = NS], and caveolin [control (n = 4) 133.4 ± 32.42 vs. resistant (n = 4) 101.1 ± 5.8; F = 0.09, P = NS] content were similar in both groups. These data suggest that β-AR expression was not altered by MI in dogs resistant to the induction of VF. In agreement with these findings, we previously demonstrated (18) that β-AR responsiveness was similar in susceptible and resistant dogs before MI; differences were only noted after the infarction.

Fig. 2. Expression of β1-adrenoceptor as determined by real-time PCR using SYBR Green. After PCR, the cycle where threshold amounts of β1-adrenergic receptor are detected (Ct) was determined and then the Ct for detection of the internal standard, 18S mRNA, was subtracted, resulting in a ΔCt. Higher values for ΔCt indicate a lower expression of the target gene. Susceptible sedentary group expressed significantly less β1-adrenoceptor mRNA in cardiac tissue than resistant group of dogs (*P < 0.05), n = No. of dogs per treatment group; each dog sample performed in triplicate. Data are plotted as means ± SD.
4.39, \( P < 0.05 \)), whereas the susceptible exercise dogs had a \( \Delta C_t \) of 0.44 ± 1.08 (a value that was not different from the resistant dogs; \( F_{15} = 4.39, P = NS \)). These data suggest that the expression of \( \beta_1 \)-AR mRNA was lower in susceptible than in resistant animals and, further, that exercise training normalized \( \beta_1 \)-AR mRNA expression such that differences were no longer detected between the susceptible and resistant groups. In contrast, \( \beta_2 \)-AR expression was similar in resistant (\( n = 8, \Delta C_t = 21.5 ± 0.8 \)) and susceptible sedentary (\( n = 4, \Delta C_t = 20.9 ± 2.2 \)), and susceptible (\( n = 4, \Delta C_t = 22.0 ± 0.4 \)) animals (Fig. 3). When \( \beta_2 \)-AR expression was compared with \( \beta_1 \)-AR expression, it became apparent that dogs susceptible to VF have a significantly higher ratio of \( \beta_2 \)-AR to \( \beta_1 \)-AR (1.30 ± 1.5) compared with either resistant (0.11 ± 0.05) or susceptible exercise dogs (0.06 ± 0.04) (\( F_{15} = 7.01, P < 0.01 \); Fig. 4).

**Effect of Exercise Training on \( \beta \)-AR Protein Abundance**

To determine whether mRNA expression changes were translated into differences in receptor protein, Western blot analysis was performed utilizing two different methods for protein solubilization. Triton X-100 preferentially extracts proteins located outside of lipid rafts or caveolae (34), whereas analysis of extracts obtained from boiling in SDS provides total protein content of the cell (34). \( \beta_1 \)-ARs were less abundant in Triton X-100 extracts in sedentary dogs susceptible to VF (\( n = 4, 54 ± 14% \)) than in resistant dogs (\( n = 8, 100 ± 34% \)) or in exercise-trained susceptible dogs (\( n = 4, 78 ± 18% \)) (\( F_{15} = 3.88, P < 0.05 \); Fig. 5A). This same tendency was apparent in the SDS extraction, but because of sample variability significant differences (\( F_{14} = 0.62, P = NS \)) were not detected (susceptible sedentary (\( n = 3 \)) 66 ± 76.2%; susceptible exercise (\( n = 4 \)), 113 ± 54%; resistant (\( n = 8 \)) 100 ± 51%) (Fig. 5B).

\( \beta_2 \)-AR content was significantly less in resistant dogs than in either susceptible treatment group when Triton X-100 was used to isolate the protein. These data suggest that \( \beta_2 \)-ARs were sequestered in nonextractable caveolae in the resistant group [susceptible sedentary (\( n = 4 \)) 318 ± 132%; susceptible exercise (\( n = 4 \)) 268 ± 52%; resistant (\( n = 8 \)) 100 ± 48.1%] (\( F_{15} = 12.87, P < 0.001 \); Fig. 6A). This pattern was not repeated with SDS extraction; the quantity of \( \beta_2 \)-ARs was equivalent in all treatment groups [resistant (\( n = 8 \)) 100 ± 45.3%; susceptible exercise (\( n = 4 \)) 82 ± 48%; susceptible sedentary (\( n = 3 \)) 88 ± 12%] (\( F_{14} = 0.27, P = NS \); Fig. 6B). These protein quantities were consistent with \( \beta_2 \)-AR mRNA expression as determined by RT-PCR reported above.

The amount of caveolin-3 extracted by Triton X-100 was significantly (\( F_{15} = 4.66, P < 0.05 \)) less in the susceptible exercise-trained group (\( n = 4, 54 ± 28% \)) than in either the resistant (\( n = 8, 100 ± 27% \)) or susceptible sedentary group (\( n = 4, 108 ± 26% \)) (Fig. 7A), although total caveolin-3 protein was not different between groups when extracted by SDS [resistant (\( n = 8 \)) 100 ± 39.6%; susceptible sedentary (\( n = 3 \)) 113 ± 42%; susceptible exercise (\( n = 4 \)) 100 ± 30%] (\( F_{14} = 0.14, P = NS \); Fig. 7B).

**DISCUSSION**

The major findings of the present study are as follows: 1) \( \beta_1 \)-AR gene expression and protein content were decreased in dogs susceptible to arrhythmias; 2) a 10-wk endurance exercise training regimen restored \( \beta_1 \)-AR expression and protein content in dogs susceptible to VF; 3) \( \beta_2 \)-AR gene expression and protein content were not different between dogs susceptible or resistant to arrhythmias; and 4) in contrast to the susceptible dogs, \( \beta_2 \)-ARs from the resistant animals resisted extraction by Triton X-100. These data indicate that the \( \beta_2 \)-ARs reside within caveolae in the resistant dogs. This partitioning into different regions of the sarcolemma could lead to alterations of \( \beta_2 \)-AR-mediated cell signaling pathways.

In both human and animal models of heart failure, \( \beta_1 \)-AR to \( \beta_2 \)-AR content ratio in LV myocardium is decreased to a extent similar to that found in the present study (19, 27, 33). As a consequence, the failing heart becomes more dependent on \( \beta_2 \)-ARs for inotropic support. However, the recruitment and activation of these “latent” \( \beta_2 \)-ARs can also alter cardiac electrical stability, increasing the propensity for the formation of malignant arrhythmias. In fact, we previously demonstrated (10, 18) that the activation of \( \beta_2 \)-ARs provoked large increases in calcium transient amplitude and aftercontractions (cellular arrhythmias) in myocytes from dogs prone to malignant arrhythmias, while the selective \( \beta_2 \)-AR antagonist ICI-118551...
prevented VF induced by acute ischemia in animals with healed MIs.

We recently demonstrated (11) that exercise training significantly reduced both the in vivo and the in vitro contractile response to β2-AR stimulation in animals known to be susceptible to VF. In fact, after training, the β2-AR response of the susceptible animals was indistinguishable from that noted in dogs resistant to malignant arrhythmias. In marked contrast, the β2-AR responsiveness increased even further in susceptible animals after the completion of a comparable sedentary period (11). The β2-AR response was also reduced in isolated myocytes; the β2-AR agonist zinterol elicited significantly larger increases in cell shortening in myocytes obtained from the susceptible sedentary group than in cells from either resistant or susceptible exercise-trained animals (11). As a consequence of the attenuated β2-AR responsiveness, exercise training also completely suppressed the VF induced by acute myocardial ischemia (7, 11). However, this study did not investigate whether the improved β2-AR responsiveness resulted from altered β-AR gene expression or β-AR protein abundance in the cell membrane. The present study extends these previous findings, demonstrating that exercise training elicited significant increases in β1-AR protein and mRNA with corresponding improvements in β2-AR to β1-AR ratio.

In agreement with the present study, exercise conditioning has been shown to reduce cardiac sympathetic activity (38) and to improve β-AR responsiveness in normal animals (4, 42), in aged animals (25), and in animals with cardiovascular disease (23). The improved β1-AR responsiveness was accompanied by either reduction (4) or no change (23, 25) in β1-AR density. For example, in normal Wistar rats, daily exercise provoked significant reductions in β1-AR density without altering either β2-AR or β3-AR density (23).

![Fig. 5. β1-Adrenoceptor protein content in canine left ventricle as determined by Western blot analysis after tissue extraction by Triton X-100 (A) or SDS (B). Protein content is expressed as % of the amount in the resistant dogs. Fifty micrograms of protein was loaded per lane on Triton X-100 gels, and 30 μg of protein was loaded per lane on SDS gels. n = No. of dogs per treatment group; each sample analyzed on 2 or 3 gels. In the Triton X-100-extracted samples, β1 protein content in susceptible (Sus) sedentary (Sed) dogs was significantly less than that in resistant (Res) dogs (*P < 0.05). Data are plotted as means ± SD.](https://www.ajpheart.org/)

![Fig. 6. β2-Adrenoceptor protein content in canine left ventricle as determined by Western blot analysis after tissue extraction by Triton X-100 (A) or SDS (B). Protein content is expressed as % of the amount in the resistant dogs. Fifty micrograms of protein was loaded per lane on Triton X-100 gels, and 30 μg of protein was loaded per lane on SDS gels. n = No. of dogs per treatment group; each sample analyzed on 2 or 3 gels. β2-Adrenoceptor protein content in Triton X-100 samples was 3-fold lower in resistant dogs compared with either susceptible sedentary or susceptible exercise-trained group (*P < 0.001). There were no differences in the groups after SDS extraction. Data are plotted as means ± SD.](https://www.ajpheart.org/)
The predicted molecular mass of β1-ARs and β2-ARs is ~51 and 47 kDa, respectively (37). On Triton X-100 extraction, immunoreactivity of β1-ARs was detectable as a band at 65 kDa (weak), a doublet at 51 kDa, and a doublet at 45 kDa (data not shown). These molecular species correspond to those reported previously (37), and all exhibit decreased levels in susceptible sedentary vs. resistant dogs. However, the 51-kDa species was the prominent band detected after SDS extraction (65 kDa was only detected after prolonged exposure to film). Therefore the data presented here are limited to the 51-kDa species, which were loaded per lane on SDS gels. A similar reduction in caveolin-3 expression (28) has been reported previously (3), while cardiac hypertrophy (subsequent to 7 wk of high-intensity exercise training in rats provoked an increase in caveolin-3 expression (34), suggesting a redistribution of caveolin to cytosolic, Golgi, and L-type calcium channel current (ICa,L) following caveolar disruption. Disruption of caveolae would result in redistribution of β2-ARs to the cell membrane, thereby allowing for a closer association and interaction with β1-ARs. As a consequence of a decreased abundance of β1-ARs, the resulting mix of β1-ARs and β2-ARs on the surface of the cell membrane could lead to an increased probability of receptor heterodimerization (30, 46). Heterodimerization of β1-ARs and β2-ARs can produce enhanced sensitivity to isoproterenol, decreased internalization of β2-ARs, and decreased signaling of β2-ARs through Gi (46).

Interestingly, exercise training decreased the amount of caveolin-3 detected in Triton X-100, but not SDS, extracted samples. These data are consistent with an exercise training-induced alteration in the distribution of caveolin within caveolae. Formation of caveolae following exercise training could restore β2-AR signaling pathways that only occur in caveolae. These results contrast with previous studies in rat that reported no change in total caveolin-3 in senescent rats 3 mo after MI but an increase in Triton-extractable caveolin-3 after infarction (34), suggesting a redistribution of caveolin to cytosolic, Golgi, or cell membrane fractions. Furthermore, voluntary exercise training in rats provoked an increase in caveolin-3 expression (3), while cardiac hypertrophy (subsequent to 7 wk of hypertension) significantly decreased caveolin-3 extracted with SDS from the canine myocardium (31). In the present study, exercise training produced a small (<10%) increase in ventricular systolic wall thickness (as measured by echocardiography) in both resistant and susceptible dogs (11), while caveolin-3 decreased in susceptible exercise-trained dogs. A similar reduction in caveolin-3 expression (28) has been reported in mice after chronic β-AR stimulation. Therefore, species differences or differences in the model of cardiac disease may account for the observed differences in caveolin-3 expression discussed above.

Limitations of Study

LV dysfunction is known to alter β-adrenergic responsiveness (1, 12). However, there was no evidence of ventricular dysfunction or dilation (determined by echocardiography) after MI in a subset of resistant or susceptible dogs. Furthermore, we previously reported (11) that neither baseline nor β-AR agonist-induced contractile changes were different in the suscep-
tible dogs before either a 10-wk sedentary or a 10-wk exercise training period; differences were only noted after the completion of the 10-wk study. Thus it is unlikely that initial differences in ventricular function can explain the results. Similarly, innate (preexisting) variability in β-AR responsiveness could contribute to the difference noted between the susceptible and resistant dogs. However, both β-AR mRNA and β-AR protein abundance were similar in control (no infarct) and infarcted (resistant sedentary) dogs. These data demonstrate that β-AR expression in the resistant dogs was similar to that in animals without infarction. Furthermore, we previously demonstrated (18) that β-AR responsiveness was similar in both resistant and susceptible dogs before MI; the differences were only noted after infarction. When considered together, these data demonstrate that the differences in β-AR expression occurred as a consequence of the MI rather than innate β-AR variability. The studies were performed with whole tissue samples rather than purified myocyte preparations. As such, the differences in β-AR expression reported in the present study could reflect exercise-induced changes in noncardiac muscle cells. However, the present results are consistent with the functional data obtained from both intact preparations and isolated myocytes (11). Thus changes in cardiac myocyte β-AR expression were most likely responsible for the functional changes induced by exercise training.

Finally, it is important to emphasize that exercise training not only alters cardiac β-AR balance but also enhances cardiac parasympathetic regulation (5, 7, 8, 11, 38). Because it is well established that cardiac vagal activation can prevent ischemically induced arrhythmias (40, 43), exercise training-induced changes in cardiac parasympathetic regulation could also contribute to the protection from VF noted in the present study. Exercise training did, in fact, improve cardiac parasympathetic regulation (increased heart rate variability), which was maintained even when the heart was stressed by either exercise or acute ischemia (7). Furthermore, exercise training both improved baroreflex sensitivity (BRS) and reduced cardiac mortality in patients recovering from MI (22). Thus exercise training augmented cardiac parasympathetic regulation that was associated with a reduced risk for cardiac mortality. Further investigation will be required to determine the relative contributions of alterations in cardiac parasympathetic regulation and/or β-AR balance for the antiarrhythmic actions of exercise training.

Clinical Implications

There are at least two findings of the present study that have potentially important clinical implications: 1) an enhanced β2-AR activation is associated with greater susceptibility to VF; and 2) exercise training can restore a more normal β-AR balance and prevents malignant arrhythmias induced by ischemia.

Clinical studies have not extensively evaluated the contribution of β2-ARs to cardiac mortality. A small clinical trial found that the β2-AR agonist salbutamol increased episodes of ventricular tachycardia in patients with congestive heart failure (26). In a similar manner, there are a few case reports in which β2-AR agonists have precipitated sudden death as a consequence of the cardiac actions of these agents in asthmatic patients (35, 36). More compelling, but indirect, evidence in support of the β2-AR hypothesis is provided by analysis of the numerous β-AR antagonist trials. There is overwhelming evidence that β-AR antagonists can protect against arrhythmia formation induced by myocardial ischemia and infarction (15, 21). Indeed, this marked reduction in cardiac mortality has been verified in at least 32 trials involving ~29,000 patients (15, 21). However, if one carefully examines the clinical studies cited above, it then becomes apparent that not all β-AR antagonists offer the same level of protection, particularly during acute MI. The majority of the studies using the β1-AR antagonist metoprolol failed to report significant reductions in the incidence of VF during acute MI (16, 17). Furthermore, although atenolol did reduce overall mortality by 15%, the number of patients who died as the result of malignant arrhythmias was not altered (20). In contrast, propranolol therapy elicited large reductions in both overall mortality (65% decrease) and sudden cardiac death (41% decrease) in post-MI patients with persistent ST segment depression (41), a group of patients known to be at a particularly high risk for subsequent cardiac events (44). Thus a better antiarrhythmic protection can be achieved with complete (i.e., β1- and β2-AR), rather than selective (i.e., β1), β-AR blockade.

The data from the present study further demonstrate that exercise training can restore a more favorable β-AR balance that is accompanied by a complete suppression of ischemically induced VF (11). In agreement with the present study, exercise training both improved cardiac autonomic regulation (as measured by an increase in BRS) and significantly reduced cardiac mortality during a 10-yr follow-up period (22). In fact, no patient in whom exercise training induced BRS increases of >3 ms/mmHg died during the study, while 23% (18 of 79) of the nontrained or trained patients that did not improve BRS died during the 10-year study period. Thus exercise training represents a potent nonpharmacological antiarrhythmic intervention.

In summary, although β2-AR gene expression and total protein content were not different in dogs resistant or susceptible to VF, it appears that the membrane compartmentalization of the β-ARs was different in the two groups; more β2-ARs were present in the noncaveolar (i.e., Triton X-100 extractable) fraction of the cardiac membrane in dogs susceptible to VF compared with animals resistant to malignant arrhythmias. Exercise did not restore β2-ARs to a sequestered site but did normalize the ratio of β1-ARs to β2-ARs, thereby restoring normal signaling pathways.

GRANTS

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REFERENCES


