Daily exercise-induced cardioprotection is associated with changes in calcium regulatory proteins in hypertensive rats

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Daily exercise-induced cardioprotection is associated with changes in calcium regulatory proteins in hypertensive rats. Am J Physiol Heart Circ Physiol 288: H532–H540, 2005. First published October 7, 2004; doi:10.1152/ajpheart.00873.2004.—Epidemiological data document that regular exercise protects against the morbidity and mortality associated with ischemic heart disease. Therefore, we tested the hypothesis that daily exercise (DE) increases the ventricular arrhythmia threshold (VAT) induced by coronary artery occlusion and alters the expression of calcium regulatory proteins. The VAT was defined as the time from coronary occlusion to sustained ventricular tachycardia resulting in a reduction in arterial pressure. To test this hypothesis, we recorded the VAT in conscious sedentary normotensive, sedentary hypertensive, and DE hypertensive rats, and we associated these thresholds with the protein expression of the L-type calcium channel, Na+/Ca2+ exchanger, phospholamban, and sarcoplasmic reticulum Ca2+-ATPase. Results document a significantly reduced time to ventricular arrhythmias (sedentary hypertensive, 3.7 ± 0.3 min vs. sedentary normotensive, 4.8 ± 0.3 min), an increased Na+/Ca2+ exchanger protein expression (47%), and a decreased phospholamban protein expression (−34%) in conscious hypertensive rats. DE increased the VAT (5.9 ± 0.2 min), decreased the protein expression of the Na+/Ca2+ exchanger, and normalized the protein expression of phospholamban in the hypertensive rats. Thus DE may be a primary prevention approach for reducing the incidence of arrhythmias by altering cardiac regulatory proteins in hypertensive rats.

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ACUTE CORONARY ARTERY OCCLUSION is the leading cause of death in industrially developed countries and will be the major cause of death in the world by the year 2020 (55). The majority of these deaths result from tachyarrhythmias that culminate in ventricular fibrillation (4, 32). The management of this epidemic should center on primary prevention programs because prevention is the most cost-effective and innocuous strategy. Daily exercise may be a safe, primary preventive approach for reducing the incidence of cardiac arrhythmias and sudden cardiac death. Epidemiological studies document that regular physical activity protects against the morbidity and mortality associated with ischemic heart disease (7, 23, 25, 38, 45, 54, 57, 60, 61, 63, 71). Furthermore, the incidence of sudden cardiac death is inversely related to the level of regular physical activity (3).

Recent evidence also suggests that daily exercise reduces the incidence of cardiac arrhythmias in individuals with cardiac disorders (38, 43, 46, 50, 57, 59, 72). For example, daily exercise improved cardiac function and reduced the arrhythmia frequency in individuals with congestive heart failure (25a, 40). Similarly, the frequency and severity of cardiac arrhythmias were reduced after an exercise training program for individuals with myocardial infarction (31).

Billman and colleagues (9) were the first to present experimental evidence documenting that 6 wk of daily exercise prevented arrhythmias induced by coronary artery occlusion in intact conscious dogs with myocardial infarction. With these exceptions in the canine model of sudden cardiac death (8), there is limited experimental evidence documenting that daily exercise reduces the susceptibility to cardiac arrhythmias in the intact conscious animal. Opie and colleagues (56, 65) reported that exercise training increased the arrhythmia threshold during coronary artery occlusion in isolated rat hearts. Other investigators (10, 11, 34, 35, 39) using isolated rat heart preparations as well as cardiomyocytes have documented that exercise training improved cardiac and metabolic function after ischemia or anoxia. Exercise training also improved myocardial tolerance to ischemia in anesthetized rats (51, 66). It is important to note that with few exceptions (56, 65), studies using isolated heart preparations or anesthetized rats do not document the incidence of arrhythmias despite prolonged periods of ischemia. This may be due to the fact that disturbances in cardiac autonomic balance play a critical role in triggering cardiac arrhythmias. Importantly, isolated hearts are devoid of cardiac autonomic innervation. Furthermore, anesthesia significantly alters the autonomous nervous system. Parenthetically, we are unable to elicit sustained ventricular arrhythmias during coronary artery occlusion in anesthetized rats.

The spontaneously hypertensive rat (SHR) is a model of neurogenic hypertension and cardiac hypertrophy. Sympathetic hyperactivity alters the expression of cardiac calcium regulatory proteins leading to disturbances in calcium homeostasis (29). Disturbances in calcium homeostasis are closely related to cardiac electrophysiological events. Sympathetic hyperactivity also increases the electrical vulnerability of the myocardium and decreases the ventricular arrhythmia threshold. In addition, the prolonged duration of repolarization, concomitant ionic alterations, and altered ion handling associated with cardiac hypertrophy also increase vulnerability (77). Thus, comparable to human hypertension, the SHR is vulnerable to developing potentially lethal cardiac arrhythmias such as ventricular fibrillation (77) and is an ideal model for examining cardiac arrhythmias (53).

Therefore, we tested the hypothesis that the increased susceptibility to ventricular arrhythmias in intact conscious hypertensive rats is associated with changes in calcium regulatory proteins.
proteins. Furthermore, we tested the hypothesis that daily exercise increases the ventricular arrhythmia threshold (VAT) induced by coronary artery occlusion and that the cardioprotection is associated with changes in calcium regulatory proteins. Conscious, chronically instrumented rats were studied to negate the confounding effects of anesthetic agents and surgical trauma (37, 67).

MATERIALS AND METHODS

Experimental Subjects

All surgical and experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the American Physiological Society “Guiding Principles in the Care and Use of Animals.” Studies determining the VAT were conducted in eight male Wistar rats (sedentary normotensive), 12–16 wk of age, and eight age-matched male SHR (sedentary hypertensive). We selected the normotensive Wistar strain because the widely used normotensive control for the SHR, the Wistar-Kyoto rat strain, develops pressure-overload independent myocardial hypertrophy with values of cell size, fibrosis, and diastolic dysfunction of a magnitude close to those of the hypertensive strain (1). Cardiac hypertrophy is a well-established major risk factor for cardiovascular disease, including sudden cardiac death (2, 48). The Wistar rat was also selected because the Wistar-Kyoto rats were established as an inbred of the Wistar colony from Kyoto that originated from the SHR strain (22).

A separate group of eight male SHR (daily exercise hypertensive), 4 wk of age, were housed in cages with free access to running wheels (Nalgene). Daily voluntary running distance (i.e., total number of revolutions of the wheel) was monitored with an optical sensor attached to the side of the running wheel interfaced with a personal computer (activity wheel counter model 86060, Lafayette Instrument).

At week 6 of voluntary running, the animals were instrumented as described below and returned to their cages with free access to running wheels. After an additional 4–6 wk of voluntary running (when running distance reached levels obtained before the instrumentation), the animals were placed in a cage without a running wheel for at least 2 days. After at least 2 days without any running activity, the rats were studied as described below. At least 3 days following the determination of the VAT, hearts were removed, and the ischemic zone was determined. Finally, whole heart homogenates from three separate groups (sedentary normotensive; n = 8, sedentary hypertensive; n = 7, and daily exercise hypertensive rats; n = 7) were used to determine relative protein expression for cardiac calcium regulatory proteins.

Surgical Procedures

Instrumentation. All surgical procedures were performed using aseptic procedures. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip) and supplemental doses (10–20 mg/kg ip) were administered if the rat regained the blink reflex or responded during the surgical procedures. The hearts were approached via a left thoracotomy through the fourth intercostal space. Subsequently, a coronary artery occluder, made from 5.0-gauge atrumatic prolene suture (8720H, Ethicon), which passed through a polyethylene-10 guide tubing (Clay Adams), was passed around the left main coronary artery 2–3 mm from the origin by inserting the needle into the left ventricular wall under the overhanging left atrial appendage and bringing it out high on the pulmonary conus (47). The guide tubing with the other end of the occluder was then exteriorized at the back of the neck. The tubing was filled with a mixture of Vasoline and mineral oil to prevent a pneumothorax. At least 1 wk was allowed for recovery (44). During the recovery periods, the rats were handled, weighed daily, and acclimatized to the laboratory and investigators. Subsequently, the animals were anesthetized as described above, and three insulated stainless steel ECG electrodes were sutured subcutaneously on the ventral side of the thorax. The ECG leads were exteriorized at the back of the neck. In addition, a telemetry device (Data Sciences International PhysioTel PA-C40) was implanted as previously described (18, 68). The sensor of the telemetry device, located within the tip of a catheter, was inserted into the abdominal aorta for continuous, nonmetered recording of pulsatile arterial blood pressure via radio telemetry. Again, at least 1 wk was allowed for recovery (44). During the recovery periods, the rats were handled, weighed daily, and acclimatized to the laboratory and investigators. Two separate surgeries, separated by at least 1 wk, were performed because the animals recover significantly better than if two major surgeries are conducted during one session.

Experimental Procedures

Ventricular arrhythmia threshold. Conscious, unrestrained rats were studied in their home cages (~13, 350 cm³) for all experiments. Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. Subsequently, the left main coronary artery was temporarily occluded by use of the prolene suture. Specifically, acute coronary artery occlusion was performed by pulling up on the suture that was around the left main coronary artery (Figs. 1 and 2). A rapid change in the ECG (S-T segment elevation or depression) and a reduction in arterial pressure occur within seconds of pulling on the suture, documenting coronary artery occlusion. Changes in the ECG and arterial pressure signals occurred within 3 s of the occlusion and within 3 s of the release (Fig. 1). The occlusion was maintained until the onset of ventricular tachycardia but no longer than 6.5 min to prevent myocardial damage. If the time to sustained ventricular tachycardia exceeded 6.5 min, the occlusion was stopped and 6.5 min was used as the VAT. Normal sinus rhythm appeared on termination of the occlusion by gently compressing the thorax. In the rare event when the animal did not resume normal sinus rhythm, cardioversion was achieved (after the rat lost consciousness) with the use of one shock (10 J) of direct current. The VAT was defined as the time from coronary artery occlusion to sustained ventricular tachycardia resulting in a reduction in arterial pressure (Fig. 2).

Determination of ischemic zone. Three days after the experiment, the rats were euthanized with an overdose of pentobarbital sodium. To determine the size of the ischemic zone, the heart was excised with the occluder intact and perfused via the aorta with 30 ml of 0.9% saline to wash out the blood. Subsequently the suture around the left main coronary artery was tied. Evans blue dye (300 μl, 1 mg/ml in 0.9% saline, Sigma Chemical) was perfused via the aorta causing the dye to infuse into the nonischemic area of the heart, leaving the ischemic regions unstained. The heart was trimmed leaving only the right and left ventricles, rinsed to remove the excess blue dye, and weighed. The heart was trimmed again leaving only the ischemic region. The weight of the ischemic zone was expressed as percentage of total heart weight.

To determine whether the occlusion produced a myocardial infarction, the heart was sliced transversally into ~1.0-mm sections and incubated in a 1% solution 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) at 37°C for 20 min. The heart sections were placed between two glass slides and immersed in 10% formalin overnight to enhance the contrast of the stain. TTC staining differentiates viable tissue by reacting with myocardial dehydrogenase enzymes to form a red brick stain. Necrotic tissue that has lost its dehydrogenase enzymes does not form a red stain and shows up as pale yellow. This stain has been shown to be a reliable indicator of myocardial infarction (28).

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Sample preparation for Western analysis. Rats were quickly decapitated without anesthetic to avoid the confounding influences of anesthetics on the cardiovascular system, including direct chronotropic effects, activation of the renin-angiotensin system, and/or...
activation of cardiovascular reflex phenomena (42, 52, 64). The hearts were rapidly removed, rinsed clean of clots, snap frozen in liquid nitrogen, and stored at −80°C for subsequent Western blotting analysis.

Each heart was subsequently pulverized to a fine powder using a liquid nitrogen-cooled stainless steel mortar and ceramic pestle (Fisher Scientific). Total protein was extracted from each heart sample by first homogenizing (4 × 10 s) the heart powder in a buffer (pH 7.5)
Daily exercise hypertensive.

Significantly higher in daily exercise SHR compared with sedentary normotensive and daily exercise hypertensive rats. A two-way ANOVA with post hoc Fisher least-significant difference method tests was used to compare mean arterial blood pressure and heart rate immediately before the occlusion (preocclusion) and immediately before the onset of ventricular arrhythmia (prearrrhythmia) among the three groups of rats.

RESULTS

During the first 6 wk of daily exercise, the animals increased their running distance, reaching a peak of 7.7 ± 1.3 km/day at week 6. At this time, the animals were instrumented to measure the VAT as well as arterial pressure and heart rate. After instrumentation, the animals were returned to their cages with free access to their running wheels. Subsequently, the running distance increased to levels near that obtained before instrumentation (week 10: 5.4 ± 0.9 km/day).

Cardiovascular Physiology

Figure 3 presents the VAT determined in sedentary normotensive rats, sedentary hypertensive, and daily exercise hypertensive rats. The one-way ANOVA revealed significant group effects. Post hoc analysis revealed that sedentary hypertensive rats had a significantly lower VAT compared with sedentary normotensive and daily exercise hypertensive rats. Importantly, daily exercise increased the VAT above that in both the sedentary normotensive and sedentary hypertensive rats. Finally, one sedentary normotensive rat and three daily exercise hypertensive rats exceeded the 6.5-min limit on coronary occlusion and therefore received VAT of 6.5 min for analysis.

Table 1 presents resting mean arterial pressure and heart rate immediately before the occlusion (preocclusion) and immediately before the arrhythmia (prearrrhythmia) in sedentary normotensive, sedentary hypertensive, and daily exercise hypertensive rats. The two-way ANOVA revealed significant group effects without significant group by treatment interactions for mean arterial pressure. These results indicate that as expected, mean arterial pressure, independent of treatment, was significantly higher in the hypertensive versus normotensive rats. Thus, there were no differences in mean arterial pressure responses to occlusion between sedentary and daily exercise hypertensive rats. Two-way ANOVA also revealed significant group and treatment effects without significant group by treatment interactions.
ment interactions for heart rate. These results indicate that daily exercise lowered heart rate independent of the treatment. In addition, prearrhythmia heart rates were higher, independent of group. Thus there were no differences in the heart rate responses to occlusion between groups.

**Molecular Cardiology**

Figure 4 presents relative difference in protein expression for phospholamban, Na\(^+\)/Ca\(^{2+}\) exchanger, SERCA2, and DHP receptor (L-type calcium channel) obtained from sedentary normotensive, sedentary hypertensive, and daily exercise hypertensive rat hearts. The relative protein expression of the Na\(^+\)/Ca\(^{2+}\) exchanger was significantly increased (47%), whereas phospholamban protein expression was significantly decreased (−34%) in sedentary hypertensive rats. Daily exercise in the hypertensive rats reduced the protein expression of the Na\(^+\)/Ca\(^{2+}\) exchanger and normalized the protein expression of phospholamban. Importantly, there was no difference in the extent of the ischemic zone between groups (sedentary normotensive, 50 ± 2%; sedentary hypertensive, 46 ± 7%; and daily exercise hypertensive, 48 ± 4%) or indication of infarction.

**DISCUSSION**

In this study, we examined the relationship between the susceptibility to ventricular arrhythmias (Fig. 3) and changes in calcium regulatory proteins (Fig. 4) in sedentary normotensive, sedentary hypertensive, and daily exercise hypertensive rats. Sedentary hypertensive rats had a significantly lower ventricular arrhythmia threshold than both sedentary normotensive and daily exercise hypertensive rats (Fig. 3). The increased susceptibility to ventricular arrhythmias in sedentary hyperten-

![Image of Western blots showing protein expression for phospholamban, Na\(^+\)/Ca\(^{2+}\) exchanger, SERCA2, and DHP receptor.](image-url)
sive rats was associated with an increased protein expression of the \( \text{Na}^{+}/\text{Ca}^{2+} \) exchanger and a reduced protein expression of phospholamban (Fig. 4). Importantly, daily exercise in the hypertensive rats reduced the susceptibility to ventricular arrhythmias, reduced the protein expression of the \( \text{Na}^{+}/\text{Ca}^{2+} \) exchanger, and normalized the protein expression of phospholamban (Figs. 3 and 4).

The effect of hypertension on the susceptibility to arrhythmias has been established in anesthetized hypertensive rats (6) as well as in isolated hearts from hypertensive rats (12, 26, 62). Furthermore, the effects of exercise training on the susceptibility to cardiac arrhythmias has been established in normotensive isolated rat hearts (56, 65). However, it is well established that disturbances in cardiac autonomic balance play a critical role in triggering cardiac arrhythmias. Specifically, reductions in parasympathetic activity or increases in sympathetic activity increase the susceptibility to cardiac arrhythmias. Importantly, isolated hearts are devoid of cardiac autonomic innervation, and anesthetics significantly alter the autonomic nervous system. Thus this study extends previous reports comparing normotensive and hypertensive rats by inducing ventricular arrhythmias with coronary artery occlusion in intact conscious rats. Furthermore, this study documents an association between ventricular arrhythmias and cardiac regulatory proteins. Finally, this is the first study in hypertensive rats documenting the effect of daily exercise on the susceptibility to ventricular arrhythmias and calcium regulatory proteins.

The results obtained in the daily exercise rats were due to the effects of daily spontaneous running and not due to the stress associated with forced exercise. With voluntary wheel running, the rats ran spontaneously during their active hours, i.e., at night and no stress or aversive stimuli was used to force the rats to run. Furthermore, because the running wheel is attached to the rats home cage, the environment is not changed in the running and nonrunning situations. Importantly, the rats were instrumented at the midpoint of the training program and allowed to resume their running activity reaching levels at week 10 similar to distances reported in previous studies in uninstrumented rats (16, 17, 19). This approach eliminates the acute effects of surgery and potential deconditioning from confounding the results. Finally, the rats were removed from the running environment at least 2 days before the study to avoid the acute effects of exercise. With the use of these approaches, daily exercise produced a resting bradycardia (Table 1). Similar daily exercise programs also resulted in a training-induced bradycardia at rest (16, 17, 19) and during exercise (16) and resulted in reduced measures of heart rate and blood pressure variability (20). Because of these approaches, we believe that we are examining the response to exercise and not the stress associated with forced activity.

The SHR is a model of neurogenic hypertension. Cardiac autonomic balance is significantly altered in hypertensive rats. Specifically, hypertensive rats have reduced cardiac parasympathetic tonus and increased cardiac sympathetic tonus (13–15). The sympathetic nervous system alters cardiac electrophysiology over seconds and minutes by activating \( \alpha \)- and \( \beta \)-adrenergic receptors. Adrenergic stimulation results in a reduction of the electrical stimulus threshold to induce ventricular fibrillation as well as an increase in the likelihood of spontaneous ventricular arrhythmias (24, 70, 76, 79). Signaling through \( \alpha \)- and \( \beta \)-adrenergic receptors also regulates the expression of calcium regulatory proteins (68). For example, Golden and colleagues (29, 30) have documented that adrenergic receptor stimulation enhances the expression of the \( \text{Na}^{+}/\text{Ca}^{2+} \) exchanger in vivo and in vitro.

**Perspectives**

Although not investigated in this study, the mechanisms mediating the increased susceptibility to ventricular arrhythmias and altered calcium regulatory proteins in sedentary hypertensive rats may be due, in part, to increased cardiac sympathetic tonus. Furthermore, the cardioprotective effects of daily exercise may be due to reductions in sympathetic activity that result in alterations in calcium regulatory proteins. Specifically, the increased cardiac sympathetic activity, higher heart rates, and changes in calcium regulatory proteins in the sedentary hypertensive rats may favor conditions of calcium overload, which increases the likelihood for ventricular arrhythmias. This is suggested because the ability of cardiac myocytes to maintain cytosolic calcium within a tightly controlled range is crucial for cardiac electrical stability (29). Furthermore, it is well documented that reductions in phospholamban protein expression result in an increased sarco(endo)plasmic reticulum calcium load (36). The sarco(endo)plasmic reticulum calcium overload may produce spontaneous calcium releases, thereby leading to ectopic activity. Similarly, triggered beats occur more frequently in the presence of increased heart rate (69).

Importantly, daily exercise lowers arterial pressure and sympathetic activity in hypertensive individuals. For example, daily exercise attenuates the developmental rise in resting arterial pressure (27, 33, 49, 58, 74, 75) and reduces sympathetic activity in the SHR (15, 20). Daily exercise alters cardiac autonomic balance in hypertensive rats favoring a greater parasympathetic influence (15). These effects of exercise may be due, in part, to an upregulation of the GABAergic inhibitory mechanisms within the hypothalamus (41, 49). This is suggested because exercise increases glutamic acid decarboxylase (the rate-limiting enzyme for GABA production from glutamate) mRNA in the hypothalamus of SHR (49). Furthermore, blockade of GABA synthesis in the hypothalamus increases arterial pressure in exercised trained but not in sedentary control SHR (41). Finally, daily exercise upregulates the nitric oxide system within the paraventricular nucleus of SHR (21). This is important because nitric oxide within the paraventricular nucleus has an inhibitory effect on arterial pressure and sympathetic activity via the release of GABA (78). Thus the daily exercise-induced reduction in sympathetic activity and heart rate (Table 1) may mediate, in part, the cardioprotection by altering calcium regulatory proteins. This proposal is supported by data documenting that adrenergic signals alter the expression of cardiac calcium regulatory proteins (30).

**Limitations**

In this study, we document a relationship among daily exercise, the susceptibility to ventricular arrhythmias, and the expression of cardiac calcium regulatory proteins. It is important to acknowledge that the association between the VAT and cardiac calcium regulatory proteins is merely correlative and does not document a cause-and-effect relationship. In addition,
other mechanisms, including alterations in the autonomic nervous system, certainly contribute to the findings reported in this study. Thus additional studies designed to examine autonomic and molecular mechanisms that mediate the daily exercise-induced cardioprotection merits consideration. Along these lines, this study would have been strengthened significantly by the inclusion of a normotensive exercise group. Inclusion of a normotensive exercise group may have provided mechanistic insights into the effects of daily exercise on ventricular arrhythmias and calcium regulatory proteins by documenting whether the effects are limited to hypertensive animals. It is also clear from discussion with colleagues as well as published reports (73) that major disagreements exist regarding the strengths and limitations of the methods used for exercise training. With limited exceptions (41), treadmill or swim training protocols force the rats to exercise during their nonactive hours and aversive stimuli are often used to force the rats to perform. However, using these methods, the quality and quantity of work is easily determined and all animals work at comparable workloads. In contrast, with voluntary wheel running, it is difficult to assess the intensity of exercise, and there is a larger variability in the work performed between animals. Finally, the arterial pressure of the daily exercise hypertensive rats was 14 mmHg lower than the arterial pressure of the sedentary hypertensive rats; however, this difference did not reach statistical significance. The lack of an exercise effect on arterial pressure may be due, in part, to the relatively short time period during each session that the animals were studied. To determine an exercise effect on arterial pressure, the studies should be designed to record arterial pressure for several hours daily.

In conclusion, this study documents an increased susceptibility to ventricular arrhythmias with concomitant changes in the expression of the Na\(^+\)/Ca\(^{2+}\) exchanger and phospholamban in conscious hypertensive rats. Daily exercise decreased the susceptibility to ventricular arrhythmias, reduced the expression of the Na\(^+\)/Ca\(^{2+}\) exchanger, and normalized the expression of phospholamban. Thus daily exercise reduced the susceptibility to ventricular arrhythmias in hypertensive rats, and this effect was associated with changes in calcium regulatory proteins.

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GRANTS

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