Time-Series Analysis of Heart Rate Variability During Submaximal Exercise
Evidence for Reduced Cardiac Vagal Tone in Animals Susceptible to Ventricular Fibrillation

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Periodic fluctuations in the R-R interval have been used as noninvasive measures of cardiac autonomic tone. For example, a reduced heart rate variability has been shown to correlate with an increased mortality in patients recovering from myocardial infarction. The effects that physiologic perturbations such as exercise have on this heart rate variability have not been investigated. Therefore, heart rate variability was measured throughout a submaximal exercise test in 36 mongrel dogs with healed anterior myocardial infarctions. The amplitude of the respiratory component (0.24–1.04 Hz) was determined by time-series analysis techniques and was used as an index of cardiac vagal tone. On a subsequent day, a 2-minute coronary occlusion was initiated during the last minute of exercise. Twenty-two animals developed ventricular fibrillation (susceptible), whereas 14 animals did not (resistant). Exercise elicited a significantly greater increase in heart rate (resistant, 205.4±7.1; susceptible, 227.0±5.4 beats/min) in susceptible animals, which was accompanied by a greater reduction in the cardiac vagal tone index (resistant, 2.7±0.3; susceptible, 1.1±0.2 ln msec²) as compared with resistant animals. Conversely, atropine sulfate (50 μg/kg) given during exercise elicited a greater heart rate increase in the resistant dogs (heart rate change: resistant, 54.2±7.0; susceptible, 18.7±4.4 beats/min). Taken together, these data suggest that exercise elicited a greater reduction in cardiac vagal tone in animals known to be susceptible to ventricular fibrillation. (Circulation 1989;80:146–157)

Disturbances in the autonomic control of the cardiovascular function can contribute significantly to cardiac mortality. For example, increased sympathetic activity has been shown to reduce cardiac electrical stability predisposing the heart to ventricular fibrillation, whereas parasympathetic activation may protect against arrhythmia formation.¹ The quantification of abnormalities in cardiac autonomic tone may prove to be useful in the identification of patients at risk for sudden death. These abnormalities may become particularly apparent in response to physiologic perturbations of the system, such as exercise.

Heart rate variability has been used as one noninvasive means of indirectly evaluating the autonomic inputs to the heart. Heart rate variability has been shown to decrease with severe coronary artery disease,² congestive heart failure,³ ⁴ and diabetic neuropathy.⁵ ³³ Recently, Kleiger and coworkers⁶ ⁷ and Martin et al⁸ reported that patients with the lowest heart rate variability, as measured by the standard deviation of the R-R interval, had the greatest risk of dying suddenly. In fact, the relative mortality risk was calculated to be 5.3-fold greater in patients with a R-R interval variability of less than 50 msec compared with patients with a variability of more than 100 msec.⁶ The authors concluded that heart rate variability probably correlated with increased sympathetic and decreased parasympathetic activity.

More recently, spectral analysis techniques have been proposed as a noninvasive means of differentiating between sympathetic and parasympathetic inputs to the heart. In particular, the oscillations in the heart period that correspond to respiration (usually >0.15 Hz; peak 0.40 Hz) were found to be mediated primarily by the vagus nerves.⁹ ¹¹ In an
analogs, Porges and coworkers\textsuperscript{12-15} developed a time-series analysis technique that accurately evaluates the amplitude of the respiratory sinus arrhythmia yet allows for real-time data analysis over short time intervals (30 seconds). Using these techniques, it would be possible to evaluate dynamic changes in autonomic tone. Indeed, dynamic changes in autonomic tone have been recently evaluated by spectral analysis techniques. Arai et al.\textsuperscript{16} found that both the high and low frequency peaks of the heart rate power spectrum decreased in response to exercise.

Billman et al.\textsuperscript{17} have previously demonstrated that the hemodynamic response to exercise was markedly different in animals prone to ventricular fibrillation compared with those dogs resistant to malignant arrhythmias. In fact, heart rate was elevated in the susceptible animals, even after $\beta$-adrenergic receptor blockade, suggesting a reduced parasympathetic activity in these animals. It was, therefore, the purpose of this series of experiments to evaluate the effect of exercise on cardiac vagal tone in animals known to be susceptible to sudden cardiac death. In particular, the hypothesis that cardiac vagal tone decreased to a significantly greater extent in the susceptible animals was tested. Time-series analysis techniques were used to quantify cardiac vagal tone both at rest and in response to exercise.

**Methods**

**Surgical Preparation**

Fifty mongrel dogs weighing 15.0–27.3 kg were used. The animals were given Innovar-Vet (0.02 mg/kg fentanyl citrate and 1 mg/kg droperidol i.v., Pittman-Moore) as a preanesthetic. Anesthesia was induced with sodium pentobarbital (10 mg/kg i.v., Harvey Laboratories). Using strict aseptic techniques, a left thoracotomy was made in the fourth intercostal space. The heart was exposed and supported by a pericardial cradle. The left circumflex coronary artery was dissected from the surrounding epicardial fat. A 20-MHz pulsed Doppler flow transducer and a hydraulic occluder were placed around this vessel. Insulated silver-coated copper wires were sutured to the epicardial surface of the left and right ventricle and later were used to record a ventricular electrogram. A precalibrated solid-state pressure transducer (Konigsberg Instruments) was placed in the left ventricle through a stab wound in the apical dimple and secured by means of a purse-string suture. The solid-state transducers were recalibrated at the end of the study and periodically checked for instrument drift in vivo using a catheter acutely inserted for this purpose. This transducer was used to measure left ventricular pressure from which the first derivative with respect to time was obtained (an index of cardiac inotropic state). An experimental myocardial infarction was then produced. A two-stage occlusion of the left anterior descending coronary artery was performed approximately one third the distance from the vessel’s origin. The vessel was partially occluded for 20 minutes and then tied off.

All the leads to the cardiovascular instrumentation were tunneled under the skin to exit on the back of the animal’s neck. Pentazocine lactate (Talwin, Winthrop-Breon Lab, 30 mg i.m.) was given as needed to minimize postoperative pain. In addition, the long-acting local anesthetic bupivacaine hydrochloride (Marcaine, Winthrop-Breon Lab) was used to block the intercostal nerves in the area of the incision to minimize discomfort to the animal. Each animal was placed on antibiotic therapy (penicillin $G \times 10^6$ units i.m., Burns Veterinary Supply) twice daily for 7 days.

The animals were placed in an “intensive care setting” for the first 24 hours and received the following antiarrhythmic therapy. The dogs received 100 mg lidocaine HCl i.m. (Xylocaine, Astra Laboratories) before surgery, which was supplemented (60 mg i.v.) before each stage of the two-stage occlusion. In addition, the animals received 600 mg tocainide HCl (Tonocard, Merek, Sharpe & Dohme) every 12 hours beginning the day before surgery and continuing for the next 4 days. Ten dogs died acutely during surgery or within the first 72 hours. Two additional animals were found to be heartworm positive and were eliminated from the study. Finally, two animals could not be classified due to rupture of the coronary occluder (see below) and were also eliminated from the study. Thus, of the original 50 animals, 36 completed the studies described below. The principles governing the care and treatment of animals as expressed by the American Physiological Society were followed at all times during this study. In addition, the procedures used in this study were approved by The Ohio State University Institutional Animal Care and Use Committee.

**Classification of Animals: Sudden Death Testing**

The study began 3–4 weeks after the production of the myocardial infarction. The animals were walked on a motor-driven treadmill for several days to familiarize them with the laboratory and extinguish any orienting responses associated with the novel environment. The cardiac response to submaximal exercise was then evaluated before and after $\beta$-adrenergic receptor blockade, as described below. On a subsequent day (i.e., after all exercise data had been collected), the susceptibility to ventricular fibrillation was assessed by the combination of exercise and acute ischemia, as has been previously described.\textsuperscript{18,19} Briefly, the animals ran on a motor-driven treadmill while workload increased every 3 minutes (4.8 mph, 0% grade during the first 3 minutes; 6.4 mph, 16% grade during the last 3 minutes) or until a criterion heart rate of approximately 210 beats/min had been reached. During the last minute of exercise, the left circumflex coronary artery was occluded, the treadmill was then stopped, and the occlusion maintained for an additional
minute. The occlusion, therefore, lasted a total of 2 minutes. Large metal plates were placed across the animal's chest so that electrical defibrillation could be achieved with a minimal delay but only after the animal was unconscious (10–20 seconds after ventricular fibrillation began). Electrocardiogram, heart rate, left circumflex coronary blood flow, and left ventricular pressure were recorded throughout the exercise plus ischemia test. Left circumflex coronary blood flow was measured to confirm that the coronary occlusion had been made (i.e., blood flow velocity fell to zero).

Exercise Protocol

The response to exercise was assessed by an exercise protocol as previously described by Stone.20 Briefly, the treadmill exercise lasted a total of 18 minutes and was divided into 3-minute blocks. The protocol began with a 3-minute warm-up period during which the animal ran at 4.8 kph at 0% grade. The speed was increased to 6.4 kph and the grade of the treadmill was increased every 3 minutes as follows: 0%, 4%, 8%, 12%, and 16%. Each animal received three exercise tests that were averaged such that only one set of data was obtained for each animal. Once the control response was determined, the exercise test was repeated after β-adrenergic receptor blockade with propranolol hydrochloride (1 mg/kg i.v., Sigma Chemical, St. Louis, Missouri). The β-adrenergic receptor agonist isoproterenol hydrochloride (Isuprel 1 μg/kg i.v., Winthrop) was injected before and 5 minutes after propranolol to confirm the completeness of the blockade. This dose of propranolol completely eliminated the heart rate and dP/dt increase elicited by the isoproterenol injections. On subsequent days, a catheter was percutaneously placed in a cephalic or external jugular vein so that atropine sulfate (50 μg/kg, Lypho-Med) could be administered while the animal was running. Atropine sulfate was given during the last exercise level during both control and β-adrenergic receptor blockade exercise tests.

Data Analysis

All data were recorded on a Gould Model 2800S eight-channel chart recorder and a Teac Model MR-30 FM cassette tape recorder. Vagal tone was evaluated with a Delta Biometrics cardiac vagal tone monitor triggering off the electrocardiogram (R-R interval). This device used the time-series signal-processing techniques developed by Porges.34 This method deals with many of the statistical problems associated with extracting the amplitude of the respiratory sinus arrhythmia superimposed on a complex and changing baseline of heart rate. The electrocardiographic signal was digitized at 1 KHz, and sequential R-R intervals were timed to the nearest msec. The nonperiodic baseline fluctuations were removed with a moving third-order 21-point polynomial function.14,15,34 This procedure prevented the leakage of trends (i.e., transient changes) onto the respiratory frequency components. An output was obtained every 30 seconds of heart rate, R-R interval, R-R interval variance (from which standard deviation was calculated), and vagal tone index. The vagal tone monitor was set to evaluate the 0.24–1.04 Hz frequency component of the heart period function. This frequency band was selected because it was inclusive of the breathing frequencies noted in the animals used in this study. Control data were obtained before exercise began with the animal standing on the treadmill (last 3 minutes before exercise began). In a similar manner, recovery data were obtained 3 minutes after the cessation of exercise. The six 30-second intervals for a given level of exercise were averaged and reported as one value for that level. As noted above, these data were then averaged across exercise presentations so that only one set of values was used for a given animal (i.e., the three control exercise tests were averaged for each dog).

All data were analyzed using a two-factor analysis of variance mixed design with repeated measures on one factor.21 When the F ratio was found to exceed a critical value (p<0.05), Scheffe’s test was used to compare the means. The kurtosis and skewness of the vagal tone index and heart rate variability (standard deviation of the R-R interval) data were first evaluated with an Anderson Bell statistical package (AB STAT) and an IBM PC XT. These variables were found to be within the limits of a normal distribution and, therefore, did not violate the conditions necessary for analysis of variance techniques. All data are reported as the mean±SEM unless otherwise indicated.

Results

The dogs could be divided into two groups based on the response to the exercise plus ischemia test: 22 animals exhibited ventricular flutter that rapidly deteriorated to ventricular fibrillation (susceptible), whereas 14 animals did not (resistant). The time to onset for ventricular fibrillation was 52.5±3.0 seconds with 12 animals developing ventricular fibrillation shortly after the treadmill was stopped, while the remaining 10 had ventricular fibrillation while running. Ventricular fibrillation could be reproducibly induced in the susceptible animals with each presentation of the exercise plus ischemia test (once a week for a total of 4 weeks). Conversely, malignant arrhythmias were never associated with the exercise plus ischemia test in the resistant animals.

Heart Rate Response to Exercise

Representative examples of the electrocardiogram and heart rate for one susceptible and one resistant animal are displayed in Figure 1. The data were obtained during the last 3 minutes of exercise (6.4 kph/16% grade). Animals were selected that had similar values of heart rate (susceptible, 157; resistant, 173.5 beats/min). Note that despite similar heart rate values, the heart rate variability was
much greater in the resistant animal (R-R interval standard deviation: susceptible, 7.8; resistant, 49.4 msec; cardiac vagal tone index: susceptible, 2.2; resistant, 4.4 ln msec). The composite heart rate data before (Figure 2A) and after (Figure 2B) \( \beta \)-adrenergic receptor blockade are shown in Figure 2 and Table 1. As would be expected, heart rate increased significantly \( p<0.001 \) in response to exercise for both susceptible and resistant animals. Heart rate, however, increased significantly \( p<0.01 \) more in response to exercise in susceptible animals compared with resistant animals (Figure 2A). Heart rate remained elevated in the postexercise (recovery) period in the susceptible animals.

\( \beta \)-Adrenergic receptor blockade (Figure 2B) significantly \( p<0.01 \) attenuated the heart rate response to exercise in both groups of animals. Heart rate, however, remained significantly higher in the susceptible compared with the resistant group during the low intensity levels of exercise.

**Effect of Exercise on Cardiac Vagal Tone Index**

Accompanying the heart rate increase elicited by exercise, cardiac vagal tone (i.e., amplitude of the respiratory sinus arrhythmias), as measured by time-series analysis, significantly \( p<0.001 \) decreased in both the susceptible and resistant animals (Figure 3A, Table 1). The cardiac vagal tone index decreased to a significantly greater extent in the susceptible animals compared with resistant animals at all levels of exercise. The cardiac vagal tone index returned toward preexercise values within the first 3 minutes postexercise in the resistant animals but remained significantly depressed in the susceptible animals. The individual values of the cardiac vagal tone index for the susceptible and resistant animals are shown in Figure 4. In control (preexercise) conditions, no significant differences were noted between the groups. However, in response to exercise, the cardiac vagal tone index decreased to a significantly greater extent in the susceptible animals. In fact, during the last 3 minutes of exercise (6.4 kph/16% grade), only two susceptible animals had vagal tone values above the mean value noted in the resistant group. Conversely, only one resistant dog achieved a vagal tone value lower than the susceptible group mean. These lower vagal tone index values were noted even at similar heart rate values (see Figure 1). For example, if the cardiac vagal tone index was compared in resistant and susceptible animals with heart rates in an arbitrarily selected range (190–220 beats/min), cardiac vagal tone was still significantly depressed in the susceptible dogs. During the last 3 minutes of exercise, six susceptible and nine resistant dogs displayed heart rate values in the range above. These animals had similar heart rates (sus-
ceptible, 204.1±4.9; resistant, 207.4±2.4 beats/min), yet the susceptible dogs had significantly lower (p<0.05) values of cardiac vagal tone (susceptible, 1.6±0.5; resistant, 2.8±0.3 ln msec²).

β-Adrenergic receptor blockade accentuated the cardiac vagal tone index response to exercise in both groups of animals (Figure 3B, Table 1). That is, lower values of vagal tone were reached after propranolol pretreatment. The cardiac vagal tone index was significantly lower in the susceptible group compared with resistant animals before and during the low intensity levels of exercise. However, at the higher level of exercise, differences were no longer noted between the susceptible and resistant groups.

These data suggested that susceptible animals exhibited a lower cardiac vagal tone during exercise, indicating a more complete withdrawal of cardiac parasympathetic activity. This hypothesis was tested by administering atropine sulfate during the last level of exercise. Atropine sulfate (Figure 5A) elicited a significantly greater heart rate increase in the resistant animals (54.2±7.0 beats/min) compared with susceptible animals (18.7±4.4 beats/min). In a similar manner, atropine elicited a greater reduction in the vagal tone index in the resistant (control, 3.0±0.5; atropine, 0.1±0.1 ln msec²) compared with susceptible animals (control, 1.5±0.7; atropine, 0.1±0.1 ln msec²). After β-adrenergic receptor blockade (Figure 5B), heart rate did not significantly change in either the susceptible (8.5±3.3 beats/min) or resistant (14.9±5.1 beats/min) groups. The vagal tone index did not significantly change in either the susceptible (control, 0.3±0.3; atropine,
0.2±0.1 ln msec²) or resistant animals (control, 0.9±0.4; atropine, 0.7±0.3 ln msec²).

**Effect of Exercise on Heart Period Variability**

The heart period variability (as measured by the standard deviation of the R-R interval) response to exercise is shown in Figure 6. The SD of the R-R interval decreased significantly (p<0.01) in response to exercise in both resistant and susceptible groups. This heart period variability decreased to a significantly (p<0.01) greater extent in the susceptible compared with the resistant animals (Figure 6A, Table 1). The heart period variability remained depressed in the susceptible animals during the postexercise (recovery) period.

After β-adrenergic receptor blockade, the SD of the R-R interval decreased to a greater extent in response to exercise (Figure 6B, Table 1). Differences in heart period were noted between the susceptible and resistant animals during the lower levels of exercise but not during the higher levels of exercise.

**Discussion**

The cardiac autonomic response to submaximal exercise varied markedly between animals shown to be susceptible to and those that were resistant to ventricular fibrillation. Submaximal exercise elicited significantly greater increases in heart rate in the susceptible animals that were accompanied by correspondingly greater reductions in the parasympathetic (frequency, 0.24–1.04 Hz) component of the R-R variation. Heart rate variability as measured by the SD of the R-R intervals also decreased more rapidly and to a greater extent in response to exercise in the susceptible dogs. These data suggested that an almost complete withdrawal of parasympathetic tone was elicited by exercise in the susceptible animals, while high levels of cardiac vagal tone remained in the resistant dogs. Indeed, atropine administered while the animals were running evoked large heart rate increases in the resistant but not in the susceptible animals. In contrast, after β-adrenergic receptor blockade with propranolol, exercise elicited significantly greater reductions in cardiac vagal tone despite lower absolute values of heart rate for both the susceptible and resistant groups. Heart rate remained significantly elevated while the cardiac vagal tone index was significantly reduced in the susceptible group compared with the resistant animals during the early levels of exercise. As exercise progressed, however, these differences disappeared. The cardiac vagal tone index, in fact, was not significantly different from zero in both groups of animals, suggesting that essentially all parasympathetic influences had been withdrawn from the heart during the higher levels of exercise. Indeed, atropine injection after β-adrenergic receptor blockade failed to alter significantly heart rate in either group. When considered together, these data suggest that cardiac vagal tone was withdrawn as exercise progressed in both susceptible and resistant animals with the greatest reductions noted in the susceptible dogs. Furthermore, when the sympathetic effects of exercise were eliminated by β-adrenergic receptor blockade, an almost complete removal of parasympathetic activity was elicited by exercise, thereby attenuating the differences noted in the susceptible and resistant dogs. These data suggest that physiologic perturbations such as exercise provoke greater reductions in cardiac vagal tone in animals that were particularly at risk for ventricular fibrillation.

**Table 1. Effect of Exercise on Heart Rate Variability**

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>6.4 kph/0% grade</th>
<th>6.4 kph/8% grade</th>
<th>6.4 kph/16% grade</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>130.3±26.5</td>
<td>205.4±33.7†‡</td>
<td>214.1±25.4†‡</td>
<td>227.0±25.5†‡</td>
<td>141.9±17.7†</td>
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<tr>
<td>Resistant</td>
<td>122.8±22.1</td>
<td>176.3±21.2*</td>
<td>191.0±25.2*</td>
<td>205.4±26.7*</td>
<td>128.0±16.4</td>
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<tr>
<td>Susceptible + BB</td>
<td>117.6±18.8‡</td>
<td>169.1±16.2†‡</td>
<td>178.2±19.4*</td>
<td>185.3±21.8*</td>
<td>124.7±20.4</td>
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<tr>
<td>Resistant + BB</td>
<td>105.6±16.2</td>
<td>152.3±23.8*</td>
<td>167.7±24.7*</td>
<td>177.8±22.8*</td>
<td>114.5±25.0</td>
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Cardiac vagal tone index (in msec²)

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>6.4 kph/0% grade</th>
<th>6.4 kph/8% grade</th>
<th>6.4 kph/16% grade</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>6.12±1.34</td>
<td>2.43±1.46†‡</td>
<td>1.69±1.07†‡</td>
<td>1.07±1.15†‡</td>
<td>3.74±0.81†‡</td>
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<td>Resistant</td>
<td>6.33±1.29</td>
<td>3.93±0.87*</td>
<td>3.14±0.71*</td>
<td>2.71±1.04*</td>
<td>5.26±1.34</td>
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<tr>
<td>Susceptible + BB</td>
<td>4.60±2.26</td>
<td>1.41±1.02†‡</td>
<td>1.31±1.08*</td>
<td>1.01±1.15*</td>
<td>5.12±1.57</td>
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<tr>
<td>Resistant + BB</td>
<td>6.39±1.50</td>
<td>2.08±1.37*</td>
<td>1.15±1.15*</td>
<td>0.93±0.83*</td>
<td>4.65±1.81</td>
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</table>

Cardiac vagal tone index (in msec²)

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>6.4 kph/0% grade</th>
<th>6.4 kph/8% grade</th>
<th>6.4 kph/16% grade</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>56.4±31.5</td>
<td>14.5±7.7†‡</td>
<td>9.4±4.7†‡</td>
<td>7.1±3.6†‡</td>
<td>23.3±10.7†‡</td>
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<tr>
<td>Resistant</td>
<td>52.2±26.6</td>
<td>27.0±10.9*</td>
<td>19.8±6.8*</td>
<td>17.5±7.0*</td>
<td>34.3±9.1*</td>
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<tr>
<td>Susceptible + BB</td>
<td>40.1±30.1</td>
<td>7.2±4.3†‡</td>
<td>5.4±3.6*</td>
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<tr>
<td>Resistant + BB</td>
<td>50.8±24.4</td>
<td>14.6±2.3*</td>
<td>7.3±4.2*</td>
<td>6.0±4.5*</td>
<td>24.6±17.8*</td>
</tr>
</tbody>
</table>

Values given as mean±SD.

BB, β-adrenergic receptor blockade; preexercise, 3 minutes before exercise; recovery, 3 minutes after end of exercise.

*p<0.01 from preexercise values; ‡p<0.05, †p<0.01 susceptible compared with resistant for a given drug treatment.
Respiratory Sinus Arrhythmia as an Index of Cardiac Vagal Tone

Spontaneous oscillations in heart rate corresponding with respiration, the so-called respiratory sinus arrhythmia, are believed to be mediated primarily by the vagus nerves. In anesthetized animals, for example, the magnitude of R-R interval fluctuations correlated with total cardiac nerve activity. Recently, it has been established (with spectral analysis techniques) that oscillations in the R-R interval above 0.15 Hz were mediated predominately by respiratory driven vagal activity (peak, usually 0.40 Hz), whereas lower frequency peaks reflected the combination of sympathetic, parasympathetic, and renin-angiotensin system activity. Porges and coworkers developed a time-series technique that allowed for the real-time analysis of heart period variability. They found that the amplitude of the respiratory sinus arrhythmia as measured by this technique correlated with cardiac vagal activity. For example, atropine sulfate eliminated this respiratory frequency component (0.24–1.04 Hz peak), whereas phenylephrine enhanced it. Bilateral vagotomy has also been shown to eliminate this respiratory component, whereas drugs that antagonize the effects of acetylcholinesterase enhance the amplitude of the respiratory sinus arrhythmia. The time-series analysis
technique used calculated the amplitude of the respiratory sinus arrhythmia over periods as short as 30 seconds, allowing for the evaluation of dynamic changes in cardiac vagal tone. When considered together, the data suggest that cardiac vagal tone was indeed appropriately measured by power spectral and time-series analysis techniques.

**Autonomic Disturbances and Cardiac Death**

Disturbances in the autonomic control of the heart are often associated with cardiac disease. Eckberg et al. demonstrated that patients with the most advanced disease states exhibit the greatest impairment in parasympathetic activity. In an analogous fashion, Billman et al. showed that baroreceptor-mediated reductions in heart rate were impaired by myocardial infarctions, with the greatest impairment (smallest heart rate response) noted in animals particularly susceptible to sudden cardiac death. Recently, heart rate variability has been used as a noninvasive index of cardiac autonomic tone. In particular, heart rate variability was significantly reduced in patients with healed myocardial infarction, advanced coronary artery disease, and congestive heart failure. Wolf et al. for example, found that patients with the smallest heart rate variance at admission to the coronary care unit had the highest long-term mortality. In a more comprehensive study, Kleiger and coworkers found that in patients recovering from myocardial infarctions, those with the smallest heart rate variability as measured by the standard deviation of the R-R interval (over a 24-hour period) had the greatest risk of dying suddenly. In fact, the relative risk of mortality was 5.3-fold greater in patients with R-R interval variability of less than 50 msec compared with patients with a variability of more than 100 msec. Similar findings have been reported in patients with documented episodes of ventricular fibrillation. A reduced heart rate variability was reported in patients who died suddenly (30±10 msec) compared with normal subjects (76±14 msec). Recently, spectral analysis techniques have yielded similar results. Myers et al. found that a reduction in the 0.35–0.50 Hz (respiratory sinus arrhythmia) frequency peak of the power spectrum was associated with a greater risk of sudden cardiac death. In a similar manner, Saul et al. showed that the power spectrum was reduced in patients with congestive heart failure with the virtual elimination of the frequency components associated with parasympathetic activity. Gordon et al. found that the ratio of the low frequency to the respiratory frequency peaks was significantly reduced in pediatric patients who suffered a cardiac arrest. These studies demonstrate that reductions in cardiac parasympathetic
FIGURE 6. Plots of effect of exercise on heart rate variability before (A) and after (B) β-adrenergic receptor blockade. Variability was measured by the standard deviation of the R-R interval. **p<0.01 susceptible compared with resistant for a given exercise level.

control correlates with the severity of cardiac disease and with the risk for mortality and, while reflecting baseline changes in autonomic control, did not evaluate the dynamic response to a physiologic perturbation. Arai et al.16 recently demonstrated that exercise elicited reductions in both the high and low frequency peaks in the heart rate power spectrum. In agreement with these findings, we found that exercise elicited large reductions in cardiac vagal tone and that these reductions were accentuated after β-adrenergic receptor blockade. Most important, exercise in the present study evoked significantly greater reductions in cardiac vagal tone in animals known to be susceptible to ventricular fibrillation. Differences in cardiac vagal tone that were absent at rest (3 minutes before exercise) became obvious as exercise progressed. This observation suggests that subtle differences that were not obvious at rest became pronounced when the heart was "stressed" by exercise.

Limitations of the Study

There is at least one alternate explanation for the data described above. It is well established that exercise elicits large increases in both respiratory rate and tidal volume.36-38 Hirsch and Bishop39 demonstrated that respiratory rate and tidal volume alter the amplitude of respiratory sinus arrhythmia, decreasing respiratory sinus arrhythmia as respiratory frequency increases and increasing respiratory sinus arrhythmia as tidal volume increases. Furthermore, Saul et al.51 demonstrated that "the respira-
atory sinus arrhythmia was a frequency-dependent phenomenon with the magnitude and phase characteristics of a low pass filter.” It is, therefore, possible that differences in the respiratory response induced by exercise could contribute to the differences in the cardiac vagal tone index noted in the susceptible and resistant animals. The effect of these respiratory factors on the cardiac vagal tone index were not directly measured in the present study.

However, an estimate of respiratory frequency was obtained by counting the oscillations in systolic pressure or determining the respiratory driven peak in the R-R interval frequency spectrum. The estimated respiratory rate increased significantly and to the same extent in both the susceptible (control, 18.6±0.8; peak exercise, 28.9±1.0 breaths/min) and the resistant (control, 19.3±1.1; peak exercise, 29.4±1.1 breaths/min) animals. Furthermore, respiratory frequency was not affected by β-adrenergic receptor blockade in either susceptible (control, 19.3±1.4; peak exercise, 27.0±1.0 breaths/min) or resistant (control, 20.1±1.4; peak exercise, 29.1±1.6 breaths/min) animals, yet the vagal tone index decreased to a greater extent after this intervention. Indeed, this observation was confirmed by the absence of a heart rate increase after atropine injection. These data suggest that differences in the respiratory rate could not explain the large differences in the cardiac vagal tone index noted between the susceptible and resistant dogs.

These data cannot exclude the effects of exercise-induced changes in tidal volume on the cardiac vagal tone index. However, tidal volume probably increased during exercise that would, in turn, tend to increase the amplitude of the respiratory sinus arrhythmia, an effect opposite to what was observed. Furthermore, Eckoldt and Schubert found that during sleep, respiratory sinus arrhythmia varied independent of changes in tidal volume, suggesting that these two factors were not causally related. In a similar manner, Arai et al found that in humans, exercise elicited a 2.5-fold increase in respiratory frequency and a more than fourfold increase in the tidal volume. When taken together, they estimated that the high frequency peaks (vagal component) of the heart rate power spectrum would increase approximately 25% if only respiratory effects were considered. Yet, they found a 90% reduction in these peaks, which suggested that cardiac vagal activity was markedly reduced during exercise. Finally, it must be emphasized once again that atropine administered during exercise elicited large heart rate increases in animals with high values of the cardiac vagal tone index (resistant animals) but failed to elicit heart rate changes in animals with low values of cardiac vagal tone index (susceptible animals, resistant animals after propranolol). It, therefore, seems unlikely that the differences in cardiac vagal tone could be attributed to differences in the respiratory response to exercise.

In a similar manner, panting cannot explain the response differences for the following reasons. First, spontaneous occurrences of panting at rest did not shift either cardiac vagal tone index or the 0.24–1.04 Hz frequency peaks as measured by fast Fourier transform analysis. Second, similar panting responses were elicited in both the susceptible and the resistant dogs, yet exercise elicited a significantly greater reduction in cardiac vagal tone in the susceptible dogs. Finally, the cardiac vagal tone index was significantly reduced after β-adrenergic receptor blockade yet panting responses were not, suggesting that this respiratory effect alone could not account for the reduction in the cardiac vagal tone index.

Speculation on the Mechanisms

The mechanism underlying the observation that cardiac parasympathetic tone was reduced to a greater extent in susceptible animals remains to be determined. One might speculate that differences in ventricular function may contribute to the autonomic response differences noted in the susceptible and resistant animals. It is well established that myocardial infarction results in diminished left ventricular ejection fraction and, thereby, stroke volume. Compensatory adjustments would, therefore, be necessary to maintain cardiac performance, particularly during exercise. These adjustments would include alterations in cardiac autonomic activity. For example, because cardiac output is the product of heart rate and stroke volume, an increase of heart rate may partially compensate for the fall in stroke volume. Thus, exercise may evoke an increased sympathetic activity coupled with a more complete withdrawal of parasympathetic tone in the susceptible animals, factors known to predispose the heart to ventricular fibrillation. Therefore, one would predict that the susceptible dogs may have a relatively greater left ventricular dysfunction that becomes particularly apparent during exercise. Indeed, previous studies demonstrated a relatively greater ventricular dysfunction in animals susceptible to ventricular fibrillation. In an analogous manner, left ventricular dysfunction has been shown to increase the risk of sudden death in patients recovering from myocardial infarction. If the left ventricular function–autonomic tone hypothesis is correct, one would predict that interventions that either improve cardiac function or enhance parasympathetic activity should also decrease the incidence of sudden cardiac death. Exercise training has, in fact, been shown to improve cardiac parasympathetic control and prevent ventricular fibrillation.

The mechanisms by which alterations in parasympathetic tone affect cardiac electrical stability also remain to be determined. Anatomic studies indicate a rich cholinergic innervation of ventricular tissue (conductile pathways) in both humans and animals. Electrical stimulation of the vagus nerves has been shown to increase ventricular fibril-
lation threshold,1,42 decrease the incidence of spontaneous ventricular fibrillation during myocardial ischemia,1,42 and prevent reperfusion arrhythmias.43 Conversely, bilateral vagotomy or atropine have been shown to increase arrhythmia formation.1,46 In humans, carotid massage46 or phenylephrine injections45 have been shown to terminate ventricular tachycardia due to direct effects on the ventricles as confirmed in some of the patients.46 In a similar manner, pharmacologic studies in humans suggest that cholinergic tone lengthens ventricular refractory period47,48 and can antagonize the ventricular effects of β-adrenergic receptor stimulation.49 Prystowsky and coworkers,47 therefore, concluded that vagal activation could prevent or terminate reentrant arrhythmias by increasing the refractory period in one limb of the reentrant circuit. As a result, the propagating wave front (depolarization) could encroach on incompletely recovered tissue and thereby terminate the reentrant excitation. Clearly, the electrophysiologic effects of cardiac parasympathetic activation and its relation to sudden death merit further investigation.

In summary, submaximal exercise elicited significantly greater reductions in cardiac vagal tone as measured by time-series analysis in animals susceptible to ventricular fibrillation. β-Adrenergic receptor blockade provoked a greater reduction in the vagal tone index attenuating the differences noted in the susceptible and resistant animals. These data suggest that animals with the greatest reductions in cardiac vagal tone in response to physiologic perturbation such as exercise are also at the greatest risk for ventricular fibrillation. If these findings can be extrapolated to the clinical setting, a similar reduction in cardiac vagal tone may occur in individuals particularly at risk for sudden cardiac death. This intriguing possibility clearly merits further investigation.

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References


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