Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity

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Houle, Melanie S., and George E. Billman. Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H215–H223, 1999.—The low-frequency component of the heart rate variability spectrum (0.06–0.10 Hz) is often used as an accurate reflection of sympathetic activity. Therefore, interventions that enhance cardiac sympathetic drive, e.g., exercise and myocardial ischemia, should elicit increases in the low-frequency power. Furthermore, because an enhanced sympathetic activation has been linked to an increased propensity for malignant arrhythmias, one might also predict a greater low-frequency power in animals that are susceptible to ventricular fibrillation than in resistant animals. To test these hypotheses, a 2-min coronary occlusion was made during the last minute of exercise in 71 dogs with healed myocardial infarctions: 43 had ventricular fibrillation (susceptible) and 28 did not experience arrhythmias (resistant). Exercise or ischemia alone provoked significant heart rate increases in both groups of animals, with the largest increase in the susceptible animals. These heart rate increases were attenuated by β-adrenergic receptor blockade. Despite the sympathetically mediated increases in heart rate, the low-frequency power decreased, rather than increased, in both groups, with the largest decrease again in the susceptible animals: 4.0 ± 0.2 (susceptible) vs. 4.1 ± 0.2 ln ms^2 (resistant) in preexercise control and 2.2 ± 0.2 (susceptible) vs. 2.9 ± 0.2 ln ms^2 (resistant) at highest exercise level. In a similar manner the parasympathetic antagonist atropine sulfate elicited significant reductions in the low-frequency power. Although sympathetic nerve activity was not directly recorded, these data suggest that the low-frequency component of the heart rate power spectrum probably results from an interaction of the sympathetic and parasympathetic nervous systems and, as such, does not accurately reflect changes in the sympathetic activity.

cardiac parasympathetic activity; exercise; myocardial infarction; myocardial ischemia

THE HEART RATE VARIABILITY (HRV) spectrum, as measured by the standard deviation of the R-R interval, is routinely used as a noninvasive means of quantifying the cardiac autonomic input. Analysis of the spectrum reveals a high-frequency (>0.20 Hz) and a low-frequency (<0.10 Hz) component. The high-frequency peak or respiratory sinus arrhythmia is a reliable indicator of parasympathetic efferent activity (9, 22, 25). The administration of atropine and surgical selective sinoatrial nodal parasympathectomy have been found to abolish the high-frequency peak (22, 25). The underlying control of the low-frequency power has yet to be fully elucidated. Some studies indicate that the low-frequency peak or the ratio of low to high frequency may be an adequate reflection of sympathetic activity (13, 19, 21). Pagani et al. (21) found that dogs with a bilateral stellectomy were unable to elicit a reflex increase in the low-frequency power. These same dogs were capable of such an increase before the surgery. However, other investigators, using experimental means to increase the sympathetic activity, such as exercise or the addition of an adrenergic agonist, failed to see a significant increase in the low-frequency power (1, 3, 11, 22, 30, 32). In fact, in some cases the low-frequency power appeared to reflect the actions of the parasympathetic nervous system (22, 25, 30).

By use of the technique of Porges et al. (24), it is possible to perform real-time data analysis of the HRV spectrum and thereby detect dynamic changes in the tone. With this technique, Billman and Hoskins (4) demonstrated, in a canine model with a healed myocardial infarct, that exercise decreased the amplitude of the high-frequency component. Furthermore, there was a significantly greater reduction in animals found to be susceptible to ventricular fibrillation than in those determined to be resistant to malignant arrhythmias. Therefore, the authors concluded that exercise provoked a greater parasympathetic withdrawal in animals at risk for ventricular fibrillation (4). In a similar manner, acute myocardial ischemia also elicited a significantly greater increase in heart rate accompanied by a greater reduction in the high-frequency component of HRV in the susceptible animals (7). The effects of these interventions on the low-frequency component remain to be determined.

It is well established that myocardial ischemia (15, 20, 29) and exercise (26, 27) elicit an increase in efferent sympathetic nerve activity to the myocardium. In the case of myocardial ischemia, this alteration in the autonomic balance has been shown to reduce the cardiac electrical stability, thereby increasing the propensity for ventricular arrhythmias and the incidence of sudden death (8). Therefore, one would predict that if the low-frequency power accurately reflects alterations in sympathetic tone, then animals susceptible to ventricular fibrillation should also exhibit marked elevation in the low-frequency component of the HRV spectrum.

It was the purpose of this series of experiments to analyze the significance of the low-frequency power in animals that are susceptible and resistant to ventricular fibrillation. It is hypothesized that, along with an increased parasympathetic withdrawal, the susceptible animals also experience an increase in the sympathetic drive to the myocardium. If the low-frequency

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power is an adequate measure of sympathetic activity, then one would predict that exercise and myocardial ischemia at rest would elicit an increase in the low-frequency peak, and this increase should be greater in the high-risk animals. Likewise, the addition of a β-adrenergic receptor antagonist, propranolol, would be expected to attenuate the increase in the low-frequency peak brought about by both interventions that augment cardiac sympathetic activation.

METHODS

The principles governing the care and treatment of the 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.

Surgical procedure. Seventy-one mongrel dogs were used in this study (13.4–20.9 kg). The dogs received Innovar-Vet (0.02 mg/kg fentanyl citrate and 1 mg/kg droperidol iv; Pittman-Moore) as a preanesthetic followed by pentobarbital sodium (10 mg/kg iv; Harvey Laboratories) to induce anesthesia. With use of 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 free of the surrounding tissue. A 20-MHz pulsed Doppler flow transducer and a hydraulic occluder were placed around the vessel. Insulated silver-copper wires were sutured to the epicardial surface of the left and right ventricles for the later recordings of a ventricular electrogram. 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 min and then tied off. All leads to the cardiovascular instruments were tunneled under the skin to exit on the back of the animal's neck.

Pentazocine lactate (Talwin, Winthrop-Breon Lab, 30 mg im) was given as needed for postoperative pain. In addition, the long-lasting 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, 10^6 U im; Burns Veterinary Supply) twice daily for 7 days.

The animals were then placed in an "intensive care setting" for the first 24 h. To minimize the incidence of arrhythmias, the dogs received lidocaine hydrochloride (100 mg im; Xylocaine, Astra Laboratories) before surgery, which was supplemented (60 mg iv) before each stage of the two-stage occlusion. The animals also received 600 mg of tocainide hydrochloride (Tonocard, Merck, Sharpe & Dohme) every 12 h beginning on the day before surgery and continuing for the next 4 days.

Experimental protocol. The study began 3–4 wk after the production of the myocardial infarction. The animals were trained to walk on a motor-driven treadmill. For several days before the experiments, the animals were brought to the laboratory to familiarize them with the setting. The cardiac response to submaximal exercise was then evaluated before and after β-adrenergic receptor blockade. The response to exercise was assessed by a protocol previously described by Stone (31). Briefly, the treadmill exercise lasted a total of 18 min and was divided into 3-min blocks. The protocol began with a 3-min warm-up period, during which the animal ran at 4.8 km/h and 0% grade. The speed was then increased to 6.4 km/h, and the grade of the treadmill was increased every 3 min as follows: 0, 4, 8, 12, and 16%. Once the control response was determined, on a subsequent day the exercise test was repeated after β-adrenergic receptor blockade with propranolol hydrochloride (1 mg/kg iv; Sigma Chemical). The effect of propranolol during exercise was determined in 16 of the initial 71 animals. The β-adrenergic receptor agonist isoproterenol hydrochloride (1 μg/kg iv; Isuprel, Winthrop) was injected before and 5 min after propranolol to confirm the completeness of the blockade. This dose of propranolol completely eliminated the heart rate increase elicited by the isoproterenol injection.

On a subsequent day, with the animal lying quietly on a table, a 2-min coronary occlusion was made in 63 (40 susceptible and 23 resistant) animals. After 24–48 h, the occlusion was repeated in the presence of the β-adrenergic blocker propranolol (1.0 mg/kg). The β-adrenergic receptor blockade studies were performed on a subset of the initial 63 dogs (i.e., 9 susceptible and 8 resistant dogs received propranolol). Heart rate, left ventricular pressure, left circumflex coronary blood flow, and vagal tone were monitored during both occlusions.

Finally, the muscarinic antagonist atropine sulfate (Fujisawa) was given as an intravenous bolus (50 μg/kg, $n = 9$) and was used to evaluate the parasympathetic contribution to the low- and high-frequency components of HRV.

Seventy-two hours after the completion of the studies described above, the susceptibility to ventricular fibrillation was assessed in all 71 animals by the combination of exercise and acute ischemia (5, 28). Briefly, the animals ran on a motor-driven treadmill while the workload increased every 3 min, as described above. During the last minute of exercise, the left circumflex coronary artery was occluded. The treadmill was then stopped, and the occlusion was maintained for an additional minute. The total occlusion time was 2 min. Large metal plates (11 cm diameter) were placed across the animal's chest so that electrical defibrillation could be achieved with minimal delay, but only after the animal was unconscious. Electrocardiogram, heart rate, left ventricular pressure, and left circumflex coronary blood flow were recorded throughout the exercise + ischemia test. Left circumflex coronary blood flow was measured to confirm that the coronary occlusion was made.

All data were recorded on an eight-channel recorder (model 2800S, Gould) and an FM cassette tape recorder (model MR-30, Teac). 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 technique developed by Porges (23). 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 millisecond. The nonperiodic baseline fluctuations were removed with a moving third-order 21-point polynomial function (23, 24). This procedure prevented the leakage of trends (i.e., transient changes) onto the respiratory frequency components. An output was obtained every 30 s of heart rate, R-R interval variance (from which standard deviation was calculated), and vagal tone index. The vagal tone monitor was set to evaluate the high-frequency (0.24–1.04 Hz) or the low-frequency (0.06–0.10 Hz) components of the heart period function. Control data were obtained before exercise began with the animal standing on the treadmill.

All data were analyzed using a two-factor ANOVA mixed design with repeated measures on one factor [submaximal

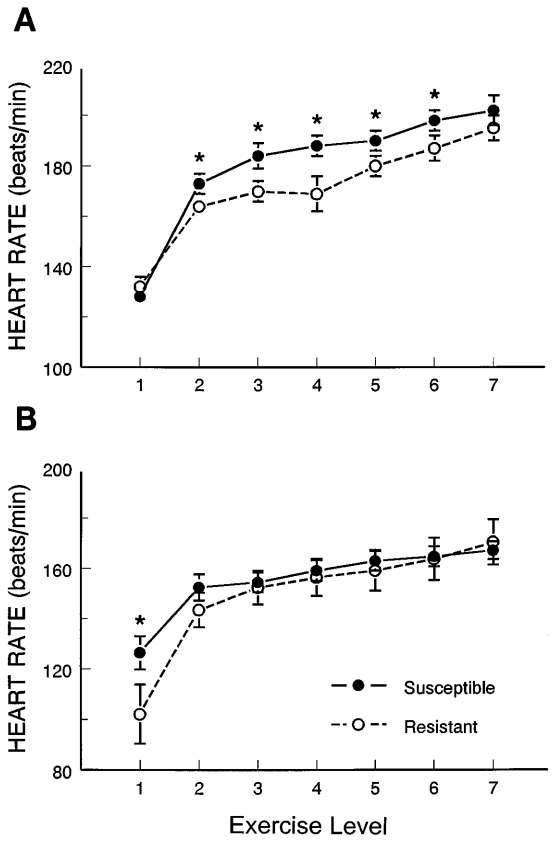

Fig. 1. Analysis of heart rate changes with increasing exercise levels in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). Exercise *level 1*, control value with animal standing quietly on treadmill before commencement of exercise; exercise *level 2*, 4.8 km/h; exercise *level 3*, 6.4 km/h; exercise *levels 4–7*, 6.4 km/h with grade increasing to 4, 8, 12, and 16%, respectively. $*P < 0.05$, susceptible vs. resistant.

exercise: group (2 levels) × exercise (7 levels); coronary occlusion: group (2 levels) × occlusion time (6 levels)]. When the F ratio was found to exceed a critical value ($P < 0.05$), Tukey's test was used to compare the means. The atropine sulfate data were analyzed with a paired *t*-test. Values are means ± SE unless otherwise indicated.

RESULTS

Effects of submaximal exercise. The exercise + ischemia test elicited ventricular fibrillation in 43 animals (susceptible), whereas 28 did not have arrhythmias (resistant). The cardiovascular response to exercise is displayed in Figs. 1–3. Exercise elicited a significant increase in heart rate in both groups of animals, with a greater increase (exercise × group interaction $F_{6/414} = 3.68$, $P < 0.001$) in the susceptible animals (Fig. 1*A*). In agreement with previous studies (4), exercise elicited large and significant reductions in the high-frequency power as exercise progressed, with larger reductions (exercise × group interaction $F_{6/414} = 2.20$, $P < 0.05$) again noted in the susceptible animals (Fig. 2*A*). In a similar manner, low-frequency power significantly decreased, rather than increased, in both groups, with a greater decrease (exercise × group interaction $F_{6/414} = 2.66$, $P < 0.02$) in the susceptible animals (Fig. 3*A*). The effects of the β-adrenergic receptor antagonist propranolol are displayed in Figs. 1–3. β-Adrenergic receptor blockade reduced the heart rate increases elicited by submaximal exercise (Fig. 1*B*), diminishing the differences noted between susceptible and resistant animals (group × exercise interaction $F_{6/84} = 4.27$, $P < 0.001$). After β-adrenergic receptor blockade, exercise still provoked significant reductions in high-frequency power (Fig. 2*B*). In fact, lower absolute values were achieved after this treatment in both groups. Although the differences between the groups were reduced by β-adrenergic receptor blockade, exercise provoked significantly greater reductions (exercise × group interaction $F_{6/84} = 2.21$, $P < 0.05$) in the susceptible animals. In a similar manner, β-adrenergic receptor blockade reduced preexercise and exercise values of the low-frequency power (Fig. 4*A*). Exercise provoked significant reductions in the low-frequency power in both groups, with greater reductions still noted in the susceptible animals (group × exercise effect $F_{6/84} = 2.62$, $P < 0.05$).

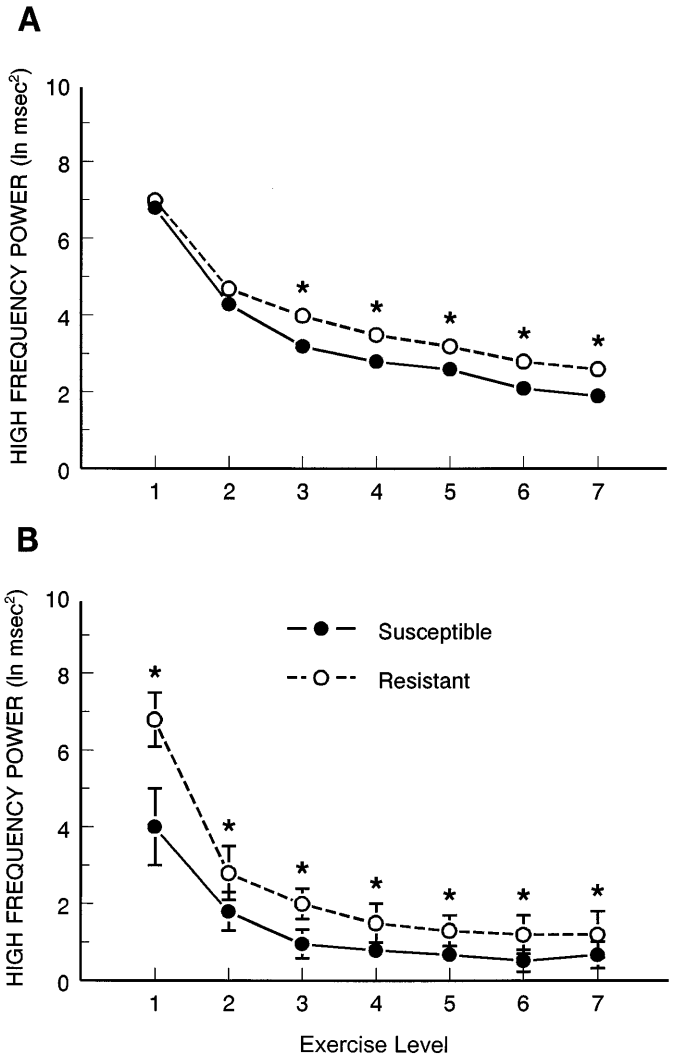

Fig. 2. Alterations in high-frequency component of heart rate variability spectrum with increasing exercise levels in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). $*P < 0.05$, susceptible vs. resistant.

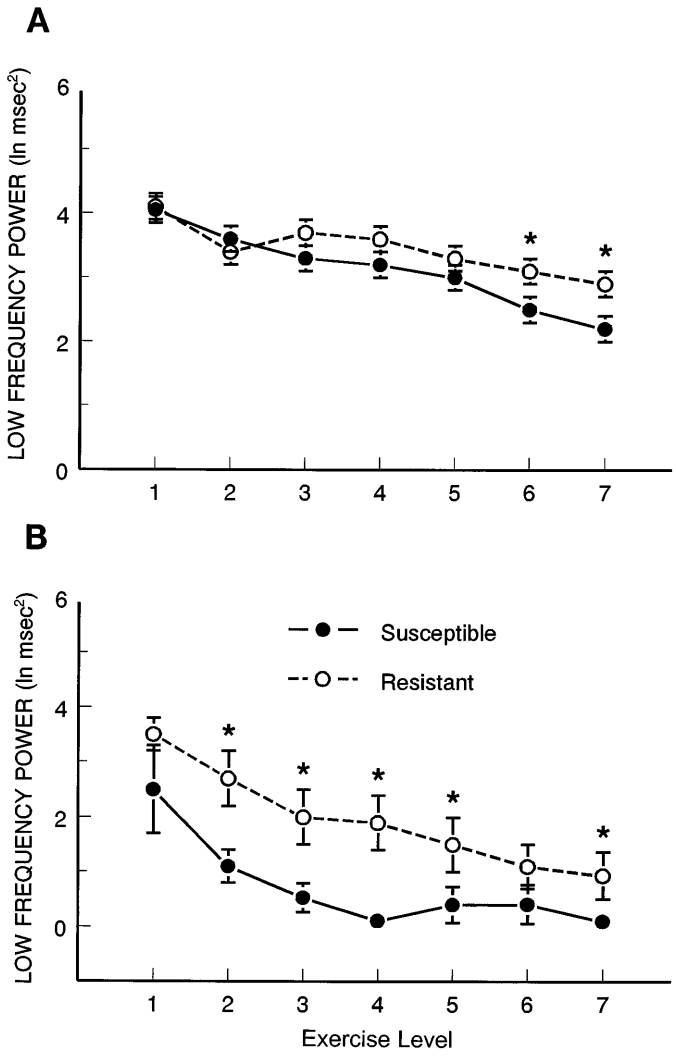

Fig. 3. Low-frequency component of heart rate variability spectrum with increasing exercise intensity in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). *$P < 0.05$, susceptible vs. resistant.

Effects of coronary artery occlusion alone. The response elicited by a coronary artery occlusion was evaluated in 63 (40 susceptible and 23 resistant) animals. The coronary artery occlusion that was performed with the animal lying quietly on a laboratory table induced ventricular fibrillation ($n = 8$) and ventricular arrhythmias ($n = 5$) in a total of 13 susceptible animals (32.5%). Arrhythmias were not induced in any of the resistant animals. The response to the coronary artery occlusion is displayed in Figs. 4–6. As expected, myocardial ischemia elicited significant increases in the heart rate in both groups of animals (Fig. 4*A*). Before the occlusion the heart rate was similar in the two groups of animals. In agreement with previous studies (7), myocardial ischemia provoked significantly greater increases in heart rate in the susceptible animals (group × occlusion interaction $F_{5/305} = 9.15$, $P < 0.001$). Myocardial ischemia provoked large and significant reductions in high-frequency power (Fig. 5*A*), with the larger reductions (group × occlusion interaction $F_{5/305} = 4.42$, $P < 0.001$) in the susceptible animals. Once again, low-frequency power decreased, rather than increased, in response to the coronary artery occlusion (Fig. 6*A*). A significantly greater reduction (occlusion × group interaction $F_{5/305} = 2.92$, $P < 0.01$) was noted in the susceptible animals.

The effects of the β-adrenergic receptor antagonist propranolol on the response to coronary artery occlusion is displayed in Figs. 4–6. β-Adrenergic receptor blockade attenuated the heart rate increase elicited by coronary artery occlusion (Fig. 4*B*). Myocardial ischemia elicited significant increases in heart rate in both groups of animals (heart rate effect $F_{5/305} = 15.74$, $P < 0.001$). However, the maximum heart rates achieved were lower after β-adrenergic receptor blockade. Furthermore, the heart rate response no longer differed (group × occlusion interaction $F_{5/305} = 1.03$, not significant) between the groups after propranolol pretreatment. β-Adrenergic receptor blockade attenuated the high-frequency power response to the coronary artery occlusion (Fig. 5*B*). However, the occlusion still provoked significantly greater (group × occlusion interaction $F_{5/305} = 4.42$, $P < 0.001$) reductions in high-frequency power in the susceptible animals. In a similar

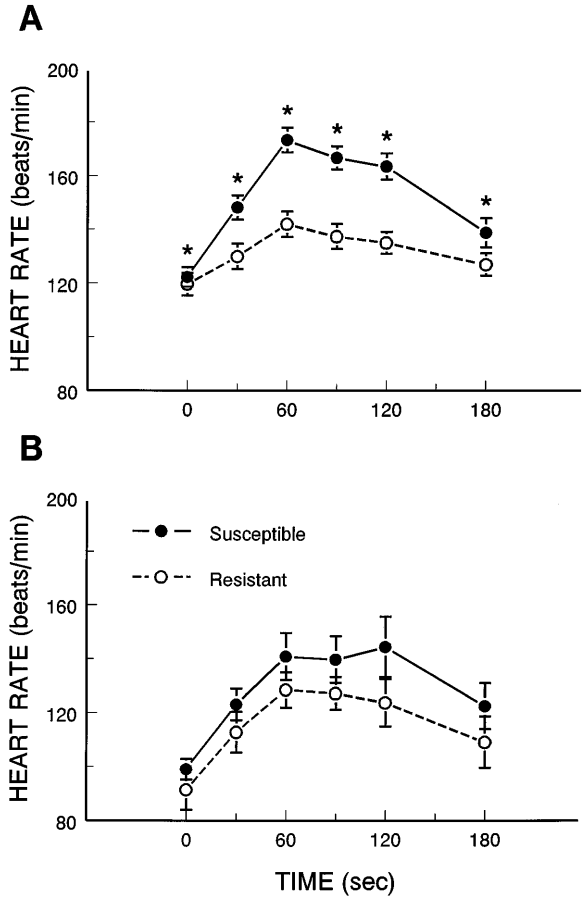

Fig. 4. Analysis of heart rate response to 2 min of myocardial ischemia alone in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). *Time 0*, control value before initiation of occlusion. Heart rate is recorded at 30, 60, 90, and 120 s into occlusion; 180-s time point is after release of occlusion. *$P < 0.05$, susceptible vs. resistant.

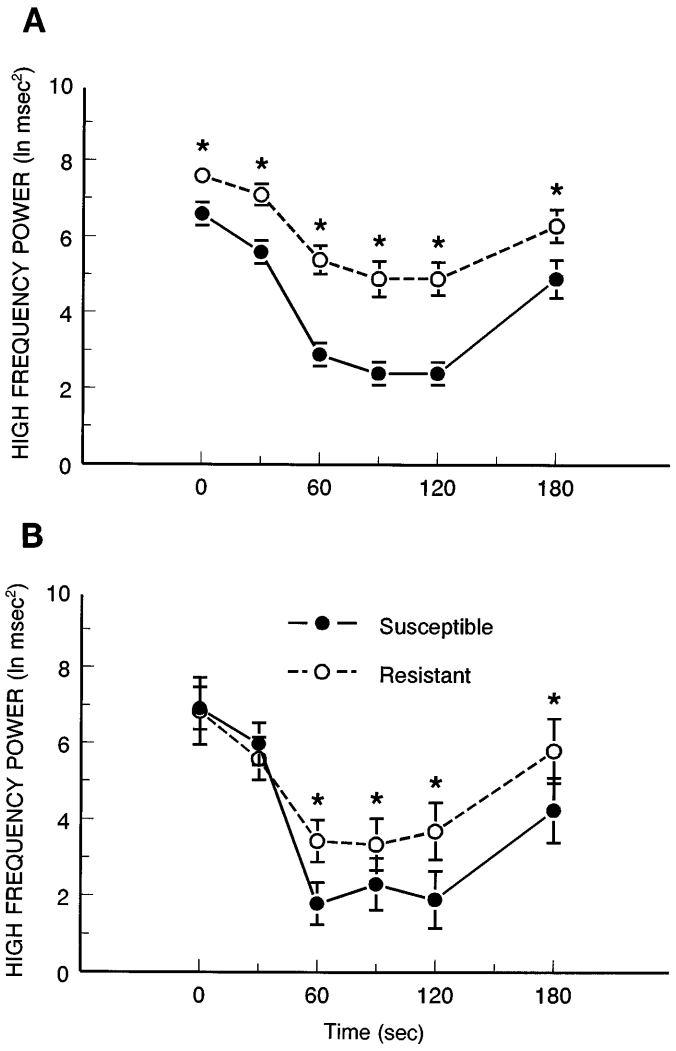

Fig. 5. High-frequency component of heart rate variability spectrum during a 2-min coronary occlusion alone in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). *$P < 0.05$, susceptible vs. resistant.

manner, β-adrenergic receptor blockade attenuated the low-frequency power response to the coronary occlusion (Fig. 6*B*). Myocardial ischemia provoked significant reductions in the low-frequency power (group × occlusion interaction $F_{5/305} = 2.3$, $P < 0.05$) in both groups, with significantly greater reductions still noted in the susceptible animals.

Effect of the cholinergic antagonist atropine. As noted above, exercise or acute myocardial ischemia elicited decreases in the high- and low-frequency powers with an increased heart rate. It is therefore possible that these changes reflect withdrawal or reductions in parasympathetic regulation of the heart. Indeed, an enhanced sympathetic activity should increase, rather than decrease, low-frequency power if the prevailing view is correct. To test this hypothesis, the muscarinic antagonist atropine sulfate was given to nine dogs lying quietly on a laboratory table. The heart rate was determined before the administration of the atropine and ~3 min after atropine once a new steady state had been achieved. All the animals studied experienced an increase in the heart rate with the addition of atropine (heart rate = 112 ± 4 and 181 ± 7 beats/min before and after atropine, respectively, $t = 7.26$, $P < 0.001$). As expected with cholinergic blockade, the high-frequency power decreased (7.5 ± 0.5 and 0.5 ± 0.2 ln ms² before and after atropine, respectively, $t = 11.34$, $P < 0.001$). However, the low-frequency power also decreased with the addition of atropine (5.1 ± 0.4 and 0.6 ± 0.2 ln ms² before and after atropine, respectively, $t = 10.05$, $P < 0.001$). These data indirectly suggest that a major component of the low-frequency component of the HRV results from parasympathetic, rather than sympathetic, activation.

DISCUSSION

In the present study, animals found to be susceptible to ventricular fibrillation consistently showed a greater increase in heart rate than resistant animals in response to interventions that are known to increase

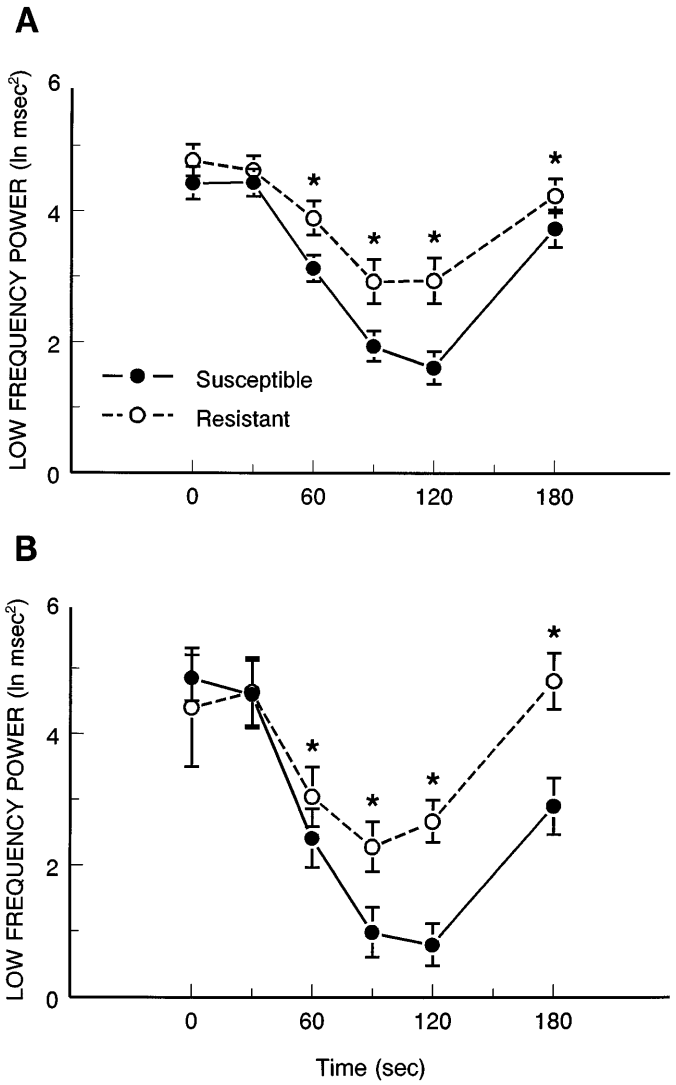

Fig. 6. Effect of a 2-min coronary occlusion alone on low-frequency power of heart rate variability spectrum in susceptible and resistant animals in absence (*A*) and presence (*B*) of propranolol (1.0 mg/kg iv). *$P < 0.05$, susceptible vs. resistant.

cardiac sympathetic activity, such as exercise and acute myocardial ischemia (Figs. 1*A* and 4*A*). This increase in heart rate was coupled with a significantly greater withdrawal of vagal tone, as demonstrated by a decrease in the high-frequency component of the HRV spectrum. The low-frequency component, which is suspected to be a marker of sympathetic activity (13, 18, 19, 21), decreased, rather than increased, during exercise and acute myocardial ischemia in the susceptible and resistant animals. Finally, the parasympathetic antagonist atropine significantly reduced the high- as well as the low-frequency component of the HRV spectrum. The low-frequency component of the HRV spectrum was consistently higher in the resistant animals. β-Adrenergic receptor blockade was capable of decreasing the low-frequency component in both groups of animals during exercise and acute myocardial ischemia. The fact that the two groups of animals responded so differently to such manipulations indicates a possible disturbance in the autonomic balance. A thorough analysis of the HRV spectrum may explain these results.

The high-frequency power is widely accepted as a marker of cardiac parasympathetic control (2, 9). Thus it is often used as a noninvasive means of studying the autonomic balance or, more specifically, the vagal tone of individuals. However, Kollai and Mizsei (16) caution against the use of the respiratory sinus arrhythmia as an exact measure of cardiac vagal tone, especially in conscious human subjects. As previously demonstrated when examining the high-frequency power (4, 7), the response to exercise and the response to a 2-min occlusion at rest differ between animals that are susceptible and resistant to ventricular fibrillation (Figs. 2*A* and 5*A*). The susceptible animals experienced a greater reduction in the high-frequency power as exercise intensity increased, indicating greater parasympathetic withdrawal in these animals (Fig. 2*A*). This same disparity was also seen during the 2-min occlusion at rest (Fig. 5*A*). In this case, the susceptible animals had an attenuated vagal tone before the occlusion that was accompanied by an even greater parasympathetic withdrawal as the ischemia progressed. Thus the susceptible animals may experience disruption in autonomic balance that predisposes the heart to an increased risk for malignant arrhythmias.

As previously mentioned, exercise (26, 27) and myocardial ischemia (15, 20, 29) have been shown to elicit an increase in sympathetic drive to the myocardium. If the low-frequency power is a marker of sympathetic activity, then the exercise test or the acute myocardial ischemia would be expected to increase this measurement. When the low-frequency power has been studied, exercise has consistently been used as a method of augmenting the sympathetic drive to the heart. In humans, sympathetic activity increases with exercise once the heart rate reaches 100 beats/min or 56% of the maximum. Until that point, the observed increase in heart rate is largely due to parasympathetic withdrawal (26, 27). If the susceptible animals have an increased sympathetic drive, then a greater increase in the low-frequency power should be noted in these animals than in the resistant animals. In both sets of animals the low-frequency peak not only did not increase with exercise, but rather as exercise progressed it decreased (Fig. 3*A*). In fact, the resistant animals had a significantly higher low-frequency power at the highest exercise levels. As noted above, if low frequency is a reliable measure of sympathetic activity, then the exercise test should have evoked an increase, a conclusion that is not supported by the present study (Fig. 3*A*). Yamamoto et al. (32), using healthy humans, reported similar results. They found that the high- and low-frequency peaks progressively decrease during exercise. An increase in the ratio of low to high frequency was not observed until the heart rate was 141–167 beats/min, long after the sympathetic system should have been activated (32). Ahmed et al. (1), again studying humans, saw no significant increase in the low frequency or the ratio of low to high frequency during exercise. The heart rate range in that study was similar to that reported by Yamamoto et al. (i.e., 150–170 beats/min). An infusion of epinephrine at a concentration and rate chosen to mimic that seen with exercise or acute myocardial ischemia was also unable to cause a change in the low-frequency peak (1). Likewise, in our study a coronary artery occlusion failed to cause an increase in the low-frequency peak (Fig. 6*A*). In fact, both groups of animals experienced a significant decrease in the low-frequency component, with the susceptible animals experiencing a greater attenuation. Interestingly, Ahmed et al. did report an increase in the low frequency and the ratio of low to high frequency during upright tilt and an increase in this ratio with an infusion of isoproterenol. Therefore, they concluded that the HRV response to sympathetic input may depend on the type of stimulation. This theory was supported by Skyschally et al. (30), who found that humans going from a supine to a standing position experienced an increase in the low-frequency peak, but exercise was unable to elicit this same increase. Therefore, perhaps the two interventions used in the present study, exercise and acute myocardial ischemia, activate the autonomic nervous system differently than does postural change.

The β-adrenergic receptor antagonist propranolol was used to characterize further the factors contributing to the low-frequency component of HRV. If the low-frequency power has a large sympathetic component, one would predict that β-adrenergic receptor blockade should abolish any increase in the low-frequency power previously observed. Because an increase in the low-frequency power was not noted, it was still of interest to determine what effect the β-adrenergic receptor blockade may have. β-Adrenergic blockade decreased the heart rate in the two groups of animals during exercise and acute myocardial ischemia. The reduction was more pronounced in the susceptible animals during either intervention (Figs. 1*B* and 4*B*). This would tend to support the conclusion that the susceptible animals rely more heavily on an increased sympathetic drive to control heart rate. Likewise, β-ad-

renergic receptor blockade elicited a reduction in the low-frequency power in the susceptible and resistant animals during exercise and the ischemia at rest. This would be expected if the low-frequency power was a measure of sympathetic activity. However, it must be emphasized that acute myocardial ischemia and exercise failed to increase the low-frequency power when the blockade was not present, data inconsistent with a significant sympathetic component to the low-frequency power. Rather, the data are more consistent with the hypothesis that the interventions provoked an even greater withdrawal of parasympathetic tone after β-adrenergic receptor blockade. In other words, the only mechanism available to increase heart rate in response to the increased metabolic demands placed on the animal was a further reduction in vagal activity. In fact, the low-frequency power was significantly reduced by the cholinergic antagonist atropine. Thus these data provide further indirect support for parasympathetic withdrawal, rather than sympathetic inhibition, as an explanation for the lower low-frequency power noted after β-adrenergic receptor blockade.

The discrepancies in the low-frequency component of HRV between the susceptible and the resistant animals may best be explained by a study done by Randall et al. (25). They found, after selective sinoatrial parasympathectomy, that the low-frequency power in dogs is a reflection of multiple components. The authors concluded that the total low-frequency power is composed of 50% parasympathetic activity, 25% sympathetic activity, and 25% "other" yet to be identified factors (25). If that is true, then β-adrenergic receptor blockade would be expected to have a greater effect on the low-frequency component of the susceptible dogs. As demonstrated in this study (Figs. 2*A* and 5*A*) and past studies (4, 7), susceptible animals have diminished vagal tone during exercise and acute myocardial ischemia. Therefore, one might expect that reductions in parasympathetic activity triggered by exercise or acute ischemia could reduce the low-frequency power by ≥50%. During the β-adrenergic receptor blockade, the 25% of the low-frequency component due to sympathetic input is also abolished, leaving only the 25% due to "other" to be measured. The resistant animals have greater vagal tone. Therefore, in the presence of β-adrenergic receptor blockade, the 25% of the low-frequency component that is controlled by the sympathetic system is abolished, whereas the 50% of the low-frequency component that is controlled by the parasympathetic nervous system should remain intact. As a result, these animals would tend to have a consistently greater low-frequency peak. As noted above, as a consequence of the β-adrenergic receptor blockade, the animals must withdraw vagal tone to an even greater extent to increase heart rate during exercise. Therefore, if there is a large parasympathetic component to the low-frequency power, then the greater withdrawal of vagal tone would also lead to a greater reduction in the low-frequency power. Furthermore, atropine sulfate, a cholinergic antagonist, significantly reduced low-frequency power, an observation consistent with a large parasympathetic component to the low-frequency power. Thus our results support those of Randall et al. and others (11, 22) that contend that the low-frequency ratio cannot be used as reliable measures of sympathetic activity. In a similar manner, Pomeranz et al. (22) found in healthy humans that the parasympathetic nervous system influenced heart rate fluctuations at all frequencies studied, but the sympathetic nervous system only influenced the low-frequency power and then only when the subject was in a standing position. Furthermore, Grasso et al. (11) concluded that all fluctuations in the low-frequency peak resulted from changes in the parasympathetic nervous system and failed to note any postural influence of the sympathetic nervous system.

In contrast, Malliani et al. (18, 19) argue that the low-frequency peak is, indeed, a reliable marker of sympathetic activity. Pagani et al. (21) found that a bilateral stellectomy in dogs was able to abolish any sympathetic-induced increase in low-frequency power (normalized units). Similarly, Inoue et al. (13) observed in resting quadriplegic patients in the supine position that the low-frequency peak was absent, whereas the high-frequency peak was observed. Guzzetti et al. (12), also studying quadriplegic patients, found that the low-frequency component was absent in ~46% of patients. When present, the low-frequency component (normalized units) decreased during tilt and increased with controlled respiration again, suggesting a possible vagal influence (12). However, the authors point out that if the low-frequency component did have a significant vagal influence, it should not have disappeared in the quadriplegic patients, especially so soon after injury, when vagal tone predominates.

Jaffe et al. (14) offers an explanation that might account for some of the discrepancies noted above. They investigated the optimal frequency range for delineating sympathetic activity. They found by experimentation and through the analysis of previously published papers by other investigators that restricting the sympathetic frequency band to >0.05 Hz and <0.1 Hz and then dividing this by the total spectral amplitude (0.004–0.5 Hz) gave the best sympathetic indicator. In fact, the frequency range of 0.10–0.15 Hz, which is often included in the low-frequency measurements, has a significant negative correlation with heart rate (14). This suggests a strong parasympathetic influence in this range. The frequency range of 0.06–0.10 Hz used in our study is below the range of presumed parasympathetic dominance. Therefore, these data further indicate the complexity of the low-frequency power.

Limitations of the study. First, it must be acknowledged that sympathetic nerve activity was not directly measured in the present study. Therefore, any conclusions about changes in autonomic nerve activity can only be inferred on the basis of changes in the low-frequency component of the R-R interval variability. However, a number of studies (15, 20, 26, 27, 29) have demonstrated that sympathetic nerve activity is increased during myocardial ischemia or exercise. Second, Lombardi et al. (17), studying the normalized low-frequency power (LF/LF + HF, where LF is low

frequency and HF is high frequency) in patients after myocardial infarction, found that the sympathovagal interaction varied depending on the amount of time that had passed since the infarction. These authors found an increase in the low-frequency component (normalized) and a decrease in the high-frequency component (normalized) at rest in postmyocardial infarction patients compared with control subjects. However, interventions used to augment sympathetic activity failed to result in an increase in the low-frequency component (normalized) 2 wk after the infarction. These data suggest that the sympathovagal balance may be disrupted during acute healing after ischemic injury and, as such, makes accurate quantifications of changes in autonomic balance problematic. In the present study, animals were examined >1 mo after myocardial infarction. Thus it is unlikely that the lack of sympathetic component to the low-frequency power resulted from the healing process.

As demonstrated, an accurate representation of cardiac sympathetic activity using the HRV spectrum is difficult. Changes in the low-frequency power appear to be dependent on the method used to augment sympathetic activity as well as the method of analysis. The blood pressure power spectrum may, in fact, offer a better indication of cardiovascular sympathetic activity. Brown et al. (6) found a strong coherence between sympathetic nerve activity and arterial pressure at 0.4 Hz in conscious and anesthetized rats. When the sympathetic nerve activity was compared with the heart rate spectrum, little coherence was observed throughout the spectrum. Therefore, cardiovascular sympathetic activity may more accurately be reflected in the blood pressure power spectrum.

In summary, the present study demonstrates that low-frequency (0.06–0.10 Hz) fluctuations in the R-R interval variability may not accurately reflect changes in sympathetic activity. Changes in sympathetic activity may contribute to the low-frequency peak, but a large influence of the parasympathetic activity is also present. As such, the interpretation of the low-frequency component of the HRV spectrum becomes problematic and, under most circumstances, cannot be taken as an accurate measurement of cardiac sympathetic input. The present study supports a review by Eckberg (10) in which the author outlines the complexity of the HRV spectrum. Eckberg argues that often the language and the mathematical manipulations used to describe the parameters associated with the HRV spectrum may be in danger of obscuring the physiology behind the spectrum. The author states that there is little doubt that sympathetic neural mechanisms contribute to the low-frequency component, yet this does not necessarily mean that the low-frequency component is a "quantitative probe for sympathetic traffic." Therefore, care should be taken when evaluating such information, inasmuch as the data appear to vary depending on the frequency range used as well as the experimental means used to augment the sympathetic activity.

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