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A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: Implications for future anti-arrhythmic drug development

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Abstract

Sudden cardiac death resulting from ventricular tachyarrhythmias remains the leading cause of death in industrially developed countries, accounting for between 300,000 and 500,000 deaths each year in the United States. Yet, despite the enormity of this problem, the development of safe and effective anti-arrhythmic agents remains elusive. The identification of effective anti-arrhythmic agents is critically dependent upon the use of appropriate animal models of human disease. During the last 25 years, a canine model of sudden cardiac death has proven to be useful in both the identification of factors contributing to ventricular fibrillation (VF) and the evaluation of potential anti-arrhythmic therapies. The present review provides a detailed retrospective analysis of the data obtained with this model. Briefly, VF was reliably and reproducibly induced by the combination of acute myocardial ischemia at site distant from a previous myocardial infarction during submaximal exercise (to activate the autonomic nervous system). This exercise plus ischemia test identified 2 stable populations of dogs: those that development malignant arrhythmias (susceptible, $n=303$) and those that rarely developed even single premature ventricular activation (resistant, $n=209$). The susceptible animals exhibited an elevated sympathetic activation (due to an enhanced β_2-adrenoceptor responsiveness) and a subnormal parasympathetic regulation. Several interventions have proven to be particularly effective in preventing VF in the susceptible dogs; including calcium channel antagonists, left stellate ganglion disruption, ATP-sensitive potassium channel antagonists, β-adrenoceptor antagonists, and non-pharmacological interventions (endurance exercise training and dietary omega-3 fatty acids).

Keywords: Ventricular fibrillation; Myocardial ischemia; Myocardial infarction; Heart rate variability; Autonomic nervous system; Calcium channel antagonists; ATP-sensitive potassium channel antagonists

Abbreviations: CBF, coronary blood flow; LVP, left ventricular pressure; QTc, QT interval corrected for heart rate; Vcf, velocity of circumferential fiber shortening; VF, ventricular fibrillation.

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1. Introduction

Sudden cardiac death resulting from ventricular tachyarrhythmias remains the major cause of death in most industrially developed countries, accounting for between 300,000 and 500,000 deaths each year in the United States (Zipes & Wellens, 1998). Yet despite the enormity of this problem, the identification of the mechanisms responsible for these untimely deaths, as well as the development of safe and effective anti-arrhythmic therapies, remains elusive. Indeed, many initially promising anti-arrhythmic medications were subsequently shown to promote rather than abolish arrhythmias in some patient populations (Echt et al., 1991; Waldo et al., 1996; Sager, 1999). Furthermore, even the best currently available therapies have not been completely successful in the suppression of malignant arrhythmias and also frequently exhibit untoward side effects. To date, only β-adrenergic receptor antagonists and amiodarone, a class III antiarrhythmic drug that also blocks β-adrenergic receptors, have been shown to reduce sudden cardiac death (Held & Yusuf, 1989, 1993; Kendall et al., 1995; Amiodarone trials meta-analysis investigators, 1997; Zipes & Wellens, 1998). However, mortality following myocardial infarction remains high among patients with substantial ventricular dysfunction, even with β-adrenergic receptor antagonist therapy. The 1-year mortality is 10% or higher, with sudden death accounting for approximately 1/3 of the deaths in these high-risk patients (Buxton et al., 1999). In a similar manner, the long-term use of amiodarone is limited due to adverse side effects, including pulmonary fibrous and thyroid toxicity (Nattel, 2000). Clearly, the "ideal" anti-arrhythmic drug has yet to be discovered.

The use of appropriate animals models of ventricular arrhythmias is critical, not only for discovering the mechanisms that trigger lethal cardiac rhythm disorders, but also for the pre-clinical evaluation of potential anti-arrhythmic drugs. The model used must mimic, as closely as possible, the underlying pathological conditions most often associated with the high risk of sudden cardiac death. The present review provides a detailed retrospective analysis of one such model, a canine model of sudden cardiac death that has proven useful in both the identification of factors contributing to ventricular fibrillation (VF) and the evaluation of potential anti-arrhythmic therapies.

2. A canine model of ventricular fibrillation

The development of safe and effective anti-arrhythmic drugs is critically dependent upon the choice of an appropriate animal model for the pre-clinical evaluation of these medications. The model used must mimic, as closely as possible, the underlying pathological conditions associated with a high risk of sudden death in patients. Several clinical studies indicate that among the most important factors associated with a high risk of sudden death are the following: previous myocardial ischemic injury (Davis et al., 1979), acute myocardial ischemia at a site distant from this injury (Schuster & Bulkley, 1980), and alterations in cardiac autonomic regulation (Corr et al., 1986; Schwartz & Zipes, 2000). Post-mortum studies indicate that the vast majority of the cases of sudden death result from tachyarrhythmias in patients with underlying, and often undetected, coronary artery disease (Kuller et al., 1972; Weaver et al., 1976; Abildstrom et al., 1999). The observation that many victims of sudden death have coronary artery thombi (Kuller et al., 1972; Weaver et al., 1976; Abildstrom et al., 1999) suggests that acute ischemia may contribute significantly to the development of VF. Indeed, post-myocardial patients that experienced ischemia at a site distant from the infarction had a much greater mortality rate than those patients that did not (Schuster & Bulkley, 1980). Finally, reductions in cardiac parasympathetic tone coupled with elevations in sympathetic activity are also associated with a greater risk for sudden death (La Rovere et al., 1988; Bigger, 1997; Hohnloser et al., 1997; La Rovere et al., 1998). In particular, it has been shown that myocardial infarction will reduce heart rate variability and, further, that the patients with the greatest reduction in this variable also have the greatest risk for sudden death (Kjellgren & Gomes, 1993; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997). Kleiger et al. (1987) found that in patients recovering from myocardial infarction, those with the smallest heart rate variability (standard deviation of R–R interval) had the greatest risk of dying suddenly. The relative risk of mortality was 5.3 times greater in patients with a R–R interval variability less than 50 msec compared to patients with a variability greater than 100 msec. This observation has been subsequently confirmed by numerous clinical studies (La Rovere et al., 1988; Bigger et al., 1992; Bigger, 1997; Hohnloser et al., 1997; La Rovere et al., 1998). To cite just one example, La Rovere et al. (1998), reporting for the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) group, found that post-myocardial infarction patients with either low heart rate variability or a small heart rate response to an increase in blood pressure (baroreceptor reflex sensitivity) had a much greater risk of sudden death than those with well preserved

cardiac vagal regulation. The greatest risk for mortality was observed in patients with a large reduction in both markers of cardiac vagal regulation (La Rovere et al., 1998). Thus, the ideal model should incorporate as many of these clinically important factors as possible.

In addition, it is essential that the lethal arrhythmias be reliably and reproducibly induced in the model before an accurate assessment of a given compound can be made. For example, sample size can dramatically influence the results obtained for a compound. Trolese-Mongheal et al. (1985) investigated the effects of sample size on the reproducibility of sudden death using a large series of dogs ($n=648$) in which acute myocardial ischemia was produced by ligation of the left anterior descending coronary artery. They then randomly divided these animals into groups of various sizes ($n=10$ to $n=100$) and found that the pretreatment rate of sudden death varied widely depending on the sample size. For example, the sudden death rate ranged 0–70% and 5–55% if the sample size was 10 or 20 dogs, respectively. In contrast, a much smaller range (14–36%) was found in groups that contained 100 animals. Stable values were obtained with a sample size of between 50 and 60 dogs. This wide disparity in the incidence of sudden death may explain the often-conflicting results that have been obtained with the same compound. In other words, the risk for a false positive result was inversely related to the sample size; that is, the smaller the sample size, the greater the risk was to conclude falsely that a given treatment protected against sudden death. These authors (Trolese-Mongheal et al., 1985), therefore, concluded that a reliable assessment of the anti-arrhythmic potential for given drug could only be made with at least 50 animals per treatment group. However, it is not always possible to use large numbers of animals in the pre-clinical evaluation of test compounds, due to both the high costs and the long periods of time that would be necessary to complete studies. Approaches that employ the same animals in both the control and treated groups (i.e., provide an internal control) would reduce the total number of animals required and thereby expedite the evaluation of a compound or set of compounds. Thus, an appropriate model must not only closely mimic the pathology responsible for sudden death in patents but also use a sufficient number of animals so that the anti-arrhythmic properties of a given drug can be accurately assessed.

In 1980, I joined the laboratory of Dr. H. Lowell Stone, and together with Dr. Peter Schwartz, we developed a canine model of sudden cardiac death (Billman et al., 1982; Schwartz et al., 1984; Billman, 2005). This model combines several factors that have been clinically proven to contribute significantly to the induction of VF including, a brief and *reversible* left circumflex occlusion in animal with healed anterior myocardial infarctions, such that acute ischemia was induced at site distant from previous myocardial damage (Billman et al., 1982; Schwartz et al., 1984). Since, as note above, alterations in cardiac autonomic regulation have been linked to a greater propensity for sudden death (Corr et al., 1986; La Rovere et al., 1988; Bigger, 1997; Hohnloser et al., 1997; La Rovere et al., 1998), submaximal exercise was used as a physiological relevant means of activating the autonomic nervous system.

A detailed description of this canine model has been recently published (Billman, 2005). Therefore, the following paragraph contains only a brief description of the model. After 3–4 weeks of recovery period following the surgery and myocardial infarction, the animals learn to run on a motor-treadmill (usually over 3 or 4 days). The susceptibility to VF is then assessed using an exercise plus ischemia test. The animals run on a motor-driven treadmill for 15–18 min until a criterion heart rate of 210 beats/min has been achieved (~70% of maximum heart rate). The workload is increased every 3 min during this test (initial level, 0% grade 4.8 kph, 0% grade 6.4 kph, 4% grade 6.4 kph, 8% grade 6.4 kph, 12% grade 6.4 kph, and finally, 16% grade 6.4 kph). During the last minute of the exercise the left circumflex occluder is inflated, the treadmill is then stopped and the occlusion maintained for an additional minute. The occlusion therefore lasts 2 min: 1 min during exercise and 1 min post exercise. This allows for the differentiation of arrhythmias induced during exercise, post exercise, and post occlusion release. The occlusion is immediately released in those animals that exhibit ventricular tachyarrhythmias (most frequently ventricular flutter that rapidly deteriorates into VF). Large flexible metal pads (approximately 11-cm diameter) are placed across the animal's chest and are connected to an external defibrillator. A major advantage of this model is the identification of 2 highly reproducible and stable populations of animals: those resistant and those susceptible to VF (Billman et al., 1982; Schwartz et al., 1984; Billman, 2005).

3. Characteristics of the model

I have used this exercise plus ischemia test to investigate the mechanisms responsible for VF for a little over 25 years. In this review, I present the first comprehensive analysis of data collected with this model, first as a post-doctoral fellow in Dr. H. Lowell Stone' laboratory at the University of Oklahoma (1980–1984) and then after joining the faculty of The Ohio State University (1984 to present).

To date, I have produced an anterior wall myocardial infarction in a total of 768 animals (male, $n=287$; female, $n=481$) (Fig. 1). Two hundred and thirteen (27.7%) dogs died acutely either during surgery or usually within the next 4 days following surgery. The distribution of the timing of the early mortality is displayed in Fig. 2. The majority of the dogs died either during surgery ($n=90$) or within the first 24 hr ($n=52$) after the myocardial infarction. Over the years the survival rate has improved, as highlighted by comparing the results from the first 100 animals with studies completed on the most recent set of 100 dogs. Thirty of the first 100 dogs died before studies began (13 died during surgery) while only 19 dogs in the most recent set of 100 dogs died acutely (only 2 died during surgery). Thus, like mastering a complex piano sonata or learning a new language, practice makes perfect.

Following the myocardial infarction, 555 dogs survived to be studied; of these dogs, 4 were eliminated (heart worm positive) and 39 experienced instrumentation failure (left circumflex coronary occluder rupture) and could not be classified. The exercise plus ischemia test has been performed on 512 animals.

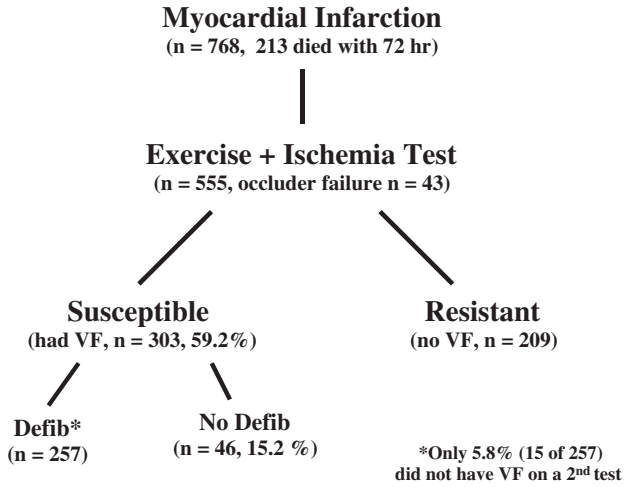

Fig. 1. A flow chart illustrating the classification of animals that have been studied using a canine model of sudden cardiac death. VF = ventricular fibrillation, defib = defibrillation.

Three hundred and three dogs developed ventricular flutter that rapidly deteriorated to VF (susceptible, 59.2%) during this test, while the remaining 209 did not (resistant, 40.8%). There were no obvious gender differences (susceptible: female 59.5%, male, 58.7% vs. resistant: female 40.5%, male 41.3%). Representative examples of 1 susceptible and 1 resistant dog are shown in Fig. 3. Only 15.2% (n=46) of the susceptible animals were not successfully resuscitated. The timing of the VF onset was analyzed in 215 of these dogs. VF was induced during the first minute of the occlusion (i.e., during exercise) in 109 dogs (50.6%), during the second minute of the occlusion (i.e., after the treadmill was stopped) in 93 dogs (43.2%), and following occlusion release in 3 dogs (1.4%). In addition, 1 dog developed VF during exercise before the occlusion and 11 dogs had the malignant arrhythmia with a 2-min occlusion at rest (5.1%). The lethal arrhythmias were highly reproducible in the susceptible animals; that is, VF could be repeatedly induced with *each* presentation of the exercise plus ischemia test. The time to VF onset was very similar between the first (56.1±2.6 sec) and second (55.4±2.4 sec) occlusion. In initial studies, VF was induced once a week for up to 8 weeks (n=8 at 8 weeks, the longest period studied). In fact, only 15 of 257 (5.8%) of the susceptible animals failed to develop VF during either a second or a third exercise plus ischemia test. Conversely, a resistant dog *never* developed VF during repeated presentations of the exercise plus ischemia test. In fact, these dogs rarely even developed single premature ventricular activation during the exercise plus ischemia test. Arrhythmias were only induced in18.7% of the resistant dogs (1–3 arrhythmias, n=12; 4 or more arrhythmias, n=27). Therefore, unless some other interventions are applied, the vast majority of susceptible animal will remain prone to VF, while the resistant dogs never develop malignant arrhythmias throughout the course of a given study.

The exercise plus ischemia test was also performed in a few dogs (n=33) without prior myocardial infarctions. This test provoked VF in a much smaller proportion of these non-infracted dogs. Lethal tachyarrhythmias were elicited in only 12 dogs (36.4%) of the non-infarcted dogs. Interestingly, this number compared very favorable with the percentage (27.7%) of animals that either died during surgery or the first few days following myocardial infarction. Thus, there would appear to be a population of animals that are prone to lethal arrhythmias induced by ischemia even without previous injury. Furthermore, these animals most likely constitute the group of dogs that die acutely as a consequence of the initial myocardial infarction procedure.

3.1. Hemodynamic responses

The effect of either exercise or coronary artery occlusion (both at rest or during exercise) on left ventricular pressure

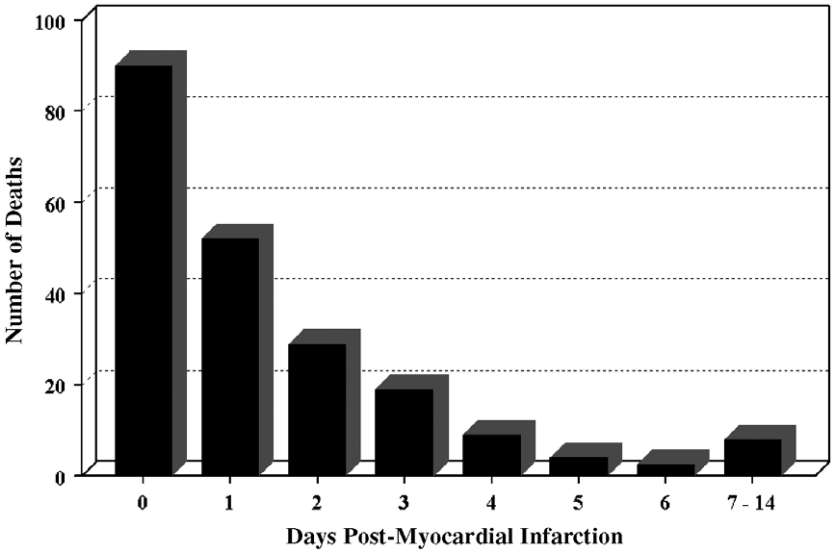

Fig. 2. The time course for the animals that died acutely following myocardial infarction. The majority of the dogs died either during the surgery (n=90) or within the first 24 hr following the (n=52, 15 died within the first 6 hr) myocardial infarction.

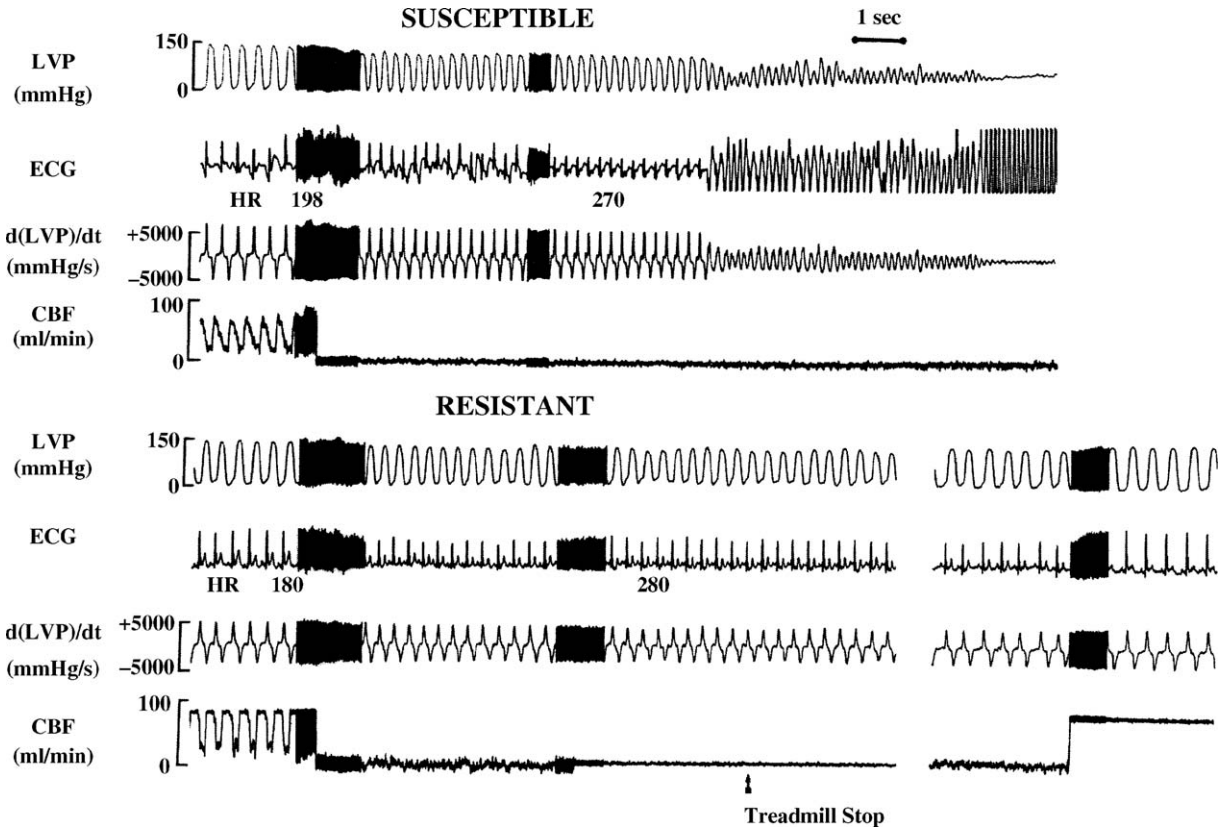

Fig. 3. Representative recordings from 1 susceptible and 1 resistant animal. The exercise plus ischemia test induced ventricular flutter (that rapidly progresses to ventricular fibrillation) in the susceptible animal. Note the smaller heart rate increase in response to the coronary occlusion in the resistant animal. LVP = left ventricular pressure, CBF = coronary blood flow, HR = heart rate (beats per min).

(LVP) was measured in both susceptible (n = 59) and resistant (n = 55) dogs. The left ventricular response to exercise is displayed in Fig. 4. There were very few differences noted between the animals before the onset of exercise (i.e., standing on the treadmill), only heart rate was slightly higher in the susceptible dogs before exercise onset. Exercise elicited significantly greater increases in heart rate and left ventricular diastolic pressure in the susceptible as compared to more modest changes the resistant dogs (Billman et al., 1985). In a similar manner, myocardial ischemia provoked much larger increases in heart rate in the susceptible dogs as compared the resistant animals either alone (Fig. 5) or when combined with exercise (the exercise plus ischemia test, Fig. 6). In a similar manner, the coronary artery occlusion, when performed during exercise, provoked significantly greater increases in left ventricular diastolic pressure in the susceptible dogs as compared to the resistant animals (Fig. 6). In contrast, myocardial ischemia provoked similar reductions in left ventricular systolic pressure and left ventricular dP/dt maximum in both groups of animals (Figs. 5 and 6). When considered together, these data suggest that left ventricular function was compromised to a greater extent (larger increases in left ventricular diastolic pressure) in the susceptible dogs than in the resistant animals (Billman et al., 1985). As a consequence, heart rate had to increase to a greater extent in the susceptible dogs than in the resistant animals in order to maintain cardiac output when the heart was stressed by either exercise (Fig. 5) or ischemia. These differing heart rate responses have potentially profound implications with regards to the autonomic regulation of the heart and subsequent susceptibility to VF as shall be discussed in Section 3.3.

The hemodynamic response to coronary occlusion was highly reproducible for a given animal. For example, the exercise plus ischemia test provoked very similar changes in the susceptible dogs during the first and the second occlusion (Fig. 7). As such, neither VF nor the electrical defibrillation procedures exacerbated the hemodynamic impairment in these animals, providing further evidence for the reproducibility of the model. In addition, there were no obvious gender differences. The hemodynamic responses to either exercise or the coronary occlusion were nearly identical in both male and female dogs (for either susceptible or resistant dogs) (data not shown). In contrast, myocardial infarction size probably contributes to some of the hemodynamic response differences noted between susceptible and resistant dogs. Animals with larger infarctions would be expected to have poorer ventricular function and a higher risk for VF. Myocardial infarction size was determined in 143 of the dogs. This analysis revealed that the susceptible dogs had larger infarctions (n = 93, 17.7 ± 0.9%) compared to the resistant dogs (n = 50, 12.6 ± 1.4%). The susceptible dogs also exhibited, diffuse mottled infarctions with frequent anterior papillary muscle involvement, while the resistant animals exhibited well localized mid wall myocardial infarctions. Once again there were no obvious gender

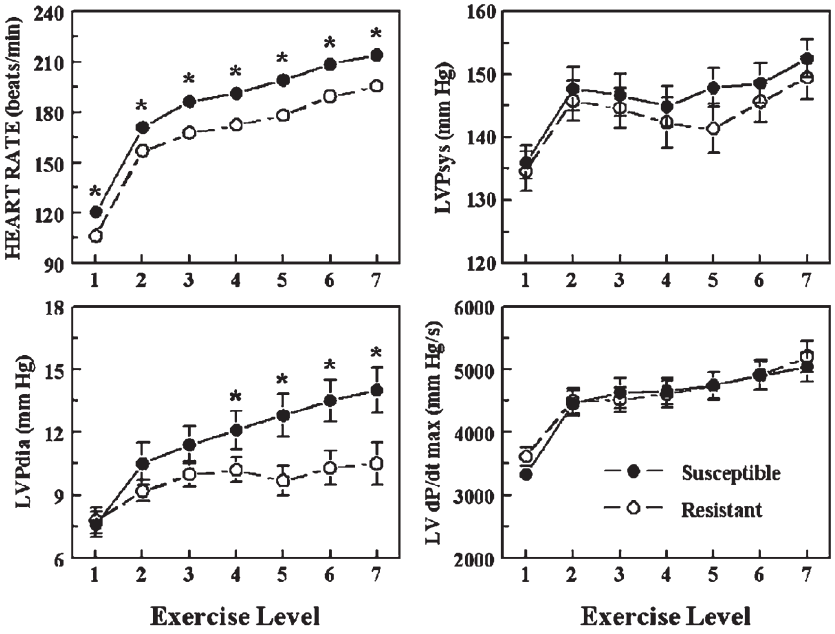

Fig. 4. The hemodynamic response to submaximal exercise in dogs susceptible ($n=59$, solid line/filled circles) and resistant ($n=55$, dashed line/open circles) to ventricular fibrillation. Note that exercise elicited larger increases in heart rate and left ventricular diastolic pressure in the susceptible compared to the resistant animals. Exercise levels: level 1=control 1 min before the onset of exercise; level 2=4.8 kph/0% grade; level 3=6.4 kph/0% grade; level 4=6.4 kph/4% grade; level 5=6.4 kph/8% grade; level 6=6.4 kph/12% grade; and level 7=6.4 kph/16% grade. *$P<.01$ susceptible versus resistant, LVP=left ventricular pressure.

differences (Fig. 8). Since the left anterior descending artery ligation was made at roughly the same location in the all the dogs, the larger myocardial infarction noted in those dogs susceptible to VF suggests that these animals may have relatively fewer coronary collateral vessels than in the dogs subsequently shown to be resistant to malignant arrhythmias.

3.2. Electrocardiographic responses

The susceptible and resistant dogs also exhibit a number of interesting response differences both at rest in response to acute myocardial ischemia. At rest, programmed electrical stimulation induced non-sustained ventricular tachycardia in 19 of 25 susceptible animals but failed to induce even single extrasystoles

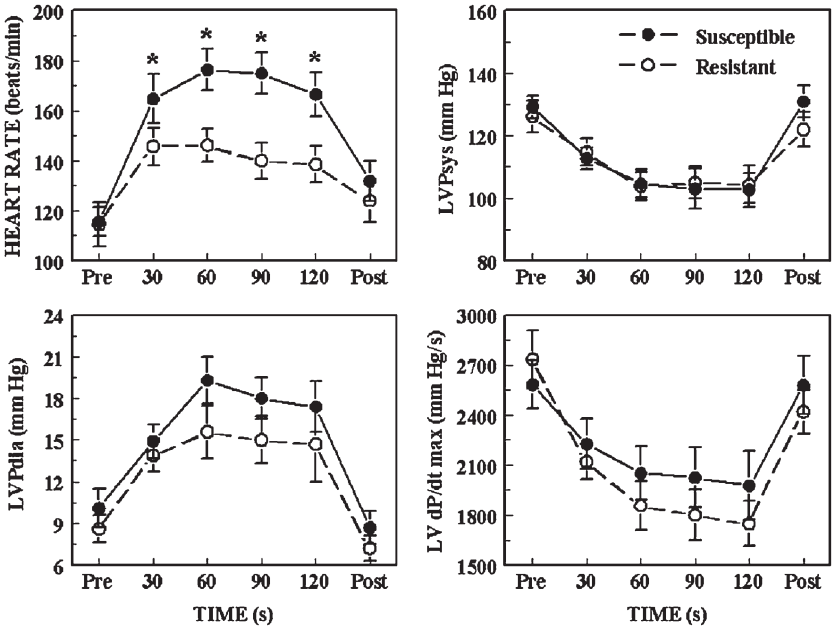

Fig. 5. The hemodynamic response to left circumflex coronary occlusion at rest submaximal exercise in dogs susceptible ($n=59$, solid line/filled circles) and resistant ($n=55$, dashed line/open circles) to ventricular fibrillation. Note that exercise elicited larger increases in heart rate in the susceptible compared to the resistant animals. *$P<.01$ susceptible versus resistant dogs, Pre=control 1 min before the occlusion, Post=3 min after the occlusion release. Time indicated is seconds.

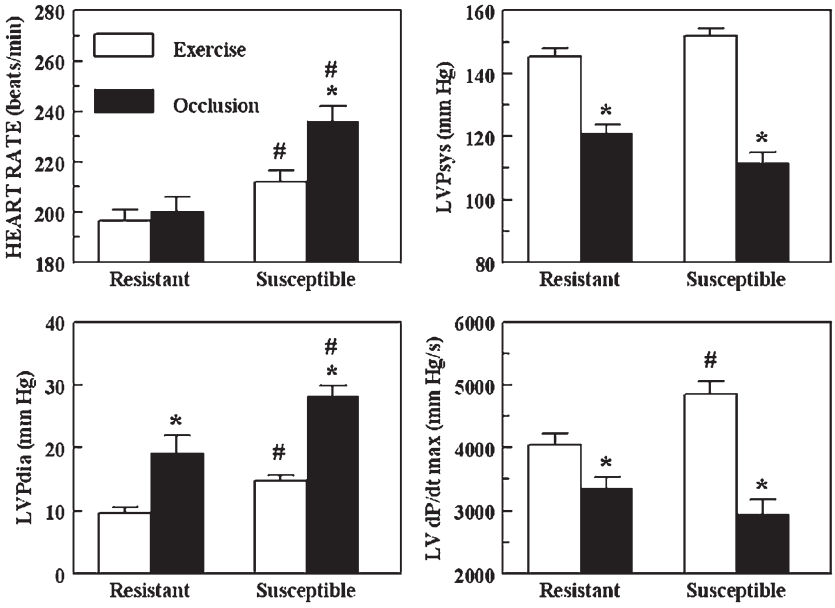

Fig. 6. The hemodynamic response to the exercise plus ischemia test. The coronary artery occlusion provoked significant reductions in left ventricular systolic pressure and left ventricular d*P*/d*t* maximum for the both the resistant ($n=55$) and susceptible ($n=59$) dogs. Note further that the coronary artery occlusion provoked larger increases in heart rate and left ventricular diastolic pressure in the susceptible animals. *$P<.01$ exercise versus occlusion (60 sec or last 5 sec before ventricular fibrillation) resistant dogs, $^{\#}P<.01$ susceptible versus resistant dogs, LVP=left ventricular pressure.

in any resistant animal ($n=23$) (Billman & Hamlin, 1996). As programmed electrical stimulation activates re-entrant pathways, these data suggest that the larger more diffuse myocardial infarction in the susceptible animals may provide anatomical subtract for re-entrant arrhythmias. The activation of these re-entrant pathways may become particularly important during myocardial ischemia and have important therapeutic implications that will be discussed in Section 4.2.

There were no obvious differences in ECG parameters at rest or in response to exercise between the susceptible and resistant dogs. However, differences between the groups became apparent when the heart was stressed by myocardial ischemia. The effects of the exercise plus ischemia test on ECG parameters are displayed in Fig. 9 (susceptible $n=45$, resistant $n=31$). The coronary occlusion did not alter QRS duration in either group and provoked similar reductions in P–R interval (probably due to the activation of sympathetic efferent nerves in the AV nodal region) in both the susceptible and resistant dogs. In contrast, the coronary occlusion provoked significantly greater increases in heart rate in the susceptible compared to the

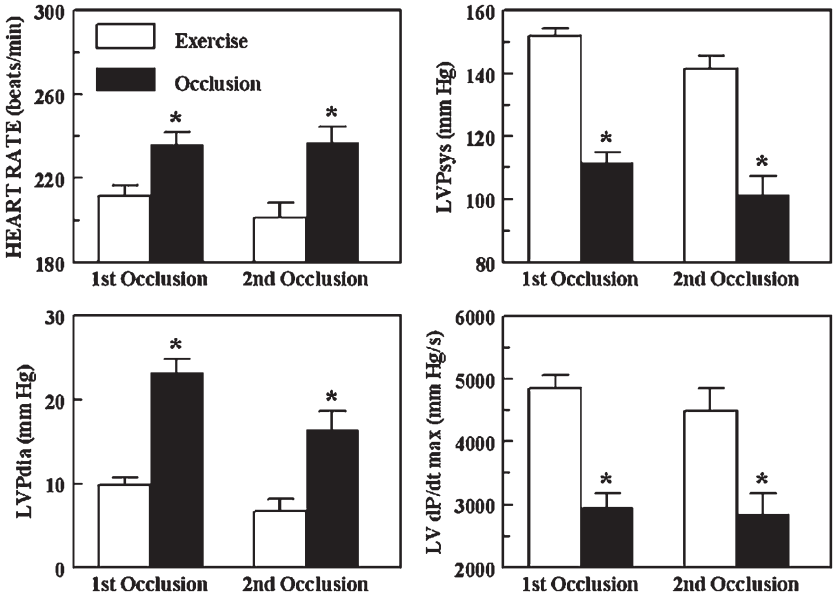

Fig. 7. A comparison of the hemodynamic response to the first and second exercise plus ischemia test in dogs susceptible ($n=21$) to ventricular fibrillation. Note that coronary artery occlusion elicited similar changes in both the first and second occlusions. *$P<.01$ occlusion versus exercise. LVP=left ventricular pressure.

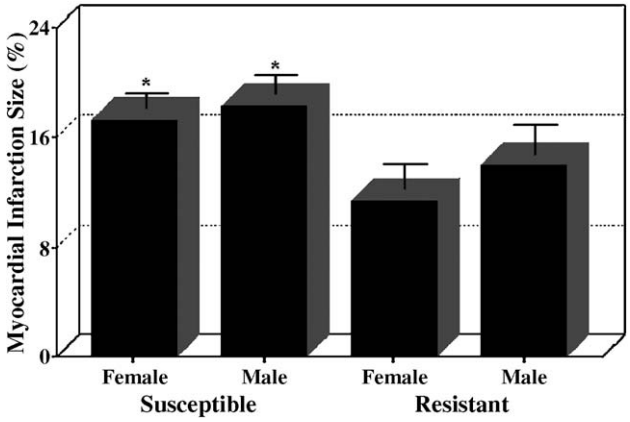

Fig. 8. The effect of gender on myocardial infarction in animals susceptible ($n=93$) or resistant ($n=50$) to ventricular fibrillation. Note that the myocardial infarction was larger in susceptible (male $n=34$, female $n=59$) animals of either gender as compared to resistant (male $n=30$, female $n=25$) dogs. *$P<.01$ susceptible versus resistant dogs.

resistant dogs. In addition, the occlusion provoked a significantly greater increase in the QT interval corrected for heart rate (QTc) in the susceptible animals. The T wave morphology also differed in the 2 groups of animals. The ischemia induced a notching or bifurcation in the T wave (i.e., a biphase T wave) in more susceptible dogs (22 of 45 animals, 48.9%) than in resistant animals (5 of 31 animals, 16.1%). The duration of the T wave also increased to a greater extent in the susceptible animals. In particular, the descending portion of the T wave (T peak–T end) increased to a much greater extent in the susceptible animals (pre-occlusion 29.1±1.6 msec, occlusion 44.2±2.0 msec) than in the resistant dogs (pre-occlusion 29.0±1.3, occlusion 30.6±1.3 msec). The descending portion of the T wave provides an indication of the transmural dispersion of repolarization (Yan & Antzelevitch, 1998). Furthermore, the dispersion (inhomogeneities) of repolarization can promote the formation of re-entrant circuits and is important factor in the induction of VF (Wit & Janse, 1993). In fact, a marked heterogeneity of left ventricular repolarization has been recorded in susceptible dogs while resistant animals displayed no such regional differences in repolarization (Swann et al., 2003). It would appear that the susceptible dogs are more sensitive to ischemia-induced changes in repolarization than are the resistant dogs perhaps as a consequence of there being relatively fewer coronary collateral vessels in the susceptible dogs (i.e., more tissue became ischemic in these animals during the coronary occlusion). Indeed, the coronary occlusion provoked a larger depression in the ST in the susceptible dogs (−7.2±11.0 mm) than in the resistant dogs (−3.5±0.8 mm). As previously noted, left anterior descending artery ligation produced larger myocardial infarctions in the susceptible dogs than in the resistant animals. These data further suggest that the susceptible dogs may have relatively fewer coronary collateral vessels than the resistant dogs.

3.3. Alterations in cardiac autonomic responses

As previously noted, alterations in the autonomic regulation of the heart can play an important role in the susceptibility to VF. In particularly, it is now widely accepted that elevations in cardiac sympathetic activity and/or reduction in cardiac parasympathetic activity greatly increase the risk for malignant arrhythmias (Schwartz & Zipes, 2000). During the past 25 years, my laboratory has completed a number of studies that demonstrate that the susceptible animals exhibit an abnormal autonomic regulation of the heart.

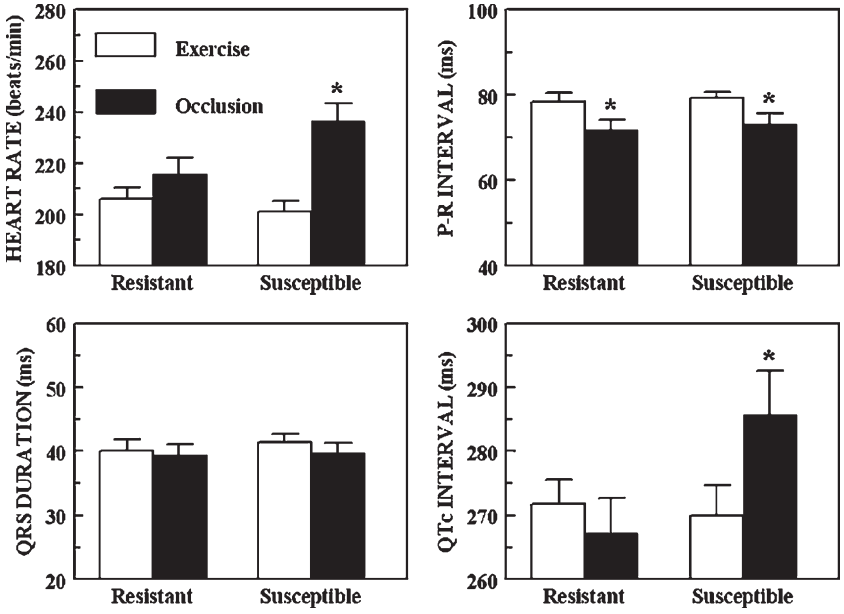

Fig. 9. The effects of the exercise plus ischemia test on electrocardiographic parameters in dogs susceptible ($n=45$) or resistant to ventricular fibrillation ($n=31$). The coronary occlusion provoked similar reductions in P–R interval in both groups of dogs. In contrast, the coronary occlusion provoked larger increases in both heart rate and Q–T interval corrected for heart rate (QTc). *$P<.01$ susceptible versus resistant dogs.

The initial studies (Billman et al., 1982) examined the heart rate response to pharmacologically induced increases in arterial pressure (baroreceptor reflex sensitivity). These studies provide an example as to how serendipity can play an important role in the advancement of science. As Louis Pasteur once said "Chance favors the prepared mind". LVP was measured in a number of the animals. This pressure was recorded from solid-state pressure transducers placed in the left ventricle via the left ventricular apex. These transducers must be calibrated on a regular basis (weekly) as the baseline can "drift" over time. In order to perform this calibration, a catheter was placed into the cephalic vein to administer the vasoactive drug phenylephrine while arterial pressure was recorded percutaneously from a femoral artery. The change in arterial pressure was then used to calibrate ventricular pressure (i.e., matching the peak pressure changes). Thus, by adding an ECG recording it became easy to evaluate the change in heart rate or R–R interval induced by increase in arterial pressure. Regression analysis was then performed to calculate the slope of the line obtained by plotting the change in pressure against changes in either heart rate or R–R interval. This slope value provides an index of the baroreceptor regulation of the heart (i.e., cardiac efferent nerve responses).

As it happened, an interesting report by Takeshita et al. (1980) was published about the time I began studies with the canine model of sudden death. These authors found that acute myocardial ischemia significantly reduced the baroreceptor mediated heart rate response to changing arterial pressure. Since I was routinely measuring the arterial pressure response to phenylephrine to calibrate the LVP transducer, I examined the these records in order to determine whether or not animals with healed myocardial infarction also displayed reduced baroreflex regulation. This was, in fact, the case; dogs with myocardial infarction exhibited a reduced baroreceptor response when compared to non-infarcted dogs that were used in other studies in the laboratory. An abstract of these findings was prepared and presented at the Federated American Societies of Experimental Biology (FASEB) meeting in 1981 (Billman et al., 1981).

It soon became apparent that phenylephrine did not elicit the same heart rate response in all the post-myocardial infarction animals, some animals exhibited robust reductions in heart rate while other dogs showed very modest reductions in heart rate. When I matched the baroreceptor response to the exercise plus ischemia test outcome, it became immediately obvious that all the dogs with a low baroreceptor sensitivity (i.e., small heart rate decrease to an increase in arterial pressure) had subsequently developed VF while almost all the dogs with a robust response proved to resistant to malignant arrhythmias. This retrospective analysis was very encouraging. However, before breaking the news to my post-doctorial advisor (H. Lowell Stone), I decided that I should first try to predict outcome based upon the baroreceptor sensitivity results. I calculated the baroreceptor sensitivity on the next set of animals in advance of the exercise plus ischemia test and entered the predicted arrhythmia outcome based upon these results in my laboratory notebook. I then anxiously waited for the day of testing, would these predictions prove to be correct? The first dog was tested and matched the prediction, and then the second once again matched the predicted results, and then the third, and so on. At the end of the day we had tested 6 dogs and each had matched the predicted outcome. I now felt confident enough to present my hypothesis and the initial analysis of the results to Dr. Stone. These results were so exciting that we decided to publish even before the description of the model. The rest, as they say, is history. The manuscript was accepted (Billman et al., 1982) and a much more detailed analysis with a larger set of animals was also subsequently published (Schwartz et al., 1988). Both the heart rate and R–R interval response to increases in arterial pressure were significantly reduced in susceptible ($n=40$) as compared to resistant animals ($n=27$) (Fig. 10). For example, the heart rate reduction induced by a 30 mm Hg increase in arterial pressure was -40 ± 12.2 beats per min in resistant animals and -2.9 ± 5 beats per min in susceptible animals. Similar findings have been reported in a number of recent clinical studies (La Rovere et al., 1988; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). To cite just one example, La Rovere et al. (1998) reporting for the ATRAMI group found that post-myocardial infarction patients with either low heart rate variability or a small heart rate response to an increase in blood pressure had a much greater risk of sudden death than those with well preserved cardiac vagal tone. The greatest risk for mortality was observed in patients with a large reduction in both markers of cardiac vagal tone.

The determination of baroreflex sensitivity requires the administration of potent vasoactive drugs as well as the measurement of arterial pressure and the ECG. As such, this procedure is invasive and may not be appropriate for all patients. As early as the mid 1970s, a number of studies

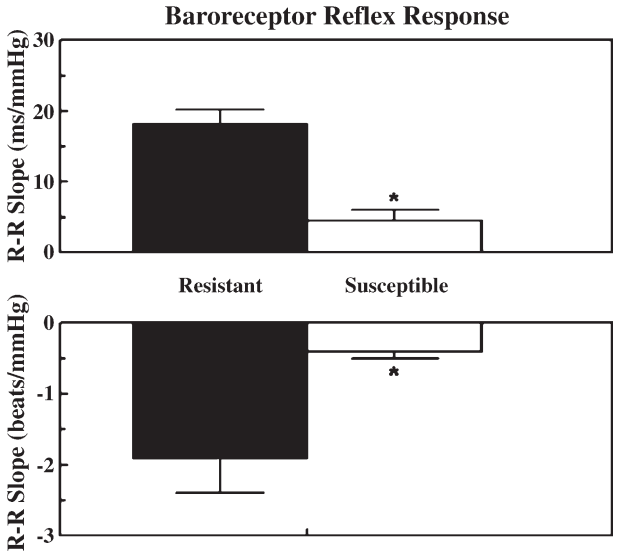

Fig. 10. A comparison of baroreceptor reflex sensitivity in dogs susceptible ($n=40$) or resistant ($n=27$) to ventricular fibrillation. The data are plotted either as changes in R–R interval versus systolic arterial pressure (top panel) or changes in heart rate versus systolic arterial pressure (bottom panel). *$P<.01$ susceptible versus resistant dogs.

suggested that the analysis of the periodic oscillations in the heart rate, the so-called respiratory sinus arrhythmia could provide information about the autonomic regulation of the heart (Chess et al., 1975; Akselrod et al., 1981; Pomerantz et al., 1985; Billman & Dujardin, 1990). Time series analysis of these periodic fluctuations in R–R interval (0.24–1.04 Hz) has been shown to provide a non-invasive marker of cardiac vagal activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). I have used this technique to evaluate the heart rate response to exercise (Billman & Hoskins, 1989; Halliwill et al., 1998; Houle & Billman, 1999; Smith et al., 2005; Billman & Kukielka, in press). The heart rate and the heart variability response to submaximal exercise for susceptible (n=69) and resistant (n=42) animals are shown in Fig. 11. Exercise induced larger increases in heart rate in the susceptible animals as compared to the resistant animals. This greater heart rate increase was accompanied by correspondingly greater reductions in several indices of the cardiac vagal activity in the susceptible animals. Conversely, atropine given during exercise elicited a much greater heart rate increase in the resistant dogs (heart rate change resistant 45.2±7 vs. susceptible 18.7±4.4 beats per min). β-Adrenoceptor blockade attenuated the heart rate increase induced by exercise but exacerbated the reductions in the cardiac vagal tone index. Lower values of cardiac vagal activity were still noted even after β-adrenoceptor blockade in the susceptible dogs (n=41) compared the resistant dogs (n=26) (Fig. 12). Once again gender did not appear to affect the response to submaximal exercise. Exercise provoked similar changes in both male and female dogs for either the susceptible of resistant animals (Fig. 13).

The time series analysis was also performed to evaluate the cardiac response to acute myocardial ischemia at rest (Collins & Billman, 1989; Halliwill et al., 1998; Houle & Billman, 1999; Billman & Kukielka, in press). The heart rate and heart rate variability responses to a 2-min occlusion of the left circumflex coronary artery for susceptible (n=83) and resistant (n=48) animals are shown in Fig. 14. The coronary artery occlusion elicited significantly greater increases in heart rate in the susceptible dogs (control 123.7±2.3, occlusion 174.8±3.5 beats/min) compared to resistant animals (control 118.4±3.2, occlusion 142.5±3.5 beats per min). Correspondingly, the indices of cardiac vagal activity were reduced to a much greater extent in the susceptible animals. β-Adrenoceptor blockade reduced but did not eliminate the heart rate differences noted between the groups. In fact, this intervention provoked even greater reductions in the cardiac vagal activity index (Fig. 15). Once again gender did not appear to affect the response to the coronary occlusion. The coronary occlusion provoked similar changes in both male and female dogs for either the susceptible of resistant animals (Fig. 16).

Recently, we have also performed a retrospective of analysis of heart rate recovery following the termination of exercise (Smith et al., 2005). Time series analysis of heart rate variability was measured 30, 60, and 120 sec after submaximal exercise (treadmill running) for susceptible (n=66) and resistant (n=39) dogs. Heart rate recovery was significantly greater in resistant dogs than in susceptible dogs at all 3 times, with the most dramatic difference at the 30-sec mark (resistant, 48.1±3.6; susceptible, 31.0±2.2 beats per min, change from the last minute of exercise). Correspondingly, indices of parasympathetic regulation increased to a

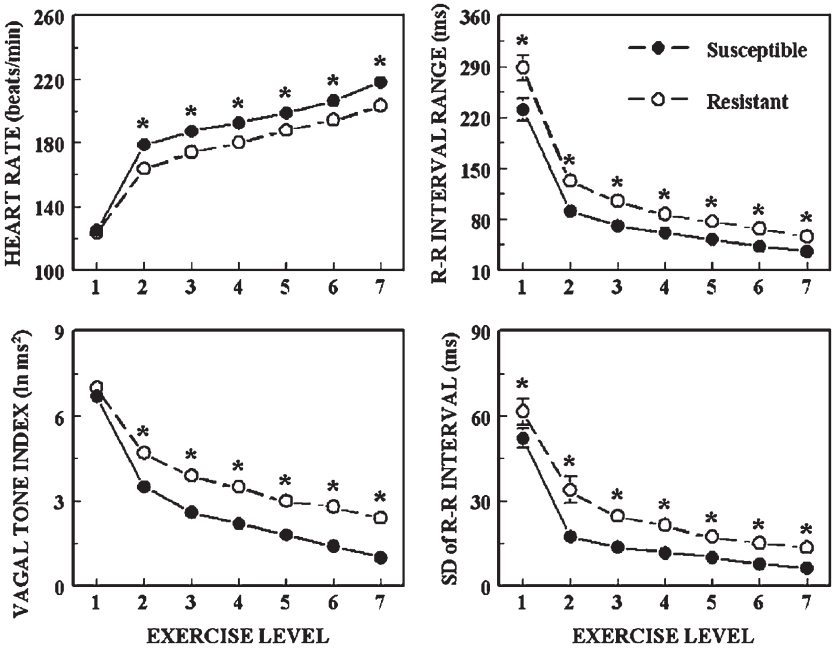

Fig. 11. The heart rate and heart variability response to submaximal exercise in susceptible (n=69, solid line/filled circles) and resistant (n=42, dashed line/open circles) animals. Note the higher heart rate and greater reductions in all 3 indices of cardiac parasympathetic activity in response to the exercise for the susceptible animals. *P<.01, Exercise levels: level 1=control 1 min before the onset of exercise; level 2=4.8 kph/0% grade; level 3=6.4 kph/0% grade; level 4=6.4 kph/4% grade; level 5=6.4 kph/8% grade; level 6=6.4 kph/12% grade; and level 7=6.4 kph/16% grade.

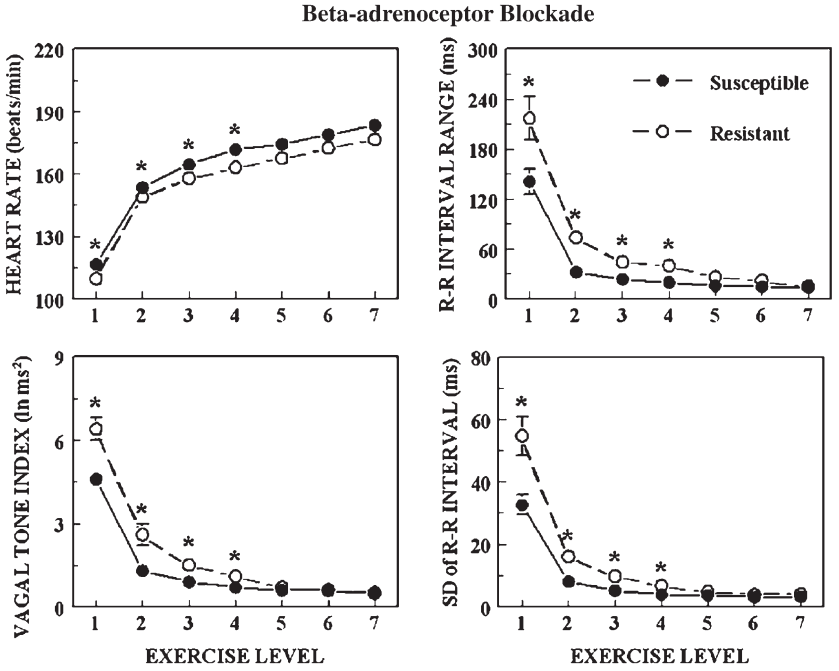

Fig. 12. The effect of the beta-adrenoceptor antagonist (propranolol HCl 1.0 mg/kg, i.v.) on the heart rate and heart variability response to submaximal exercise in susceptible (n=41, solid line/filled circles) and resistant (n=26, dashed line/open circles) animals. Note the higher heart rate and greater reductions in all 3 indices of cardiac parasympathetic activity in response to the exercise (lower levels of exercise) for the susceptible animals. *P<.01, Exercise levels: level 1=control 1 min before the onset of exercise; level 2=4.8 kph/0% grade; level 3=6.4 kph/0% grade; level 4=6.4 kph/4% grade; level 5=6.4 kph/8% grade; level 6=6.4 kph/12% grade; and level 7=6.4 kph/16% grade.

significantly greater extent in resistant dogs at 30 and 60 sec post-exercise. These differences were eliminated by atropine pre-treatment. Thus, the resistant dogs exhibited a more rapid recovery of vagal activity after exercise than those animals that were subsequently shown to be susceptible to VF. Similar result have obtained in patients, once again patients with poor heart rate recovery following exercise had the greatest cardiovascular mortality (Cole et al., 1999; Jouven et al., 2005).

Heart rate variability can also be evaluated using wavelet transform analysis to partition the heart rate fluctuations into the various frequency components of the signal (Addison, 2005).

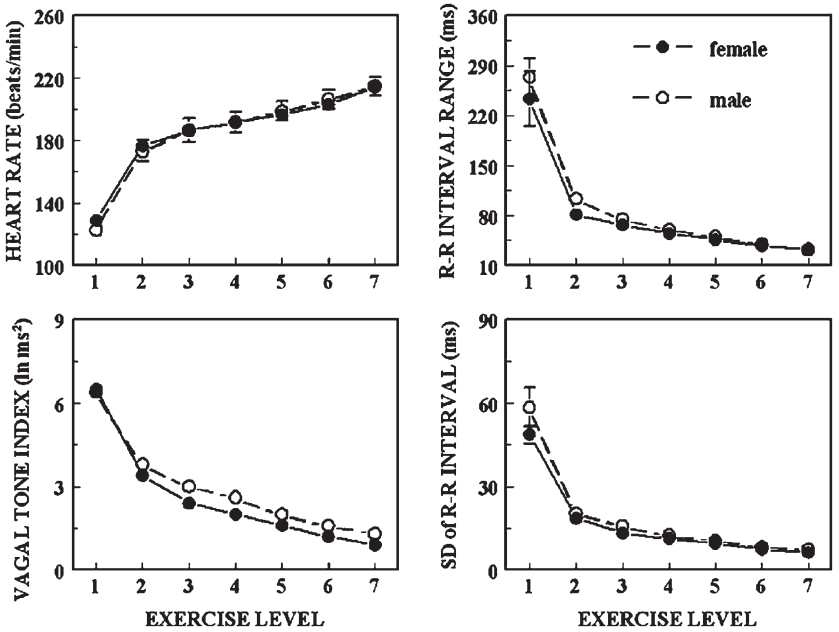

Fig. 13. The effect of gender on the heart rate and heart variability response to submaximal exercise in susceptible animals. There were no differences between male (n=23) or female (n=46) dogs. Exercise levels: level 1=control 1 minute before the onset of exercise; level 2=4.8 kph/0% grade; level 3=6.4 kph/0% grade; level 4=6.4 kph/4% grade; level 5=6.4 kph/8% grade; level 6=6.4 kph/12% grade; and level 7=6.4 kph/16% grade.

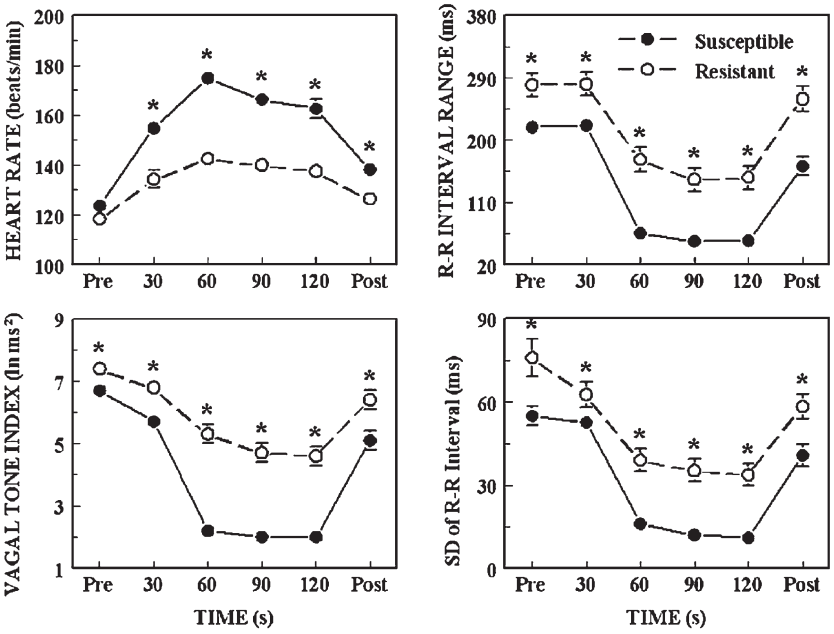

Fig. 14. The heart rate and heart variability response to left circumflex coronary artery occlusion at rest in susceptible ($n=83$, solid line/filled circles) and resistant ($n=48$, dashed line/open circles) animals. Note the higher heart rate and greater reductions in all 3 indices of cardiac parasympathetic activity in response to myocardial ischemia in the susceptible animals. *$P<0.01$, Pre=control 1 min before the occlusion, Post=3 min after the occlusion release. Time indicated is seconds.

The resulting 3-D plots graphical illustrate the complexity of the heart rate variability in the susceptible and resistant animals. Representative examples of composite data obtained from dogs either resistant ($n=6$) or susceptible ($n=6$) are show in Fig. 17. The resistant dogs exhibited considerably more variability than the susceptible dogs both before and during the coronary occlusion. The data for the resistant dogs more closely resembled the Rocky Mountains of Colorado while the data for susceptible animals resembled the wheat fields of Kansas. The complexity of the signal noted for the resistant dogs is indicative of a robust cardiac electrical system that can rapidly adapt to changing environmental demands

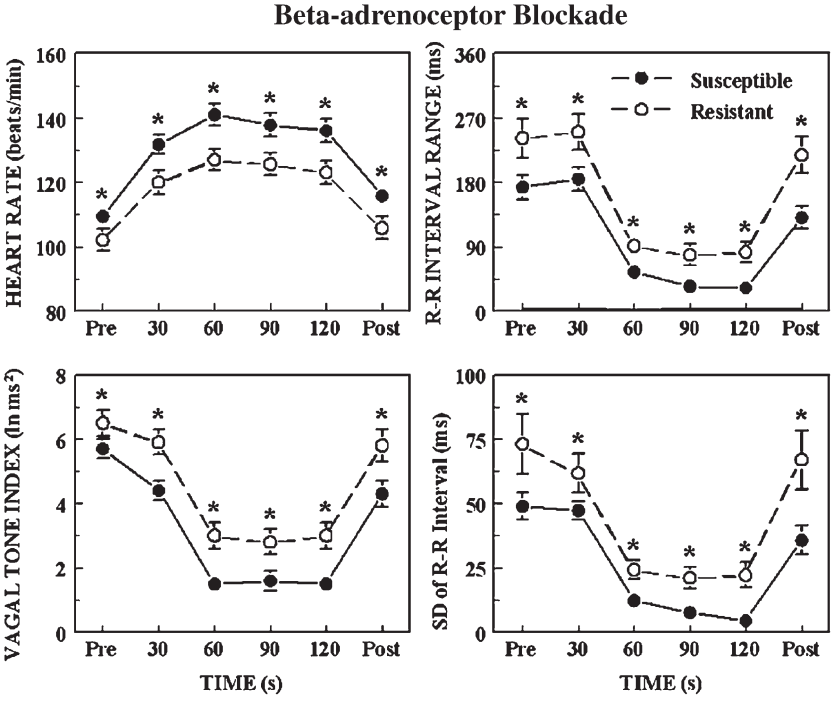

Fig. 15. The effect of the beta-adrenoceptor antagonist (propranolol HCl, 1.0 mg/kg, i.v.) on the heart rate and heart variability response to left circumflex coronary artery occlusion at rest in susceptible ($n=44$, solid line/filled circles) and resistant ($n=32$, dashed line/open circles) animals. Note the higher heart rate and greater reductions in all 3 indices of cardiac parasympathetic activity in response to myocardial ischemia in the susceptible animals. *$P<.01$, Pre=control 1 min before the occlusion, Post=3 min after the occlusion release. Time indicated is seconds.

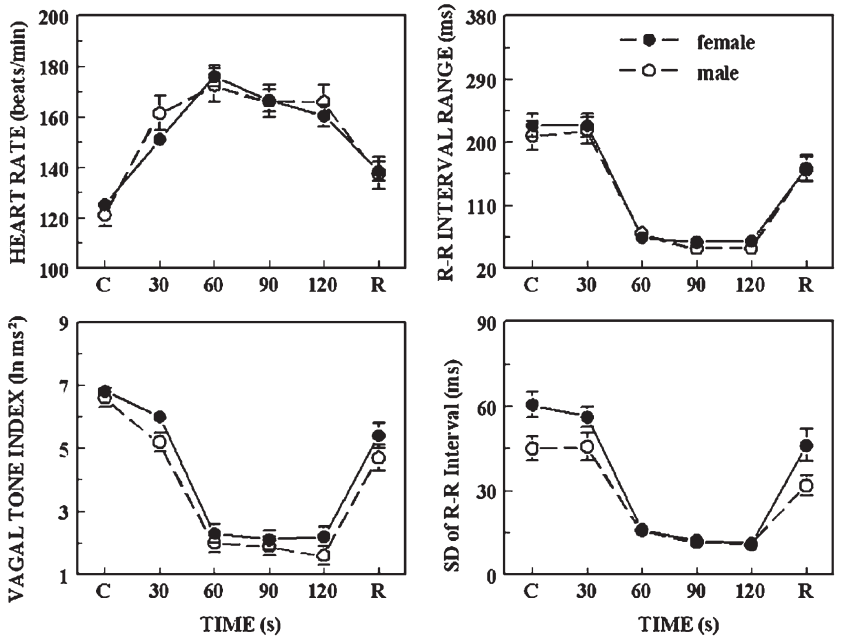

Fig. 16. The effect of gender on the heart rate and heart variability response to left circumflex coronary artery occlusion at rest in susceptible (male $n=29$, female $n=54$) animals. There were no effects of gender on the response to the coronary artery occlusion. Pre=control 1 min before the occlusion, Post=3 min after the occlusion release. Time indicated is seconds.

(i.e., negative feedback regulation buffers against changes). In contrast, the monotonic heart rate response noted for the susceptible dogs may indicate the potential electrical instability of the hearts of these animals. These animals are balancing on the edge of a precipice, a slight nudge and the heart slides into the chaos of VF.

When all the studies described in this section are considered together, then these data clearly demonstrate that animals

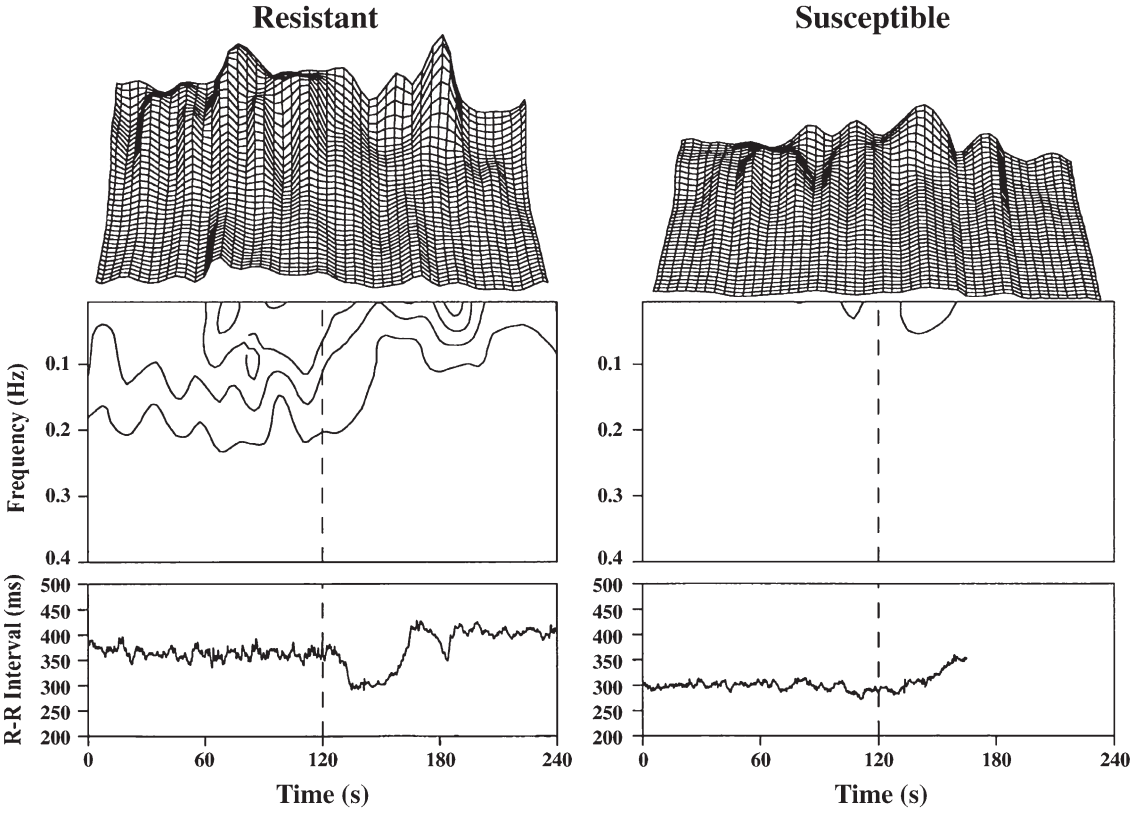

Fig. 17. Wavelet transform analysis of the heart rate variability during the exercise plus ischemia test in animals susceptible ($n=6$) or resistant ($n=6$) to ventricular fibrillation. Note the greater variability in the resistant animals.

susceptible to malignant arrhythmias exhibit alterations in the autonomic control of the heart: namely, an elevated sympathetic activity accompanied by reductions in parasympathetic activity.

4. Effect of pharmacological interventions

This canine model has been used to evaluate the therapeutic potential of a number of test compounds, both investigational drugs and drugs routinely used in the clinic. The following sections will describe some of the results obtained with model. At this point it is important to emphasize once again a major advantage of the model currently under discussion. In this model each animal acts as its own control, thereby reducing the number of animals needed to complete a study.

4.1. Autonomic interventions

The data described above suggest that alterations in the autonomic control of the heart contribute significantly to the development of arrhythmias. If this hypothesis were to be correct, then one would predict that interventions designed either to reduce sympathetic activity or to enhance parasympathetic activity would protect against VF. Conversely, an increase in sympathetic or a reduction in parasympathetic activity should provoke arrhythmias in resistant animals. Over the years, my laboratory has completed a number of studies that largely confirms these predictions.

4.1.1. Parasympathetic interventions

Cardiac vagal activity was enhanced by the cholinergic agonist carbachol (20 μg/kg, i.v.; Billman, 1990). This intervention prevented VF in 11 of 14 susceptible animals. This drug also reduced heart rate. Therefore, studies were repeated with heart rate held constant by means of ventricular pacing. Carbachol prevented VF in 5 of 6 susceptible animals even with heart rate held constant. These data suggest that heart rate alone cannot account for this drug's protection. In a similar manner, the effects of the cholinergic intracellular 2nd messenger cyclic GMP on susceptibility to VF were examined using the long acting cyclic GMP analogs, 8-bromo cyclic GMP ($n=9$, infused at the rate of 100–150 μg/kg/min, i.v. throughout the exercise test) or dibutyry cyclic GMP ($n=5$, infused at the rate 100–150 μg/kg/min. i.v. throughout the exercise test). Representative recordings from the same susceptible animal before and after 8-bromo cyclic GMP treatment are shown in Fig. 18. Increasing cyclic GMP levels attenuated the heart rate response to the coronary artery occlusion and protected 13 of the 14 susceptible animals from malignant arrhythmias (Billman, 1990). In a similar manner, electrical stimulation of the vagus nerves also prevented VF, protecting 27 of 30 the susceptible dogs tested (Vanoli et al., 1991). When heart rate was controlled by atrial pacing, vagal stimulation only protected 5 of 9 susceptible animals.

In contrast, the cholinergic antagonist atropine sulfate (50 μg/kg, i.v.) was given 3 min before the coronary occlusion (i.e., while the animals were running) to 21 resistant animals; 3

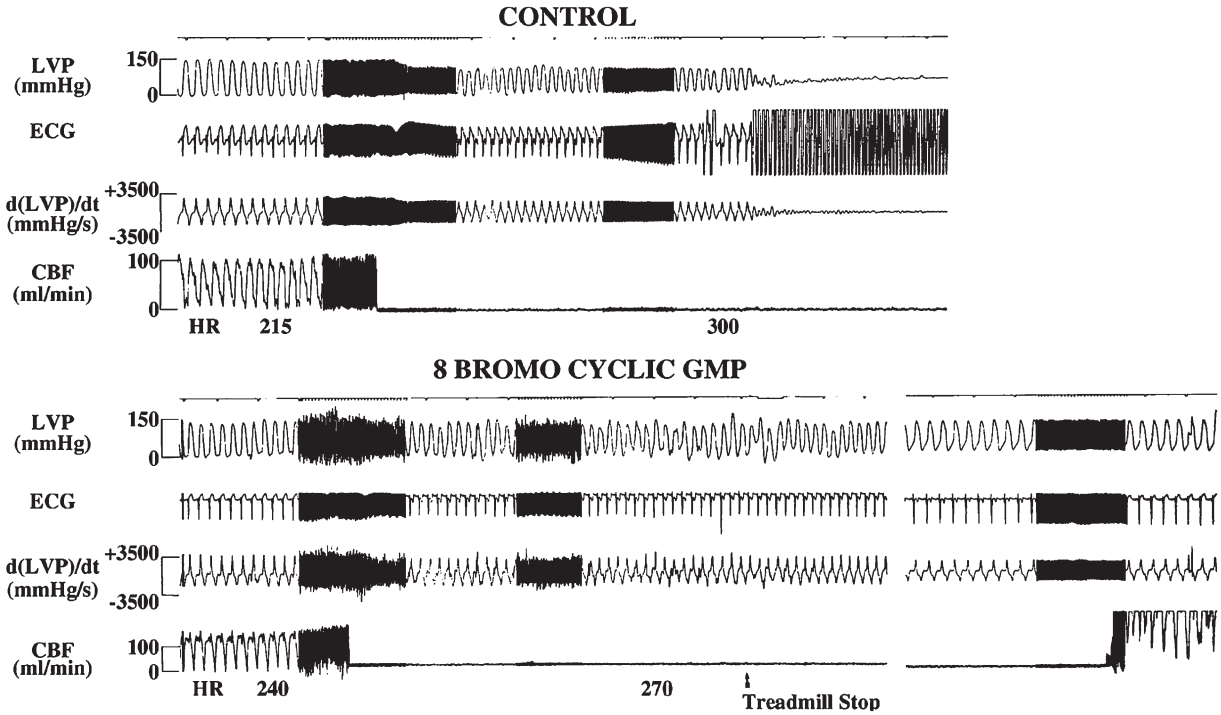

Fig. 18. Representative recordings obtained from the same susceptible animal before and after treatment with 8-bromo cyclic GMP (long acting analog of cyclic GMP, parasympathetic intracellular 2nd messenger). Note the absence of ventricular arrhythmias during the 8-bromo cyclic GMP treatment. 8-Bromo cyclic GMP prevented ventricular fibrillation in 8 of 9 susceptible animals, dibutyryl cyclic GMP protected 5 of 5 susceptible animals. Thus, cyclic GMP protected 13 of 14 animals tested. LVP = left ventricular pressure, CBF = coronary blood flow, HR = heart rate (beats per min). (Reprinted with permission from Billman, 2005.)

animals developed VF while 6 additional dogs showed an increase in arrhythmia formation (unpublished observation). DeFerrari et al. (1991) reported similar findings using the same canine model of sudden death. They found that exercise plus ischemia test only induced VF in 24% (11 of 45) of the resistant dogs. Therefore, a reduction in parasympathetic activity is probably not solely responsible for VF.

These data demonstrate that subnormal cardiac parasympathetic regulation increases the risk for malignant arrhythmias while interventions that enhance cardiac vagal function can protect against VF. However, it is important to emphasize that the improvement of autonomic balance during baseline conditions (i.e., at rest) might not provide sufficient protection against arrhythmias when the heart is stressed during dynamic situations. Indeed, the observation that low doses of cholinergic antagonists paradoxically increased the level of cardiac vagal activity (Kottmeier & Gravenstein, 1968) led to the proposal that this treatment could provide an acceptable means of enhancing cardiac parasympathetic activity in patients (Casadei et al., 1993; Vybiral et al., 1993). However, we (Billman et al., 1994b; Halliwill et al., 1998) and others (Hull et al., 1995) demonstrated that although low doses of cholinergic antagonists (atropine sulfate 2 μg/ kg, i.v. or scopolamine 3 μg/ kg, i.v.) increased baseline cardiac vagal activity (R–R interval variability), this treatment failed to prevent VF induced by myocardial ischemia. We (Billman et al., 1994b; Halliwill et al., 1998) further demonstrated that the enhanced baseline vagal activity was not maintained when the heart was stressed by either exercise or myocardial ischemia. As such, it was not surprising that this therapy failed to prevent VF. It would appear that in order for an effective antiarrhythmic therapy, an intervention must not only increase baseline vagal activity but, more importantly, must also maintain this enhanced activity when the heart is stressed.

4.1.2. Sympathetic interventions

Several anti-adrenergic interventions have been shown to protect against VF induced in this model. Removal of the left stellate ganglion has proven to be one of the most effective of these interventions (Schwartz et al., 1984). Representative recordings from the same susceptible animal before and after removal of the left stellate ganglion are displayed in Fig. 19. This intervention prevented VF in all 11 susceptible animals tested. The cardiac sympathetic efferent nerves primarily release norepinephrine as the neurotransmitter that then binds to post-synaptic receptors (both to β- and α-adrenoceptors) to activate the myocardium.

The α_1-adrenoceptors antagonist, prazosin HCl (0.5 mg/kg, i.v.) elicited significant reductions in left ventricular systolic pressure (control 157.0±6.5 vs. prazosin 118.5±2 mm Hg) and prevented VF 13 of 14 susceptible dogs (Billman, 1994c). However, this drug also had profound sedative (central nervous system) effects that could have contributed to the protection noted for this drug. The drug also must be given slowly in order to avoid a profound reflex tachycardia due to reductions in arterial pressure. WB4101 a drug that is selective for the α_{1a}-adrenoceptors did not alter any hemodynamic parameter nor did it have any central nervous system (sedative effects) yet prevented malignant arrhythmias in 7 of 9 susceptible dogs. In contrast to these findings, Vanoli et al. (1994) reported that a lower dose of prazosin (0.1 mg/kg, i.v.) and the selective α_{1a}-adrenoceptor antagonist, abanoquil (1.0 μg/kg, i.v.) failed to

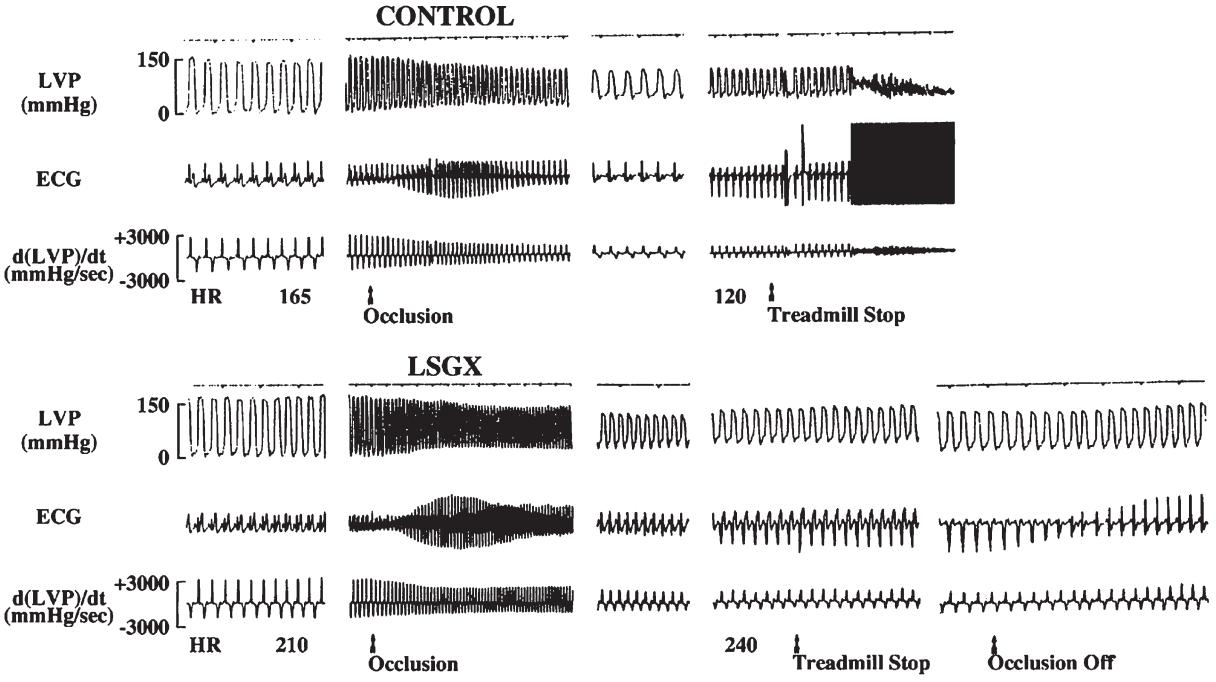

Fig. 19. Representative recordings obtained from the same susceptible animal before and after removal of the left stellate ganglion (LSGX). Note that despite the large ischemic ECG changes, the removal of the left stellate ganglion prevented ventricular fibrillation during the second exercise plus ischemia test. LSGX protected 11 of 11 susceptible animals tested. LVP=left ventricular pressure, HR=heart rate (beats per min). (Reprinted with permission from Billman, 2005.)

prevent VF in any susceptible animals tested with these compounds. Both drugs increased heart rate and exacerbated the response to the coronary artery occlusion, an effect that would compete with any anti-arrhythmic effects of these agents. Given these conflicting findings, it is difficult to ascertain the role that activation of myocardial α-adrenoceptors play in ischemically induced ventricular arrhythmias.

In contrast to α-adrenoceptor antagonists, studies using β-adrenoceptor antagonists have yielded more consistent results. β-adrenoceptor blockade with propranolol HCl (1 mg/kg, i.v.) has proven to be less effective than destruction of the cardiac sympathetic nerves, protecting only 11 of 18 animals tested (unpublished observation). It should be noted that 3 of the animals went into acute ventricular pump failure (i.e., ventricular systolic pressure fell to low levels while diastolic pressure increased). As propranolol also decreased heart rate, it is not possible to differentiate between the cardioprotection that results from direct effects on the ischemic myocardium from indirect effects due to reductions in heart rate. Similar results have been reported using the same canine model of sudden death. Adamson et al. (1998) and Vanoli et al. (1995) reported that propranolol prevented VF in 8 of 11 and 5 of 9 susceptible dogs, respectively. These studies did not control for the β-adrenoceptor blockade mediated reductions in heart rate. If the data from these 3 studies are combined, then the non-selective β-adrenoceptor antagonist propranolol protected 63.2% (24 of 38) of the susceptible dogs from malignant arrhythmias.

The mammalian myocardium contains both β_1- and β_2-adrenoceptors (Altschuld & Billman, 2000). In the normal heart, the β_1-adrenoceptor is the dominant receptor subtype and mediates the inotropic response to the activation of sympathetic nerves. Under certain pathological conditions, however, the activation of β_2-adrenergic receptors may become particularly important (Bristow et al., 1986; Altschuld & Billman, 2000). During heart failure, β_1-adrenoceptor sensitivity decreases substantially, whereas β_2-adrenoceptor density remains relatively constant (Bristow et al., 1986; Altschuld & Billman, 2000). As a consequence, the failing heart becomes more dependent upon the activation of β_2-adrenoceptors for inotropic support. The activation of these receptors may help maintain cardiac function in diseased hearts but not without potentially adverse consequences. β_2-adrenoceptor activation promotes an increase in the calcium current without altering calcium reuptake by the sarcoplasmic reticulum (Altschuld et al., 1995). The resulting elevation in intracellular calcium could provoke oscillations in membrane potential that, in turn, could trigger arrhythmias (Billman, 1991). Thus, in the diseased heart, β_2-adrenoceptor activation would tend to reduce the cardiac electrical stability and increase the propensity for the formation of malignant arrhythmias.

Recently, we (Billman et al., 1997b, Houle et al., 2001, Billman et al., in press) demonstrated in dogs with healed myocardial infarctions that the non-selective β-adrenoceptor agonist isoproterenol provoked significantly larger increases in both heart rate and in the velocity of circumferential fiber shortening (Vcf, an index of contractility) in those animals that were susceptible to VF induced by myocardial ischemia as compared to those animals that were resistant to these malignant arrhythmias (Fig. 20). The selective β_2-adrenoceptor antagonist, ICI 118,551, reduced the isoproterenol response to a much greater extent in the susceptible animals eliminating any differences noted between the groups (Billman et al., 1997b, Houle et al., 2001, Billman et al., in press). In a similar manner, both the calcium transient amplitude and the single-cell isotonic shortening responses to isoproterenol were larger in myocytes obtained from the hearts of susceptible compared to resistant dogs, differences that were also eliminated by β_2-adrenoceptor blockade but not by β_1-adrenoceptor blockade (Billman et al., 1997b, Houle et al., 2001). In the intact dog, β_2-adrenoceptor blockade almost completely suppressed VF induced by acute myocardial ischemia, protecting 10 of 11 susceptible animals (Billman et al., 1997b). Furthermore, isoproterenol induced after-transients in ventricular myocytes obtained from susceptible dogs and these cellular surrogates of arrhythmias were suppressed by β_2-adrenoceptor blockade but not by β_1-adrenoceptor blockade (Fig. 21). The β_2-adrenoceptor mediated increases in intracellular calcium did not result from global increase in cAMP (Altschuld et al., 1995) and interventions that increased cAMP levels in intact dogs failed to induce arrhythmias in these animals (Avendano & Billman, 1994). When considered together, these data demonstrate that an enhanced β_2-adrenoceptor responsiveness is associated with an increased propensity for VF most likely as the result of abnormal intracellular calcium regulation.

Conversely, one would predict that interventions that enhance cardiac sympathetic regulation should also increase the risk for malignant arrhythmias. I tested this hypothesis using low dose of the sympathomimic drug, cocaine (1 mg/kg, i.v. given 3 min before the coronary occlusion). Representative recording from 1 resistant animal before and after pre-treatment are displayed in Fig. 22. After cocaine pretreatment, the exercise plus ischemia test induced VF in 76.4% (42 of 55) of the

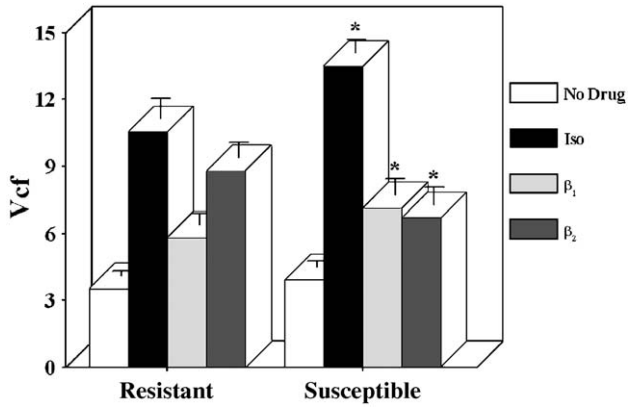

Fig. 20. Effects of selective β-adrenoceptor antagonists on the maximum isoproterenol induced Vcf responses in dogs susceptible ($n=12$) or resistant ($n=15$) to ventricular fibrillation. Isoproterenol elicited a greater increase in the susceptible compared to the resistant dogs. The β_2-adrenoceptor antagonist ICI 118,551 (0.2 mg/kg, i.v.) elicited a significantly greater reduction in the isoproterenol response in the susceptible compared to the resistant animals. β_1-adrenoceptor antagonist=bisoprolol (0.6 mg/kg, i.v.), isoproterenol=0.5 μg/kg/min. *$P<.01$ susceptible versus the corresponding drug treatment for the resistant dogs.

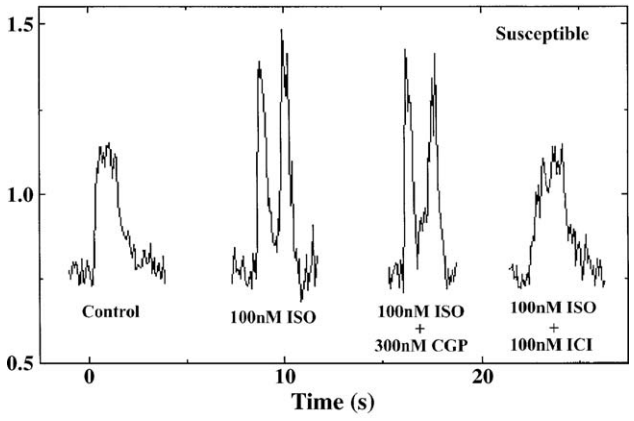

Fig. 21. Representative recordings of calcium transients recorded in ventricular myocytes obtained from 1 susceptible dog in which β-adrenoceptor agonist isoproterenol (ISO, 100 nmol/L) induced aftertransients. Treatment with the β_2-adrenoceptor antagonist ICI 118,551 (ICI, 100 nmol/L) but not β_1-adrenoceptor antagonist CGP-20712A (CGP, 300 nmol/L). Completely suppressed these after transients. (Reprinted with permission from Billman et al., 1997b.)

resistant dogs, even in animals without the presence of a pre-existing myocardial infarction (Billman & Hoskins, 1988; Billman, 1989a, 1993a, 1993b, 1994a). In a similar manner, infusion of the α-adrenoceptor agonist, phenylephrine HCl (10 μg/kg/min, 2 min before the occlusion) induced malignant arrhythmias in 10 of 17 susceptible dogs. Atropine sulfate (50 μg/kg, i.v.) was injected prior to the infusion to prevent reflex decreases in heart rate; atropine alone did not induce VF in these animals (Billman, 1989b). Both the cocaine and the phenylephrine-induced arrhythmias could be reduced by the prior treatment with the intracellular calcium chelator, BAPTA-AM (Billman, 1989b, 1993b).

4.2. Interventions that alter intracellular calcium

The studies mentioned above clearly indicate that the autonomic nervous system plays an important role in the genesis of life-threatening arrhythmias. The cellular mediators of this autonomic imbalance probably contribute significantly to VF. Over the last several years a number of studies have focused on the role that changes in intracellular calcium play in the induction of VF during myocardial ischemia.

The role that changes in intracellular calcium play in the induction of VF was evaluated using the intracellular calcium chelator BAPTA (delivered as BAPTA-AM, 1.0 mg/kg, i.v.). This drug readily enters the cell where esterases rapidly remove the AM (acetoxymethyl ester) group, thereby trapping the calcium chelator within the cell. As a result, one can selectively buffer against changes intracellular calcium. As would be expected, this drug provoked large reductions in left ventricular d*P*/d*t* (Billman et al., 1991). More importantly, intracellular calcium chelation also prevented VF induced by either myocardial ischemia (protecting 8 of 12 susceptible animals) or by calcium overload induced by the calcium channel agonist BAY K 8644 (30 μg/kg, i.v., preventing VF in 5 of 5 resistant animals) (Billman et al., 1991). Intracellular calcium could increase as the result of either change in calcium entry through voltage gated channels or from enhanced calcium release from

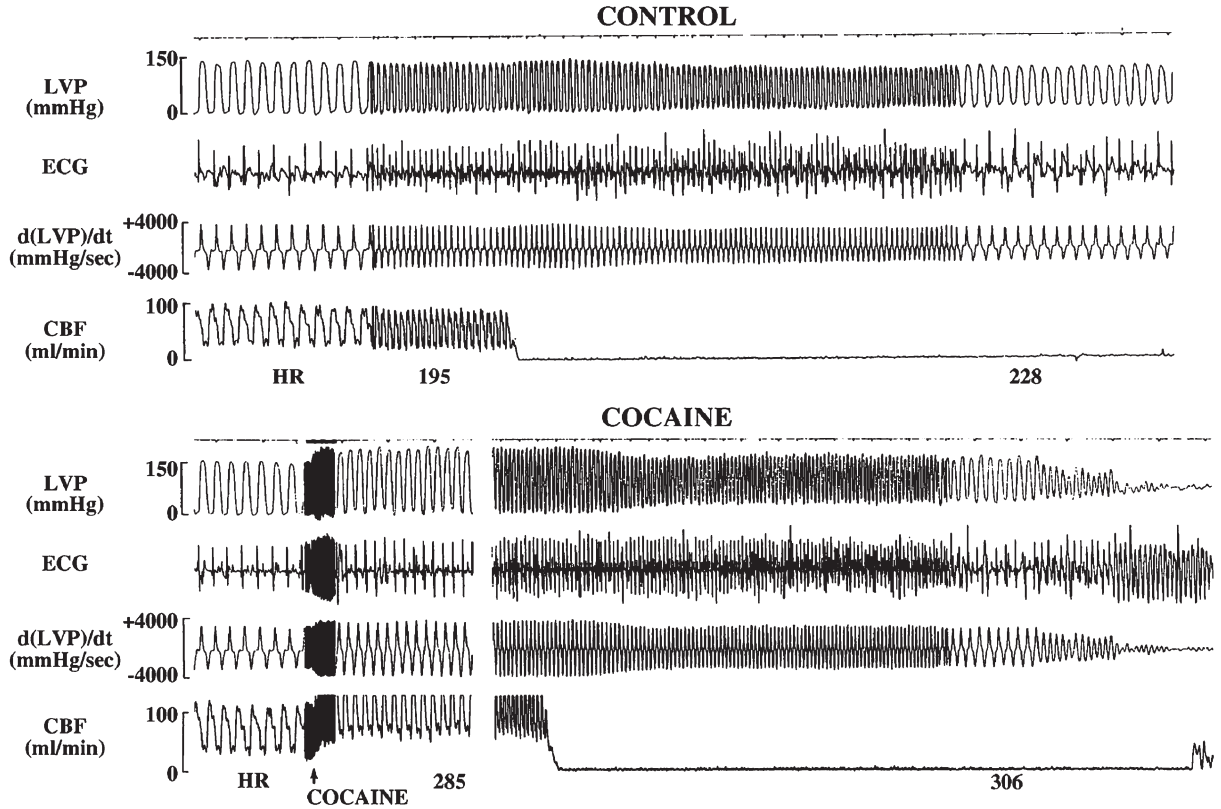

Fig. 22. Representative recording from 1 resistant dog with and without pretreatment with cocaine (1.0 mg/kg, i.v., given 3 min before the coronary artery occlusion).

the sarcoplasmic reticulum. Agents that alter calcium entry into the cell or calcium release from the sarcoplasmic reticulum were also used to evaluate the relative contribution of each in the induction of VF.

The calcium channel antagonists (verapamil, 250 μg/kg, i.v.; diltiazem, 1.0 mg/kg, i.v.; mibefradil, 1.0 mg/kg, i.v.; high doses of nifedipine, 100 μg/kg, i.v.; and magnesium, 100 mg/kg, i.v.) prevented VF in susceptible animals (verapamil 17 of 17, diltiazem, 8 of 8, mibefradil 13 of 17, nifedipine 8 of 9, flunarzine 13 of 13, and magnesium 7 of 9 dogs) while the calcium channel agonist BAY K 8644 (30 μg/kg, i.v.) induced VF in all 9 resistant animals tested (Billman, 1989a; Billman et al., 1991; Billman, 1992a, 1992b, 1993a; Billman & Hamlin, 1996). The calcium channel antagonist studies also demonstrated the important role that acute myocardial ischemia plays in the induction of malignant arrhythmias, which has important implications with regards to the evaluation of potential anti-arrhythmic drugs.

As was previously noted, programmed electrical stimulation induced non-sustained ventricular tachycardia in 19 of 25 susceptible animals but failed to induce even single extrasystoles in any resistant animal ($n=23$) (Billman & Hamlin, 1996). Lidocaine (2 mg/kg, i.v., injected 3 min before the occlusion) completely suppressed these electrically-induced arrhythmias, whereas calcium channel antagonists (mibefradil, verapamil and diltizam) not only failed to prevent these arrhythmias, but also increased the duration of the ventricular tachycardia in about half the susceptible animals. In contrast, the same dose of lidocaine failed to prevent arrhythmias induced by the exercise plus ischemia test, while pretreatment with the calcium channel antagonists protected against VF (Billman & Hamlin, 1996). This observation may help explain why so many promising anti-arrhythmic medications have proven to be such dismal failures in the clinical setting. The drugs may have been developed to prevent the wrong arrhythmias; that is, drugs were selected to prevent re-entrant arrhythmias induced by programmed electrical stimulation rather than those potentially life-threatening arrhythmias associated with myocardial ischemia. It is, therefore, crucial to identify what factor or factors that render the *ischemic* myocardium vulnerable to arrhythmia formation in order to develop effective anti-arrhythmic interventions. Once this has been accomplished, it should then be possible to develop therapeutic interventions that correct these ischemically induced changes in cardiac electrical stability and thereby prevent sudden death. The "ideal" drug would be one that only affects the ischemic tissue with little or no action on the normal cardiac tissue. As a consequence of this selectivity, one would expect that these drugs should also have a low propensity for pro-arrhythmic events.

The role of calcium release from the sarcoplasmic reticulum was evaluated using ryanodine (10 μg/kg, i.v.) to deplete sarcoplasmic reticular calcium. This drug did not prevent VF induced by ischemia (only 1 of 10 animals was protected), but it significantly decreased left ventricular dP/dt max (Lappi & Billman, 1993). In contrast, the same dose of ryanodine prevented ventricular tachycardia ($n=10$) induced by ouabain (40 μg/kg, i.v., bolus followed by 0.076 μg/kg/min infusion over 1 hr, then a second 20 μg/kg bolus) in anesthetized dogs (Lappi & Billman, 1993). Thus, alterations in sarcoplasmic reticular calcium release may contribute to arrhythmias resulting from digitalis toxicity but not those arrhythmias induced by myocardial ischemia.

When considered together, these data suggest that alterations in intracellular calcium that result from enhanced calcium entry, rather than from increased calcium release from the sarcoplasmic reticulum, are responsible for VF induced by myocardial ischemia.

4.3. ATP-sensitive potassium channel antagonists

It is now generally accepted that the activation of the ATP-sensitive potassium channel during myocardial ischemia provokes a potassium efflux and reductions in action potential duration that lead to dispersion of repolarization (Billman, 1994b). Since heterogeneity of repolarization plays a crucial role in the induction of VF (Wit & Janse, 1993), drugs that prevent ATP-sensitive potassium channel activation should be particularly effective in the suppression of malignant arrhythmias induced by ischemia. The non-selective ATP-sensitive potassium channel antagonist glibenclamide either at a high (10 mg/kg, i.v.) or at a low dose (1.0 mg/kg, i.v.), prevented VF in 13 of 15 (Billman et al., 1993) and 6 of 7 (Billman et al., 1998) animals, respectively. However, this drug also reduced exercise-induced increases in mean coronary blood flow (CBF), depressed left ventricular function, and produced profound hypoglycemia (particularly at the high dose).

Recently, drugs selective for the cardiac sarcolemmal cardiac ATP-sensitive potassium channel have been developed (Goegelein et al., 1999; Englert et al., 2001; Goegelein, 2001). As such, these drugs would attenuate ischemically induced changes in cardiac electrical properties, thereby preventing malignant arrhythmias without the untoward effects of non-selective drugs. HMR 1883 (or its sodium salt HMR 1098) has been shown to block only cardiac sarcolemmal ATP-sensitive potassium channels (Goegelein et al., 1998). This drug attenuated ischemically-induced ST segment changes and prevented VF in 16 of 20 susceptible animal tested without altering blood glucose levels, mean CBF, or ventricular function (Billman et al., 1998, 1999b). Similar results were obtained with HMR 1402, another cardioseletive ATP-sensitive potassium channel antagonist that is approximately 6 times more potent than HMR 1098 (Goegelein et al., 1998; Billman et al., 2004). This drug prevented VF in 7 of 8 susceptible dogs without altering plasma insulin, plasma glucose, or coronary vascular responses (Billman et al., 2004). Since the ATP-sensitive potassium channel only becomes active as ATP levels fall, drugs like HMR 1883 or HMR 1402 should *only* have effects on ischemic tissue with little or no effect noted on normal tissue. Thus, selective antagonists of the cardiac cell surface ATP-sensitive potassium channel may represent the first truly "ischemia selective" anti-arrhythmic medications, and as such, should be free of the pro-arrhythmic effects that have plagued many of the currently available anti-arrhythmic drugs.

4.4. Other potassium channel antagonists

A number of other potassium channel antagonist have also been tested with this model yielding mixed results. The selective inhibitor of the rapid component of the delayed rectifier current (I_{Kr}), D-sotalol (2.0 mg/kg, i.v.) was given to 3 resistant and 8 susceptible dogs. Pretreatment with this drug elicited a dose-dependent increase in QTc and, not only failed to prevent VF in any of the susceptible animals, but also induced arrhythmias in 2 of the resistant dogs. In agreement with these findings, Vanoli et al. (1995) reported that D-sotalol (8.0 mg/kg, i.v.) only protected 1 of 10 susceptible dogs tested. In contrast, the selective inhibitor of the slow component of the delayed rectifier current (I_{Ks}), L-768,673 (0.03 mg/ kg, i.v.), elicited only modest increases (7%) in QTc and prevented VF in 5 of 6 susceptible dogs (Lynch et al., 1999). This drug did not induce ventricular arrhythmias in resistant animals but also suppressed arrhythmias (single extra systoles) in 2 of these dogs (Lynch et al., 1999). Thus, depending on the agent used, potassium channel antagonists may anti-arrhythmic or pro-arrhythmic properties. Drugs that selectively block I_{Kr} were ineffective (and perhaps even pro-arrhythmic) while drugs that inhibited I_{Ks} significantly reduced the incidence of VF induced by the exercise plus ischemia test.

5. Non-pharmacological interventions

As previously noted, the development of safe and effective anti-arrhythmic therapies remains elusive. Many initially promising anti-arrhythmic medications were subsequently shown to promote rather than abolish arrhythmias in some patient populations (Echt et al., 1991; Waldo et al., 1996; Sager, 1999) and even the most effective agents fail to protect all individual and often have serious side effects (Buxton et al., 1999; Nattel, 2000). Therefore, the development of non-pharmacological approaches to management of life threatening arrhythmias merits consideration. My laboratory has used the canine model of sudden cardiac death to evaluate the therapeutic potential of 2 such approaches: endurance exercise training and dietary omega-3 fatty acids.

5.1. Effect of endurance exercise training

It is now well established that aerobic exercise conditioning can alter cardiac autonomic tone, both reducing cardiac sympathetic activity and increasing cardiac parasympathetic activity (Billman, 2002) and may, thereby, protect against malignant arrhythmias. In order to test this hypothesis, susceptible animals were assigned to either an exercise (6 weeks treadmill running, $n=8$) or a sedentary (6 week cage rest, $n=8$) group (Billman et al., 1984). At the end of the 6-week period, the animals were re-tested with the exercise plus ischemia test; all 8 exercise-treated animals but only 1 of the 8 sedentary animals were protected from VF. The baroreceptor reflex control of heart rate was evaluated every 2 weeks during the test period. The heart rate response to an increase in arterial pressure was increased in the exercise treated group but not in the sedentary group; the heart rate response to submaximal exercise was also reduced in the exercise group. Two of the exercise-trained animals were then tested after a 6-week cage rest. The heart rate response to an increase in arterial pressure declined toward pre-exercise training levels and the exercise plus ischemia test induced VF in 1 animal and non-sustained ventricular tachycardia in the other animal. This initial observation was subsequently confirmed. Hull et al. (1994) found that a similar 6-week exercise program improved baroreflex sensitivity, increased baseline heart rate variability, and also produced protection for all 7 susceptible dogs that completed the training program. The positive effect of exercise training on baroreflex sensitivity has recently been confirmed in patients (La Rovere et al., 2002). Exercise training improved baroreflex sensitivity in patients recovering from myocardial infarction and further, this improvement was associated with a reduced risk for sudden cardiac death (La Rovere et al., 2002). These data suggest that daily exercise may alter autonomic tone and thereby protect against VF induced by myocardial ischemia. However, the contributions of changes in cardiac autonomic balance to the protection afforded by exercise training were not extensively examined in these studies.

I have just completed a series of studies that comprehensively examined the effects of exercise training both the parasympathetic (Billman & Kukielka, in press) and the β-adrenoceptor (Billman et al., in press) regulation of cardiac function in dogs susceptible or resistant to VF induced by the exercise plus ischemia. The susceptible ($n=20$) and resistant ($n=13$) dogs were randomly assigned to either a 10-week exercise program (susceptible, $n=9$; resistant $n=8$) or an equivalent sedentary period (susceptible, $n=11$; resistant $n=5$). Heart rate variability was evaluated at rest, during exercise, and during a 2-min occlusion at rest, before and after the 10-week period. As in our previous studies, pre-training, the occlusion provoked significantly greater increases in heart rate (susceptible 54.9±8.3 vs. resistant 25.0±6.1 beats per min) and greater reductions in heart rate variability (susceptible −6.3±0.3 vs. resistant −2.8±0.8 ln $msec^2$) in the susceptible dogs as compared to the resistant animals. Similar response differences between susceptible and resistant dogs were noted during submaximal exercise. Exercise training significantly reduced the heart rate and heart rate variability response to either the coronary occlusion (Fig. 23) or submaximal exercise (Fig. 24) to a greater extent in the susceptible animals compared to the resistant dogs (data not shown). In contrast, these variables were not altered in the sedentary susceptible dogs (Figs. 23 and 24).

Representative ECG recording from 1 susceptible dog before and at the end of the 10-week exercise training period and a second animal before and at the end of the 10-week sedentary period are displayed in Fig. 25. After completing the 10-week exercise-training program, VF could no longer be induced in the susceptible dogs, while 4 sedentary susceptible dogs died during the 10-week control period and the remaining 7 animals still had malignant arrhythmia when tested. The exercise plus ischemia test was then repeated after the injection of atropine to

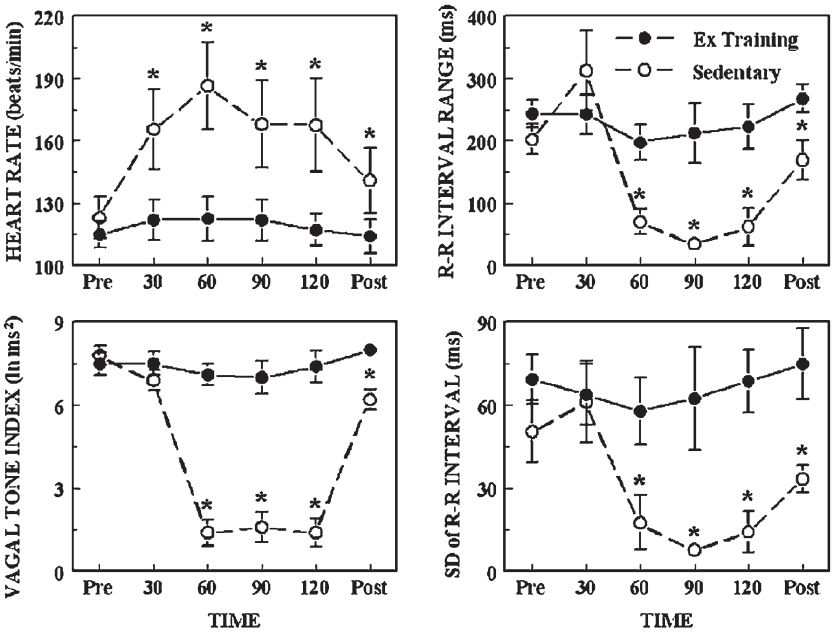

Fig. 23. The effect of the 10-week exercise training ($n=9$) or 10-week sedentary ($n=7$) period on the heart rate and the heart rate variability responses to a 2 min coronary occlusion in animals susceptible to ventricular fibrillation. The coronary occlusion elicited significantly smaller increase in heart rate and smaller reductions in the various indices of cardiac vagal regulation in the exercise-trained dogs as compared to animals that received a similar sedentary period. The post-training response in the susceptible exercise trained animals was no longer different from that noted for the resistant (either exercise trained or sedentary) dogs. *$P<0.01$ exercise-trained versus sedentary. Pre = last 30 sec before the coronary occlusion, Post = 1 min following coronary occlusion release (i.e., average over 30–60 sec post release). (Reprinted with permission from Billman & Kukielka, in press.)

abolish any exercise training-induced enhancement of cardiac vagal regulation. This intervention increased heart rate and provoked reductions in the various indices of heart rate variability but only induced VF in 1 of 8 trained susceptible dogs. Thus, exercise training-induced increases in cardiac vagal activity were not solely responsible for the training-induced

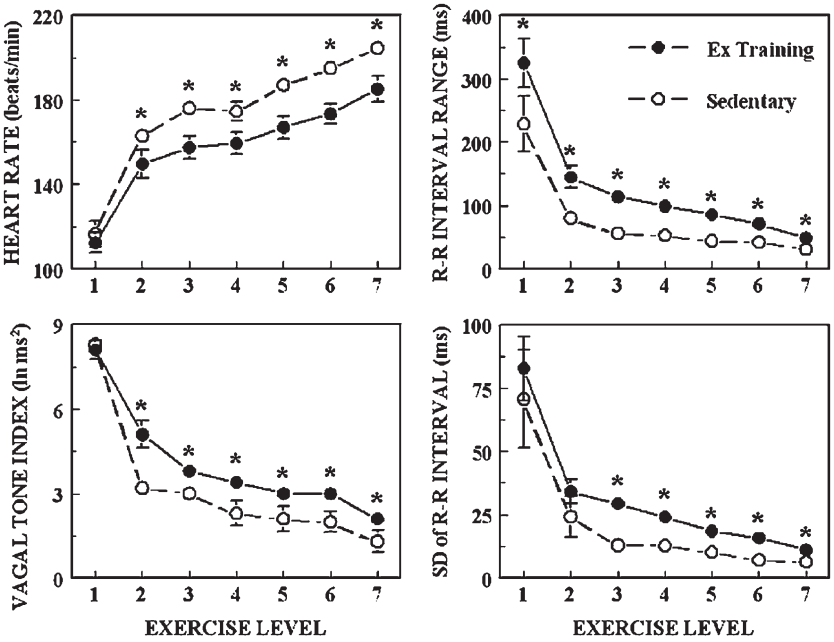

Fig. 24. The effect of the 10-week exercise training ($n=9$) or 10-week sedentary period ($n=7$) on the heart rate and the heart rate variability responses to submaximal exercise in animals susceptible ventricular fibrillation. Exercise elicited significantly smaller increase in heart rate and smaller reductions in the various indices of cardiac vagal activity in the exercise-trained dogs as compared to animals that received a similar sedentary period. The post-training response in the susceptible exercise-trained dogs was no longer different from that noted for the resistant (exercise trained or sedentary) dogs. *$P<0.01$ exercise-trained versus sedentary, Exercise levels: 1 = 0 kph/0% grade, 2 = 4.8 kph/0% grade, 3 = 6.4 kph/0% grade, 4 = 6.4 kph/4% grade, 5 = 6.4 kph/8% grade, 6 = 6.4 kph 12% grade, 7 = 6.4 kph/16% grade. (Reprinted with permission from Billman & Kukielka, in press.)

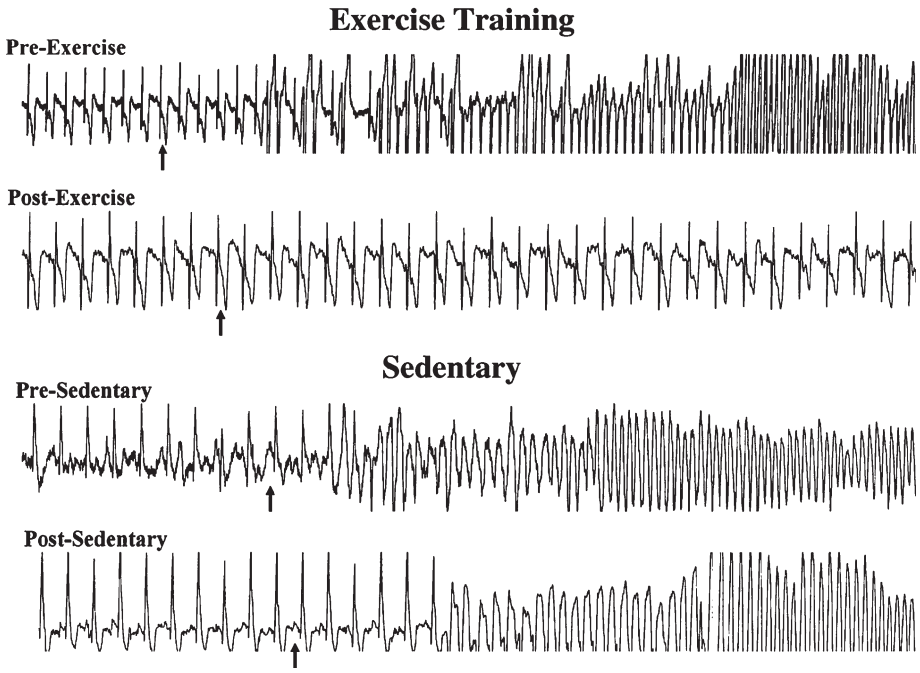

Fig. 25. Representative ECG recordings from 2 different susceptible dogs, 1 before after completion of a 10-week endurance exercise program and 1 before and after an equivalent 10-week sedentary period. Note arrhythmias were no longer induced by the exercise plus ischemia test in the exercise-trained animal. The arrow indicates the time at which the treadmill was stopped. (Reprinted with permission from Billman et al., in press.)

protection from VF. Other factors, including alterations in the β-adrenoceptor regulation of the heart, also contributed to the protection from VF.

In a second set of studies (Billman et al., in press), the effects of exercise training on β-adrenoceptor function were also evaluated in these same animals. Before exercise training, the β_2-adrenoceptor antagonist ICI 118,551 (0.2 mg/kg) significantly reduced the peak contractile (by echocardiography) response to isoproterenol more in the susceptible animals (susceptible −45.5±6.5% vs. resistant −19.2±6.3%) compared to the resistant dogs (Fig. 26). After exercise training, the resistant and the susceptible dogs exhibited similar responses to the β_2-AR antagonist (susceptible −12.1±5.7% vs. resistant −16.2±6.4%) (Fig. 26). In contrast, ICI 118,551 provoked even greater reductions in the isoproterenol response in the sedentary susceptible dogs (−62.3±4.6%) (Fig. 26). The β_2-adrenoceptor agonist zinterol (1μM) elicited significantly smaller increases in isotonic shortening in ventricular myocytes (Fig. 27) from

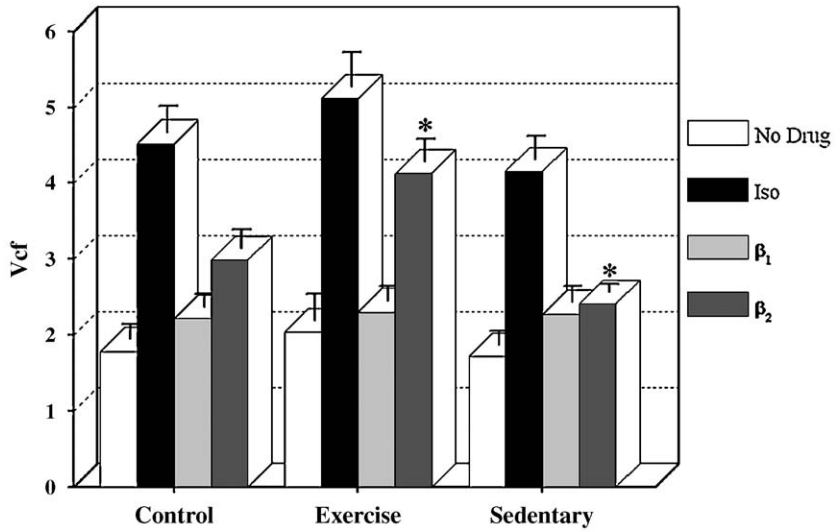

Fig. 26. Effects of selective β-adrenoceptor antagonists on the maximum isoproterenol induced Vcf response in susceptible animals before (control $n=18$) and after either exercise training ($n=8$) or a sedentary ($n=10$) time period. The β_2-adrenoceptor antagonist ICI 118,551 (0.2 mg/kg, i.v.) elicited a significantly greater reduction in the isoproterenol response in the sedentary animals. In contrast, the β_2-adrenoceptor response was significantly reduced in the exercise-trained animals. β_1-adrenoceptor antagonist=bisoprolol (0.6 mg/kg, i.v.), isoproterenol=0.5 μg/kg/min. *$P<0.01$ control drug treatment response compared to the corresponding drug treatment response for either the 10-week exercise-trained or the 10-week sedentary group. (Reprinted with permission from Billman et al., in press.)

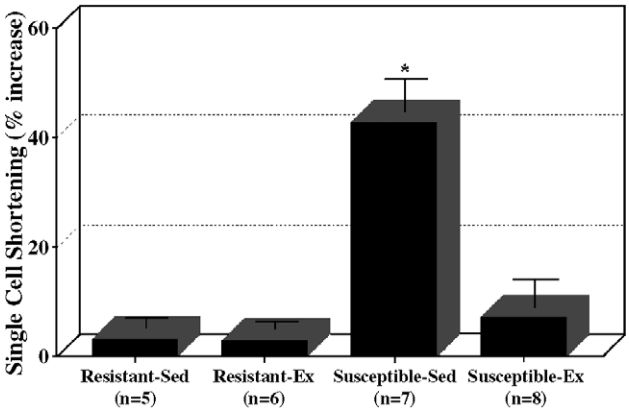

Fig. 27. The effect of exercise training on the single-cell shortening (% change from control) response to the selective β-adrenoceptor agonist (zinterol, 100 nM). The responses were compared between ventricular myocytes (up to 15 cells per dog, averaged such that only value was reported for a given animal) obtained from susceptible (sedentary $n=7$, exercise training $n=8$) and resistant (sedentary $n=5$, exercise training $n=6$) animals. Note the much larger response in the sedentary susceptible animals. *$P<.01$ sedentary versus exercise training. (Reprinted permission from Billman et al., in press.)

susceptible dogs after training ($n=8$, 7.2±4.8%) than in those from sedentary dogs ($n=7$, 42.8±5.8%), a response similar to that noted in the resistant dogs (trained, $n=6$, 3.0±1.4%; sedentary, $n=5$, 3.2±1.8%). Thus, exercise training can restore cardiac β-adrenoceptor balance (by reducing β_2-adrenoceptor responsiveness) and could, thereby, prevent VF. The mechanisms by which exercise training induced a restoration in cardiac β-adrenoceptor balance remain to be determined.

5.2. Effect of omega-3 fatty acids (fish oil)

There is an increasing body of evidence that demonstrates that the consumption of the long chain omega-3 fatty acids found in fish or diets enriched with omega-3 fatty acids supplements are associated reduce the cardiac mortality in post myocardial infarction patients (Leaf et al., 2003a, 2003b). For example, the GISSI-Prevenzione trial treated 11,324 patients with a recent myocardial infarction with fish oil capsules (850 mg of docosahexaenoic acid, DHA; eicosapentaenoic EPA) and reported a 45% reduction in sudden cardiac death GISSI-Prevenzione Investigators, 1999). The hearts from animals (rats or marmosets) fed diets enriched with fish oil also exhibited a lower incidence of ventricular tachyarrhythmias during myocardial ischemia (McLennan et al., 1985, 1992). We investigated the effects of the acute intravenous infusion (to avoid the complications associated with dietary supplementation) in dogs susceptible to VF. We first investigated the effects of an emulsion prepared from concentrated fish oil (Billman et al., 1994a, 1997a). One to ten grams of the fish oil concentrate was slowly infused over a 1-hr and then the exercise plus ischemia test was performed in 13 susceptible animals. This intervention prevented VF in 10 of 13 susceptible dogs. In contrast, the infusion of a similar volume of an emulsion made from soybean oil (intralipid $n=7$) failed

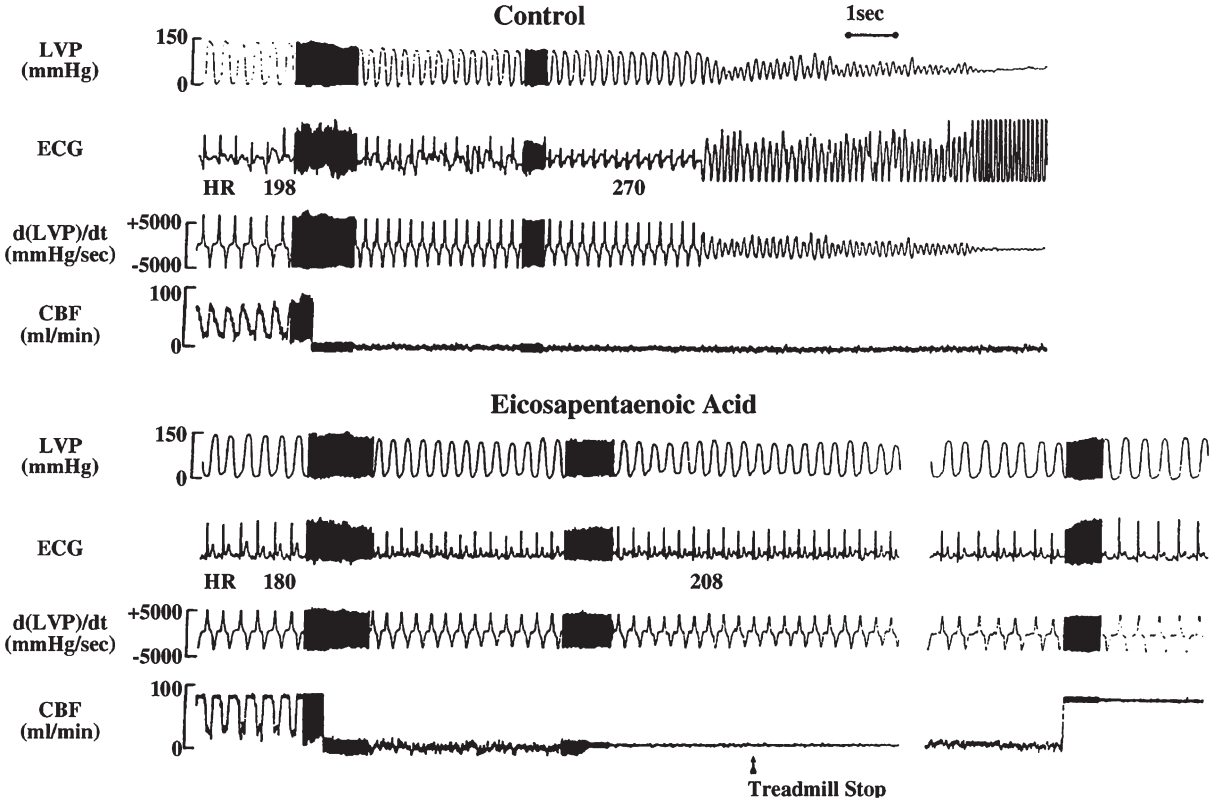

Fig. 28. Representative recordings of 1 susceptible dog before and after pretreatment with the omega-3 fatty acid, eicosapentaenoic acid (1 g in 100 mL infused over 1 hr). Note the absence of arrhythmias after treatment with the omega-3 fatty acid. This treatment prevented ventricular fibrillation in 5 of 7 susceptible dogs.

to protect any of these animals. Fish oil is composed of a number of fatty acids with the EPA and DHA serving as the active ingredients. We next investigated the effects of purified omega-3 fatty acids (Billman et al., 1999a). Representative recordings from 1 susceptible dog with and without treatment with DHA are shown in Fig. 28. Both DHA and EPA significantly reduced the incidence of VF protecting 6 of 8 and 5 of 7 susceptible dogs, respectively. The mechanisms responsible for this protection remain to be determined in both human subjects and intact animal models. However, studies performed on rat neonatal cell cultures or isolated cell preparations demonstrate the omega-3 fatty have potent affects on many ion channels (Leaf et al., 2003a, 2003b). These substances shift the inactivation threshold for sodium channels to a more negative potential (thereby decreasing cell excitability), inhibit calcium entry through the L-type calcium channels, and inhibit several potassium channels (transient outward current, I_{to}, and slow component of the delay rectifier current, I_{Ks}) (Leaf et al., 2003a, 2003b). It seems reasonable that omega-3 fatty acids exert their anti-arrhythmic action via interaction with these membrane channels.

6. Summary and conclusions: lessons learned and future directions

In order to be an effective, the animal model must closely mimic the pathology responsible for sudden death in patients. The model must also use a sufficient number of animals so that the anti-arrhythmic properties of a given drug can be accurately assessed. As such, an important advantage of the present model is that the exercise plus ischemia identifies 2 highly reproducible populations of animals. As a consequence, the same animals can be used in both the control and treated groups (i.e., provide an internal control), thereby reducing the total number of animals required for a given study (reducing the cost and expediting the evaluation of a compound or set of compounds).

As has been detailed in the preceding sections, many studies have been completed using this canine model of sudden cardiac death. As we have seen, this canine model has many clinically relevant characteristics, including recent ischemia at site distant from a previous injury and abnormalities in cardiac autonomic regulation. Many important observations that were first described in this model have been subsequently confirmed in patients in the clinic. Of particular importance, was the observation that animals that were susceptible to VF also exhibited abnormal autonomic regulation of the heart. Non-invasive markers of this autonomic dysfunction have proven to be highly effective in prospectively identifying not only the animals, but also patients, at the greatest risk for VF/sudden cardiac death.

The model has also provided important insight into factors that may play an important role in triggering malignant arrhythmias potentially facilitating the development of novel therapeutic approaches for the management of these potentially life threatening cardiac rhythm disturbances. A summary of the results of the various studies can found in Figs. 29 and 30. When considered together a number of broad conclusions can be made from these studies.

The most effective anti-arrhythmic drugs should target the ischemic myocardium. The studies completed with various calcium channel antagonist highlight the important role that acute myocardial ischemia plays in the induction of malignant arrhythmias. As was previously noted, programmed electrical stimulation induced non-sustained ventricular tachycardia in the majority of the susceptible animals tested but failed to induce even single extrasystoles in any of resistant dogs (Billman &

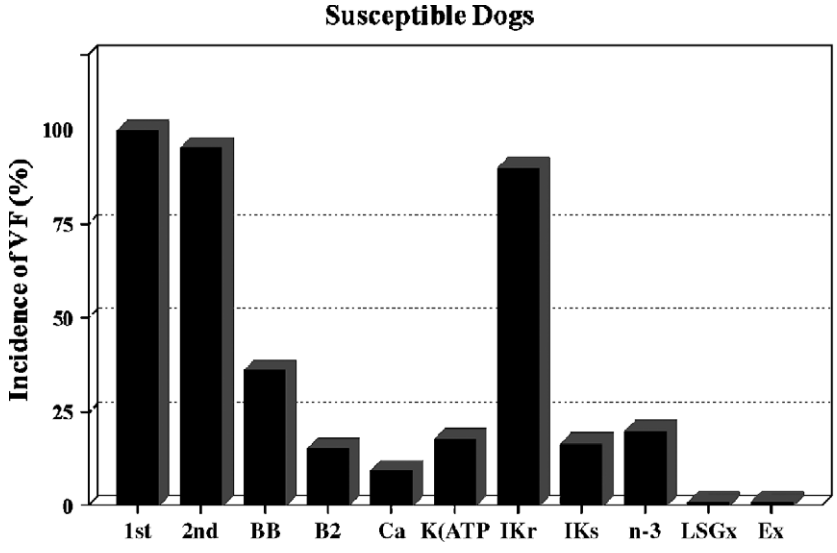

Fig. 29. Summary of the results of some of the interventions tested in dogs susceptible to ventricular fibrillation. 1st and 2nd control (no drug treatment) $n=257$; BB=β-adrenoceptor blockade (propranolol HCl., 1.0 mg/kg, i.v., $n=38$); B2=β_2-adrenoceptor blockade (ICI 118,551; 0.2 mg/kg, i.v., $n=11$); Ca=calcium channel antagonists (average 90.5%, range 76.5–100% depending on agent used, see text for details); K(ATP)=ATP-sensitive potassium channel antagonist (HMR 1883/1098 and HMR 1402, $n=28$); IKs=slow component of the delayed rectifier current (L-768,673, 0.03 mg/kg, i.v., $n=6$); IKr=rapid component of the delayed rectifier current (D-sotalol, 2.0 to 8.0 mg/kg, i.v., $n=18$); n-3=omega-3 fatty acids ($n=21$); LSGx=left stellate ganglionectomy ($n=11$); Ex=endurance exercise training ($n=24$).

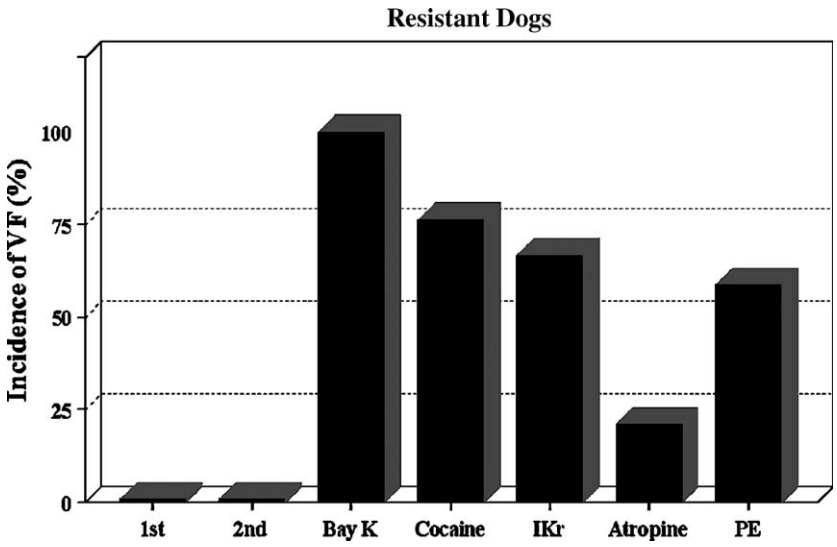

Fig. 30. Summary of results some of the interventions tested in dogs resistant to ventricular fibrillation. 1st (n=209) and 2nd (n=133)=control no drug occlusions (n=209); Bay K=the calcium channel agonist Bay K 8644 (30 μg/kg, i.v., 3 min before the coronary occlusion, n=14); cocaine (1.0 mg/kg, i.v., 3 min before the occlusion, n=55); Atropine (50 μg/kg, i.v., n=66), PE=phenylephrine, 10 μg/kg/min 2 min before occlusion onset, n=17) IKr=rapid component of the delayed rectifier current (D-sotalol 2.0 mg/kg, i.v., induction of arrhythmias in 2 of 3 dogs tested).

Hamlin, 1996). The class I, anti-arrhythmic drug, lidocaine completely suppressed these electrically induced arrhythmias, whereas calcium channel antagonists (mibefradil, verapamil and diltizam) not only failed to prevent these arrhythmias, but also increased the duration of the ventricular tachycardia in about half the susceptible animals. However, completely different results were obtained during acute myocardial ischemia. Lidocaine failed to prevent arrhythmias induced by the exercise plus ischemia test, while pretreatment with the calcium channel antagonists protected against VF (Billman & Hamlin, 1996). It is, therefore, crucial to identify what factor or factors render the *ischemic* myocardium vulnerable to arrhythmia formation in order to develop effective anti-arrhythmic interventions. Once this has been accomplished, it should then be possible to develop therapeutic interventions that correct these ischemically induced changes in cardiac electrical stability and thereby prevent sudden death. The "ideal" drug would be one that only affects the ischemic tissue with little or no action on the normal cardiac tissue. As a consequence of this selectivity, one would expect that these drugs should also have a low propensity for pro-arrhythmic events.

Selective antagonists of the cardiac sarcolemmal ATP-sensitive potassium channel antagonists may prove to be "ischemia" selective agents. The activation of cardiac cell membrane ATP-sensitive potassium channels during myocardial ischemia promotes potassium efflux, reduction in action potential duration, and inhomogenieties in repolarization, thereby creating a substrate for re-entry (Billman, 1994a). Drugs that selectively block this channel were found to be highly effective in the present canine model of sudden death (Billman et al., 1998, 2004). Two different cardioselective ATP-sensitive potassium channel antagonists (HMR 1883/1098 and HMR 1402) have been tested in this canine model of sudden cardiac death. Each drug provided significant protection form malignant arrhythmias. Together, these drugs protected 82% (23 of 28) of the susceptible dogs and never induced arrhythmias in resistant animals. It is interesting to note that many currently available anti-arrhythmic drugs, including verapamil, mibefradil, quinidine, lidocaine and amiodarone, have also been reported to inhibit ATP-sensitive potassium channels at therapeutic concentrations (Haworth et al., 1989; Colatsky et al., 1990; Olschewski et al., 1996; Holmes et al., 2000). Therefore, the inhibition of the ATP-sensitive potassium channel may be required for anti-arrhythmic actions during myocardial ischemia. Since, as noted above, the ATP-sensitive potassium channel only becomes active as ATP levels fall, ATP-sensitive potassium channel antagonists have the added advantage that this drug would *only* have effects on ischemic tissue with little or no effect noted on normal tissue. One would predict that these agents should reduce cardiac mortality in high-risk patients with advanced ischemic heart disease with little or no pro-arrhythmic potential.

Alterations in cardiac autonomic regulation also contribute significantly to the susceptibility to VF. The susceptible dogs exhibit an enhanced β_2-adrenoceptor responsiveness coupled with reduced cardiac vagal regulation. Interventions that either reduce sympathetic activity and/or increase parasympathetic regulation generally protect against VF. For example, β-adrenoceptor antagonist prevented about 63% of the death while disruption of the left stellate ganglion protected 100% of susceptible dogs (Fig. 29). In similar fashion, interventions that increased parasympathetic activity also protected against sudden death. However, as atropine pretreatment only modestly increased arrhythmia frequency in resistant animals and did not alter the protection induced by exercise training, it is likely that impairments in parasympathetic activity probably are solely not responsible for the enhanced risk for arrhythmias noted in the susceptible dogs.

The mechanism linking alterations in cardiac autonomic function, particularly with regards to the apparent enhanced β_2-

adrenoceptor responsiveness, to an increased susceptibility to ventricular function remain to be determined.

Neurotransmitters released from the cardiac autonomic nerves must bind to post-synaptic receptors, thereby triggering a cascade of intracellular events. Alterations in intracellular calcium represent the final common pathway for most of these second messenger systems. Abnormalities in cellular calcium regulation would disrupt cardiac electrical stability and ultimately culminate in the genesis of malignant arrhythmias. Thus, disruption of myocyte calcium homeostasis almost certainly plays a central role in provoking lethal cardiac rhythm changes particularly as a consequence of myocardial ischemia. Both the intracellular calcium chelator BAPTA-AM and several different calcium channel antagonists have been evaluated with the canine model of sudden death. These agents have proved to be highly effective in the prevention of VF; BAPTA-AM protected 75% (8 of 12) susceptible dogs while the calcium channels agonists prevented VF in between 76.5% (mibefradil) to 100% (verapamil, diltiazem, flunarizine) of the animals depending on the agent used (overall average for all the calcium channel antagonists was 90.5%). In contrast, the calcium channel agonist, BAY K 8644 induced intractable VF in all 8 resistant dogs tested (Fig. 30). In marked contrast, ryanodine, a drug that disrupts sarcoplasmic reticular calcium regulation, only prevented malignant arrhythmias in 1 of 10 susceptible dogs. When considered together, these data suggest that an enhanced calcium entry, rather than altered calcium release from the sarcoplasmic reticulum, is responsible for VF induced by myocardial ischemia.

Finally, non-pharmacological interventions may offer a particularly promising approach for the management of malignant arrhythmias. Both regular exercise and nutritional approaches (diets enriched with omega-3 fatty acids) could improve both the quality of life and reduce cardiac mortality in myocardial infarction patients. As previously noted, 3 studies have investigated the effects of exercise training on the susceptibility to VF in the same canine model (Billman et al., 1984; Hull et al., 1994; Billman & Kukielka, in press; Billman et al., in press) yielding very consistent results. Endurance exercise training protected 100% of the susceptible animals in each study, abolishing VF in all 24 susceptible dogs that completed the exercise program. In contrast, the exercise plus ischemia test only failed to induce malignant arrhythmias in one of 18 dogs after the completion of an equivalent sedentary period. In addition, 4 dogs died prematurely during the sedentary period while no animals died during the course of the exercise program. Thus, endurance training has proven to be one of the most effective interventions for the prevention of VF induced by acute myocardial ischemia. The most recent studies cited above (Billman & Kukielka, in press) clearly demonstrate that exercise training induced alterations in cardiac parasympathetic regulation are not solely responsible for this protection. However, the exercise training induced restoration of a more normal cardiac β-adrenoceptor balance (by reducing β_2-adrenoceptor responsiveness) could improve intracellular calcium homeostasis increasing cardiac electrical stability. This improvement in β_2-adrenoceptor responsiveness could result from either a reduction in β_2-adrenoceptor density (or distribution) or from alterations in β_2-adrenoceptor signaling (i.e., events downstream from the receptors). The mechanisms by which exercise training induced a restoration in cardiac β-adrenoceptor balance remain to be determined.

Although, during the last 25 years, the canine model of sudden cardiac death described in this article has provided invaluable information concerning factors involved in VF, there remain many unanswered questions. The mechanisms responsible for VF at the cellular and subcellular level remain largely to be determined. It, therefore, is very likely that this canine model for sudden death will continue to stimulate new research and produce interesting results for the next 25 years.

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