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The Effect of Autonomic Mediators Released by Electrical Stimulation of the Ventricles on Contractile Force and Vulnerability to Fibrillation

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THE EFFECT OF AUTONOMIC MEDIATORS RELEASED BY ELECTRICAL
STIMULATION OF THE VENTRICLES ON CONTRACTILE FORCE
AND VULNERABILITY TO FIBRILLATION

by

David Euler

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
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1980

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VITA

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CHAPTER I

INTRODUCTION

The innervation of the mammalian heart by both the sympathetic and parasympathetic divisions of the autonomic nervous system has been firmly established on the basis of both anatomical and functional evidence (59, 92, 93). Thus, experimental procedures which involve direct electrical stimulation of the myocardium may result in the excitation of local autonomic fibers. Electrical stimulation of isolated preparations of cardiac muscle has been shown to release substantial quantities of autonomic neuromediators (1, 6, 7, 123, 129). However, the phenomena has not been demonstrated in the intact ventricles. Therefore, part of the purpose of the present study was to determine if electrical stimuli delivered to the intact myocardium would release autonomic neuromediators in sufficient magnitude to alter local contractile function.

Since electrical testing of cardiac vulnerability to fibrillation involves the application of high intensity current pulses (either trains or single stimuli) to the myocardium during the vulnerable period of the cardiac cycle, it is feasible that these stimuli could release local autonomic neuromediators, thereby altering the susceptibility of the heart to fibrillation. Furthermore, ventricular fibrillation thresholds measured with trains of stimuli might be affected to a greater magnitude by local autonomic neuromediator release than

thresholds measured with single stimuli, since a train of stimuli would presumably result in the liberation of more neurotransmitter. Tamargo et al. (117) discounted the importance of a local adrenergic discharge from the observation that the fibrillation threshold measured with trains of stimuli did not change relative to the single-stimulus fibrillation threshold in response to propranolol. However, no quantitative data were reported by these investigators to substantiate this conclusion (117). Thus, a second purpose of the present investigation was to quantitatively evaluate the effects of propranolol, atropine and chronic cardiac denervation on ventricular fibrillation thresholds to determine if local release of autonomic mediators significantly influences ventricular vulnerability to fibrillation

Although reentry is probably the mechanism responsible for the maintenance of ventricular fibrillation, the mechanism responsible for the initiation of fibrillation following electrical stimulation of the heart during its vulnerable period has not been fully elucidated. Moe et al. (83) demonstrated that fibrillation began with a series of multiple extrasystoles originating from the vicinity of the stimulating electrodes. By mapping the spread of activation of the initial extrasystoles with bipolar electrograms they eliminated the possibility of a macroreentrant circuit and suggested that the initial impulses originated from an automatic center created by the stimulus current. However, since the direct bipolar recordings made by Moe et al. (83) were never closer than six millimeters from the stimulating electrodes, they were unable to eliminate the possibility of local reentry. Therefore, the third goal of the present experiments was to record multiple

bipolar electrograms from muscle directly adjacent to a pair of stimulating electrodes to further elucidate the mechanism responsible for the initiation of fibrillation following the delivery of either trains of stimuli or single stimuli to the ventricles during their vulnerable period.

CHAPTER II

LITERATURE REVIEW

A. Ventricular Fibrillation and Defibrillation

Ventricular fibrillation was first reported in 1842 by Erichsen (22) following ligation of the coronary arteries in a dog. Eight years later, Hoffa and Ludwig (40) observed ventricular fibrillation in cold-blooded and mammalian hearts in response to strong, rapidly repeated galvanic shocks. These investigators (40) noted that the ventricles became dilated with blood as the incoordinated quivering movement of the muscle became insufficient to expel the contents of the ventricles. In 1874, Vulpian (124) first used the word fibrillation (movement fibrillaire) to describe the incoordinated contractions following faradization of the canine ventricles. Vulpian (124) also noted that regardless of the area to which a faradic stimulus was applied, both ventricles were thrown into fibrillation. Furthermore, the incoordinated contractions were not transmitted to the atria, which continued to show rhythmic contractions long after the fibrillary contractions of the ventricles has ceased. In 1887, McWilliam (76) reported that the ventricles of small animals (cat, rabbit, mouse, hedgehog and fowl) frequently recovered spontaneously from fibrillation while such recovery was infrequently observed in the dog. A systematic investigation of the importance of tissue mass in the maintenance of

ventricular fibrillation was conducted by Garrey in 1914 (26). Garrey (26) demonstrated that any piece of tissue cut away from the fibrillating canine ventricles would cease fibrillating if its surface area was less than four square centimeters. The functional integrity of the excised pieces of tissue was indicated by the observation of coordinated contractions when the muscle was stimulated with single pulses.

In 1940, Wiggers (130) made a detailed analysis of the initiation and progression of ventricular fibrillation using cinematographic and electrocardiographic techniques. The progression of fibrillation following direct faradization of the canine ventricles was divided into four stages. The first stage, or "undulatory stage," lasted one to two seconds and consisted of three to six undulating contractions, having the characteristics of normal premature systoles. The second stage lasted 15 to 40 seconds and was designated "convulsive incoordination." This stage was characterized by more frequent contractions with different regions contracting asynchronously. The third stage, that of "tremulous incoordination," was marked by progressive fragmentation of the surface of the ventricles into smaller and smaller independently contracting units. The third stage continued for two to three minutes until the onset of the final stage, that of "atonic fibrillation." The complete loss of contractility characteristic of this stage was considered to result from increasing anoxia due to the lack of adequate coronary perfusion (130).

The induction of fibrillation in the dog, as noted by Hoffa and Ludwig (40), ultimately led to the death of the animal. Although

McWilliam (76) found that cooling the fibrillating ventricles would arrest the incoordinated contractions of fibrillation, he was unable to restore the normal pumping ability of the myocardium. Other early methods of terminating fibrillation consisted of the administration of potassium chloride by either injection into the carotid artery under pressure (46) or injection directly into the ventricular cavities (131). The termination of ventricular fibrillation was presumably related to the ability of the potassium to suppress excitability and conduction, resulting in total asystole. Following defibrillation with potassium chloride, the washout of the excess potassium from the coronary circulation with an excess of calcium injected into the coronary circulation sometimes led to a resumption of spontaneous, coordinated beats (46, 131).

The most effective means of resuscitating a heart from fibrillation is by electrical defibrillation. In 1933, Hooker et al. (47), expanding on the work of Prevost and Battelli (1899), demonstrated that a dog's ventricle could be successfully defibrillated with a 60 HZ AC current of 0.1 to five seconds delivered through electrodes placed directly on the heart. If countershock was instituted in less than two minutes, then an effective circulation was reestablished. However, if fibrillation had lasted more than two minutes, then the recovery of spontaneous, effective beats did not follow countershock defibrillation. In the latter case, cardiac massage or injection of adrenaline was found to be of some benefit in restoring an effective circulation (47).

In a detailed study of electrical defibrillation performed by Wiggers in 1940 (131), it was found that following long periods of

fibrillation (five minutes) the difficulty in resuscitation of the heart was not due to the fact that the ventricles could not be defibrillated, but was due to the incapacity of the myocardium to resume vigorous beating. Therefore, Wiggers (131) advocated the use of cardiac massage immediately following the onset of fibrillation until the initiation of countershock defibrillation. Wiggers (131) also advocated the use of serial defibrillation with three to seven shocks applied at intervals of one to two seconds rather than a single shock. Each consecutive shock was thought to cause a coarser type of fibrillation contained in a larger mass of muscle, until the final shock arrested fibrillation completely.

In addition to defibrillation with AC current, the work of Gulvich and Yuniev (31) first demonstrated that defibrillation was possible with DC current generated from a single capacitor discharge. Lown et al. (70) compared the two techniques and found that DC countershock was more successful in defibrillating the canine heart and produced less post-defibrillation arrhythmias than AC countershock. Further work done by Yarbough et al. (135) showed that AC shocks applied directly to the heart resulted in a significant deterioration of ventricular function as compared to DC shocks.

B. Theories of the Mechanism of Ventricular Fibrillation

The various hypotheses which have been promulgated to account for ventricular fibrillation have centered around two main ideas: (1) that the rapid impulses arise from a single or multiple center of automaticity and (2) that the rapid incoordinated activity is due to

repetitive reentry of impulses.

The concept that altered automaticity was responsible for fibrillation originated with Engelman in 1896 (21). Engelman (21) suggested that since ventricular muscle fibers appear to contract independently during fibrillation then, due to a state of altered excitability, the fibers must have become independently rhythmic. In 1900, Hering (32) suggested that ventricular fibrillation represented the most advanced degree of automatic impulse formation in the ventricles while a single extrasystole represented the lowest degree of ventricular automaticity. Since fibrillation invariably appeared to start as a rapid series of extrasystoles, it was only logical for Hering (32) to consider that a common mechanism (abnormal ventricular automaticity) was responsible for both.

The first indication that the continuous rapid movement of fibrillation might involve reentrant excitation came from the work of McWilliam in 1887 (76). Since adjacent muscle bundles were connected by anastomosing branches, McWilliam (76) suggested that contraction waves arriving at one fiber might propagate again over an adjacent fiber which had recovered from a previous contraction.

The first experimental demonstration that a single excitation wave may pass more than once through the same tissue was given by Mayer in 1908 (75). Using a ring of subumbrella tissue cut from a jelly fish, Mayer created a transient conduction block by applying graded pressure near the site of stimulation. By release of the block at the proper moment, Mayer (75) observed that a contraction wave

traveling the opposite direction would continue propagating around the ring. It was further observed that the "single wave going constantly in one direction around the circuit may maintain itself for days at a uniform rate" (75).

In 1913, Mines (78) extended the observations of Mayer (75) to rings of tissue composed of auricles and ventricles from the tortoise heart. A single stimulus delivered to any portion of the ring resulted in a single contraction which propagated in both directions. After a series of rapid, successive stimuli, a contraction wave appeared which continuously circulated around the ring. Mines (78) ruled out an automatic focus as a mechanism for the circulating contraction since the application of an external stimulus to either chamber which was out of phase with the cycle stopped the contractions. Furthermore, Mines (78) recognized that the factors essential for the initiation of the circulating contraction were a unidirectional conduction block, slow conduction and a short refractory period. Mines (78) also suggested that in the normal heart reentry did not occur because the conduction velocity was so rapid and the refractory period so long that the entire muscle mass was excited before any portion had recovered from refractiveness. However, during faradic stimulation of the ventricles the rapid stimulation frequency would shorten refractory periods and decrease conduction velocity such that it would become possible for one wave of excitation to spread more than once over the same group of fibers.

In 1914, Garrey (26) confirmed the work of Mines (78) by inducing continuous, circulating contraction waves in rings of ventricular

muscle cut from the hearts of marine turtles. These "circus contractions" which developed in the presence of unidirectional conduction blocks were considered by Garrey (26) to represent the essential phenomena of fibrillation. In contrast to the uniform "circus contractions" observed in isolated rings of muscle, the abnormal contractions of fibrillation in the intact heart were attributed to the number and complexity of bypass pathways open to a circulating impulse. Furthermore, Garrey (26) indicated that fibrillation was impossible in small tissue masses because the time required for an impulse to propagate through all possible circuits was less than the refractory period of the tissue. In larger masses of tissue the length and number of potential circuits would be greater and thus the greater the probability of an impulse circulating until it reexcites tissue that has passed out of refractoriness.

Further support for the role of reentry in the production of fibrillation was added by the studies of Lewis and his collaborators (65, 67) on atrial flutter and fibrillation. By determining the order of electrical activation of the right atrium using direct bipolar leads these investigators suggested that during atrial flutter and fibrillation an excitation wave circulated in the atria along a path consisting of the taenie terminalis and around the great veins. Because of the similar appearance of local electrograms in atrial and ventricular fibrillation, Lewis (64) reasoned that a similar mechanism was probably responsible for both arrhythmias. However, Lewis (64) pointed out that there was no direct evidence for excitation waves circulating in the ventricles.

Renewed interest in the automatic focus theory of ventricular fibrillation was provided by Scherf et al. in 1950 (104). The topical application of aconitine crystals to a localized area of the ventricular surface in dogs resulted in the appearance of ectopic beats and ventricular tachycardia. Cooling the site of application arrested the tachycardia which reappeared upon rewarming. Once the ventricular tachycardia had degenerated into sustained fibrillation, cooling the site of aconitine application or any other area of the ventricular surface had no effect on the fibrillation. The authors (104) concluded that the aconitine created a single center of automatic impulse formation which could be suppressed by cooling. Furthermore, the degeneration of the ventricular tachycardia into fibrillation was considered to be due to the formation of multiple centers of automaticity, such that the cooling of any one center could no longer abolish the arrhythmia.

The experiments of Scherf et al. (104) indicated that fibrillation could be initiated from a single, rapidly firing, ectopic focus, but they did not demonstrate that the maintenance of fibrillation depended upon the appearance of multiple centers of automaticity. If the aconitine-induced focus fired more rapidly than all of the surrounding fibers were capable of following, then wave fronts would fractionate and the conditions necessary for reentrant excitation would be established.

Further studies on the mechanism of aconitine-induced fibrillation were undertaken by Sano and Sawanobori (102) using cultured

ventricular muscle from the rat embryo. Each preparation consisted of a mass of tissue a few cell layers in thickness and several hundred microns in diameter. In 50% of the preparations the addition of aconitine resulted in an irregular, rapid movement in all parts of the tissue. The action potential recorded by a single microelectrode was similar with that of adult, mammalian cardiac fibrillation and thus, the authors suspected that fibrillation was present in the cultured cells. Furthermore, the fibrillatory activity was considered to arise from a single automatic focus. The possibility of reentry was eliminated because of the small size of the preparation and because action potentials recorded simultaneously from different parts of the muscle mass were roughly synchronous. The authors emphasized the fact that the synchronism throughout the fibrillating mass of cultured cells was in marked contrast to the chaotic, asynchrony of different fibers in adult ventricular fibrillation (101). Therefore, the authors indicated that the arrhythmia in the cultured cells might correspond to the initiating focus in adult fibrillation with the maintenance of the arrhythmia involving some other mechanism (102).

The absence of diastolic depolarization in either normal ventricular muscle or fibrillating ventricular muscle (41) makes it difficult to accept a mechanism for the maintenance of fibrillation based on automatic foci. Although it is possible that pacemakers within the His-Purkinje system might become sites of rapid impulse formation during fibrillation, fibrillatory activity has not been observed in isolated papillary muscles containing bundles of Purkinje fibers

capable of multifocal pacemaker activity (41). Furthermore, it is difficult to account for the relationship between tissue mass and the ability of cardiac muscle to sustain fibrillation on the basis of multiple centers of automaticity. Although it has been suggested that small hearts will not sustain fibrillation due to an insufficient number of automatic centers (104) the experiments of Garrey showed that both small hearts and small pieces of tissue excised from large hearts would sustain fibrillation as long as local faradic current was applied.

Although there is no direct evidence for reentrant excitation as the mechanism responsible for the maintenance of fibrillation, the indirect evidence of Mines (78) and Garrey (26) strongly suggest that this arrhythmia depends upon multiple, asynchronous reentry. The concepts of Mines (78) and Garrey (26) have been tested in a mathematical model developed for a digital computer by Moe et al. (84). The model consisted of a two-dimensional matrix containing 992 hexagonal units. The units were similar to cardiac cells in the following manner:

- (1) each unit was assigned a refractory period,
- (2) each unit, when not in its refractory phase, could be fired by a neighboring unit and
- (3) each unit could transmit excitation to a non-refractory neighboring unit.

Different units were assigned slightly different refractory periods to stimulate the normal inhomogeneity of refractory periods in cardiac tissue. Rapid excitation of a group of units resulted in self-sustained turbulent activity resembling fibrillation. The activity was not due to fixed reentry circuits, but rather was supported by multiple, irregular wavefronts which varied in position,

size and number. Increasing the refractory periods of all of the units or reduction of the area of the model resulted in arrest of the activity. Although direct test of the model in cardiac tissue was not possible, the model confirmed the earlier concepts of both Mines (78) and Garrey (26) regarding the mechanism of fibrillation. Furthermore, the model demonstrated that multiple reentrant activity need not depend upon anatomical obstacles or functional abnormalities of the myocardium; the intrinsic inhomogeneity of recovery among myocardial cells should provide the substrate for the maintenance of multiple reentry circuits and fibrillation.

C. Measurement of Ventricular Vulnerability to Fibrillation

The first evidence for the existence of cardiac "vulnerable" period during which applied stimuli are likely to produce fibrillation was provided by the experiments of DeBoer in 1921 (19). DeBoer (19) found that a stimulus applied to the ventricle of a frog at the end of the refractory period resulted in a series of extrasystoles while the same stimulus delivered later in the cycle resulted in only a single response. More definitive studies in 1936 by Ferris et al. (23) demonstrated that shocks of .03 seconds were effective in fibrillating the canine ventricles provided the shocks were delivered during the T-wave of the electrocardiogram. In 1940 Wiggers and Wegria (132) provided conclusive evidence that the last .06 seconds of systole in the canine heart constituted a cardiac vulnerable period. It was shown that during this time a single induction shock or condenser discharge applied to a small portion of the myocardium could elicit fibrillation. A later study by the same investigators (127) demonstrated that brief shocks (40 msec or less) applied outside of the vulnerable period never resulted in fibrillation regardless of their strength. Wiggers and Wegria (133) went on to define the ventricular "fibrillation threshold" as the current strength of a brief DC shock of constant duration (10-30 msec) which is just able to induce fibrillation when applied during the vulnerable period of late systole. Despite repeated fibrillations and defibrillations, the fibrillation threshold was highly reproducible with no abrupt changes over a period of four to five hours in the open-chest animal (133).

In 1941, Moe, Harris and Wiggers (83) expanded the initial observations of Wiggers and Wegria (132, 133) on the nature of the vulnerable period. They demonstrated that a strong stimulus delivered during the vulnerable period resulted in a series of multiple, accelerating extrasystoles which would either be followed by a pause and resumption of normal rhythm or would degenerate into sustained fibrillation. By mapping the spread of activation of the accelerating extrasystoles on the surface and interior of the ventricles, they concluded that the initial tachycardia which preceded the development of fibrillation originated from a single focus located close to the site of the stimulating electrodes.

The experiments of Hoffman et al. (42) in 1951 suggested that the tendency for fibrillation to develop following the delivery of a single stimulus during the vulnerable period could be related to the basic excitability of cardiac tissue during the relative refractory period. They found that during a very brief portion of the relative refractory period the excitability threshold of a bipolar stimulus was lower than the immediately preceding or following periods of the cardiac cycle. The period of enhanced excitability during the relative refractory period was termed "dip" in the excitability curve. In addition to enhanced excitability, the period of time corresponding to the "dip" portion of the curve was associated with the lowest ventricular fibrillation thresholds (42). Cranefield et al. (17) determined separate excitability curves for both anodal and cathodal stimuli, and found that the abrupt dip in excitability during the relative refractory

period was only characteristic of anodal stimuli.

The greater susceptibility of the heart to anodal current during the vulnerable period is consistent with the finding that the current required to produce ventricular fibrillation with cathodal stimuli is 2.5 times greater than current required by anodal stimuli (25). Although there has never been an adequate explanation of the dip phenomenon or the greater vulnerability of the heart to anodal currents, the two phenomena may be related to the ability of anodal currents to shorten the duration of the action potential during phase 3 (16).

In addition to the measurement of cardiac vulnerability to fibrillation by the delivery of single stimuli during the vulnerable period, other techniques have been reported involving the delivery of multiple stimuli. In 1964, Han (33) introduced a technique employing a train of constant current rectangular pulses (100 Hz) that scanned the T-wave of the electrocardiogram. This technique eliminated the need to repeatedly scan the relative refractory period with single stimuli to determine the point of maximum vulnerability, and thus minimized the time necessary to carry out a fibrillation determination. In the same report (33), the author described an alternative method for the quantitation of ventricular vulnerability involving the continuous application of a train of 100 Hz rectangular pulses of twice the diastolic threshold. The number of premature beats evoked by the train was shown to be directly related to the duration of the train. The index of vulnerability was taken as the minimum duration of a train which could produce a sufficient number of accelerating premature beats to elicit fibrillation.

In addition to the measurement of fibrillation thresholds with rectangular pulses of current, some investigators have tested cardiac vulnerability to fibrillation using 60 Hz alternating current (39, 115, 119). The quantities measured have been both the magnitude of the current (39) and the duration of stimulation (119) necessary to evoke ventricular fibrillation. Sugimoto et al. (115) found that the magnitude of the current required to fibrillate the heart with a 60 Hz AC train (5 sec) was about 30 times less than the current required with a single rectangular stimulus (10 msec). By varying the duration of the AC train, they found that the current required to fibrillate was directly related to the number of premature responses evoked by the train. When the duration of a train was adjusted to produce five or six repetitive premature beats, the fibrillation threshold was less than one milliampere and approached the diastolic threshold. The authors (115) concluded that during 60 Hz stimulation the fibrillation threshold is progressively reduced after the first five or six premature beats, thus making it possible to induce fibrillation with very weak current.

In addition to the nature of the testing pulses, a number of other physical factors have been shown to influence the absolute magnitude of current necessary to induce ventricular fibrillation. The number of heart beats between successive test stimuli (118), the size and configuration of the testing electrodes (11), the distance of separation between the electrode poles (118), and the position of the electrodes on the ventricles (48, 107, 109) have all been shown to

significantly affect the fibrillation threshold. Shumway et al. (107) reported that fibrillation thresholds were least when measured on the right ventricular surface followed by the posterior surface of the left ventricle and then the anterior surface of the left ventricle. More recent studies (48, 109) have indicated that the fibrillation threshold measured in the left ventricular endocardium is significantly less than the epicardial threshold, but not different from the threshold measured at either the epicardium or endocardium of the right ventricle.

D. Physiological and Pharmacological Influences on Ventricular Vulnerability to Fibrillation

The quantitative nature of a ventricular fibrillation threshold measurement has made it possible to evaluate the effects of a wide spectrum of physiological and pharmacologic interventions on the vulnerability of the heart to fibrillation (85, 138). Because of the large number of physical factors (enumerated in Section C) which affect the absolute current required to evoke fibrillation, the absolute ventricular fibrillation threshold has little physiological significance. Therefore, the relative change in the fibrillation threshold from its control value provides the only meaningful index of evaluating the effect of various interventions on the vulnerability of the heart to fibrillation.

The validity of the use of the ventricular fibrillation threshold in determining changes in the vulnerability of the heart to fibrillation is supported by the fact that the majority of experimental interventions which alter the ventricular fibrillation threshold result in a similar directional change in the spontaneous susceptibility of the heart to fibrillation (85, 138). Myocardial ischemia produced by ligation of a coronary artery in the experimental animal decreases the ventricular fibrillation threshold (33, 54, 107, 112, 134) and results in the appearance of spontaneous ectopic activity and fibrillation (38, 134). Generalized myocardial hypothermia in the experimental animal decreases the ventricular fibrillation threshold and increases the likelihood of spontaneous ectopic activity and fibrillation (13, 14). Metabolic acidosis predisposes the human heart to

ventricular fibrillation and decreases the ventricular fibrillation threshold in experimental animals (28, 99). In addition, the increase in the ventricular fibrillation threshold following the administration of therapeutic doses of quinidine (126), lidocaine (30) or procaine amide (133) to experimental animals is consistent with the ability of these agents to suppress spontaneous ventricular arrhythmias in both man and experimental animals (5, 82).

Some insight into the mechanism by which various influences alter the ventricular fibrillation threshold was provided in a series of papers by Han, Moe and co-workers (33 - 37). These reports indicated that there was a close correlation between the amount of asynchrony of recovery of excitability of closely adjacent areas of the myocardium and the ventricular fibrillation threshold. Recovery of excitability was determined by the measurement of functional refractory periods using multiple (four to eight) pairs of bipolar electrodes inserted into closely adjacent areas of the myocardium. The asynchrony of recovery of excitability was expressed as the range of variation between the refractory periods determined at the different test sites. The authors evaluated the effect of heart rate (36), premature stimulation (34), and adrenergic mediators (35) on both asynchrony of recovery of excitability and the ventricular fibrillation threshold measured with a single stimulus delivered during the vulnerable period.

In the case of heart rate, it was shown that, as cardiac cycle length was increased from 300 msec to 700 msec, refractory periods

lengthened, the dispersion of refractory periods increased by about 20 msec and the fibrillation threshold fell by about 30% (36).

Following an early premature beat, refractory periods shortened, temporal dispersion of refractoriness increased by 10 to 15 msec and the fibrillation threshold decreased by about 50% (34). The changes in dispersion of refractoriness and fibrillation threshold were maximal within a 12 millimeter radius of the electrode site used to elicit the premature response. At greater distances from the electrode site, there was little change in either dispersion of refractoriness or fibrillation threshold between the control beats and the premature beats.

In the experiments dealing with adrenergic influences, Han et al. (35) found that stimulation of the left stellate ganglion shortened refractory periods by up to 15 msec, increased temporal dispersion of refractoriness by five to 10 msec and decreased the multiple response threshold by 34%. The threshold for multiple ventricular responses was determined instead of the fibrillation threshold to avoid the delay required for fibrillation and countershock defibrillation. In contrast to the effects of sympathetic nerve stimulation, an intravenous infusion of epinephrine or norepinephrine (2 ug/kg/min) resulted in a transient decrease in the multiple response threshold followed by a sustained increase above control (35). Although refractory periods shortened throughout the duration of the infusion, the initial period of enhanced vulnerability was accompanied by an increase in temporal dispersion of refractoriness, while the dispersion of refractory periods decreased as the fibrillation thresholds increased. The

biphasic effects of the catecholamines on both vulnerability and temporal dispersion of refractoriness were attributed to the myocardial distribution of the catecholamines during the infusion (35). It was presumed that during the start of an infusion, when catecholamine levels in the blood were rapidly increasing, the myocardial distribution was nonuniform, becoming more uniform with time as the blood levels of catecholamine stabilized.

The importance of the effect of nonuniform recovery of excitability on the ventricular fibrillation threshold was further emphasized by Spear et al. (112). These investigators measured the temporal dispersion of refractoriness following a single premature beat induced by either a single stimulus or train of stimuli delivered during the vulnerable period. Refractory periods were determined from a circular array of six bipolar electrodes surrounding a central bipolar electrode used to deliver current during the vulnerable period. Increasing the intensity of a train or single stimulus at the central site resulted in an increase in the temporal dispersion of refractoriness following the premature beat at the peripheral electrodes. In addition, the fibrillation threshold of the premature beat, measured at the central electrode, decreased as the intensity of the train or single stimulus delivered during the preceding vulnerable period was increased. Since all hearts contain a certain degree of intrinsic asynchrony of recovery of excitability, the authors (112) suggested that the fibrillation threshold was a measure of the additional asynchrony of recovery of excitability that must be added to the myocardium by the stimulating

current to cause multiple, asynchronous reentry and fibrillation. Furthermore, it was hypothesized that interventions which alter the intrinsic level of homogeneity of recovery of excitability would likewise affect the fibrillation threshold by increasing or decreasing the amount of "extra inhomogeneity" that must be added to the myocardium by the stimulating current to elicit fibrillation (112).

Although asynchrony of recovery of excitability may be an important determinant of the ventricular fibrillation threshold, the level of resting excitability may also affect the current required to induce fibrillation. The ability of therapeutic doses of quinidine, procaine amide or aprindine to elevate the ventricular fibrillation threshold may be related in part to the ability of these three antiarrhythmic agents to decrease the resting level of excitability (27, 126, 136). Guam et al. (27) have shown that changes in the resting level of excitability produced by infusion of potassium chloride correlate quite well with changes in the ventricular fibrillation threshold. An increase in serum potassium ion concentration of about 40% resulted in a significant decrease in both the excitability threshold (measured in end-diastole) and the fibrillation threshold. Further infusion of potassium chloride to increase the potassium ion concentration by 120% of control resulted in an excitability threshold and fibrillation threshold which were significantly greater than control. Since there were no changes in the dispersion of refractory periods associated with either of the two hyperkalemic states, the authors concluded that the changes in the fibrillation threshold were

most likely mediated by changes in the resting level of excitability (27).

Because of the potential importance of the autonomic nervous system in the genesis of sudden cardiac death in man, the effects of altered autonomic states on the ventricular fibrillation threshold have received considerable attention. Verrier et al. (120) demonstrated that stimulation of the posterior hypothalamus resulted in a 40% reduction of the ventricular fibrillation threshold in closed-chest dogs. The increase in the vulnerability of the heart to fibrillation was shown to be due to a direct effect of sympathetic nerves on the ventricles and independent of hemodynamic changes. The fibrillation threshold in the experiments of Verrier et al. (120) was measured with a special technique described as sequential R/T pulsing. The R/T pulsing technique involved the delivery of two low intensity stimuli in rapid succession resulting in two premature systoles. A third stimulus of variable intensity was used to scan the vulnerable period of the second premature systole to determine the fibrillation threshold. Using the R/T pulsing technique in closed-chest animals the same group of investigators (121) demonstrated that reflex changes in cardiac sympathetic tone, mediated by carotid sinus baroreceptors, also resulted in marked changes in the fibrillation threshold.

More recent studies by Matta et al. (73) have documented the importance of changes in sympathetic tone on ventricular vulnerability to fibrillation in the conscious animal. Since the use of conscious animals precluded the induction of fibrillation, the authors used

the R/T pulsing technique to measure the threshold for the production of multiple ventricular extrasystoles. An earlier study from the same laboratory (74) in anesthetized animals had shown that, regardless of the level of sympathetic tone, the current required to produce multiple ventricular extrasystoles was a constant fraction (66%) of the current required to produce fibrillation. Changes in sympathetic tone in the conscious animal were elicited by creating a stressful environment via instrumental aversive conditioning. Exposure of the animals to such an environment resulted in a 50% decrease in the multiple response threshold. Administration of the cardioselective, beta-adrenergic blocking drug, tolamolol, eliminated the stress-induced changes in threshold (73).

In addition to a sympathetic influence on ventricular vulnerability to fibrillation, there is also evidence that the parasympathetic nervous system may alter the ventricular fibrillation threshold. Kent et al. (53) provided the first conclusive evidence that stimulation of the cervical vagosympathetic trunk increases the ventricular fibrillation threshold. In the same study, the parasympathetic innervation of the ventricles was histologically mapped using acetylcholinesterase staining. Only sparse cholinergic innervation was identified in ventricular muscle, while a dense innervation of the proximal portion of the Purkinje system was observed. From their histological studies, the authors concluded that the decreased vulnerability of the ventricles to fibrillation produced by vagal stimulation was mediated by cholinergic nerve fibers which supply the specialized ventricular conduction system (53).

The experiments of Kolman et al. (56) provided further insight into the mechanism by which the vagus alters vulnerability to fibrillation. These experiments showed that in closed-chest, chloralose-anesthetized animals, vagal stimulation had no effect on the ventricular fibrillation threshold. However, when sympathetic tone was augmented by thoracotomy or by electrical stimulation of the left stellate ganglion, vagal stimulation produced a significant increase in the fibrillation threshold. Furthermore, the effect of the vagus on the fibrillation threshold was eliminated following beta-adrenergic blockade. The authors concluded that the vagal effect was indirect, and was expressed by counteracting the effects of elevated adrenergic tone on ventricular vulnerability.

The findings of Kolman et al. (56) have been confirmed by Rabinowitz et al. (91) and Yoon et al. (137); thus, it would appear that a parasympathetic-sympathetic interaction is responsible for the effect of vagal activation on the fibrillation threshold. The absence of a direct effect of the vagus on cardiac vulnerability is consistent with the absence of a direct effect of acetylcholine on ventricular transmembrane action potentials (44). Furthermore, the ability of the vagus to antagonize the effects of sympathetic tone on the fibrillation threshold is consistent with the ability of the vagus to antagonize the effects of sympathetic tone on ventricular contractile force.

E. Release of Autonomic Mediators by Electrical Stimulation of the Heart

The first experimental evidence indicating that electrical stimulation of the heart causes release of autonomic mediators was provided by Lewis in 1921 (66). Lewis recorded direct bipolar leads from different areas of the right atrium, while a faradic current (45 Hz) was applied to the right atrial appendage. A local area with a radius of eight to 20 millimeters surrounding the point of stimulation achieved rates of 1,500 to 2,400 b/m during the stimulation, while the remainder of the atria responded at rates of only 300 to 500 b/m. Following atropine, the rapid local response was eliminated, with the entire atrium responding to faradic stimulation with rates of 300 to 400 b/m. The author concluded that the faradic shocks excited local vagal nerve endings which shortened local refractory periods, allowing a more rapid rate of response.

In 1943, Knowlton (55) examined the response of isolated turtle atria to single induction shocks applied during the absolute refractory period. After a single strong induction shock, there was a decrease in contractile strength of the isolated atrium. The response required several heart beats to fully develop and lasted for a period of up to a minute. With stronger induction shocks, a negative chronotropic response was also observed. The prevention of the negative inotropic and chronotropic responses by atropine and their prolongation by eserine led the author to conclude that acetylcholine was liberated at vagal terminals by the induction shocks.

In 1958, Whalen (128) studied the response of isolated feline atria and papillary muscles to various intensity driving stimuli delivered by means of large platinum electrodes lying alongside the muscle. When the stimulus voltage used to drive the papillary muscles was suddenly increased, (2-10 times threshold) there was a gradual increase in the force of the contractions after a few seconds latency. When the voltage was returned to threshold, the force of the contractions gradually returned to baseline. Strips of atrial muscle under the same conditions showed a depressed contractile force during the high-voltage drive followed by a positive rebound when the voltage was returned to threshold. Atropine eliminated the depression and unmasked an augmentation of atrial contractile force during the high-voltage drive. In a subsequent study, Whalen et al. (129) repeated the high-voltage stimulation experiments on muscles that were obtained from cats which had been previously subjected to thoracic sympathectomy (8-12 days). In the denervated atrial muscles, the depression was more pronounced than in innervated muscles, and there was no positive rebound following termination of the high voltage drive. The authors (129) concluded that supramaximal driving stimuli liberated both acetylcholine and norepinephrine in both atrial and ventricular muscle, the predominant response (potentiation or depression) depending upon the relative amount of each transmitter released.

In contrast to the high-voltage driving stimuli employed by Whalen et al. (129), Brady et al. (7) attempted to stimulate nerve elements within an isolated feline papillary muscle by the application

of a train of high-intensity pulses during the absolute refractory period of the muscle. These investigators demonstrated that a train of supramaximal stimuli exerted a biphasic effect on contractile force: a depression of contractile force while the stimulus train was applied and a large potentiation of subsequent contractions. The potentiation was eliminated by the beta-adrenergic blocking agent, dichlorophenyl isopropyl aminoethanol, while the depression was not altered. The authors concluded that the depression was due to a direct membrane depressant effect of the current, while the potentiation was attributed to the liberation of norepinephrine from sympathetic nerve endings.

The most complete pharmacologic testing of the response of isolated cardiac muscle to electrical stimulation was performed by Blinks (6). Blinks (6) employed trains of high-voltage stimuli to drive isolated atria of guinea pigs along with atria and papillary muscle of kittens. An adrenergic response (increased contractile force) was observed in both atrial and papillary muscles, and was eliminated by either propranolol, pretreatment of the animals with reserpine, or chronic cardiac denervation (regional nerve ablation). Furthermore, the maximum positive inotropic response obtainable by electrical stimulation of a papillary muscle was equal to the maximum response obtainable by superfusion of the tissue with norepinephrine. A cholinergic response (decreased contractile force) was pronounced in the atrial muscle, but was only minimal in the ventricular muscle. Furthermore, a cholinergic response in the papillary muscle was only evident in the absence of the adrenergic response. The cholinergic

responses in both tissues were eliminated by atropine.

In addition to inotropic changes associated with electrical stimulation of isolated cardiac tissues, experiments by West and co-workers (2, 123) have demonstrated marked chronotropic and dromotropic responses to electrical stimulation of isolated heart preparations. Amory and West (1) observed hyperpolarization, bradycardia, and a secondary tachycardia in isolated rabbit sinoatrial nodes driven with supramaximal stimuli (12 volts). The hyperpolarization and bradycardia were eliminated by atropine and enhanced by physostigmine. The secondary tachycardia was blocked by guanethidine, bretylium, dichloroisoproterenol or pretreatment of the animals with reserpine. In a subsequent study, Vincenzi and West (123) demonstrated that electrical stimuli which were subthreshold for myocardial excitation were capable of exciting intramyocardial nerve fibers. Bursts of high-frequency stimuli (100 Hz) with a short pulse duration (less than .5 msec) were applied to the region of either the sinoatrial node or the atrioventricular node of the isolated rabbit heart. The stimuli resulted in the appearance of both negative and positive chronotropic and dromotropic responses. The negative responses were facilitated by physostigmine and blocked by atropine, while the positive responses were facilitated by cocaine and blocked by dichloroisoproterenol. Furthermore, both negative and positive inotropic responses were also observed when subthreshold stimuli were applied to isolated left atrial strips from dogs, guinea pigs, or rabbits. In feline papillary muscles, bursts of high-frequency stimuli resulted in a positive inotropic response only (123).

The observations of Vincenzi and West (123) that stimuli sub-threshold for the excitation of cardiac muscle may excite intramyocardial nerve fibers raises the question of whether autonomic nerves are ever excited by normal cardiac action potentials. Vincenzi and West (123) could find no evidence for autonomic excitation resulting from the rapid drive of an isolated preparation through an intracellular electrode. Furthermore, Blinks (6) found that although cocaine or propranolol had marked effects on the contractions of isolated papillary muscles driven with suprathreshold stimuli, the drugs had little effect on muscles driven with threshold stimuli. In the absence of any direct anatomical connections between myocardial cells and autonomic nerve fibers, it would seem unlikely that the extracellular current generated by depolarization of myocardial cells would be of sufficient magnitude to excite the terminal nerve fibers.

CHAPTER III

MATERIALS AND METHODS

A. Acute Surgical Procedure

Adult mongrel dogs of either sex weighing between 17 and 24 kg were anesthetized with sodium pentobarbital (30 mg/kg). A femoral artery was cannulated with polyethylene tubing (PE 240) for measurement of arterial blood pressure. Blood pressure was recorded via a Statham transducer (P2D3B) and a Grass polygraph (model 7). A femoral vein was also cannulated for injection of drugs or lactated Ringer's solution.

The dogs were ventilated with 40% O₂ using a positive pressure ventilator (Bird Mark 7) while a left thoracotomy was performed at the level of the fifth intercostal space. The ventilation of the thoracotomized animal was adjusted to maintain an arterial pO₂ of greater than 100 torr and an arterial pCO₂ of less than 40 torr. Decamethonium (10 mg/kg) was administered to produce neuromuscular blockade during the surgery, and supplemental doses (1 mg/kg) were administered as needed to maintain the blockade throughout the duration of the experiment. Supplemental doses of sodium pentobarbital (1-2 mg/kg) were also administered every one to two hours.

Following thoracotomy, the ansae subclavia originating from the right and left stellate ganglia were isolated and transected. The right and left stellate cardiac nerves were also transected in those

animals in which the nerves originated from the stellate ganglia rather than the ansae subclavia. Since the majority of cardiac sympathetic nerves funnel into the stellate ganglia and exit via the ansae subclavia (62,79,94), these denervation procedures should have produced a nearly complete sympathetic decentralization of the heart. Following sympathetic decentralization, total autonomic decentralization of the heart was produced by bilateral transection of the cervical vagosympathetic trunks.

Fat tissue overlying the pericardium was removed, the pericardium was incised and the heart was suspended in a pericardial cradle. The temperature of the myocardium was monitored via a YSI probe (model 425) sutured to the epicardium and connected to a YSI telethermometer (model 46). The temperature of the heart was held constant (36-38°) by means of an infrared heating lamp placed above the thoracic cavity.

B. Chronic Surgical Procedure

Chronic ventricular sympathectomy was attempted in 11 dogs, while nine dogs served as sham-operated controls. The denervation procedure used was a modification of the technique described by Kaye et al. (51). The animals were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with a positive pressure respirator (Bird Mark 7). Under aseptic conditions, a left thoracotomy was performed at the level of the fourth intercostal space. The pericardium over the pulmonary artery was opened and the heart suspended in cradle fashion. The adventitia surrounding the complete circumference of the pulmonary artery (approximately one centimeter distal to the pulmonary valve annulus) was transected along with the adventitia on the medial surface of the

aorta. The ventrolateral cervical cardiac nerve was transected at the junction of the pulmonary veins and left atrium. The circumference of the left superior pulmonary vein was dissected free, and connective tissue lying between the left atrium and right pulmonary artery was transected. Following these intrapericardial denervation procedures, the pericardium was loosely approximated with sutures, and the denervation was continued in the thorax. The ventromedial, ventrolateral, dorsal and innominate cardiac nerves were severed on the left side, while the recurrent cardiac nerve and ansae subclavia were transected on the right side of the animal. The nerves were identified according to the anatomical descriptions given by Mizeres (80).

In the sham-operated animals, the pericardium was opened and the heart was left undisturbed for a period of time (30 minutes) approximately equal to the amount of time required for the intrapericardial denervation. In addition, the thoracic cardiac nerves were isolated, but were not transected.

Following completion of the denervation or sham procedure, the chest was closed and the animals were treated with antibiotics. Two of the denervated animals died within the first postoperative week. The remaining nine denervated animals and the nine sham-operated animals were used in an acute experiment, two to four weeks after the initial surgery. The acute surgical procedures used on these animals were the same as described earlier in Section A. At the end of the acute experiment, tissue samples were taken from both ventricles and immediately frozen in liquid nitrogen for subsequent determination of norepinephrine levels using the trihydroxyindole-fluorometric method (18).

(Analyses were generously performed by Dr. S. B. Jones, Department of Physiology, Loyola University Medical Center.) Analyses of myocardial norepinephrine levels were completed in six of the chronically denervated animals and six of the sham-operated animals.

C. Walton-Brodie Strain Gauge Arch

The Walton-Brodie strain gauge arch used was a small U-shaped piece of brass with a variable resistor exposed to its back surface. The arch measured eight millimeters long and three millimeters wide and was sutured directly to the myocardium through two holes in each foot. The arches were modified so that one foot of each arch contained a unipolar electrode which was insulated from the arch. The electrode consisted of the tip of a 23 gauge stainless steel needle extending for a distance of three millimeters from the foot of the arch.

To obtain a maximal response from a strain gauge arch, the muscle fibers beneath the arch must be stretched from their resting length by about 40 to 50% (12). However, stretching the muscle fibers by more than 25% of their control length has been shown to result in damage of the fibers (98). In the present experiments, the arches were applied so that the muscle fibers beneath the arches were stretched by 20 to 30% of their control length.

The arches were sutured to the epicardium so that the long axes of each arch was parallel to the epicardial muscle fibers. Furthermore, the sutures were placed approximately two to three millimeters deep to try to encompass fibers which were nearly parallel to the surface fibers.

The absolute force developed by a strain gauge arch depends upon three important variables: (a) the stretch of the fibers beneath the arch (12), (b) the number of muscle fibers encompassed by the sutures used to anchor the arch (12), and (c) the orientation of the muscle fibers encompassed by the sutures relative to the long axis of the arch (88,106). Because it is impossible to account for all of these variables, only relative magnitudes of force can be accurately measured with the strain gauge arch. Quantitation of the strain gauge arch response in the present experiments was obtained by utilization of the percentage change of the force of contraction from its control level.

Since relative comparisons of contractile force are only valid if a strain gauge arch responds in a linear fashion, each arch used was calibrated to determine its linearity. One foot of each arch was clamped in a vice, and weights ranging from five to 100 grams were suspended from the other foot. Changes in resistance of the strain element of the arch were converted to a voltage signal using a Wheatstone bridge circuit and a Grass preamplifier (model 7P1). Using amplification settings similar to those used in actual experiments, the strain gauge arches were found to respond in a linear fashion to loads of five to 100 grams.

D. Electrical Stimulation of the Myocardium

To determine if electrical stimulation of the intact myocardium results in the release of autonomic mediators, trains of rectangular pulses were delivered at various points on the epicardial surface via

the electrodes embedded in the strain gauge arches. To prevent excitation of the myocardium during the stimulations, the trains were delivered during the absolute refractory period of the cardiac cycle. The absolute refractory period was rapidly located by recording a unipolar electrogram from the arch electrode with the indifferent electrode in the left hind limb. The electrogram, along with a lead II ECG, were amplified with a Grass (model 7P5) AC preamplifier (time constant .45 sec) and were displayed on a storage oscilloscope (Tektronix D13). Left atrial pacing was employed to maintain a constant heart rate (120-150 b/m). A synchronizing pulse from the pacing stimulator (Grass SD9) was used to trigger the sweep generator of the oscilloscope so that the time relationship between the pacing stimulus and the depolarization and repolarization complexes of the unipolar electrogram could be determined.

Once the time of excitation and repolarization of the muscle beneath the arch electrode had been determined, the arch electrode was switched to a stimulation mode. A synchronizing pulse from the pacing stimulator triggered a digital stimulator (Haer 4i) to generate a 100 msec rectangular pulse (40 volts) which was applied to the modulating input of a third stimulator (Grass SD9). The third stimulator generated a train of 10 rectangular pulses (100 Hz) which were delivered to the myocardium through the arch electrode. An 18 gauge needle placed in the left hind limb completed the circuit of current flow. The arch electrode was made cathodal with respect to the hind limb electrode. The delay control of the digital stimulator was set so that a train of stimuli was initiated after the muscle beneath the arch had depolarized,

and was terminated before the muscle started to repolarize. The current intensity of each train was determined by monitoring the voltage drop across a precision 100 ohm resistor in series with the stimulating electrodes. The voltage drop was measured with a Tektronix differential amplifier (model 5A20N) and was displayed on a storage oscilloscope (Tektronix D13).

Figure 1 shows an oscilloscope trace which illustrates the position of a train of stimuli relative to a surface ECG (lead II) and a unipolar electrogram recorded from an electrode in a strain gauge arch sutured to the outflow tract of the right ventricle (RVE). The oscilloscope was triggered to sweep by the pacing stimulator. The stimulus train seen at the bottom of the figure consisted of 10 pulses (4 msec, 100 Hz) delivered through a dummy load (500 ohms) instead of the myocardium. Figure 1 clearly demonstrates that the train started after the excitation wave had reached the arch electrode and terminated before the onset of local repolarization.

While recording local myocardial contractile force, trains of stimuli were delivered each cardiac cycle to the myocardium through strain gauge arch electrodes sutured to either the right or left ventricle. The approximate location of the arches used in most of the experiments is shown in Figure 2. In several experiments multiple arches were attached to either ventricle. In some animals the frequency, duration and intensity of individual pulses within a train were varied to determine the effect of these parameters on local myocardial contractile force. In another series of experiments, the

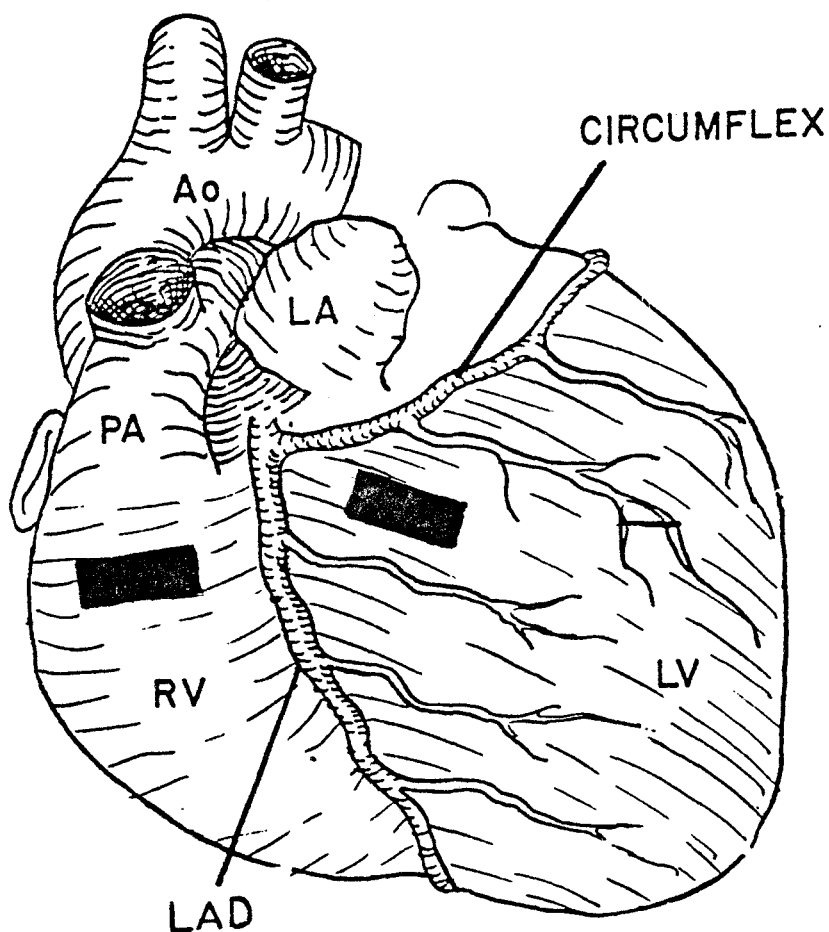
FIGURE 1

EXAMPLE OF A STIMULUS TRAIN USED TO ELICIT
CHANGES IN CONTRACTILE FORCE



A lead II ECG is shown along with a unipolar electrogram (RVE) recorded from an electrode embedded in a strain gauge arch sutured to the right ventricular outflow tract. The third channel (STIM) shows the timing of a 10 pulse stimulus train (100 Hz, 4 msec) delivered through a 500 ohm resistor in place of the myocardium. The train started after local excitation of tissue beneath the arch electrode and terminated before the onset of local repolarization.

FIGURE 2
THE POSITION OF STRAIN GAUGE ARCHES OR ELECTRODES
ON THE EPICARDIAL SURFACE



A schematic diagram of the heart illustrating the placement of strain gauge arches or electrode plaques for testing cardiac vulnerability to fibrillation. The solid rectangles represent the arches or electrode plaques. The landmarks labeled are the aorta (Ao), the left atrial appendage (LA), the pulmonary artery (PA), the right ventricle (RV), the left ventricle (LV) and the left anterior descending coronary artery (LAD).

internal cycle time on the digital stimulator was set so that the synchronizing pulses from the pacing stimulator were only effective in triggering the digital stimulator and generating a train once every six seconds. Changes in contractile force in response to trains of stimuli were measured during control conditions and following administration of either propranolol (.5-3 mg/kg) or atropine (.2 mg/kg). In addition, the effect of current trains on local myocardial contractile force was evaluated in chronically denervated and sham-operated control animals.

E. Ventricular Fibrillation Thresholds

Ventricular fibrillation thresholds were determined by using both trains of pulses and single pulses delivered during the vulnerable period of the cardiac cycle. The electrode used to measure the fibrillation threshold consisted of two 23 gauge stainless steel needles embedded in an acrylic plaque. The two needles were separated from each other by a distance of one centimeter and extended for a distance of three millimeters from the surface of the plaque. Small holes at either end of the plaque allowed the electrode to be sutured directly to the epicardial surface. A plaque electrode was sutured to both the right and left ventricles at the locations indicated in Figure 2.

The train method of testing cardiac vulnerability to fibrillation involved the delivery of a train of 14 rectangular pulses (4 msec, 100 Hz) during the vulnerable period of the cardiac cycle. While heart rate was held constant (120-150 b/m) by left atrial pacing, the pacing stimulator (Grass SD9) triggered a digital stimulator (Haer 4i) to generate the train. To correctly time the train of pulses to fall

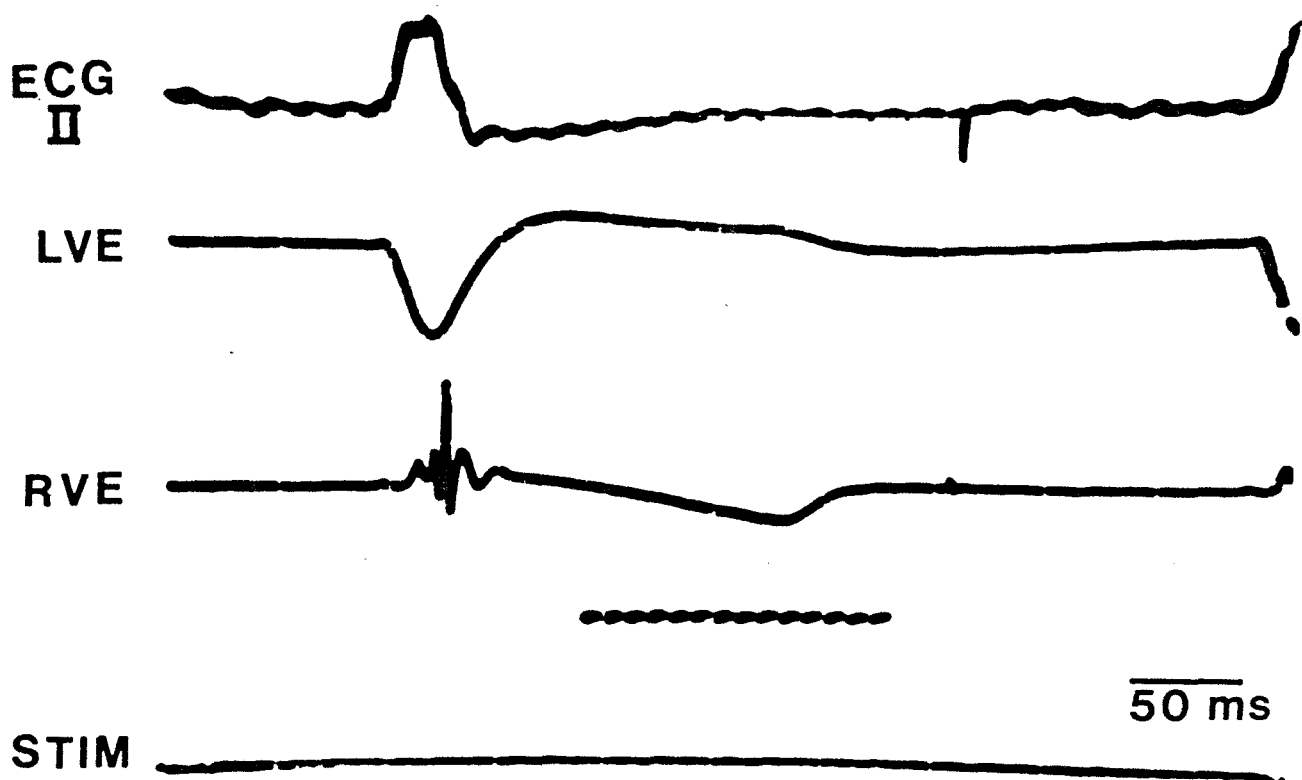
during the vulnerable period of the cardiac cycle, the stimulating electrodes were first used to record a local bipolar electrogram. The signal was amplified with a Grass (model 7P5) AC preamplifier (time constant .45 sec) and displayed on a storage oscilloscope (Tektronix D13). The oscilloscope sweep was synchronized to the pacing stimulator to determine the delay between the pacing stimulus and the T-wave of the bipolar electrogram. The appropriate delay was programmed into the digital stimulator so that the train would start during the ST-segment and terminate 20 msec beyond the T-wave of the local electrogram.

Figure 3 shows an oscilloscope trace which illustrates the timing of a train relative to the cardiac cycle. The right ventricular electrogram (RVE) in this figure was obtained from an electrode plaque sutured to the right ventricular outflow tract. The left ventricular electrogram (LVE) was recorded between a 33 gauge wire hooked into the endocardium of the left ventricle and a needle inserted into the left hind limb. The stimulus train shown in the bottom channel was delivered through a dummy load (500 ohms) to avoid excitation of the myocardium. As shown in the figure, the stimulus train started during ST-segment of the bipolar electrogram (RVE) and terminated 20 msec beyond the end of the local bipolar T-wave.

The current intensity of the trains used to test cardiac vulnerability to fibrillation was determined by monitoring the voltage drop across a precision resistor in series with the stimulating electrodes as described earlier in Section D. The trains were delivered to the

FIGURE 3

AN EXAMPLE OF A STIMULUS TRAIN USED TO TEST
CARDIAC VULNERABILITY TO FIBRILLATION



Shown are a lead II ECG, a unipolar electrogram from the left ventricle (LVE) and a bipolar electrogram recorded from a plaque electrode sutured to the right ventricular outflow tract. The stimulus train in the bottom channel (STIM) consisted of 14 pulses (4 msec, 100 Hz) delivered through a 500 ohm resistor instead of the myocardium. The stimulus train started during the ST-segment of the bipolar electrogram (RVE) and terminated 20 msec beyond the end of the local bipolar T-wave. The artefact in the ECG following the T-wave was due to the atrial pacing stimulus.

myocardium starting at intensities below the level necessary to evoke multiple extrasystoles and were increased by .2 to 1 ma increments until ventricular fibrillation occurred. A time interval of six seconds (12 to 15 heart beats) was used to separate successive trains.

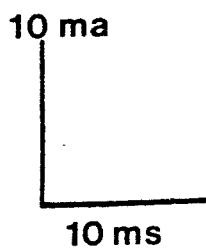
The current intensity of individual pulses within a train usually tended to decrease during the train, indicating the presence of electrode polarization. Figure 4 shows an oscilloscope trace of the current levels of a single stimulus (panel A) and a train of stimuli (panel B) delivered to the right ventricle. In panel B, the first pulse in the train had an intensity of 31 ma while the last pulse had an intensity of 30 ma. Changes in current intensity during the single pulse (panel A) were less obvious than during the train. Regardless of the absolute current level of a train, changes in current intensity from the first pulse to the last pulse were never more than 10% of the current intensity of the initial pulse. To minimize permanent electrode polarization due to the buildup of electrolyte deposits, the stimulating electrodes were immersed for several seconds in a mixture of concentrated nitric and hydrochloric acids following each experiment.

The method of testing cardiac vulnerability to fibrillation using a single stimulus involved scanning the vulnerable period of the heart with a 10 msec rectangular pulse. The method used for the generation and timing of the stimulus was similar to the method described for the generation and timing of the trains of stimuli. The single stimulus was delivered to the myocardium beginning in the absolute refractory period and moving by 5 msec increments through the

FIGURE 4

THE METHOD OF MONITORING STIMULUS CURRENT DURING
A FIBRILLATION THRESHOLD DETERMINATION

A



B

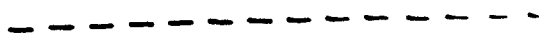
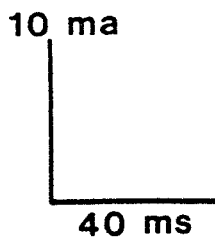


FIGURE 4

LEGEND

The figure shows an oscilloscope display of the voltage drop across a 100 ohm resistor in series with the stimulating electrodes. The stimulating electrodes were sutured to the outflow tract of the right ventricle. A current and time calibration are shown in the lower right hand corner of each panel. In panel A, a single stimulus (30 ma, 10 msec) was delivered to the myocardium, while in panel B, a train of stimuli (4 msec, 100 Hz, 14 pulses) was applied. The current intensity of the train fell from 31 ma during the first pulse to 30 ma during the last pulse indicating the presence of electrode polarization. In contrast to the slight amount of polarization during the train, the current level of single stimulus remained constant.

relative refractory period. Successive stimuli were separated by a six second recovery period. The stimuli were started at current intensities below the level necessary to evoke multiple extrasystoles or fibrillation and were increased by .5 to 1 ma increments. At each current intensity, the relative refractory period was scanned at 5 msec intervals to insure that the stimuli would fall during the most vulnerable phase of the cardiac cycle.

Once fibrillation had been initiated with either a train or single stimulus, defibrillation was achieved within 15 seconds by the delivery of a DC 30 watt-second pulse (Burdick DC/150) through paddles placed across the myocardium. To minimize damage to myocardial muscle, the paddles were applied outside of the pericardium. A 10 to 15 minute recovery period was allowed between successive determinations of fibrillation thresholds, and all thresholds were determined in triplicate. A fibrillation threshold was considered stable when three successive measurements did not deviate from each other by more than 20%. Since metabolic acidosis has been shown to significantly decrease ventricular fibrillation thresholds (28,99), arterial pH was measured in each experiment. When the arterial pH fell below 7.35, sodium bicarbonate was infused in sufficient doses to maintain the pH within a range of 7.35 to 7.45.

Ventricular fibrillation thresholds were determined with both trains of stimuli and single stimuli at both a right and left ventricular stimulating site (Figure 2). Following the establishment of stable, control thresholds, propranolol (.5 mg/kg) was administered and the

measurements were repeated. In one series of animals, right ventricular fibrillation thresholds were determined using trains before and after administration of atropine (.2 mg/kg). Fibrillation thresholds were also determined in the animals which had been subjected to either chronic, surgical denervation or sham thoracotomy. In some of the chronically denervated animals, fibrillation thresholds were determined before and after administration of propranolol (.5 mg/kg).

In addition to fibrillation thresholds, the excitability threshold for a single response was measured late in diastole in several animals. The threshold was measured via a pair of electrodes sutured to the anterior surface of the left ventricle (not the same electrodes used to test cardiac vulnerability to fibrillation). A single stimulus (10 msec) was delivered to the ventricle 20 to 40 msec before the arrival of the normal wave of excitation. The excitability threshold was taken as the current intensity of a stimulus which was just sufficient to evoke a single propagated response.

F. Local Electrogram Recordings During the Induction of Fibrillation

In a series of 10 animals, bipolar electrograms were recorded from different areas of the ventricles to determine if abnormal activation patterns could account for the genesis of multiple extrasystoles and fibrillation resulting from stimulation of the heart during its vulnerable period. The recording electrodes consisted of a 25 gauge needle threaded with a single Teflon-coated stainless steel wire (.005 inch diameter) bent at the tip to form a small hook. The tip of the wire was stripped of its insulation for one to two millimeters to

lower the electrode impedance and increase the area of contact with the myocardium. The needles were inserted into the myocardium for about two to four millimeters and then withdrawn, leaving the wires hooked into the heart muscle. Two to six pairs of recording wires were inserted in each animal.

The position of the recording electrodes relative to the stimulating electrode is shown in Figure 5. The solid circles represent the position of the bipolar needles used for delivering current. The needles were embedded in an acrylic plaque and sutured to the epicardium as described earlier in Section E. The open circles in Figure 5 represent the position of the bipolar recording electrodes. The two wires in each pair were separated by a distance of about seven to ten millimeters.

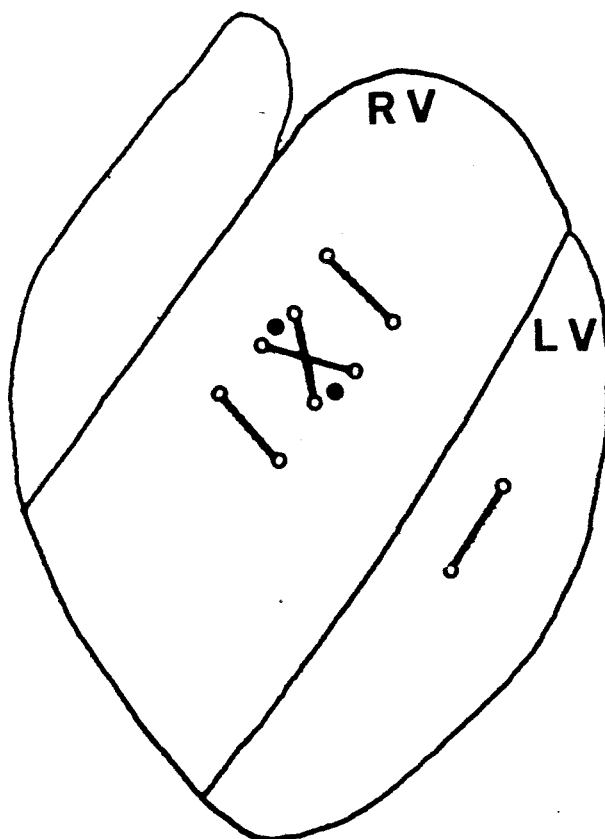
Each bipolar electrogram was amplified with a Gould differential AC preamplifier (model 13-4615-56). The preamplifier filters were set to pass frequencies of 30 Hz to 3 kHz. The bipolar electrograms along with a lead II ECG were displayed on a Gould-Brush direct writing recorder (model 2800) at a paper speed of 200 mm/sec. The recorder pens had a rise time of less than four milliseconds and would respond to frequencies of greater than 125 Hz.

G. Data Analysis

Where possible, all results were expressed as the mean plus or minus the standard error of the mean (\pm SE). Statistical comparison of means was performed using the Student's t-test for paired or unpaired samples. When it was necessary to make multiple group comparisons, a one-way or two-way analysis of variance was performed.

FIGURE 5

LOCATION OF STIMULATING AND RECORDING ELECTRODES FOR
ANALYSIS OF THE INITIATION OF FIBRILLATION



The solid circles indicate the position of a pair of bipolar stimulating electrodes on the right ventricle used to evoke multiple extrasystoles and fibrillation. The open circles indicate the position of bipolar recording electrodes. The two recording electrodes in each bipolar pair were separated by a distance of about seven to ten millimeters.

Following the analysis of variance, an F-test, t-test or Scheffe test was used to determine the significance of differences between individual means. The results of all statistical tests were considered to indicate the presence of a significant difference or variation when $p < .05$. The statistical methods and tables used were taken from Downie and Heath (20) and Sokal and Rohlf (108).

CHAPTER IV

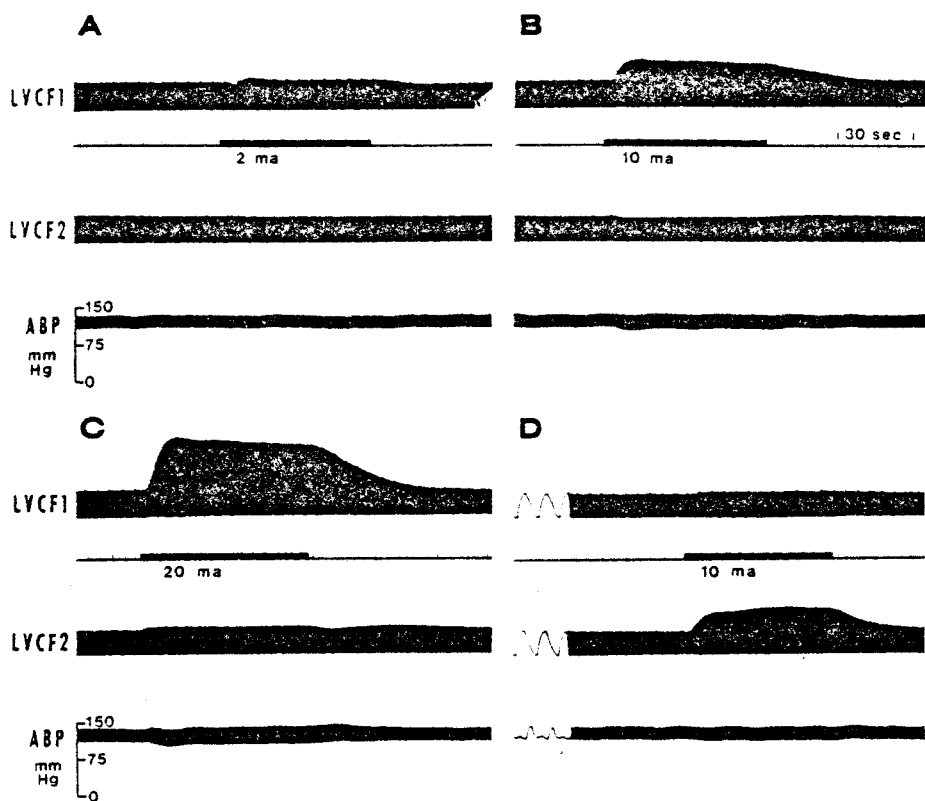
RESULTS

A. Contractile Force Changes in Response to Electrical Stimulation of the Myocardium

Figure 6 shows the response of an animal to trains of stimuli delivered to the myocardium through two strain gauge arches sutured to the left ventricle. The proximal arch (LVCF1) was sutured to the base of the left ventricle as shown in Figure 2. The distal arch (LVCF2) was sutured between the first and second diagonal branches of the left anterior descending coronary artery and was separated from the proximal gauge by a distance of about two centimeters. The trains consisted of 10 pulses (2 msec, 100 Hz) delivered once every 400 msec (heart rate 150 b/m). In panel A, 2 ma trains were delivered through the electrode in the proximal arch resulting in a 9% increase in local contractile force. In panel B, a 65% increase in contractile force resulted from the delivery of 10 ma trains, while in panel C, 20 ma trains resulted in a 160% increase in contractile force. In panels A and B there was little change in either arterial blood pressure or contractile force measured by the distal arch (LVCF2). In panel C, the distal arch showed a 15% increase in contractile force while pulse pressure increased by 10 mm Hg. In panel D, 10 ma trains were delivered through the electrode in the distal arch. Although the distal arch responded with a 90% increase in contractile force, the

FIGURE 6

TYPICAL RESPONSE OF THE LEFT VENTRICLE TO STIMULUS
 TRAINS OF VARIOUS INTENSITY



Two strain gauge arch recordings are shown from the anterior surface of the left ventricle (LVCF1 and LVCF2) along with an arterial blood pressure trace. A time line with a stimulus indicator is shown between the two contractile force recordings. In panels A thru C stimulus trains were delivered beneath the proximal arch (LVCF1) at current intensities of 2, 10 and 20 ma, respectively. In panel D, 10 ma trains were delivered beneath the distal arch (LVCF2). Since the two arches were separated by only a distance of about two centimeters, the highly localized changes in contractile force indicate a highly local excitation of autonomic fibers.

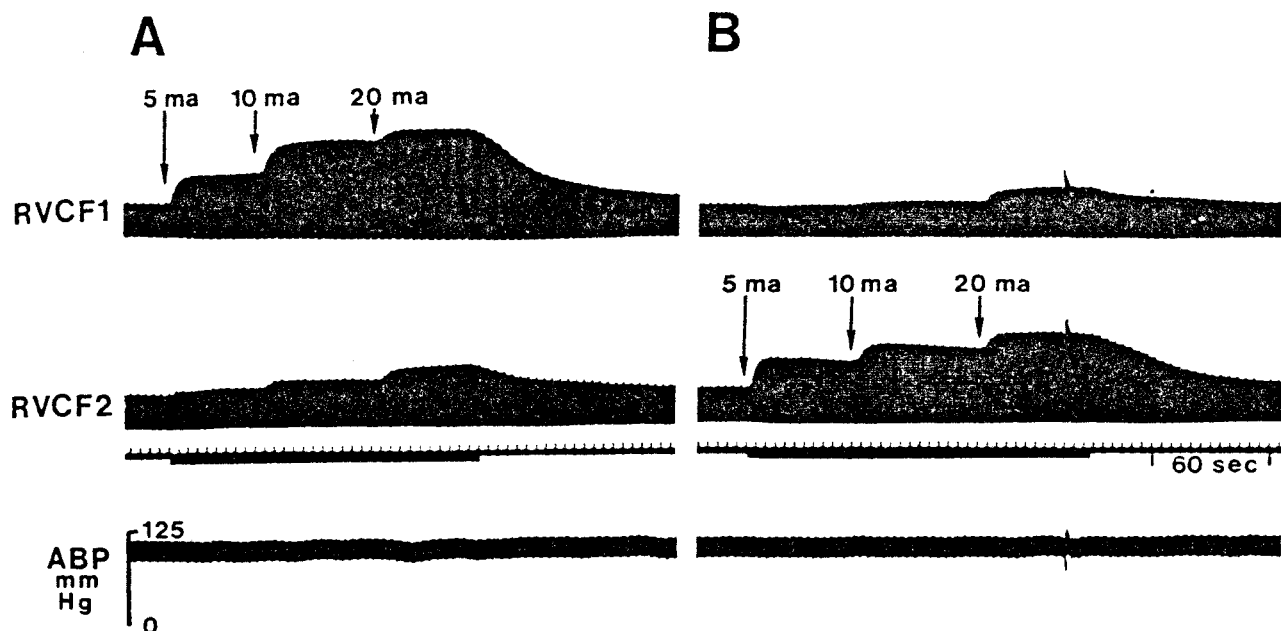
proximal arch showed only a 5% increase. Furthermore, there was no change in arterial blood pressure.

Figure 7 shows the response of the right ventricle to trains of stimuli. The record was obtained from an animal different from the one described in Figure 6. Two strain gauge arches were sutured to the right ventricular outflow tract, separated by a distance of approximately seven millimeters. The trains of stimuli consisted of 10 pulses (2 msec, 100 Hz) delivered once every 460 msec (heart rate 130 b/m). In panel A, the trains were delivered through an electrode in the proximal arch (RVCF1) while in panel B the distal arch (RVCF2) served as the site of stimulation. In both panels, there was an increase in local contractile force as current was increased from 5 to 20 ma. The responses were highly localized such that the greatest increase in contractile force was obtained from the arch which served as the stimulation site. The sudden surge in contractile force during the 20 ma trains in panel B was due to postextrasystolic potentiation following a premature contraction.

Results similar to those shown in Figures 6 and 7 were observed in six different animals in which multiple strain gauge arches were sutured to either the right or left ventricle. With current intensities of 20 ma or less, a strain gauge arch sutured more than 30 millimeters away from the site of stimulation consistently failed to record an increase in contractile force.

The effects of train intensity on myocardial contractile force were systematically studied in eight animals in which an arch was

FIGURE 7
 TYPICAL RESPONSE OF THE RIGHT VENTRICLE TO
 STIMULUS TRAINS OF VARIOUS INTENSITY

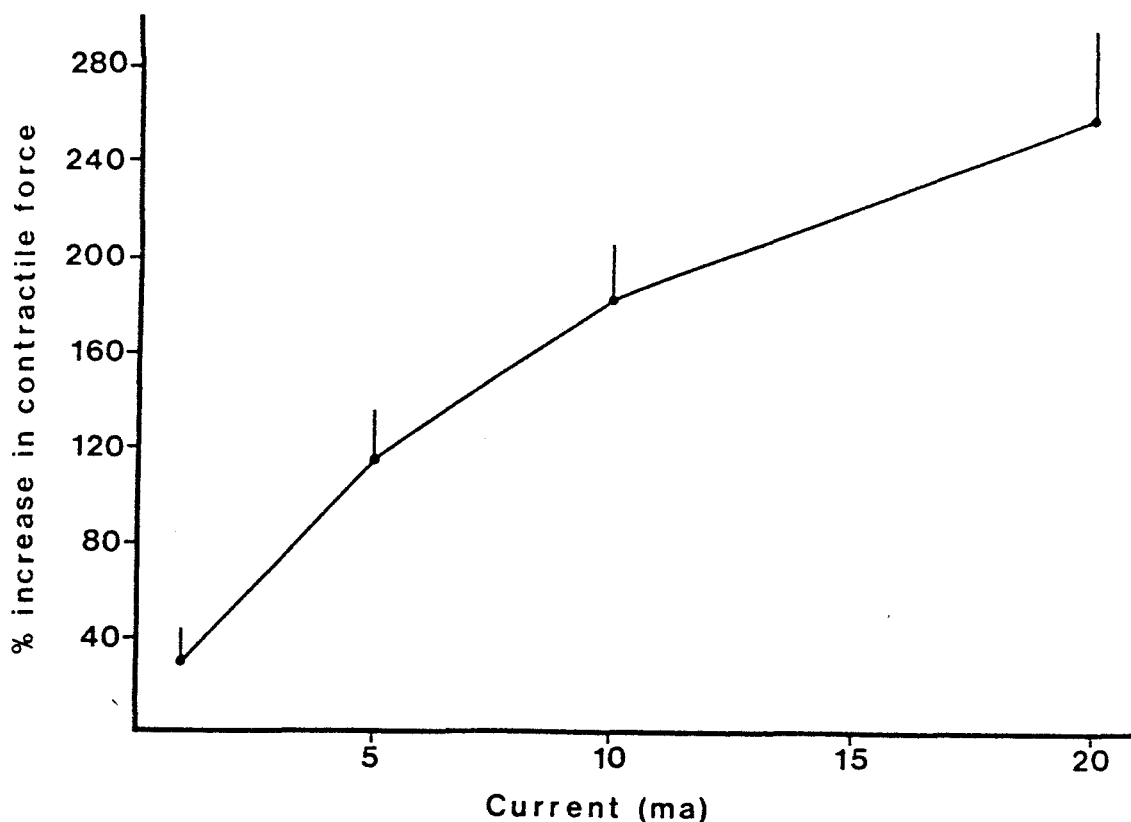


Two strain gauge arch recordings are shown along with an arterial blood pressure trace. The proximal arch (RVCF1) was sutured to the right ventricular outflow tract (Figure 2). The distal arch (RVCF2) was placed about seven millimeters below the proximal arch. In panel A, stimulus trains of 5, 10 and 20 ma were delivered through the proximal arch, while in panel B, the same trains were delivered through the distal arch. The responses were highly localized such that the greatest increase in contractile force was obtained from the arch which served as the stimulation site. The sudden surge in contractile force during the 20 ma trains in panel B was due to post-extrasystolic potentiation following a premature contraction.

sutured to approximately the same location on the right ventricular outflow tract (Figure 2). The trains consisted of 10 pulses (2 msec, 100 Hz) and were delivered during each cardiac cycle. The intensity of the trains was set initially at 1 ma and increased every 30 seconds to a maximum of 20 ma. Figure 8 shows a plot of the mean \pm SE increases in contractile force as a function of the current intensity of the trains. It is evident that contractile force increased in a nonlinear manner with each step increase in current. The data in Figure 8 were subjected to a one-way analysis of variance, and the resultant F value showed significance at the .01 level. A paired t-test was used to compare individual means. The t-test indicated that the increase in contractile force in response to 1 ma trains ($31 \pm 14\%$) was significantly less ($p < .005$) than the increase produced by 5 ma trains ($116 \pm 20\%$). Furthermore, the increase at 10 ma ($183 \pm 23\%$) was significantly different ($p < .005$) from both the increase at 5 ma and the increase at 20 ma ($260 \pm 37\%$).

The group of animals used to evaluate the effects of current intensity on right ventricular contractile force was also used to determine the effects of variations in stimulus duration, polarity, and frequency. With the current level of the trains held constant at 10 ma, pulse durations were varied between .01 msec and 5 msec. Contractile force started to increase at an average pulse duration of $.3 \pm .1$ msec and became maximal at a duration of 2 msec. With pulse duration, frequency, and current intensity held constant, changes in polarity of the strain gauge arch electrode (cathodal to

FIGURE 8
QUANTITATIVE EVALUATION OF THE EFFECT OF STIMULUS
STRENGTH ON CONTRACTILE FORCE



The abscissa shows the current strength of stimulus trains delivered to the right ventricular conus through a strain gauge arch, while the ordinate depicts the percentage increase in local contractile force recorded by the arch. Each point represents the mean \pm SE increase determined in eight different animals. Paired t-tests following a one-way analysis of variance indicated that the increase in relative contractile force associated with each step increase in current was statistically significant ($p < .005$).

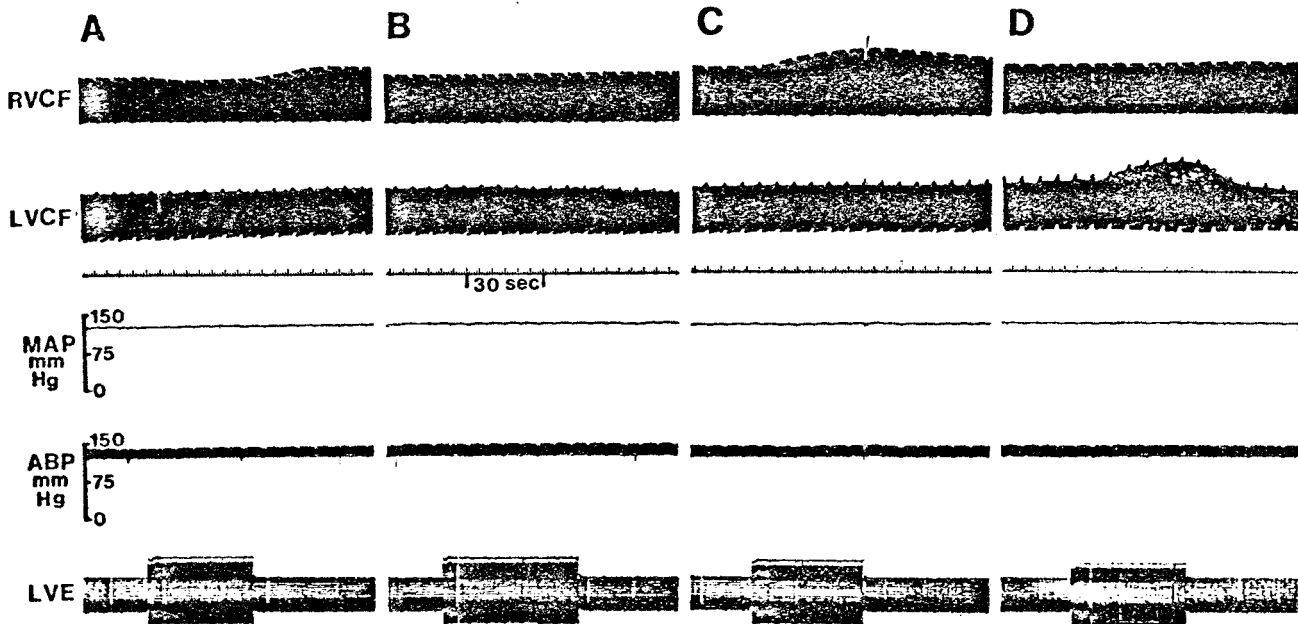
anodal) did not alter the increases in contractile force. In three animals the increases in contractile force produced by trains of five pulses (2 msec, 10 ma) at a frequency of 50 Hz were compared to changes produced by the same trains delivered at a frequency of 100 Hz. Identical increases in contractile force were observed at both stimulation frequencies.

Figure 9 shows the response of an animal to trains of stimuli following blockade of beta-adrenergic receptors with propranolol (2.5 mg/kg). The artefacts in the left ventricular electrogram (LVE) at the bottom of the figure indicated the onset of the trains of stimuli. In panel A, delivery of 10 ma trains (10 pulses, 2 msec, 100 Hz) to the right ventricle resulted in a 15% decrease in contractile force (RVCF). Although not shown in the figure, prior to beta-adrenergic blockade, the same trains resulted in a 187% increase in contractile force. In panel B, the trains were delivered to the left ventricle resulting in little change in local contractile force (LVCF). However, before propranolol the same trains produced a 135% increase in left ventricular contractile force. Panels C and D were recorded in the same animal following the administration of atropine (.2 mg/kg). An increase in contractile force of about 40% was observed in response to trains delivered to the right ventricle (panel C) and the left ventricle (panel D). Since the responses shown in panels C and D were obtained within 15 minutes following the responses in panels A and B, it was likely that the responses observed were related to the muscarinic blocking effects of atropine rather than a decrease in the plasma concentration of propranolol.

FIGURE 9

TYPICAL RESPONSE OF THE VENTRICLES TO STIMULUS

TRAINS FOLLOWING BETA-ADRENERGIC BLOCKADE



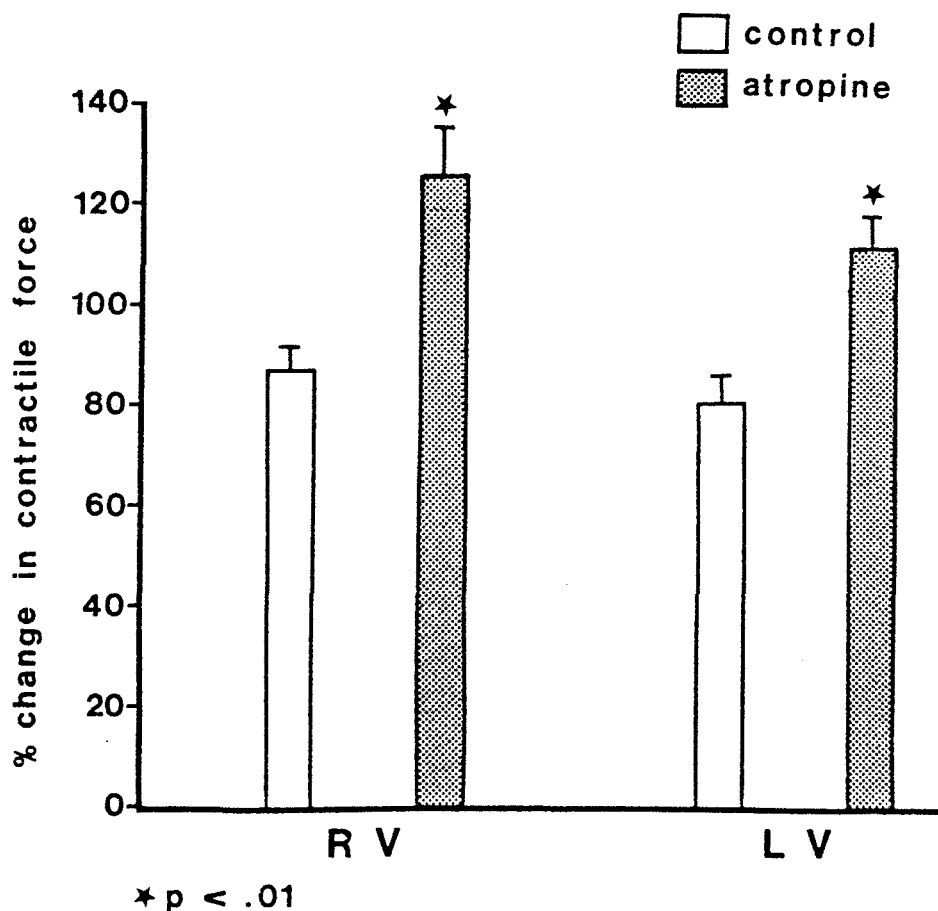
Strain gauge arch recordings are shown from the right (RVCF) and left (LVCF) ventricle along with pulsatile and mean arterial blood pressure. The artefacts in the left ventricular electrogram (LVE) in the bottom channel indicate the timing of the stimulus trains. Panels A and B were obtained in the presence of propranolol (2.5 mg/kg), while panels C and D were obtained in the presence of both propranolol and atropine (.2 mg/kg). In panel A, 10 ma trains delivered to the right ventricle resulted in a 15% decrease in local contractile force. In panel B, the same trains delivered to the left ventricle did not alter contractile force. Following the administration of atropine, the 10 ma trains produced a 40% increase in both right (panel C) and left (panel D) ventricular contractile force. The responses shown in panels C and D were obtained within 15 minutes of the responses shown in panels A and B.

Administration of propranolol resulted in a dose-dependent reduction in the magnitude of the positive inotropic response to trains of stimuli in eight different animals. Doses of 2 to 3 mg/kg were required to totally abolish the positive inotropic responses to 10 ma trains of stimuli. At these high doses of propranolol, a small negative inotropic responses (5-20%) was observed in the right ventricle in six of eight animals, while a decrease in left ventricular contractile force was observed in only three of the eight animals.

The effect of atropine (in the absence of propranolol) on the inotropic responses to trains of stimuli was also evaluated in eight animals. Figure 10 shows the mean \pm SE contractile force changes of the two ventricles in response to trains of stimuli delivered before and after atropine (.2 mg/kg). The trains consisted of 10 pulses (2 msec, 100 Hz) and were delivered for 30 seconds at a current intensity which was sufficient to produce an increase in contractile force of 70 to 100% under control conditions. To insure reproducibility, each stimulation was repeated twice and the sequence in which the two ventricles were stimulated was randomized. The duplicate trials did not vary by more than 10%. As shown in Figure 10, the increase in contractile force under control conditions was $87 \pm 4\%$ in the right ventricle and $81 \pm 5\%$ in the left ventricle. Following atropine, the same trains of stimuli increased right ventricular contractile force by $121 \pm 9\%$ and left ventricular contractile force by $112 \pm 6\%$. A paired t-test showed both changes to be highly significant ($p < .01$).

In eight of the chronically denervated animals and eight of the sham-operated animals, 10 ma trains of stimuli were delivered to both

FIGURE 10

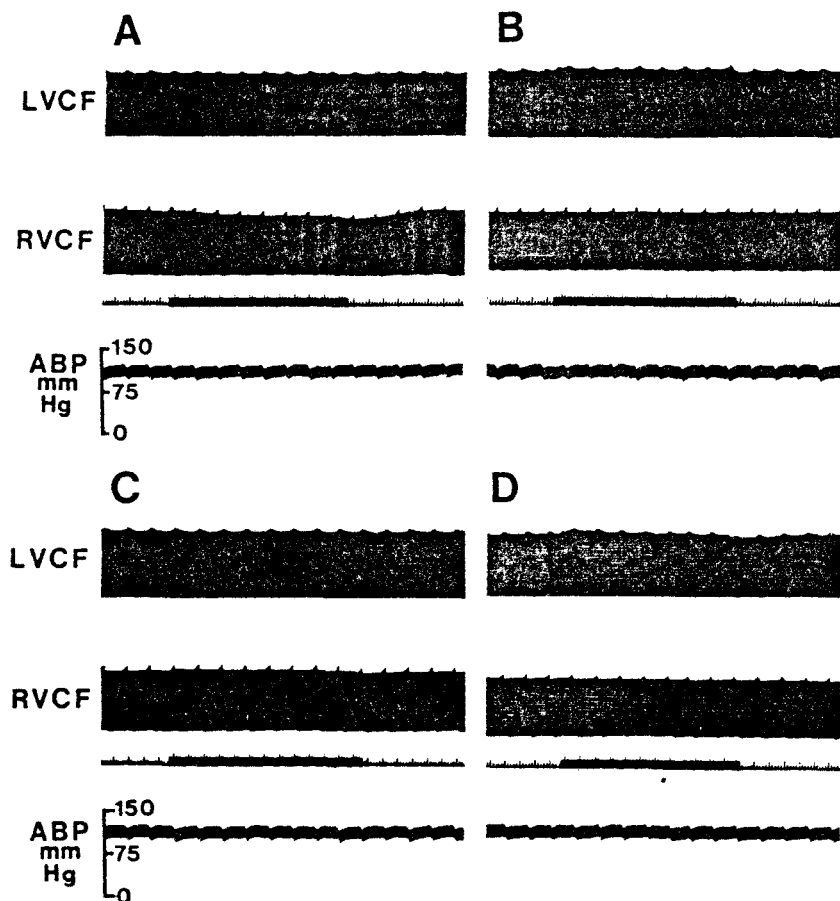
QUANTITATIVE EFFECT OF ATROPINE ON THE INOTROPIC RESPONSE
OF THE VENTRICLES TO TRAINS OF STIMULI

The bars represent the mean \pm SE increase in contractile force of both the right (RV) and left (LV) ventricle in eight animals. The open bars represent the control response to trains of stimuli, while the stippled bars represent the response following atropine (.2 mg/kg). A paired t-test showed that following atropine, the stimulus trains resulted in a significantly greater ($p < .01$) contractile force change in both chambers.

the right and left ventricle. Figure 11 shows the contractile force changes in response to the trains in one of the denervated animals. In panel A the trains were delivered to the right ventricle resulting in a 14% decrease in right ventricular contractile force (RVCV). Application of the trains to the left ventricle resulted in a 2% increase in contractile force (LVCF) as shown in panel B. Since the increase in contractile force in panel B developed immediately with the onset of the stimuli and disappeared immediately upon termination of the stimuli, it is doubtful that local autonomic mediators were involved. Panels C and D were obtained in the same animal five minutes after the administration of atropine (.2 mg/kg). Delivery of trains of stimuli to the right ventricle after atropine (panel C) did not result in the negative inotropic response observed under control conditions (panel A). The response of the left ventricle following atropine (panel D) was not different from the control response (panel B).

The absence of a significant positive inotropic response to trains of stimuli was a consistent finding in all of the denervated animals. In three of the eight denervated animals a slight negative inotropic response (5-15%) was observed in response to trains of stimuli applied to the right ventricle, while none of the animals displayed a negative inotropic response to trains applied to the left ventricle. The sham-operated control animals all demonstrated positive inotropic responses of at least 50 to 100% in response to 10 ma trains. Catecholamine analysis of multiple tissue samples taken from both ventricles in six of the denervated animals showed norepinephrine levels of .01 to .05 ug/g. Similar samples taken from six of the sham-operated

FIGURE 11
 INOTROPIC RESPONSE OF A CHRONICALLY DENERVATED
 HEART TO TRAINS OF STIMULI



Strain gauge arch recordings from the left (LVCF) and right (RVCF) ventricles are shown along with arterial blood pressure. A time line with a stimulus mark is shown below the RVCF recording. In panel A, 10 ma trains were delivered to the right ventricle resulting in a 14% decrease in contractile force. In panel B, the same trains, delivered to the left ventricle, resulted in little change in contractile force. Panels C and D were recorded during the delivery of trains of stimuli to the right (C) and left (D) ventricles five minutes following the administration of atropine (.2 mg/kg). Atropine eliminated the negative inotropic response in the right ventricle and did not alter the response in the left ventricle.

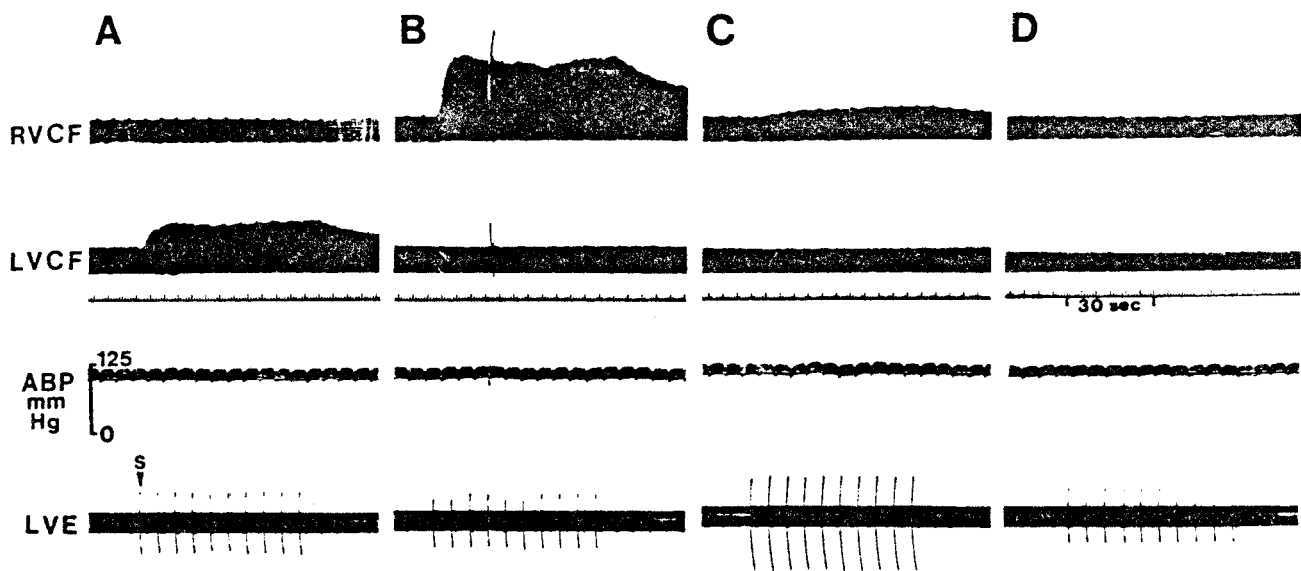
control animals showed norepinephrine levels of .5 to 1.2 ug/g.

Since the electrical testing of cardiac vulnerability to fibrillation involves the intermittent delivery of current to the myocardium, several experiments were performed to determine if contractility changes were associated with the delivery of trains of stimuli or single stimuli to the myocardium at six second intervals. Figure 12 shows a typical experiment in which trains of stimuli and single stimuli were delivered to the ventricles during a single cardiac cycle once every six seconds. In panel A, a 10 pulse train (10 ma, 4 msec, 100 Hz) was delivered to the left ventricle. The artefacts in the left ventricular electrogram (LVE) indicated the timing of the trains. After the first few trains, left ventricular contractile force (LVCF) increased to a steady state level which was 70% greater than control. In panel B, the same trains as in A were delivered to the right ventricle resulting in a 194% increase in local contractile force (RVCF). Single stimuli (10 msec, 10 ma) were delivered to the right ventricle in panel C resulting in a 30% increase in right ventricular contractile force. Panel D was obtained five minutes following the administration of propranolol (.5 mg/kg) and illustrates the total absence of changes in contractile force in response to trains of stimuli delivered to the right ventricle. Although not shown in the figure, propranolol also eliminated the contractile force changes shown in panels A and C.

Figure 13 shows a quantitative comparison of the changes in contractile force produced by trains (10 ma, 4 msec, 100 Hz) to the changes produced by single stimuli (10 ma, 10 msec). Each train or single stimulus was delivered once every six seconds for a total period of

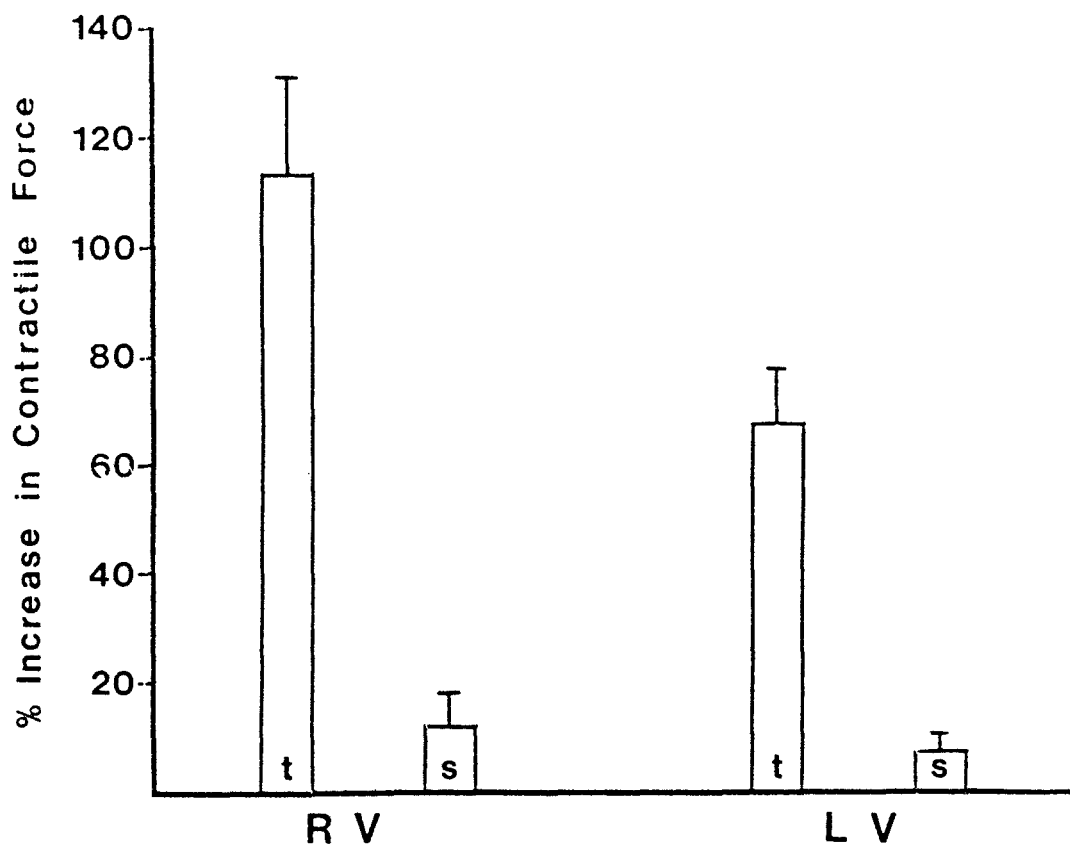
FIGURE 12

TYPICAL INOTROPIC RESPONSE TO STIMULUS TRAINS AND SINGLE
STIMULI DELIVERED AT SIX SECOND INTERVALS



Contractile force recordings are shown from both the right (RVCF) and left (LVCF) ventricles along with an arterial blood pressure trace. The artefacts in the left ventricular electrogram (LVE) in the bottom channel mark the onset of the stimuli. In panel A, 10 ma trains, delivered to the left ventricle, resulted in a 70% increase in local contractile force. In panel B, 10 ma trains were delivered to the right ventricle resulting in a 194% increase in local contractile force. In panel C, 10 ma single stimuli were delivered to the right ventricle resulting in a 30% increase in local contractile force. Panel D was obtained five minutes following the administration of propranolol (.5 mg/kg) and shows the total elimination of a positive inotropic response during the delivery of 10 ma trains to the right ventricle. Although not shown in the figure, the positive inotropic responses in panels A and C were also eliminated by propranolol.

FIGURE 13
COMPARISON OF THE INOTROPIC EFFECTS OF
STIMULUS TRAINS TO SINGLE STIMULI



Each bar represents the mean \pm SE increase in local contractile force during a 60 second period of stimulation. Successive trains (t) or single stimuli (s) were separated by a six second recovery period as shown in Figure 12. The data were obtained from a total of 16 animals with eight animals used to compare the two types of stimuli in each ventricle. A two-way analysis of variance followed by a Scheffe test indicated that in both ventricles the relative increase in contractile force in response to trains was significantly greater ($p < .01$) than the increase produced by single stimuli. Furthermore, the increase in right ventricular contractile force in response to trains was significantly greater than the increase in left ventricular contractile force ($p < .05$), while no significant difference ($p > .05$) was found between the response of the two ventricles to single stimuli.

60 seconds. The sequence in which the stimulations were performed was randomized, and each period of stimulation was repeated at least twice. Contractile force began to increase approximately two seconds following the first stimulus and achieved a steady state after the first three or four stimuli. The bars in Figure 13 represent the mean \pm SE increases of steady state contractile force in eight animals. The results were subjected to a two-way analysis of variance. The analysis of variance indicated that a significant interaction ($p < .05$) was present between the variables; therefore, the significance of differences between means was determined using a Scheffe test. The increase in right ventricular contractile force in response to trains ($117 \pm 18\%$) was significantly greater ($p < .01$) than the increase produced by single stimuli ($12 \pm 3\%$). Likewise, the increase in left ventricular force in response to trains ($68 \pm 10\%$) was significantly greater than the increase produced by single stimuli ($8 \pm 3\%$). With trains of stimuli, the right ventricular response was significantly greater ($p < .05$) than the left while no significant difference ($p > .05$) was found between the response of the two ventricles to single stimuli.

B. Ventricular Fibrillation Thresholds

The observation of changes in local myocardial contractile force in response to electrical stimulation of the myocardium raises the question of whether release of local autonomic mediators influence the current required to evoke ventricular fibrillation. Therefore, ventricular fibrillation thresholds were determined by the delivery of a train of stimuli to the myocardium during its vulnerable period and compared to the thresholds obtained by scanning the vulnerable period

with a single stimulus.

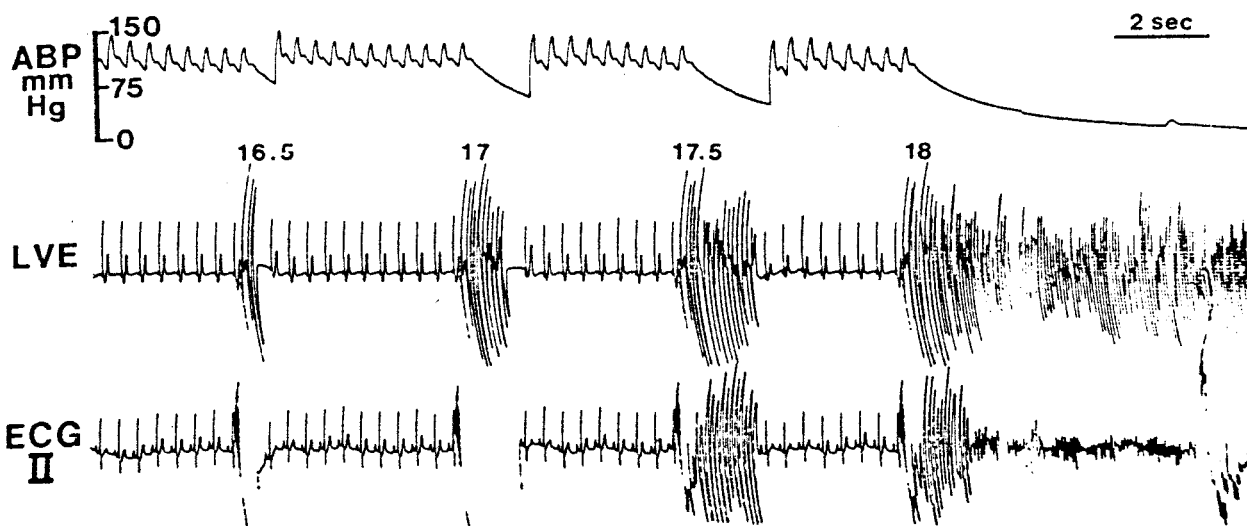
Figure 14 illustrates a typical experiment in which a ventricular fibrillation threshold was determined by the delivery of trains of stimuli (14 pulses, 4 msec, 100 Hz) to the left ventricle. The current intensity (milliamperes) of each train is indicated by the numbers above the premature beats in the left ventricular electrogram (LVE). It is evident that the number of multiple extrasystoles evoked by a train increased as the intensity of the trains increased until fibrillation was produced with a train of 18 ma. The record shows only the last four trains in a series of 27 trains which were started at 5 ma and increased by .5 ma increments. Following defibrillation of the animal and a 15 minute recovery period, the identical fibrillation threshold was obtained when only a total of nine trains were delivered starting at a current intensity of 14 ma. In general, it was found that the fibrillation threshold, measured with either trains of stimuli or single stimuli, was independent of the total number of stimuli delivered as long as at least six to 10 stimuli were delivered before fibrillation occurred. When fibrillation was evoked with less than six stimuli the thresholds tended to be unstable.

Figure 15 shows a quantitative comparison of fibrillation thresholds measured with trains to thresholds measured with single stimuli at both a right and left ventricular stimulating site. The data in the figure were obtained from a total of 32 animals, with each bar representing the mean \pm SE fibrillation threshold for eight different animals. Five animals were excluded from the study due to unstable

FIGURE 14

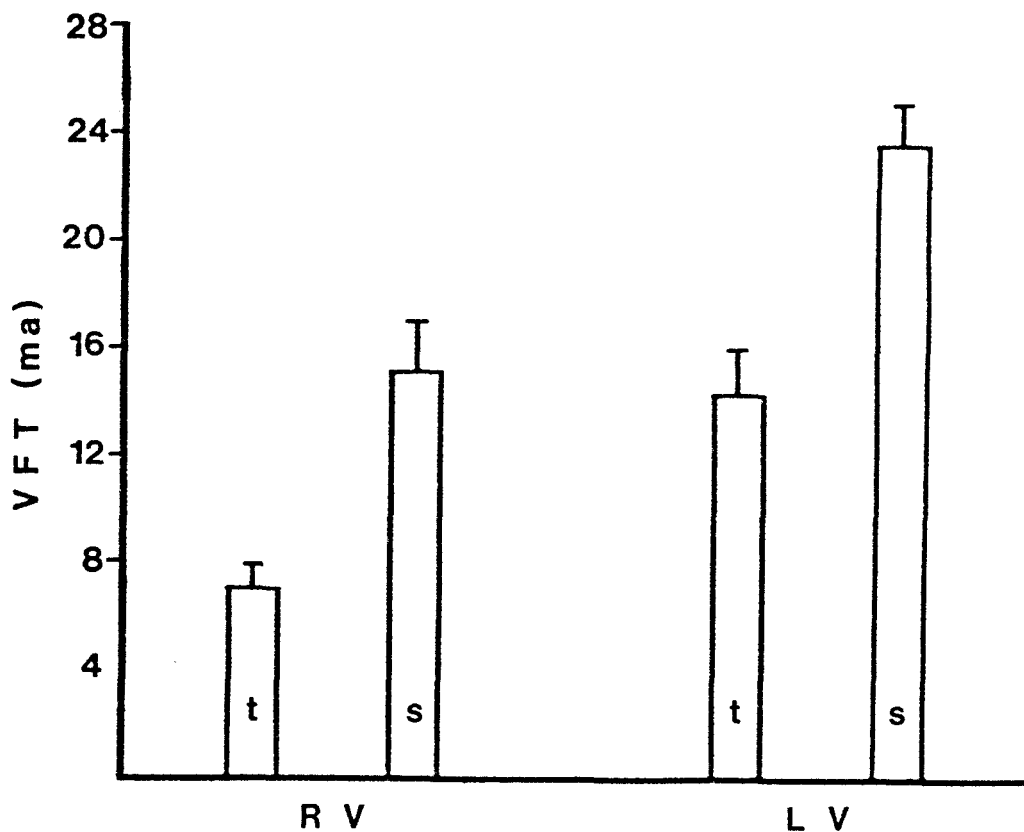
A TYPICAL EXAMPLE OF A FIBRILLATION THRESHOLD

DETERMINED WITH TRAINS OF STIMULI



A lead II ECG and a unipolar left ventricular electrogram (LVE) are shown along with an arterial blood pressure trace. The numbers above the left ventricular electrogram indicate the current intensity of stimulus trains (14 pulses, 4 msec, 100 Hz) delivered at six second intervals to the left ventricle. The number of multiple extrasystoles evoked by the trains increased as the intensity of the trains was increased until fibrillation was evoked with a train of 18 ma. The record shows only the last four trains in a series of 27 trains that were started at 5 ma and increased by .5 ma increments.

FIGURE 15
COMPARISON OF TRAIN FIBRILLATION THRESHOLDS
TO SINGLE-STIMULUS THRESHOLDS



Each bar represents the mean \pm SE fibrillation threshold (ma) determined in eight animals. A separate group of eight animals was used for each of the four determinations. The four groups of data were subjected to a two-way analysis of variance followed by an F-test. The fibrillation thresholds measured with trains (t) were found to be significantly less ($p < .01$) than the thresholds measured with single stimuli (s), while the thresholds measured at the right ventricular site (RV) were found to be significantly less ($p < .01$) than the thresholds measured at the left site (LV).

thresholds. The four groups of data in Figure 15 were subjected to a two-way analysis of variance to test the significance of effects due to the site of stimulation (RV vs LV) and method of stimulation (trains vs single stimuli). Fibrillation thresholds measured at the right ventricular site were found to be significantly less ($p < .01$) than the thresholds measured at the left ventricular site, while the thresholds measured with trains were significantly less ($p < .01$) than the thresholds measured with single stimuli. Furthermore, there was no significant interaction ($p > .05$) between the two variables.

Following the control measurements shown in Figure 15, propranolol (.5 mg/kg) was administered and the fibrillation threshold measurements were repeated. Beta-adrenergic blockade increased the right ventricular train threshold from $7.0 \pm .9$ ma to 21.6 ± 1.9 ma and the single stimulus threshold from 15.2 ± 1.9 ma to 19.4 ± 2.1 ma. At the left ventricular site, the train threshold increased from 14.4 ± 1.6 ma to 39.5 ± 2.4 ma, while the single stimulus threshold increased from 23.7 ± 1.5 ma to 30.2 ± 1.8 ma. A paired t-test indicated that each change in threshold produced by propranolol was statistically significant. The adequacy of the beta-adrenergic blockade was tested in each animal by the delivery of 10 ma trains (10 pulses, 4 msec, 100 Hz) at six second intervals to the right ventricle via a strain gauge arch electrode. The presence of beta-blockade was indicated in each animal by the absence of a change in contractile force during a 60 second stimulation period (Figure 12).

To compare the relative increases in fibrillation threshold following administration of propranolol, the change in each animal was

expressed as a percentage increase above the control threshold. The mean \pm SE percentage increases in fibrillation threshold are depicted by the bar graph in Figure 16. At both stimulation sites the relative increase in the threshold measured with trains was about seven times greater than the relative increase in the threshold measured with single stimuli. A two-way analysis of variance applied to the four groups of data in the figure indicated that the difference due to the method of stimulation (trains vs single stimuli) was highly significant ($p < .01$) and independent of the stimulation site (probably of variable interaction $> .05$). The analysis of variance also showed that there was no significant difference ($p > .05$) between the relative increase in the right ventricular threshold and the relative increase in the left ventricular threshold.

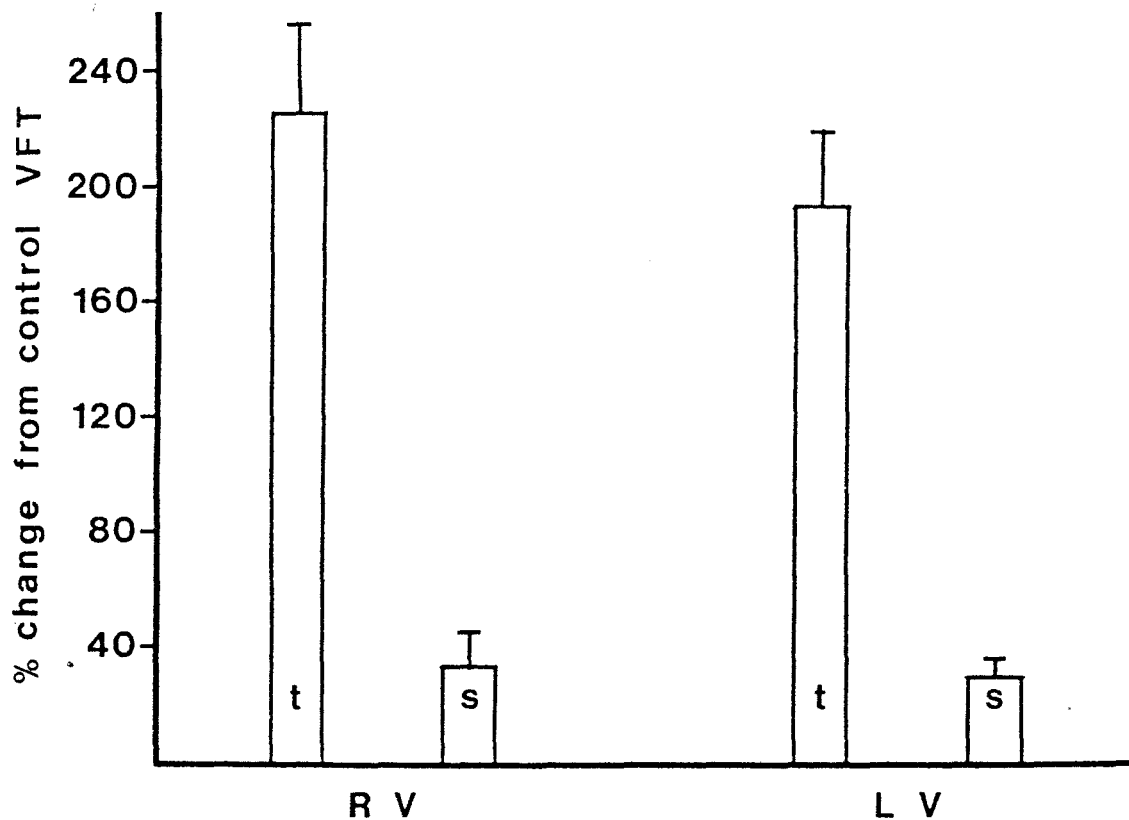
In addition to an elevation of the ventricular fibrillation threshold, administration of propranolol resulted in some slight hemodynamic changes. In the 32 animals used to obtain Figures 15 and 16, propranolol resulted in a decrease in spontaneous heart rate from 127 ± 4 to 120 ± 5 b/m ($p < .05$). With heart rate held constant, there was a decrease in right ventricular contractile force of $19 \pm 3\%$, and a slight decrease in mean arterial blood pressure from 110 ± 3 to 108 ± 4 mm Hg ($p > .1$). In nine of the 32 animals, the diastolic excitability threshold was measured before and after propranolol. The threshold increased from $.071 \pm .06$ to $.076 \pm .06$ ma; however, the increase was not statistically significant ($p > .1$).

In addition to the effects of beta-adrenergic blockade, the effects of cholinergic blockade on vulnerability to fibrillation was

FIGURE 16

EFFECT OF PROPRANOLOL ON TRAIN FIBRILLATION THRESHOLDS

COMPARED TO SINGLE-STIMULUS THRESHOLDS

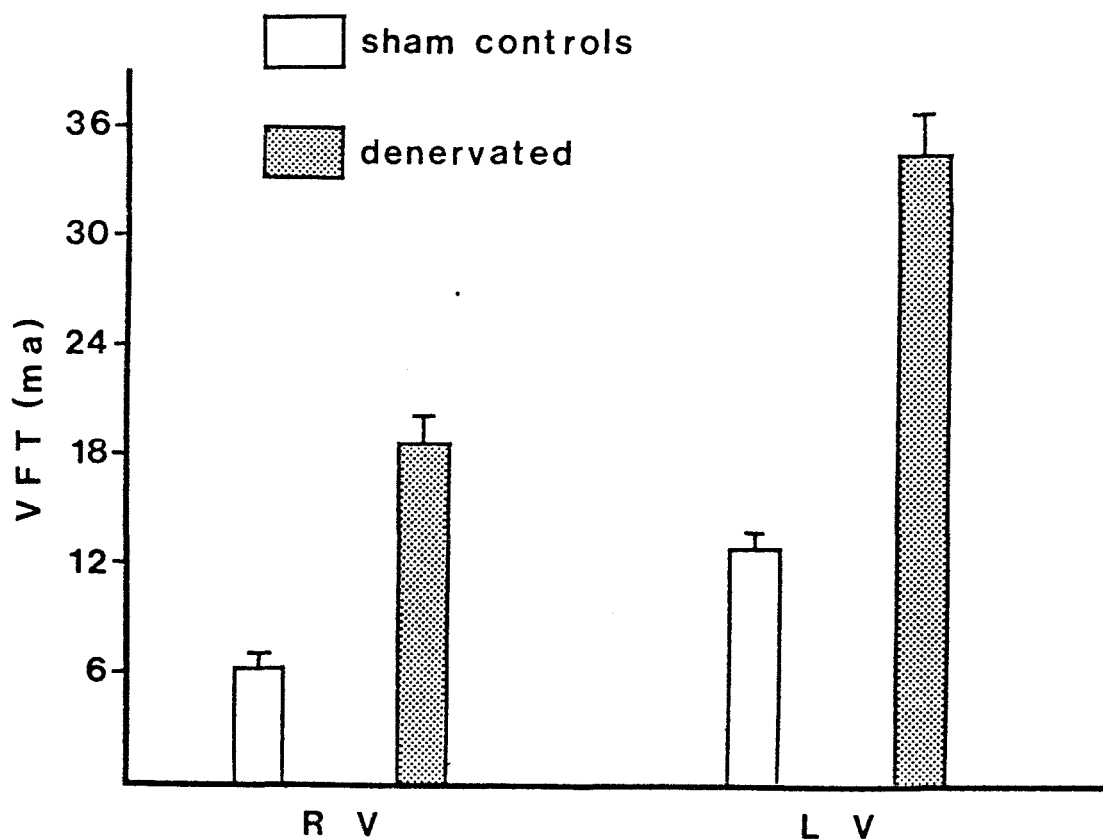


Each bar represents the mean \pm SE percentage increase in the fibrillation threshold above control in response to propranolol (.5 mg/kg). A two-way analysis of variance followed by an F-test indicated that the relative increase in the fibrillation threshold measured with trains was significantly greater ($p < .01$) than the relative increase in the single-stimulus threshold. Furthermore, with either stimulation technique there was no significant difference ($p < .05$) between the relative increase in the fibrillation threshold measured at the right ventricular site compared to the left ventricular site.

determined in eight animals. These animals were a separate group from those used to obtain the data shown in Figures 15 and 16. The fibrillation thresholds were determined with trains of stimuli at the right ventricular site before and after administration of atropine (.2 mg/kg). Under control conditions, the fibrillation threshold was found to be $7.8 \pm .8$ ma, a value not significantly different ($p > .1$) from the corresponding control threshold shown in Figure 15. Following the administration of atropine, the fibrillation threshold fell to $6.0 \pm .8$ ma. However, the difference between the two means did not reach statistical significance ($.1 > p > .05$). The adequacy of muscarinic blockade was indicated in each animal by the lack of a heart rate change in response to a 10 second stimulation of the distal end of the right cervical vagus (10 volts, 20 Hz, .5 msec).

The results of fibrillation threshold determinations with trains of stimuli in the chronically denervated and sham-operated control animals are shown in Figure 17. Each bar represents the mean \pm SE fibrillation threshold determined for eight animals. The same animals were used to determine the fibrillation threshold at both a right and left ventricular site. The results from one denervated and one sham-operated animal were not included due to unstable fibrillation thresholds. The paced heart rate (150 ± 6 b/m) and mean arterial blood pressure (107 ± 3 mm Hg) of the denervated animals were not significantly different ($p > .1$) from the paced heart rate (149 ± 4 b/m) and mean arterial blood pressure (103 ± 3 mm Hg) of the sham-operated control animals. Furthermore, the diastolic excitability threshold of the denervated hearts ($.067 \pm .005$ ma) was not significantly

FIGURE 17
EFFECT OF CHRONIC CARDIAC DENERVATION ON
TRAIN FIBRILLATION THRESHOLDS



Each bar represents the mean \pm SE fibrillation threshold (ma) determined in eight animals. In each denervated or sham-operated animal the thresholds were determined at both a right (RV) and left (LV) ventricular site. The four groups of data were subjected to a two-way analysis of variance followed by an F-test. The fibrillation thresholds of the denervated hearts were found to be significantly greater ($p < .01$) than the sham-operated hearts, while the thresholds measured at the right ventricular site were significantly less ($p < .01$) than thresholds at the left site.

different ($p > .1$) from the diastolic threshold of the sham-operated hearts ($.068 \pm .008$ ma). As shown in Figure 17, the fibrillation threshold measured at either site in the denervated animals was about three times greater than the threshold of the corresponding ventricle in the sham-operated animals. A two-way analysis of variance indicated that the elevation of the ventricular fibrillation threshold produced by chronic cardiac denervation was highly significant ($p < .01$) and independent of the site of stimulation (probability of variable interaction $> .05$). Furthermore, in both groups of animals the fibrillation threshold measured at the right ventricular site was significantly less ($p < .01$) than the threshold measured at the left ventricular site.

In six of the chronically denervated animals, fibrillation thresholds were determined at the right ventricular site following the administration of propranolol (.5 mg/kg). Beta-adrenergic blockade in these animals increased the fibrillation threshold from 17.8 ± 1.4 ma to 19.5 ± 2.4 ma. A paired t-test indicated that the increase was not statistically significant ($.1 > p > .05$). In contrast to the small change in fibrillation threshold, propranolol significantly slowed the spontaneous heart rate of the denervated animals from 140 ± 10 to 116 ± 9 b/m ($p < .05$). With the heart rate held constant, right ventricular contractile force fell by $23 \pm 5\%$, and mean arterial blood pressure showed a slight decrease (109 ± 6 to 108 ± 5 mm Hg) which did not achieve statistical significance ($p > .1$). The adequacy of beta-blockade was indicated in each animal by the absence of a heart rate response following the administration

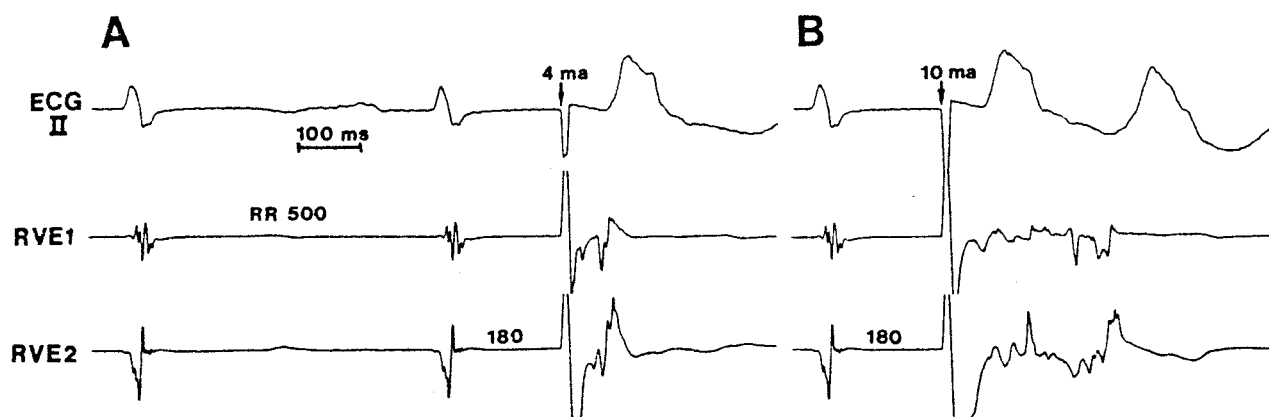
of norepinephrine (.2 ug/kg).

C. Electrographic Analysis of the Initiation of Fibrillation

The application of high intensity currents to the ventricles during the vulnerable period of the cardiac cycle resulted in the appearance of considerable abnormal extracellular electrical activity. Figure 18 illustrates a typical experiment in which abnormal local electrical activity was observed following the delivery of a single stimulus (10 msec) to the right ventricle during the vulnerable period of the cardiac cycle. The record shows a surface ECG (lead II) and two bipolar electrograms (RVE1 and RVE2) recorded from electrodes contiguous to the stimulating electrode (Figure 5). In panel A, while heart rate was paced at 120 b/m, a 4 ma stimulus was delivered 180 msec after the intrinsic deflection of RVE2. A stimulus artefact can be seen in all three traces with the largest artefact occurring in the local bipolar electrograms. Following the stimulus artefact, a single premature ventricular complex was observed in the surface ECG while both local electrograms showed fractionated complexes which lasted three times as long as the preceding control complexes. In panel B, the current intensity of the stimulus was increased to 10 ma resulting in the production of two closely coupled extrasystoles in the surface ECG. Both of the local electrograms displayed multiple disorganized electrical complexes which spanned the interval from the stimulus to the onset of the R-wave of the second extrasystole in the ECG.

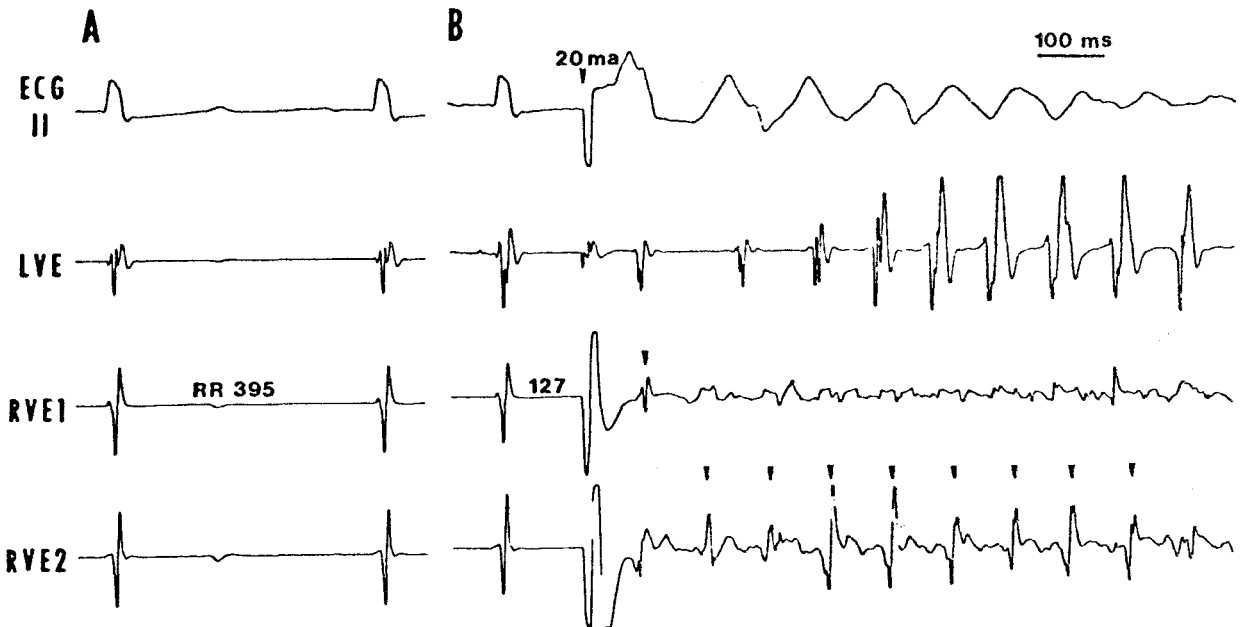
Figure 19 shows a record from another experiment in which abnormal, local electrical activity resulted from ventricular stimulation during the vulnerable period of the cardiac cycle. Panel A

FIGURE 18
 ABNORMAL LOCAL ELECTRICAL ACTIVITY FOLLOWING
 VULNERABLE PERIOD STIMULATION



Two bipolar electrograms from the right ventricle (RVE1 and RVE2) are shown along with a lead II ECG. The bipolar electrograms were recorded from electrodes contiguous to a pair of stimulating electrodes (Figure 5). The atria were driven at a basic cycle length of 500 msec. Following the control cycle in panel A, a 4 ma stimulus was delivered to the right ventricle 180 msec after the intrinsic deflection of the basic response in RVE2. A single premature systole appeared in the ECG while both bipolar electrograms showed some abnormal electrical activity lasting for about 75 msec following recovery from the stimulus artefact. In panel B, the stimulus strength was increased to 10 ma resulting in two premature ventricular systoles. The bipolar electrograms showed continuous, disorganized electrical activity which spanned the interval in the ECG between the two premature beats.

FIGURE 19
 ABNORMAL LOCAL ELECTRICAL ACTIVITY PRECEDING
 THE DEVELOPMENT OF FIBRILLATION



The electrograms shown are the same as in Figure 18 with the addition of a bipolar electrogram recorded from the anterior surface of the left ventricle (LVE). The atria were driven at a basic cycle length of 395 msec as shown in panel A. In panel B, a 20 ma stimulus was delivered 127 msec following the intrinsic deflection of the basic response in RVE1. The stimulus resulted in a series of accelerating extrasystoles which degenerated into sustained fibrillation. The left ventricular electrogram showed only uniform activation complexes during the initial cycles of the arrhythmia. The first activation complex that could be identified in RVE1 (arrow) was followed 50 msec later by continuous, disorganized electrical activity. Although some uniform complexes were identifiable in RVE2 (arrows), the complexes were bridged by continuous, disorganized electrical activity.

shows an ECG (lead II) and three local bipolar electrograms recorded during a control cycle (cycle length 395 msec). The two right ventricular electrograms (RVE1 and RVE2) were recorded from electrodes contiguous to the stimulating electrodes, while the left ventricular electrogram (LVE) was recorded from a pair of electrodes located in the anterior epicardium of the left ventricle (Figure 5). In panel B, a 20 ma stimulus (10 msec) was delivered to the right ventricle 127 msec following the intrinsic deflection of RVE1. The stimulus resulted in a series of multiple extrasystoles which degenerated into sustained fibrillation. Following a large stimulus artefact, a single uniform activation complex was observed in RVE1 (arrow) which was followed 50 msec later by continuous, disorganized electrical activity. In contrast to the total disorganization of electrical activity in RVE1, some uniform activation complexes could be identified in RVE2 (arrows). However, the uniform activation complexes were bridged by continuous, disorganized electrical activity. Continuous, local electrical activity was not observed in the left ventricle (LVE), although the activation complexes demonstrated a marked increase in amplitude and duration as the initial tachycardia degenerated to fibrillation.

Results similar to those shown in Figures 18 and 19 were observed in 10 different animals. As the current intensity of single stimulus delivered during the vulnerable period was increased, there was an increase in the duration of disorganized electrical activity recorded from the vicinity of the stimulating electrode. When the intensity of the stimuli became sufficient to evoke multiple

extrasystoles, the local electrical activity became continuous, bridging the interval between successive premature beats. Disappearance of the local disorganized electrical activity was always followed immediately by termination of the extrasystoles. Continuation of the local, disorganized electrical activity led to sustained fibrillation.

To determine if the disorganized electrical activity associated with multiple extrasystoles and fibrillation was confined to the local vicinity of the stimulating electrode, several experiments were performed in which multiple electrograms were recorded from the right ventricle. Figure 20 shows a record from an experiment in which four electrograms were monitored from the right ventricle. Two of the electrograms were recorded from electrodes placed six millimeters above (RVE1) and 15 millimeters below (RVE2) the stimulating electrode. The remaining two electrograms (RVE3 and RVE4) were obtained from electrodes contiguous to the stimulating electrodes. A left ventricular electrogram (LVE) and ECG are also shown. The figure shows a control cycle (cycle length 465 msec) followed by a 15 ma stimulus delivered 150 msec after the intrinsic deflection of RVE3. The stimulus resulted in three premature ventricular systoles followed by a premature supraventricular systole. The first ventricular extrasystole was a direct result of the stimulus current, while the second and third extrasystole were spontaneous. The arrows above the three local electrograms recorded away from the direct site of stimulation (LVE, RVE1 and RVE2) indicate the activation complexes associated with each ventricular extrasystole. Although these complexes demonstrated some

FIGURE 20
AREA OF TISSUE INVOLVED IN THE GENESIS OF
ABNORMAL ELECTRICAL ACTIVITY

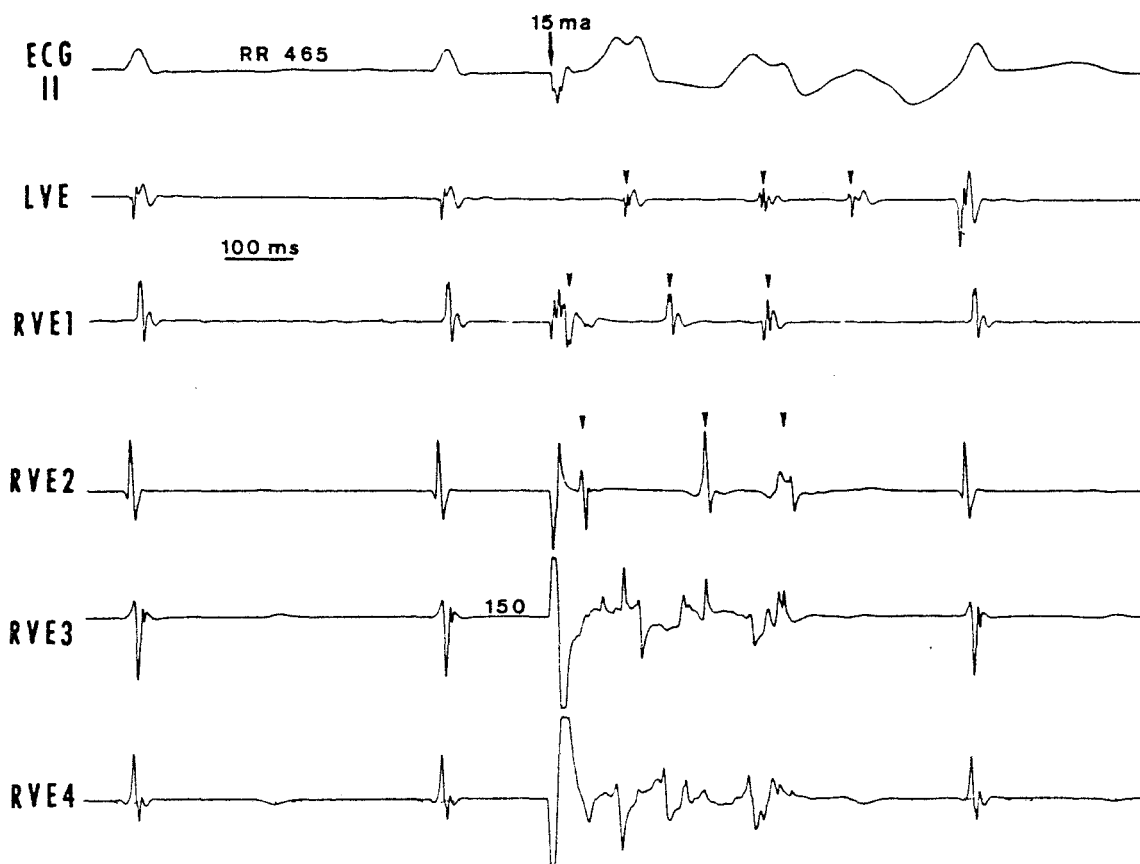


FIGURE 20

LEGEND

Five bipolar electrograms are shown along with a lead II ECG. The electrodes used to record RVE3 and RVE4 were placed in close proximity to the stimulating electrodes (Figure 5). The two other right ventricular electrograms were recorded from electrode pairs placed six millimeters above (RVE1) and 15 millimeters below (RVE2) the stimulating electrodes. The remaining electrogram (LVE) was recorded from the anterior surface of the left ventricle. Following a control cycle (cycle length 465 msec), a 15 ma stimulus was delivered to the right ventricle 150 msec after the intrinsic deflection of the control response in RVE3. The stimulus resulted in three premature ventricular systoles followed by a premature supraventricular systole. The arrows in LVE, RVE1 and RVE2 indicate the activation complexes associated with each ventricular extrasystole. The appearance of continuous, disorganized complexes in RVE3 and RVE4 suggests that the abnormal electrical activity induced by the stimulus was confined, at least initially, to a small area surrounding the stimulating electrodes.

degree of aberrancy compared to the control complexes, the electrical potentials recorded returned rapidly to baseline following each complex. The similar activation sequence (RVE1-RVE2-LVE) for each extrasystole suggested that all of the ventricular extrasystoles arose from foci near the stimulating electrodes. In contrast to the uniform activation complexes in RVE1, RVE2 and LVE, both RVE3 and RVE4 showed continuous, disorganized electrical activity which appeared immediately following the stimulus artefact and preceded the development of two spontaneous extrasystoles.

The highly local nature of the disorganized electrical activity (Figure 20) resulting from the application of high intensity currents during the vulnerable period suggested that the abnormal activity may have been an artefact of the stimulus. This possibility was tested in four animals, but was eliminated by the finding that local, disorganized electrical activity did not appear following stimulation of the heart outside of its vulnerable period. Figure 21 shows a record typical of the responses observed in these four animals. Panel A shows a control trace of five local electrograms along with a surface ECG. The first two right ventricular electrograms (RVE1 and RVE2) were recorded from the vicinity of the stimulating electrode, while the third and fourth right ventricular electrograms were recorded 20 millimeters below (RVE3) and six millimeters above (RVE4) the stimulating electrodes. In panel B, a 10 ma stimulus was delivered 190 msec following the intrinsic deflection of the control complex in RVE2. The appearance of a closely coupled spontaneous extrasystole following the stimulus-induced extrasystole indicated that the stimulus

FIGURE 21
RELATIONSHIP OF ABNORMAL LOCAL ELECTRICAL
ACTIVITY TO THE VULNERABLE PERIOD

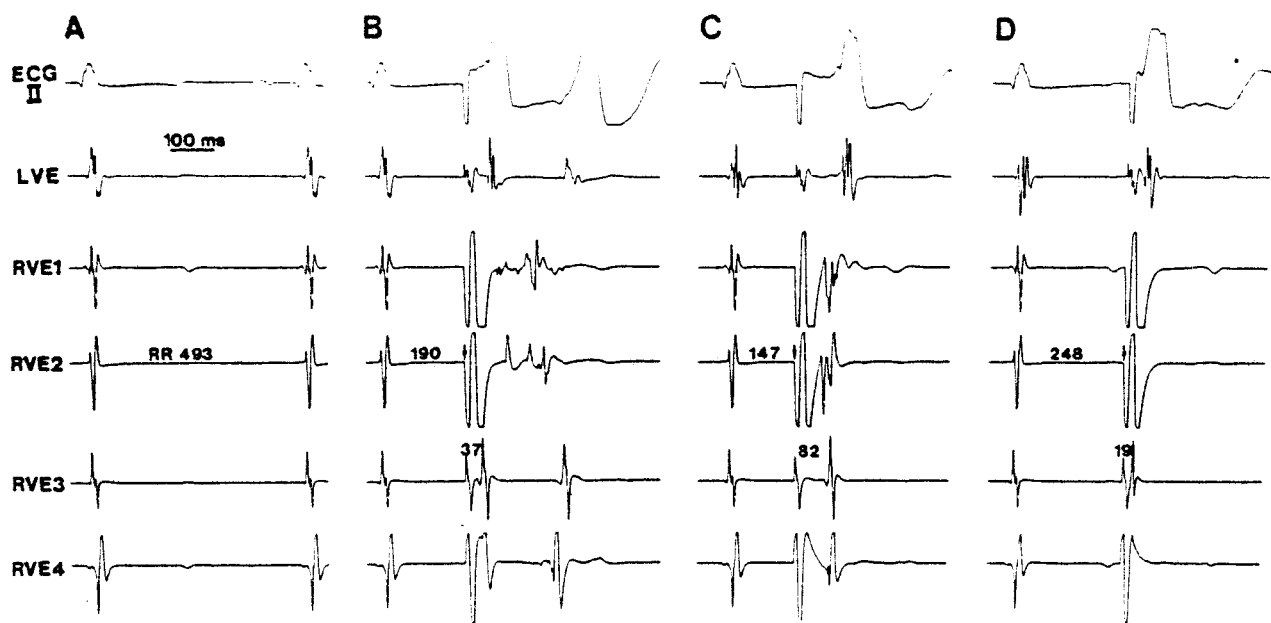


FIGURE 21

LEGEND

Five bipolar electrograms are shown along with a lead II ECG. RVE1 and RVE2 were recorded from the immediate vicinity of the stimulating electrodes. The two other right ventricular electrograms were recorded from electrode pairs placed six millimeters above (RVE4) and 20 millimeters below (RVE3) the stimulating electrodes. The remaining electrogram (LVE) was recorded from the anterior surface of the left ventricle. A control cycle is shown in panel A, while panel B shows two ventricular extrasystoles in response to a 10 ma stimulus delivered during the vulnerable period (coupling interval 190 msec). Disorganized electrical activity bridging the interval between the two extrasystoles was apparent only in RVE1 and RVE2. In panel C, the same stimulus was delivered earlier in the relative refractory period (coupling interval 147 msec) resulting in only a single ventricular response. The latency from the stimulus to the onset of negativity in RVE3 was 82 msec which was 45 msec longer than the stimulus-response latency in panel B. Although some disorganized electrical activity was recorded in RVE1 and RVE2 in panel C, the activity disappeared shortly following the onset of negativity in RVE3 and RVE4. In panel D, the 10 ma stimulus was delivered at the end of the T-wave (coupling interval 248 msec) resulting in a single premature response. The longer coupling interval, as compared to panels B and C, was associated with a shorter stimulus-response latency (19 msec in RVE3). Furthermore, in panel D, no disorganized electrical activity could be identified in RVE1 or RVE2 following recovery from the stimulus artefact.

fell during the vulnerable period of the cardiac cycle. The latency from the onset of the stimulus to the intrinsic deflection of the first extrasystole in RVE3 was 37 msec. Disorganized electrical activity, bridging the interval between the two extrasystoles, was observed only in RVE1 and RVE2. In panel C, the same stimulus was delivered earlier in the relative refractory period at a coupling interval of 147 msec. The stimulus was followed by a single ventricular extrasystole. The latency from the stimulus to the onset of the local activation complex in RVE3 was 87 msec. This was 45 msec longer than the stimulus-response latency shown in panel B. The longer stimulus-response latency in panel C probably resulted from extremely slow impulse conduction away from the stimulation site due to excitation of myocardial cells very early in their relative refractory period (49). Although some disorganized electrical activity was recorded in RVE1 and RVE2 immediately following the disappearance of the stimulus artefact, the activity disappeared shortly following the onset of negativity in RVE3 and RVE4. In panel D, the 10 ma stimulus was delivered at a coupling interval of 248 msec resulting in a single ventricular extrasystole. The longer coupling interval as compared to panels B and C was associated with a shorter stimulus-response latency (19 msec in RVE3). The shorter stimulus-response latency was probably due to the greater excitability and conduction velocity of the more completely repolarized fibers. In contrast to the disorganized electrical activity following the stimulus in panel B, the RVE1 and RVE2 electrograms in panel D returned to isopotential following the stimulus artefact.

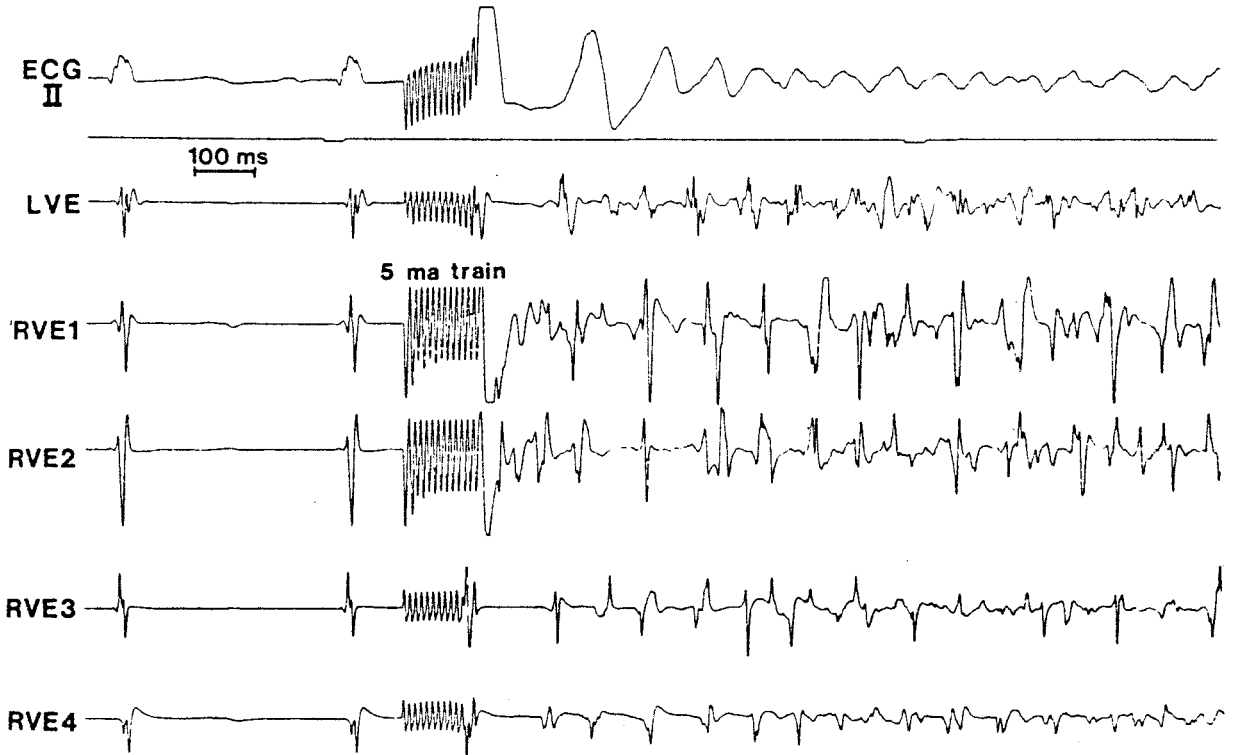
Local disorganized electrical activity similar to that shown in

Figures 18-21 was also observed in four animals in which trains of stimuli were used to evoke multiple extrasystoles and fibrillation. Figure 22 shows the response of the same animal used to obtain the record for Figure 21 to a train of 14 pulses (4 msec, 100 Hz) delivered at a current intensity of 5 ma. The two pairs of recording electrodes contiguous to the stimulating electrodes (RVE1 and RVE2) showed continuous disorganized electrical activity between the first and second extrasystoles, while the electrograms recorded away from the direct site of stimulation (LVE, RVE3 and RVE4) showed only discrete activation complexes. Once the multiple extrasystoles had degenerated into sustained fibrillation, all of the local electrograms displayed continuous disorganized electrical activity.

FIGURE 22

ABNORMAL LOCAL ELECTRICAL ACTIVITY EVOKED

BY A TRAIN OF STIMULI



The electrical recordings are the same as described in the legend of Figure 21. Following a control cycle, a train of 14 pulses (4 msec, 100 Hz) was delivered to the right ventricle with a current intensity of 5 ma. The train resulted in a series of accelerating extrasystoles which degenerated into sustained fibrillation. The electrodes in close proximity to the stimulating electrodes (RVE1 and RVE2) recorded continuous electrical activity between the first and second extrasystoles, while the remaining electrograms showed no electrical activity between the first and second extrasystoles. Once the extrasystoles had degenerated into sustained fibrillation, all of the local electrograms showed continuous, disorganized electrical complexes.

CHAPTER V

DISCUSSION

A. Contractile Force Changes In Response to Electrical Stimulation of the Myocardium

A substantial body of evidence has been accumulated indicating that electrical stimuli applied to preparations of isolated ventricular muscle may cause the release of autonomic mediators by excitation of intramyocardial nerve fibers (6, 7, 123, 128, 129). The present set of experiments provides the first demonstration of this phenomenon in the intact ventricles. The positive inotropic responses elicited by trains of stimuli (Figures 6-8) were most likely due to the local liberation of norepinephrine. Although direct evidence for the release of norepinephrine was not obtained, the elimination of the positive inotropic responses following beta-adrenergic blockade or chronic cardiac denervation provides strong indirect evidence for the local release of norepinephrine. The enhancement of the positive inotropic response following atropine (Figure 10), and the appearance of a negative inotropic response in some animals following beta-adrenergic blockade (Figure 9) or chronic cardiac denervation (Figure 11) provides indirect evidence for the local release of acetylcholine. Presumably, the two neuromediators were liberated from autonomic nerve fibers within the myocardium as nerve fibers are the

only structures in the ventricles known to contain the neuromediators in appreciable quantities.

The method used to stimulate intramyocardial nerves in the present study involved the application of trains of rectangular pulses to the myocardium during its absolute refractory period. This method is similar to the method used by Brady et al. (7) and by Blinks (6) to study the effects of electrical stimuli on contractions of isolated feline papillary muscles. Brady et al. (7) found that the application of a train of supramaximal stimuli during the absolute refractory period of a papillary muscle resulted in a depression of the contraction during which the stimuli were applied and a large potentiation of subsequent contractions. The potentiation was eliminated by pretreatment of the animals with reserpine, while the depression was not susceptible to pharmacological blockade. The magnitude of the depression was as large as 50% of the control contractile strength and was a direct function of the amount of charge (current x time) delivered to the tissue. The magnitude of the potentiation effect in response to trains of stimuli in the present study was similar to the magnitude of the potentiation effect reported by Brady et al. (7). However, no consistent evidence could be found in the present experiments for a direct depressant effect of the stimulus current on local myocardial contractile force. These results are consistent with the observations of Blinks (6) that the direct depressant effect of current on contractile force was never greater than 5% of control.

The beta-adrenergic blocking agent, propranolol, has been shown

to possess direct membrane depressant activity in addition to beta-adrenergic blocking properties (4, 24, 72). The high doses of propranolol (2-3 mg/kg) used in the present study (Figure 9) probably exerted some direct depressant effects upon the myocardium (4, 24, 72). However, high doses of propranolol were only necessary to totally abolish the positive inotropic responses to trains of stimuli; lower doses (.5-1 mg/kg) consistently resulted in a significant attenuation of the response. Furthermore, when the myocardium was stimulated with trains of stimuli delivered at six second intervals (Figure 12), the positive inotropic responses were totally abolished at a .5 mg/kg dose of propranolol. Presumably, a dose of this magnitude is free of direct membrane effects (24, 72).

Although the 100 Hz trains of stimuli delivered to the myocardium in the present study appeared to liberate local stores of norepinephrine, it was not possible to determine if the release of norepinephrine from the terminal sympathetic nerve fibers was able to follow the high stimulation frequency. Brown and Gillespie (9) stimulated the postganglionic sympathetic fibers to the cat spleen and found that the output of norepinephrine per stimulus showed a progressive decrease at frequencies greater than 40 Hz. At frequencies of 100 Hz, they could not measure norepinephrine in the perfusate. Roshe et al. (100) studied the pressor response to stimulation of the left stellate ganglion and found a reversal in the response at frequencies above 15 Hz, with both systolic and pulse pressures regressing from peak levels as frequency was increased to 80 Hz. In contrast to

the deleterious effects of high frequency stimuli on autonomic function in the intact animal, both Brady et al. (7) and Blinks (6) found that the positive inotropic response to a single train of stimuli with a constant number of pulses was independent of frequency at frequencies of up to 100 Hz. At frequencies greater than 100 Hz, the positive inotropic response was attenuated. In the present experiments decreasing the frequency of pulses within a train from 100 Hz to 50 Hz, while maintaining a constant number of pulses per train, did not alter the magnitude of the positive inotropic response. These results along with the observations of Blinks (6) and Brady et al. (7) suggest that the postganglionic sympathetic fibers are capable of following high stimulation frequencies when the stimuli are delivered in brief bursts or trains. It may well be that the deleterious effects of high frequency stimuli (100 Hz) on norepinephrine output are only demonstrative under conditions of continuous postganglionic stimulation.

The highly localized nature of the positive inotropic responses to trains of stimuli (Figures 6 and 7) is consistent with the observations of Randall and co-workers (50, 96, 97, 116). These investigators found that excitation of individual thoracic nerve trunks induced positive inotropic responses in highly localized regions of the ventricles. Furthermore, stimulation of the stellate ganglia, following ablation of narrow strips of epicardium resulted in obliteration of contractility changes in very discrete areas of the ventricles. In the present study, trains of stimuli were applied to different areas of the ventricles through unipolar electrodes. Thus, it was likely

that during each stimulation there was current flowing throughout the entire myocardium. However, the efficacy of a stimulus is primarily determined by the density of current flowing within an irritable tissue rather than by the absolute magnitude of the current delivered. Since current density is always maximal at an electrode-tissue interface, sympathetic fibers close to the electrode site were presumably excited with lower intensity trains of stimuli than more distant fibers. Therefore, at low current intensities (5 ma or less) contractility changes were only observed at the site of stimulation, while at high current intensities (20 ma) responses were more generalized (Figures 6-7). Furthermore, recruitment of sympathetic fibers at greater distances from the electrode site is the most likely explanation for the positive correlation between the magnitude of a local inotropic response and the current intensity of the stimuli (Figure 8). Increases in current intensity above 20 ma (Figure 8) resulted in little further change in contractility which suggests that the 20 ma stimuli resulted in near maximal recruitment of local sympathetic fibers.

Previous studies on the sympathetic control of regional ventricular function have shown that the percentage increase in contractile force of the right ventricular conus in response to right or left stellate stimulation is consistently greater than the response of other regions of the right or left ventricles (3, 50). In the present study, the relative increase in contractile force of the right ventricular conus in response to 10 ma trains of stimuli was almost twice the magnitude of the response of the left ventricular base to

the same stimuli (Figure 12). Since the left ventricular base and right ventricular conus contain similar levels of norepinephrine (2), it is doubtful that the difference in the response of the two regions to trains of stimuli is related to the amount of norepinephrine liberated. The greater responsiveness of the right ventricular conus might well be due to the parallel nature of the muscle fibers which comprise this area (3, 52). In contrast to the fibers of the conus, histologic examination of the left ventricle has shown that fiber angles begin to change within one millimeter of the epicardium (114). In the present study, it is doubtful that all of the muscle fibers encompassed by the sutures used to anchor a strain gauge arch to the left ventricle were parallel. Thus, only a fraction of the total force developed by some of the muscle fibers beneath an arch may have been registered by the strain gauge.

In addition to differences in the positive inotropic response of the two ventricles to trains of stimuli, there was also a difference in the magnitude of the negative inotropic response of the two ventricles to trains of stimuli in the absence of adrenergic influences. Following beta-adrenergic blockade with propranolol, a decrease in right ventricular contractile force in response to trains of stimuli was observed in 75% of a group of eight animals, while a decrease in left ventricular contractile force was observed in only 38% of the animals. In the chronically denervated animals, a decrease in right ventricular contractile force was observed in 38% of the animals, while none of the animals demonstrated a decrease

in left ventricular contractile force. The negative inotropic responses to trains of stimuli in both the beta-blocked and chronically denervated animals were eliminated by atropine (Figures 9 and 11), indicating that the responses were probably mediated by local acetylcholine release. The more frequent negative inotropic response in the right ventricle is consistent with the observations of Priola and Fulton (89). These investigators showed that efferent vagal stimulation produced a greater relative decrease in right ventricular systolic pressure (4-12%) in the isovolumetric canine heart than left ventricular systolic pressure (1-10%). Furthermore, Schmid et al. (105) found that choline acetyltransferase activity in the right ventricle of the guinea pig heart was twice as great as the enzyme activity in the left ventricle. Thus, the more consistent depression of right ventricular contractile force observed in the present experiments may well be due to a greater cholinergic nerve density in the right ventricle.

The inconsistent negative inotropic response in the chronically denervated animals was somewhat surprising in light of the findings of Priola et al. (90); that postganglionic parasympathetic fibers surviving chronic cardiac denervation are capable of depressing both right and left ventricular contractility in isovolumetric paced hearts. The lack of a more consistent and pronounced negative inotropic response in the chronically denervated ventricles in the present experiments may be related to peripheral vagolytic effects of pentobarbital (95), or possibly a change in local levels of

acetylcholine following chronic cardiac denervation.

The experiments of Levy et al. (61) and Stanton and Vick (113) have demonstrated that the depressant effects of vagal stimulation on ventricular contractility are accentuated in the presence of an elevated background of sympathetic activity. Furthermore, the antagonism was shown to be specific for the type of enhanced contractility produced by adrenergic interventions (63). In the present study, a parasympathetic-sympathetic interaction may have been responsible for the significant increase in contractile force in response to trains of stimuli after blockade of only muscarinic receptors. As shown in Figure 10, trains of stimuli delivered following atropine (.2 mg/kg) resulted in a further increase in right ventricular contractile force of 34% and a further increase in left ventricular contractile force of 31%. These responses were more consistent and of a greater magnitude than the negative inotropic responses to trains of stimuli following the elimination of adrenergic influences.

The mechanisms which have been proposed to account for parasympathetic-sympathetic interactions in cardiac tissue are shown schematically in Figure 23. Loffelholz and Muscholl (68, 69) and Levy (61) have shown that vagal stimulation acts through a muscarinic receptor to inhibit the release of norepinephrine in response to sympathetic stimulation. This type of interaction is represented in Figure 23 by an inhibitory axo-axonal synapse of the vagus on the sympathetic fiber. In addition to a reduced neuronal release of norepinephrine, acetylcholine has also been shown to antagonize (through a muscarinic receptor) the inotropic effects of exogenous

FIGURE 23

MECHANISMS PROPOSED FOR PARASYMPATHETIC-SYMPATHETIC INTERACTION

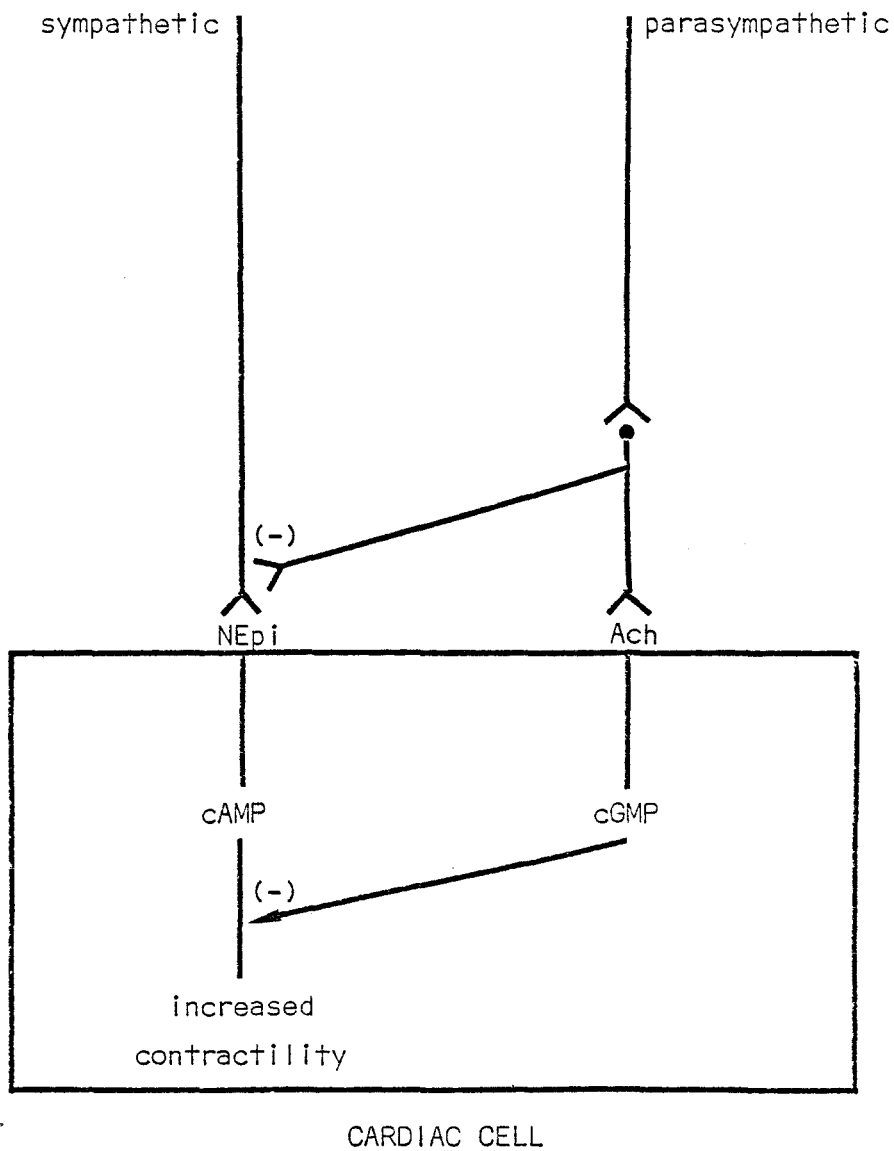


FIGURE 23

LEGEND

Schematic diagram showing the mechanisms which have been proposed to account for the antagonism of sympathetic responses by the vagus. As shown in the diagram, parasympathetic fibers may presynaptically inhibit the release of norepinephrine through an axo-axonal synapse. Alternatively, vagal activation may increase cellular levels of cyclic guanosine monophosphate which may inhibit the effects of cyclic adenosine monophosphate on cellular function.

catecholamine (45, 125). The mechanism for this antagonism, as shown in Figure 23, may be an inhibition of the stimulatory effect of cyclic AMP on the contractile force of the cell by cyclic GMP (125).

B. Ventricular Fibrillation Thresholds

The experiments of Wiggers and Wegria (132) were the first to demonstrate that an appropriately timed stimulus of sufficient energy delivered to the ventricles in late systole could result in ventricular fibrillation. These same investigators (133) later suggested that the minimum current intensity of a single stimulus, delivered during the vulnerable period and resulting in ventricular fibrillation, provided a quantitative measure of ventricular vulnerability to fibrillation. To minimize the amount of time necessary to precisely locate the vulnerable period with a single stimulus, Han (33) introduced a technique in which a gated series of rectangular pulses (100 Hz) was applied to the ventricles during the T-wave of the electrocardiogram. It was assumed that the 100 Hz train of pulses would be comparable to a scan of the vulnerable period with a single pulse at 10 msec intervals, and that the fibrillation threshold obtained would be comparable to the value obtained with the single-pulse technique.

Tamargo et al. (117) compared the two techniques and found that the fibrillation threshold measured with a train was consistently less than the threshold measured with a single stimulus. These investigators considered the possibility that trains of stimuli might excite adrenergic fibers close to the stimulating electrodes, thereby reducing the level of current necessary to evoke fibrillation. However, this possibility was eliminated by the finding that propranolol did not

alter the fibrillation threshold measured with trains relative to the threshold measured with single stimuli.

Verrier et al. (122) suggested that the current necessary to produce fibrillation with a train of stimuli depended upon the time relationship of the train relative to the protective zone of the cardiac cycle. The protective zone was defined as a period starting 10 to 15 msec after the nadir of the vulnerable period and lasting for approximately 50 msec. It was shown that a stimulus delivered during this period of time could inhibit or prevent the development of ventricular fibrillation resulting from an earlier stimulus delivered during the vulnerable period. Verrier et al. (122) showed that trains of stimuli which scanned the vulnerable period and ended during the protective zone had substantially higher fibrillation thresholds than trains of stimuli which ended before the onset of the protective zone or extended beyond the end of the protective zone boundary.

In the present study, fibrillation thresholds measured with trains of stimuli were found to be significantly less than thresholds measured with single stimuli (Figure 15). The single stimuli used to scan the vulnerable period had a duration of 10 msec, which was considerably longer than the 4 msec duration of individual pulses within a train. Had equal pulse durations been employed with both techniques, it is likely that the difference between the fibrillation thresholds would have been even greater. The trains of stimuli used to test cardiac vulnerability to fibrillation extended beyond the T-wave of

the electrocardiogram, and thus, presumably extended beyond the boundaries of the protective zone as defined by Verrier and co-workers (122).

Although the timing of a train relative to the protective zone of the cardiac cycle may be an important determinant of the fibrillation threshold, the results of the present study indicate that the level of current necessary to fibrillate the heart with trains of stimuli is markedly altered by the local excitation of sympathetic nerves. The importance of a local excitation of sympathetic nerves was indicated by the observation that propranolol increased the fibrillation threshold measured with trains by a greater relative magnitude than the threshold measured with single stimuli. In contrast to the findings of Tamargo et al. (117), the relative increase in the fibrillation threshold measured with trains at either ventricular site was approximately seven times greater than the relative increase in the single-stimulus threshold determined at the same site (Figure 16). Furthermore, with either method of stimulation, the relative increase in the fibrillation threshold determined at the right ventricular site was not significantly different from the relative increase in the threshold determined at the left ventricular site (Figure 16).

Stimulation of thoracic, cardiac, sympathetic nerves has been shown to reduce the ventricular fibrillation threshold measured with either trains of stimuli (29, 54) or single stimuli (35). In the present study, since the hearts were autonomically decentralized, a local excitation of sympathetic fibers by the stimulus current could have accounted for the effects of propranolol on the fibrillation threshold

measured with both trains of stimuli and single stimuli. A local excitation of sympathetic fibers was indicated by the observation that both trains of stimuli and single stimuli increased local myocardial contractile force (Figures 12 and 13). Trains of stimuli delivered to the myocardium at six second intervals through a strain gauge arch electrode resulted in a much greater increase of local myocardial contractile force than single stimuli delivered in a similar fashion (Figure 13). Since the positive inotropic response to both trains and single stimuli was eliminated by propranolol (Figure 12) it is likely that the stimuli liberated local stores of norepinephrine, with trains of stimuli releasing a greater amount of the catecholamine than single stimuli. Thus, the much greater effect of propranolol on the fibrillation threshold determined with trains than on the threshold determined with single stimuli was probably due to the greater liberation of local norepinephrine by the trains.

The latency from the onset of sympathetic nerve excitation to a measurable change in sinus rate or atrioventricular conduction has been shown to be about one to two seconds (111, 112). In the present study a similar latency was observed between the onset of a train of stimuli and an increase in local myocardial contractile force. Since adrenergic influences on ventricular vulnerability have been shown to be mediated by cyclic adenosine monophosphate (71), it is doubtful that activation of local sympathetic fibers would start to alter vulnerability to fibrillation in less than one to two seconds. However, the latency from the onset of a train of stimuli delivered during the vulnerable period to the development of multiple extrasystoles leading

to fibrillation was invariably less than one second (Figures 14 and 22). Therefore, it is doubtful that any norepinephrine liberated during a given train could alter the vulnerability of the heart to that particular train. It is possible, though, that norepinephrine released during a given train might remain in sufficient quantities to alter the vulnerability of the heart to subsequent trains. A local buildup of norepinephrine lasting between successive trains was indicated by the maintained increase in local myocardial contractile force observed during a series of 10 trains (Figure 12). A series of 10 single stimuli also resulted in a measurable increase in local myocardial contractile force which was maintained between successive stimuli (Figure 12). Thus, a local buildup of norepinephrine might also influence the level of current necessary to fibrillate the heart with single stimuli. If a longer time interval were allowed between successive trains or single stimuli, then more time would be available for removal of norepinephrine and thus the adrenergic influence on cardiac vulnerability would presumably be diminished. The failure of Tamargo et al. (117) to demonstrate a local adrenergic influence on ventricular fibrillation thresholds measured with trains of stimuli may have been due to a lack of precise control over the time interval between successive trains.

In addition to beta-adrenergic blockade, the direct membrane depressant effects of high doses of propranolol have been shown to prevent non-catecholamine mediated arrhythmias (72). However, it is doubtful that the dose of propranolol which elevated ventricular

fibrillation thresholds in the present study was sufficient to exert a membrane anesthetic effect (4, 24, 72). The absence of a significant change in the diastolic excitability threshold following propranolol argues for the lack of a significant membrane effect. The significant decrease in heart rate and contractile force in response to propranolol was probably due to the blockade of the beta-stimulatory effects of circulatory catecholamines. Although high levels of circulatory catecholamines have been shown to increase the ventricular fibrillation threshold (35, 43), a recent study by Rabinowitz et al. (91) showed that the infusion of sub-pressor amounts of norepinephrine resulted in an 18% reduction in the threshold for multiple extrasystoles that was sustained throughout the duration of the infusion. Thus, it is conceivable that a small part of propranolol's ability to elevate the fibrillation threshold measured with either trains or single stimuli may have been due to blockade of the beta-stimulatory effects of circulating catecholamines.

Further evidence for the influence of stimulus-induced, local release of norepinephrine on ventricular vulnerability to fibrillation was indicated by the significant elevation of the ventricular fibrillation threshold following chronic cardiac denervation. Compared to sham-thoracotomized animals, depletion of myocardial catecholamines by surgical denervation resulted in a three-fold elevation of the ventricular fibrillation threshold measured with trains of stimuli (Figure 17). The difference in the mean fibrillation threshold between the two groups of animals could not be accounted for by differences in heart rate, diastolic threshold, or arterial pH.

Furthermore, in contrast to the pronounced effects of propranolol on the fibrillation threshold measured with trains in innervated hearts, beta-blockade in the denervated heart resulted in little change in the fibrillation threshold. Although circulating catecholamines were present in the denervated animals, as indicated by the changes in heart rate and contractile force in response to propranolol, their influence on cardiac vulnerability to fibrillation appeared to be minimal. The absolute ventricular fibrillation thresholds in the chronically denervated animals were not significantly different ($p > .1$) from the thresholds (measured with trains in normal animals with intact innervation following beta-adrenergic blockade (unpaired t-test)). Furthermore, the thresholds in the sham-operated animals were not significantly different ($p > .1$) from the thresholds in normal animals with intact innervation prior to beta-blockade. Thus, it can be concluded that depletion of myocardial catecholamines by surgical denervation produces the same increase in ventricular fibrillation threshold as does beta-adrenergic blockade in animals with an intact, terminal, sympathetic innervation.

The results of the present study shed some doubt on the validity of the use of the ventricular fibrillation threshold measured with a train of pulses as an index of vulnerability of the ventricles to fibrillation. Since trains of stimuli produce an "artificial" enhancement of sympathetic tone to local areas of the myocardium, the technique is not appropriate to monitor changes in cardiac vulnerability associated with reflex changes in autonomic tone. In addition, the train of pulses technique may not be appropriate to test the effect

of an antiarrhythmic drug on ventricular vulnerability to fibrillation if the drug antagonizes the release of norepinephrine or its effect on cardiac beta-receptors.

In addition to stimulus-induced release of catecholamines, it is possible that stimulation of parasympathetic nerve endings may influence the current required to evoke ventricular fibrillation. Cervical vagal stimulation has been shown to increase the ventricular fibrillation threshold measured with either trains of stimuli (53, 137) or single stimuli (56). However, in the presence of beta-adrenergic blockade, the fibrillation threshold measured with either method was not affected by vagal stimulation (56, 137). Thus, it would appear that the vagal effect on cardiac vulnerability is indirect and works by antagonizing sympathetic effects. In the present study, blockade of muscarinic receptors with atropine resulted in a 15% decrease in the ventricular fibrillation threshold measured with trains of stimuli. However, the decrease did not achieve statistical significance at the .05 level of probability. Because of the rapid hydrolysis of acetylcholine and the six second time interval between successive trains, it is doubtful that local levels of acetylcholine reached sufficient magnitude to significantly antagonize the effects of locally released norepinephrine.

The precise electrophysiological mechanisms by which norepinephrine, released from sympathetic terminals, alters the vulnerability of the heart to fibrillation have yet to be fully elucidated. Han et al. (35) showed that left stellate stimulation could shorten the functional refractory period of ventricular muscle by as much as 15 msec.

The decrease in refractory period is consistent with the ability of norepinephrine to increase the slope of phase 3 repolarization in isolated ventricular muscle cells (41). Han et al. (35) also found that stellate stimulation did not shorten refractory periods measured at closely adjacent points on the epicardial surface by an equal magnitude. Presumably the nonuniform effects of sympathetic nerve activation on refractory periods was the result of a nonuniform distribution of sympathetic nerve terminals to different myocardial cells. Cells closer to a nerve terminal would see a greater concentration of norepinephrine and undergo a greater shortening of refractory period than cells further away. The resulting increase in temporal dispersion of refractoriness might facilitate the induction of fibrillation by causing fractionation of a premature wave front. In the present experiments, the localized release of norepinephrine by the stimulus current probably created a marked temporal dispersion of refractoriness. The abbreviation of refractory periods presumably followed the distribution of liberated norepinephrine.

In addition to effects on dispersion of refractoriness, activation of sympathetic nerves has been shown to alter the ventricular excitability threshold. Han et al. (35) demonstrated a decreased diastolic excitability threshold during left stellate stimulation, although other investigators (8, 57, 58) have failed to demonstrate a consistent effect of sympathetic nerve stimulation on the resting level of excitability. However, Burgess et al. (10) have shown that during the relative refractory period the dip in the bipolar or anodal

strength-interval curve is lowered in response to sympathetic activation. Since the dip in the strength-interval curve coincides in time with the vulnerable period (42), it is possible that changes in excitability during this period may exert a parallel influence on the ventricular fibrillation threshold.

C. Abnormal Electrical Activity During the Induction of Fibrillation

There is substantial evidence indicating that interventions which alter the ventricular fibrillation threshold do so by altering the conditions necessary for the production of reentrant excitation. Several studies have indicated that changes in ventricular fibrillation thresholds are closely correlated to the amount of asynchrony of recovery of excitability among adjacent myocardial fibers (34-36, 77, 112). Absolute changes in conduction velocity and refractory period appear to be less important than the dispersion of these properties between different areas of the myocardium (34-36, 77). Presumably, nonuniformity of excitability during the propagation of a premature impulse would facilitate the likelihood of unidirectional conduction block and reentrant excitation.

Although the ventricular fibrillation threshold is affected by interventions which alter the susceptibility of the heart to reentry, direct recordings from the myocardium during the induction of fibrillation have never been able to substantiate a reentrant mechanism. Moe et al. (83) suggested that a single stimulus of sufficient intensity delivered during the vulnerable period gave rise to a single rhythmic center from which several impulses were discharged at an accelerating rate. These investigators hypothesized that the progressive

decrease in refractory periods associated with the accelerating rate and the conduction delay of the premature response would establish the conditions necessary for reentry and fibrillation. The conclusion that the initial, accelerating extrasystoles originated from a single rhythmic center was supported by the observation of a similar spread of activation of each extrasystole on the surface and interior of the ventricles. The possibility of a macroreentrant circuit was eliminated since there was a period of time between each of the initial beats when no area of the ventricles was excited (83).

The results of the present study do not support the concept of a single rhythmic center as the mechanism responsible for the genesis of multiple extrasystoles and fibrillation in response to either a single stimulus or train of stimuli delivered during the vulnerable period. The observation of continuous, disorganized electrical activity spanning the interval between the first premature beat and subsequent premature beats (Figures 19-2) suggests that the mechanism is much more complex than a single, accelerating ectopic focus. In the experiments of Moe et al. (83) electrograms were recorded from very close bipolar electrodes (interelectrode distance 1.5 mm) attached to the ventricles at least six millimeters from the site of stimulation. Presumably, the failure to observe continuous, disorganized electrical activity was due to their recording from a sufficient mass of tissue in close proximity to the stimulating electrodes. As shown in Figures 20-22, disorganized electrical activity was recorded from the electrodes placed within a few

millimeters of the stimulating electrodes. Electrograms recorded from electrodes placed six to 20 millimeters above or below the stimulating electrodes did not record disorganized electrical activity during the initial cycles of the arrhythmia.

Although it was not possible to determine the precise origin of the continuous electrical activity recorded in the vicinity of the stimulating electrodes, the amplitude and rate of change of the majority of complexes would suggest that they were associated with asynchronous depolarization of local myocardial fibers. It is possible that some of the electrical complexes were due to asynchronous repolarization of adjacent fibers, although the high pass filters employed presumably attenuated the potentials generated by repolarization to a much greater degree than those generated by depolarization.

It is possible that the continuous, disorganized electrical activity recorded following the first extrasystole elicited by a train or single stimulus was not the direct cause of subsequent, spontaneous extrasystoles, but was merely associated with their occurrence. However, the fact that local, disorganized electrical activity always preceded the appearance of a spontaneous extrasystole argues for a cause and effect relationship. Furthermore, the appearance of disorganized electrical activity did not depend upon the appearance of spontaneous extrasystoles since the abnormal activity was observed at stimulus intensities below the threshold for the production of multiple ventricular response (Figure 18). In each animal, the duration of the disorganized electrical activity increased

as the intensity of the stimulus current was increased. Thus, the appearance of spontaneous extrasystoles following the initial stimulus-induced extrasystole was probably the result of the formation of multiple local wavefronts, outlasting the refractory period of the surrounding myocardium. If the local wavefronts survived long enough, then the multiple extrasystoles degenerated into sustained fibrillation.

Although the precise mechanism responsible for the continuous, disorganized electrical activity could not be determined, possible mechanisms include either multiple ectopic foci discharging asynchronously or localized reentry. Moe et al. (83) suggested that a strong stimulus delivered during the vulnerable period created a local state of tissue polarization which acted as a persisting stimulus resulting in multiple premature responses. It is conceivable that in the present experiments a long-lasting state of polarization created by a train or single stimulus could have produced multiple, asynchronous firing of closely adjacent fibers resulting in the appearance of continuous electrical activity in the local electrograms. Alternatively, it is possible that a series of oscillatory afterpotentials (15) could have been triggered in many different fibers by the intense stimulus current. However, if the initiation of fibrillation following a single electrical stimulus depended upon a persisting stimulus due to tissue polarization, or on a series of oscillatory afterpotentials, it would be difficult then to account for the presence of a discrete vulnerable period. As shown in Figure 21, only stimuli which fell during the vulnerable period resulted in local, disorganized electrical activity which preceded the appearance of spontaneous extrasystoles.

When stimuli were delivered earlier or later than the ventricular vulnerable period, there was little disorganized electrical activity extending beyond the single premature response evoked by the stimulus (Figure 21).

The appearance of local, disorganized electrical activity resulting only from stimulation of the myocardium during its vulnerable period can be explained on the basis of multiple reentry circuits. The conditions necessary for reentry to occur have been defined as: (1) the presence of a unidirectional conduction block, (2) activation of tissue distal to the block over an alternate pathway and (3) reexcitation of tissue proximal to the block (81). Since not all adjacent myocardial cells recover from refractoriness at the same moment in time, a stimulus delivered during the vulnerable period could result in a propagated response in some cells while other cells would act as a temporary block to the spread of excitation. The greater the strength of the stimulus, the larger the mass of tissue exposed to the excitatory effects of the stimulus and the greater the number of local areas of block which could form. In addition, the stimulus current itself may act to increase asynchrony of recovery of excitability, thereby increasing the probability of local conduction block formation (112).

In order for reexcitation of fibers proximal to a site of block to occur, the conduction time over an alternate route must exceed the refractory period of the tissue to be reentered. The rapid appearance of disorganized electrical activity in the present study following a

single stimulus delivered during the vulnerable period suggests that the refractory period of some fibers must have been exceedingly brief. Sasynuk and Mendez (103) have shown that whenever conduction block occurs in terminal Purkinje fibers due to premature activation, there is a marked decrease in the duration of action potentials and refractory periods of cells proximal to the site of block. Presumably, this shortening of action potential duration and refractory period is due to the effect of repolarizing current flowing from neighboring unexcited areas. Furthermore, Sasynuk and Mendez (103) demonstrated that the shortening of refractory periods proximal to a site of block was sufficient to allow reentry to occur over a very short pathlength.

In the present study, it is possible that multiple areas of local conduction block could have shortened the refractory period of some fibers enough to allow reentry to occur in a small mass of tissue. For the local reentrant activity to survive long enough to produce multiple extrasystoles and fibrillation, the local sites of conduction block must have frequently shifted location, resulting in the formation of new reentry circuits. At the fibrillation threshold a critical number of reentry circuits would form such that local reentry could be sustained long enough to spread to other regions of the ventricles resulting in sustained fibrillation.

In addition to conduction block and reentry within ventricular muscle fibers, terminal Purkinje fibers may also have been responsible for perpetuation of the reentrant excitation following a single stimulus or train of stimuli delivered during the vulnerable period. The

distal Purkinje fibers, two to three millimeters proximal to their termination in ventricular muscle, have been shown to possess much longer action potential durations and refractory periods than either proximal Purkinje fibers or ventricular muscle cells (86,87). Furthermore, both Kakihana et al. (48) and Spear et al. (109) have shown that an equal magnitude of current is required to fibrillate the ventricles at either the right ventricular epicardium, right ventricular endocardium or left ventricular endocardium, while a significantly greater amount of current is required at the left ventricular epicardium. Since there is a high degree of asynchrony of recovery of excitability at the Purkinje-muscle junction, it is possible that the proximity of the stimulating electrodes to the terminal Purkinje fibers may be an important determinant of the fibrillation threshold.

CHAPTER VI

SUMMARY

Trains of electrical stimuli were applied to the intact ventricles of anesthetized dogs during the absolute refractory period of the cardiac cycle. The stimulus trains were delivered to the epicardial surface via unipolar electrodes embedded in the feet of modified Walton-Brodie strain gauge arches. The delivery of a train during each cardiac cycle while heart rate was held constant by atrial pacing resulted in highly localized potentiation of ventricular contractile force as measured by the strain gauge arches. The magnitude of the potentiation and the mass of ventricular tissue showing a positive inotropic response were directly related to the intensity of the stimulus trains. Trains of equal intensity resulted in a greater increase in right ventricular contractile than left ventricular contractile force. The positive inotropic response of either ventricle to trains of stimuli was abolished following chronic cardiac denervation or beta-adrenergic blockade with propranolol indicating that the response was probably mediated by the excitation of local sympathetic nerve fibers. Following the elimination of adrenergic influences, a slight negative inotropic response to trains of stimuli was observed in some animals. The magnitude and consistency of the negative inotropic response appeared to be greater in the right ventricle than in the left ventricle. Administration of atropine blocked the negative inotropic response. Furthermore, when the adrenergic system was

intact, the administration of atropine resulted in a significant augmentation of the positive inotropic response of both ventricles to trains of stimuli. Thus, in addition to norepinephrine release, it also appeared that electrical stimulation of the ventricles resulted in activation of parasympathetic fibers with subsequent release of acetylcholine.

The influence of norepinephrine, liberated by electrical stimulation of the ventricles, on vulnerability to fibrillation was determined by comparing the effects of beta-adrenergic blockade on the fibrillation threshold measured with a train of stimuli to the effect of beta-adrenergic blockade on the fibrillation threshold measured with a single stimulus. Prior to beta-adrenergic blockade, ventricular fibrillation thresholds determined by scanning the vulnerable period with a train of 14 pulses (4 msec, 100 Hz) were significantly less than fibrillation thresholds determined by scanning the vulnerable period with a single 10 msec stimulus. Following beta-adrenergic blockade with propranolol, the relative increase in fibrillation thresholds measured with trains was about seven times greater than the relative increase in the single-stimulus threshold. The relative increase in the fibrillation threshold measured with either method was the same at both a right and left ventricular stimulation site. Furthermore, fibrillation thresholds measured with trains of stimuli in a group of animals subjected to chronic cardiac denervation were about three times greater than the thresholds in a group of animals subjected to a sham thoracotomy. In addition, the train fibrillation thresholds of the catecholamine-depleted hearts were not different

from the train thresholds of beta-blocked, innervated hearts. Thus, it was concluded that the norepinephrine released during the measurement of a ventricular fibrillation threshold with trains of stimuli, exerted a profound influence on the level of current necessary to evoke fibrillation.

In another series of experiments the initiation of fibrillation by the application of either a train of stimuli or single stimulus to the myocardium during the vulnerable period of the cardiac cycle was analyzed by means of multiple bipolar electrograms. Electrograms recorded from electrodes contiguous to a pair of stimulating electrodes in the right ventricle showed a considerable amount of abnormal electrical activity following the delivery of an intense single stimulus or train of stimuli during the vulnerable period. At stimulus intensities below the threshold for multiple ventricular response, the abnormal activity consisted of low-amplitude complexes which lasted considerably longer than the control activation complexes. When the stimulus intensity was sufficient to evoke multiple extrasystoles or fibrillation, the local electrograms showed continuous electrical activity, bridging the interval between the first premature beat and subsequent premature beats. Electrograms recorded several millimeters away from the direct site of stimulation did not show similar abnormal electrical activity. Furthermore, the abnormal local electrical activity resulted only from stimulation of the heart during its vulnerable period; stimuli applied earlier or later in the cardiac cycle did not result in continuous local electrical activity. The presence of continuous local electrical activity preceding the development of multiple extrasystoles and fibrillation suggested that the stimulus induced the formation of multiple,

local reentry circuits, although a mechanism involving abnormal automaticity could not be ruled out.

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