structure resembling a telegraph cable (Fig 3).

Cardiac conduction velocity is determined by many factors. There are four important physiological variables to be considered: (1) action potential amplitude, (2) the rate of rise of the action potential, (3) threshold, and (4) internal and external electrical resistances.

Action potential amplitude, because it is related to the size of the depolarizing current, also determines the distance ahead that the depolarized tissue can initiate a propagated action potential. Increasing the amplitude of the action potential projects currents that exceed threshold, and thus are able to excite quiescent tissue, a greater distance ahead of the wave front. Similarly, conduction is accelerated by an increased rate of depolarization because of the more rapid spread of depolarizing currents into the excitable, resting tissue ahead of the wave front. Decreased threshold accelerates conduction by reducing the amount of current needed to initiate a propagated action potential in the resting tissue ahead of the wave front. Longitudinal currents are influenced by extra- and intra-cellular resistances, whereas current flow across the membrane (Shaded in Fig 4) is determined by membrane capacitance and membrane resistance. Conduction is slowed when extracellular or intracellular resistance is increased, but is accelerated by increased membrane resistance.

Unidirectional block means block of impulse conduction in only one direction. It occurs when a premature impulse meets a border between two areas with different refractory periods. The impulse propagates through the excitable area and is blocked in the refractory area. When the impulse turn back from the other side, this refractory area may be excitable, resulting in reentry. Unidirectional block is an important factor in reentry formation (Mines, 1914).

Conduction velocity can be calculated by the linear regression of electrode distance over activation time, for a series of electrodes along the line of propagation.

1.2.3 Myocardial refractoriness

The delayed reactivation of the sodium channels accounts for the atria and

ventricles' refractoriness, which means that the atria and ventricles cannot be reexcited during and immediately after the passage of an action potential. Although able to generate a local response, the heart cannot develop a propagated action potential during its refractory period (Fozzard & Arnsdorf, 1992). This implies that the refractory period prevents cells from responding to excessively rapid activation or premature excitation. Thus, the long refractory period of cardiac myocytes has a major physiologic significance in preventing cardiac arrhythmias, and many antiarrhythmic drugs increase refractory period to stop reentrant arrhythmias (Wang et al., 1992; Wang et al., 1993; Kirchhof et al., 1991).

Two degrees of refractoriness are commonly described in myocardial cells (Fig 5). During the absolute refractory period, which begins with depolarization, no stimulus, whatever its magnitude, can produce a propagated response. The relative refractory period, which begins after the end of the absolute refractory period, is an interval in which only stimuli that exceed the normal threshold can initiate a propagated response. Even though abnormally strong stimuli can initiate propagated action potentials during the heart's relative refractory period, these responses are not normal. They are generally slow-rising and of low amplitude. As these small action potentials conduct slowly, they are of considerable importance in the arrhythmias associated with reentry. For example, Dawes and Vane (1951) produced repetitive discharges in the isolated auricles of guinea pigs by stimulating the preparation at a rate slightly greater than the normal rate, and introducing another stimulus after every fourth beat. When this fell just outside the absolute refractory period, the auricle responded by repeated discharges at a much greater rate than the normal.

Usually, the effective refractory period (ERP) is measured by the extrastimulus technique, with a train of basic stimuli (S1) followed by a premature stimulus (S2). ERP is defined as the longest S1S2 interval failing to produce a propagated response.

1.3 Detection of cardiac electrical activity

The detection of cardiac electrical activity relies on the fact that the body in which

the heart lies can be considered as a salt solution that conducts electricity. Therefore, the electrical currents generated during the heart beat can be detected from the surface of the body a long distance away from the heart itself by the electrocardiogram (ECG). Each time the heart beats, the ECG exhibits a small slow wave (P wave) which is followed by a fast series of changes (QRS complex) followed by another slow wave (T wave). These waves can be associated with events occurring in the heart itself (Fig 6). The P wave corresponds to atrial excitation, the QRS complex to ventricular excitation or phase 0 depolarization. Repolarization of the ventricles generates the T wave, which corresponds to the end of phase 2 and phase 3 of the cardiac action potential (Katz: Chapter 20, 1992).

Cardiac electrical activity is initiated by a propagating electrical wavefront which activates cardiac cells according to a given sequence. To identify such sequences requires a detailed knowledge of the spatial distribution of membrane activity. To accomplish this, simultaneous recording of electrical activity at a large number of points over the heart is necessary. The sequence of cardiac electrical activation can thus be mapped. The use of intracellular electrodes to record accurately the intracellular potentials simultaneously from a sufficiently large number of sites has proven to be impractical because of tissue movement, cell damage, and inaccessibility (Spach et al, 1972; 1973). Therefore, extracellular potential recordings have been used. Spach et al. have provided a theoretical rationale and experimental evidence suggesting good agreement between activation as determined from extracellular and intracellular recordings in the two-dimensional structure, Purkinje fibers (Spach et al., 1973), and in multidimensional anisotropic cardiac muscle (Spach et al.,1979). Extracellular electrodes have been extensively employed in cardiac mapping to record potential changes at multiple sites in the heart.

The technique of epicardial mapping in dogs was first reported by Rothberger et al, in 1913 (Rothberger et al.,1913) and Lewis et al. in 1915 (Lewis et al., 1915). This technique was then used to record the excitatory process in the exposed heart of a patient (Barker et al., 1930). This method has been extensively applied to localize accessory pathways associated with the Wolff-Parkinson-White syndrome (Gallagher et al., 1978), to study normal and abnormal atrial activation (Puech et al., 1954; Wellens et al., 1971), to delineate the course of the atrioventricular (A-V) conduction system (Kaiser et al.,

1970; Dick et al., 1979), and to identify areas of ischemia and infarction (Durrer et al., 1964) so as to further understand the mechanism of ventricular arrhythmia (Kramer et al., 1985).

Cardiac mapping is a method by which potentials recorded directly from the heart are spatially depicted as a function of time in an integrated manner (Gallagher et al., 1978). The recording can be achieved by employing a mesh sock with an array of electrodes over the heart. Extracellular potentials then can be recorded at many electrode sites. A unipolar recording from the epicardial surface gives rise to a positive deflection, followed by a rapid intrinsic deflection in the negative direction, with a final return to the baseline (Gallagher et al., 1978). The fast negative deflection, the "intrinsic deflection", in the unipolar electrode lead corresponds to activation at each electrode site (Durrer et al., 1954). Unipolar recordings are generally recorded at frequencies settings of 0.1-1200 Hz. The low frequency response tends to make the unipolar electrogram unstable. One of the ways to solve this problem is by using two closely spaced unipolar electrodes, a bipolar electrode, and recording differential voltage between them. The configurations of the two unipolar leads recorded in contiguous areas differ only in the detail of the electrogram recorded at the moment of local activation (Gallagher et al., 1978). Therefore, if one of the unipolar leads is electrically inverted and the two leads are algebraically summated, the two unipolar electrograms will thus cancel each other except where voltage differences occur (Gallagher et al., 1978). This voltage difference will produce a differential spike that is coincident in time with the rapid intrinsic deflection of a unipolar electrogram (Durrer et al., 1954), and is referred to as a bipolar electrogram. The bipolar electrogram is recorded at filter frequencies , with a lower limit usually $\geq$ 5Hz and a higher limit $\geq$ 200Hz, and provides a sharp deflection that is readily identifiable. Both the peak amplitude (Durrer et al., 1954; Gallagher et al., 1978; Kramer et al., 1985) and the slope of the differential spike have been used as indicator of local activation.

Because of the extensive analysis needed to generate an activation map, computers are used for data handling. All the electrogram signals are converted into digital form and transferred to a computer system. The timing of electrogram signals by computer analysis

has been shown to correlate well with local activation time expressed relative to a standard ECG reference (Spach & Dolber, 1986). A continuous scan of simultaneously acquired electrograms can then be performed, showing the local activation time of each electrode. The sequence of surface activation is then depicted as isochrone lines. Conduction time to each surface electrode site can be calculated as the difference between the earliest activation time and the activation time at each surface site.

2. Neural control of the heart

2.1 Anatomy of autonomic innervation to the heart

The dog has been used widely in physiological investigations of cardiac neural control since the turn of the century (Cyon, 1907; Keng, 1893; Schurawlew, 1928). Mizers (1955, 1957,1958), by combining physiological analysis with gross anatomical descriptions, presented the first widely accepted study of canine cardiac innervation. Although the knowledge of the anatomy of the innervation of the heart remains incomplete, the present knowledge of the gross anatomy of cardiopulmonary nerves and the intrinsic innervation of the heart has been well reviewed (Armour & Hopkins, 1984; Levy & Martin, 1979).

Both sympathetic and parasympathetic divisions of the autonomic nervous system have been shown to be under direct control of the hypothalamus. The hypothalamus is regulated by certain areas of the cerebral cortex and in turn reciprocally influences the cortex (Levy and Martin, 1979).

Parasympathetic preganglionic axons leave the brain stem as part of cranial nerves III, VII, IX, and X. The axons in nerves III, VII, and IX are distributed to the head region, whereas the vagus nerve (cranial nerve X) distributes autonomic fibers to the cervical, thoracic, and abdominal viscera as far caudally as the left colic flexure. The right vagus nerve, distal to the origin of the recurrent nerve, gives off two or more fine branches called the cranial and caudal cardiac vagal nerves. The cranial cardiac vagal nerves go mainly to the pretracheal plexus. The caudal cardiac vagal nerves distribute to

the dorsal wall of the right atrium. Electrical stimulation experiments conducted on the left vagus of the dog indicate that cardioinhibitory fibers leave the left recurrent laryngeal nerve as it passes around the arch of aorta and go to the left atrial region. Other inhibitory fibers, like cranial and caudal cardiac vagal nerves, leave the left vagus distal to the origin of the recurrent nerve and are also distributed to the left atrium. (Armour and Hopkins, 1984)

Sympathetic preganglionic axons take origin from small cells in the intermediolateral cell column of spinal cord segments T1 through L4 or L5. These fibers leave each spinal nerve and then attach to the sympathetic trunk. The trunk is a paired strand of preganglionic and postganglionic sympathetic nerve fibers located ventrolateral to the bodies of the vertebrae in the thoracic and lumber regions. The stellate is the largest autonomic ganglion in the dog and is located on the lateral surface of the longus coli muscle at the level of the first intercostal space. Axons terminating in or passing through the stellate ganglion originate in preganglionic neurons in the spinal cord from the C7 to the T5 segments (Kostreva, et al., 1977; Lichtman, et al., 1980; Norris, et al., 1974; Wiesman, et al., 1966). On the right side (Fig 7) the ansae subclavia originate at the cranial pole of the stellate ganglion. The ventral ansa courses ventral to the subclavian artery and interconnects with the thoracic vagus at the same level as the stellate ganglion. The dorsal ansa lies dorsal to the subclavian artery and courses cranially for a few centimeters before entering the middle cervical ganglion. The ventral ansa usually gives rise to three branches. The first branch is a small one, named right interganglionic nerve, because of its origin and the fact that it contains axons from cell bodies in both the middle cervical and stellate ganglia. Another branch of the ventral ansa usually courses horizontally and unites with a vagal branch arising at the same level of the vagus called cranial cardiac vagal nerve. A third branch of the ventral ansa courses cranially just lateral to the vagus with which it is interconnected. At the level of the middle cervical ganglion this branch together with the dorsal ansa, joins the above-mentioned ganglion. The left-sided thoracic autonomic nerves and ganglia in the dog appear to differ from right-sided ones but with respect to their origins from the ganglia and their connections there are certain similarities. The left stellate ganglion is thick with an enlarged cranial

region. It gives ventral and dorsal ansae which horizontally join the middle cervical ganglion. Thus, the majority of the left cardiopulmonary nerves arise from the middle cervical ganglion and have been named in accordance with their site of origin. (Armour and Hopkins, 1984)

2.2 Action of autonomic nervous system in the heart

Autonomic regulation of the heart is effected mainly by two systems: the sympathetic and parasympathetic nervous systems. In general, these two systems have opposite effects on the heart. For example, sympathetic stimulation increases heart rate and myocardial contractility (Levy et al, 1966), whereas parasympathetic stimulation slows the heart rate, and causes a negative inotropic effect on the atria (Levy et al., 1969).

As early as 1955, Burn et al. (1955) and Loomis et al. (1955) showed shortening and inhomogeneity of refractoriness were caused by acetylcholine (ACh) in vitro. In 1958, Alessi et al. (1958) found a nonuniform distribution of vagal effects on the atrial refractory period in anaesthetized dogs. They measured the refractory period (RP) at several points on the right atrial surface. During stimulation of the vagus nerves, the RP varies widely compared to the values under control conditions. Reflex excitation of the vagi, induced by increased arterial pressure, yielded similar results. Eight years later, Ninomiya (1966) used suction electrodes and recorded the duration of the monophasic action potential (APD) at several sites from both atria. He also obtained data which supported the nonuniform distribution of vagal effects on dog atria. In terms of the location of vagal effect on refractory period, Zipes et al (1974) demonstrated that the effects from right and left vagi were unevenly distributed on both atria.

In 1951, DiPalma et al. (1951) reported a shortening of atrial muscle refractory period duration following epinephrine administration. Kralios et al. (1981) showed right and left stellate ganglion stimulation in dogs significantly shortened atrial ERP. They also concluded that stimulation of the right stellate ganglion produced localized shortening in the right atrium, particularly at the sinus node area, and the left stellate ganglion affected

only left atrial sites. Takei et al. (1991) found that stimulation of the discrete intracardiac sympathetic nerves to the sinoatrial (SA) nodal region uniformly shortened ERPs at three sites in the right atrium after administration of atropine. He stimulated right ansa subclavia instead and observed similarly-shortened ERPs in the absence of atropine.

Thus, we can conclude that both sympathetic and parasympathetic nervous systems have the effects on the sinus rate and the atrial ERP shortening. Sympathetic nervous system may uniformly shortens ERP, whereas vagal nerves have nonuniform effects.

It is generally recognized that the two divisions of the autonomic nervous system have complicated interactions on various aspects of the performance of the heart. The additive nonuniform shortening of atrial refractory period by sympathetic and parasympathetic stimulation was reported by Takei et al. (1991). The right ansa subclavia and the discrete intracardiac parasympathetic nerves to the sinus node region were stimulated. The results recorded at three sites in the right atrium showed the effects of combined stimulation to be an algebraic sum of the individual responses to each stimulation, and the parasympathetics to exert the principal neural control over ERP.

Parasympathetic stimulation elicits a progressively greater reduction in heart rate as the level of tonic sympathetic stimulation is increased (Takei et al., 1991). The exaggerated inhibitory effect has been termed "accentuated antagonism" (Levy. 1971). Accentuated antagonism also exists in atrial (Furukawa et al, 1980; Stuesse et al., 1979) and ventricular (Levy et al., 1966) muscle contractility and ventricular ERP (Nattel et al., 1981), whereas the effects of cardiac sympathetic and vagus stimulation on atrioventricular nodal conduction are algebraically additive ("additive antagonism") (Levy et al., 1969; Wallick et al., 1982).

3. Mechanisms of cardiac arrhythmia

3.1 Automaticity

One of the three main differences between cardiac muscle and most other kinds of

excitable cell is automaticity, which is the spontaneous, intrinsic rhythm generated by some specialised cells (pacemaker cells). Pacemaker activity is normally found only in nodal and conducting tissue. These cells share a common characteristic: during diastole, the membrane potential slowly declines to less negative values. This slow potential decline is called diastolic depolarization, and it has a different slope in different pacemaker tissues. The fastest rate of diastolic depolarization is found in the sinus node cells, which acts as the normal pacemaker for the heart (Vassalle, 1982). The functional determinants of spontaneous automaticity are the maximum diastolic potential, the rate of phase 4 depolarization, the threshold potential and the action potential duration.

In the category of arrhythmias due to abnormal impulse generation (Zipes, 1983), disturbances of rhythm result either from a normal mechanism for impulse generation, such as the one normally present in the His-Purkinje system when its spontaneous rate is enhanced by catecholamines (Gilmour et al, 1986), or from some abnormal mechanisms in diseased cardiac tissue, for example, in the early phase of acute ischemia (Euler et al., 1981). The former is referred to as "enhanced automaticity", and the latter as "abnormal automaticity".

3.2 Triggered activity

Triggered activity is the term used to describe impulse initiation in cardiac fibers that is dependent on afterdepolarizations (Cranefield et al., 1974, 1977, 1988). It is separate and distinct from automaticity, which results from the spontaneous diastolic depolarization caused by the pacemaker current (Di Francesco et al., 1981, 1985). The basis for triggered activity is an afterdepolarization, that is, an oscillation of the membrane potential that occurs near the time that the cell is repolarizing. The oscillations occurring during phase 2 or 3 of the cardiac action potential are known as early afterdepolarizations (EADs) (Roden and Hoffman, 1985) and those occurring just after repolarization or during phase 4 of the cardiac action potential are known as delayed afterdepolarizations (DADs) (January and Fozzard, 1988) (Fig 8). When afterdepolarizations are large enough to reach the threshold potential for activation of a

regenerative inward current, the resultant action potentials are referred to as "triggered".

EAD-induced triggered activity is generally cycle-length dependent (Damiano et al., 1984). Any factors which cause excessive lengthening of APD, particularly at slow activation rates and promote inward Na^+ or / and Ca^{++} current, may induce EADs (Singh, 1993; Roden, 1993). Early afterdepolarizations in the ventricles are now thought to be important in the genesis of Torsade de Pointes in the setting of prolonged cardiac repolarization (Singh, 1993; Roden, 1993). Thus, drugs that increase APD not only have the ability to prevent fibrillation but, given the appropriate clinical setting, they also have a proclivity to induce Torsade de Pointes ventricular arrhythmias (Singh, 1988).

DADs usually occur under a variety of conditions in which there appears to be a large increase in Ca in the myoplasm and the sarcoplasmic reticulum (sometimes referred to as "Ca overload") (January and Fozzard, 1988). This explains the tendency of inotropic drugs like digitalis to cause DADs (Ferrier et al., 1973). The ionic mechanism responsible for DAD is the result of a net inward current known as the transient inward current (TI) (Lederer et al., 1976) which occurs when the intracellular calcium concentration $[Ca]_i$ increases beyond its normal range. How does this rise in $[Ca]_i$ cause an inward current? One possibility is that it results from activation of sodium-calcium exchange across the cell membrane (Mechmann and Pott, 1986; Lipp and Pott, 1988). This counter-transport system acts to transfer one Ca^{2+} ion out of the cell in exchange for three Na^+ ions, resulting in a net influx of one positive charge which acts to depolarize the cell. Increasing the intracellular calcium concentration will thus produce an increase in net inward current, and therefore membrane depolarization. Whatever the mechanism is, the importance of the transient inward current in the genesis of dysrhythmias, and its close relation to intracellular calcium concentration, is now well-established.

3.3 Reentry

In the normally-activated heart, the propagating impulse initiated by the sinus node stops after sequential activation of atria and ventricles because it is surrounded by

refractory tissue that it has just excited and because it meets the inexcitable fibrous annulus. Under special conditions (see below), the impulse may not die out after complete activation of the heart but may persist to reexcite atria or ventricles after the end of the refractory period. This is called reentrant excitation. Reentrant excitation as a cause of cardiac arrhythmias has been studied since the second decade of this century. The characteristics of reentrant excitation and the conditions necessary for initiation and maintenance of reentrant excitation were well-defined by Mines (1913, 1914), and Garrey (1914). For many decades, however, reentry as a cause of arrhythmias was considered by many a theoretical possibility, and only some 50 years later did it become generally accepted that reentry could be responsible for a wide variety of clinically and experimentally observed arrhythmia, such as atrial fibrillation (Wang et al., 1992; Cox et al., 1991), atrial flutter (Rensma et al., 1988), atrioventricular reentry (Gallagher et al., 1976), ventricular tachycardia (Pagwizd, 1992), and ventricular fibrillation (Chen et al., 1993).

Different Forms of Reentry

3.3.1 Reentry Involving an Anatomic Obstacle

The simplest concept of reentry in cardiac tissue was introduced by Mines in 1913, based on the presence of an area of unidirectional block caused by differences in refractory periods in adjacent regions on a ring of excitable heart tissue (Fig 9). In fig 9, the circus moves around a gross anatomic obstacle. A stimulated impulse leaves in its wake absolutely refractory tissue (black area) and relatively refractory tissue (stippled area). In both A and B, the impulse conducts in one direction only. In A, because of a long refractory period, the tissue is still absolutely refractory when the impulse has returned to its site of origin. In B, because of a short refractory period, the tissue has recovered excitability by the time the impulse has reached the site of origin, and the impulse continues to circulate. Thus, in an anatomically defined circuit, Mines recognized that the duration of the refractory period, the conduction velocity and unidirectional block

determined whether or not reentrant excitation would occur. In atria, the impulse may find an anatomical obstacle in its path, possibly the orifice of the vena cava or a pulmonary vein. The anatomical obstacle then defines a pathway allowing the propagation of the excitation wave in a circus movement fashion. An important feature of this model of circus movement is the existence of an excitable gap (the white part of the circle in Fig 9B or Fig 10A) between the crest of the excitation wave and its tail of relative refractoriness (dotted area). This implies that impulse originating outside the reentrant circuit may enter it and influence the reentrant rhythm by terminating or resetting it.

3.3.2 The Leading-Circle Model

Allessie et al. (1973, 1976, 1977) developed a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle, on the basis of observation of circus movement tachycardia induced in small pieces of isolated left atria from rabbit hearts via a properly timed premature stimulus. In this preparation, no anatomic obstacles are present, and the reentrant circuit is completely determined by the electrophysiologic properties of the tissue involved. The leading circle was defined as "the smallest possible pathway in which the impulse continues to circulate, and in which the stimulating efficacy of the wavefront is just enough to excite the tissue ahead which is still in its relative refractory phase". (Allessie et al., 1977). Fig 10B is a diagram for "leading circle" reentry. The arrow indicates the direction of impulse propagation. The darkened area represents the absolute refractory period and dotted area the relative refractory period. Since the circuit is the minimum pathlength for reentry, the crest of the wavefront just catches its tail which is still in its relative refractory period, so that there is no excitable gap between the crest and the tail. Reentrant tachycardia based on the leading circle model is not thought to be influenced by a premature impulse initiated outside the reentry circuit because of the low stimulating efficacy of the propagating impulse and lack of an excitable gap existed.

According to this "leading circle" mechanism, the length of the reentry circuit is equal to the wavelength of the impulse. The wavelength is defined as the distance

travelled during a period of time equal to the refractory period, and corresponds to the product of conduction velocity times the effective refractory period (WL=ERP×CV) (Wiener and Rosenblueth, 1946). Shortening of the functional refractory period or slowing of conduction velocity will result in a smaller reentrant circuit, whereas when the refractory period is long or if conduction velocity is rapid, the dimensions of the reentrant circuit to sustain circus movement become larger. However , because the crest of the wave front impinges on the relative refractory tail of the previous wave, the circulating wave travels through partially refractory tissue. Therefore, conduction velocity is reduced. In fact, during circus movement in the leading-circle model, refractory period and conduction velocity are interdependent. The final effect on the WL therefore depends on the changes in both ERP and CV.

4. Previous concepts and experimental studies of AF mechanisms

4.1 Early ideas about AF mechanisms

Fibrillary contraction of heart muscle was first reported by Hoffa and Ludwig (1850). They described ventricular fibrillation, produced in cold-blooded and mammalian hearts by electrical stimulation. They described a limited region of "tetanus", or rather a condition of persisting contraction immediately about the electrode; while in the rest of the heart contractions were observed which were weak and absolutely irregular. Vulpian (1874) was the first to note that local faradization of the auricles abolishes normal systole, which is replaced by a strong, incoordinate "flutter".

At first, people believed that fibrillation is caused only by agents which increase the excitability of the cardiac musculature. They thought that in the fibrillation the muscle fibers contract independently, because owing to increased excitability they have become independently rhythmic (Engelmann, 1895). Each ectopic focus may be beating at its maximal rate (Hofmann FB, 1905). This was felt to imply that multiple ectopic foci of impulse formation, "heterogenesis" (Winterberg, 1906), must have developed. Later, the conception of circus movement, as demonstrated by Mines and Garrey, was adopted.

From his studies on rings of excitable tissue, Mines (1913) recognized one of the essential requirements for the initiation of a reentrant rhythm, namely, the presence of an area of unidirectional block, caused by differences in refractory periods in adjacent regions. He also was the first to consider reentrant excitation as a cause for arrhythmias occurring in humans. In 1914, Garrey made observations similar to those of Mines on ringlike preparations and became convinced that reentrant excitation was the basis for fibrillation. When he cut away small pieces of the fibrillating muscle, or functionally severed them from the main fibrillating mass by clamping, each severed piece in turn ceased to fibrillate and came to rest, though remaining excitable and contractile. When enough of these pieces had been successively cut away to make a sufficient reduction in the mass of the remaining tissue, it too ceased to fibrillate and again contracted coordinately in response to the normal rhythmic impulses. This experiment was crucial. It showed that the individual fibers are not independently rhythmic. An important contribution of Garrey was that he proved that a minimal mass of tissue is required to maintain fibrillation. Also, he was the first to consider that reentry could occur without the involvement of an anatomic obstacle around which the circulating wave front could circulate, and he thought that differences in refractory periods would create temporal barriers for conduction, necessary to maintain circus movement (Garrey, 1924). He realized that the circus movement in fibrillation was much more complex than in a single ring.

Lewis (1925) provided further evidence for Mines' and Garrey's ideas. He clearly formulated that the reentrant circuit was governed by three factors: 1) the length of the muscle path, 2) the rate at which the wave travels, 3) the duration of the refractory state at any given point. Although he considered an anatomic obstacle necessary for reentrant excitation to occur, which was advocated by Mines, he came close later to defining the conditions necessary for reentrant circuits that are wholly determined by the functional properties of the tissue involved, as hypothesized by Garrey.

So in the early part of the twentieth century, three predominant theories of AF existed: 1) the presence of multiple, non coordinated ectopic foci; 2) the presence of a single, rapid reentry circuit which, because of its rate and variable conduction to the rest of the atria, produces the irregular activity typical of AF; and 3) the presence of multiple

simultaneous reentering wavefronts (Garrey, 1924).

4.2 The "multiple wavelet" concept

On the basis of earlier studies, in late 1950s and early 1960s, Moe (1959, 1962) formulated the so-called "multiple wavelet" hypothesis to explain the mechanisms of true fibrillation. Since, at that time, simultaneous recording from a sufficient number of atrial sites to document the complex excitation pattern was impossible, Moe et al. (1964) developed a computer model of AF which was based on multiple reentrant wavelets in a two-dimensional sheet with realistic properties. The key feature of the model was a nonhomogenous distribution of refractory periods in otherwise identical elements.

According to Moe's finding based on his computer model, fibrillation is maintained by the presence of a number of independent wavelets that wander randomly through the myocardium around islets or strands of refractory tissue. Each of these wavelets may accelerate or decelerate as it encounters tissue in a more or less advanced state of recovery of excitability. They may extinguish, divide, or combine with a neighbour wavelet, (which is different from Garrey's coexistence of multiple wavelets without interaction within them,) and they continuously fluctuate in size and change direction of propagation. In Moe's theory, the number of wavelets present at one time could vary, and could be changed by the changing of electrophysiologic properties of the atrial tissue. The likelihood of spontaneous termination of fibrillation depends on the average number of wavelets present. If many wavelets exist ("fine" fibrillation), the chance that all wavelets will extinguish simultaneously is small. On the other hand, with only a small number of wavelets present ("coarse" fibrillation), at a certain moment the waves may die out or fuse into a broad single wave front. This may result in either transition into atrial flutter or resumption of normal sinus rhythm.

A direct test of the multiple-wavelet hypothesis was performed by Allessie et al. (1985). They inserted two egg-shaped multipolar electrodes, containing 480 recording terminals in each, into the atrial cavities of isolated, Langendorff-perfused dog hearts. Atrial fibrillation was induced by a single premature stimulus while acetylcholine was

administered continuously to the perfusion fluid. During maintained fibrillation, excitations of right and left atria were mapped consecutively. As Moe's theory mentioned, the presence of multiple independent wavelets was demonstrated. Each individual wavelet existed for a short time, not longer than several hundred milliseconds. Extinction of a wavelet could be caused by fusion or collision with another wavelet, by reaching the border of the atria, or by meeting refractory tissue. New wavelets could be formed by division of a wave at a local area of conduction block or by an offspring of a wave, travelling toward the other atrium. This kind of reentry is defined as random reentry. However, the critical number of wavelets in both atria necessary to maintain fibrillation was between three and six found by Allessie et al.(1985), much less than the number which was estimated by Moe's computer model.

4.3 The role of WL in AF

According to Allessie's "leading circle" model, the length of the circuit is defined by the electrophysiologic properties of atrial tissue and is equal to the wavelength of the impulse. The wavelength can be shortened by a shortening of the refractory period or by a decrease in conduction velocity, or by both. The shorter the wavelength is, the more reentry circuits could exist in a given area. Since for perpetuation of AF a critical number of wandering wavelets is required (Allessie et al., 1985), the WL is an important determinant for the degree of stability of fibrillation. If the WL during fibrillation is relatively long, the reentry circuits will be larger, fewer waves can circulate through the atria, and fibrillation will be short-lasting. If, however, the WL during fibrillation is short, a greater number of wavelets will be present, and fibrillation will tend to be stable and long-lasting (Allessie et al., 1990). In this sense, WL has a crucial role in determining the maintenance of AF.

The relevance of this concept was supported by the observations of Rensma et al (1988), who found that the types of atrial arrhythmias induced by programmed stimulation in conscious dogs depended on the wavelength. At long values of wavelength, reentry was not seen, whereas as wavelength progressively decreased, repetitive

responses occurred, followed by atrial flutter, and finally AF at the shortest wavelength values (< 8 cm). So, currently, WL is believed to be the main determinant of AF.

4.4 Pharmacologic studies of AF determinants

The most widely used classification of antiarrhythmic drugs, formulated by Singh and Vaughan Williams (1970, 1972), divides antiarrhythmic agents into four categories. This classification divides drugs according to four major pharmacological actions: (I) sodium channel blockers; (II) β-blocking agents; (III) drugs that prolong action potential duration; and (IV) calcium channel blockers.

During the last 10 years, there have been a number of studies on the mechanisms of antiarrhythmic drug action in atrial fibrillation. Rensma et al (1988), implanted multiple stimulating and recording electrodes on both atria in chronically instrumented conscious dogs, and showed that the wavelength was an accurate predictor of the inducibility of atrial arrhythmias in conscious dogs. Drugs that prolonged the wavelength of early premature impulses above this critical value effectively prevented induction of atrial fibrillation. They used quinidine (class Ia) and *d*-sotalol (class III) which increased the wavelength and prevented the induction of atrial fibrillation, while propafenone (Ic) and lidocaine (Ib) had little effect on wavelength and apparently did not alter the ability to induce AF. Quinidine markedly prolonged the refractory period (K^+ channel blocker), but prolongation of wavelength was less because of a simultaneous decrease in conduction velocity (Na^+ channel blocker). d-Sotalol also increased refractory period (K^+ channel blocker), but because it had no appreciable effect on conduction velocity, this drug was the most effective in prolongation of wavelength. Both propafenone and lidocaine had strong but opposite effects on refractoriness and conduction (Na^+ channel blocker), and consequently, little effect on the wavelength.

In a subsequent study from the same laboratory, the experimental class Ic compound ORG 7797 was found to reduce the inducibility of AF (Kirchhof et al, 1991). This action was associated with a tachycardia-dependent increase in atrial refractory period. The drug limited the minimum wavelength that could be produced by rapid

pacing, thus presumably decreasing the number of simultaneous atrial reentry circuits possible and the likelihood of AF.

Wang et al (1992) evaluated the efficacy and mechanisms of action of flecainide (class Ic) by using a model of sustained AF, which was produced by a brief burst of atrial pacing in the presence of vagal stimulation and persisted spontaneously until vagal stimulation was stopped, and used epicardial mapping to address antiarrhythmic mechanisms. Flecainide increased atrial ERP and reduced conduction velocity in a tachycardia-dependent manner. Doses of flecainide that converted AF resulted in larger changes in ERP than in conduction velocity, increasing the minimum pathlength capable of supporting reentry (wavelength). Under control conditions, multiple small zones of reentry coexisted. Flecainide progressively increased the size of reentry circuits, decreased their number, and slowed the frequency of atrial activation until the arrhythmia finally terminated; all changes were compatible with an increase in wavelength. The above observations consistent with the leading circle model (Allessie et al,. 1977) and the multiple wavelet reentry concept (Moe, 1962; Moe et al., 1964).

Subsequent studies (Wang et al, 1993, 1994) showed that propafenone (class Ia), procainamide (class Ic), sotalol (class III) and ambasilide (class III) are all capable of terminating atrial fibrillation and preventing its induction in a dog model of vagotonic sustained atrial fibrillation. Doses of all these agents that terminated AF increased the wavelength at short cycle lengths, slowed atrial activation during atrial fibrillation, and increased the size of reentry circuits. These results support the role of wavelength in mediating antiarrhythmic action in atrial fibrillation.

4.5 Autonomic effects on AF

4.5.1 Experimental studies

There are a long series of remarkable studies which have revealed so much about the autonomic nervous system and the atrium (see section 2.2). It is well known that vagal nerve stimulation or ACh shortens atrial ERP and causes heterogeneity of refractoriness

in atria. According to Moe's "multiple wavelet reentrant" hypothesis, vagal effect creates conditions that favour AF which has, in fact, been shown by numerous experimentors.

The earliest study showing AF with vagal nerve stimulation was produced by Andrus and Carter (1930), who introduced a single stimulus shortly after the end of the refractory period in the dog's heart. Scherf and Chick (1951) showed that when a 5% solution of acetylcholine was applied to the area of the sinus node, flutter or fibrillation started immediately. Later on, numerous investigators have demonstrated the ability of vagal nerve stimulation or cholinergic agonists (Burn et al, 1955; Hoff et al, 1955; Moe, et al, 1959) to promote the occurrence of sustained AF. Vagus nerve stimulation abbreviates atrial refractoriness, which shortens WL, and increases the variability of refractoriness, which increases the possibility for unidirectional block, hence greatly increases the ability of single atrial extrasystoles (Andrus et al., 1930) and fast pacing (Burn et al., 1955) to induce sustained AF.

Sustained AF induced by vagal nerve stimulation in anaesthetized dogs has been used as a model to study the mechanisms of antiarrhythmic drug action (Wang et al, 1992; Wang et al, 1993, 1994). In this model, the cervical vagi were transected, and the distal ends stimulated at 2/3 of the asystole threshold. During vagal stimulation, AF could readily be induced and was sustained for as long as vagal stimulation was continued. Upon the cessation of vagal stimulation, sinus rhythm returned quite rapidly, allowing for electrophysiologic measurements to be made. Atrial epicardial mapping with a 112-electrode atrial array was used to study the pattern of atrial activation during vagal AF and the mechanism of the drug's action on AF. The activation pattern of AF was consistent with "leading circle" reentry. The drugs gradually increased the size of reentry circuits, decreased their number, and slowed the frequency of atrial activation until the arrhythmia finally terminated; all changes compatible with an increase in wavelength .

Recently, radiofrequency catheter ablation of the atria (RFCA) in open-chest dogs has been found to produce partial vagal denervation and to reduce the inducibility and duration of AF (Elvan et al., 1995), which helps us to further understand vagal effects on AF.

Relatively little work has been performed to establish the effects of sympathetic nerve stimulation or adrenergic agonists on the occurrence and properties of AF. Andrus and Carter (1930) noted that the infusion of epinephrine in atropinized dogs reduced the atrial refractory period by a mean of 40%, but that while single premature stimuli regularly induced AF in the presence of vagal stimulation, this never occurred during the infusion of epinephrine. Hashimoto et al (1968) showed that norepinephrine infusion into the sinus node artery could potentiate AF induction by concomitantly administered acetylcholine. This could be explained by the additive nonuniform shortening of atrial refractory period by sympathetic and parasympathetic actions (see section 2.2). No studies of the effects of sympathetic nerve stimulation alone on the maintenance of AF have been reported.

4.5.2 Clinical information

Atrial fibrillation is the most common sustained cardiac arrhythmia in clinical practice, and is likely to become more common with the aging of the population (Kannel et al, 1992; Pritchett, 1992; Kannel et al, 1982). As mentioned in the previous section, many experimental observations during AF point towards an essential role for multiple circuit reentry. Recent mapping studies in patients suggest the importance of multiple wavelet reentry in man (Ferguson et al., 1993; Konings et al., 1994). The success of the surgical maze procedure in preventing AF by electrically isolating zones of atrial tissue too small to support the arrhythmia (Cox et al., 1991 (2)) support the clinical relevance of the multiple wavelet reentry concept. Indirect evidence points towards atrial wavelength as a significant determinant of the ability of AF to sustain itself in man (Asano et al., 1992; Botteron et al., 1995; Botteron et al., 1996). Autonomic tone, especially vagal tone, may play an important role in clinical AF (Coumel, 1994).

Clinical cases of paroxysmal atrial tachyarrhythmia suggesting a predominant vagal mechanism often display a pattern of atrial fibrillation that alternates with a typical atrial flutter and its sawtooth electrical activity. In contrast, aspects suggesting ectopic automatic foci by their ECG appearance and their electrophysiologic behaviour

predominate in adrenergic dependent atrial fibrillation (Coumel, 1992). There are consistent studies to suggest that, in normal atria, vagal influences predominate (Takei et al., 1991), and macro-reentry in the form of common flutter is favoured by shortening of refractoriness. Diseased atria are more sensitive to adrenergic influences that tend to favour automatic activities or micro-reentry (Coumel, 1994). The onset of atrial fibrillation that occurs in the setting of rest or digestive periods, and is preceded by a progressive heart rate decrease, are often ascribed to a vagal mechanism. In contrast, palpitations starting at exercise or stress are likely to be adrenergically mediated. Frequently, however, an imbalance, rather than the definite predominance of either limb of the autonomic nervous system, may be important. These clinical notions remain, for the moment, speculative because clear proof for the role of autonomic influences in clinical AF is lacking.

5. Goal of present studies

Vagal nerve stimulation is known to abbreviate atrial refractoriness, decrease the reentrant wavelength, and facilitate the maintenance of AF, but the role of the sympathetic nervous system in AF is less clear. Activation of cardiac sympathetic input by ansa subclavia stimulation reduces atrial refractory period (DiPalma et al., 1951; Kralios et al., 1981). Reductions in atrial refractory period decrease the wavelength for reentry and should (according to current thinking) promote the occurrence of AF. Increases in cardiac sympathetic tone would therefore be expected to augment the likelihood and duration of AF, an effect similar to the well-recognized effect of cardiac vagal activation; however, relatively little is known about the effect of cardiac sympathetic stimulation on the properties of AF in experimental models.

The present experiments were designed to assess 1) the effect of bilateral stellate ansa stimulation at various frequencies on the wavelength for atrial reentry and the duration of AF, and 2) the relative effects of sympathetic and vagal stimulation on AF duration for a comparable degree of wavelength abbreviation.

METHODS

1. General Methods

Adult mongrel dogs of either sex weighing 21.5 to 41.0 kilograms (mean±SE, 30.7±1.5 kilograms) were anaesthetised with morphine (2 mg/kg i.m.) and α-chloralose (120 mg/kg i.v.) and ventilated via an endotracheal tube with room air supplemented with oxygen (NSH 34RH respirator, Harvard Apparatus, South Natick, MA) at a rate of 20 to 25/min and a tidal volume obtained from a nomogram. Arterial blood gases were measured to ensure adequate oxygenation (SaO_2>90%) and physiological pH (7.38 to 7.45). Catheters were inserted into the right femoral artery and both femoral veins and kept patent with heparinized saline solution (0.9%). Body temperature was maintained at 37 to 39°C with a homeothermic heating blanket.

A median thoracotomy was performed. An incision was made into the pericardium extending from the cranial reflection to the ventricular apex, and a pericardial cradle was created. Four bipolar Teflon-coated stainless steel electrodes were inserted into the right and left atrial appendages for recording and stimulation, and one was inserted into the left ventricle for electrogram recording. A programmable stimulator (Digital Cardiovascular Instruments, Berkeley, CA) was used to deliver 4-ms pulses at twice-threshold current. A demand pacemaker (GBM 5880, Medtronic Inc., Minneapolis, MN) was used to pace the right ventricle when the ventricular rate was less than 90/min. A P23 ID transducer (Statham Medical Instruments, Los Angeles, CA), electrophysiological amplifiers (Bloom Associates Ltd, Flying Hills, PA), and a paper recorder (Astromed MT-95000, Toronto, Ontario) were used to record six standard surface ECG leads, both atrial and left ventricular electrograms, stimulus artifacts and arterial pressure.

2. Nerve Stimulation

Both cervical vagal trunks were isolated, doubly ligated and divided. Bipolar hook

electrodes (stainless steel, insulated with Teflon except for the distal 10 to 15 mm) were inserted into the middle of each nerve with the electrode running within and parallel to vagal fibers for several centimeters. Bilateral vagal nerve stimulation was delivered by a Grass SD-9F stimulator (Grass Instruments, Inc., Quincy, MA) with a pulse width of 0.2 ms and amplitudes and frequencies as indicated below. The adequacy of vagal nerve stimulation was verified at the beginning of the experiment by ensuring that 6 V stimuli at 10 Hz reduced the sinus rate by at least two-thirds. The vagal frequency-response relation was assessed before each series of measurements and the vagal stimulation frequency was adjusted to produce consistent sinus node slowing.

The stellate ganglia were isolated and decentralized as previously described.[9] Insulated bipolar electrodes were attached to the dorsal and ventral ansae, which were stimulated with square-wave pulses of 2-ms duration, 6 V intensity and frequencies indicated below as necessary. The adequacy of stellate ansa stimulation was determined from the heart rate and blood pressure response to unilateral stimulation at 10 Hz of the right and left ansae. Overall, heart rate increased from 136 ± 5/min to 254 ± 3/min ($P<.001$) during right ansa stimulation, and blood pressure increased from 109 ± 7 / 76 ± 5 mm Hg to 157 ± 6 / 103 ± 6 mm Hg ($P<.001$, $P<.01$ for systolic and diastolic pressure respectively) during left ansa stimulation. During each stimulation period for electrophysiologic measurement and evaluation of arrhythmia response, bilateral ansa stimulation was applied and the constancy of effect was confirmed by observing constant actions on blood pressure and heart rate.

3. Activation Mapping

Five thin plastic sheets containing 112 bipolar electrodes with 1-mm interpolar and 6-mm interelectrode distances were sewn into position on the atrial epicardial surface (Fig 11). The electrode arrays were similar to those we have previously used,[3,4] except that the disposition of stimulating electrode sites was somewhat altered. One sheet was placed under the root of the aorta to cover the anterior aspect of the atrial appendages and Bachmann's bundle. Three sheets were sewn to the posterior aspects of the atrial

appendages and to the free walls. The parietal pericardium was gently separated, and a fifth plaque was placed between the pulmonary arteries and veins. Each signal was filtered (30 to 400 Hz), digitized with 12-bit resolution and 1-kHz sampling rate, and transmitted into a microcomputer (model 286, Compaq Computer, Houston, TX). Software routines were used to amplify, display, and analyze each electrogram signal as well as to generate activation maps. Each electrogram was analyzed with computer-determined peak-amplitude criteria and was reviewed manually. The data were downloaded on high-density diskettes for subsequent off-line analysis. Isochrone maps and activation times for each activation were recorded by the use of an IBM ink jet printer. Hardware and software for the mapping system were obtained from Biomedical Instrumentation, Inc. (Markham, Ontario).

4. Electrophysiologic Measurements

Atrial conduction velocity (CV) and effective refractory period (ERP) were assessed after at least 2 minutes of constant pacing at a basic cycle length (BCL) of 400, 300, 250, 200, and 150 ms under control, sympathetic and vagal stimulation conditions. A programmable stimulator was used to deliver square constant current pulses of 4 ms duration and two times diastolic threshold. The ERP was measured with a train of 15 basic (S1) stimuli followed by a premature (S2) stimulus and then a 1-second pause. The S1S2 coupling interval was decreased in steps of 10 ms and the ERP was defined as the longest S1S2 interval failing to produce a propagated response. Activation maps during steady-state pacing were generated off-line after the experiment. Conduction velocity was measured in the vicinity of the stimulating electrode (beginning at least 8 mm from the stimulation site to avoid virtual cathode effects (Wikswo et al, 1991) by analyzing activation times at a series of electrode sites in the direction of longitudinal propagation as determined from activation mapping. Distance of each site from the first of the series of electrodes was plotted against activation time, and conduction velocity determined by linear regression. A minimum of three electrode sites was used for each analysis (mean 4.3, range 3-6 sites for all analyses), and the correlation coefficient always exceeded

0.99. Electrograms were manually reviewed to ensure that a corresponding point on the electrogram was used to time activation at each cycle length and for each condition in a given dog. Fig 12 shows an example of activation maps, electrogram recordings, and regression analysis of conduction velocity at three cycle lengths in one dog. The same sites were used for conduction velocity measurements without and with sympathetic or vagal stimulation, after ensuring a constant pattern of impulse propagation. Results from at least three beats were used to calculate CV for each determination. The wavelength for reentry (WL) was determined by multiplying the ERP as determined at a given site by the local CV measured during stimulation at the same site, according to the formulation described by Wiener and Rosenblueth (1946).

Under each condition, AF was induced 10 times by stimulating the right atrial appendage at 10 Hz with four times threshold, 4-ms duration pulses. If AF lasted for ≥10 minutes on two occasions, and no briefer episodes of AF occurred, mean AF duration was based on these two episodes and no further AF induction was attempted under that condition, in order to avoid excessive prolongation of the experiment. In some cases, particularly when refractory periods at BCL 150 ms were less than 100 ms, stimulation at 10 Hz was associated with 1:1 conduction rather than AF. In such cases, stimulation for AF induction was applied at a frequency greater than 10 Hz. AF was defined as a rapid (more than 450/min under control conditions), irregular atrial rhythm with varying atrial electrogram morphology. AF duration was measured from the time of discontinuation of rapid atrial stimulation to the time of spontaneous termination of AF. Sustained AF was defined by a duration of more than 10 minutes. An 8-second window of electrogram data was obtained during AF, and the duration of the arrhythmia was recorded for each AF episode. The AF frequency was determined at each electrode site as previously described (Wang et al, 1992; Wang et al, 1993) on the basis of the number of atrial activations over a 2-second interval. Correlation with the surface ECG was used to eliminate ventricular electrograms. Double potentials due to propagation adjacent to a site of block were treated as single activations.

5. Experimental Design

In seven dogs, the stimulation frequency-dependence of the effects of sympathetic stimulation on electrophysiologic variables and AF duration was assessed. ERP, CV, WL and AF duration were determined as described above during pacing at the right atrial appendage (site 1 in Fig 11) under the following conditions: prior to stellate ganglion and vagal decentralization (autonomically intact); after autonomic decentralization and without nerve stimulation; and during bilateral stellate ansa stimulation at 1, 4, and 10 Hz.

The next series of studies were designed to compare the effects of vagal and sympathetic stimulation at frequencies producing comparable effects on WL, and was performed in six dogs. Control measurements were obtained following stellate and vagal decentralization. In addition to measuring AF duration and ERP, CV, and WL during stimulation at site 1 at five BCL's as described above, we determined ERP, CV, and WL at a BCL of 200 ms at sites 2 to 7 in Fig 4. The stellate ganglia were then stimulated at 10 Hz and 6 V, and the same measurements repeated. After results had been obtained during sympathetic stimulation, we selected a combination of vagal stimulation frequency and intensity that produced the same degree of ERP abbreviation at site 1 and a BCL of 150 ms as sympathetic stimulation. The ranges of frequencies and intensities selected for vagal stimulation in individual dogs were 2 to 8 Hz and 3 to 6 V, with mean values of 4.7 ± 0.8 Hz and 4.5 ± 0.7 V. The measurements obtained under control and sympathetic stimulation conditions were then repeated during vagal stimulation.

Subsequent studies were performed in 10 dogs to exclude the possibility that spontaneous acetylcholine or norepinephrine release from vagal or sympathetic nerve endings during rapid stimulation or AF could be contributing to the results obtained. Electrophysiologic measurements and AF duration determinations were obtained after vagal and stellate decentralization and in the absence of nerve stimulation during pacing at site 1, and then atropine (five dogs) or nadolol (five dogs) was administered intravenously at doses (1 mg for atropine, 0.5 mg/kg for nadolol) we have previously shown to block the effects of vagal or sympathetic nerve stimulation respectively in the dog (Talajic et al, 1990). The measurements obtained under control conditions were then repeated.

A final series of three dogs was studied to evaluate possible time-dependent

changes in the key variables analyzed. We measured atrial ERP at a cycle length of 200 ms under control conditions (autonomic decentralization) and then determined the mean duration of AF during 10 episodes induced by burst pacing. The same measurements were then repeated during sympathetic and then during vagal stimulation. Thirty minutes after these measurements were completed, the same procedures were repeated for all three conditions.

6. Data Analysis

Group data are presented as the mean$\pm$SE. Analysis of variance with a range test (Scheffé contrast) was used to test the statistical significance of differences between group means. Two-tailed tests were used, with a $P<.05$ taken to indicate statistical significance.

RESULTS

1. Effects of Bilateral Stellate Ansa Stimulation on AF Duration and Electrophysiologic Variables

Changes in ERP and wavelength (measured during stimulation at the right atrial appendage at a BCL of 150 ms) and AF duration induced by autonomic decentralization and sympathetic stimulation are shown in Table 1. When dogs were studied prior to autonomic decentralization (autonomically-intact condition), AF was sustained for an average of 67±25 seconds. When the vagi and stellate ganglia were decentralized, there was a significant increase in ERP and wavelength, accompanied by a substantial decrease in AF duration. Sympathetic stimulation produced a decrease in ERP and wavelength that became larger with increased nerve stimulation frequency. At a sympathetic stimulation frequency of 10 Hz, the ERP and wavelength were reduced to values not significantly different from those observed in autonomically-intact dogs. Despite this, sympathetic stimulation did not restore AF duration to values under autonomically-intact conditions. Furthermore, while sympathetic stimulation significantly reduced ERP and WL compared to baseline values after decentralization, it failed to increase AF duration.

2. Comparative Effects of Sympathetic and Vagal Stimulation on AF Duration

We observed that sympathetic stimulation at frequencies that significantly reduced the wavelength for reentry at site 1 failed to alter AF duration. This finding is in clear contrast to our previous observation that bilateral vagal nerve stimulation produces a frequency-dependent decrease in wavelength associated with corresponding increases in AF duration (Wang et al, 1996). We therefore compared directly the effects of bilateral stellate ansa and vagal nerve stimulation at frequencies producing similar changes in the wavelength for reentry at site 1 in six dogs.

Sympathetic and vagal stimulation decreased ERP, while tending to produce slight increases in CV. Fig 13 shows examples of activation maps, electrogram recordings, and

regressions of distance on activation time to obtain conduction velocity under control, vagal, and sympathetic stimulation conditions in a representative dog. Activation patterns and electrogram morphologies remained constant in the presence of nerve stimulation. Fig 14 shows the values of ERP, CV and WL obtained at various atrial pacing cycle lengths during stimulation at site 1 under control conditions and in the presence of vagal and sympathetic stimulation. ERP decreased as a function of BCL, and was decreased at all cycle lengths by both sympathetic and vagal stimulation. Both vagal and sympathetic stimulation produced small increases in CV, which were of a similar order at cycle lengths of 150 and 200 ms. Sympathetic and vagal stimulation decreased wavelength significantly and to a similar extent at cycle lengths of 150 and 200 ms. Because it accelerated sinus node automaticity, sympathetic stimulation effects during atrial pacing could only be studied at cycle lengths $\leq$250 ms.

Fig 15 shows values for ERP and wavelength (measured at site 1 at a BCL of 150 ms), along with AF duration under each condition. Sympathetic and vagal stimulation reduced both ERP and wavelength significantly and to a similar extent. While small, statistically nonsignificant increases in AF duration were produced by sympathetic stimulation, vagal stimulation caused important and highly-significant increases in the duration of AF. Sustained AF occurred reproducibly during vagal stimulation in four dogs, but was never observed under control or sympathetic stimulation conditions.

Although vagal and sympathetic stimulation produced very similar changes in ERP and wavelength at site 1, we considered the possibility that vagal actions may have been much greater at other sites, making the value at site 1 not truly representative. We therefore measured ERP, CV and WL at all seven sites indicated in Fig 4 at a BCL of 200 ms. This cycle length was selected, rather than 150 ms, because during rapid stimulation at certain sites (particularly in the presence of vagal stimulation) prolonged AF tended to be induced, making the protocol difficult to complete. Fig 16 shows mean values of ERP, CV and WL at all seven sites. Under control conditions (left), ERP, CV and WL averaged respectively 102 ± 2 ms, 117 ± 5 cm/s and 12.0 ± 0.6 cm overall at all sites. Sympathetic stimulation (middle) reduced ERP to 88 ± 2 ms ($P<.05$), increased CV to 123 ± 5 cm/s ($P<.01$) and shortened WL to 10.9 ± 0.5 cm ($P<.05$). Vagal stimulation

decreased ERP to 83±3 ms (P<.01 versus control, P=NS versus sympathetic stimulation), increased CV to 126±5 cm/s (P<.01 versus control), and shortened WL to 10.6±0.7 cm (P<.05 versus control, P=NS versus sympathetic stimulation). Thus, the differences in AF duration during sympathetic and vagal stimulation cannot be attributed to nonrepresentativity of electrophysiologic variables measured during stimulation at site 1 of effects on atrial refractoriness.

The next possibility that we considered was that the differences in AF duration were due to changes in the heterogeneity of atrial refractory properties. The standard deviation in refractory period (SD-ERP) at the seven sites studied was used as an index of the heterogeneity in atrial refractoriness. Fig 15D shows values of SD-ERP under each condition. While values under sympathetic and control conditions were very similar, vagal stimulation caused a highly-significant increase in SD-ERP compared to values under other conditions. Further looking at Fig 16, sympathetic stimulation shortened ERP to almost the same extent at all sites, nevertheless, vagal stimulation shortened ERP quite differently from site to site. Since there were no different effects on the CV variety under both interventions, vagal nerve stimulation shortened ERP as well as WL markedly at S3 and S4, which made it being more susceptible to the formation of reentry.

Atrial ERP was measured at seven sites during 1:1 atrial pacing at a single cycle length of 200 ms. In order to obtain an indication of variability in atrial refractory and activation properties at all recording sites during AF, we measured the frequency of activation at each of the 112 recording sites during the arrhythmia. The number of activations over a 2-second interval at each site was counted, with careful review of data to reject low-amplitude potentials and to detect double potentials associated with block. Fig 17 displays the activation frequency at each recording site under all three conditions in a representative dog, along with electrograms recorded at three adjacent electrode sites to illustrate raw data. Under control conditions (Fig 17A), the mean activation frequency was 471/min, and the standard deviation of frequency was 18/min, reflecting the relative constancy of activation frequencies throughout the atria. Recordings from individual adjacent sites show relatively synchronous activation. During sympathetic stimulation (Fig 17B), the activation frequency during AF was somewhat greater, but as under control

conditions there was relatively little variability in frequency at different sites. Vagal stimulation (Fig 17C) was associated with greater activation rates, but also strikingly increased the variability in local activation frequency, which had a standard deviation several times the value under both control and sympathetic stimulation conditions. Marked differences in activation frequency were noted during vagal stimulation for sites as little as 6 mm apart. For example, the three adjacent sites whose recordings are shown in the bottom of Fig 17C had activation frequencies of 935, 633, and 784/min respectively, and activations clearly occur at individual sites without corresponding activation at one or two other sites, indicating substantial differences in local refractory properties.

Overall, the mean activation frequency averaged 552 ± 22/min under control conditions, and was increased to 624 ± 19/min by sympathetic stimulation (P=NS). In the presence of vagal stimulation, the mean frequency was increased further, to 732 ± 39/min, a 33% increase over control values ($P < .01$). The standard deviation of activation frequency averaged 32 ± 5 ms under control conditions and was not altered by sympathetic stimulation, under which it averaged 32 ± 9 ms. In contrast, vagal stimulation increased the standard deviation of frequency to 83 ± 16 ms, a 155% increase over control ($P < .05$ versus both control and sympathetic stimulation conditions). These findings are consistent with the results of direct ERP measurements, in suggesting greater heterogeneity in atrial refractory properties in the presence of vagal stimulation compared to control or sympathetic stimulation conditions.

3. Differences in Atrial Activation Patterns

The differences in AF duration and local activation rates between control, sympathetic stimulation and vagal stimulation conditions would lead one to expect differences in activation pattern during AF. This possibility was tested by creating activation maps from data obtained during individual cycles of AF. A cycle was defined as the shortest interval containing at least one activation at all sites showing clear atrial activity. Examples of activation maps constructed from two consecutive cycles for each

condition are shown in Fig 18. Since some sites were activated twice in each cycle, two maps were generated for the first cycle. The first map (left panels of figure) shows activation with times set at the time of first activation at each electrode site, and the second (middle panels) uses the time of the second activation at all sites activated twice. The panels at the right of the figure show maps constructed with the first activation at each site during the second cycle.

Under control conditions, a large area of early activation is present in the lateral right atrium (top left panel). Activation spreads across Bachmann's bundle toward the left atrial appendage, and in a counterclockwise direction into zones of slow conduction. This results in reactivation of the early-activating areas indicated by the asterisks in the top left panel, as shown in the top middle panel. The next cycle (top right panel) begins with activation near the AV ring, corresponding to an area of early activation (asterisk in top middle panel) adjacent to a zone of late activation in the preceding cycle. An additional area of early activation in the second cycle is present in the lateral left atrium, the source of which is not evident. In the presence of sympathetic stimulation (middle), several activation wavefronts are present, with one zone of reentry in the lateral left atrium. The second cycle has large wavefronts beginning in the region of the septum, and radiating laterally in both atria. Under both control and sympathetic stimulation conditions, the isochrones are relatively large, reflecting considerable organization of activity. The activation pattern is markedly different during vagal AF (bottom). In contrast to control and sympathetic stimulation conditions, which show two or three discrete zones of early activation, at least six discrete zones of early activation are seen in the bottom left panel. At widely-dispersed sites, areas of early activation are located next to late-activating areas, leading to reactivation of the sites indicated by the asterisks in the bottom left panel in the second half of the cycle (bottom middle panel). Four discrete reentry wavefronts were identified. The asterisks in the bottom middle panel indicate areas activated early in the first cycle, adjacent to areas reactivated late in the same cycle. The reactivation of these early-activated areas initiate wavefronts of reentry in the next cycle (bottom right). Overall, activation during vagal stimulation is more heterogeneous and shows more functional reentry circuits than under control or sympathetic stimulation conditions.

We quantified the number of functional reentry wavefronts/cycle under each condition in each dog by identifying the number of regions activated early in the cycle that were reactivated by a reentry wavefront later in the same cycle. For the examples shown in Fig 18, there were one, one, and four reentry wavefronts in the first cycle shown under control, sympathetic, and vagal stimulation conditions respectively, with reexcitation wavefronts indicated by the arrows in the middle panel for each condition. Three consecutive cycles were analyzed for each condition and the average of the three was taken to be representative. Overall, there were 1.4 ± 0.2 reentry circuits/cycle under control conditions, 1.8 ± 0.2 reentry circuits/cycle in the presence of sympathetic stimulation (P=NS versus control), and 3.3 ± 0.3 reentry circuits/cycle during vagal stimulation ($P < .001$ versus control and sympathetic conditions, all results are for six dogs under each condition).

4. Effects of Atropine and Nadolol on Properties of AF

One possible explanation of the greater effect of vagal stimulation than that of sympathetic stimulation on the duration of AF is that spontaneous acetylcholine release from vagal nerve endings occurs at the rapid rates of AF, resulting in synergistic effects with vagal nerve stimulation. To test this hypothesis, AF duration and activation frequency were measured before and after the administration of atropine in five autonomically-decentralized dogs. AF duration averaged 17.5 ± 7.8 seconds before and 19.9 ± 8.9 seconds after atropine (P=NS), and mean AF activation frequency averaged 568 ± 23/min before and 569 ± 20/min after atropine. To exclude a possible role for endogenous norepinephrine release, similar studies were performed before and after the administration of nadolol in five dogs. The duration of AF averaged 21 ± 8 seconds before and 21 ± 8 seconds after nadolol (P=NS), and AF frequency averaged 486 ± 24/min before and 490 ± 25/min after nadolol.

5. Time Course of Sympathetic and Vagal Effects

We considered the possibility that the effects of sympathetic nerve stimulation may desensitize rapidly, so that although ERP appears to be significantly decreased when measured during brief periods of sympathetic stimulation, the effect is not sustained during continued sympathetic stimulation and AF consequently terminates. To test this possibility, we performed repeated measurements of ERP at a basic cycle length of 200 ms during sympathetic and vagal nerve stimulation. The results are shown in Fig 19A, which indicates that the effects of sympathetic stimulation on atrial refractoriness were quite constant over a 10-minute observation period in five dogs. Vagal effects on ERP, studied in four dogs, showed a tendency to desensitization which was not statistically significant. Time-dependent vagal desensitization should, if anything, have decreased the ability of vagal stimulation to maintain AF over time. Since an ERP determination takes close to 30 seconds, we could not exclude the possibility of rapid desensitization over the first 30 seconds of stimulation. To evaluate this, we recorded atrial (sinus) rate continuously during vagal and sympathetic stimulation. Fig 19B provides values for sinus rate as measured at 10-second intervals during vagal and sympathetic stimulation, and indicates that the effects of nerve stimulation were rapid in onset and constant over 60 seconds of observation from the initiation of stimulation.

The final series of experiments were performed to evaluate possible time-dependent changes in electrophysiologic variables or effects. The results are presented in Table 2, and show that repeated measurements over a time frame equivalent to that of our other experiments provided highly reproducible results under control, sympathetic stimulation and vagal stimulation conditions. No significant differences were noted between a first set of measurements and a second, later series of determinations.

DISCUSSION

We have found from the results that: increasing heart rate shortens atrial ERP and decreases conduction velocity; intact basal autonomic tone contributes to AF maintenance; sympathetic and parasympathetic nerve stimulation both shorten atrial ERP and increase CV significantly; when vagal nerve sitmulation frequencies are adjusted to make the WL comparable at BCL 150 ms under both interventions, sympathetic nerve stimulation is much less effective than vagal stimulation in promoting the persistence of AF; the above results were not explained by time dependent changes in effects on atrial refractoriness or by release of neurotransmitters from nerve endings during AF; vagal stimulation caused important increases in the heterogeneity of atrial ERP and in the variability in local activation frequency during AF, while sympathetic stimulation did not alter the heterogeneity of atrial refractory properties.

ERP shortening by increasing heart rate could possibly be explained by two ionic mechanisms. First, $I_{Ca,L}$, an important current to keep the plateau of APD, recovers relatively slowly from inactivation (Li et al., 1995). Increasing rate decreases $I_{Ca,L}$, and this contributes significantly to the decreases in APD and atrial refractory period caused by increasing heart rate. Second, there is some evidence that I_{Ks}, a slowly-activated repolarizing current, may accumulate at rapid rates, contributing to rate-dependent APD abbreviation (Sanguinetti et al., 1990; Jurkiewicz et al., 1993). Rate-dependent shortening of ERP is an important contributor to the induction and maintenance of AF, by shortening the WL of premature beats and during AF. At rapid rates, conduction velocity is also decreased, which can contribute to AF by decreasing the wavelength.

Both sympathetic and parasympathetic systems normally exert a tonic effect on the heart at rest. They additively shorten the atrial ERP (Takei et al., 1991). In our experiments, autonomic denervation of the heart increased atrial ERP and WL, and decreased the AF duration significantly. Since shorter WLs are required for AF perpetuation, intact autonomic tone contributes to the maintenance of AF.

Compared to control conditions, both sympathetic and vagal nerve stimulation increased atrial CV significantly. This phenomenon could be explained by their ionic

mechanisms . Increasing CV with parasympathetic nerve stimulation is probably due to the hyperpolarization of the cell caused by ACh activated potassium channel opening (I_{KAch}), which, in turn, increases the rate of depolarization (Hartzell, 1988). There is also evidence that β-adrenergic stimulation can increase I_{Na} (Matsuda et al., 1990), a possible underlying mechanism by which sympathetic stimulation increases CV. Since both sympathetic and parasympathetic could activate K^+ channels (Kass, 1992), which are important repolarizing currents in the atrial cells, increasing sympathetic and parasympathetic tone shortens atrial ERP.

By comparing the effects of bilateral stellate ansa stimulation and of bilateral vagal nerve stimulation on AF duration, for an equal degree of refractory period and wavelength abbreviation, we found that vagal stimulation promoted AF much more strongly than did sympathetic stimulation. By calculating the standard deviation of ERPs measured at 7 widely spreaded sites on the atria and of AF frequencies counted from 112 electrodes we found that sympathetic stimulation did not increase the heterogeneity in atrial refractory properties, whereas vagal stimulation strongly enhanced it, similar to observation made by Takei et al. (1991) and Alessi et al. (1958). The analysis of the atrial activation mapping showed, under vagal nerve stimulation condition, multiple and smaller wavelets in the atria compared to the control and sympathetic stimulation conditions, which further supported the increased heterogeneity by vagal nerve stimulation. We therefore concluded that inhomogeneities in ERP are important to the ability of vagal stimulation to promote AF. The role of ERP heterogeneity in AF will be further discussed in the later paragraphs.

One of the other possible reasons that vagal stimulation had greater effects than sympathetic stimulation on the duration of AF is that spontaneous acetylcholine release from vagal nerve endings occurs at the rapid rates (Euler et al., 1987) of AF. The results showing no difference in AF duration before and after administration of atropine and nadolol eliminate the possibility of local release of ACh or NE at the nerve endings during the fast rates to facilitate AF.

Zang et al. (1993) demonstrated that because of phosphorylation / dephosphorylation reactions in atrial cells, I_{KAch} desensitized rapidly which was proposed

to account for the rapid fade of action of ACh. This led us to consider the possibility that the effects of sympathetic nerve stimulation may desensitize even more rapidly, so that although ERP appears to be significantly decreased when measured during brief periods of sympathetic stimulation, the effect is not sustained during continued sympathetic stimulation and AF consequently terminates. The repeated measurements of sinus rate at the first minute and ERP over ten-minute duration indicated that the effects of sympathetic stimulation on atrial refractoriness were quite constant, whereas vagal effects on ERP showed a tendency to desensitization which was not statistically significant. We can thus conclude that no apparent desensitization occurred during sympathetic nerve stimulation, and the brief AF duration during sympathetic stimulation is not due to time-dependent decreases in effects of stimulation of the sympathetic nerves.

1. Comparison to Previous Experimental Studies of the Effects of Sympathetic and Vagal Stimulation on AF

Numerous investigators have demonstrated the ability of vagal nerve stimulation (Andrus and Carter, 1930) or cholinergic agonists (Burn et al, 1955; Hoff et al, 1955; Moe et al, 1959) to promote the occurrence of sustained AF. Surprisingly, relatively little work has been performed to establish the effects of sympathetic nerve stimulation or adrenergic agonists on the occurrence and properties of AF. Andrus and Carter noted that the infusion of epinephrine in atropinized dogs reduced the atrial refractory period by a mean of 40%, but that while single premature stimuli regularly induced AF in the presence of vagal stimulation, this never occurred during the infusion of epinephrine (Andrus and Carter, 1930). Hashimoto et al (1968) showed that norepinephrine infusion into the sinus node artery could potentiate AF induction by concomitantly administered acetylcholine. These investigators did not determine whether the interaction was due to an electrophysiologic mechanism or to some other factor such as changes in arterial tone, regional ischemia, or electromechanical interactions. Rensma et al. (1988) found that neither isoproterenol infusion nor propranolol administration significantly altered atrial refractoriness and conduction properties in a conscious dog model. Our study is the first

of which we are aware to study in detail the effects of cardiac sympathetic stimulation on the ability of AF to sustain itself.

2. The Role of Heterogeneity in Atrial Refractoriness

Numerous studies have documented the nonuniform effects of vagal nerve stimulation on atrial refractoriness. Alessi et al (1958) studied local vagal effects on canine right atrial refractoriness and Ninomiya (1966) evaluated changes in atrial action potential duration caused by vagal stimulation in dogs. Both studies reported marked increases in the heterogeneity of atrial repolarization during vagal nerve stimulation, even between contiguous sites in the same atrium. In the present study, as in previous reports (Wang et al, 1992; Wang et al, 1996), we found that bilateral cervical vagal nerve stimulation significantly increases the heterogeneity in atrial refractory period. Furthermore, we noted that vagal stimulation enhanced the variability in local activation frequency during AF. Since variation in local activation rate is an index of the dispersion of refractoriness during fibrillation (Ramdat et al, 1992; 1995), this finding provides additional evidence for increased spacial nonuniformity in atrial refractory properties during vagal activation. The local activation rate analysis suggests that significant variations can occur over relatively small distances during vagal stimulation (Fig 9), a concept supported by prior direct observations (Alessi et al, 1958; Ninomiya, 1966).

Several experimental observations have suggested that heterogeneous atrial repolarization properties can contribute to the occurrence and maintenance of AF. Sato et al reported that increased dispersion of atrial refractoriness and increased conduction time characterized dogs with AF induced by single atrial extrastimuli after cardiopulmonary bypass (Sato et al., 1992). We noted that the duration of AF induced by graded vagal stimulation correlated more closely with the standard deviation of atrial refractoriness than with the absolute value of the refractory period or the wavelength (Wang et al, 1996). Variability in atrial refractoriness is increased in dogs with idiopathic AF (Wang et al, 1995), and flecainide doses that terminate AF reduce the variability in atrial refractoriness (Wang et al, 1992; 1995). Increased inhomogeneity of ERP has also been

observed in patients with atrial fibrillation (Boutjdir et al., 1986; Ramdat Misier et al., 1992; Luck et al., 1979; and Michelucci et al., 1982).

In Gordon Moe's classical computer model of AF, variability in atrial refractoriness was essential for the production of typical AF when refractory properties were kept uniform, the number of reentering wavefronts was dramatically reduced and the activation became "more characteristic of flutter than of fibrillation" (Moe et al, 1964). Moe believed that the nonuniformity in atrial repolarization induced by vagal stimulation is important in its ability to promote sustained AF (Moe, 1962).

In all of the experimental studies described above, changes in the heterogeneity of atrial refractoriness were accompanied by alterations in determinants of the wavelength, such that when atrial heterogeneity was increased and AF more likely to be sustained, atrial refractoriness (and/or wavelength) was abbreviated. Thus, these studies do not allow the role of heterogeneity in atrial refractory properties to be dissociated from that of changes in absolute refractoriness and the wavelength for reentry. The contrasting effects of sympathetic and vagal stimulation on AF in the present study were accompanied by differing effects on indices of refractoriness dispersion but similar changes in atrial refractory period and the wavelength for reentry. These results point directly to an important role of the variability in atrial refractory properties in determining the perpetuation of AF.

3. Potential Clinical Relevance of our Findings

The mechanisms underlying a variety of clinical arrhythmias, including idiopathic AF, remain unclear. There is evidence that patients with idiopathic AF have an increased dispersion of atrial refractoriness (Ramdat et al, 1992). Additional data suggest that patients with post-infarction ventricular fibrillation have a greater dispersion of refractoriness in noninfarcted tissue than patients with ventricular tachycardia (Ramdat et al, 1995). Our results point directly the potential mechanistic importance of the dispersion of refractoriness in promoting AF.

In extensive studies of the role of the autonomic nervous system in clinical AF,

Dr. Coumel noted that evidence for adrenergic involvement in AF is much rarer than evidence for vagal involvement (Coumel, 1992). Murgatroyd and Camm have similarly observed that the onset of attacks of AF much more commonly occurs in situations of enhanced vagal tone than sympathetic tone (Murgatroyd and Camm, 1992). The findings in the present manuscript may explain these observations, by showing that sympathetic effects on the heart are much less effective in promoting AF than vagal actions, even when refractoriness and wavelength are abbreviated to a similar extent.

Our results highlight the importance of heterogeneity in atrial refractoriness as a determinant of the persistence of AF. This concept has potential implications for the development of novel therapeutic approaches to the prevention of AF. The prolongation of atrial refractoriness is currently the primary target of new antiarrhythmic drug therapies for AF. It is conceivable that drug effects on the variability of atrial refractoriness are as important as effects on the absolute value of the refractory period, and that considering effects on refractoriness dispersion might allow for the identification of novel and/or more effective therapies.

There is increasing interest in ablation procedures for the termination and prevention of AF (Haisaguerre et al, 1994; Swatz et al, 1994; Seifert et al, 1994; Morillo et al, 1995; Elvan et al, 1995). While most of these procedures use multiple linear lesions patterned after the surgical "maze" procedure (Cox et al, 1991), in at least one experimental model of AF characterised by an increased dispersion of refractoriness, the probability of AF was substantially reduced by a discrete cryoablation lesion in the posterior left atrium (Morillo et al, 1995). By ablating atrial tissue with the briefest refractory period, this procedure reduced the dispersion of atrial ERP, a result which may have contributed to its success. The use of ablation to eliminate zones of brief refractoriness, increasing the mean wavelength and reducing the disparity in refractoriness, may prove to be an interesting approach to the management of AF. While the "maze" procedure is believed to prevent AF by dividing the atria into zones without the critical mass to maintain fibrillation, it is possible that changes in atrial functional properties such as the dispersion of refractoriness play an important role. In any case, it is clear that further work is necessary in order to improve our understanding of how tissue

modification procedures alter atrial functional properties and the likelihood of AF, and through this understanding to optimise the effectiveness of such procedures.

4. Potential Limitations

We produced sympathetic or vagal effects by stimulating both stellate ansae or both cervical vagal nerves. It is possible that under physiologic conditions in awake animals, the pattern of cardiac nerve firing is more discrete, and the effects of sympathetic activation on atrial refractoriness are more heterogeneous. This is an interesting possibility that goes beyond the scope of the present manuscript.

Beta-adrenergic receptor blockers appear to be effective in preventing postoperative AF, implying a role for cardiac sympathetic actions in this syndrome of uncertain mechanism (Kowey et al, 1992; Lauer et al, 1992). While our results argue against the ability of uniform cardiac sympathetic stimulation to promote AF, possible explanations for sympathetic involvement in postoperative AF include nonuniform activation of cardiac sympathetic nerves, an interaction between sympathetic effects and some other factor (such as cardiac vagal activation or pericarditis), and an augmentation of atrial automaticity which initiates intra-atrial reentry and AF. Favoring the latter possibility is the clinical observation that, while beta-blockers are effective in preventing postoperative AF, they are relatively ineffective in terminating it (Lauer et al, 1992).

There is an analysis of the distribution of acetylcholinesterase(AChE) in human atrial biopsies obtained from individuals without arrhythmias and in patients with atrial fibrillation, showed a decrease in the amount of the globular forms of AChE from atria during AF (Gonzalez et al, 1993). In our experiments, we compared the AF duration and activation frequency before and after atropine was given. The results excluded the possibility of spontaneous acetylcholine release from vagal nerve endings and changes in acetylcholinesterase contents. The differences in results from those of Gonzalesz et al. are probably due to the fact that they made atrial biopsies from chronic AF patients with mitral rheumatic valvulopathy. In our experimental model, only acute AF was studied, and chronic structural changes were absent.

While we noted, as have others, the important spacial variability in vagal effects on atrial refractoriness, the mechanisms underlying such effects remain largely unknown. It is possible that fibers immediately adjacent to vagal postganglionic endings are exposed to relatively high concentrations of the cholinergic mediator and are profoundly affected, while fibers more remote from sites of acetylcholine liberation are influenced to a much lesser degree (Alessi et al., 1958; Zipes et al., 1974). On the other hand, the high concentration of AChE in atria makes the degeneration of transmitters fast, and localizes the effect of ACh in atria. The other potential possibilities include local variability in the density of vagal nerve endings and/or muscarinic receptors, discrepancies in the coupling of muscarinic receptors to ion channel effectors, variations in cholinergic K^+ channel density, and variation in the concentration of other ion channels. There is evidence that action potential variability in the human atrium is due, at least in part, to variations in the proportion of different types of time-dependent K^+ channels (Wang et al, 1993).

Although the different effects of vagal and sympathetic stimulation on the heterogeneity of atrial refractoriness are a logical explanation of their differing actions on AF duration, a possible role of other factors cannot be excluded. Differences in actions on coronary blood flow, on hemodynamic variables (including differences in atrial distension), and on ventricular response rate could all contribute in presently unknown ways to the greater AF-promoting effects of vagal compared to sympathetic nerve stimulation.

Finally, there are limitations to the mapping approach that was used. Septal activity is not recorded. In addition, we cannot exclude microreentry below the resolution limits of our electrode arrays (6 mm).

CONCLUSIONS

We have shown that bilateral stellate ansa stimulation is much less effective in promoting the perpetuation of AF than is bilateral cervical vagus nerve stimulation. These differences are observed despite comparable changes in mean refractory period and wavelength, and are associated with important discrepancies in effects on the heterogeneity of atrial refractoriness. These observations point towards a significant role for the variability in atrial refractory properties in determining the maintenance of AF, and have potentially important consequences for our understanding of the factors that may promote clinical AF and the therapeutic interventions that can be used to prevent its occurrence.

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FIGURE LEGENDS

Figure 1. Conduction system of the human heart, showing anatomical features of the heart (labels at left) and the conducting structures (labels at right). (Adapted from: Katz AM (1992): Chapter 1. Structure of the heart. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp9).

Figure 2. The cardiac action potential (shown here for a Purkinje fiber) lasts over 300 msec and consists of five phases. (Adapted from: Katz AM (1992): Chapter 19.The cardiac action potential. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp439).

Figure 3. Cable properties in a strand of cardiac muscle transmitting a propagated impulse from left to right. Currents between the depolarized tissue (shaded, left) and resting tissue (unshaded, right), viewed here as electron flux, are indicated by arrows. Current flows away from the depolarized tissue along the outside of the cell, and the circuit is completed when current returns toward the depolarized region inside the cell. (Adapted from: Katz AM (1992): Chapter 21. The arrhythmias. I. Introduction and Mechanisms. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp518).

Figure 4. Current flow along and across the cardiac cell membrane (thin arrows). Longitudinal currents are influenced by extra- and intracellular resistances, whereas current flow across the membrane (shaded) is determined by membrane capacitance and membrane resistance. Conduction is slowed when extracellular or intracellular resistance is increased, but is accelerated by increased membrane resistance. (Adapted from: Katz AM (1992): Chapter 21. The arrhythmias. I. Introduction and Mechanisms. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp519).

Figure 5. Excitability during the cardiac action potential. The absolute refractory period (APD), during which no stimulus regardless of its strength is able to initiate a

propagated action potential, is followed by the relative refractory period (RRP) during which only stimuli that exceed the normal threshold can cause a propagated action potential. (Adapted from: Katz AM (1992): Chapter 19. The cardiac action potential. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp458).

Figure 6. Temporal relationships between the ECG (top) and a representative cardiac action potential (bottom). The QRS complex is produced by the upstrokes (phase 0) of all of the action potentials throughout the ventricles; the isoelectric S-T segment corresponds to the plateaus (phase 2), whereas the T wave is inscribed during repolarization (phase 3) of the ventricular mass. The isoelectric segment which comes after the T wave corresponds to ventricular diastole (phase 4). (Adapted from: Katz AM (1992): Chapter 20. The electrocardiogram. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp473).

Figure 7. Schematic drawing of the right canine cardiopulmonary nerves seen from the ventral aspect. The outlines of smaller ganglia are indicated in the nerves. (Adapted from: Armour JA, and Hopkins DA (1984): Anatomy of the extrinsic efferent autonomic nerves and ganglia innervating the mammalian heart. In Randall WC, eds: Nervous control of cardiovascular function. Oxford university press, Inc., pp. 22).

Figure 8. Different types of afterdepolarization. A: Early afterdepolarizations showing a subthreshold afterdepolarization that does not reach threshold (1), and larger afterdepolarizations cause a single (2) and repetitive (3-4-5) triggered depolarizations. B: Late afterdepolarizations showing a subthreshold afterdepolarization that does not reach threshold (1) and afterdepolarizations that reach threshold so as to produce one (2) or a series (3-4-5) of triggered depolarizations. (Adapted from: Katz AM (1992): Chapter 19. The cardiac action potential. In: Katz AM, eds: Physiology of the Heart. 2nd ed. Raven Press, pp438).

Figure 9. Mines' diagram to explain that reentry will occur if conduction is slowed and the refractory period duration is decreased. A stimulated impulse leaves in its wake absolutely refractory tissue (black area) and relatively refractory tissue (stippled area). In both A and B, the impulse conducts in one direction only. In A, because of fast conduction and a long refractory period, the tissue is still absolutely refractory when the impulse has returned to its site of origin. In B, because of slow conduction and a short refractory period, the tissue has recovered excitability by the time the impulse has reached the site of origin, and the impulse continues to circulate. (Adapted from: Mines GR (1913). See references.)

Figure 10. Differences in characteristics of circus movement reentry in an anatomically defined circuit and a functionally defined circuit. (Adapted from: Allessie et al., 1977. See references)

Figure 11. Schematic diagram of electrode arrays. Each black circle indicates a bipolar recording electrode. The numbers indicate bipolar stimulation sites. Anatomic landmarks are provided as follows: LAA, RAA: left and right atrial appendages; IVC, SVC: inferior and superior vena cavae; PV: pulmonary vessels; AVR: AV ring.

Figure 12. Activation maps (left), electrograms at sites used to determine conduction velocity (middle), and regression analysis used to determine conduction velocity (right), at three cycle lengths in a representative dog. Anatomic format is as in Fig 1, and points represent electrode sites. Stimulation was performed in the right atrial appendage. The duration of the window shown for electrogram recordings is 150 ms. The lines in the maps at left are 10-ms isochrones (numbers at top indicate isochrone times), and the numbers near the larger dots indicate activation times (relative to the stimulus) corresponding to the time points indicated by the arrows (middle panels) at the electrode sites (larger dots on maps) which were used for the conduction time analyses in the right panels.

Figure 13. Activation maps (left), electrograms (middle), and regression analysis used to determine conduction velocity (right) under control conditions (top) and in the presence of sympathetic (middle) and vagal (bottom) stimulation in a presentative dog. Anatomic format is as in Fig 1, and points represent electrode sites. Results shown were obtained during pacing at the site indicated with an "X" at a cycle length of 200 ms. The duration of the window shown for electrogram recordings is 150 ms. The lines in the maps at left are 10-ms isochrones, and the numbers indicate activation times (relative to the stimulus) corresponding to the time points indicated by the arrows (middle panels) at the electrode sites (larger dots on the maps) which were used for the conduction time analyses at the right.

Figure 14. Atrial effective refractory period (ERP), conduction velocity (CV) and wavelength (WL) as a function of basic cycle length (BCL) in six dogs. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ versus control at same BCL.

Figure 15. Effects of sympathetic (S) and vagal (V) stimulation on atrial effective refractory period (ERP, panel A) and wavelength (WL, panel B), both measured at a basic cycle length of 150 ms at site 1, along with changes in the mean duration of AF induced by atrial burst pacing (panel C) and the standard deviation of atrial refractory period (SD-ERP, panel D). $^{**}P<.01$, $^{***}P<.001$ versus control (C) values; $^{++}P<.01$ versus values in the presence of sympathetic stimulation.

Figure 16. Mean ($\pm$SE) values of atrial effective refractory period (ERP), conduction velocity (CV), and wavelength (WL) as measured at each of the seven sites shown in Fig 1 at a cycle length of 200 ms under control, sympathetic stimulation (SYMP STIM), and vagal stimulation (VAGAL STIM) conditions.

Figure 17. Activation frequencies at each recording site are shown under control (panel A), sympathetic stimulation (panel B), and vagal stimulation (panel C) conditions, along with recordings at three adjacent sites in the right atrial appendage. A time scale

representing 0.5 seconds is provided. Anatomic representation and landmarks for the atria are as in Fig 1.

Figure 18. Activation patterns during two cycles of AF under control conditions (top), in the presence of sympathetic stimulation (middle), and in the presence of vagal stimulation (bottom). Some sites were activated twice during each cycle. The left panels show maps obtained using the first activation at each site during one cycle, and the middle panels show maps using the second activation time at all sites activated twice within the same cycle. The right panels show activation during the next cycle, with first activation time used for all sites activated twice during the second cycle. The lines show 10-ms isochrones, and the arrows indicate activation wavefronts. Asterisks indicate early activated zones which are reactivated in the second part of the first cycle (asterisks in left panels, reactivation wavefronts in middle panels) or at the beginning of the second cycle (asterisks in middle panels correspond to early-activated zones in the first cycle that are adjacent to late-activated zones in the same cycle, and whose reactivation initiates activity in the second cycle as shown in right panels). The anatomic representation is as in Fig 11.

Figure 19. Time course of changes in atrial effective refractory period at a basic cycle length of 200 ms (ERP, panel A) and sinus rate (panel B) during sympathetic stimulation in five dogs and vagal stimulation in four dogs. While both interventions significantly altered ERP and sinus rate, no statistically significant time-dependent changes in effects occurred over the observation periods indicated.

Figure 1

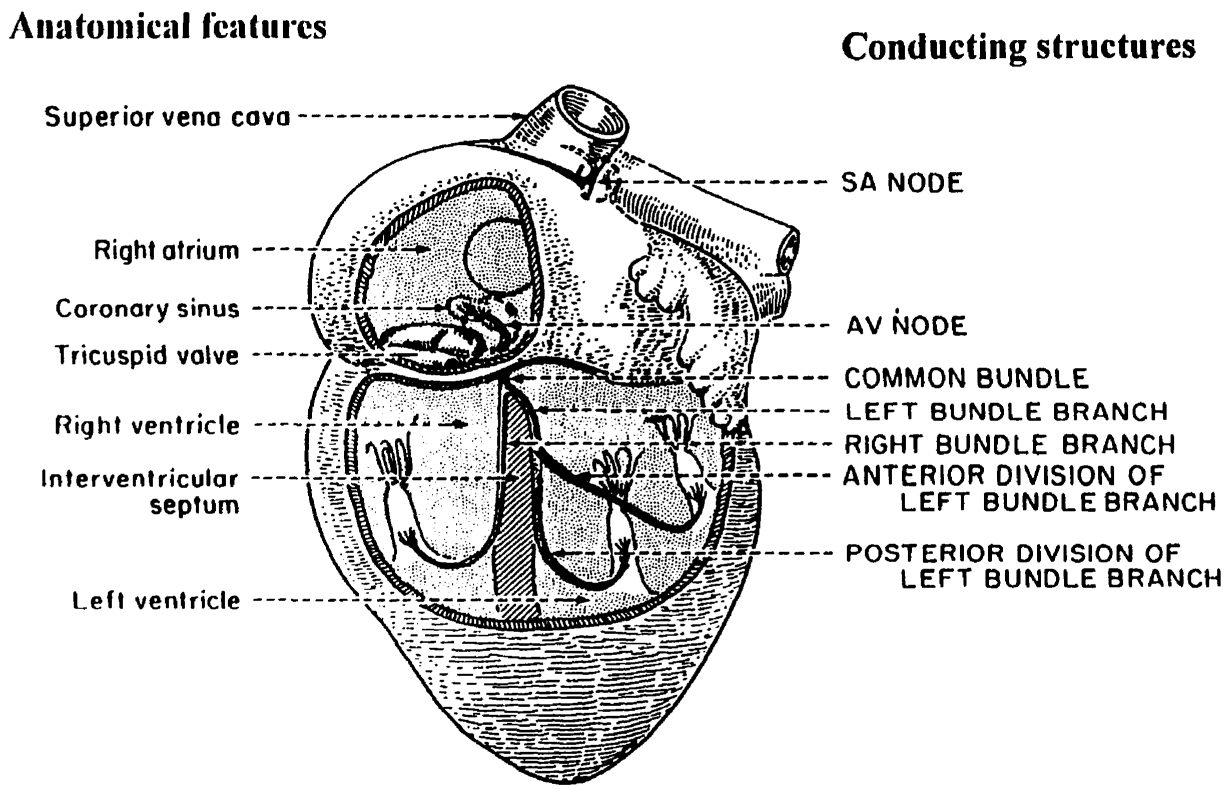

Anatomical features
Conducting structures
Superior vena cava
Right atrium
Coronary sinus
Tricuspid valve
Right ventricle
Interventricular septum
Left ventricle
SA NODE
AV NODE
COMMON BUNDLE
LEFT BUNDLE BRANCH
RIGHT BUNDLE BRANCH
ANTERIOR DIVISION OF LEFT BUNDLE BRANCH
POSTERIOR DIVISION OF LEFT BUNDLE BRANCH

Figure 2

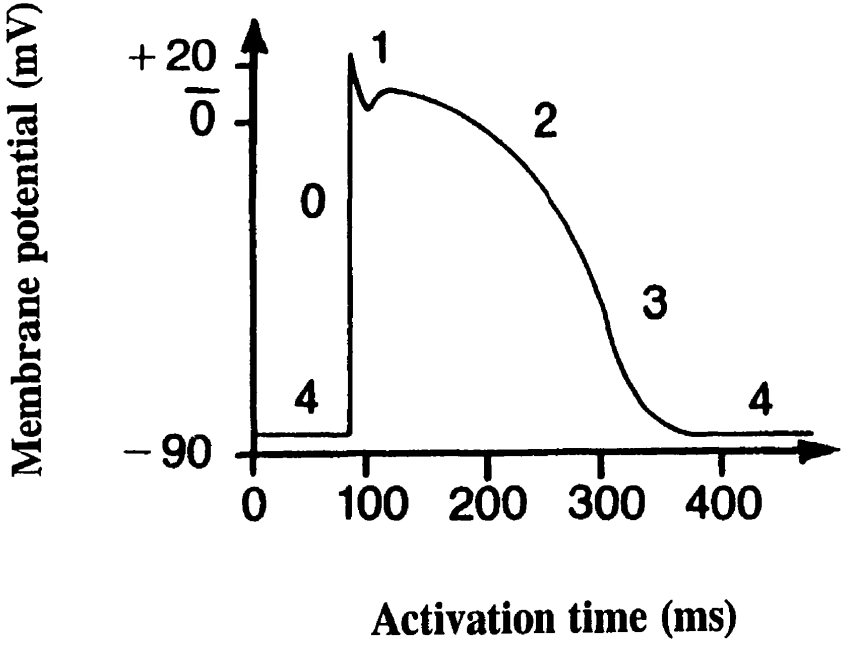
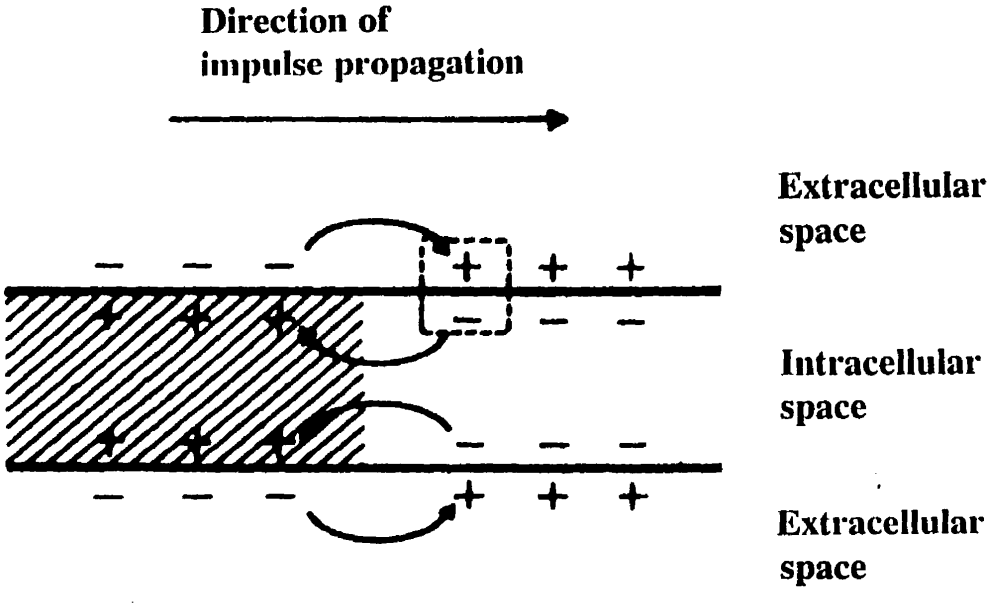

Membrane potential (mV)
+20
0
−90
0
1
2
3
4
4
0
100
200
300
400
Activation time (ms)

Figure 3



Cable properties in a strand of cardiac muscle

Figure 4

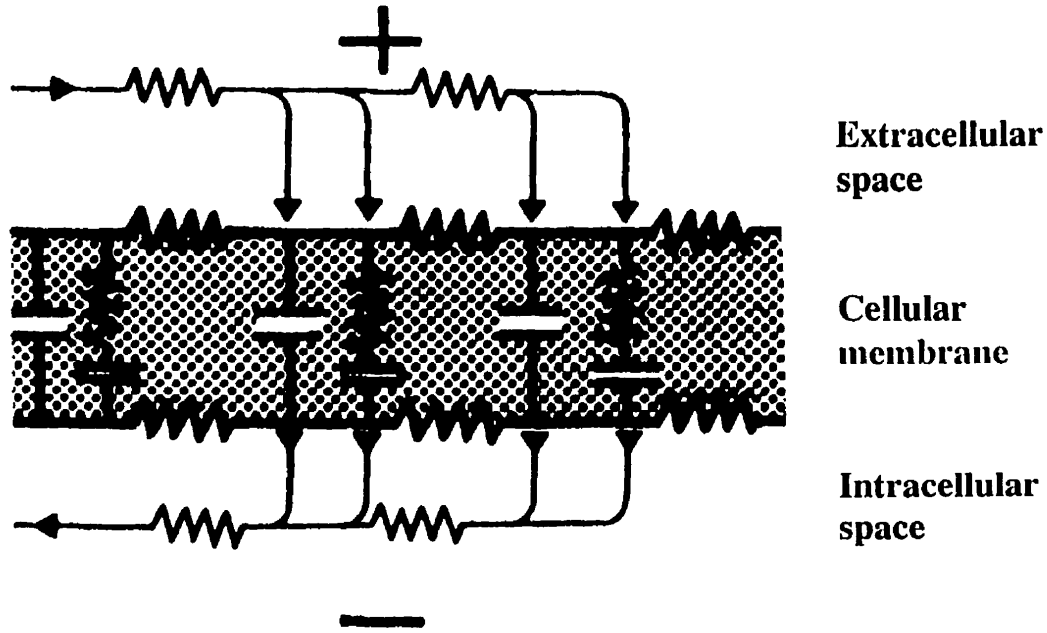

+
−
Extracellular space
Cellular membrane
Intracellular space

Figure 5

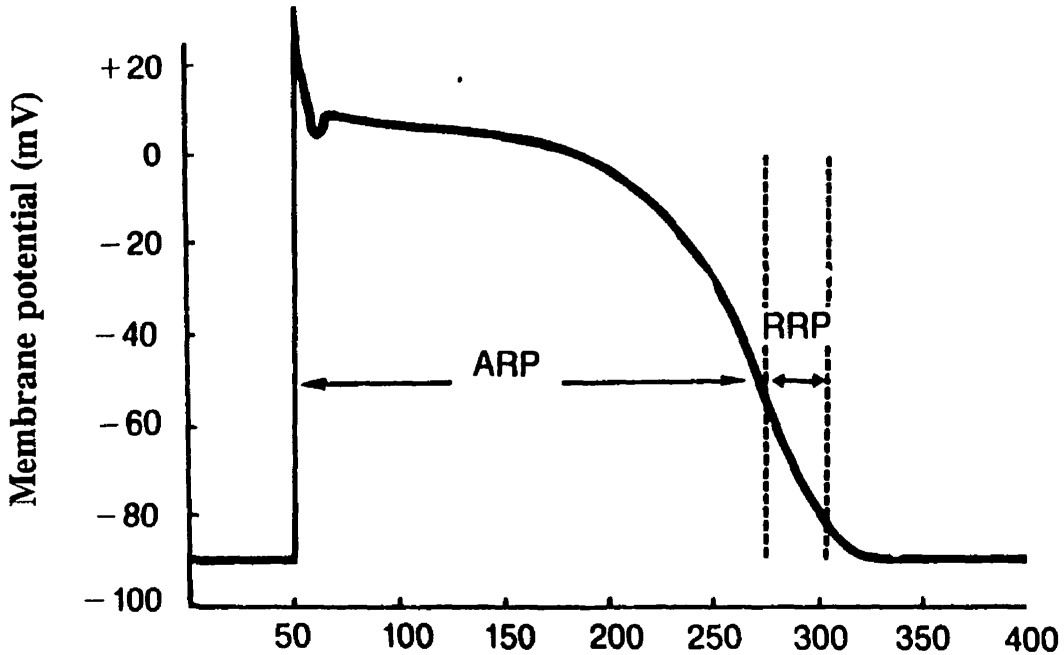

Membrane potential (mV)
+20
0
−20
−40
−60
−80
−100
ARP
RRP
50
100
150
200
250
300
350
400
Activation time (ms)

Figure 6

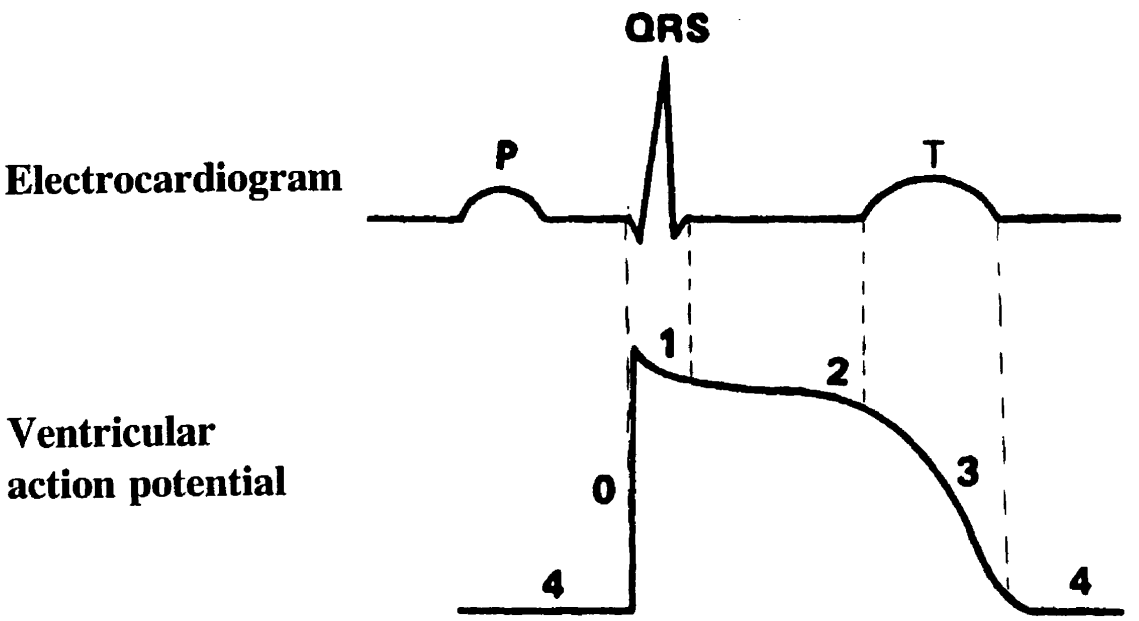

QRS
Electrocardiogram
P
T
Ventricular
action potential
1
2
0
3
4
4

Figure 7

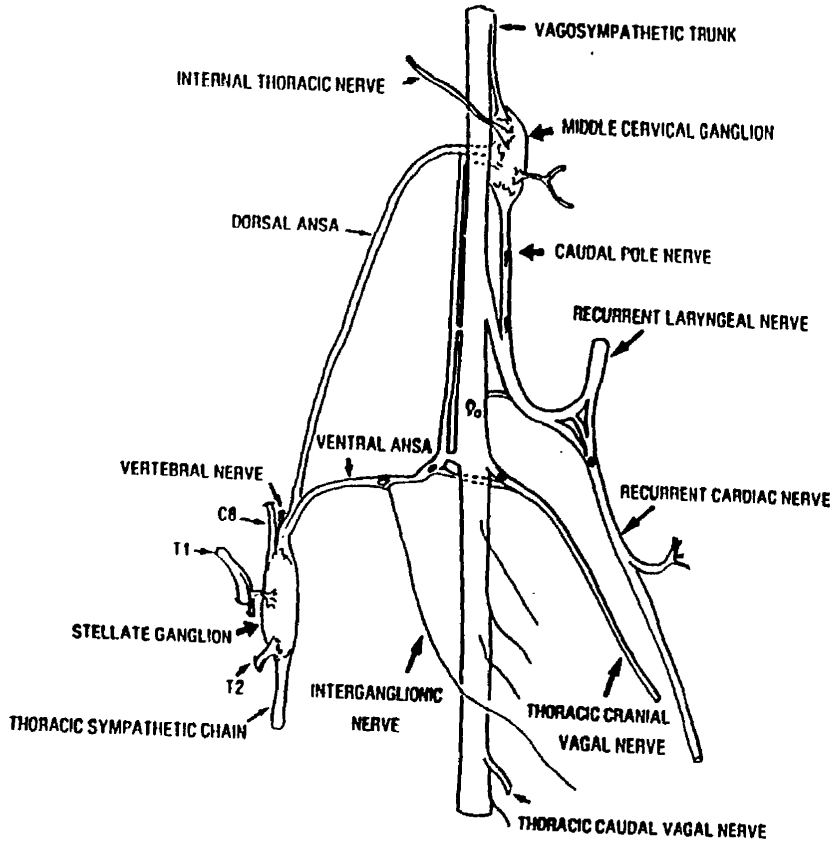

VAGOSYMPATHETIC TRUNK
INTERNAL THORACIC NERVE
MIDDLE CERVICAL GANGLION
DORSAL ANSA
CAUDAL POLE NERVE
RECURRENT LARYNGEAL NERVE
VENTRAL ANSA
VERTEBRAL NERVE
RECURRENT CARDIAC NERVE
C8
T1
STELLATE GANGLION
T2
INTERGANGLIONIC NERVE
THORACIC CRANIAL VAGAL NERVE
THORACIC SYMPATHETIC CHAIN
THORACIC CAUDAL VAGAL NERVE

Figure 8

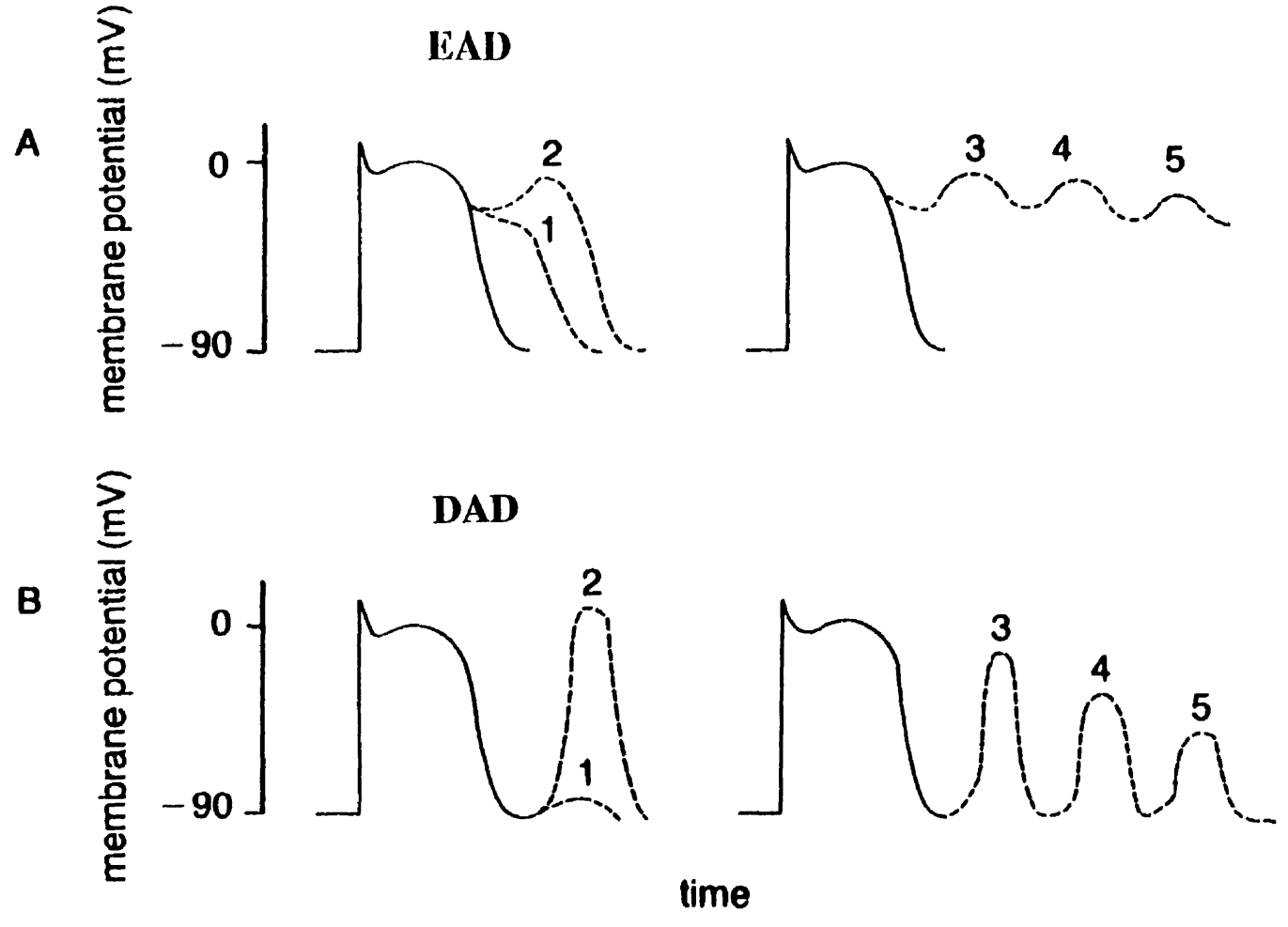

A
EAD
membrane potential (mV)
0
−90
1
2
3
4
5
B
DAD
membrane potential (mV)
0
−90
1
2
3
4
5
time

Figure 9

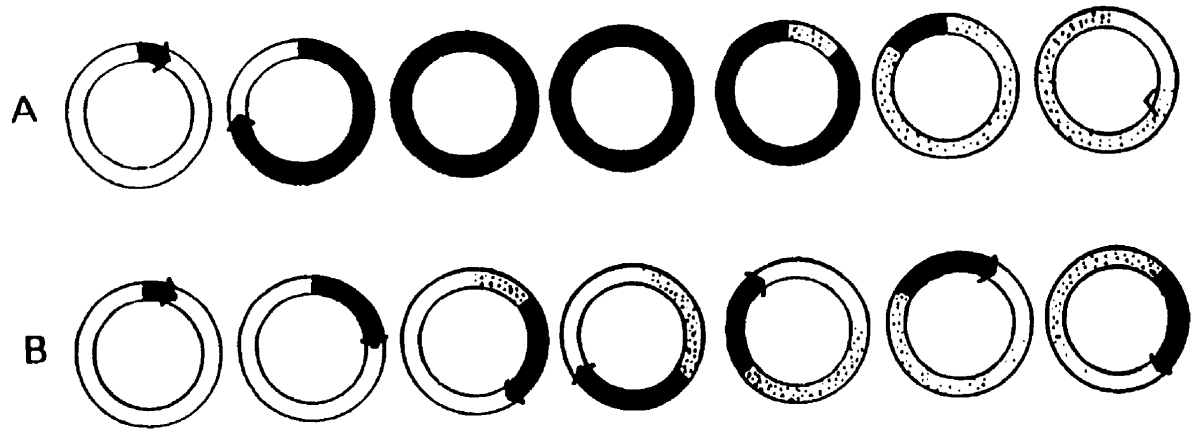


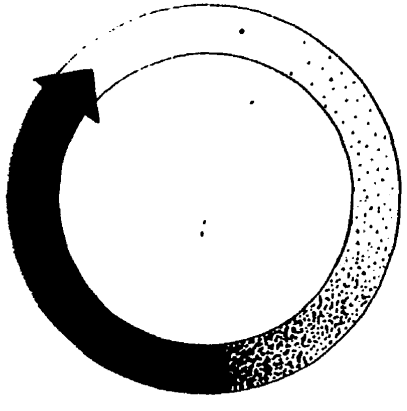
Figure 10

Anatomiclly defined circuit

1. FIXED LENGTH OF CIRCUIT.

2. USUALLY EXCITABLE GAP BETWEEN HEAD AND TAIL OF IMPULSE.

2. INVERSE RELATION REVOLUTION TIME AND CONDUCTION VELOCITY.



Functionally defined circuit

1. CIRCUIT LENGTH DEPENDENT ON COND. VEL. REFR. PER.

2. NO GAP OF FULL EXCITABILITY.

3. REVOLUTION TIME PROPORTIONAL TO LENGTH REFRACTORY PERIOD.

4. SHORTCUT OF CIRCUIT POSSIBLE.

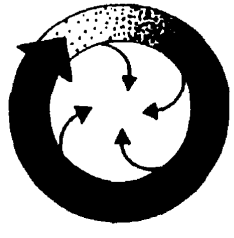


Figure 11

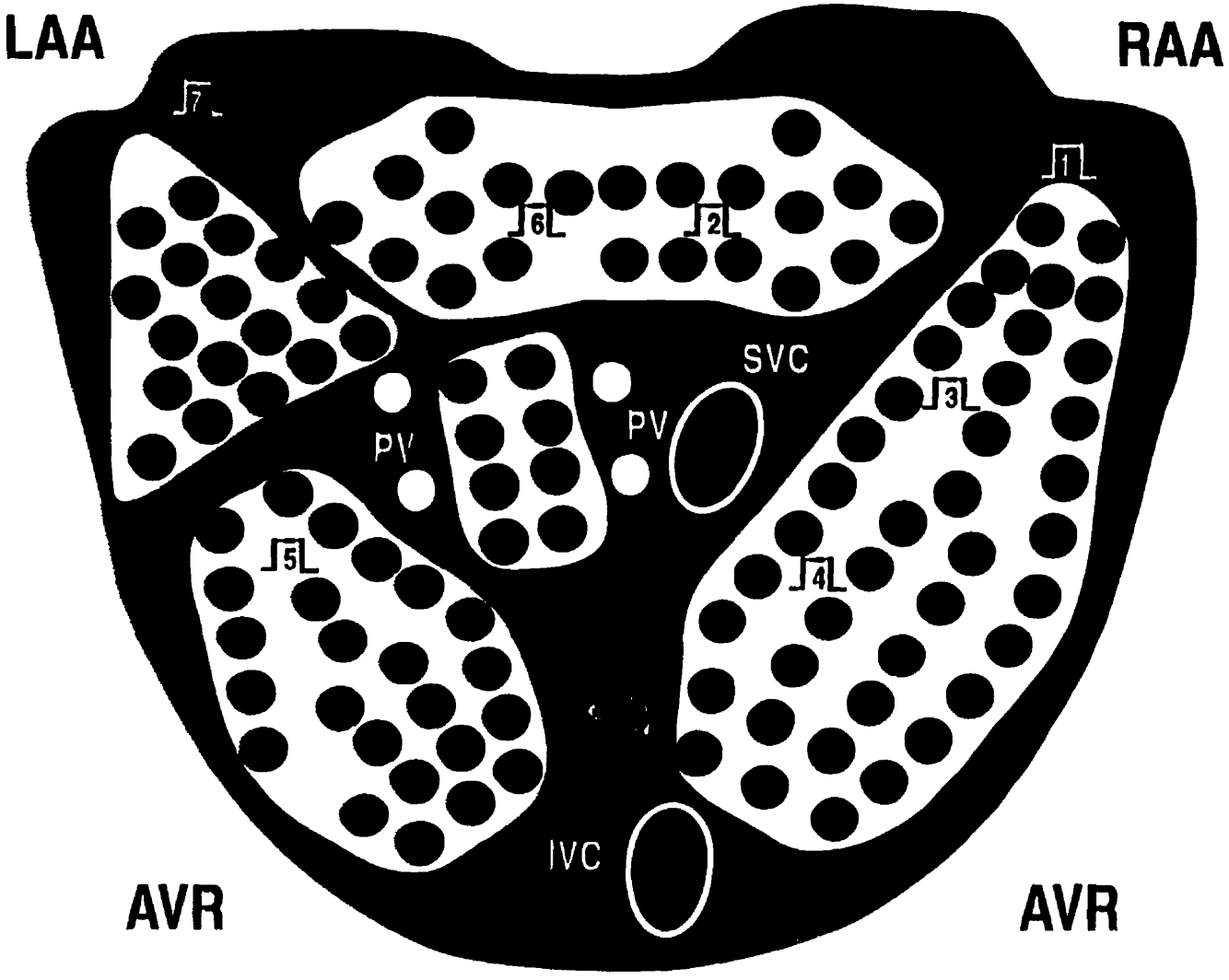


Figure 12

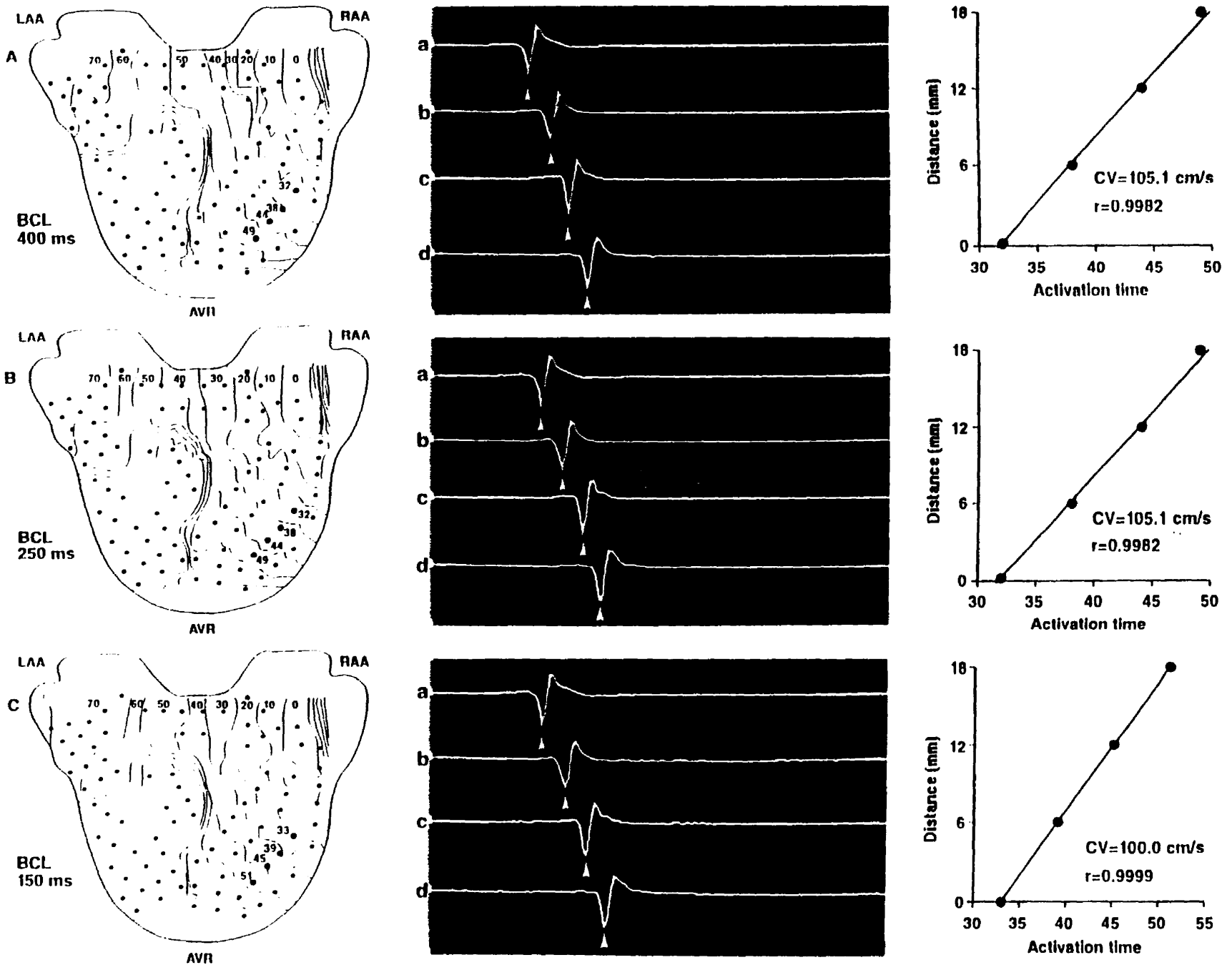

LAA
RAA
A
70 60 50 40 30 20 10 0
32
38
44
49
BCL
400 ms
AVR
a
b
c
d
Distance (mm)
18
12
6
0
30 35 40 45 50
Activation time
CV=105.1 cm/s
r=0.9982
LAA
RAA
B
70 60 50 40 30 20 10 0
32
38
44
49
BCL
250 ms
AVR
a
b
c
d
Distance (mm)
18
12
6
0
30 35 40 45 50
Activation time
CV=105.1 cm/s
r=0.9982
LAA
RAA
C
70 60 50 40 30 20 10 0
33
39
45
51
BCL
150 ms
AVR
a
b
c
d
Distance (mm)
18
12
6
0
30 35 40 45 50 55
Activation time
CV=100.0 cm/s
r=0.9999

Figure 13

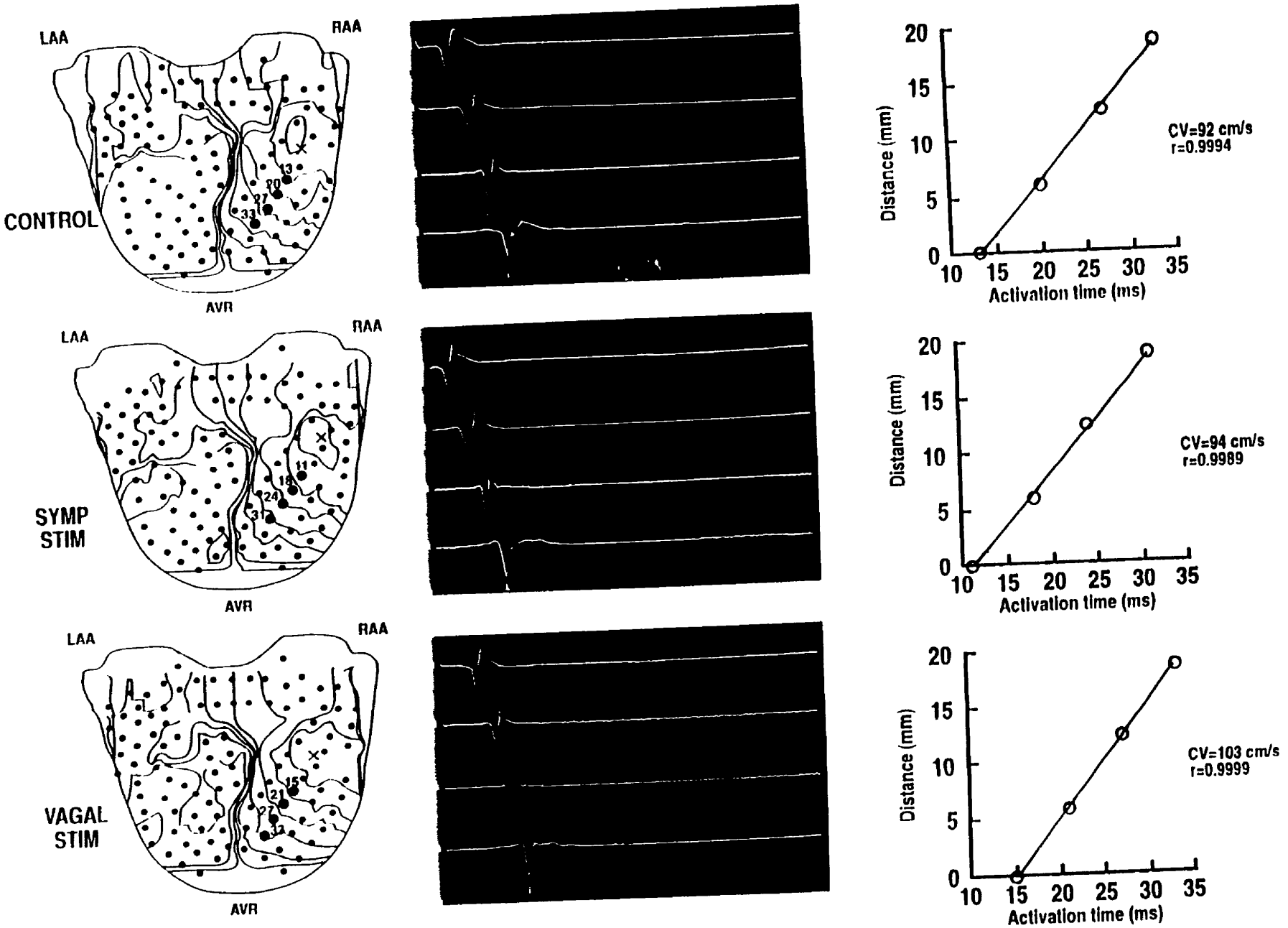

LAA
RAA
CONTROL
AVR
13
20
27
33
SYMP
STIM
11
18
24
31
VAGAL
STIM
15
21
27
31
Distance (mm)
Activation time (ms)
CV=92 cm/s
r=0.9994
CV=94 cm/s
r=0.9989
CV=103 cm/s
r=0.9999

Figure 14

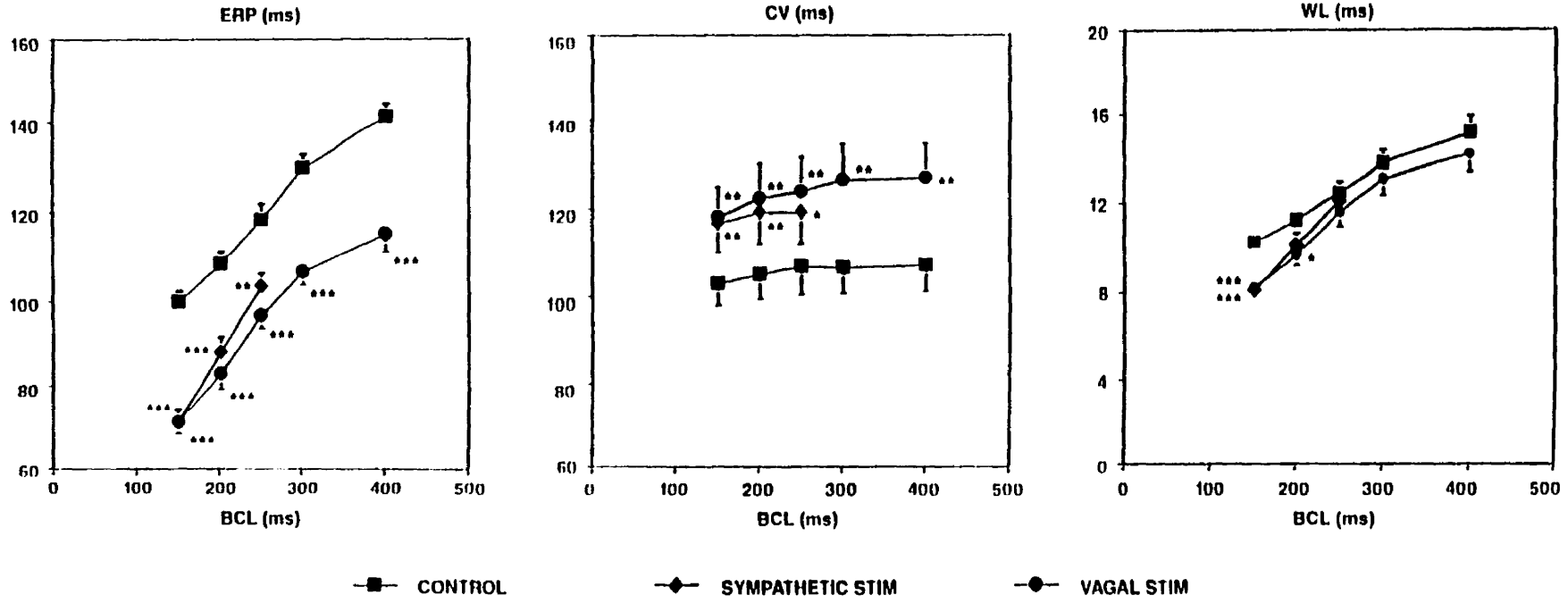

ERP (ms)
160
140
120
100
80
60
0
100
200
300
400
500
BCL (ms)
CV (ms)
160
140
120
100
80
60
0
100
200
300
400
500
BCL (ms)
WL (ms)
20
16
12
8
4
0
0
100
200
300
400
500
BCL (ms)
CONTROL
SYMPATHETIC STIM
VAGAL STIM

Figure 15

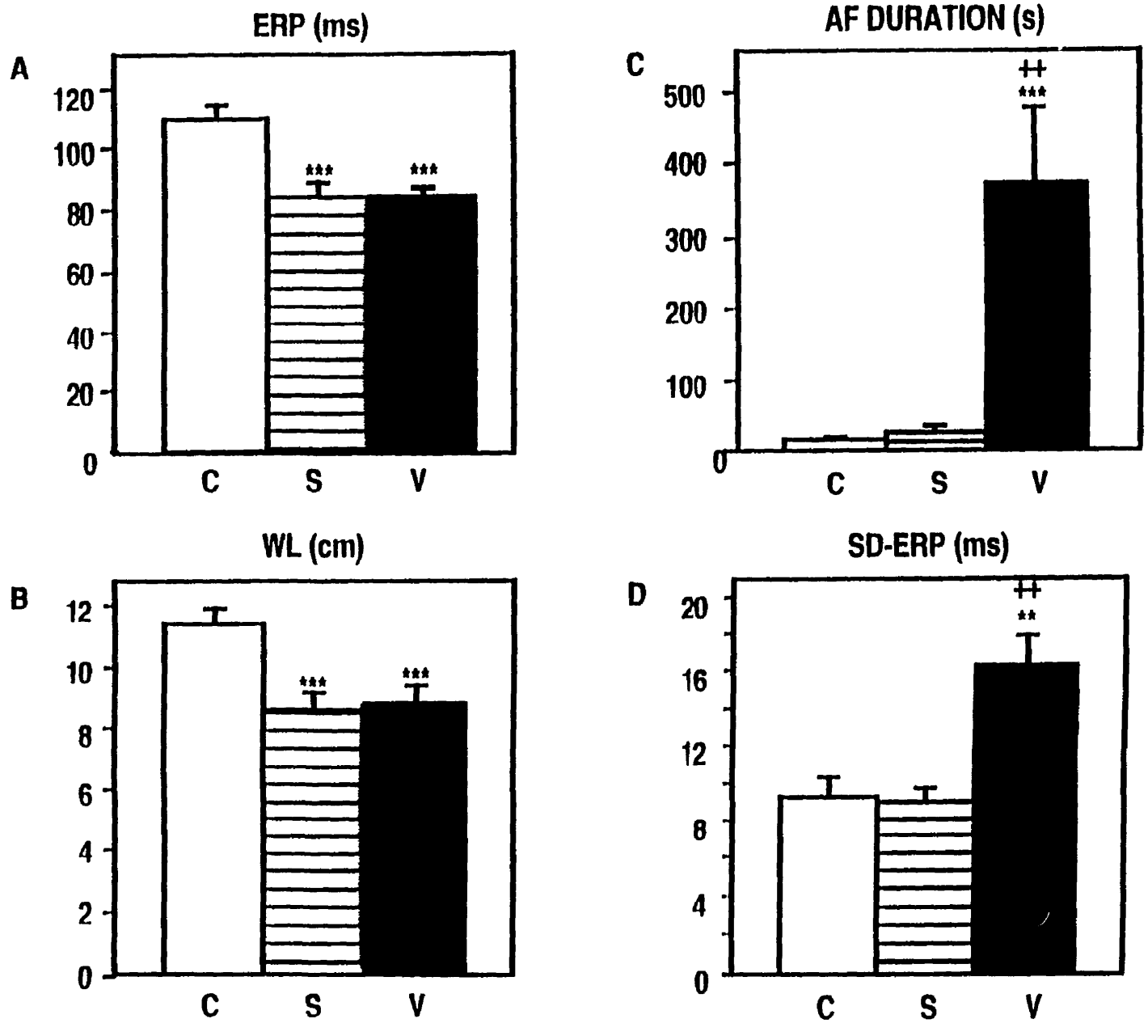

A
ERP (ms)
120
100
80
60
40
20
0
***
***
C
S
V
C
AF DURATION (s)
500
400
300
200
100
0
++
***
C
S
V
B
WL (cm)
12
10
8
6
4
2
0
***
***
C
S
V
D
SD-ERP (ms)
20
16
12
8
4
0
++
**
C
S
V

Figure 16

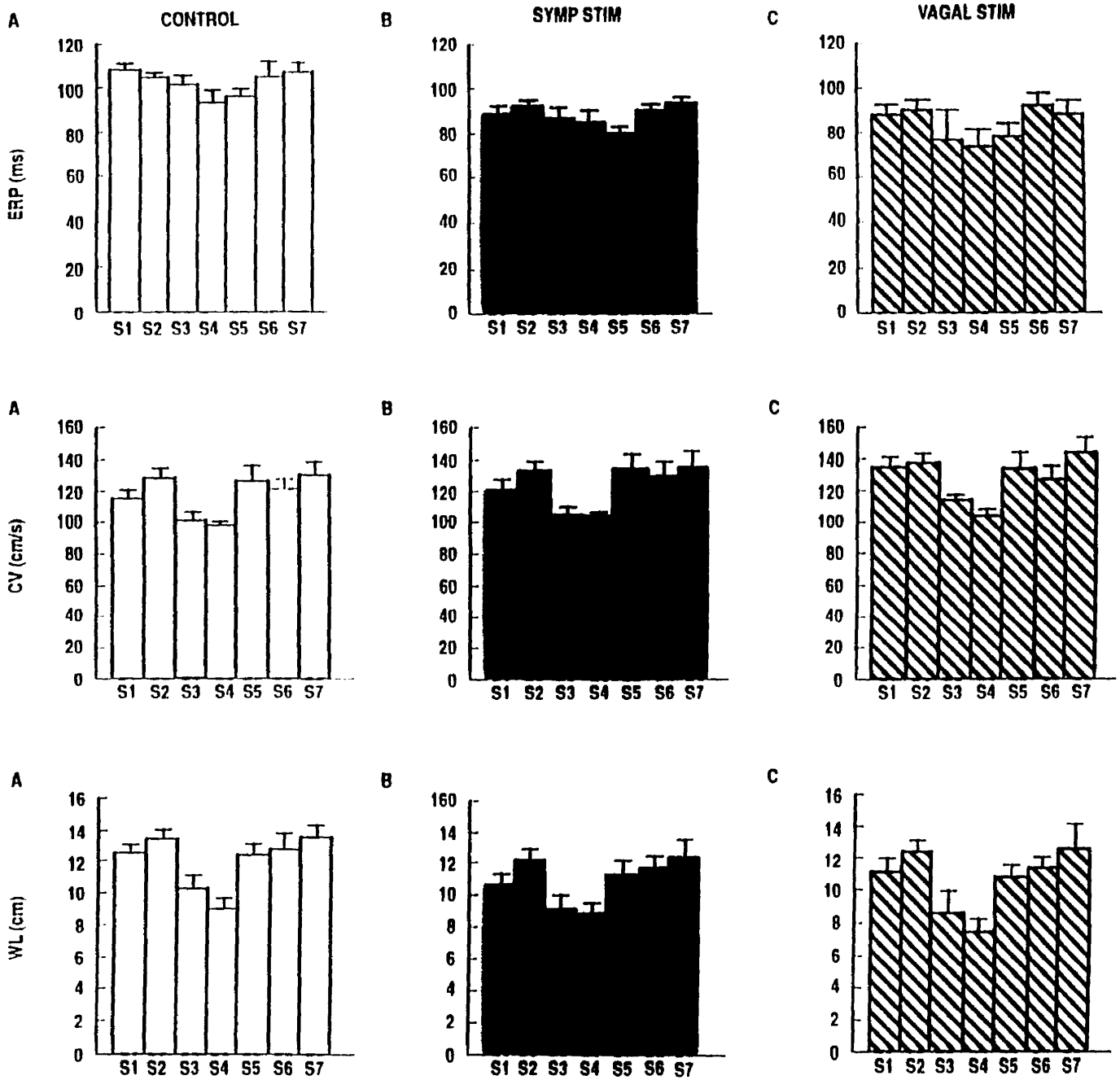

A
CONTROL
B
SYMP STIM
C
VAGAL STIM
ERP (ms)
CV (cm/s)
WL (cm)
S1 S2 S3 S4 S5 S6 S7

Figure 17

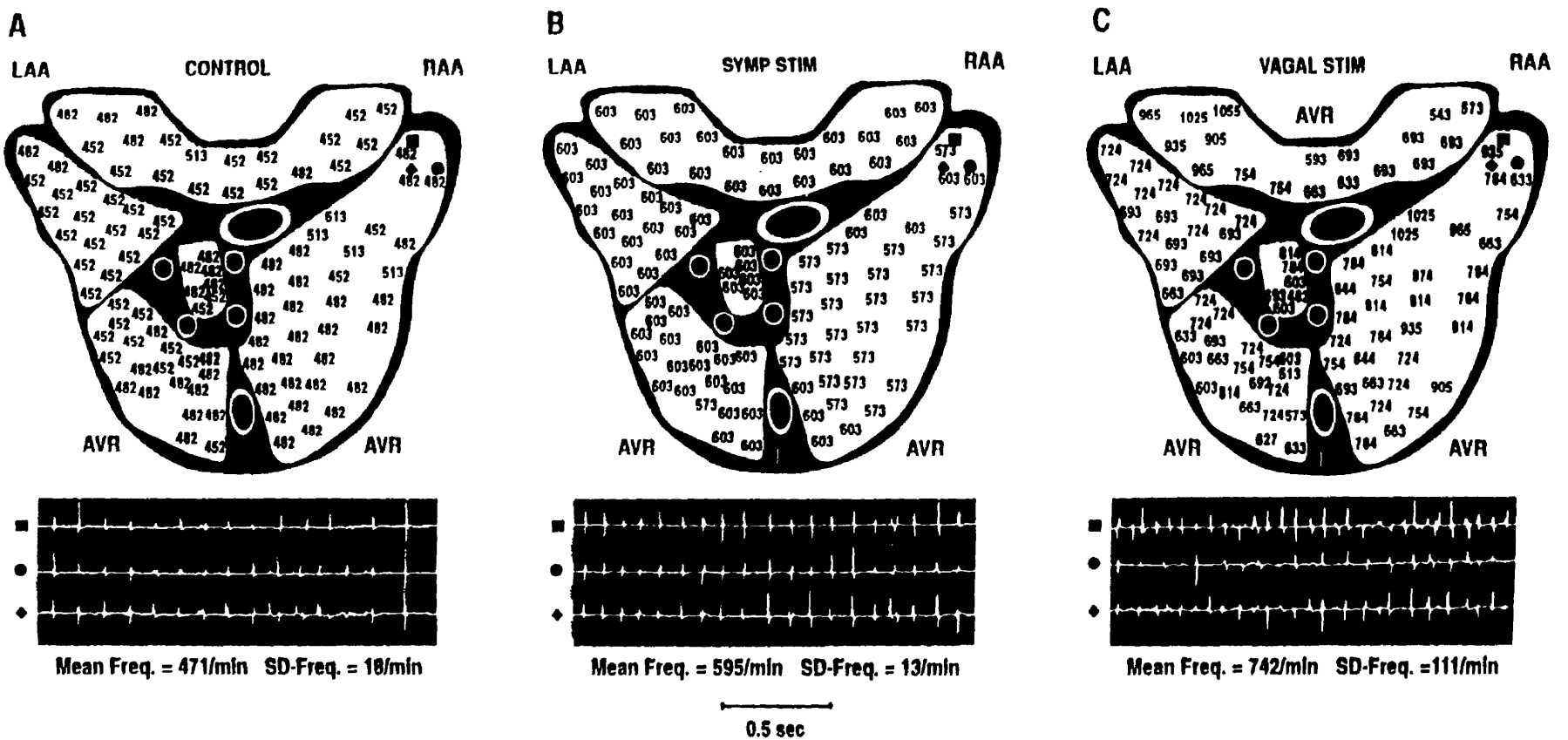
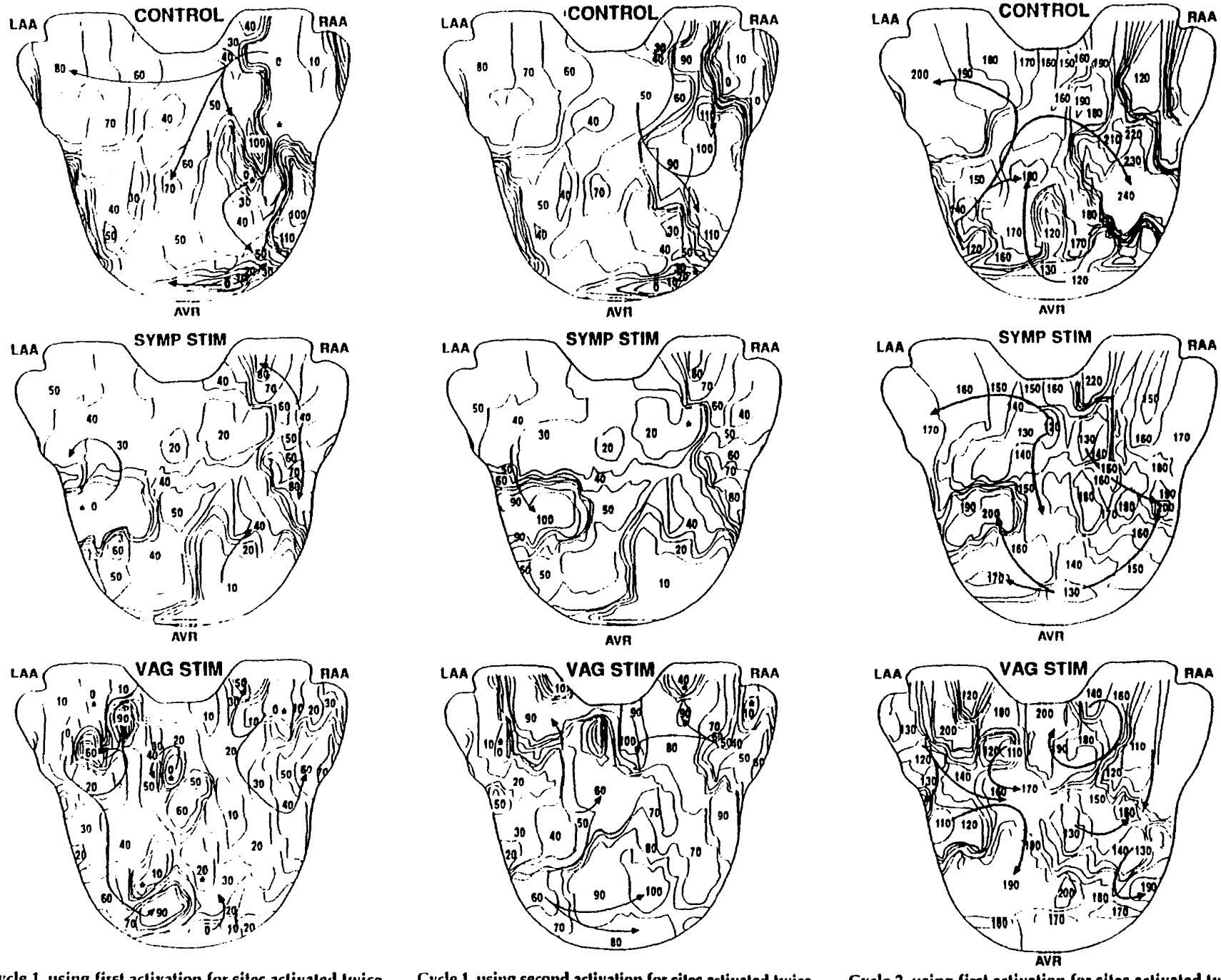

A
LAA
CONTROL
RAA
AVR
AVR
Mean Freq. = 471/min SD-Freq. = 18/min
B
LAA
SYMP STIM
RAA
AVR
AVR
Mean Freq. = 595/min SD-Freq. = 13/min
0.5 sec
C
LAA
VAGAL STIM
RAA
AVR
AVR
Mean Freq. = 742/min SD-Freq. =111/min

Figure 18



Cycle 1, using first activation for sites activated twice

Cycle 1, using second activation for sites activated twice

Cycle 2, using first activation for sites activated twice

Figure 19

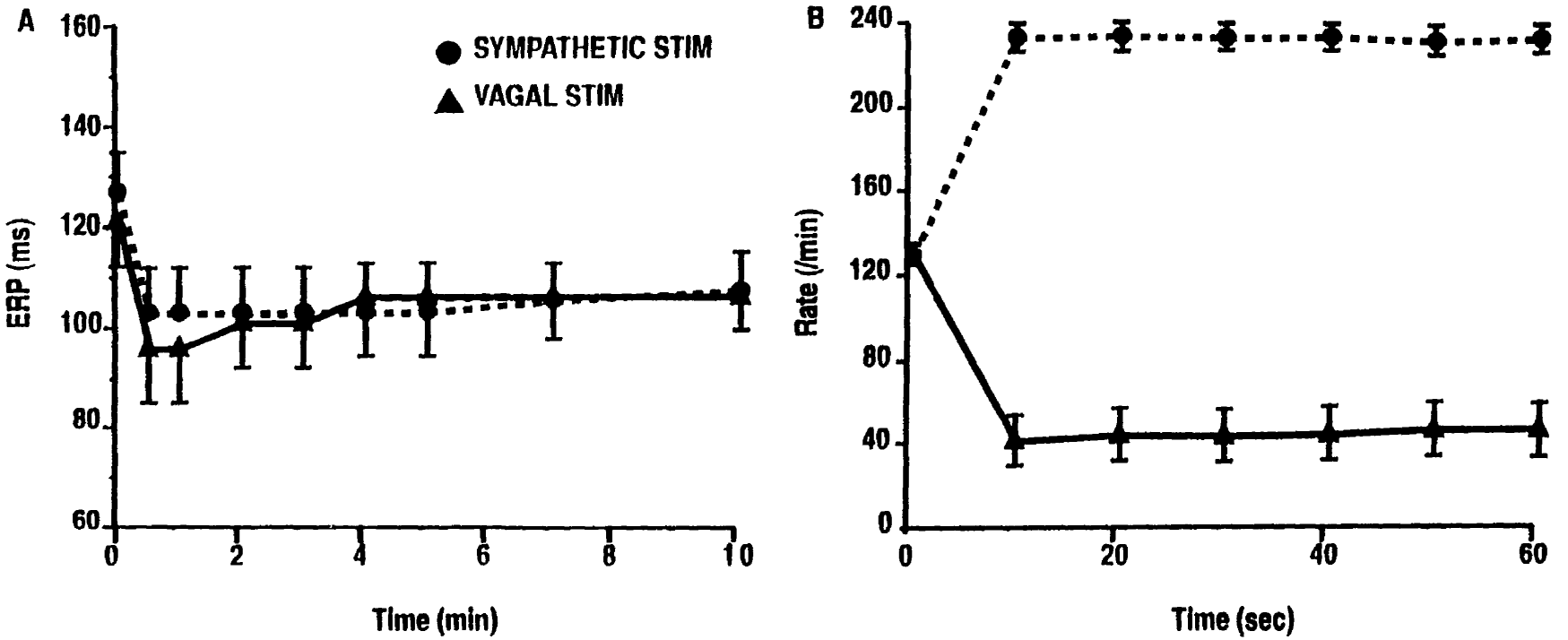

A
B
SYMPATHETIC STIM
VAGAL STIM
ERP (ms)
Rate (/min)
Time (min)
Time (sec)
160
140
120
100
80
60
240
200
160
120
80
40
0
0
2
4
6
8
10
20
40
60

TABLE 1. Effects of Combined Vagal and Stellate Ganglion Decentralization, and of Graded Stellate Ansa Stimulation, on Electrophysiologic Variables and AF Duration

	ERP (ms)	Wavelength (cm)	Duration of AF (seconds)
Autonomically intact	91±7	8.1±0.5	67±25
Vagal/stellate decentralization	114±4**	10.5±0.4**	12±4*
Sympathetic outflow stimulation			
1 Hz	99±6	9.3±0.6	10±3*
4 Hz	91±8†	8.3±0.6	19±7*
10 Hz	83±8††	8.0±0.7†	17±7*

*$P<.05$, **$P<.01$ vs results in autonomically intact condition. †$P<.05$, ††$P<.01$ vs results in absence of nerve stimulation after bilateral vagal and stellate ganglion decentralization.

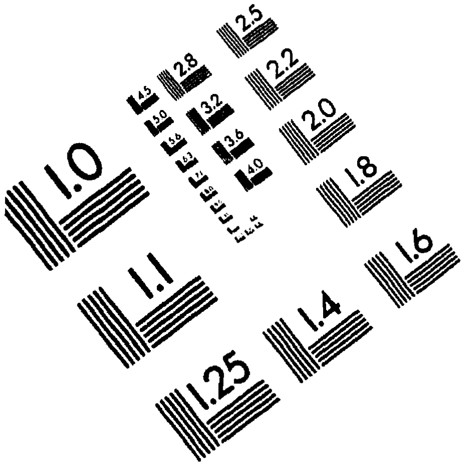
Results are mean±SE in seven dogs, with ERP and wavelength measured at a basic cycle length of 150 ms.

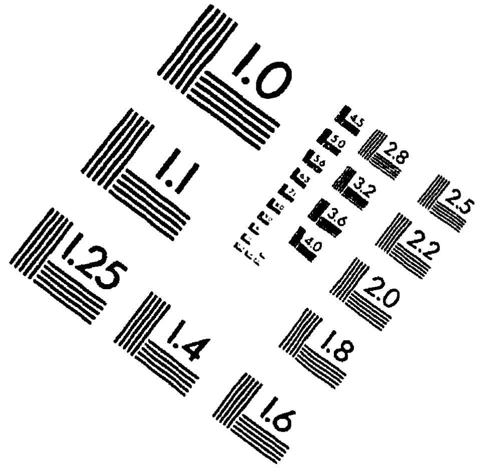
TABLE 2. Results of Repeated Measurements of ERP and AF Duration

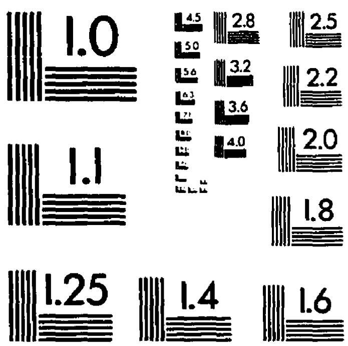
	First Measurement Period			Second Measurement Period		
	Control	Symp. Stim.	Vagal Stim.	Control	Symp. Stim.	Vagal Stim.
ERP (ms)	113±5	90±5	90±5	113±5	90±5	90±5
AF duration (seconds)	63±25	68±41	456±118	53±20	62±33	431±138

Results are mean±SE in three dogs.

IMAGE EVALUATION TEST TARGET (QA-3)

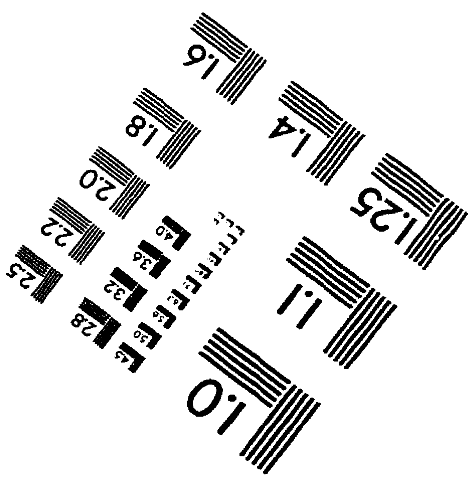


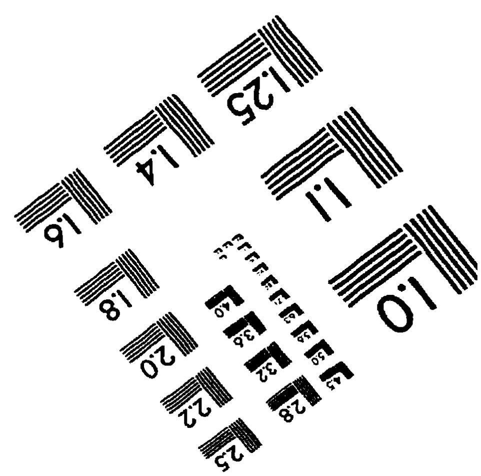




150mm

6″





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