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An Analysis of Pacemaker Activity in the Canine Right Atrium

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AN ANALYSIS OF PACEMAKER ACTIVITY IN THE CANINE RIGHT ATRIUM

By

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VITA

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CHAPTER I

INTRODUCTION

The mammalian heartbeat is governed by a group of specialized cells in the right atrium that spontaneously activate the heart in a rhythmic fashion: the sinoatrial node (129). While it is apparent that the sinoatrial node is the primary pacemaker of the heart (141, 58), it is also known that certain fibers outside the anatomic confines of the sinoatrial node are capable of self-excitation. These secondary pacemakers are referred to as "latent" or "subsidiary" because it is believed they normally are activated by propagated impulses from the sinoatrial node before they are able to spontaneously activate (19). Thus it is hypothesized that subsidiary pacemakers become dominant in the event the sinoatrial node fails to discharge.

Functional pacemaker activity of atrial origin has been demonstrated in the conscious dog to persist for months following excision of the entire sinoatrial node (120,184,56). Furthermore, these subsidiary atrial pacemakers are innervated functionally by both divisions of the autonomic nervous system. They are initially unstable, compared to the sinoatrial node,

and autonomic influences are known to contribute to this instability (184 , 56). However, due to limitations of *in vivo* models of subsidiary atrial pacemaker activity, the mechanisms by which autonomic neuromediators regulate rate and rhythm are not entirely understood.

The cellular electrical properties of the sinoatrial node have been studied extensively in recent years but at present, little is known of the electrophysiological characteristics of subsidiary atrial pacemaker tissue. Fibers that exhibit diastolic depolarization have been recorded from regions outside the classically described sinoatrial node (173 , 94 , 254). However, it is uncertain under what conditions these or other fibers become spontaneously active and whether autonomic neuromediators play a role in regulating impulse formation and conduction. Information in this regard would be of importance in understanding the basis of autonomically-mediated pacemaker instability observed *in vivo*.

The present investigation was undertaken to 1) characterize the functional properties of subsidiary pacemakers in the right atrium and compare them with those of the sinoatrial node and 2) determine the cellular electrical events which underlie the functional characteristics. The characterization of functional properties was made on a multicellular level using extracellular electrodes to assess pacemaker activity. In addition, a second series of experiments was conducted to determine the cellular electrical properties of fibers in subsidiary

atrial pacemaker tissue. This series utilized microelectrodes to study pacemaker automaticity plus extracellular electrodes to assess the conduction of spontaneous impulses.

CHAPTER II

LITERATURE REVIEW

A. ORIGIN OF THE MAMMALIAN HEARTBEAT

The anatomical site of origin of pacemakers in the mammalian heart and the mechanisms governing their rhythmic behavior have been a scientific focus of interest for many years. By the late 1800's, Gaskell (68) and Engelmann (19) had established the location of the primary pacemaker in amphibian hearts within the sinus venosus, a chamber anatomically separate from the atrium. At this time however, the origin of the mammalian heartbeat was not as well defined. The early attempts addressing this question generally involved the use of crude methods and subjective observations. Nevertheless, these initial observations provided the foundation for subsequent, more systematic investigations.

One of the first approaches to determine the pacemaker site in the mammalian heart was the visual identification of that region in the excised heart which continued beating the longest, the so-called "ultimum moriens". It was assumed that the region which exhibited the highest degree of automaticity *in vivo*, the primary pacemaker, would continue beating the longest in the dying heart. Using this technique, MacWilliam in 1888 (157) and later Hering (19) stated that

the ultimum moriens was located near the mouth of the vena cava. However, several other related studies failed to confirm these observations which led to a divergence of opinion as to where the primary pacemaker was located. Fredericq (64) found the ultimum moriens between the two vena cavae while Erlanger and Blackman (54) simply stated that it was near the great veins. Koch (19) observed the dying hearts of still-born human fetuses and concluded that the last region to continue contracting was at the mouth of the coronary sinus. Finally, Hirschfelder and Eyster (86) observed dissociated contractions of various parts of the atria. The results obtained from locating the ultimum moriens generally were inconclusive but these studies did establish the anatomic correlation of pacemaker and caval tissue as well as describe the occurrence of pacemaker activity in a number of different atrial regions.

Perhaps the first systematic study to locate the primary pacemaker site in the mammalian heart was conducted by MacWilliam (157) using a heating method described previously by Gaskell (68). He observed that mild heat applied to the terminal portion of the vena cava <u>in situ</u> accelerated the rate of the entire heart whereas rate did not change when heat was applied to ventricular tissue. This finding indicated that the primary pacemaker was located at the junction of atrial and caval tissue. Other studies confirmed this observation and further showed that excision of tissue in the vicinity of the great veins led to a slower heart rate (54, 19).

In 1906, Keith and Flack (129) discovered an unusual structure in histological sections of the superior vena cava-right atrial junction. Although muscular, this structure was quite different from the surrounding tissue and resembled the atrioventricular node which had been described earlier by Tawara (130). This structure subsequently was observed in a number of different mammalian hearts at the same anatomical location. As a result of Tawara's work, Keith and Flack inferred that the group of "primitive fibers" at the "sino-auricular junction of the mammalian heart was where the rhythm of the heart normally begins" (129).

Soon after Keith and Flack's histological description of the sinoatrial node, a number of physiological studies emerged which supported their hypothesis that this structure is the primary pacemaker of the heart. Erlanger and Blackman (54) found that experimental procedures such as cooling, clamping, pinching, excision, and application of atropine and muscarine before and during extrinsic nerve stimulation, were without effect on cardiac rhythm unless performed on the sinoatrial node. The first electrophysiologic studies of the sinoatrial node were performed by Lewis et al. (141) in 1910. They found that stimulating the canine heart from the sinoatrial node region most nearly duplicated the atrial electrical complex of the normal heart beat. Later that year, Lewis et al. (141) and Wybauw (258) introduced the method of determining the point of primary negativity as a means of locating the primary pacemaker site. This technique is based on the

premise that the tissue immediately adjacent to the pacemaker depolarizes before any other region. Using paired surface electrodes, Lewis et al. (141) found that the point of primary negativity was directly above the sinoatrial node, as verified histologically. Eyster and Meek in 1914 (57) confirmed the findings of Lewis et al. and in a series of extensive experiments (58,59) showed that the point of initial negativity normally appears in the upper part of the sinoatrial node. Furthermore, they found that manipulations such as vagal stimulation, potassium chloride infusion or cooling the upper part of the node shifted the point of initial negativity to the lower part.

The now-classical concept of a single site of origin of pacemaker activity in the sinoatrial node has been accepted for over 50 years. However, recent studies of canine atrial activation by Boineau et al. (12,13,14) and Schuessler et al. (199) have altered this concept. Using a mapping procedure with 100 to 144 bipolar epicardial and endocardial recording sites, they concluded that atrial depolarization originates from a widespread multicentric pacemaker system rather than being unifocal in origin. They described three to five origin or O-points which are located at sites beyond the dimensions of the classically described sinoatrial node. It has been hypothesized that each pacemaker site dominates the spontaneous excitation of the heart within a specific range of heart rates. A change in spontaneous rate due to alterations in neural tone

or infusion of neuromediators was found to shift the O-points within the multicentric system that in turn altered the activation pattern of the right atrium and changed the morphology of the ECG P wave.

B. HIERARCHY OF ATRIAL PACEMAKERS

During the initial attempts to ascertain the location of the primary pacemaker of the mammalian heart, it was observed by many that more than one region of the heart possesses the property of automaticity. This was evident from observations on the rhythmicity of excised segments of atrial tissue (54 , 55) as well as the variety of pacemaker sites obtained from studies attempting to locate the ultimum moriens. Furthermore, it was known that although many parts of the atrium are endowed with independent rhythmic activity, the rhythmicity of various parts are not equal (54, 68) and that there is an order or hierarchy of automaticity. Perhaps the first to convey the concept of pacemaker hierarchy was Gaskell in 1884 (68) who stated that the highest degree of rhythmicity in amphibian hearts is within the sinus venosus and that "the rhythmical power of each segment of the heart varies inversely as its distance from the sinus". Similar observations were made in the mammalian heart by MacWilliam (157) and Erlanger and Blackman (54).

Just as it is important to determine the site of primary pacemaker activity so too is it important to locate

the regions of the atria which functionally exhibit automaticity in the absence of the sinoatrial node; the so-called ectopic or subsidiary atrial pacemakers. An early clue in the elucidation of the atrial pacemaker hierarchy came from experiments conducted by Englemann (19) which indicated that the atrioventricular nodal region is a functionally important site of subsidiary pacemaker activity. He found that the atria and ventricles of frog hearts contracted simultaneously, or nearly so, when a Stannius ligature isolated the sinus venosus from the atrium. A year later, Lohmann (19) observed that vagal escape beats in the rabbit heart often showed reduced As-Vs (atrial systole-ventricular systole) intervals that were negative, zero or positive in value. Using the initial negativity technique of Lewis et al. (141), Meek and Eyster (59) located the site of subsidiary pacemaker activity at the atrioventricular node after the sinoatrial node had been suppressed by application of formalin, vagal stimulation, clamping, crushing or excision of nodal tissue. They also found evidence for pacemaker activity near the coronary sinus, confirming the earlier work of Zahn (59).

More recent electro-physiologic and -cardiographic investigations have distinguished between at least five different pacemaker sites in the atrioventricular nodal region: the ostium of the coronary sinus (60, 16, 32,200) the upper, middle and lower atrioventricular node (195,196) and His bundle (196,228,119). The complexity of the atrioventricular nodal region as a site of subsidiary atrial pacemaker activity

has prompted the use of the term "atrioventricular junction" as a semantic compromise to include pacemaker regions of the coronary sinus, the atrioventricular node itself and the His bundle (196).

Urthaler et al. (226,227,228) have characterized two distinct forms of atrioventricular junctional rhythms in the canine heart following suppression of the sinoatrial node by direct perfusion of physostigmine into the sinoatrial node artery. The first form of junctional rhythm termed AVJ-1, is characterized by simultaneous atrial and ventricular depolarization and a spontaneous rate which is 66% of the control sinoatrial node spontaneous rate. The second junctional rhythm termed AVJ-2 is established after complete atrioventricular block by perfusion of physostigmine through the atrioventricular node artery. This rhythm, believed to originate from the His bundle (119), is characterized by an insensitivity to high concentrations of acetylcholine and by a spontaneous rate that is 22% of the control sinoatrial node spontaneous rate. A similar differentiation of atrioventricular junctional rhythms has been reported in clinical studies (196) and in the experimental studies of Motomura (166,167) using the excised blood-perfused canine atrioventricular node preparation.

Electrophysiologic investigation of the atrioventricular junction with microelectrodes has provided evidence for automatic cells in those regions which have been shown by in vivo methods to possess pacemaker activity. It is generally

accepted that fibers in the N region of the AV node (174) do not exhibit transmembrane potentials characteristic of pacemaker cells (89 , 90,245). However, fibers demonstrating diastolic depolarization have been recorded from the AN and NH regions of the atrioventricular node (41, 89, 90,245). According to Hoffman and Cranefield (90) and Watanabe and Dreifus (245) automaticity in the AN and NH regions may correspond to the upper and middle rhythms described by investigators using extracellular recording techniques (196). Automatic fibers also have been recorded from atrial tissue outside the ostium of the canine coronary sinus, confirming observations of earlier studies that have implicated this region as a subsidiary atrial pacemaker site (16,200,254). In the presence of norepinephrine, these fibers exhibit action potential configurations characteristic of primary pacemaker cells: a smooth transition from phase 4 to phase 1. However, in relation to pacemaker fibers of the sinoatrial node (256,257) those of the coronary sinus are characterized by a higher maximum diastolic potential and more rapid upstroke (254).

From the classic studies of Englemann (19) and Meek and Eyster (158) to more contemporary investigations by Urthaler et al. (226,227,228), the definition of atrial pacemaker hierarchy has stated that the atrioventricular junction is the second most automatic region below the sinoatrial node. Recent studies (120,121,148,122) have presented evidence which re-defines atrial pacemaker hierarchy to include pacemaker

activity in the inferior right atrium. Jones et al. (120, 121,122) excised the canine sinoatrial node and used electrophysiologic mapping techniques to locate the site of earliest activation. They found that in 80% of the animals prepared in this fashion, the subsidiary site of earliest activation was located at the junction of the inferior vena cava-inferior right atrium. This anatomic location is clearly on the anterior free wall of the atrium away from the atrioventricular junction. Furthermore, they found that the inferior right atrial pacemaker spontaneous rate is approximately 73% of the sinoatrial node spontaneous rate prior to excision.

It has been shown in the dog that excision of the entire sinoatrial node (120,184,56,185) or embolization of the sinoatrial node artery (147,148) results (after a variable period of pacemaker instability following surgery) in a rhythm characterized by a definitive P wave in lead II. Although not as precise as invasive epicardial mapping techniques, clinical electrocardiographic analysis of P wave morphology has also indicated that subsidiary or ectopic atrial pacemaker activity exists in regions outside the atrioventricular junction. Mirowski et al. (162,163) presented evidence to support the existence of an ectopic focus located on the anterior-superior aspect of the right atrium. The ectopic rhythm was characterized by a normal P wave in the extremity leads and left precordial lead plus inversion of the P waves in leads V1 through V4. Vectorial analysis of atrial activation resulted in a mean P vector direct inferiorly, anteriorly and slightly to the left. Leon

et al. (137) artifically induced ectopic right atrial rhythms in normal hearts by endocardial pacing from a variety of sites. They concluded that the P wave contour and polarity was specifically determined by the site of stimulation thus suggesting that precise analysis of the ECG P waves could be used clinically to determine the site of primary atrial activation. MacLean et al. (151) paced the hearts of patients following open-heart surgery from a number of atrial sites utilizing temporary implanted bipolar electrodes. They concluded however that the P wave polarity and morphology were quite variable and therefore unreliable in determining the origin of an ectopic atrial rhythm. Sherf and James (203) stated that alterations in P wave morphology occurring with a simultaneous change in heart rate indicated the presence of an ectopic pacemaker. Similar ECG changes without a concomitant change in heart rate were said to indicate abnormal conduction in one of the internodal pathways.

The cellular electrophysiologic basis of pacemaker activity in the right atrium outside the classically described sinoatrial node region is poorly understood. This stems from the fact that spontaneously active subsidiary pacemaker fibers have not been characterized electrically. The present belief is that specialized conducting fibers, possible components of internodal tracts, become spontaneously active when the sinoatrial node fails to discharge. Earlier studies in the rabbit right atrium by Paes deCarvalho (173) and DeMello and Hoffman (51) demonstrated specialized fibers along the

crista terminalis comprising the so-called sinoatrial ring bundle, which are characterized by diastolic depolarization as well as a resistance to elevated potassium concentrations. Sano and Iida (192) showed evidence of spontaneous pacemaker activity in the caudal region of the rabbit crista terminalis after removal of the sinoatrial node, superior and inferior vena cavae and the ostium of the coronary sinus. They hypothesized that under certain conditions, such as acetylcholine administration, the pacemaker shifts from the sinoatrial node down the crista terminalis toward the atrioventricular node. In the dog right atrium, Hogan and Davis (95) and Davis (48 , 49) described fibers located along the caval border of the crista terminalis which resemble Purkinje fibers in their action potential configuration. Similar in location to those fibers of the rabbit sinoatrial ring bundle, these "plateau" fibers are believed to be a part of the posterior internodal tract described by James and Sherf (117). Certain plateau fibers were found to exhibit diastolic depolarization which steepened and spontaneously depolarized when either isoproterenol or epinephrine was applied locally to the impaled fiber. Specialized atrial fibers demonstrating spontaneous activity also have been recorded from human atrial tissue obtained during cardiac surgery (220 , 71 , 153, 154).

In addition to the atrioventricular junctional and inferior right atrial sites of subsidiary pacemaker activity, automatic properties have been demonstrated in a number of

other atrial regions. The functional role of these potential pacemaker regions is not entirely understood but it is believed they may be significant in relation to the genesis of atrial dysrhythmias. Generally, they are found in tissue adjacent to venous or valvular structures. In the right atrium, action potentials exhibiting phase 4 depolarization have been recorded from the right atrioventricular ring (92) and associated tricuspid valve leaflets (10). Pacemaker activity of left atrial origin is considered rare (54 , 55) however Mirowski et al. (162,163) have presented clinical and experimental evidence to support its existence. The cellular electrical basis for left atrial rhythms has been suggested from microelectrode studies which demonstrate phase 4 depolarization in fibers at the junction of the pulmonary veins as well as in the left atrioventricular ring (92). Finally, extracellular mapping studies in the dog heart have demonstrated pacemaker shifts to regions of the anterior internodal tract near Bachmann's bundle (74,120,121). However, microelectrode studies of this region in the dog heart have yet to substantiate the existence of potential pacemaker fibers (97).

C. REGULATION OF PACEMAKER ACTIVITY

1. Autonomic Regulation

The mammalian atria receive abundant innervation from both divisions of the autonomic nervous system which modulate not only automaticity but conductivity and contract-

ility as well. The sympathetic innervation originates from preganglionic fibers whose cell bodies are located in the intermediolateral cell columns of segments T1-T8 of the spinal cord (183,138). These fibers exit the spinal cord through the white ramus communicantes and, in the dog, ascend in the paravertebral chain to the stellate ganglion. Synapse with postganglionic fibers generally occurs in the upper thoracic ganglia including the stellate ganglion or the caudal cervical ganglion, by way of the ansae subclavia (183,138). From this point, sympathetic fibers project onto the atria by way of the superior vena cava, the superior left atrium and the great arteries (70,183).

In the dog, preganglionic parasympathetic fibers originate in the nucleus ambiguus and dorsal motor nucleus of the vagus. These fibers descend by way of the vago-sympathetic trunk and project onto the atria along the superior vena cava, great arteries, superior left atrium, interatrial groove and the junction of the inferior vena cava and inferior left atrium (73,183,138).

Details of the terminal innervation of the right atrium has been obtained recently by a number of biochemical, histochemical and electronmicroscopic techniques that have contributed greatly to an understanding of the autonomic control of atrial automaticity. In most species that have been studied, the atrial concentration of norepinephrine, presumably reflecting the density of sympathetic nerve fibers, is two to four times greater than in ventricular tissue (7,

138,198,139). Moreover, the distribution of fibers within the atrium is not uniform, as evidenced by regional differences in norepinephrine concentration (103,205, 36,198) and by histochemical fluorescence (46,103). Coglianese et al. (36), using radioactive tracers to map the relative densities of sympathetic nerve terminals, found a gradient of [^{3}H] norepinephrine that was highest in the tip of the atrial appendage and lowest in the interatrial septum. They also found, as others have (7,138,139) that the concentration of norepinephrine in the sinoatrial node is not significantly different from working atrial myocardium. In the region of the atrioventricular node, a high density of sympathetic fibers is found also (46) although distribution of sympathetic fibers within the node is uneven (215).

As in the case for sympathetic nerves, the density of parasympathetic fibers as measured by enzymatic markers, is greater in atrial than in ventricular tissue (8, 37,113, 188,189,197). Furthermore, there are regional differences in acetylcholinesterase concentration within the right atrium with the sinoatrial node possessing the greatest concentration (37,113,131, 26,197,207) and presumably the greatest vagal innervation. Histologic and electronmicroscopic analyses of atrial tissue reveal that parasympathetic ganglia associated with both sinoatrial and atrioventricular nodes are located predominantly adjacent to these structures rather than within them (103,113). The overall nonuniform distribution of parasympathetic fibers in the right atrium probably contributes

to the dispersion of refractoriness and development of fibrillation with vagal stimulation (2, 85).

The chronotropic actions of nerve stimulation or the application of neuromediators on sinoatrial node automaticity have been documented extensively. The positive chronotropic effects of sympathetic nerve stimulation (216, 69, 74, 75, 120) or application of catecholamines (113,116, 33, 38, 65, 150) are well established as are the negative chronotropic effects of vagal stimulation (68,159,250, 85,138) or application of acetylcholine (248,110,111, 33). Complex chronotropic responses are obtained with direct subthreshold electrical stimulation of sinoatrial node tissue which presumably stimulates both sympathetic and parasympathetic postganglionic nerve fibers (225, 3 ,140,240,250,134). It has been shown that an initial negative chronotropic phase is followed by a phase of acceleration. The initial phase is abolished by atropine while the later phase is eliminated by beta adrenergic blocking agents (3,240,250,134).

The positive chronotropic action of sympathetic stimulation or applied catecholamines is mediated through beta adrenergic receptors (138). Stimulation of alpha adrenergic receptors also has been shown to modulate spontaneous rate although the precise mechanism is uncertain. James et al. (114) and others (82) have shown that alpha adrenergic stimulation decreases the spontaneous rate of the sinoatrial node. While James et al. (114) attribute this negative chronotropic effect to direct alpha stimulation on the pacemaker cells,

Hashimoto et al. (82) conclude that alpha stimulation causes release of endogenous acetylcholine which suppresses spontaneous rate. Experiments conducted by Mary-Rabine et al. (154) suggested that the chronotropic changes induced by epinephrine on human atrial specialized fibers are mediated by both alpha and beta receptors: alpha stimulation decreasing and beta stimulation increasing spontaneous rate.

In addition to their chronotropic effects, autonomic nerve stimulation or applied neuromediators often shift the pacemaker to sites within (159, 17,127, 75, 12,150) or outside (158, 69, 32) the sinoatrial node. During left stellate ganglion stimulation in the dog, Geesbreght and Randall (69) found that the pacemaker had shifted to the region of the coronary sinus and atrioventricular node. Stimulation of the vagus nerve has also been shown to shift the pacemaker to the atrioventricular junction (158,179). These studies indicate that functional subsidiary atrial pacemaker activity can be unmasked either by adrenergic stimulation, which selectively enhances its automaticity above that of the sinoatrial node, or by cholinergic stimulation that suppresses the sinoatrial node. It is likely that the observed shifts are the result of non-homogeneous distribution of autonomic nerve endings or differences in the sensitivities of pacemaker fibers to neuromediators (17, 69, 75,150).

The autonomic regulation of subsidiary atrial pacemakers has been studied independently with the use of experimental models in which sinoatrial node automaticity is either

transiently suppressed or totally eliminated. Jones et al. (120) and Goldberg et al. (74) excised the canine sinoatrial node and demonstrated that pacemaker activity in the inferior right atrium is responsive to adrenergic stimulation. Either isoproterenol infusion or stellate ganglion stimulation markedly enhances the subsidiary spontaneous rate but not to the same degree as the sinoatrial node before excision. These studies also showed pacemaker activity to exist in the region of Bachmann's bundle. In the conscious dog model (120, 184, 56,148), subsidiary pacemaker activity, not of junctional origin, was found to be influenced strongly by the parasympathetic nervous system. Disturbances in rate and rhythm characterized by severe bradycardia, brady-tachydysrhythmia and periods of asystole, were abolished by intravenous administration of atropine or by exercise. Differences in the response of subsidiary atrial and sinoatrial pacemakers to brief periods of vagal stimulation also has been documented by Spear and Moore (210) in acute experiments. They found however, that "ectopic" atrial pacemakers are less sensitive to the effects of a single vagal stimulus than either the sinoatrial node or atrioventricular junctional pacemakers.

Pacemaker activity in the atrioventricular junctional region also has been shown to be regulated by autonomic nerves. In the isolated atrioventricular node of the rabbit, Vincenzi and West (240) functionally demonstrated both adrenergic and cholinergic modulation of automaticity. They

observed a biphasic chronotropic response to subthreshold stimuli applied directly to the tissue that was similar to that of the sinoatrial node (3) and was also the result of endogenously released acetylcholine and norepinephrine. Urthaler et al. (226) induced atrioventricular junctional rhythms in the dog heart by injecting physostigmine into the sinoatrial node artery to suppress sinoatrial node automaticity. They found that atrioventricular automaticity is dependent upon adrenergic neural tone to maintain pacemaker stability. Periods of asystole and slow erratic beats were observed when adrenergic neural tone was impaired by stellectomy, beta blockade with propranolol or pretreatment with reserpine. Spear and Moore (210) did not observe such an instability in atrioventricular junctional rate after crushing the sinoatrial node but in their preparations the stellate ganglia had not been decentralized. Other reports using the technique of selective perfusion of the atrioventricular node artery have documented the occurrence of distinctive junctional rhythms which differ in their response to perfused neuromediators (31,166,167). These studies coincide with clinical findings of two types of junctional pacemakers which differ in their response to cholinergic stimulation or cholinergic-blocking agents (196).

2. Interrelationships of Pacemakers

Under normal conditions, the fibers of the sinoatrial node act as the dominant pacemaker of the heart by

virtue of their inherently faster rate of spontaneous depolarization (92,235,170). The slope of diastolic depolarization of these fibers is the steepest and as a result they reach threshold before other fibers outside the sinoatrial node that possess a smaller degree of diastolic depolarization, i.e. subsidiary pacemakers (235,236). When the sinoatrial node fails to discharge, a subsidiary pacemaker then assumes dominance but usually after a period of quiescence that is longer than the cycle length of the newly established subsidiary rhythm (235). This period of quiescence indicates that subsidiary pacemakers are suppressed normally by the more rapid spontaneous rate of the sinoatrial node, a phenomenon known as overdrive suppression. This phenomenon may explain partially, the overall hierarchial dominance of cardiac pacemakers (233).

Studies investigating the chronotropic response of pacemaker activity to rapid pacing indicate that the sensitivity of sinoatrial node pacemakers to overdrive pacing is less than for subsidiary pacemakers (135,149,185). Lange (135) electrically drove the canine sinoatrial node at rates greater than 20% above the spontaneous rate and observed a temporary suppression of automaticity on termination of the pace. When the rate of overdrive was less than 20% above the spontaneous rate, the rate accelerated upon termination. After crushing the sinoatrial node, overdrive of the dominant subsidiary atrial pacemaker resulted in a more pronounced

suppression. Lange also determined that the amount of suppression is a function of the rate of overdrive as well as its duration. In the cat right atrium superfused *in vitro*, Lu *et al*. (149) found that subsidiary pacemakers within the sinoatrial node also exhibit greater degrees of suppression upon overdrive pacing than primary pacemakers. Subsequent investigations have documented significant degrees of overdrive suppression in pacemakers of the inferior right atrium (56,147,144,186) atrioventricular junction (227,144) and idioventricular pacemakers (239,233,123,133). Prolonged suppressions also have been documented clinically in patients exhibiting dysfunction of the sinoatrial node, the so-called sick sinus syndrome. This pathologic condition is marked by dysrhythmias in the form of severe bradycardia and periods of asystole. When compared to patients with normal sinoatrial node function, pacemaker suppression in patients exhibiting the above mentioned dysrhythmias is significantly longer (152,123,29,259,35). This characteristic has lead to the use of overdrive pacing as a means of diagnosing sick sinus syndrome.

The mechanism of overdrive suppression in atrial pacemakers is partially explained by the release of endogenous neuromediators upon direct stimulation of atrial tissue (3). In the sinoatrial node, West (249) found that the hyperpolarization of pacemaker cells due to release of endogenous acetylcholine accounted for the temporary suppression of

automaticity following overdrive. Other investigations both *in vivo* and *in vitro* have demonstrated that in general, manipulations which enhance acetylcholine's stimulation of muscarinic receptors (135,149) or reduce the effects of norepinephrine on beta adrenergic receptors (135,227,56,185) lengthen the duration of suppression. On the other hand, suppression is reduced but not totally abolished by either administration of atropine (68,135,149,56,185) or by manipulations that enhance beta adrenergic stimulation (135). With idioventricular pacemakers, it has been shown that alterations in beta adrenergic stimulation influence the duration of overdrive (180,181,182) in a qualitatively similar fashion as subsidiary atrial pacemakers.

Lu *et al*. (149) determined that the cat sinoatrial node is suppressed slightly by overdrive even in the presence of atropine. Studies of subsidiary atrial pacemaker activity in the dog (56,185) also have shown that a residual suppression remains in the presence of atropine. These studies indicate that factors, other than neural tone or stimulus-induced release of endogenous neuromediators are involved in the overdrive suppression of automaticity. Lu *et al*. (149) and others (132) have concluded that the neuromediator-independent suppression of sinoatrial node automaticity is partially the result of ion shifts during the period of overdrive, such as extracellular potassium accumulation, which decrease the slope of diastolic depolarization (231,182). In spontane-

ously active Purkinje fibers, it also has been shown that suppression is the result of a decreased slope of diastolic depolarization (1,233,239). The mechanism of this change is believed to be the result of accumulation of extracellular potassium (1,233,235) and a stimulus-induced increase in electrogenic pump activity (233,235).

Another possible mechanism of overdrive suppression is the accumulation of intracellular calcium. In atrial tissue, the exchange of calcium is proportional to the rate of stimulation (76) and it has been shown that intracellular calcium modulates membrane potassium conductance (124). Thus it is hypothesized that the effects of an increase in intracellular calcium concentration on enhancing potassium conductance as well as raising the excitation threshold (235) may contribute to the observed suppression. However, differences exist between various pacemakers in relation to the calcium hypothesis. While it has been shown that verapamil reduces the suppression of Purkinje fiber automaticity following overdrive (96) this compound also has been shown to lengthen the suppression of atrial pacemaker activity (29). In general, the cellular mechanism of overdrive suppression, as studied in sinoatrial node and Purkinje fiber pacemakers, involves a complex interaction of active and passive membrane properties. To date however, little information is known of the cellular basis of overdrive suppression in subsidiary atrial pacemakers.

3. Electrotonic Interactions

The functional syncytial nature of cardiac tissue lies in the electrical coupling of individual cells by an intercellular structure known as the nexus (9,52,202,176). This specialized low resistance junction allows the electrical activity of a cell to be influenced by the activity of adjacent cells by way of current flow through the nexus (63). In the case of pacemaker activity, it has been shown that electrotonic depolarizations of subthreshold amplitude can modify the rhythm of pacemakers depending upon the timing of the depolarization (193,104,105,106). Jalife and Moe (104) demonstrated in Purkinje fibers that stimulated responses applied proximal to a region of block produced electrotonic depolarizations in a spontaneous segment distal to the block. These depolarizations, which were the result of electrotonic current spread, modified the rhythm of the spontaneous segment in a consistent fashion. Specifically, electrotonic depolarizations which occurred early in the spontaneous cycle delayed while those that occurred later in the cycle accelerated the subsequent spontaneous action potential. They constructed phase response curves which are qualitatively similar to those obtained from experiments studying the phasic effects of vagal stimulation on sinoatrial node (20,138,105) and atrioventricular junctional pacemakers (243,244). Furthermore, the spontaneous activity in the distal segment could be entrained by stimuli applied to the proximal segment even though conduction was blocked. Thus the phasic response of cardiac pacemakers to premature

stimuli, whether the result of propagated responses or subthreshold electrotonic depolarizations, is a means other than prolonged overdrive by which pacemaker activity may be suppressed (147).

D. CELLULAR MECHANISMS OF SPONTANEOUS ACTIVITY

The rhythmicity of the heart has long been an object of curiosity for scientists and philosophers alike and a great number of theories have been proposed to explain the mechanism of this property. One of the earliest theories, which prevailed up to the 19th century, was that the origin of the heartbeat was the result of activity of nerves. This "neurogenic theory", proposed by Willis in 1664 (87), stemmed from anatomical studies which showed that nerves entered the heart and from analogy with other muscles of the body. A "myogenic theory" of rhythmicity, proposed by Haller in 1754 (19), later replaced the neurogenic concept as it was shown that rhythmic contractions of the heart continued after the extrinsic nerves were cut. This observation along with evidence that the embryonic heart begins beating before being innervated (177) led to a greater acceptance of the concept that automaticity of the heart is the result of an inherent irritability of myocardial cells.

An important development in ascertaining the precise mechanism of spontaneous activity was the introduction of the microelectrode technique (145) which provided a means of recording the electrical activity of individual cells. Using this technique in cardiac tissue, Draper and Weidmann in 1951 (53) found that fibers of the canine false tendon exhibit slow depolarization during diastole. In addition, it was determined that the rate of diastolic depolarization is proportional to

the extracellular sodium concentration. They concluded that "autorhythmicity of heart muscle is probably connected with the existence of slow depolarization during diastole" and that depolarization is due to an increase in net inward sodium current. Although Draper and Weidmann established diastolic depolarization as the hallmark of pacemaker tissue, earlier studies using extracellular electrodes (53,18) demonstrated prepotentials in pacemaker regions during diastole. More recent investigations using a unipolar extracellular electrode also have demonstrated charateristic potential changes during diastole (i.e. diastolic depolarization)in pacemaker regions of the sinoatrial node (39,40,80) and the ostium of the coronary sinus (168).

The concept that pacemaker fibers undergo diastolic depolarization led to microelectrode investigations of other regions of the heart known to possess the property of automaticity. Subsequently, fibers exhibiting diastolic depolarization were recorded in the primary pacemaker regions of the amphibian sinus venosus (217,99) and the sinoatrial node of mammalian hearts (247). Recently, studies of the cellular electrophysiology of pacemaker tissue have expanded to include the subsidiary atrial pacemaker regions of the atrioventricular junction (89,245), crista terminalis (173,94 192,49) ostium of the coronary sinus (254) and from the anterior free wall of the human right atrium (220,71,154, 155). To date, transmembrane action potentials have not been recorded from pacemaker fibers of the inferior right atrium (120,121). As is the case for the other subsidiary

atrial pacemakers, little is known of the mechanism of impulse generation in this area nor are the effects of neuromediators on spontaneous activity completely understood.

1. Ionic Currents

A significant understanding of the ionic currents underlying diastolic depolarization has come largely from investigations using the voltage-clamp tehcnique. In Purkinje fibers, Deck and Trautwein (50) found a brief inward current followed by a decaying outward current when the membrane was clamped from the plateau back to the resting potential. The outward-current tail appeared at potentials more negative than -50 mV and reversed direction at potentials negative to E_k. Vassalle (232) clamped the membrane of a spontaneous active fiber at the maximum diastolic potential and recorded a time-dependent inward current that was associated with an increase in membrane resistance. These findings along with those of Deck and Trautwein (50) suggested that diastolic depolarization in Purkinje fibers is due to a time-dependent decrease in potassium conductance (219,170,237), a mechanism which differs from an increase in inward sodium current hypothesized by Draper and Weidmann (53).

Noble and Tsien (169) studied the kinetic properties of the pacemaker current, which they termed i_{k2}, and found that it was fully activated at -60 mV and inactivated at -90 mV. Furthermore, they determined that the channel carrying i_{k2} displays inward-going rectification at more positive levels of the pacemaker potential. Thus it is apparent that the pacemaker potential in Purkinje fibers is a combination of two events: an initial phase that is due to a time-dependent de-

crease in i_{k_2} and a later phase that is due to a voltage-dependent decrease in i_{k_2} and the initial activation of i_{Na} (170). The contribution of i_{Na} during the later phase of diastolic depolarization may explain the changes in spontaneous rate observed by Draper and Weidmann (53) in low sodium solutions and the suppressing effects of tetrodotoxin on pacemaker activity (170).

In some respects, the mechanism of the pacemaker potential in the sinoatrial node is similar to that in Purkinje fibers. Voltage-clamp experiments conducted in rabbit sinoatrial node (172) and frog sinus venosus (23, 25) indicate that diastolic depolarization results from inactivation of a potassium current. Analysis of this current has shown that it resembles i_x and is similar to the pacemaker current obtained in frog atrial tissue upon application of a steady depolarizing current (21, 24). In contract to the mechanisms of diastolic depolarization mentioned above, recent experiments in the rabbit sinoatrial node (170) have identified an inward current, termed i_f, that is activated by hyperpolarizing pulses. Thus diastolic depolarization in the sinoatrial node may be due to an activation of an inward current rather than inactivation of an outward current.

The pacemaker range of potentials in sinoatrial node cells, unlike Purkinje fibers, lies at a depolarized level and the "resting potential" in a quiescent state generally occurs between -40 and -50 mV (171,170). This low level of membrane potential is believed to be due to a steady inward current

(220,171) that depolarizes the membrane to a potential range in which the normal pacemaker currents are present (170). The steady inward current, believed to be due to a high sodium conductance (171), accounts for the insensitivity of sinoatrial node cells to elevated external potassium concentrations (28).

Another important inward current participating in the electrogenesis of sinoatrial node cells is a background current carried primarily by calcium ions. Alterations in extracellular calcium concentration significantly change the spontaneous rate of sinoatrial node cells whereas it has little effect on Purkinje fiber automaticity (19,165). Seifen et al. (201) found that the rate of diastolic depolarization increases in proportion to the extracellular calcium concentration between 0.3 and 7 mM but above this concentration, the rate decreases. Further evidence to support the hypothesis that calcium is an important current carrier during diastolic depolarization comes from a variety of studies that have found that verapamil (252, 62, 34) and manganese ions (102, 28) slow or even stop spontaneous depolarization.

In summary, diastolic depolarization of sinoatrial node cells occurs 1) by the decay of a potassium current which progressively enhances the effect of a background inward current or 2) by activation of an inward current upon membrane repolarization. As depolarization continues during diastole, the membrane reaches the potential range which activates the slow inward current that in turn causes depolarization to continue until threshold is reached (170). While

the ionic mechanisms of spontaneous activity in sinoatrial node and Purkinje fibers have been studied extensively, little is known of the excitatory events occurring in subsidiary atrial pacemakers. An approach to this question recently has been taken by Mary-Rabine et al. (155) investigating the spontaneous activity of human atrial specialized fibers. They found the slope of diastolic depolarization to be highly sensitive to verapamil and to a lesser extent tetrodotoxin. It remains to be determined whether pacemaker activity in subsidiary sites relies on mechanisms that resemble the pacemaker currents of Purkinje fibers or sinoatrial node cells.

2. Influence of Neuromediators

Intracellular recordings of spontaneously active sinus venosus or sinoatrial node cells reveal that vagal stimulation or exogenously applied acetylcholine inhibits pacemaker activity by decreasing the slope of diastolic depolarization and by hyperpolarizing the membrane (99,248,100,216,93). The mechanism of the effects of acetylcholine is believed to be due to an increase in potassium permeability (27) and several lines of evidence support this hypothesis. Trautwein et al. (218), measuring passive membrane properties, demonstrated that acetylcholine decreases membrane resistance. Studies specifically measuring ion movements using radioactive potassium have shown an increased efflux of potassium with acetylcholine that is mediated through a muscarinic receptor (100, 146). Lastly, voltage-clamp experiments (170) indicate that

acetylcholine increases an outward current whose reversal potential lies near the equilibrium potential for potassium.

In addition to membrane hyperpolarization, acetylcholine has been shown to markedly shorten the duration of the atrial action potential (88, 99,248). Voltage-clamp experiments on frog atrium (101,170) have indicated that acetylcholine decreases the slow inward current even at concentrations which do not hyperpolarize the membrane. This decrease in the slow inward current contributes to the decreased slope of diastolic depolarization in sinoatrial node cells and partially accounts for the negative inotropic action of acetylcholine (170).

The negative chronotropic effect of acetylcholine is mediated through a muscarinic receptor which in turn modifies the ionic currents discussed above. It is hypothesized that the guanylyl cyclase system plays an important role in changing potassium and calcium conductances through its regulation of intracellular cyclic GMP. This hypothesis has been supported by experiments which have shown that dibutyryl cyclic GMP decreases the spontaneous rate of cultured heart cells as well as attenuating the positive chronotropic actions of epinephrine (211). Taniguchi *et al.* (213) have shown that the concentration of cyclic GMP in the sinoatrial node of the rabbit heart is greater than other regions of the atria and the ventricles. This regional distribution coincides with other studies which conclude that the sinoatrial node region contains the highest density of cholinergic nerve

fibers in the heart (37,113,197).

Sympathetic nerve stimulation or application of catecholamines steepens the slope of diastolic depolarization of both atrial and ventricular pacemaker fibers and thus increases the level of automaticity (91, 93). In some instances, catecholamines also hyperpolarize the maximum diastolic potential, an effect attributable to increased activity of an electrogenic sodium pump (234,170). Furthermore, the positive chronotropic actions of catecholamines are independent of changes in resting membrane conductance or threshold potential (28,170).

Voltage-clamp analysis of the action of epinephrine on Purkinje fiber and sinoatrial node cell automaticity indicate that the positive chronotropic mechanisms differ in these two pacemaker tissues. In Purkinje fibers, epinephrine shifts the activation curve of i_{k_2} in a depolarizing direction (84,224). The net effect is an accelerated inactivation of the pacemaker current and thus a steepening of diastolic depolarization. In sinoatrial node cells, epinephrine enhances automaticity by its actions on the slow inward current. Unlike the pacemaker potential in Purkinje fibers which occurs at potentials more negative to the slow inward current threshold, sinoatrial node cells undergo diastolic depolarization in a voltage range near this threshold (170). Brown and Noble (22) found that epinephrine increases the amplitude of a potassium current, i_x, and greatly enhances the slow inward current presumed to be carried by calcium. Collectively, these current changes account for epinephrine's effects on the sino-

atrial node action potential: shortened duration, increase in the maximum diastolic potential and increased slope of diastolic depolarization and amplitude (170).

The increase in the slow inward current brought about by the interaction of catecholamines with a beta adrenergic receptor is thought to be mediated by the adenylyl cyclase system (211). Specifically, beta adrenergic stimulation increases the synthesis of cyclic AMP (212) which, through a protein kinase step, acts on calcium channels to increase the slow inward current and hence automaticity. This hypothesis is supported by experiments which have shown that the spontaneous rate of sinoatrial node cells increases when dibutyryl cyclic AMP is applied extracellularly or injected intracellularly (260). As is the case for cyclic GMP, the regional distribution of cyclic AMP in the heart is such that the concentration in the sinoatrial node and right atrium is greater than in any other region (212).

3. Afterdepolarizations

A unique form of spontaneous impulse generation which has mechanisms different from automatic activity develops from afterdepolarizations that occur either early or late during the cardiac action potential (43, 44, 45). Early afterdepolarizations in Purkinje fibers generally occur during phases 2 or 3 of the cardiac action potential and represent a transient inhibition of repolarizing current which may reactivate the slow inward current and result in a regenerative second upstroke (44 ,255). This form of spontaneous activity is an

example of the type which is dependent upon a preceding action potential; thus it is said to be triggered (43).

Triggered activity also can develop from late or delayed afterdepolarizations which occur during phase 4 of the cardiac action potential (43). This type of spontaneous activity has been observed experimentally in a number of atrial and ventricular preparations under conditions of increased rate of stimulation (190) and in the presence of increased levels of catecholamines (253,254) or cardiac glycosides (61, 83). Under these conditions, the "triggerable" fibers display a delayed afterdepolarization which, when threshold is reached, initiates a coupled premature action potential or a series of sustained spontaneous beats. As is the case for activity developing from an early afterdepolarization, delayed afterdepolarizations occur only after a preceding action potential, either initiated by an externally applied stimulus or from a propagated impulse generated by an automatic focus (43 ,254). Wit and Cranefield (254) observed triggered activity of the latter type in the canine coronary sinus that developed spontaneously when norepinephrine was superfused. Automatic fibers located outside the ostium of the coronary sinus served as the triggering stimulus to fibers within the coronary sinus. When norepinephrine was applied, the pacemaker site shifted from outside to within the coronary sinus as a more rapid triggered activity dominated.

Clues as to the mechanism of the delayed afterdepolarizations have been provided by voltage-clamp studies analyzing

transient inward currents in the presence of cardiac glycosides (136,125,126). These studies have demonstrated that transient inward currents correlate with transient depolarizations following depolarizing clamps. The amplitude of the transient inward current and hence the transient depolarization is a function of the amplitude and duration of depolarizing clamp pulses in addition to the concentration of extracellular calcium (136,126). Agents which block the slow inward current such as D600, verapamil or manganese ions inhibit the transient inward current (126). It has been hypothesized that this current is due either to a transient increase in sodium or calcium permeability or a phasic release of calcium from an intracellular store which transiently enhances an inward current carried by another ion (126). The norepinephrine-induced delayed afterdepolarization also may be explained by the mechanism determined for cardiac glycosides since norepinephrine is known to increase the slow inward current and hence intracellular calcium concentration (187). Vassalle and Mugelli (238) recently have shown in sheep Purkinje fibers that an oscillatory current or transient inward current is enhanced by any of a number of conditions which increase intracellular calcium. These conditions include lowered extracellular potassium, elevated extracellular calcium, norepinephrine or strophanthidin.

E. EMBRYOLOGY

Studies of the embryologic development of myocardial tissue indicate that the intrinsic heart beat starts very early.

In the chick embryo, the heart begins to beat at a slow rate at approximately thirty hours of incubation. Initial signs of automaticity in the rat and rabbit heart occur at approximately nine and eight and one half days respectively (19). During fusion of the paired cardiac primordia and formation of the cardiac tube a "cephalocaudal gradient" of pacemaker dominance is established beginning at the caudal or ventricular end, proceeding with the atrium and ending with the formation of the sinus venosus (178,230). Fragmentation studies of Patten (178) and others (175, 19) indicate that the embryonic pacemaker progresses in location and rate toward the newest-formed chamber. If ventricular and atrial segments are separated at a stage when ventricular myocardium is complete but atrial myocardium is unfused, the ventricle continues beating at a rate of the intact cardiac tube while the atrium does not beat. When the last portion of the heart has formed, the sinus venosus, the spontaneous rate of the cardiac tube is faster than at earlier stages. If the sinus venosus, atrial and ventricular segments now are separated, the sinus venosus beats at a rate of the intact cardiac tube while the atrial and ventricular segments beat at an intermediate and slower rate respectively (178). Thus there is an hierarchy of pacemaker activity of the cardiac tube such that the intrinsic rate of the caudal-most portion, the sinus venosus, is greater than the atrial portion which is in turn greater than the ventricular tissue.

The cells which ultimately make up the sinoatrial

node in the adult heart originate from the sinus venosus of the embryonic heart (230). While pacemaker potentials have been obtained from cells in the sinoatrial nodal region at early stages of development (229), the sinoatrial node cannot be demonstrated morphologically until much later (230). These findings suggest that the cells of the sinoatrial node are not primitive remains of the embryonic heart (19) but represent special development of a specific region (47,115). This region lies at the right horn of the sinus venosus which ultimately becomes the superior vena cava in the adult heart (178).

The development of pacemaker tissue at the atrioventricular junction is at present uncertain. Patten (178) hypothesized that the atrioventricular node originates from the left horn of the sinus venosus and later migrates to its final position as the sinus venosus shifts to the right. Patten further states that, for a time, the embryonic heart possesses bilaterally symmetrical pacemaking areas, located at the right and left horns of the sinus venosus. This postulate represents the embryonic basis for the role of the atrioventricular junction as a subsidiary pacemaker region. More recently however, Van Mierop and Gessner (229) demonstrated the presence of a unilateral, right-sided sinoatrial node in a mouse embryo (10.5 days gestational age) and found no evidence for pacemaker activity in the left sinus horn.

The embryologic origin of other atrial pacemaking regions is more uncertain than that of the atrioventricular

junction. This may stem from the fact that specialized fibers in the embryo virtually are indistinguishable histologically from working myocardium (230). At present it is believed that specialized conducting tissues in the adult mammalian heart represent remnants of sinoatrial valves present in the embryonic heart (261,164,143). Electrophysiologic studies in the chick heart (142,164) have demonstrated fibers with diastolic depolarization in the musculature of the sinoatrial valves. These valves (right and left) appear to divide the sinus venosus and the atrium (261). In the adult mammalian heart, the most prominent remnant of the right sinoatrial valve is the crista terminalis. At its caudal portion, the remains of the right sinus valve are represented by structures located at the orifice of the inferior vena cava, the Eustachian valve, and at the mouth of the coronary sinus, the Thebesian valve (261). Finally, remnants of the left sinus valve are believed to be found in the interatrial septum (261,115).

Microelectrode recordings from specialized tissues in the adult right atrium have indicated that fibers possessing pacemaker potential are located frequently at the junctions of cardiac structures where embryonic valves were once present (6 , 72). Such fibers have been identified along the crista terminalis (173, 174, 48 , 192, 49 , 95) and at the mouth of the coronary sinus (254) which are believed to be remnants of the right sinus valve (261). Also, evidence for pacemaker activity near Bachmann's bundle (74, 120, 121) co-

incides with the development of this region from an embryonic structure known as the septum spurium which is formed by the fusion of the right and left valves of the sinus venosus (178, 143).

F. HISTOLOGY

Histological examination of atrial tissue under the light microscope reveals an heterogeneous collection of cells differing in size and staining characteristics. Cells of the sinoatrial node appear as slender fusiform muscle fibers which form an interweaving network within a collagen frame (73,222, 112,118). These cells are smaller than working atrial myocardial cells (about 5-8 μm in diameter), contain fewer myofibrils, a disproportionately large nucleus and exhibit a paler staining characteristic that is the basis of the term P cell often used to describe them (111). The fibers of the sinoatrial node in the dog and man are organized about a central artery which prompted Soderstrom (208) to describe the node as resembling an enormous adventitia of this artery. In other animals such as the rabbit (112) and guinea pig (5) no central artery is present.

Transitional cells are a second type of nodal cells which are found between P cells and working atrial cells. These are larger than P cells (10-12 μm in diamater) but smaller than ordinary atrial cells and contain more myofibrils which are oriented in a more organized fashion (118,102). The working atrial cells are relatively large and contain more

myofibrils which are oriented in a longitudinal array (156). As the atrioventricular node is approached, the atrial fibers become smaller and bear many similarities to cells of the sinoatrial node (47,223). Also, P cells have been found in the atrioventricular node which have similar histological characteristics and relations to transitional cells as in the sinoatrial node (113).

Electron microscopic studies recently have provided a more detailed distinction between the many cell types in the atrium. Sherf and James (203,204) studying the fine structure of cells in the Eustachian ridge and Bachmann's bundle have described six cell types distinct from one another electron microscopically. The most prevalent cell type, the working myocardial cell, contains densely packed myofilaments highly organized in a longitudinal fashion. The specialized cellular junctions observed in ventricular tissue, the desmosome, fascia adherens and nexus also are observed between working atrial cells (156,209).

In contrast to cells which are rich in myofibrillar content are fibers which resemble Purkinje fibers of the ventricular myocardium. These myofibril-poor cells have few, randomly oriented myofilaments, a clear cytoplasm with large amounts of glycogen and few mitochondria (203,204). These fibers, particularly those located toward the endocardium, lack a T system (209) and are believed to be part of internodal pathways (113,161,241,203,204) even though they are dispersed and are not found in discrete tracts as in ventric-

ular tissue (204,209).

P cells have been observed not only in the sinoatrial node region (114,241,223,118) but in extranodal regions of the Eustachian ridge and Bachmann's bundle (203,204) as well as in the atrioventricular node (114). These cells generally are small and have a clear or pale cytoplasm due to few myofibrils. In the sinoatrial node of the dog, P cells are found in grape-like clusters surrounded by a single basement membrane (118). Structure-function studies in the rabbit (221,214) and dog (256) sinoatrial node indicate that P cells are the true pacemaker fibers of the heart. Although no intercalated discs are observed under the light microscope, specialized cellular junctions have been observed between P cells (241,204,209). In general, few nexi are present and desmosomes and fascia adherens are less frequent in occurrence than in working atrial cells (127,203,204,209). The fewer number and smaller size of intercellular connections may account for the slow conduction within the sinoatrial node (102). It has been estimated (15) that nexi represent 0.2 percent of the rabbit sinoatrial node cell surface area which is about 10 times less than in working myocardial cells.

While it is generally agreed that P cells in the sinoatrial node are the true pacemaker cells of the heart, it is not known what cell type is responsible for subsidiary atrial pacemaker activity. It is tempting to conclude that extranodal P cells such as those found in the Eustachian ridge and Bachmann's bundle are the basis for subsidiary rhythms (203).

However, action potentials resembling sinoatrial node cells have not been demonstrated in extranodal sites. Others have postulated that specialized conducting fibers associated with internodal pathways are responsible for subsidiary pacemaker activity (173, 94,154). The " plateau fiber" described by Hogan and Davis (94) was shown to depolarize spontaneously under catecholamine influence. Although no structure-function relationships have been made for the plateau fiber, it is likely these fibers are the myofibril-poor cells described by Sherf and James (203,204). It remains to be determined whether subsidiary atrial pacemaker activity is the result of electrical activity of cells similar to those found in the sinoatrial node (P cells) or specialized conducting cells resembling ventricular Purkinje fibers (plateau fibers or myofibril-poor cells).

G. ARTERIAL SUPPLY OF THE RIGHT ATRIUM

The arterial blood supply to the mammalian right atrium originates from major branches of the right and/or left coronary arteries (160, 77,108). In the dog, the sinoatrial node region and much of the right atrium is supplied by a major branch of the right coronary artery in 80-90 percent of hearts and by branches of the left coronary artery in the remainder (160, 77,108). The major atrial branch of the right coronary artery is referred to as the ramus atrialis dexter intermedius in the earlier literature (160) but because of its distribution to the sinoatrial node, it is frequently

termed the sinoatrial node artery in contemporary studies (110,148). In man, the sinoatrial node and right atrium are supplied by a major branch of the right coronary artery in about 55 percent of hearts and by a branch of the left circumflex artery in about 45 percent (109, 98,118). Venous drainage of the atrial myocardium is by short Thebesian channels which empty directly into the right atrium (118, 4).

In the dog heart, the sinoatrial node region often is perfused by arteries which originate from both the right and left coronary arteries (160, 77). Numerous arterial anastomoses have been observed histologically (77) which explain why, under in vivo conditions, it is difficult to suppress sinoatrial node activity by ligating the sinoatrial node artery (107, 11). The interarterial anastomoses also account for the substantial retrograde pressure (between 30-50 mmHg) measured from the sinoatrial node artery (110, 81). However, under in vitro conditions in which sinoatrial node activity is maintained by perfusion through the cannulated sinoatrial node artery, ligation of this artery or cessation of perfusion results in marked suppression of activity (30,256).

Studies assessing the distribution of the sinoatrial node artery using dyes (78, 79), contrast medium (160, 98) or radioactive microspheres (251) agree that the distribution is not discrete but is widespread. It has been suggested by Hutchinson (98) that the large size of this artery probably is not related to the metabolism of the nodal tissue but rather to the considerable volume of the surrounding myocardium it

supplies. Hardie et al. (78 , 79) found that dyes injected into the sinoatrial node artery extend well beyond the sinoatrial node region and include the atrial sections of the superior and inferior vena cavae and portions of the interatrial septum. They also found that the dye-distribution patterns averaged 44 percent of the total endocardial surface but did not include the atrioventricular junction which is supplied by its own nutrient artery (226,228,166,119,167).

CHAPTER III

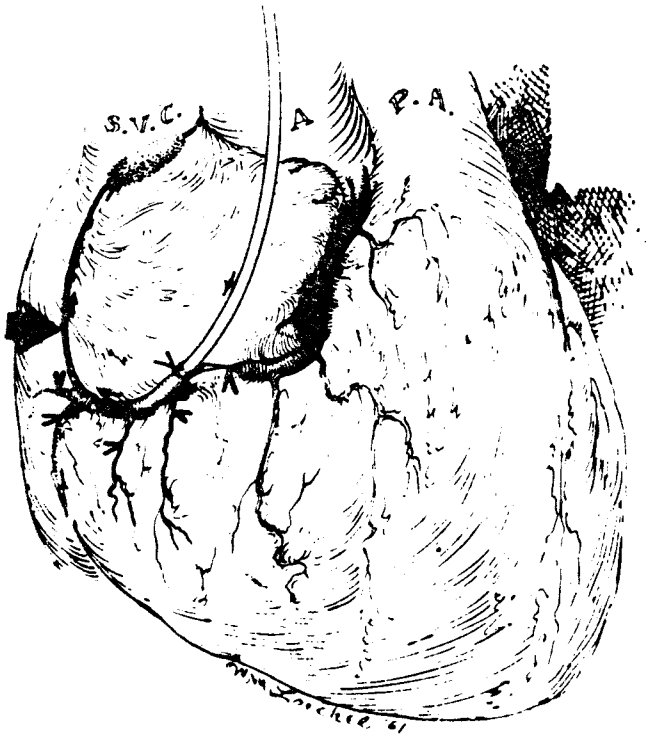
METHODS

A. SINOATRIAL NODE ARTERY CANNULATION *IN VIVO*

Mongrel dogs of either sex weighing from 13 to 20 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and maintained by positive pressure respiration (Bird, Mark 7). A right thoracotomy was performed through the fourth intercostal space and the heart suspended by a pericardial cradle. Atria to be used for *in vitro* perfusion were selected for the presence of a major branch of the right coronary artery leading to the sinoatrial node region; the sinoatrial node artery. This anatomical criterion was met in 57 of 74 hearts, the remaining hearts being used in another capacity (to be discussed). The cannulation of the sinoatrial node artery was carried out using the technique described by James and Nadeau (108) and is represented schematically in Figure 1. A 2 cm segment of the right coronary artery was isolated near the origin of the sinoatrial node branch and ligatures placed both proximal and distal to this point. A fluid-filled cannula (PE 50, Clay Adams) was introduced into the proximal right coronary artery, advanced into the first 1 cm of the sinoatrial node artery and tied in place. The

FIGURE 1

IN VIVO CANNULATION OF THE SINOATRIAL NODE ARTERY



Cannulation of the sinoatrial node artery was carried out by inserting a fluid-filled polyethylene tube into the right coronary artery and advancing it into the first 1 cm of the sinoatrial node artery. The position of the tip of the cannula is shown by the large arrow. (From James and Nadeau (108)).

distribution to the sinoatrial node region was assessed visually and was tested for the occurrence of injection bradycardia (110,81). The injection of 0.5 ml of indocyanine green (cardio-green, H.W.D., Inc.) was used to ascertain the arterial distribution in cases where visual inspection and the test for injection brady cardia proved inconclusive (79).

B. DISSECTION AND MOUNTING

Following cannulation, the heart with at least 2 cm of each vena cava attached was quickly excised and immersed in room temperature Tyrode's solution equilibrated with 95% O_2, 5% CO_2 (Ohio Medical). The composition of the Tyrode's solution used in all experiments is shown in Table 1. During dissection, the sinoatrial node artery was perfused with Tyrode's solution at 2-4 ml/min by a variable speed roller pump (Cole Parmer). Ventricular tissue was removed by a cut made 1-2 cm below the atrioventricular groove (coronary sulcus). The right atrium was then opened by an incision made along the tricuspid valve and up along the superior vena cava. The coronary sinus was removed as was all left atrial tissue up to the interatrial septum. The resultant preparation thus consisted of the anterior free wall of the right atrium including portions of each vena cava and was devoid of the coronary sinus. A small rim of right ventricular tissue containing the isolated right coronary artery was included also. The isolated right atrium was then trans-

TABLE 1

COMPOSITION OF TYRODE'S SOLUTION (mM)

NaCl	-	137
KCl	-	2.7
$NaHCO_3$	-	11.9
NaH_2PO_4	-	0.33
$CaCl_2$	-	1.8
$MgCl_2$	-	1.05
Glucose	-	11.0

pH - 7.37 ± .02 (n=13)*

Osmolarity - 297.1 ± 1.9 mOsm/L (n=5)**

*Measured by Beckman pH meter

**Measured by freezing point method (Advanced Instruments Osmometer, Model 3L)

ferred to a plexiglass chamber (capacity - 200 ml) and pinned to the Sylgard (Dow Corning) floor epicardial side up.

C. IN VITRO PERFUSION

The perfusion cannula was switched to a second pump which delivered oxygenated Tyrode's solution at a PO_2 of 550 ± 25.7 mm Hg (Instrumentation Laboratories) to the tissue from a reservoir. The conditions that were established to maintain pacemaker activity in this preparation were similar to those used in related studies by Woods et al. (256,257) and Chiba et al. (30). The perfusate was passed through a water-heated thermostatic coil the temperature of which was set to yield a tissue temperature of 36 ± 1°C (YSI Model 47 Tele-thermometer) as monitored by an implantable thermistor. Perfusion pressure was measured by a transducer (Statham P23 Gb) from a T-junction located in the perfusion line and was displayed on a polygraph (Grass Model 7). The perfusion flow was maintained at a constant rate (4.7 ± 0.1 ml/min) which was set at the beginning of each experiment to yield a perfusion pressure of 100 mm Hg. In addition to tissue perfusion, the atria were superfused with Tyrode's solution at a flow rate of 10-12 ml/min. The superfusate was warmed by passing through coiled polyethylene tubing located in a temperature-controlled water bath beneath the tissue chamber. The PO_2 of the superfusate as sampled near the tissue was 386 ± 21.2 mm Hg. The level of Tyrode's

solution in the tissue chamber was maintained constant by a curved glass aspiration tube that continuously removed excess solution and emptied it into a large collection bottle.

To test whether the perfusion and superfusion conditions adequately met the metabolic requirements of the tissue, adenosine (3.7×10^{-4}M) was perfused continuously in five preparations while pacing above the spontaneous rate. This adenosine concentration was found to produce maximal vasodilation of the perfused vasculature as indicated by the maximal drop in perfusion pressure. Changes in vascular resistance due to adenosine were calculated from known values of pressure and flow. A decrease in vascular resistance was interpreted as indicating that the arterial vasculature was not maximally vasodilated as would be expected if the tissue was hypoxic.

The area of the right atrium perfused by the sinoarterial node artery was assessed qualitatively in five atria using Microfil (Canton Bio-Medical Products) injection techniques. At the end of the experiment, a silicone rubber compound was injected manually through the cannulated sinoatrial node artery at a rate of approximately 0.1 ml/sec. The injection mass was allowed to cure overnight and the tissue treated by a sequential series of ethyl alcohol dehydration steps. A final immersion in methyl salicylate (Sigma) cleared the tissue allowing the distribution of the vasculature to be visualized easily.

D. EXTRACELLULAR MAPPING

Extracellular recordings were used to quantitatively localize the site of earliest activation (SEA) in the presence and absence of sinoatrial node activity. The technique employed was a modification of the procedure first introduced by Lewis et al. in 1910 (141) and used in a number of recent studies (69, 74,120). The timing of epicardial activation was obtained with the use of contiguous bipolar silver electrodes (interpole distance - 0.6 mm) insulated except at the tip. Electrode holders were fashioned from curved glass capillary tubes that provided rigid support and enabled fine adjustment of electrode placement. Three reference electrodes were positioned at fixed points in the rostral, mid and caudal regions of the sulcus terminalis. Each electrode lightly touched the surface of the tissue and was oriented with the lead axis parallel to the direction of the sulcus terminalis. A fourth bipolar electrode, mounted on a micromanipulator, served as a mobile probe allowing the recording of electrical activity from any epicardial site.

All four electrograms were amplified (Grass Dual P9 AC pre-amplifier) and displayed simultaneously on a storage oscilloscope (Tektronix 5103N) to be photographed (Tektronix C-5). Pre-amplifier filter settings at 10 Hz and 40 kHz yielded the sharpest electrogram deflections with minimal baseline fluctuation. The output from the caudal reference electrode also triggered a tachograph (Grass Model 7P448) whose poly-

graph display provided a continuous record of spontaneous rate.

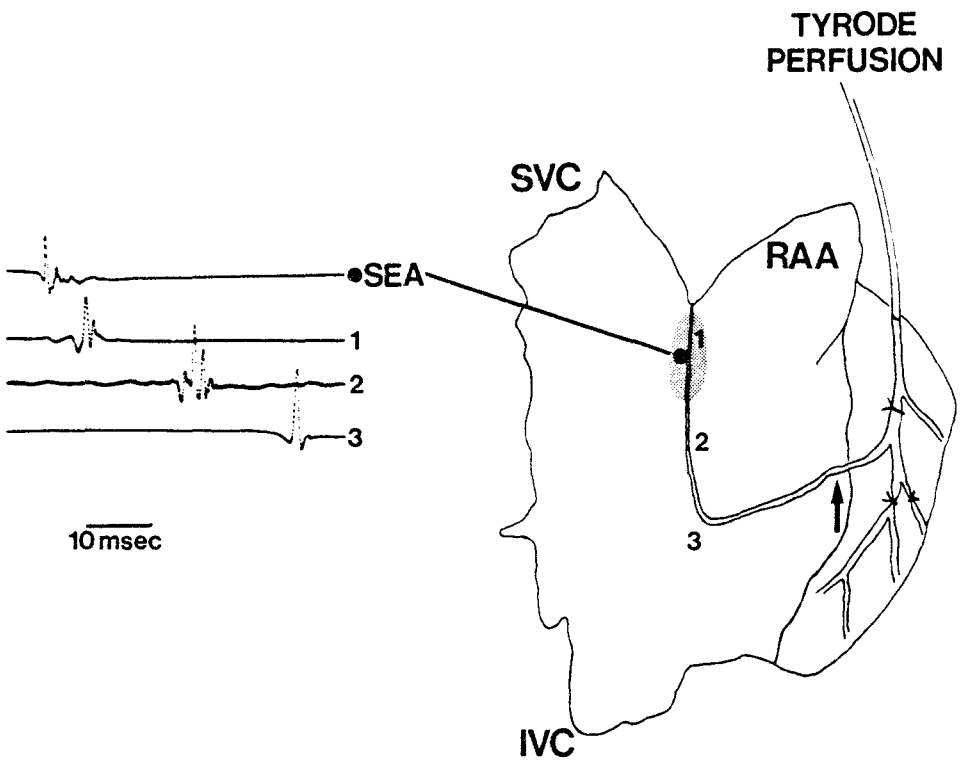
The mobile electrode scanned the surface of the preparation to locate that site which depolarized prior to the regions beneath the reference electrodes. The site of earliest activation was defined as that location in which the mobile electrogram preceded the reference electrograms by the greatest time interval as measured from the initial rapid deflection in each recording. Movement of the mobile electrode by as little as 2 mm in any direction from the site of earliest activation attenuated the maximal time interval. It was assumed that the electrode recording the earliest depolarization was closest to the origin of pacemaker activity. An example of the mapping procedure used to locate the control site of earliest activation is shown in Figure 2.

E. ELICITING SUBSIDIARY ATRIAL PACEMAKER ACTIVITY

The *in vitro* perfusion of the right atrium through the sinoatrial node artery eliminated the *in vivo* source of collateral perfusion from the left circumflex artery (118). A consequence of this condition was that automaticity was dependent solely upon one controlled source of perfusion. Interruption of perfusion led to suppression of all spontaneous activity within minutes (Figure 3). This property was applied to suppress selectively sinoatrial node automaticity and elicit or unmask subsidiary atrial pacemaker activity. A 1-3 mm length of the sinoatrial node artery was isolated proximal to the sino-

FIGURE 2

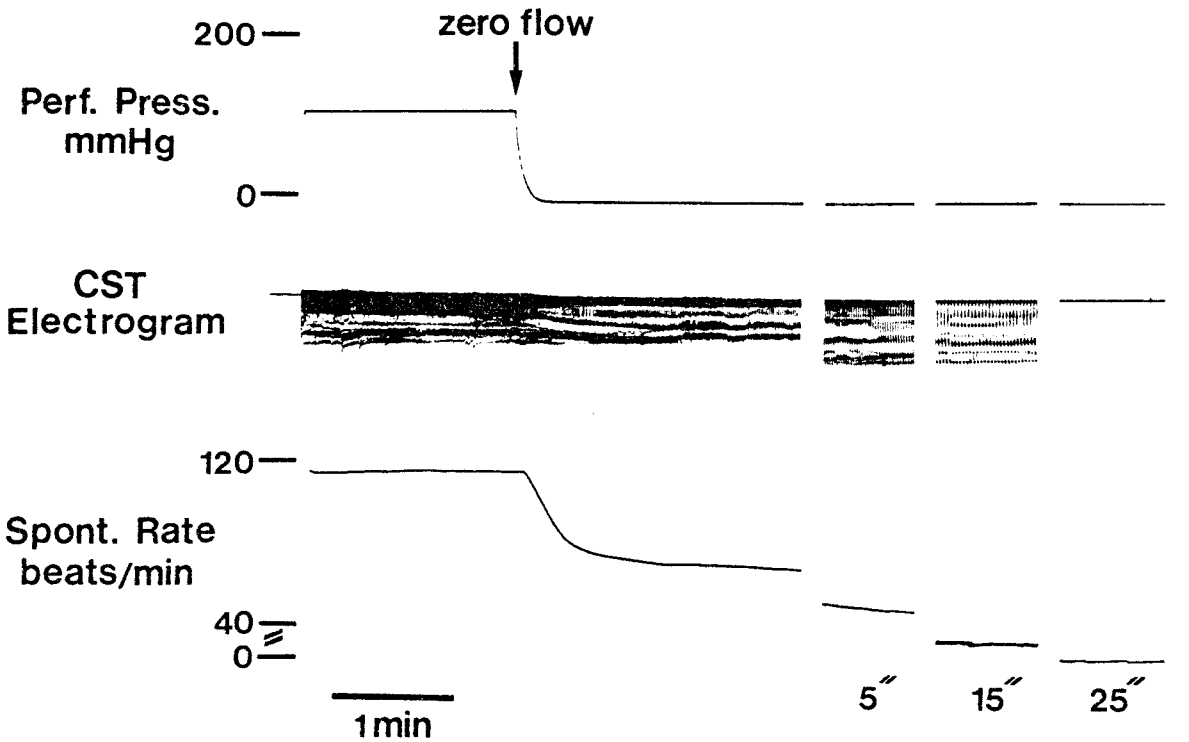
EXTRACELLULAR MAPPING OF THE CONTROL SITE OF EARLIEST ACTIVATION



In 93% of the perfused atria, the control site of earliest activation (SEA) was located within the sinoatrial node region (stippled area). The panel at the left demonstrates that in this example, the site beneath the mobile electrode (represented by the filled circle) depolarized before the three stationary reference sites. SVC=superior vena cava, IVC=inferior vena cava, RAA=right atrial appendage. The arrow marks the tip of the perfusion cannula.

FIGURE 3

SUPPRESSION OF SINOATRIAL NODE SPONTANEOUS ACTIVITY BY STOPPING PERFUSION



Top trace: perfusion pressure. Middle trace: electrogram recording from the caudal sulcus terminalis (CST) reference electrode. Bottom trace: tachograph display (triggered from CST electrogram) of spontaneous rate. At the arrow, perfusion was stopped and the spontaneous rate declined gradually until at 25 minutes, activity had ceased entirely.

atrial node region and thus the control site of earliest activation. Ligation of the artery at this point with fine silk thread resulted in a continuous decline in the sinoatrial node spontaneous rate until a subsidiary atrial pacemaker emerged (under specific conditions to be discussed in Results) from regions below the ligature which still were perfused. Thus, the ligation procedure allowed the study of subsidiary atrial pacemaker activity independent of sinoatrial node influences.

The isolation of the sinoatrial node artery for the purpose of ligation introduced the possibility that injury currents due to tissue damage could have contributed to the development of subsidiary atrial pacemaker activity. To minimize this possibility, subsidiary atrial pacemaker activity was studied only when the site of earliest activation was located greater than 6 mm or four space constants (191) away from the ligation site.

F. PACEMAKER CHARACTERIZATION

1. Chronotropic Response to Neuromediators

The chronotropic response of sinoatrial node and subsidiary atrial pacemaker activity to neuromediators was tested by perfusing varying concentrations of either norepinephrine or acetylcholine for brief time periods. The switch from control to test perfusate solution, for the desired exposure time, was done by way of a three-way stopcock. The minimal duration of exposure to test solutions which produced the greatest chron-

otropic response was determined to be 30 seconds or 1 minute depending upon whether norepinephrine or acetylcholine was perfused. For a given neuromediator, all test solutions were perfused in random order allowing time for complete recovery of the spontaneous rate between test periods. When autonomic blocking agents were administered, they were perfused continuously for at least 15 to 30 minutes and the block tested with the appropriate agonist. A change in the spontaneous rate of not more than 5 beats/min was taken as an indication of total blockade.

2. Chronotropic Response to Overdrive Pacing

Another functional property of pacemaker activity tested was the chronotropic response to external pacing at rates faster than the inherent spontaneous rate, i.e. overdrive pacing.

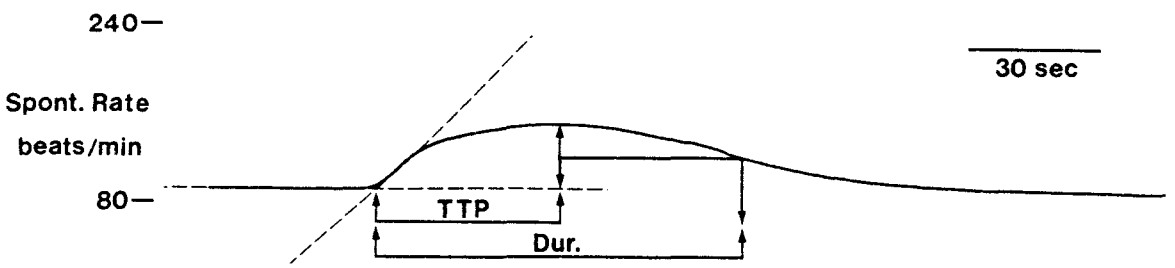
Rectangular pulses 2 msec in duration and 50% above threshold voltage were applied through bipolar pin electrodes from an isolated external stimulator (Pulsar 4i, Frederick Haer). The stimulating electrodes were placed on the inferior vena cava in all experiments. Both sinoatrial node and subsidiary atrial pacemaker activities were driven at 100, 150 and 200% above their respective steady state spontaneous rates for periods of 0.5, 1 and 2 minutes.

3. Measured Parameters

Figure 4 schematically defines the measurements made from the tachograph recording of spontaneous rate in response to a 30 second bolus of norepinephrine. Qualitatively similar

FIGURE 4

MEASURED PARAMETERS OF THE CHRONOTROPIC RESPONSE TO NEUROMEDIATORS



This figure shows a continuous tachograph trace of the chronotropic response of sinoatrial node activity to a 30 second bolus of norepinephrine. Similar traces were obtained for subsidiary atrial pacemaker activity. In addition to the peak or maximum response, the time to peak (TTP) response was measured from the onset of the effect to the peak value. The duration (Dur.) of the response was measured from the onset to where the spontaneous rate recovered by 50%. Qualitatively similar parameters were obtained for the negative chronotropic effects of acetylcholine.

parameters were measured for the negative chronotropic response to acetylcholine. The peak response was measured directly from the calibrated tachograph trace displayed on ruled polygraph paper. The time to peak response (TTP) was obtained by measuring the time of onset of the chronotropic effect to when the effect reached its peak. The onset of the response was taken from the intersection of lines drawn through control and through the steepest slope of the curve. The duration of the response was measured from the onset to the point where spontaneous rate had recovered from the peak response by 50%.

The amount of suppression following the various periods of overdrive pacing was measured directly from the polygraph traces of the reference electrogram that triggered the tachograph. A corrected recovery time (CRT) was obtained by subtracting the control cycle length (in seconds) from the duration of pacemaker suppression as measured from the last driven beat to the first return spontaneous beat.

G. DISSECTION AND MOUNTING OF TISSUE SEGMENTS FOR MICROELECTRODE STUDY

Endocardial mapping during subsidiary atrial pacemaker activity in ten preparations indicated that the site of earliest activation is associated frequently with a distinct region caudal to the crista terminalis. This region is characterized by a pale or whitish appearance distinguishable from the surrounding working atrial myocardium which is pink in

color. Tissue segments containing these pale regions were excised from 17 atria in which subsidiary atrial pacemaker activity had not been elicited. These included 11 of 17 atria that did not possess a sinoatrial node artery of right coronary artery origin. Tissue segments also were obtained from ten perfused preparations in which subsidiary atrial pacemaker activity had been elicited by the ligation procedure described previously. These segments contained at their centers the subsidiary site of earliest activation determined by extracellular mapping.

All isolated tissues (approximately 1.5 cm^2) were pinned to the Sylgard floor of a 5 ml capacity bath endocardial side up. Preparations not exhibiting spontaneous activity were paced externally at 30 or 60 beats/min with rectangular pulses, 2 msec in duration and 50% above threshold voltage, delivered through bipolar pin electrodes. Tyrode's solution warmed to 36 ± 0.5°C and equilibrated with 95% O_2, 5% CO_2 (PO_2 = 507 ± 22.5 mm Hg) was superfused continuously at 7-10 ml/min.

In four preparations, transmural strips of sinoatrial node tissue 1 to 1.5 mm thick were isolated and maintained in the same manner as subsidiary atrial pacemaker tissue. The strips were positioned flat on one side and multiple microelectrode impalements made transmurally.

H. RECORDING TRANSMEMBRANE ACTION POTENTIALS

1. Instrumentation

Transmembrane action potentials were recorded with machine-pulled (David Kofp Instruments, Model 700C) glass capillary microelectrodes (tip diameter $<1\ \mu m$) filled with 3M KCl solution. The voltage signal obtained from the recording electrode was referenced to a second electrode placed in the solution superfusing the preparation. Each electrode was coupled by a Ag - AgCl wire to a follower amplifier with capacity neutralization (Picometric 181, Instrumentation Laboratories) whose output led to a differential amplifier. The signal was displayed on a storage oscilloscope (Tektronix 5103 N) used to monitor continuously the recorded signal plus a non-storage oscilloscope (Tektronix 561 A) used for photographing. The maximum rate of rise of the action potential upstroke was electronically differentiated and displayed on both oscilloscopes.

Displayed signals of both extracellular and intracellular recordings were photographed on 35 mm film from the non-storage oscilloscope with a kymograph camera (Nihon-Kohden, Model PC-2A). Signals also were photographed directly from the screen of the storage oscilloscope with a Polaroid camera (Tektronix C-5). Microelectrode resistance (10 - 30 Mohms) was tested during the course of an experiment with an electronic circuit that measured the potential drop across the microelectrode. A 50 mV pulse was applied across a 5 Mohm resistor in series with the microelectrode resistance. The

measured voltage drop across the microelectrode was displayed on the storage oscilloscope and resistance calculated from this signal.

At the conclusion of each experiment, square wave and ramp pulses (Wavetek, Model III) were applied to the recording apparatus to calibrate voltage and differentiated signals respectively.

2. Cellular Characterization

Multiple impalements were obtained in each experiment to record the cellular electrical characteristics of cells in the subsidiary atrial pacemaker region. In some spontaneously active tissues, mapping was carried out using a silver bipolar electrode as a fixed reference. In this procedure, the microelectrode recorded from a number of different sites to identify electrophysiologically the cellular region where activity preceded the bipolar electrogram by the greatest time interval.

3. Histology

Histological identification of subsidiary atrial pacemaker tissue was carried out on segments excised from three perfused right atria documented to possess subsidiary atrial pacemaker activity. Immediately after microelectrode recordings had been obtained, these tissues were fixed in 10% formalin and later embedded in paraffin. Serial sections 6 μm thick were mounted on glass microscope slides and treated with either Masson's trichrome or hematoxylin and eosin stain for light microscope examination.

4. Measurement of Cellular Electrical Properties

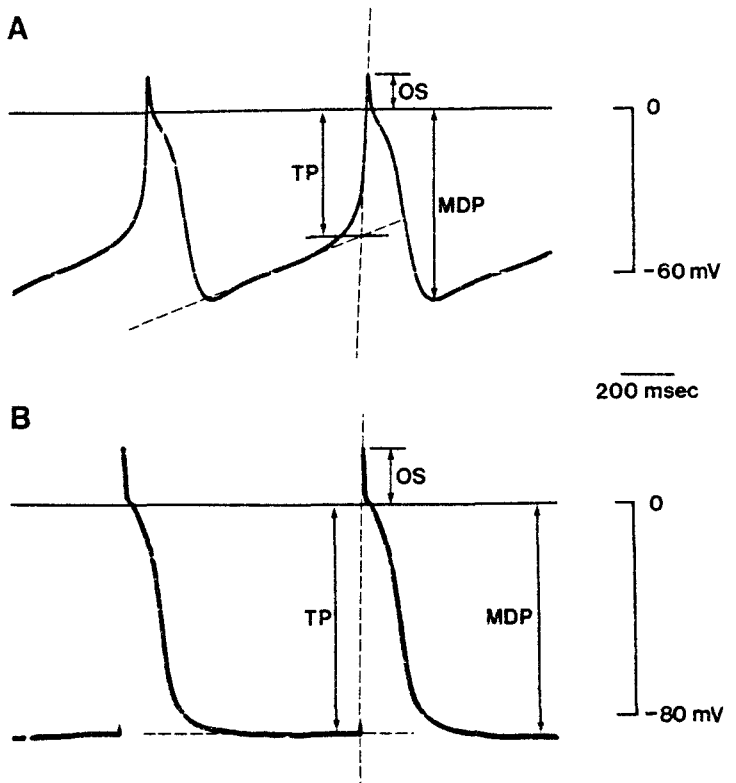
To characterize the electrical properties of fibers in the subsidiary atrial pacemaker region, various parameters were measured from enlarged action potentials recorded on 35 mm film. The parameters which were measured are defined schematically in Figure 5. Maximum diastolic potential (MDP) is defined as the level of maximum negativity attained during the course of the action potential. The overshoot (OS) was measured from the "zero" or reference potential to the peak of the action potential. The take-off potential (TP) was used as an estimate of the threshold potential. This value was obtained by measuring the potential (referenced to the zero potential) at the point of intersection of two lines: one drawn through the linear portion of the diastolic slope and the other through the steepest portion of the upstroke. The amplitude of the action potential was obtained by adding the absolute values of the maximum diastolic potential and overshoot. Finally, the slope of diastolic depolarization was measured from the slope of the line drawn through the linear portion of the diastolic slope.

I. PREPARATION OF SOLUTIONS

All solutions used in this study were prepared fresh, prior to each experiment, in Tyrode's solution. Desired concentrations of acetylcholine (acetylcholine chloride, Sigma) were prepared from cold stock solutions to reduce the hydrolysis that occurs at warm temperature. To minimize oxidation,

FIGURE 5

MEASUREMENT OF ACTION POTENTIAL PARAMETERS



Panel A: pacemaker fiber. Panel B: working atrial fiber. OS = overshoot, TP = take-off potential, MDP = maximum diastolic potential.

norepinephrine (Levophed bitartrate, Breon) solutions were prepared in Tyrode's solution containing 6 X 10^{-5}M ascorbic acid (Eastman). This ascorbic acid concentration effectively acts as an antioxidant while not altering pH (246). Other drugs used in this study were: atropine sulfate (Sigma), eserine salicylate (Sigma), methoxamine hydrochloride (Vasoxyl, Burroughs Wellcome), phentolamine (Regitine HCl, Ciba-Geigy), DL-propranolol hydrochloride (Sigma) and tetrodotoxin (Sigma).

J. DATA ANALYSIS

All data values were expressed as a mean plus or minus the standard error of the mean ($\bar{x} \pm$ SEM). Statistical comparisons of two groups, either correlated or uncorrelated, were carried out using the appropriate student's t-test. When the analysis called for a comparison of more than two experimental groups, one-way analysis of variance was used. Differences were considered statistically significant at a $p < .05$ (194, 242).

CHAPTER IV

RESULTS

A. ISOLATED PERFUSED RIGHT ATRIUM

The use of the isolated right atrium to study pacemaker activity was contingent upon the presence of a major atrial artery having its origin at the right coronary artery. In 57 of 74 (77%) hearts used in this study, the major atrial artery emanated from the right coronary artery, passed transversely and bent sharply in a rostral direction coursing along the sulcus terminals to the sinoatrial node region. The *in vivo* spontaneous rate of these hearts, 163.6 ± 3.5 beats/min, did not change significantly after artery cannulation, 153.5 ± 3.3 beats/min ($p > .05$). However, injection of a small volume of Tyrode's solution through the cannula induced bradycardia in 98.2% of the hearts, verifying the vessel as the sinoatrial node artery. In 10 hearts (13.5%) no sinoatrial node artery was observed while in 7 (9.4%) an artery was cannulated which perfused the inferior right atrium but not the sinoatrial node region as indicated by the lack of injection bradycardia.

1. *In Vitro* Perfusion

Under constant flow conditions (4.7 ± 0.1 ml/min)

at an initial perfusion pressure of 100 mmHg, the spontaneous rate of the isolated perfused right atrium after equilibration for one half to one hour was 82.9 $\pm$ 1.3 beats/min (n=55). The study of subsidiary atrial pacemaker activity (to be discussed) was conducted in the presence of a modified Tyrode's solution containing 10^{-8} M norepinephrine. Therefore, to compare the activities of the sinoatrial node and subsidiary atrial pacemakers under identical conditions, modified Tyrode's solution was perfused and superfused continuously during the equilibration period. This significantly increased the control spontaneous rate to 101.5 $\pm$ 1.9 beats/min ($p < .0005$).

The extracellular mapping procedure (Figure 2) used to estimate pacemaker location indicated that the control site of earliest activation was within the sinoatrial node region in 93% of the perfused atria. Specifically, these sites ranged from 2 to 20 mm caudal to the junction of the superior vena cava and right atrium which corresponds to the anatomical site of the canine sinoatrial node determined histologically (118, 14). Under steady state conditions, the control site of earliest activation and spontaneous rate remained stable for several hours. In seven preparations, the spontaneous rate was measured at various times for a period of four hours. There was no significant difference in the steady state spontaneous rate at the beginning of the experiment, 103.6 $\pm$ 3.1 beats/min, compared to the rate at four hours, 105 $\pm$ 2.7 beats/min ($p > .20$).

The injection of Microfil into the sinoatrial node artery of five atria indicated that the distribution of this artery was widespread. Figure 6 demonstrates a typical arterial distribution pattern as viewed from the epicardial surface. As in other studies (251, 79), the distribution was found to include not only the sinoatrial node region but the atrial portions of the superior and inferior vena cavae as well. The endocardial view of the distribution (not shown) incorporated the same regions as those seen epicardially and also showed vessels extending into the right side of the interatrial septum.

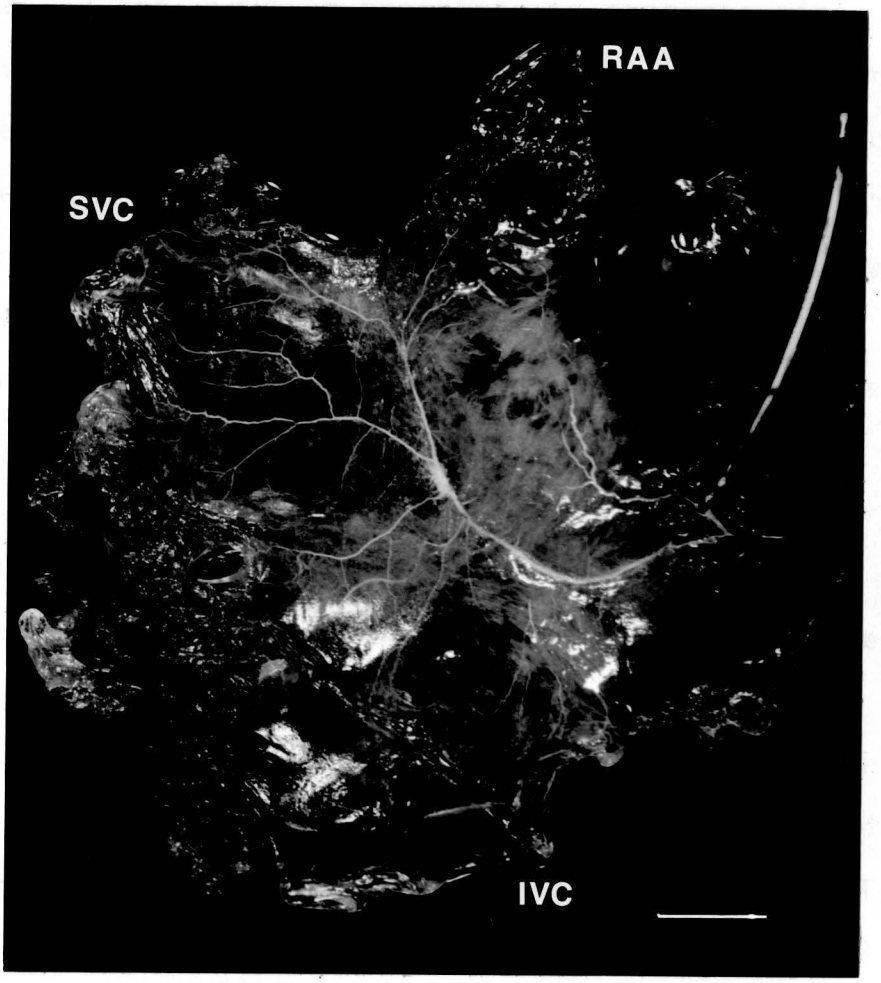
The response of the vasculature to continuously perfused adenosine (3.7×10^{-4}M) was vasodilation in all five atria tested. Vasodilation was indicated by a fall in perfusion pressure while maintaining flow at a constant rate. Resistance decreased by 7.4 ± 3.7 mmHg/ml/min which corresponded to a 17.7 ± 5.8% change. This change in resistance indicated that the vasculature of the preparation was not maximally vasodilated suggesting that the tissue was not hypoxic.

2. Subsidiary Atrial Pacemaker Model

As indicated in Figure 3, the activity of the sinoatrial node was dependent upon a continuous perfusion of Tyrode's solution through the sinoatrial node artery. When the sinoatrial node artery was ligated from 3 to 24 mm proximal to the control site of earliest activation, the activity of the sinoatrial node gradually declined until subsidiary

FIGURE 6

DISTRIBUTION OF THE SINOATRIAL NODE ARTERY



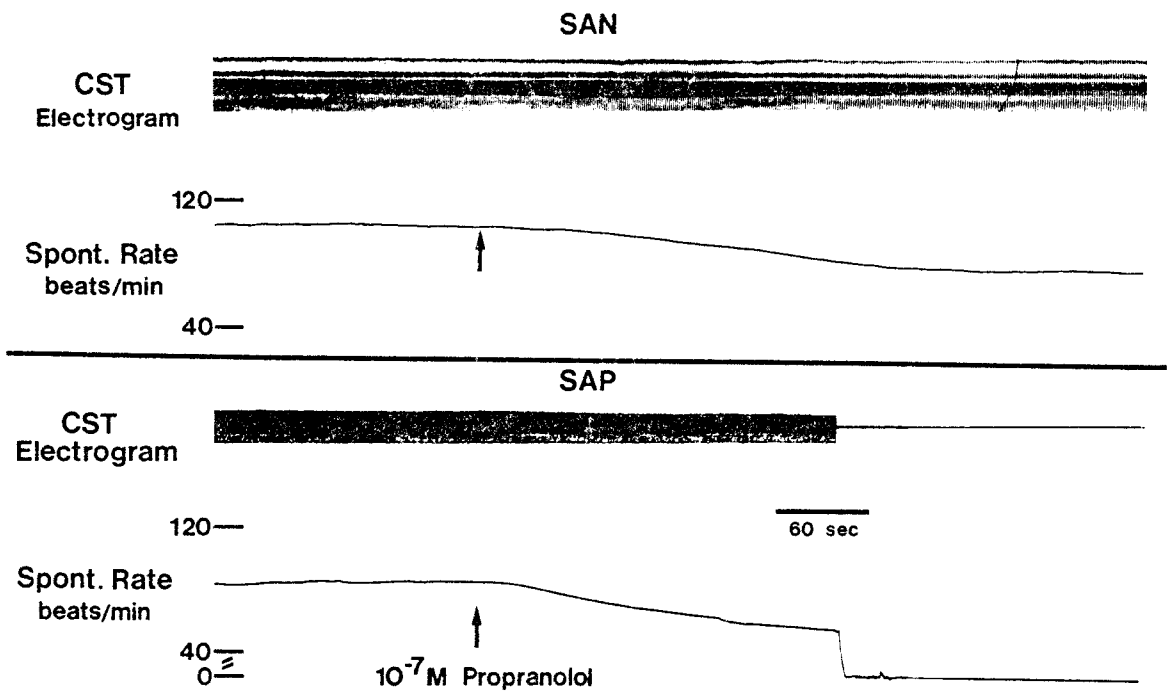
This figure shows an epicardial view of the Microfil distribution pattern after tissue dehydration and clearing in methyl salicylate. The light areas represent regions perfused by the sinoatrial node artery. RAA = right atrial appendage, SVC = superior vena cava, IVC - inferior vena cava. Calibration = 1 cm.

atrial pacemaker activity became manifest. Preliminary experiments using the ligation procedure to suppress sinoatrial node activity indicated that the frequency of occurrence of subsidiary atrial pacemaker activity was markedly increased when the perfusate was enriched with 10^{-8} M norepinephrine. As a result of this finding, all experiments were conducted in the continuous presence of 10^{-8} M norepinephrine. Under this condition, ligation was successful in unmasking subsidiary atrial pacemaker activity in 36 of 49 (73.5%) preparations. In addition, 4 of 7 atria that had an inferior right atrial artery not perfusing the sinoatrial node developed subsidiary atrial pacemaker activity in the presence of background norepinephrine without having to ligate.

The dependence of subsidiary atrial pacemaker activity on a background level of norepinephrine was tested in 26 preparations either by perfusing norepinephrine-free Tyrode's solution (n=17) or propranolol (1 - 3 X 10^{-7} M) plus 10^{-8} M norepinephrine (n=9). Figure 7 compares the effects of perfusing 10^{-7} M propranolol (in the presence of background norepinephrine) on sinoatrial node (top panel) and subsidiary atrial pacemaker activity (bottom panel). The arrow in each panel marks the onset of propranolol perfusion. In the case of sinoatrial node activity, propranolol caused a gradual decline in spontaneous rate which leveled off to a new steady state value. For subsidiary atrial pacemaker activity however, the same concentration of propranolol resulted in a decline of spontaneous rate followed by an abrupt ces-

FIGURE 7

DEPENDENCE OF SUBSIDIARY ATRIAL PACEMAKER ACTIVITY ON BETA ADRENERGIC STIMULATION



The top panel demonstrates the response of sino-atrial node (SAN) activity to continuous perfusion of propanolol (10^{-7}M) in the presence of 10^{-8}M norepinephrine. The bottom panel shows the effects of propranolol plus background norepinephrine on subsidiary atrial pacemaker (SAP) activity. The arrow in each panel marks the onset of propranolol administration. CST = electrogram recording from the caudal sulcus terminalis reference electrode.

sation of activity. Qualitatively similar results were obtained when norepinephrine-free Tyrode's solution was perfused rather than propranolol. In total, 73.1% of those preparations exhibiting subsidiary atrial pacemaker activity became quiescent or dysrhythmic when either propranolol or norepinephrine-free Tyrode's solution was perfused. Thus in a majority of cases, a background or basal level of beta adrenergic stimulation was required to sustain subsidiary atrial pacemaker activity. In contrast, sinoatrial node activity did not exhibit such a dependence.

In five subsidiary atrial pacemaker preparations, the minimal perfusate norepinephrine concentration, or threshold level, required to maintain a stable rhythm was determined. In these experiments, the perfusate norepinephrine concentration was lowered in a stepwise fashion until activity ceased entirely or until it became dysrhythmic. The concentration was then increased in small steps until rhythmic activity resumed. The "threshold" value determined in this fashion was $6.4 \pm 4.7 \times 10^{-9}$ M. In 85% of the preparations, the background level of norepinephrine previously found to sustain subsidiary atrial pacemaker activity (10^{-8} M) represented a suprathreshold level. In 15%, subsidiary atrial pacemaker activity required norepinephrine concentrations greater than 5×10^{-8} M.

Under the conditions specified above, the mean steady state spontaneous rate of subsidiary atrial pacemaker activity was 82.9 ± 3.9 beats/min (n=34). This value is significantly

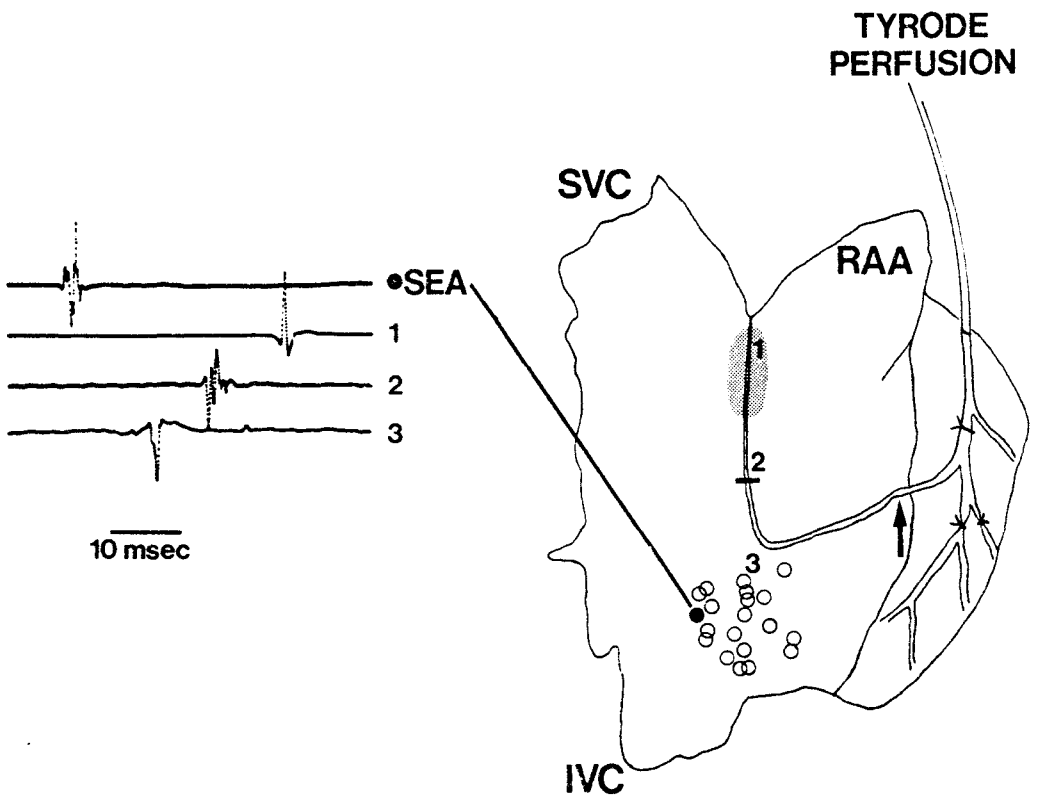
less than ($p < .001$) the mean sinoatrial node spontaneous rate with the same background level of norepinephrine, 101.5 ± 1.9 beats/min. In general, the spontaneous rate of subsidiary atrial pacemaker activity was 80.5 ± 3.7% of the sinoatrial node rate prior to suppression. As was the case for sinoatrial node activity, the model of subsidiary atrial pacemaker activity was stable for a number of hours after being elicited. In six preparations, the measured spontaneous rate at the onset of subsidiary atrial pacemaker activity, 77.8 ± 4.7 beats/min, was not significantly different ($p > .20$) than at four hours, 80.5 ± 5.3 beats/min.

3. Location of Subsidiary Atrial Pacemaker Sites of Earliest Activation

Figure 8 schematically represents the procedure used to establish the subsidiary atrial pacemaker model and summarizes the location of subsidiary sites of earliest activation. Following ligation of the sinoatrial node artery (depicted by the solid bar) and suppression of sinoatrial node activity, the extracellular mapping procedure used to locate the control site of earliest activation (Figure 2) was repeated to locate the subsidiary site of earliest activation. In the example shown in Figure 8, the site beneath the mobile electrode, represented by the filled circle, was found to depolarize earlier than any of the reference sites. Note that the activation sequence of the reference electrodes is opposite to what it was under control conditions (Figure 2).

FIGURE 8

EXTRACELLULAR MAPPING OF THE SUBSIDIARY ATRIAL PACEMAKER SITE OF EARLIEST ACTIVATION



The same procedure used to locate the control site of earliest activation (SEA) also was used after subsidiary atrial pacemaker activity was unmasked by ligating the sino-atrial node artery (solid bar). In the example shown in this figure, the subsidiary site of earliest activation (filled circle) was located at the junction of the inferior right atrium-inferior vena cava. Compare the reference electrogram activation sequence shown in the left panel above with that of the control sequence in the same atrium (Figure 2). The open circles represent 19 other subsidiary sites. SVC = superior vena cava, IVC = inferior vena cava, RAA = right atrial appendage.

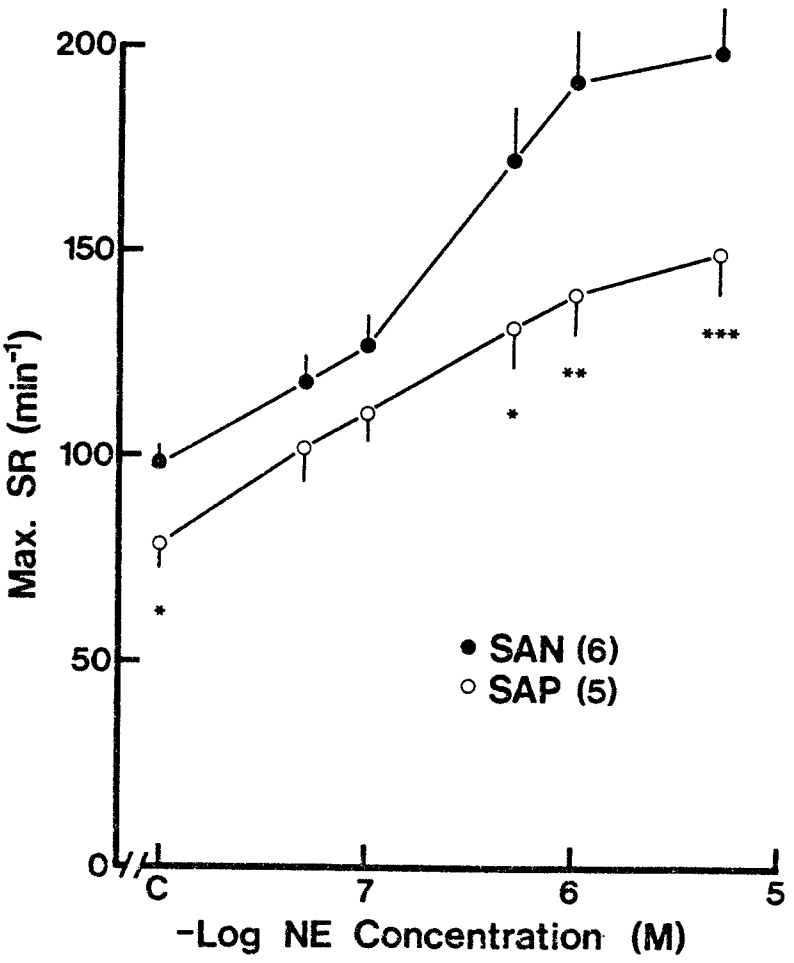
The open circles represent the subsidiary site of earliest activation in 19 other experiments (for the purpose of clarity, only 19 of the remaining 39 sites are shown) and indicate that in general, subsidiary atrial pacemaker activity developed from a region at the junction of the inferior vena cava-inferior right atrium. Collectively, these sites were 28.3 $\pm$ 1 mm caudal to the control site of earliest activation and 11.6 $\pm$ 0.6 mm caudal to the site of ligation.

In 10 preparations, the subsidiary site of earliest activation was mapped on both the epicardial and endocardial surfaces. For endocardial mapping, the preparation was turned over and the reference electrodes positioned along the crista terminalis in a similar orientation as they were along the sulcus terminalis on the epicardial surface. The positions determined on both surfaces of the same preparation differed by 3.8 $\pm$ 0.9 mm. Thus there was generally a close correlation of the site of earliest activation as determined on the endocardial and epicardial surfaces.

4. Chronotropic Response to Norepinephrine

The chronotropic response of both sinoatrial node and subsidiary atrial pacemaker activities to norepinephrine in a concentration range of 5×10^{-8} to 5×10^{-6}M is shown in Figure 9. Under control steady state conditions with background norepinephrine, the sinoatrial node had a significantly greater ($p < .025$) spontaneous rate than subsidiary atrial pacemaker activity. Near-parallel changes in the maximum spontaneous rate were obtained at concentrations less than 10^{-7}M. However, at concentrations greater than 10^{-7}M,

FIGURE 9
CHRONOTROPIC RESPONSE TO NOREPINEPHRINE



Concentration-response curves were obtained for sinoatrial node (SAN) and subsidiary atrial pacemaker (SAP) activities by perfusing varying concentrations of norepinephrine each for 30 seconds. The maximum spontaneous rate (ordinate) is plotted as a function of the negative log of the norepinephrine (NE) concentration. C = control steady state spontaneous rate. *$p < .025$. **$p < .01$. ***$p < .005$.

a highly significant difference in maximum spontaneous rate was observed as evidenced by the sharp rise in the sinoatrial node curve. At these higher levels of adrenergic stimulation, transient shifts in the activation sequence of the reference electrograms were observed, suggesting an intranodal shift in the site of earliest activation. These shifts in activation sequence occurred with an abrupt secondary increase in spontaneous rate recorded on the tachograph. Evidence of an intranodal shift in pacemaker was observed in five of six preparations with a rostral shift occurring three times and a caudal shift twice. In contrast to the sinoatrial node, subsidiary atrial pacemaker activity attained a significantly slower spontaneous rate at higher norepinephrine concentrations and shifts in the site of earliest activation were not observed.

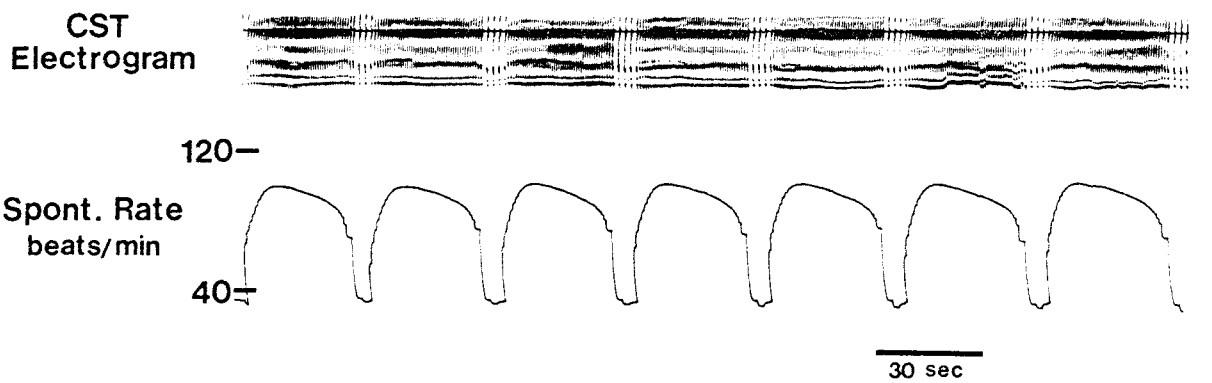
The time course of the chronotropic response to norepinephrine generally was faster for the sinoatrial node than for subsidiary atrial pacemaker activity. Over the same range of norepinephrine concentrations (5×10^{-8} - 5×10^{-6} M), the time to peak response for sinoatrial node activity was significantly less than ($p < .05$) subsidiary atrial pacemaker activity, 46.4 ± 4.8 sec vs 65.5 ± 6.7 sec respectively. As for the duration of the response, it was significantly shorter ($p < .05$) for sinoatrial node activity than subsidiary atrial pacemaker activity, 105.8 ± 14.1 sec vs 187.0 ± 29.5 sec respectively.

The chronotropic effect of alpha adrenergic stimulation on both sinoatrial node and subsidiary atrial pacemaker activities varied similarly with the concentrations of alpha agonist administered. A one minute bolus of 10^{-5} M methoxamine produced a small increase in spontaneous rate of 3 ± 2.5 beats/min (n=5) for sinoatrial node activity and 5.3 ± 0.6 beats/min (n=3) for subsidiary atrial pacemaker activity. These changes in spontaneous rate were not significantly different from each other ($p > .05$). A one minute bolus of 10^{-4} M methoxamine decreased the spontaneous rate of the sinoatrial node by 11.7 ± 0.9 beats/min (n=3) and of the subsidiary atrial pacemakers by 15.5 ± 1.7 beats/min (n=4). These changes also were not significantly different from one another ($p > .05$). The chronotropic effects of methoxamine were blocked by phentolamine (5×10^{-7} M) which by itself did not significantly change the spontaneous rate.

In five preparations, subsidiary atrial pacemaker activity was associated with a spontaneously repetitive dysrhythmia an example of which is shown in Figure 10. This type of rhythm disturbance generally occurred with concentrations of norepinephrine greater than 10^{-8} M but in one case it was observed with norepinephrine-free Tyrode's solution. The dysrhythmia qualitatively resembled triggered activity in that a slow spontaneous phase quickly developed into a rapid phase which reached a peak, gradually declined and stopped abruptly. The peak spontaneous rate of the rapid phase in four experiments was 100.5 ± 4.6 beats/min and its

FIGURE 10

SPONTANEOUSLY REPETITIVE DYSRHYTHMIA



This figure demonstrates a spontaneous dysrhythmia that occurred in the absence of sinoatrial node activity with 2×10^{-8}M norepinephrine. CST = caudal sulcus terminalis reference electrogram. Bottom trace: tachograph recording triggered from electrogram above.

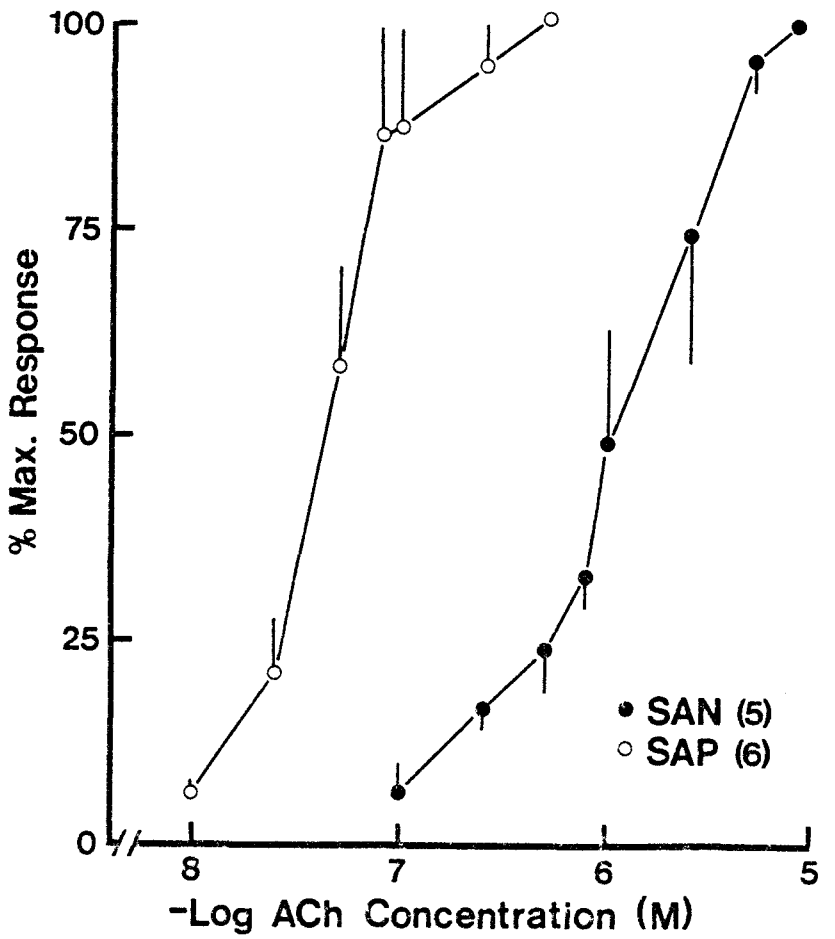
duration was 35.1 ± 4.6 seconds.

5. Chronotropic Response to Acetylcholine

The perfusion of a given concentration of acetylcholine for a brief time period resulted in a markedly different magnitude of response depending upon whether it was administered to the sinoatrial node or subsidiary atrial pacemaker. Figure 11 represents the concentration-response curves for both pacemakers in a concentration range of 10^{-8} to 7.5 X 10^{-6} M. In this figure, the chronotropic response, plotted on the ordinate, is expressed as a percent of the maximal response which for each pacemaker corresponded to total suppression of activity. The concentration-response curve for subsidiary atrial pacemaker activity is shifted far to the left of the curve for sinoatrial node activity. The EC_{50} for the individual sinoatrial node experiments, 1.2 ± 0.3 X 10^{-6} M is significantly greater than the EC_{50} obtained for the subsidiary atrial pacemaker experiments, 5.7 ± 2.1 X 10^{-8} M ($p < .005$). These data indicate that subsidiary atrial pacemaker activity exhibited a significantly greater sensitivity to acetylcholine than the sinoatrial node.

The time course of the changes brought about by acetylcholine for both subsidiary atrial pacemaker and sinoatrial node activities were compared at a concentration of 10^{-7} M. The time to peak response for sinoatrial node activity, 59.5 ± 5.5 sec, was not significantly different from subsidiary atrial pacemaker activity, 57.4 ± 12.6 sec ($p >$

FIGURE 11
CHRONOTROPIC RESPONSE TO ACETYLCHOLINE



Concentration-response curves were obtained for sinoatrial node (SAN) and subsidiary atrial pacemaker (SAP) activities by perfusing varying concentrations of acetylcholine (with 10^{-8}M norepinephrine) each for 1 minute. The response (ordinate) is expressed as a percent of the maximal chronotropic response which for each pacemaker was total suppression. The acetylcholine (ACh) concentration is expressed as a negative log of the molar (M) concentration.

.20). However, the duration of the response was significantly longer ($p < .05$) for subsidiary atrial pacemaker than for sinoatrial node activity, 181.1 $\pm$ 16.1 sec vs 109.5 $\pm$ 18.1 sec respectively. The negative chronotropic response to acetylcholine of sinoatrial node activity was blocked by the prior administration of atropine (5×10^{-8}M). When the block was tested by perfusion of 10^{-6}M acetylcholine, an acceleration of rate from 6-12 beats/min was observed. In five of six subsidiary atrial pacemaker preparations however, atropine totally suppressed activity that later returned on washout.

Eserine (3.6×10^{-5}M) was administered to five sinoatrial node and subsidiary atrial pacemaker preparations to assess the spontaneous release of acetylcholine from each respective pacemaker tissue. The results obtained from a 15 second bolus of eserine are summarized in Table 2. Eserine induced a greater reduction in the spontaneous rate of subsidiary atrial pacemakers than the sinoatrial node although the difference was not statistically significant. However, the response to eserine took longer to reach its peak level and lasted longer for subsidiary atrial pacemaker tissue than for sinoatrial node tissue.

6. Overdrive Suppression

The response of both sinoatrial node and subsidiary atrial pacemaker activities to overdrive pacing was pacemaker suppression that lasted longer than the duration of the spon-

TABLE 2

PERFUSION OF ESERINE (3.6×10^{-5}M) THROUGH SINOATRIAL NODE AND SUBSIDIARY ATRIAL PACEMAKER TISSUES

	Decrease in SR (beats/min)	TTP (sec)	Duration (sec)
SAN	27.8 ± 5.5	182.8 ± 19.9	447.4 ± 42.9
SAP	44.0 ± 11.5	450.8 ± 25.5	1226.0 ± 103.1
p	< .20	< .001	< .001

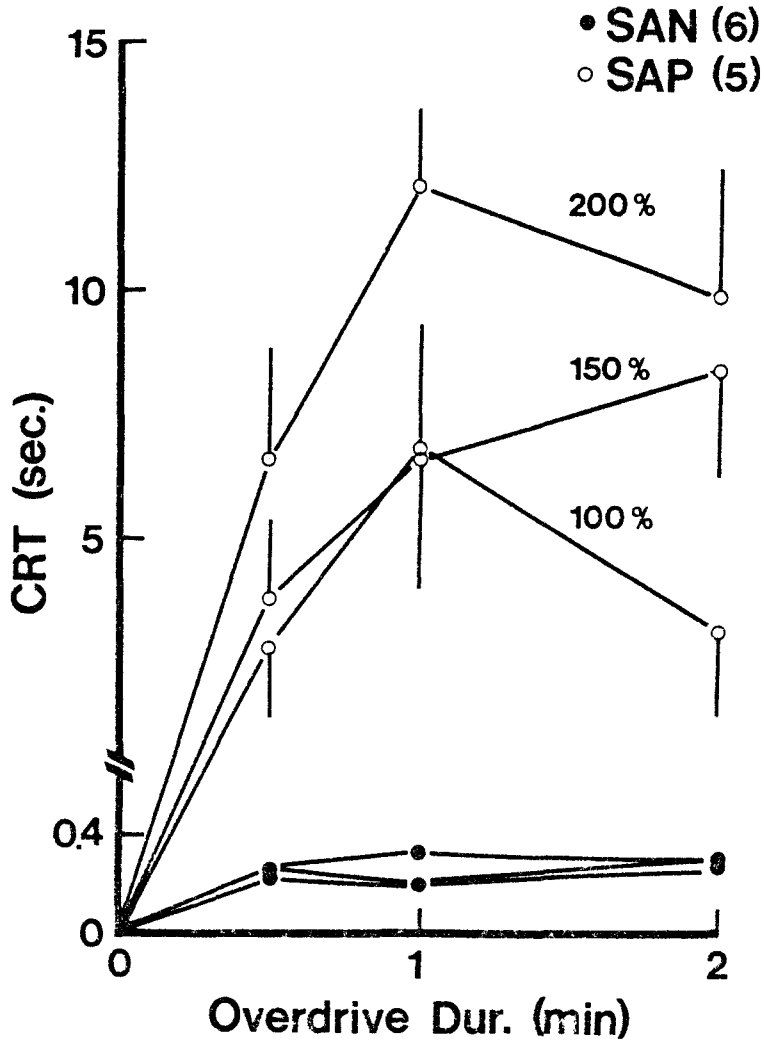
The response of sinoatrial node and subsidiary atrial pacemaker activities were both measured during a 15 second bolus of eserine. SR = spontaneous rate, TTP = time to peak response, SAN = sinoatrial node, SAP= subsidiary atrial pacemaker.

taneous cycle length. The amount of suppression was a function of the duration and magnitude of the overdrive. Furthermore, the amount of suppression at any given duration or magnitude of overdrive was far greater for subsidiary atrial pacemaker activity than for sinoatrial node activity, measured in seconds for the former and milliseconds for the latter. Figure 12 represents the amount of suppression, expressed as a corrected recovery time (CRT), of each type of pacemaker activity as a function of the duration of overdrive at pacing rates of 100, 150 and 200% above the control spontaneous rate. Note the change in scale of the ordinate. This figure demonstrates that subsidiary atrial pacemaker activity is suppressed far longer than sinoatrial node activity at a given overdrive duration. It also shows that generally, the longer the overdrive duration, the greater the suppression. A one-way analysis of variance conducted for each duration revealed a significant difference ($p < .01$) among the data points. Multiple paired comparisons were made at each overdrive duration using a least significant difference technique and the results are summarized in Table 3.

Figure 13 represents the amount of suppression as a function of the magnitude of overdrive (expressed as pacing rate above the control spontaneous rate) at overdrive durations of 0.5, 1 and 2 minutes. This figure demonstrates a proportional relationship between the amount of suppression and the magnitude of overdrive which is significantly greater for subsidiary atrial pacemaker than for sinoatrial node activity.

FIGURE 12

PACEMAKER SUPPRESSION AS A FUNCTION OF THE DURATION OF OVERDRIVE PACING



Sinoatrial node (SAN) and subsidiary atrial pacemakers (SAP) were driven at rates 100, 150 and 200% above their respective spontaneous rates for durations of 0.5, 1 and 2 minutes. Pacemaker suppression is expressed as a corrected recovery time (CRT). Standard error bars for sinoatrial node suppressions were omitted for clarity.

TABLE 3

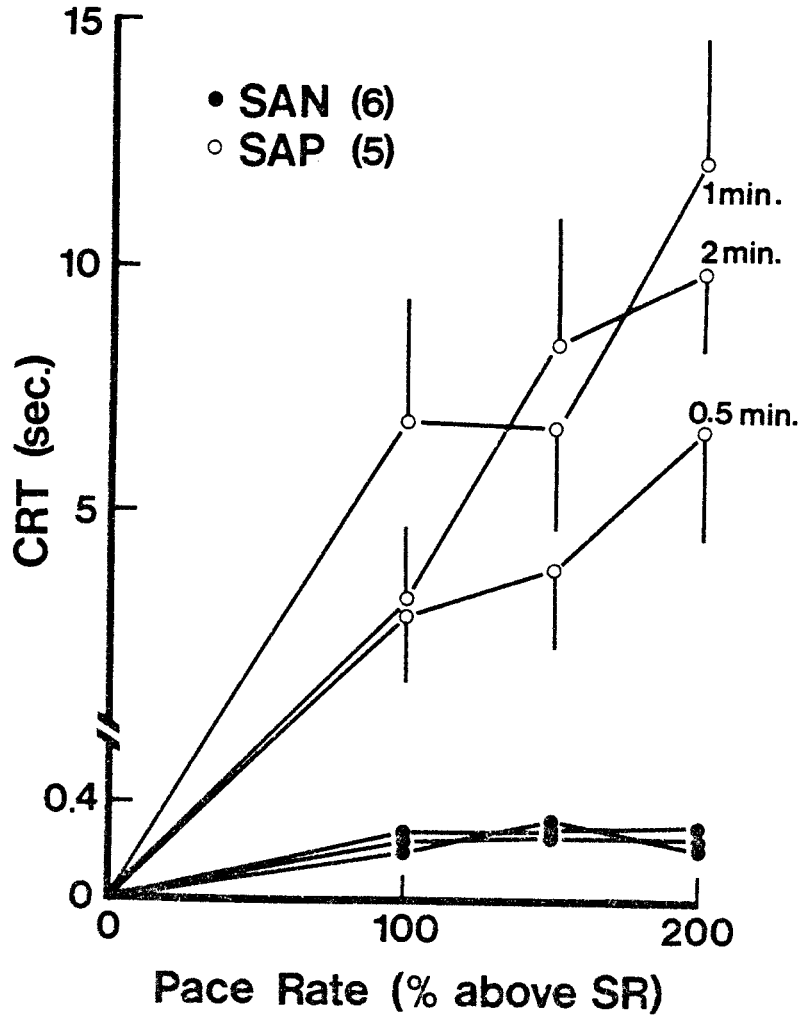
MULTIPLE COMPARISONS OF CORRECTED RECOVERY TIMES FOR OVERDRIVE PACING DURATIONS OF 30 SECONDS, 1 AND 2 MINUTES

30 sec.		1 min.		2 min.	
Comparison	p	Comparison	p	Comparison	p
SAN_{100} vs SAN_{150}	NS	SAN_{100} vs SAN_{150}	NS	SAN_{100} vs SAN_{150}	NS
SAN_{150} vs SAN_{200}	NS	SAN_{150} vs SAN_{200}	NS	SAN_{150} vs SAN_{200}	NS
SAN_{200} vs SAN_{100}	NS	SAN_{200} vs SAN_{100}	NS	SAN_{200} vs SAN_{100}	NS
SAP_{100} vs SAP_{150}	NS	SAP_{100} vs SAP_{150}	NS	SAP_{100} vs SAP_{150}	$<.05$
SAP_{150} vs SAP_{200}	NS	SAP_{150} vs SAP_{200}	$<.05$	SAP_{150} vs SAP_{200}	NS
SAP_{200} vs SAP_{100}	$<.05$	SAP_{200} vs SAP_{100}	$<.05$	SAP_{200} vs SAP_{100}	$<.05$
SAP_{100} vs SAN_{100}	NS	SAP_{100} vs SAN_{100}	$<.05$	SAP_{100} vs SAN_{100}	NS
SAP_{150} vs SAN_{150}	$<.05$	SAP_{150} vs SAN_{150}	$<.05$	SAP_{150} vs SAN_{150}	$<.05$
SAP_{200} vs SAN_{200}	$<.05$	SAP_{200} vs SAN_{200}	$<.05$	SAP_{200} vs SAN_{200}	$<.05$

The subscripts correspond to overdrive pacing at a rate of either 100, 150 or 200% above the control spontaneous rate. SAN = sinoatrial node, SAP = subsidiary atrial pacemaker, NS = not significant.

FIGURE 13

PACEMAKER SUPPRESSION AS A FUNCTION OF THE MAGNITUDE OF OVERDRIVE PACING



Sinoatrial node (SAN) and subsidiary atrial pacemakers (SAP) were driven for 0.5, 1 and 2 minutes at rates of 100, 150 and 200% above their respective spontaneous rates. CRT = corrected recovery time. Standard error bars for sinoatrial node suppressions were omitted for clarity.

A one-way analysis of variance conducted at each pace rate indicated a significant difference among the data points ($p < .05$). As in the previous figure, multiple paired comparisons were made using a least significant difference technique. The results of these comparisons are summarized in Table 4.

B. ISOLATED TISSUE SEGMENTS

1. Endocardial Location of Subsidiary Atrial Pacemaker Sites of Earliest Activation

The endocardial subsidiary atrial pacemaker sites of earliest activation (n=10) were located consistently in a distinct region represented schematically in Figure 14. This region exhibited a pale color that was distinguishable from the surrounding working atrial myocardium which is characteristically pink in color. It was found caudal to the crista terminalis approximately 2-3 cm from the sinoatrial node region and corresponded to the region of epicardial sites of earliest activation (Figure 8). The shape of the pale region varied from atrium to atrium but was generally triangular, crescent or oval and the dimensions ranged from 2 X 4 mm to 7 X 12 mm.

The tissue segments obtained for microelectrode studies were excised so that the pale region was in the center and bounded on all sides by at least 5 mm of pink tissue. The segments excised from atria in which subsidiary atrial pacemaker activity previously had been elicited contained

TABLE 4

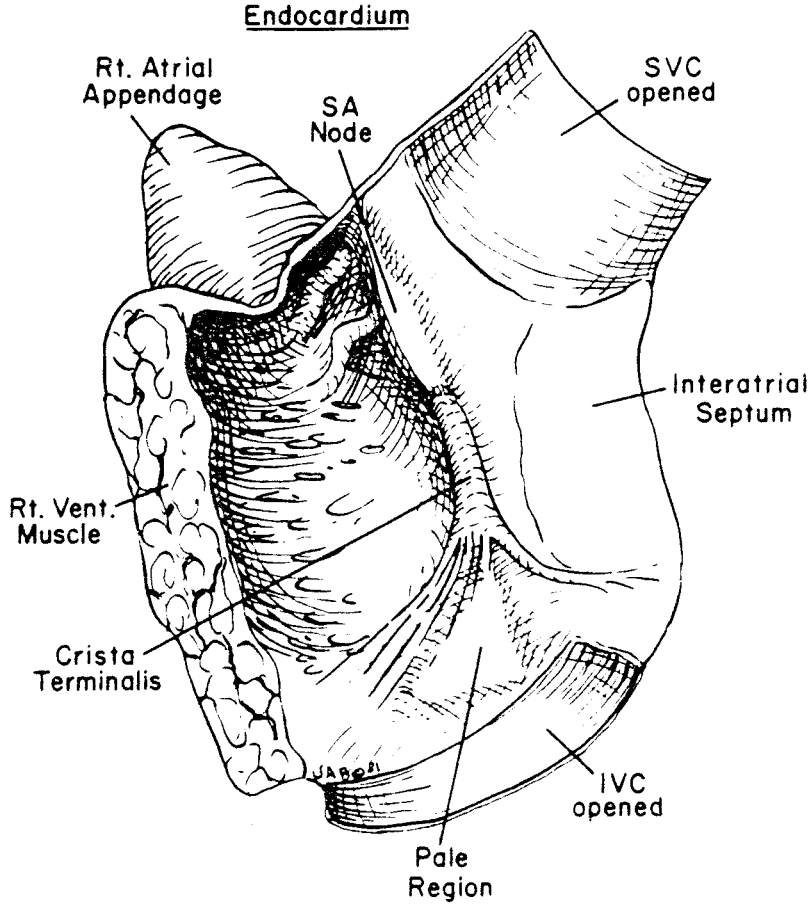
MULTIPLE COMPARISONS OF CORRECTED RECOVERY TIMES FOR OVERDRIVE PACING AT 100, 150 AND 200% ABOVE THE CONTROL SPONTANEOUS RATE

100%		150%		200%	
Comparison	p	Comparison	p	Comparison	p
$SAN_{0.5}$ vs SAN_1	NS	$SAN_{0.5}$ vs SAN_1	NS	$SAN_{0.5}$ vs SAN_1	NS
SAN_1 vs SAN_2	NS	SAN_1 vs SAN_2	NS	SAN_1 vs SAN_2	NS
SAN_2 vs $SAN_{0.5}$	NS	SAN_2 vs SAN_1	NS	SAN_2 vs $SAN_{0.5}$	NS
$SAP_{0.5}$ vs SAP_1	NS	$SAP_{0.5}$ vs SAP_1	NS	$SAP_{0.5}$ vs SAP_1	$<.05$
SAP_1 vs SAP_2	NS	SAP_1 vs SAP_2	NS	SAP_1 vs SAP_2	NS
SAP_2 vs $SAP_{0.5}$	NS	SAP_2 vs $SAP_{0.5}$	$<.05$	SAP_2 vs $SAP_{0.5}$	NS
$SAP_{0.5}$ vs $SAN_{0.5}$	NS	$SAP_{0.5}$ vs $SAN_{0.5}$	NS	$SAP_{0.5}$ vs $SAN_{0.5}$	$<.05$
SAP_1 vs SAN_1	$<.05$	SAP_1 vs SAN_1	$<.05$	SAP_1 vs SAN_1	$<.05$
SAP_2 vs SAN_2	NS	SAP_2 vs SAN_2	$<.05$	SAP_2 vs SAN_2	$<.05$

The subscripts correspond to overdrive pacing either for 0.5, 1 or 2 minutes. SAN = sinoatrial node, SAP = subsidiary atrial pacemaker, NS = not significant

FIGURE 14

ANATOMICAL LOCATION OF SUBSIDIARY ATRIAL PACEMAKER TISSUE



This figure shows an endocardial view of the canine right atrium. Tissue segments excised for microelectrode studies contained a pale region which previously had been found to be associated with subsidiary sites of earliest activation.

the subsidiary site of earliest activation at its center along with the entire pale region.

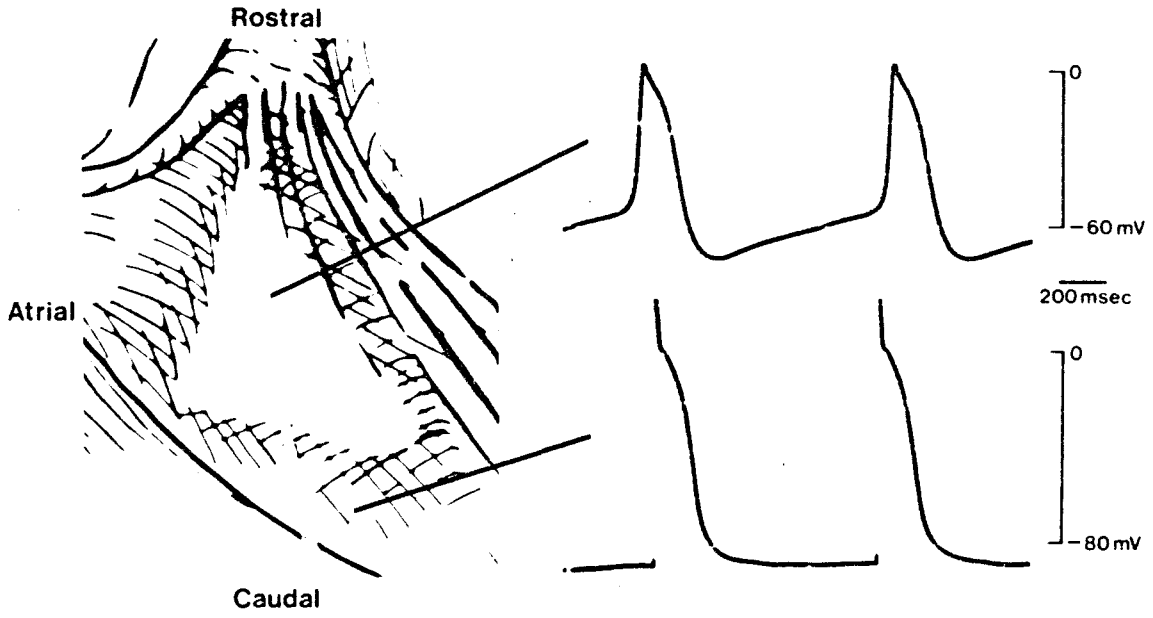
2. Action Potential Characteristics

Of the 27 tissue segments isolated for microelectrode study, 17 were excised from atria in which subsidiary atrial pacemaker activity had not been elicited under *in vitro* perfusion conditions. Ten of these (58.8%) exhibited automaticity in the presence of background norepinephrine with a spontaneous rate of 66.7 ± 7 beats/min. In 10 other experiments, tissues were excised from isolated perfused right atria in which subsidiary atrial pacemaker activity previously had been elicited by the ligation procedure. These tissues, containing the subsidiary atrial pacemaker site of earliest activation, all exhibited spontaneous activity with background norepinephrine (10^{-8} - 5 X 10^{-8} M). The spontaneous rate of these excised tissues, 74.9 ± 7 beats/min, was not significantly different ($p > .20$) from the subsidiary spontaneous rate of the perfused right atria from which they were obtained, 75.7 ± 8.3 beats/min.

Multiple impalements on the endocardial surface were made to characterize the action potentials from the pale region and the surrounding pink tissue. Slow response action potentials exhibiting diastolic depolarization were recorded frequently from fibers within the pale region (Figure 15). In contrast, fibers located in the surrounding pink tissue of the same preparation exhibited fast response action potentials with no or little diastolic depolarization. Tran-

FIGURE 15

ACTION POTENTIAL CONFIGURATIONS OF FIBERS IN THE PALE REGION AND SURROUNDING TISSUE



This figure represents a tissue segment excised for study which contained a pale region at its center. During spontaneous activity, slow response action potentials (top trace) with diastolic depolarization were recorded predominantly from the pale region. Fast response action potentials (bottom trace) were recorded from the surrounding tissue.

sitional action potentials were obtained from fibers at the junction of pale and pink tissues.

A detailed comparison of action potential parameters for fibers in pale and pink tissues revealed significant differences under spontaneous and non-spontaneous (driven) conditions (Table 5). The individual parameters measured in spontaneously active tissue segments from atria in which subsidiary atrial pacemaker activity was elicited (n=10) or not elicited (n=10) were not significantly different and therefore were pooled. In spontaneously active tissues with background norepinephrine (10^{-8} - 10^{-6} M), fibers within the pale region exhibited significantly lower values of maximum diastolic potential, overshoot, take-off potential, dv/dt_{max} and amplitude than fibers in the surrounding pink tissue. On the other hand, the magnitude of afterhyperpolarization or undershoot was significantly greater for fibers in the pale region.

As shown in the bottom half of Table 5, a similar electrophysiological distinction between fibers in pale and pink tissues was observed in preparations that were not spontaneously active with background norepinephrine (10^{-8} - 10^{-7} M) but were driven at 60 beats/min. Unlike the situation under spontaneous conditions, the difference in the magnitude of afterhyperpolarization approached but did not reach statistical significance ($p < .10$). In comparisons made of each parameter between spontaneously active and driven preparations, no significant difference was found in fibers from

TABLE 5

ACTION POTENTIAL PARAMETERS OF FIBERS IN PALE AND PINK TISSUES

		MDP (mV)	OS (mV)	TP (mV)	MDP-TP (mV)	dv/dt max (V/sec)	AMP (mV)
Spontaneous	Pale	72.8±1.5 (14)	6.1±1.2 (14)	60.0±2.2 (14)	12.7±1.6 (14)	14.6±7.2 (10)	79.6±2.4 (14)
	Pink	84.4±1.1 (10)	24.7±2.3 (10)	81.8±2.3 (10)	2.3±1.3 (10)	233.3±11.6 (7)	109.1±3.3 (10)
	p	< .001	< .001	< .001	< .001	< .001	< .001
Driven	Pale	74.8±1.6 (5)	8.9±3.0 (5)	66.5±4.6 (5)	8.3±3.4 (5)	31.6±6.9 (3)	83.8±4.5 (5)
	Pink	82.3±1.6 (6)	24.6±4.1 (6)	80.9±1.7 (6)	1.4±0.4 (6)	205.9±15.2 (5)	107.3±5.5 (6)
	p	< .01	< .02	< .02	NS	< .001	< .02

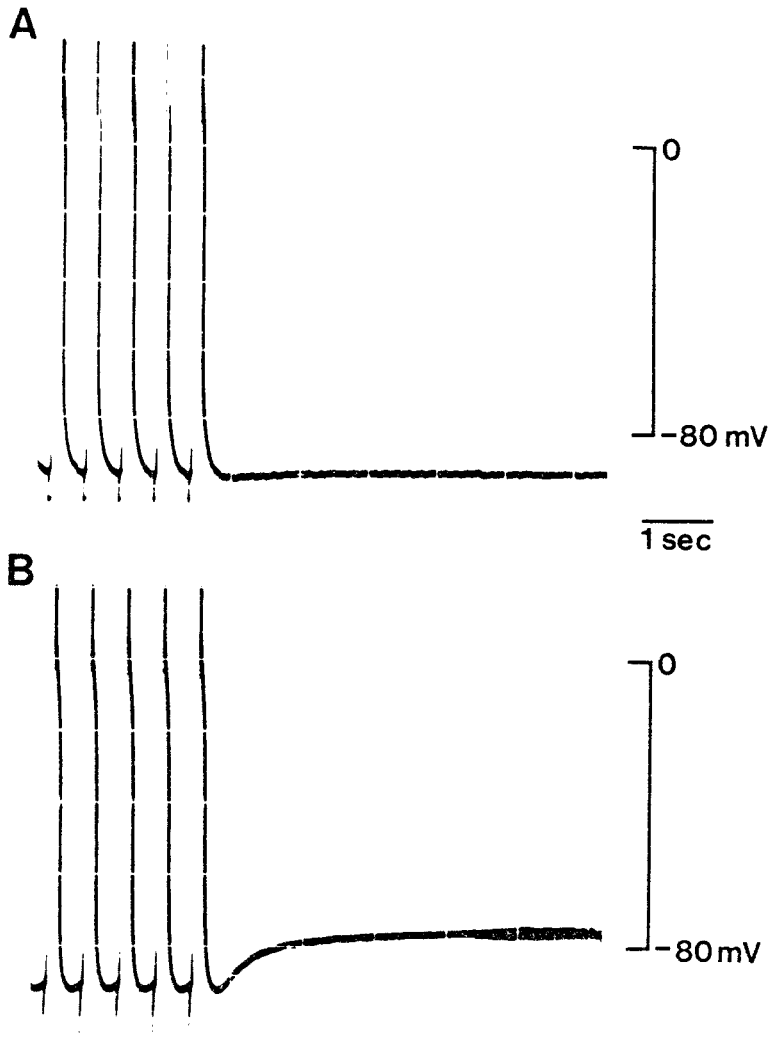
All parameters were measured in the presence of a background level of norepinephrine (10^{-8}-10^{-6}M). The mean rate of spontaneously active tissues was 65.6±6.3 beats/min. Preparations not spontaneously active were driven electrically at 60 beats/min. MDP = maximum diastolic potential, OS = overshoot, TP = take-off potential, MDP-TP = afterhyperpolarization or undershoot, dv/dt max = maximum rate of rise of the action potential upstroke, AMP = action potential amplitude obtained by adding MDP + OS. NS = not signficant.

pale or pink tissues. Thus, fibers within the pale region exhibited electrophysiological characteristics distinct from fibers in the surrounding tissue. This distinction was present whether the tissues were spontaneously active or whether driven electrically at a comparable rate.

The lower resting membrane potential of fibers in the pale region is indicated by the value of the maximum diastolic potential shown in Table 5. This characteristic was evident particularly in quiescent tissues where the basic drive stimulus was stopped momentarily to allow the membrane to reach a steady state resting value. Such an experiment is shown in Figure 16. The action potentials shown in panel A were recorded from a fiber in pink tissue in the presence of 10^{-8}M norepinephrine. Under control rate of stimulation at 60 beats/min (not shown) the maximum diastolic potential, overshoot and dv/dt_{max} were 92.3 mV, 30.5 mV and 235.3 V/sec respectively. Panel A shows the end of a one minute period of rapid pacing at 120 beats/min. The maximum diastolic potential, overshoot and dv/dt_{max} (measured from the last driven action potential) changed slightly during rapid pacing to 95.3 mV, 30.0 mV and 235.3 V/sec respectively. At the end of one minute, the pace was stopped and the membrane potential leveled off to a resting value of 93.5 mV, nearly the same as the maximum diastolic potential at 60 beats/min.

Panel B shows action potentials obtained from a fiber near the pale region of the same preparation from which the

FIGURE 16
ACTION POTENTIALS FROM QUIESCENT TISSUE



Panel A: fiber recorded from the surrounding pink tissue. Panel B: fiber recorded from the pale region of the same tissue segment. In a quiescent (not spontaneously active) preparation with norepinephrine, fibers were driven at 120 beats/min for 1 minute. Each panel shows the response of the fiber when the pace was stopped at the end of the 1 minute period.

fiber in Panel A was recorded and with the same norepinephrine concentration. Note that the action potential parameters of this "transitional-type" fiber are larger than those recorded from fibers in the center of the pale region from which the data shown in Table 5 were obtained. The maximum diastolic potential, overshoot and dv/dt_{max} at 60 beats/min (not shown) were 82.3 mV, 16.4 mV and 49.4 V/sec respectively. At the end of the one minute pacing period at 120 beats/min the values of the above mentioned parameters increased to 89.4 mV, 20.2 mV and 58.8 V/sec respectively. When the pace was stopped at the end of one minute, the membrane potential leveled off at 74.1 mV and a prominent afterhyperpolarization was observed following the last driven beat. Qualitatively, similar results as those shown in panels A and B were obtained in four other preparations and indicate that the resting membrane potential of fibers in the pale region is lower than in fibers of the surrounding pink tissue. Furthermore, it is evident that the action potential characteristics of fibers in the pale region approach those of the fast response fibers in the surrounding tissue at higher rates of stimulation.

3. Chronotropic and Dromotropic Effects of Norepinephrine

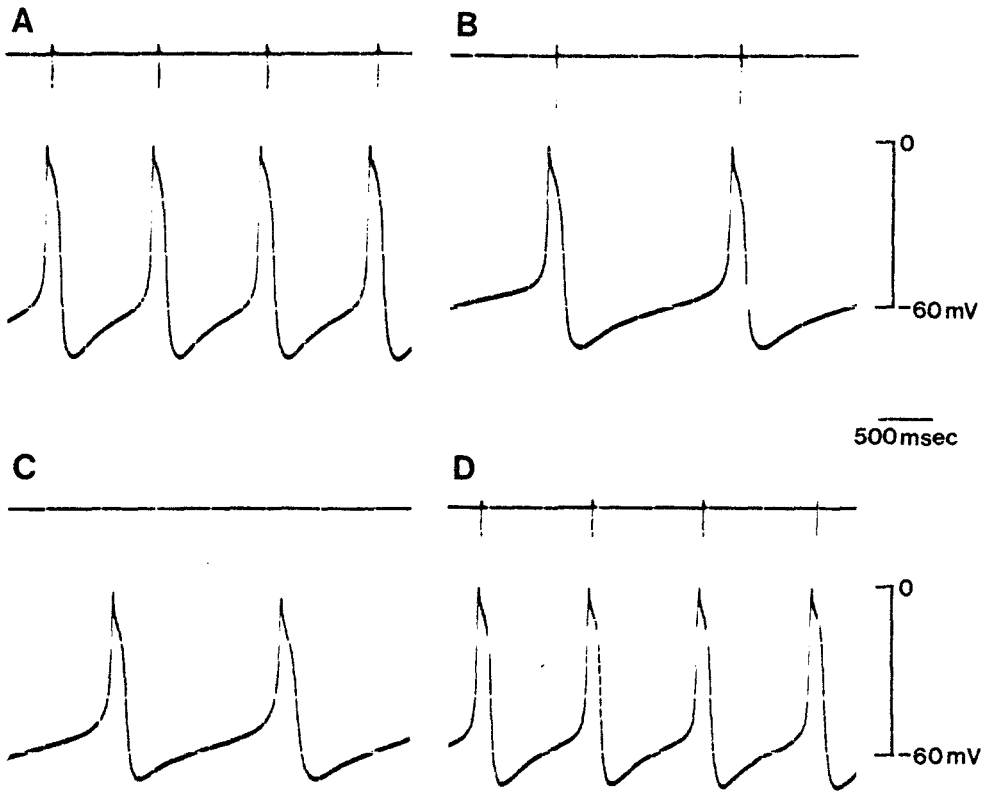
In 12 spontaneously active preparations, the subsidiary atrial pacemaker region was localized with the use of a bipolar silver reference electrode positioned outside the pale region. The simultaneous recording of intra- and extracellular electrical events provided information on the activity of fibers in the immediate subsidiary atrial pacemaker region and also allowed a qualitative assessment of

conduction of the spontaneous impulse. The dependence of subsidiary atrial pacemaker activity on norepinephrine observed in the isolated perfused preparation (Figure 7) was studied in 11 isolated tissue segments using the extracellular reference electrode. The spontaneous rate and conduction of subsidiary atrial pacemaker impulses were assessed by varying the superfusate norepinephrine concentration.

In the experiment shown in Figure 17, action potentials were recorded from a cell in the subsidiary atrial pacemaker region that was 5.5 mm from the reference electrode. The control spontaneous rate in the presence of 5×10^{-8} M norepinephrine (panel A) was 60 beats/min and each spontaneous action potential was conducted to the surrounding tissue as indicated by the bipolar electrogram. The superfusion of norepinephrine-free Tyrode's solution led to a decrease in spontaneous rate which after one minute was 37 beats/min. Panel B shows that as norepinephrine was washed out of the tissue chamber and spontaneous rate decreased, 1 to 1 conduction still was present. After five minutes of superfusion with normal Tyrode's solution (panel C), conduction block was evident although subsidiary atrial pacemaker impulses continued to be generated. In this example, the spontaneous rate during exit block was 40 beats/min. Reperfusion of norepinephrine-Tyrode's solution returned the spontaneous rate to the control value and relieved the exit block so that 1 to 1 conduction resumed (panel D). Similar results as those shown in Figure 17 were observed in six other preparations. In only one tissue was conduction of the subisidary atrial pacemaker impulse evident without norepinephrine.

FIGURE 17

EFFECT OF REDUCING NOREPINEPHRINE ON SUBSIDIARY ATRIAL PACEMAKER AUTOMATICITY AND CONDUCTION



The figure above demonstrates the chronotropic and dromotropic effects of reducing the background norepinephrine concentration from 5 x 10^{-8}M to zero. Panel A: control, B: during washout of norepinephrine, C: steady state condition with norepinephrine-free Tyrode's solution (after 5 minutes), D: recovery. All panels represent action potentials recorded from the same fiber in the subsidiary atrial pacemaker region (pale region). The top trace in each panel is a bipolar electrogram recorded from a site approximately 5.5 mm from the impaled fiber.

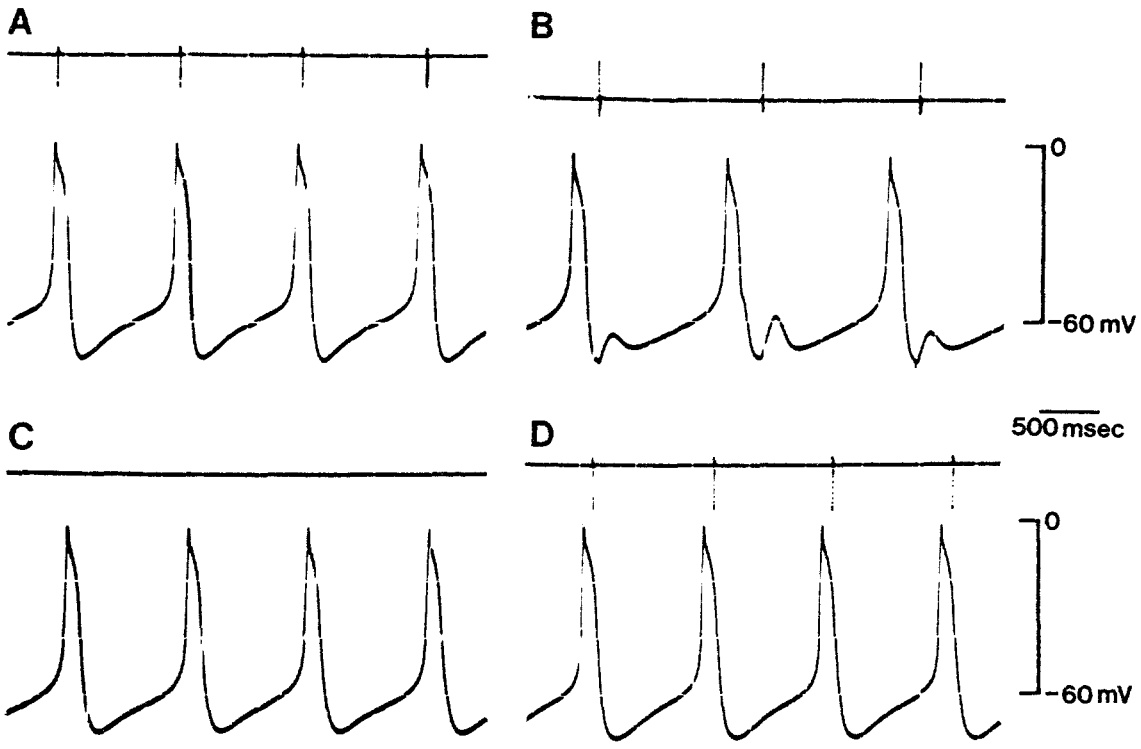
As shown in panel C of Figure 17, the removal of norepinephrine not only decreased the spontaneous rate of the subsidiary atrial pacemaker but also decreased the maximum diastolic potential and action potential amplitude as well. In five preparations, an approximately ten-fold reduction in the control background concentration of norepinephrine decreased the maximum diastolic potential and amplitude of fibers in the subsidiary atrial pacemaker region by 7.1 ± 0.6 mV and 12.2 ± 2.7 mV respectively. These changes were coincident with a 46.8 ± 12.6 beats/min reduction in spontaneous rate.

4. Chronotropic and Dromotropic Effects of Acetylcholine

The superfusion of acetylcholine for brief durations altered conduction and spontaneous rate in a qualitiatively similar fashion as removing norepinephrine from the superfusate. At acetylcholine concentrations less than 10^{-7} M, conduction of the subsidiary atrial pacemaker impulse was delayed or blocked while spontaneous action potentials continued to be generated. An example of this phenomenon is shown in Figure 18. The control spontaneous rate in the presence of 5×10^{-8} M norepinephrine was 58 beats/min (panel A). One to one conduction was present and each spontaneous impulse preceded the depolarization of the surrounding tissue (bipolar electrode was approximately 5.5 mm from the impaled fiber). At the onset of a 30 second bolus of 2.5×10^{-8} M acetylcholine (with 5×10^{-8} M norepinephrine), the spontaneous rate decreased to 45 beats/min (panel B). At this time conduction

FIGURE 18

EFFECT OF SUPERFUSING ACETYLCHOLINE ON SUBSIDIARY ATRIAL PACEMAKER AUTOMATICITY AND CONDUCTION



The effects of superfusing acetylcholine were qualitatively similar to reducing the norepinephrine concentration in that conduction block was observed. Panel A: control, with 5 x 10^{-8}M norepinephrine, B: 25 seconds after beginning superfusion of 2.5 x 10^{-8}M acetylcholine (plus norepinephrine), C: 61 seconds after beginning acetylcholine superfusion, D: recovery. The top trace in each panel is a bipolar electrogram recorded from a site approximately 5.5 mm from the impaled fiber. All action potentials were recorded from the same fiber.

was delayed and subthreshold depolarizations were present during the early portion of diastole which corresponded to depolarization of the surrounding tissue as indicated by the reference electrogram. After one minute, exit block was evident (panel C) and the spontaneous rate had now returned to the control value of 58 beats/min. Washout of acetylcholine relieved the exit block and 1 to 1 conduction returned (panel D). Similar results as those shown in Figure 18 were observed in three other preparations.

The negative chrono- and dromotropic effects of relatively low concentrations of acetylcholine occurred with small changes in action potential parameters of fibers in the subsidiary atrial pacemaker region. In fibers from four preparations, 5×10^{-8} M acetylcholine increased the maximum diastolic potential by 1.7 ± 0.4 mV while decreasing the overshoot by 3.3 ± 0.8 mV. These changes in action potential parameters occurred with a 16.8 ± 2.4 beats/min decrease in spontaneous rate. Administration of acetylchoine in concentrations greater than 10^{-7} M induced marked hyperpolarization of the membrane potential and total suppression of spontaneous impulses. In three preparations, superfusion of 10^{-6} M acetylcholine increased the maximum diastolic potential by 19.2 ± 6.6 mV and totally suppressed automaticity.

5. Overdrive Suppression

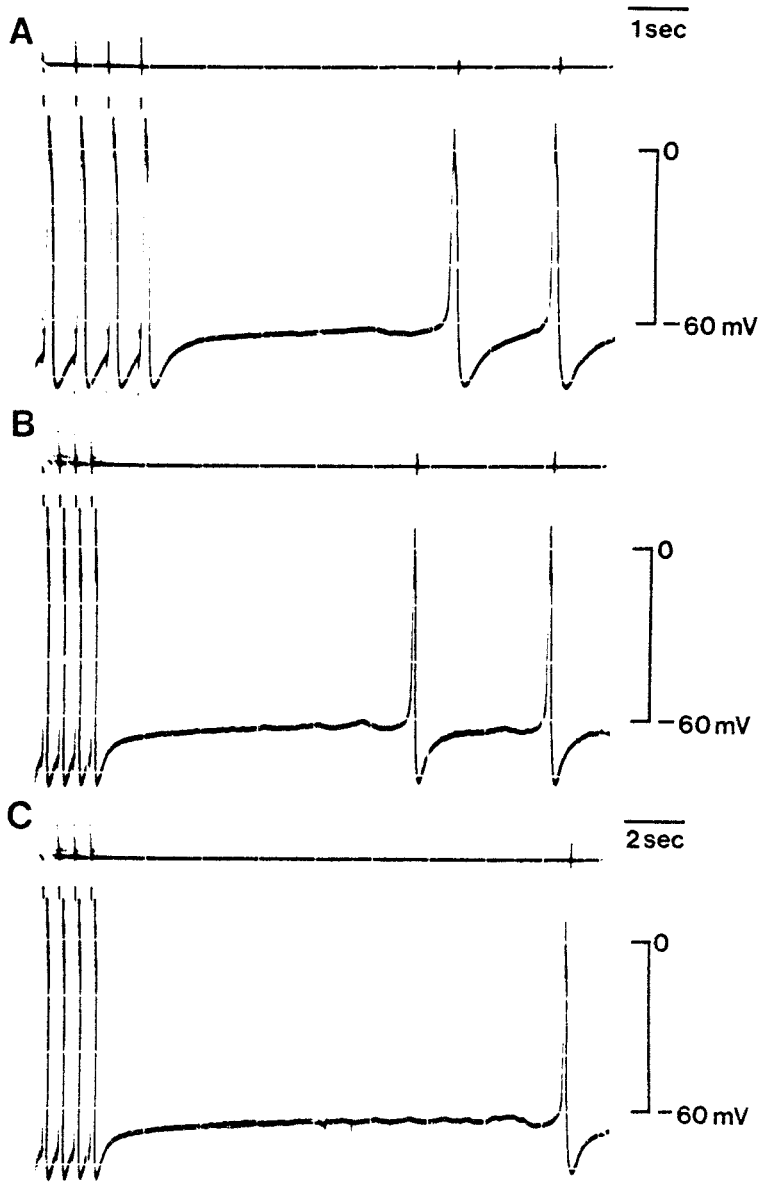
The prolonged suppression following overdrive pacing observed in the perfused subsidiary atrial pacemaker model

also occurred in spontaneously active subsidiary tissue segments. In general, the duration of suppression was a function of the magnitude as well as the duration of overdrive. Figure 19 demonstrates the amount of suppression in the same fiber paced at 100% above its control spontaneous rate for 30 seconds, 1 and 2 minutes. The respective corrected recovery times are 4.19, 9.97 and 15.27 seconds. In five preparations, pacing at 100% above the control spontaneous rate for 1 minute resulted in a corrected recovery time of 8.1 ± 0.94 seconds. In two sinoatrial node strips, the same degree of overdrive resulted in an average corrected recovery time of 0.28 seconds, far less than that obtained for subsidiary atrial pacemakers.

6. Triggered Activity

In seven tissue segments excised for microelectrode study spontaneous automaticity did not occur despite the presence of background norepinephrine. In these preparations, multiple impalements were made in both the pale region and surrounding pink tissue while pacing at 30 - 60 beats/min. With background norepinephrine (that normally would unmask subsidiary atrial pacemaker activity) fibers in the pale region exhibited marked afterhyperpolarizations that were evident when the pace was stopped momentarily (Figure 20, panel A). The same fibers exhibited delayed afterdepolarizations when norepinephrine was increased to levels greater than 10^{-7} M (panel B). Furthermore, under identical conditions fibers in the surrounding pink tissue of the same preparation did not

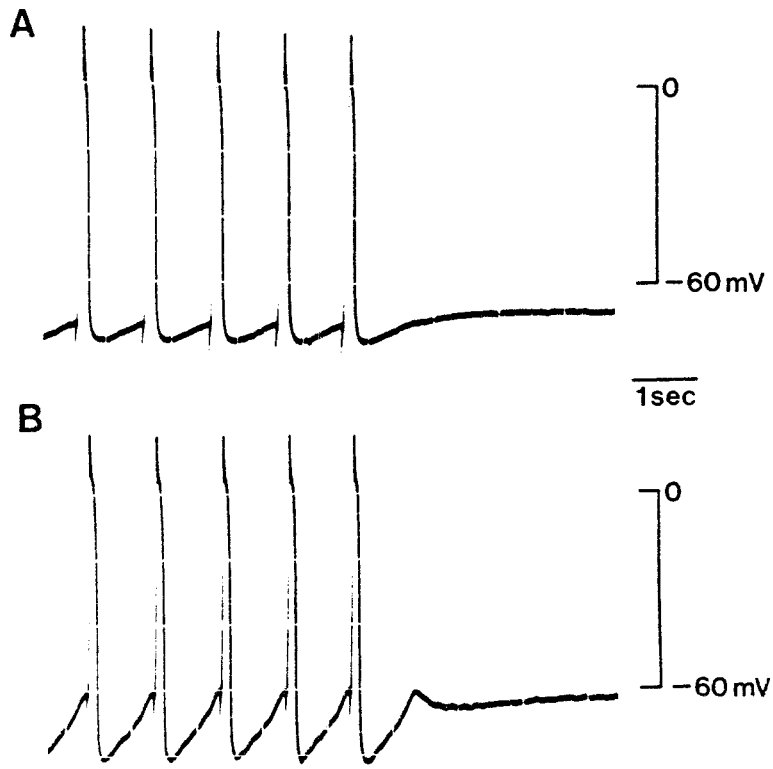
FIGURE 19
OVERDRIVE SUPPRESSION



The figure above demonstrates the chronotropic response of the same fiber to overdrive pacing at 100% above the control spontaneous rate for 30 seconds (panel A), 1 minute (panel B) and 2 minutes (panel C). Each panel shows the end of the pacing period and the first returning spontaneous impulse. Top trace: bipolar electrogram.

FIGURE 20

DELAYED AFTERDEPOLARIZATIONS WITH ELEVATED NOREPINEPHRINE



Panels A and B represent action potentials recorded from the same fiber within the pale region of a quiescent (not spontaneously active) tissue segment. In each case, the basic drive stimulus (60 beats/min) was stopped momentarily. Panel A: 10^{-8}M norepinephrine. Panel B: 7.5×10^{-7}M norepinephrine.

exhibit delayed afterdepolarizations (Figure 21). The presence of delayed afterdepolarizations with elevated norepinephrine was a characteristic shared by fibers within the pale region of six preparations.

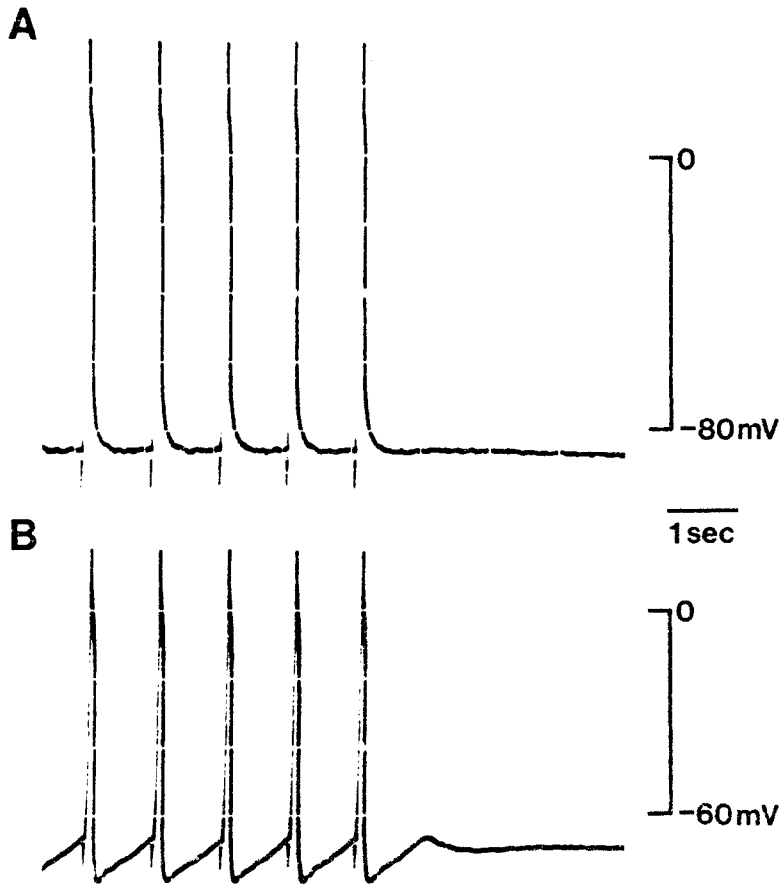
The amplitude of delayed afterdepolarizations was dependent upon the frequency of stimulation and the concentration of norepinephrine in the superfusate. Generally, the amplitude increased as either the stimulation rate or norepinephrine concentration increased. In five preparations, delayed afterdepolarizations reached threshold to induce either a coupled premature action potential or a period of sustained activity which lasted from 20 to 54 seconds and ended in damped oscillations in membrane potential (Figure 22). In the latter case, the spontaneous rate reached a peak value of 96.7 ± 19.6 beats/min and gradually declined until the activity ceased abruptly. Subsequently, the activity could be repeated but with time, the ability to induce triggered activity decreased until it would not develop even with higher norepinephrine concentrations.

7. Effects of Tetrodotoxin on Subsidiary Atrial Pacemaker Automaticity

To characterize the ionic channel participating in the development of spontaneous subsidiary atrial pacemaker impulses, the fast channel blocking agent tetrodotoxin (10^{-5} gm/ml) was superfused continuously in three different spontaneously active preparations. In two of three preparations, tetrodotoxin blocked conduction of the subsidiary

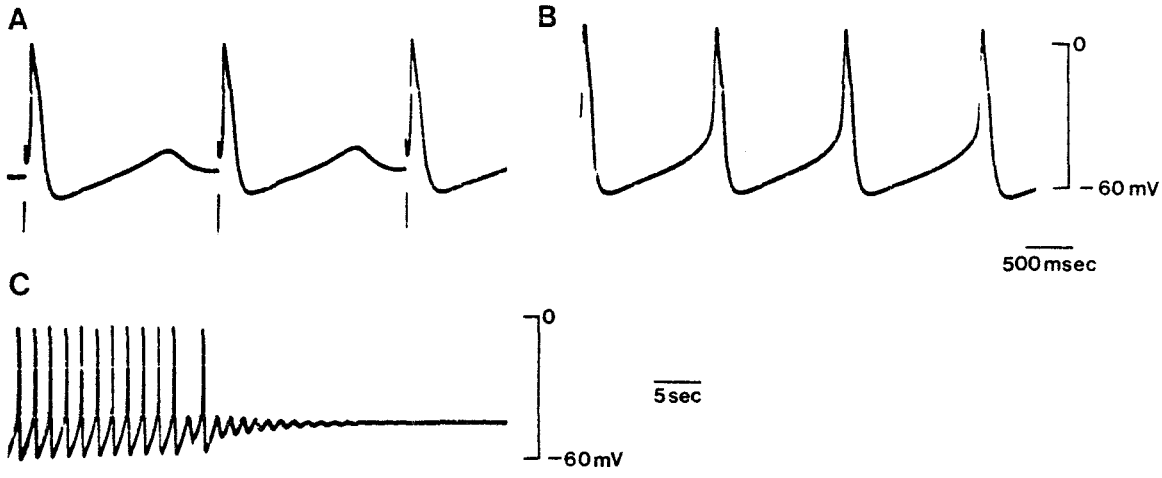
FIGURE 21

DELAYED AFTERDEPOLARIZATIONS IN FIBERS OF THE PALE REGION



In the presence of 10^{-6}M norepinephrine, fibers in the pale region (panel B) exhibited delayed afterdepolarizations (when the basic drive was stopped momentarily) while fibers in the surrounding pink tissue (panel A) did not.

FIGURE 22
TRIGGERED ACTIVITY



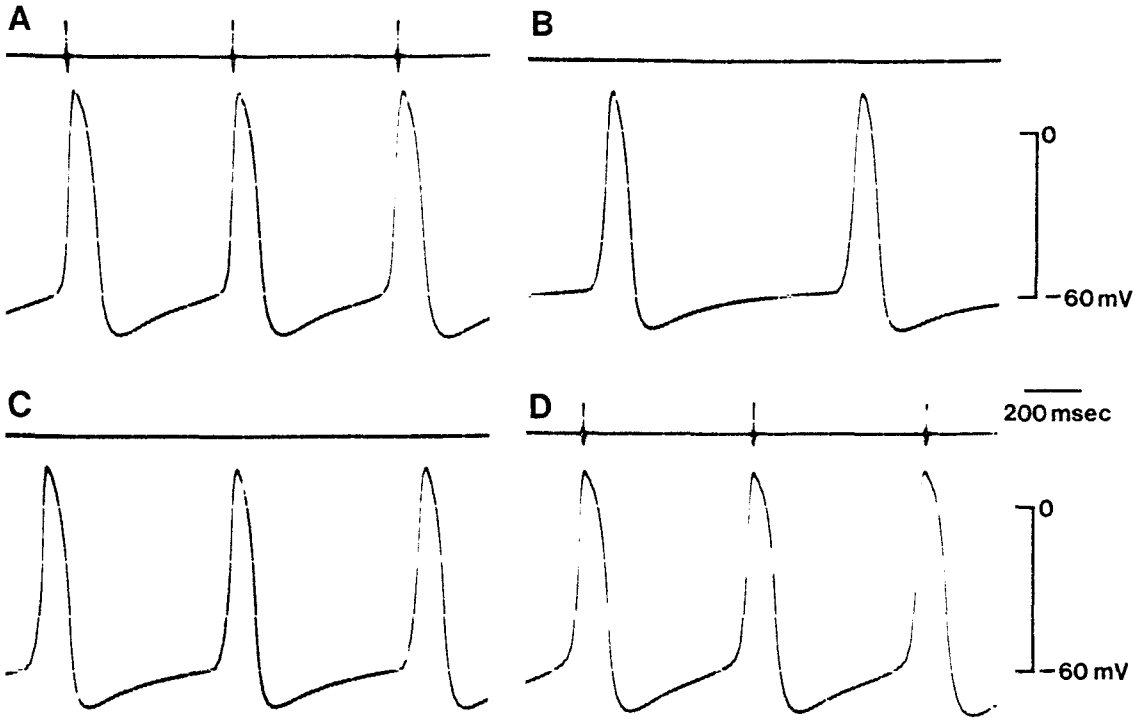
This figure shows the development and termination of sustained triggered activity from a fiber in the pale region. Panel A: stimulation rate was 30 beats/min with 5 x 10^{-8}M norepinephrine, B: as afterdepolarizations reached threshhold, spontaneous activity developed and the pace was turned off C: termination of triggered activity.

atrial pacemaker impulse but did not eliminate automaticity (Figure 23). During tetrodotoxin administration, the spontaneous rate decreased by an average of 18.1 beats/min while the action potential amplitude and maximum diastolic potential changed slightly. In one preparation, tetrodotoxin totally eliminated activity of a fiber in the pale region. These preliminary findings suggest that subsidiary atrial pacemaker impulses are generated by fibers exhibiting slow response characteristics.

8. Dysrhythmias

Alterations in the rhythm of subsidiary atrial pacemaker impulses and its manifest activity were observed during changes in neuromediator concentration and also during steady state conditions. In many instances, the manifest dysrhythmia was the result of conduction disturbances. Figure 24 provides an example of a spontaneous dysrhythmia under steady state conditions in the presence of 7.5×10^{-8} M norepinephrine. The manifest rhythm recorded by an extracellular electrode (top trace) in this example was characterized by an alternating slow and fast rate. Recording from a fiber in the subsidiary atrial pacemaker region indicated that during the slower phase, each propagated impulse was followed by a subthreshold depolarization. The time from the immediately preceding action potential to the peak of each of the first two subthreshold depolarizations is approximately equal to the cycle length of the first four conducted action potentials. The third subthreshold depolarization is greater in amplitude

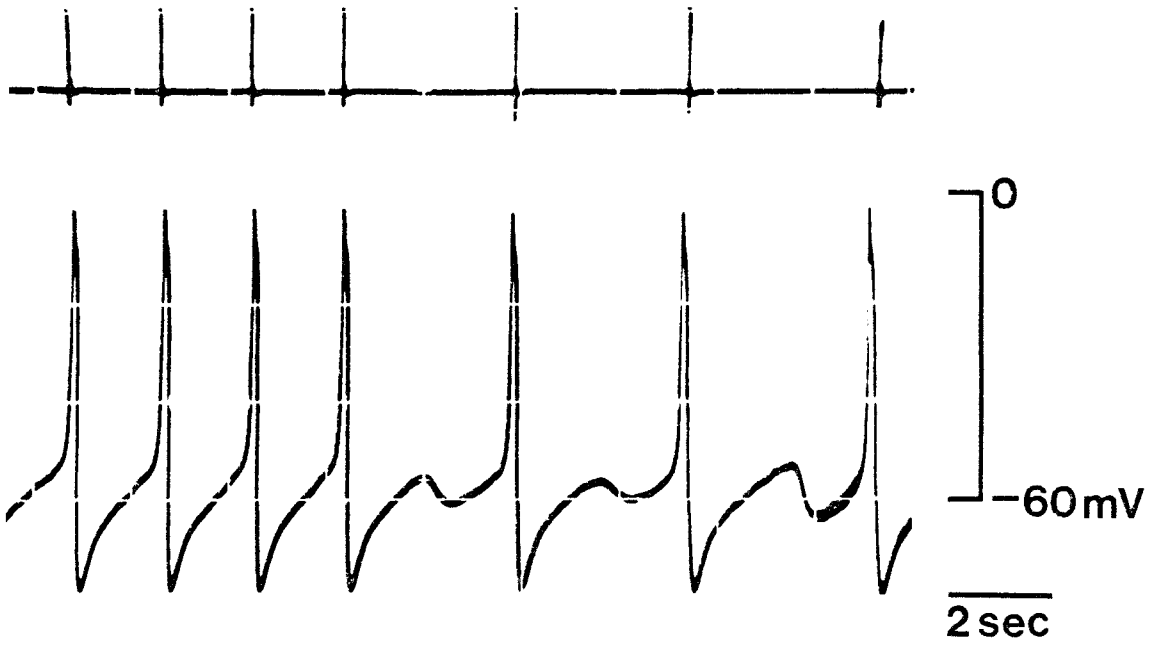
FIGURE 23
EFFECT OF TETRODOTOXIN



Tetrodotoxin (10^{-5} gm/ml) was administered to a spontaneously active tissue segment while recording from a fiber in the subsidiary atrial pacemaker region. Each panel shows action potentials recorded from the same fiber. Top trace: bipolar electrogram recorded from a site approximately 3.5 mm from the impaled fiber. Panel A: control, with 10^{-7}M norepinephrine, B: 20 seconds of tetrodotoxin (plus norepinephrine), C: 5 minutes of tetrodotoxin, D: recovery.

FIGURE 24

SPONTANEOUS DYSRHYTHMIA



The above action potentials were recorded from a fiber in the pale region in the continuous presence of 7.5 x 10-8M norepinephrine. The top trace is a bipolar electrogram recorded approximately 4.5 mm from the impaled fiber.

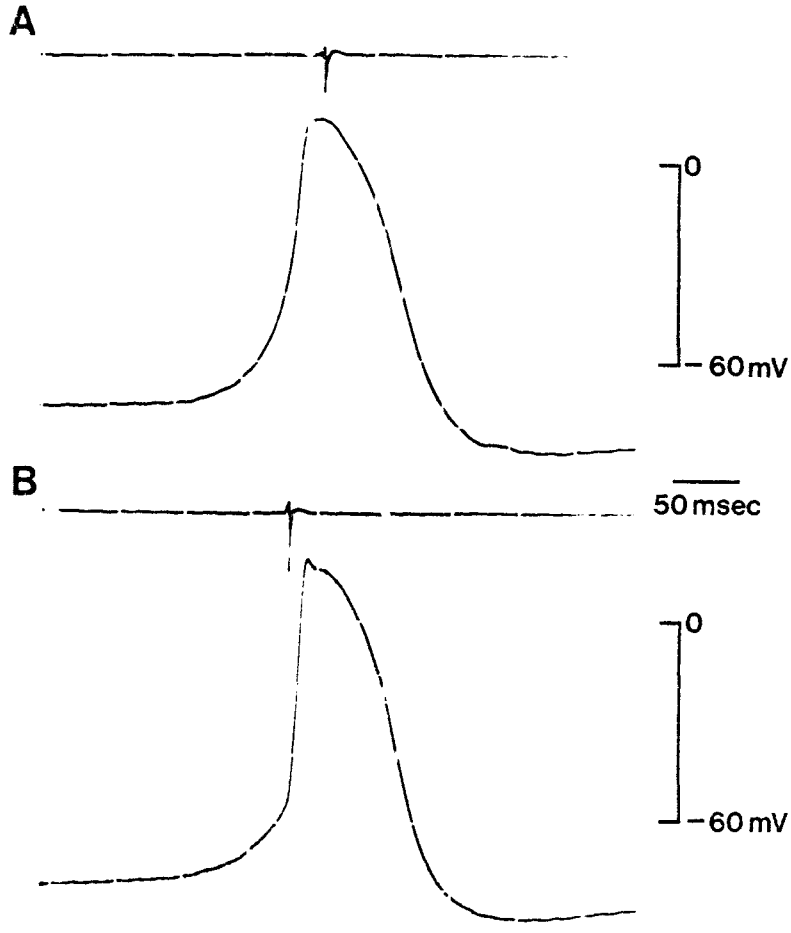
than the preceding two and came later in the cycle of the slower phase. This delayed the occurrence of the last spontaneous impulse shown in this figure.

Evidence for more than one pacemaker within the excised subsidiary tissue was obtained in five preparations during steady state conditions. In two tissues, an abrupt change in spontaneous rate occurred simultaneously with an alteration in the activation sequence between a subsidiary atrial pacemaker fiber and the reference electrode (Figure 25). In three other preparations, a second focus of pacemaker potentials was found which depolarized at a rate greater than the rest of the tissue (Figure 26). These faster foci depolarized independently but were modulated by activity of the surrounding tissue. In the example shown in Figure 26, the spontaneous cycle length of the rapid focus was 329 msec while that of the rest of the preparation was 1035 msec (as measured from the reference electrogram). The first electrogram occurred early in the cycle of the rapid focus and did not alter the duration of that cycle. The next electrogram occurred slightly later in the cycle of the rapid focus and delayed the next spontaneous impulse by 19 msec. Note also the change in slope of diastolic depolarization of this cycle.

Conduction delays which occurred mostly during changes in neuromediator concentration frequently produced notches or secondary depolarizations that in turn modified the rhythm of the original spontaneous impulse. Figure 27 demonstrates the modulating effect of delayed conduction on the spontaneous rhythm of a subsidiary atrial pacemaker fiber as the norepine-

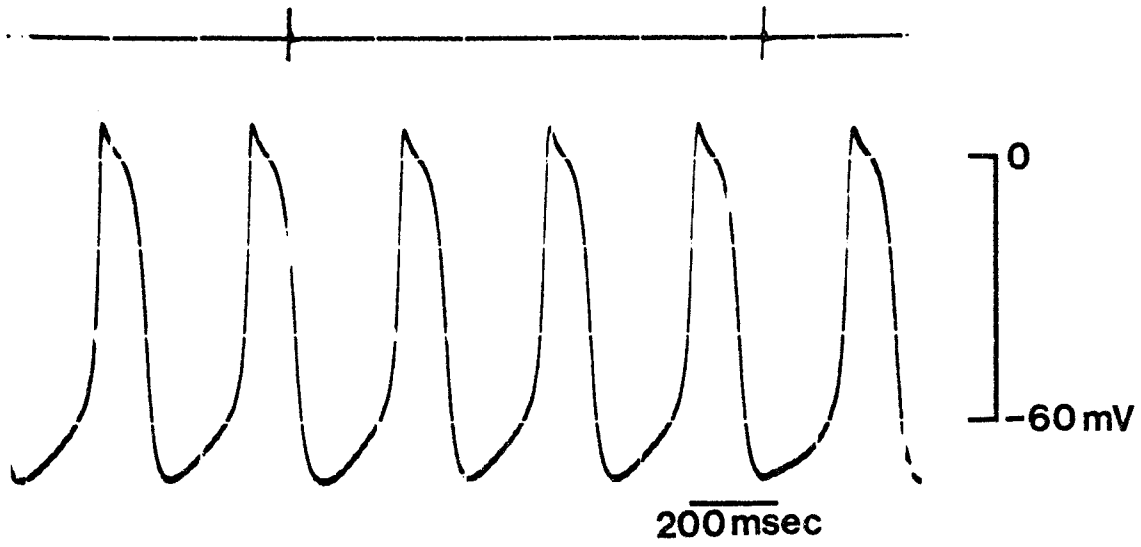
FIGURE 25

SPONTANEOUS SHIFT OF PACEMAKER WITHIN THE SUBSIDIARY REGION



Panels A and B show the same fiber at two markedly different spontaneous rates and in different sequence with a reference electrogram (top trace in each panel). With 10^{-8}M norepinephrine, the spontaneous rate was 50 beats/min and action potentials recorded from the sibsidiary atrial pacemaker site preceded the reference electrogram (panel A). In this example, a sudden increase in rate to 92 beats/min occurred concurrently with a shift in the activation sequence of the action potential and reference electrogram (panel B).

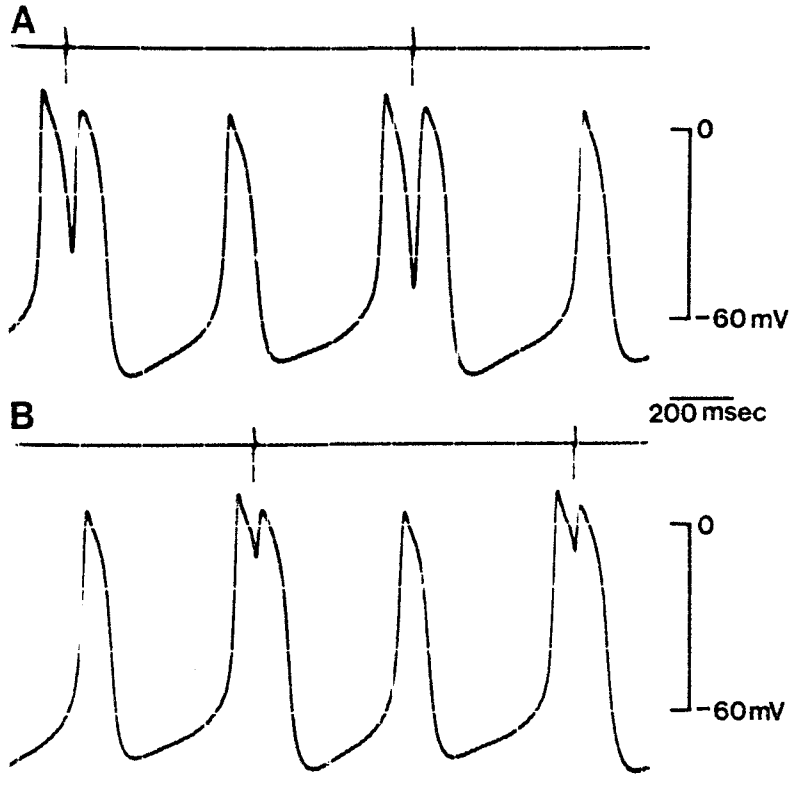
FIGURE 26
INDEPENDENT SUBSIDIARY ATRIAL PACEMAKERS



In this example, two pacemakers were acting independent of one another with the slower focus (spontaneous cycle length = 1035 msec) dominating the rhythm of the preparation. Despite this relationship, depolarization of the preparation modulated the cycle length of the fast focus (spontaneous cycle length = 329 msec). Depolarization of this surrounding tissue early in the spontaneous cycle of the faster focus delayed the next impulse by 19 msec. Depolarizations which came late in the cycle accelerated the next impulse (not shown).

FIGURE 27

ELECTROTONIC MODULATION OF SUBSIDIARY ATRIAL PACEMAKER RHYTHM



With no norepinephrine, subsidiary automaticity continued but complete exit block persisted (not shown). Panels A and B (same fiber) show 2 to 1 exit block as 5 x 10^{-8}M norepinephrine was superfused. Panel A: at 4 minutes, B: at 8 minutes. Top trace: bipolar reference electrogram recorded approximately 3.7 mm from the impaled fiber.

phrine concentration was increased from zero to 5 X 10^{-8} M. In the presence of norepinephrine-free Tyrode's solution (not shown), complete exit block occurred while automaticity continued at a cycle length was 970 msec. As 5 x 10^{-8} M norepinephrine was superfused (panel A), 2 to 1 exit block developed but each conducted impulse was delayed so that depolarization of the surrounding tissue induced a secondary electrotonic depolarization. The first conducted impulse produced a secondary depolarization which delayed the next non-conducted spontaneous impulse by 90 msec. The next subsidiary impulse was conducted but with greater delay so that the secondary electrotonic depolarization occurred later and delayed the next spontaneous action potential by 137.6 msec. After eight minutes of 5 X 10^{-8} M norepinephrine (panel B), 2 to 1 exit block persisted. However, the spontaneous cycle length of the subsidiary impulse had not changed (470 msec) but the manifest (conducted) cycle length was 59 msec shorter. This occurred because each conducted impulse was delayed less than in panel A so that its electrotonic depolarization delayed the subsequent non-conducted impulse by only 47 msec. Despite the continued presence of norepinephrine in this example, 2 to 1 exit block remained under steady state conditions.

9. Histology

Light microscopic examination of histological sections taken from subsidiary atrial pacemaker tissue did not indicate the presence of pacemaker fibers such as P cells or Purkinje-like fibers. In all sections observed, the pale re

gion was characterized by a thin layer of endocardial cells that in some sections was from two to three cells deep. The remainder of the transmural section of the pale region was composed of connective tissue. In addition, the cells of the pale region appeared smaller in size when compared to the working myocardial cells in the surrounding pink tissue.

CHAPTER V

DISCUSSION

A. ISOLATED PERFUSED RIGHT ATRIUM FOR THE STUDY OF PACEMAKER ACTIVITY

The isolated perfused canine right atrium has been used previously to study the electrophysiological and pharmacological properties of the sinoatrial node (30 , 33 ,256 , 257). These studies have shown, as has the present investigation, that when the flow of the perfusate is held constant, the spontaneous rate of the sinoatrial node is stable for several hours. Furthermore, the spontaneous rate is sensitive to relatively low concentrations of neuromediators in the perfusate as well as other pharmacological agents (257 , 30). Thus it is possible to selectively study sinoatrial node activity under controlled *in vitro* conditions.

The present investigation introduced a new model for the study of subsidiary atrial pacemaker activity using a modification of the isolated perfused right atrium. The ability to study subsidiary atrial pacemaker activity in this preparation was due to the widespread distribution of the sinoatrial node artery (Figure 6) and the ability to selectively suppress sinoatrial node activity by ligating this artery proximal to

the sinoatrial node region (Figure 8). Under the perfusion conditions described in the Results section, the subsidiary atrial pacemaker spontaneous rate was stable for several hours just as it was for sinoatrial node activity. In addition, subsidiary atrial pacemaker activity was highly sensitive to neuromediators contained in the perfusate. Therefore, the isolated perfused right atrium offers a means of independently studying sinoatrial node and subsidiary atrial pacemaker activities within the same preparation.

B. ORIGIN OF PACEMAKER ACTIVITY

The results obtained from extracellular mapping of the site of earliest activation indicate that subsidiary atrial pacemaker and sinoatrial node activities originate from distinct regions of the right atrium. Whereas the dominant pacemaker under control conditions originated at the junction of the superior vena cava-right atrium, subsidiary atrial pacemaker activity was found at the junction of the inferior vena cava-inferior right atrium. The former location corresponds to the classically described sinoatrial node region; the latter region represents a recently recognized component of the pacemaker hierarchy in the mammalian heart.

The implication of the inferior vena cava-inferior right atrial junction as a region of subsidiary pacemaker activity has also been made *in vivo* by the studies of Jones *et al.* (120,121). After excising the canine sinoatrial node and adja-

cent tissue of the sulcus terminalis, they found the site of earliest activation at the junction of the inferior vena cava-inferior right atrium in 80% of the animals mapped. In the present investigation, subsidiary atrial pacemaker activity was found in this region in 73.5% of the preparations studied. Furthermore, other known subsidiary atrial pacemaker regions such as the atrioventricular junction and coronary sinus were purposely excluded in this study to allow the assessment of pacemaker characteristics in this region without competition from other pacemakers as can occur in vivo (120,121).

Other lines of evidence in this study also support the inferior vena cava-inferior right atrial junction as a subsidiary atrial pacemaker site. In those preparations in which the site of earliest activation was determined on both epicardial and endocardial surfaces during subsidiary atrial pacemaker activity, the difference between the location of the sites was only 3.8 ± 0.9 mm. Thus it is unlikely that the pacemaker activity was being generated from a site other than the inferior right atrium. The possibility that pacemaker activity in this region was related to the trauma of isolating and ligating the sinoatrial node artery also is unlikely since the subsidiary sites of earliest activation were greater than 1 cm (eight space constants) from the ligation site. Lastly, tissue segments excised for microelectrode study containing these subsidiary sites of earliest

activation 1) exhibited spontaneous rates not significantly different from the perfused preparation before excision and 2) contained fibers demonstrating pacemaker characteristics (Figures 17, 18, 24).

C. ADRENERGIC REGULATION

1. Dependence of Subsidiary Atrial Pacemaker Activity on Norepinephrine

In a majority of preparations (73.1%), subsidiary atrial pacemaker activity required a background level of norepinephrine in the perfusate. More specifically, it required a basal level of beta adrenergic stimulation (Figure 7). In none of the preparations tested did sinoatrial node activity display such an obligatory requirement. The dependence on background norepinephrine observed in this study is qualitatively similar to that demonstrated in other subsidiary regions of the heart and may represent a common characteristic of extranodal pacemakers. Urthaler *et al.* (227) found in the *in situ* canine heart that atrioventricular junctional pacemakers are dependent upon a basal sympathetic tone. Following suppression of sinoatrial node automaticity by perfusion of physostigmine into the sinoatrial node artery, bilateral stellectomy, reserpine pretreatment or propranolol administration resulted in a slow erratic atrioventricular junction rhythm marked by periods of asystole. Wit (254) studied automatic fibers in atrial tis-

sue outside the ostium of the coronary sinus and found evidence for automaticity in this region only in the presence of norepinephrine. Randall et al. (184) and Euler et al. (56) studied subsidiary atrial pacemaker activity in the conscious dog after excision of the sinoatrial node. The resultant rhythm was atrial in origin and was characterized by marked instability in the form of prominent bradycardia and periods of asystole. This instability was relieved by procedures which either enhanced beta adrenergic stimulation (exercise or isoproterenol infusion) or eliminated vagal influences (atropine infusion).

As shown in Figure 7, the perfusion of propranolol (or norepinephrine-free Tyrode's solution) eventually led to an abrupt suppression of subsidiary atrial pacemaker activity. The abrupt nature of the suppression suggested that impulse conduction may be an important component of subsidiary atrial pacemaker activity regulated by norepinephrine. Evidence to support this hypothesis came from microelectrode experiments which electrophysiologically characterized fibers in subsidiary atrial pacemaker tissue. Under spontaneous conditions, fibers in the immediate subsidiary atrial pacemaker region characteristically exhibited slow response action potentials with significantly smaller values of dv/dt_{max} and amplitude compared to the surrounding tissue (Table 5). Since these action potential properties are important determinants of conduction in myocardial tissue (63), it is likely that

spontaneous subsidiary atrial pacemaker impulses are conducted to the surrounding tissue with delay. That norepinephrine is important in the conduction of the spontaneous impulse was demonstrated in those preparations in which background norepinephrine was either reduced or eliminated while recording from a fiber in the subsidiary atrial pacemaker region (Figure 17). These experiments demonstrate that under conditions in which subsidiary atrial pacemaker activity is not present (zero norepinephrine), spontaneous impulses may continue to be generated. Thus norepinephrine (or beta adrenergic stimulation) improves conduction of the subsidiary atrial pacemaker impulse and relieves exit block.

While norepinephrine is involved in the conduction of the subsidiary atrial pacemaker impulse, the mechanism by which it improves the "quality" of the spontaneous impulse is not entirely understood. Since norepinephrine is known to increase the slow inward current in myocardial cells (43,187, 170) it is possible that this neuromediator enhances the inward excitatory current necessary to bring the surrounding fibers to threshold. In addition, norepinephrine is known to increase the spontaneous rate of pacemaker fibers (216,150) and to hyperpolarize the membrane possibly through stimulation of an electrogenic pump (234). Together these factors would cause activation of fibers in the immediate subsidiary atrial pacemaker region at higher membrane potentials which would enhance inward current through the fast sodium channels and thus gen-

erate a greater excitatory current that would improve conduction (206, 63). In the present study, it was found that reducing the background level or norepinephrine in the superfusate decreased the maximum diastolic potential and action potential amplitude of fibers in the pale region. Thus with norepinephrine present, these parameters were larger in magnitude at the same time that exit conduction was 1 to 1.

2. Relation of Subsidiary Atrial Pacemaker and Sinoatrial Node Activities

With background norepinephrine present in both cases, subsidiary atrial pacemaker activity had an inherently slower control spontaneous rate than sinoatrial node activity. This is a common characteristic of subsidiary or latent pacemakers (135, 226, 121) but the mathematic relationship of subsidiary and sinoatrial node spontaneous rates has been used to predict which subsidiary pacemaker is the second most automatic behind the sinoatrial node. The currently held view is that the atrioventricular junctional pacemakers are the next most automatic. Urthaler *et al.* (226) mathematically analyzed the relationship of sinoatrial node and atrioventricular junctional spontaneous rates *in vivo* and determined that the junctional pacemakers possess a degree of spontaneous activity that is 66% of the sinoatrial node. Jones *et al.* (121) later determined that the acutely emerging subsidiary atrial pacemakers at the junction of the inferior vena cava-inferior right

atrium have a spontaneous rate that is 73% of the sinoatrial node. They concluded that this pacemaker region and not the atrioventricular junction, is the next most automatic focus by virtue of its faster spontaneous rate.

The present study did not include an analysis of atrioventricular junctional activity and thus it would be difficult to estimate the rank of inferior right atrial subsidiary pacemakers in the pacemaker hierarchy of the mammalian heart. However, the mathematical relationship of subsidiary atrial and sinoatrial node pacemaker activities obtained in this study is in agreement with the results of Jones et al. (121). It was found that the subsidiary atrial pacemaker spontaneous rate was 80.5 ± 3.7% of the rate of the sinoatrial node when compared in the same atrium and under identical conditions (background norepinephrine). This value is not significantly different ($p > .20$) from the 73 ± 4% figure obtained by Jones et al. in the pentobarbital anesthetized open-chest dog. Thus it is apparent that the subsidiary atrial pacemaker tissue of the inferior right atrium represents an important component in the overall pacemaker hierarchy of the heart that displays a high degree of spontaneous activity in relation to the sinoatrial node.

3. Norepinephrine Concentration-Response

The chronotropic response of both subsidiary atrial pacemaker and sinoatrial node activities to norepinephrine occurred in a dose-dependent manner over the same range of con-

centrations (Figure 9). The primary difference was that the sinoatrial node attained a significantly greater spontaneous rate especially at higher norepinephrine concentrations. Further analysis of these data revealed that the EC_{50} for sinoatrial node activity ($2.1 \pm 0.2 \times 10^{-7}$M) was not significantly different ($p > .20$) from the EC_{50} obtained for subsidiary atrial pacemaker activity ($1.4 \pm 0.3 \times 10^{-7}$M). This suggests that although sinoatrial node activity occurs at a higher absolute level than subsidiary atrial pacemaker activity, the respective chronotropic responses to norepinephrine probably occur through a similar mechanism.

A general relationship found at most all norepinephrine concentrations tested was that the overall response took longer to develop and lasted longer for subsidiary atrial pacemaker activity than for sinoatrial node activity. It is possible that these differences in response times reflect the close association of sinoatrial node tissue to the relatively large sinoatrial node artery (208,118). While the relationship of subsidiary atrial pacemaker fibers to its vasculature has not been determined histologically, it is likely that the <u>in vitro</u> flow per unit of tissue was less than that for sinoatrial node tissue which is supplied by a much larger artery (251). The lower rate of delivery of norepinephrine to subsidiary atrial pacemaker tissue would mean that it would take longer for the tissue concentration to reach a peak value and therefore take longer to wash out.

4. Triggered Activity

The spontaneously repetitive dysrhythmia observed in the perfused preparation with elevated norepinephrine concentrations (Figure 10) resembles qualitatively the spontaneous development of triggered activity (254). It is possible that in the example shown in Figure 10, slow spontaneous subsidiary atrial pacemaker impulses acted as a triggering stimulus to another focus elsewhere in the preparation. When the faster (presumably triggered) rhythm ran its course, the slow rhythm resumed and once again triggered the rapid activity.

Another finding which suggests that the rapid phase was due to triggered activity was that a single external premature stimulus applied later in the rapid phase abruptly terminated activity. This type of phenomenon has been observed during sustained rhythmic activity in fibers of the mitral valve (253). Wit and Cranefield (253) determined that a single premature stimulus applied either early or late in the spontaneous cycle was followed by a subthreshold delayed afterdepolarization which terminated the rhythmic activity.

Microelectrode experiments conducted on non-spontaneously active tissue segments demonstrated that triggered activity could be induced under appropriate conditions. Like other studies in which it has been described (253,254), the conditions found to favor the development of triggered activity were an elevated norepinephrine concentration and an increase in the frequency of stimulation. More importantly it was found

that fibers in the pale region exhibited delayed afterdepolarizations with elevated norepinephrine, but that fibers in the surrounding tissue did not (Figure 21). Thus it appears that these fibers possess distinct characteristics that enable them to spontaneously depolarize provided norepinephrine is present and that they are activated initially by either automatic impulses or externally applied stimuli.

5. Alpha Adrenergic Regulation

The chronotropic response of both sinoatrial node and subsidiary atrial pacemaker activities to alpha adrenergic stimulation (with methoxamine) were similar in magnitude and both varied with the concentration administered. A small positive chronotropic response was obtained with a methoxamine concentration of 10^{-5}M whereas a slightly larger negative chronotropic response was observed with 10^{-4}M. The precise mechanism of action of methoxamine on automaticity is not entirely understood but its actions are mediated through an alpha receptor as evidenced by the blocking effects of phentolamine. In Purkinje fibers partially depolarized by high potassium, methoxamine has been shown to depress the slow response whereas it has no effect on normally polarized fibers (42). It is possible that at higher concentrations, methoxamine decreases the inward movement of calcium into pacemaker fibers that exhibit slow response characteristics. This would tend to slow the rate of diastolic depolarization and thus slow the spontaneous rate.

D. CHOLINERGIC REGULATION

The quantitative assessment of the chronotropic response to acetylcholine in the form of concentration-response relationships indicated that subsidiary atrial pacemaker activity was significantly more sensitive to acetylcholine than sinoatrial node activity (Figure 11). This was reflected in a comparison of the mean EC_{50} value for each curve which showed that the value for sinoatrial node activity was approximately 21 times greater than that for subsidiary atrial pacemaker activity. This finding is in agreement with studies of the conscious animal model of subsidiary atrial pacemaker activity which have documented a hypersensitivity to parasympathetic nervous input (184, 56, 148). These studies have shown that subsidiary atrial pacemaker activity is unstable (immediately after eliminating sinoatrial node influences) and is characterized by severe bradycardia, brady-tachydysrhythmia and periods of asystole. Upon administration of atropine or during augmented beta adrenergic stimulation, the instability is relieved.

The precise physiologic basis for the hypersensitivity of subsidiary atrial pacemaker activity to acetylcholine is not entirely understood. However on the basis of results obtained in this and other investigations, the hypersensitivity can be hypothesized to be due to 1) a relatively low tissue acetylcholinesterase concentration 2) a relatively high density of muscarinic receptors or 3) profound alterations in impulse formation and conduction that may be related to hy-

potheses 1 and/or 2.

Evidence to support the first hypothesis comes from previous histochemical studies plus the response to eserine observed in the present investigation. In the former case, it has been reported that the tissue concentration of acetylcholinesterase is greater in the sinoatrial node region than any other region of the right atrium (26). This could explain the response of both sinoatrial node and subsidiary atrial pacemaker activities to eserine (Table 2). It was found that the same amount of eserine produced a greater negative chronotropic response that lasted significantly longer for subsidiary atrial pacemaker activity. If it is postulated that subsidiary atrial pacemaker tissue contains less acetylcholinesterase, a given amount of eserine would inhibit a greater percentage of the acetylcholinesterase present in this tissue compared to sinoatrial node tissue. Thus the effects of eserine would be expected to be greater in magnitude and to last longer. The difference in the time to peak response may represent a smaller spontaneous release of acetylcholine in subsidiary atrial pacemaker tissue. If this were to occur, it would take longer for acetylcholine to increase to the effective concentration required to produce the maximal effect. Under conditions in which the "spontaneous release" was essentially the same for both pacemakers i.e. during perfusion of 10^{-7}M acetylcholine, the time to peak response was not significantly different. However the duration of the response was longer for

subsidiary atrial pacemaker activity again suggesting that the acetylcholinesterase concentration may be lower in this tissue.

The second hypothesis states that subsidiary atrial pacemaker tissue may contain a greater density of muscarinic receptors than an equivalent amount of sinoatrial node tissue. No study has been conducted to test this specific hypothesis but radioligand studies of muscarinic receptors in cultured heart cells suggest that the receptor population is inversely related to the density of innervation. Galper and Smith (66 , 67) have shown that in the continuous presence of a muscarinic agonist such as carbamylcholine, the number of receptor sites declines. Studies of the parasympathetic innervation of the atria using enzymatic markers indicate that the density of fibers is greatest in the sinoatrial node region compared to other areas of the right atrium (197). If receptors react in the same way in an intact tissue as in culture, it can be argued that the muscarinic receptor population in sinoatrial node tissue may be smaller than in other atrial regions such as subsidiary atrial pacemaker tissue.

The third hypothesis states that acetylcholine may modify subsidiary atrial pacemaker impulse formation and conduction to a greater extent than for the sinoatrial node. The microelectrode experiments conducted in this study suggest that acetylcholine greately affects the conduction of the spontaneous subsidiary atrial pacemaker impulse to the surrounding tissue. In the example shown in Figure 18, the subsidiary spontane-

ous rate was not different from control (Panel A) compared to when exit block occurred with acetylcholine (Panel C). However, when conduction was delayed (Panel B) a slower but conducted rhythm was manifest. The delay was so great in Panel B that when the surrounding tissue finally depolarized, the subsidiary atrial pacemaker fiber had completely repolarized. The depolarization of the surrounding tissue coincided with a subthreshold electrotonic depolarization during the early half of diastolic depolarization in the subsidiary atrial pacemaker fiber. This secondary depolarization delayed the next spontaneous impulse in a fashion similar to that predicted in other pacemakers by characteristic phase response relationships (20, 104,106). Thus, acetylcholine at a relatively low concentration which does not alter pacemaker action potential parameters significantly may modify the manifest rhythm by its delaying effects on conduction of the spontaneous impulse. At higher concentrations, exit block may occur presumably due to acetylcholine-induced decrease in membrane resistance and decrease in space and time constants (218). At still higher concentrations, impulse formation is inhibited as the membrane potential hyperpolarizes.

1. Muscarinic Receptor Blockade With Atropine

The negative chronotropic effect of acetylcholine on the sinoatrial node activity was blocked by the administration of atropine thus indicating that a muscarinic receptor

mediated the response. When atropine was administered during subsidiary atrial pacemaker activity, the spontaneous rate increased at first but soon activity ceased entirely. The mechanism of this phenomenon is not entirely understood although the abrupt cessation of activity suggested that exit block may have occurred. This hypothesis was tested in one microelectrode experiment which indicated that atropine did result in complete exit block. Automaticity continued, but the action potentials exhibited a lower amplitude and maximum diastolic potential. Thus it is possible that subsidiary atrial pacemaker activity is dependent upon some degree of spontaneously released acetylcholine from the pacemaker tissue. It could be argued that spontaneously released acetylcholine maintains a higher maximum diastolic potential of fibers in pale region. This would remove some inactivation of the fast sodium channels and shift the threshold potential of fibers surrounding the pacemaker to more negative values (93). The consequence of this factor is that the subsidiary atrial pacemaker impulse would more easily bring the surrounding fibers to threshold and thus generate a conducted impulse.

E. SENSITIVITY TO OVERDRIVE PACING

Subsidiary atrial pacemaker activity was determined in this study to be highly sensitive to overdrive pacing when compared to sinoatrial node activity (Figures 12 and 13). This finding is in agreement with *in vivo* studies that have tested

the response of both types of pacemaker activities to overdrive pacing (135, 56,185). It was not possible in this study to determine the effects of stimulus-induced neuromediator release on subsidiary atrial pacemaker suppression because of the inhibitory effects of propranolol and atropine on the manifest activity. Microelectrode experiments however revealed that during the period of suppression just prior to the first returning action potential, small subthreshold oscillations in membrane potential occurred (Figure 19). These oscillations usually had cycle lengths longer than the control spontaneous cycle length. Furthermore, the first returning action potential often was preceded by the largest oscillation. While the electrophysiologic basis of these oscillations is not known, it may represent intrinsic membrane oscillations in subsidiary atrial pacemaker fibers that failed to reach threshold i.e. impulse formation failure. The failure to reach threshold may be the result of enhanced pump activity (235), an increased potassium conductance or a more positive threshold potential of the pacemaker fiber. The last two possible mechanisms would be likely to occur as a result of an increase in the intracellular calcium concentration during the period of overdrive pacing (76) since it is known that intracellular calcium proportionately modulates both membrane potassium conductance (124) and threshold of excitation (235).

F. EFFECTS OF TETRODOTOXIN

The administration of tetrodotoxin (Figure 23) to spontaneously active fibers in the subsidiary atrial pacemaker region failed to eliminate automatic activity in two of three preparations. This suggests that the excitatory current generating the subsidiary atrial pacemaker impulse may occur by a slow channel mechanism. The decrease in spontaneous rate observed indicates that a background inward current (perhaps through partially inactivated sodium channels) may play a role in diastolic depolarization. An alternative hypothesis is that the inactive fast response fibers surrounding the subsidiary atrial pacemaker fibers exert an electrotonic "pulling" effect that holds down the slope of diastolic depolarization (193).

G. HISTOLOGY

Examination of histological sections of subsidiary atrial pacemaker tissue revealed no evidence of pacemaker fibers of the kind found in the sinoatrial node or of Purkinje-like fibers associated with internodal pathways. The lack of histologically identifiable pacemaker fibers may indicate that the actual number may be quite small in relation to the surrounding tissue. This possibility may partially explain the observed requirement for background beta adrenergic stimula-

tion. Beta stimulation may be required to increase the inward excitatory current of a relatively small mass of subsidiary pacemaker fibers in order to depolarize a larger mass of surrounding tissue. The lack of identifiable pacemaker fibers also may indicate that there are fibers other than P cells or Purkinje-like fibers that possess the characteristic of automaticity. This hypothesis may explain the different characteristics of pacemaker activity found for subsidiary atrial pacemakers in comparison to sinoatrial node pacemakers.

SUMMARY

Sinoatrial node and subsidiary atrial pacemaker activities were characterized using an isolated canine right atrial preparation maintained *in vitro* by perfusion of Tyrode's solution through the sinoatrial node artery. Under control conditions it was estimated, by extracellular mapping, that pacemaker activity originated from the sinoatrial node region at the superior vena cava-right atrial junction. Subsidiary atrial pacemaker activity was unmasked under conditions of suppressed sinoatrial node activity and in the continuous presence of a background level of norepinephrine (10^{-8} M) in the perfusate. Extracellular mapping indicated that subsidiary atrial pacemaker activity was associated with a distinct pale region located at the junction of the inferior vena cava-inferior right atrium far removed (2-3 cm) from the sinoatrial node region.

In addition to estimating the anatomic location of origin of pacemaker activity, each pacemaker was characterized further by determining its chronotropic response to neuromediators and to overdrive pacing. It was found that both pacemaker activities were sensitive to norepinephrine in the same concentration range (5×10^{-8} - 5×10^{-6} M) although the sinoatrial node attained a greater spontaneous rate at each con-

centration tested. It also was determined that subsidiary atrial pacemaker activity was 21 times more sensitive to acetylcholine than that of the sinoatrial node. As for the response to overdrive pacing, subsidiary atrial pacemaker activity exhibited suppression measured in seconds compared to sinoatrial node activity in which suppression was measured in milliseconds. This indicated that subsidiary atrial pacemakers were significantly more sensitive to overdrive pacing.

A second series of experiments was conducted to determine the cellular electrical events taking place during spontaneous subsidiary atrial pacemaker activity. In these experiments, a single microelectrode and a bipolar electrode were used to record from endocardial fibers of tissue segments containing the pale region previously found to be associated with subsidiary atrial pacemaker activity in the perfused preparation. Slow response pacemaker action potentials were recorded consistently from the pale region while fast responses were recorded from the surrounding tissue. The propagation of subsidiary atrial pacemaker impulses to the surrounding tissue was found to be dependent upon the presence of norepinephrine in the superfusate. Reducing the norepinephrine concentration or administering acetylcholine produced partial or complete exit block, although pacemaker impulse formation continued. When propagation was delayed, depolarization of the surrounding tissue was coincident with secondary depolarizations of the subsidiary atrial pacemaker action potentials that modulated

the spontaneous cycle length. It is concluded that autonomic neuromediators play an important role in regulating propagation of subsidiary atrial pacemaker impulses and therefore in determining the manifest or conducted subsidiary rhythm.

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The final copies have been examined by the director of the dissertation and the signature that appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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