ABSTRACT

Transmembrane action potentials were recorded from multiple sites in isolated canine anteromedial left atrial wall preparations with the anterior mitral valve leaflet attached. The preparations were superfused with Tyrode's solution. When the left atrial wall was electrically stimulated, activity propagated into the mitral valve leaflet. Typical atrial action potentials occurred in atrial wall fibers. However, maximum diastolic potential, total action potential amplitude, and rate of depolarization decreased markedly in the atrium overlying the fibrous annulus (junctional region), and repolarization characteristics were altered in this region. Mitral valve muscle fibers demonstrated still lower maximum diastolic potential, action potential amplitude, and rate of depolarization. Electrical excitation of single discrete regions in the mitral valve leaflet did not result in conduction to the atrium; conduction block occurred in the junctional region. However, simultaneous excitation of several mitral valve sites did cause an impulse to propagate to the atrium, but transmembrane action potentials of junctional fibers were characteristically different from those recorded from the same junctional fibers during activation from the atrium. Muscle fibers in the mitral valve leaflet were capable of developing spontaneous diastolic depolarization, which resulted in automatic impulse initiation, when they were exposed to epinephrine or norepinephrine (1 × 10^-7 to 1 × 10^-6M) or when they were stretched. Spontaneous diastolic depolarization and automaticity also occurred occasionally without pharmacological or other experimental interventions; moreover, spontaneous activity originating in the mitral valve leaflet could propagate into and activate the atrial wall. Acetylcholine abolished spontaneous activity. These data suggest that the mitral valve could act as a site of ectopic impulse initiation in the left atrium.

KEY WORDS: catecholamines, atrial extrasystoles, acetylcholine, atrial tachycardia, junctional region, summation, automaticity, conduction block.

The site of origin and the mechanism of many atrial dysrhythmias in man are unknown. Ectopic atrial impulses might arise in specialized fibers of the right atrial internodal tracts (1-3) or in fibers of the coronary sinus or its surrounding areas (4-6). Atrial tachyarrhythmias—flutter and fibrillation—might result from automatic foci in these regions or from reentry within the atrium or the atrioventricular (AV) node (5-9). Little consideration has been given to left atrial sites of origin for atrial dysrhythmias, possibly since only a few studies have demonstrated the ability of left atrial muscle to beat spontaneously (10-12). There is controversial electrocardiographic evidence, however, suggesting left atrial sites of origin for certain atrial dysrhythmias (13-19).

Left atrial muscle extends into the leaflets of the mitral valves (20-23). Although valve muscle is a direct extension of left atrial muscle, transmembrane action potentials recorded from muscle fibers in the canine valve leaflet are characteristically different from action potentials of working left

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atrial muscle cells (22). In the present study, we used microelectrode recording techniques to demonstrate that the mitral valve muscle could be a site of spontaneous impulse initiation. We also delineated a junctional region of muscle cells between valve muscle and atrial wall muscle which could act as a site of conduction block for impulses arising in the valve. Our results suggest that these regions might be a site of origin for certain atrial dysrhythmias.

Methods
Electrophysiological investigations were conducted on preparations isolated from 25 canine hearts. Healthy mongrel dogs weighing 15–20 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). The hearts were rapidly removed through a thoracotomy and dissected at room temperature in a modified Tyrode's solution, the composition of which has previously been described (22). Tyrode's solution was equilibrated with a gas mixture of 95% O₂-5% CO₂. For dissection of the anterior (septal) mitral leaflet, the left atrium was opened posteriorly by an incision extending from a pulmonary vein to the atrial appendage, and the left ventricle was opened laterally by an incision through the free wall. The chordae tendineae were severed at their attachments to the anterior and posterior papillary muscles. The anterior leaflet was separated from the posterior leaflet by incisions through the anterolateral and posteromedial commissures. A bit of the commissural leaflets on both lateral borders of the anterior leaflet was included in the preparation. When the mitral valve leaflet alone was studied, the leaflet was detached 2–3 mm below the annulus to ensure the absence of atrial wall muscle from the preparation. In other preparations, anteromedial left atrial wall remained attached to the valve leaflet. For these latter preparations the incision through the anterolateral and posteromedial commissures was complete, the microelectrode was forced down into the tissue; the tip and shank were broken off and left in the preparation. When the mitral valve leaflet alone was studied, the leaflet was detached 2–3 mm below the annulus to ensure the absence of atrial wall muscle from the preparation. In other preparations, anteromedial left atrial wall remained attached to the valve leaflet. For these latter preparations the incision through the anterolateral and posteromedial commissures was complete, the microelectrode was forced down into the tissue; the tip and shank were broken off and left in the preparation. The preparation was electrically stimulated through bipolar Teflon-coated silver wire electrodes located either on the atrial wall, the mitral valve leaflet, or both. Stimuli were rectangular pulses 3–6 msec in duration and one and a half to two times threshold, generated by Tektronix 160 pulse generators. The stimuli were suitably isolated from ground.

Transmembrane action potentials were recorded through machine-pulled glass capillary microelectrodes filled with 3M KCl (tip resistance 15–30 megohms). These microelectrodes were coupled by Ag-AgCl wires to high input impedance amplifiers with capacitance neutralization (type NPI Bioelectric Instruments Inc.). Three identical assemblies were used, and the outputs of the amplifiers were displayed on a Tektronix 565 oscilloscope and photographed with a Grass C-4 oscillographic camera or a Polaroid camera. Extracellular electrograms were recorded through bipolar silver wire electrodes and a suitable d-c amplifier.

The maximal rate of depolarization (Vmax) of atrial wall transmembrane action potentials was determined by electronic differentiation as previously described (22). For muscle fibers in the mitral valve Vmax was exceedingly low and was therefore determined by oscilloscopic display of the upstroke at a rapid sweep speed (5 msec/cm) and direct measurement of the slope of the rapid component of depolarization from photographs.

In ten experiments the sequence of excitation was determined during both electrical stimulation of the valve and spontaneous rhythm. During both driven and spontaneous rhythms, an extracellular electrogram recorded from a fixed site on the atrial wall was used as a time reference. Transmembrane action potentials were recorded from 30–50 locations in the mitral valve leaflet and the left atrial wall. The time of activation of each site relative to the reference site was determined at an oscilloscope sweep speed of 5–20 msec/cm. The distance between each recording site and the reference site was determined using the calibrated lateral stage movement on the micromanipulator. The times of activation relative to the distance from the reference electrode were then plotted on a two-dimensional diagram of the preparation.

In five experiments the anatomical location of the microelectrode was correlated with the electrophysiological characteristics of the recorded transmembrane action potential. After the electrophysiological study was complete, the microelectrode was forced down into the tissue; the tip and shank were broken off and left in

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Location of the three electrophysiologically different areas in the anteromedial left atrial wall and the mitral valve leaflet. Left: Enlarged photograph of a transverse section containing the mitral valve leaflet (MV), the fibrous annulus (An), the anteromedial left atrial wall (At) overlying the aorta (Ao), and the noncoronary posterior aortic cusp (Av). The tissue space between the atrial wall and the adventitia of the aorta is delineated by the white arrows. Right: Three photomicrographs (X, Y, and Z) of equal magnification corresponding to the regions marked X, Y, and Z in the left section of the figure. Locations of microelectrode recording sites are indicated by E. The diminishing thickness of the muscle (M) from the atrial wall (X), through the atrial muscle over the annulus (Y) (designated as the electrophysiological functional area in this study), and into the mitral valve leaflet (Z) is clearly demonstrated. In the valve leaflet and over the annulus, the muscle is separated from the fibrosa (F) by a loose connective tissue stroma (S). The muscle of the atrial wall is separated from the aorta by a naturally occurring tissue space. Tissue folds in the fibrosa are a sectioning artifact due to swelling of the collagen after prolonged superfusion with Tyrode’s solution. The physiological solution was then replaced with 10% neutral buffered formaldehyde. After fixation, the electrode was removed leaving a hole in the endocardial surface. The preparation was then embedded in paraffin by standard techniques and sectioned transversely so that each section contained a transverse view of the atrial wall and the mitral valve leaflet (Fig. 1). Sections were stained with hematoxylin-phloxine-saffarine and examined by light microscopy at magnifications between 10x and 100x to locate the hole made by the microelectrode. 

**Results**

**LOCATION OF MICROELECTRODE RECORDING SITES**

Regions of the left atrium and the mitral valve leaflet demonstrated dissimilar transmembrane action potential characteristics. On this basis, three
electrophysiologically different areas were delineated and correlated with the location of the recording site (see Methods). These areas were: (1) the anteromedial atrial wall (Fig. 1X), (2) an electrophysiological junctional region between the atrial wall and the mitral valve leaflet which was composed of the atrial muscle overlying the fibrous annulus (this region corresponds to the free-hanging flap of the atrial wall) (Fig. 1Y), and (3) the mitral valve leaflet (Fig. 1Z). The density and the thickness of the atrial muscle fibers decreased markedly in the junctional region and decreased even further to a thin ribbon of fibers in the mitral valve leaflet (Fig. 1). We emphasize that the electrophysiological junctional region is anatomically not considered part of the valve leaflet (22, 25) (see below for a description of action potentials recorded in this area and the rationale for the definition of a junctional region). This subdivision, although arbitrary, enables this region to be referred to separately from the atrial wall and the mitral valve leaflet.

CHARACTERISTICS OF TRANSMEMBRANE ACTION POTENTIALS RECORDED FROM ANTEROMEDIAL LEFT ATRIAL WALL AND JUNCTIONAL REGION

Electrical stimulation of the anteromedial left atrial wall results in activation of the entire left atrial musculature and the cardiac muscle in the valve leaflet (22). Stimulation of the valve leaflet results in electrical activity in the valve which propagates from the valve leaflet into the left atrial wall only under certain conditions.

We recorded transmembrane action potentials at intervals of approximately 1 mm, progressing from the anteromedial left atrial wall toward and eventually into the valve leaflet. Action potentials recorded from left atrial muscle in the atrial wall extending to but not including the atrial muscle overlying the fibrous annulus (i.e., electrophysiological junctional area) were typical of potentials reported elsewhere for atrial muscle (1, 2, 26). The repolarization phase was concave with an initial rapid repolarization that became progressively slower (Fig. 2A). Values for total action potential duration, maximum diastolic potential, total action potential amplitude, and Vmax of left atrial muscle fibers are included in Table 1. Transmembrane action potential characteristics of left atrial muscle fibers did not vary whether the excitatory impulse was initiated by stimulation of the atrium or the mitral valve leaflet. Activation time in left atrial muscle was rapid; activity spread from the stimulating electrode to the low atrium, a distance of approximately 20 mm, in 6-10 msec.

Transmembrane action potential characteristics changed markedly in the atrial muscle overlying the fibrous annulus. Muscle fibers in this region (when activated by impulses propagating from the atrial wall) did not have the concave repolarization time course of atrial wall muscle fibers but, rather, exhibited a short plateau (Fig. 2B and C). In addition, fibers in this region had lower maximum diastolic potential, total action potential amplitude, and Vmax compared with the values recorded for muscle fibers in the anteromedial atrial wall (Table 1). Maximum diastolic potential, total amplitude, and Vmax progressively decreased as the recording electrode was moved closer to the mitral valve leaflet. Depolarization of the muscle fibers nearest the base of the valve (but not in the valve leaflet) often occurred in two phases, an initial rapid depolarization followed by a slower depolarization...
ACTION POTENTIALS OF MITRAL VALVE LEAFLET

| TABLE 1 |

<table>
<thead>
<tr>
<th>Action Potential Characteristics of Atrial, Junctional, and Mitral Valve Fibers</th>
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<td>No. of impalements</td>
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All values are means ± sd for 15 preparations. LAt = left atrium, Mj = mitral junction, MV = mitral valve, MDP = maximum diastolic potential, APA = total action potential amplitude, APD = action potential duration at 50% and 100% repolarization, and Vmax = maximum rate of depolarization.

Transmembrane action potential characteristics of atrial fibers in the region overlying the fibrous annulus were intermediate between those noted for the atrial wall and the valve (22, also see below); since the atrial fibers exhibiting such characteristics were interposed between the atrium and the valve proper, for convenience we will refer to this area as the (electrophysiological) junctional region.

CHARACTERISTICS OF TRANSMEMBRANE ACTION POTENTIALS RECORDED FROM THE ANTERIOR MITRAL VALVE LEAFLET

Transmembrane potentials of muscle fibers in the mitral valve leaflet proper showed still lower maximum diastolic potentials, total action potential amplitudes, and Vmax compared with those for left atrial and junctional fibers (Table 1). Transmembrane action potentials in the mitral valve leaflet demonstrated several different contours. Some action potentials had a prominent phase 1 and plateau phase (Fig. 2F), as we have previously described (22). Other transmembrane action potentials recorded in the mitral valve leaflet lacked this prominent phase 1 (Fig. 2E). Depolarization of mitral valve leaflet fibers sometimes occurred in several phases; an initial rapid depolarizing phase was followed by a slower depolarization in some cells (Fig. 2E). Maximum diastolic potential of muscle fibers in the valve leaflet occurred immediately after repolarization, whereupon diastolic potential decreased slowly to less negative values; we have previously described this characteristic change in diastolic potential as an afterpotential (22), and it is further discussed below. Excitation spread very slowly (40-60 msec) throughout the muscle in the valve.

RETROGRADE CONDUCTION FROM MITRAL VALVE TO LEFT ATRIUM

When close bipolar electrodes (interelectrode distance approximately 1 mm) were used to stimulate the mitral valve leaflet at one localized site, excitation of mitral valve leaflet fibers at this site did not result in impulse propagation to left atrial muscle or to other regions of the valve leaflet (Fig. 3I). Only threshold stimuli were used to excite the mitral valve fibers. Slow, low-amplitude depolarizations of fibers in the junctional area suggested that conduction block of the impulse between valve leaflet and atrial wall occurred near to or in this region. This phenomenon was confirmed by microelectrode recordings from multiple sites between the stimulating electrodes and the junctional region. Action potential amplitude diminished markedly as the junctional region was approached until only these low-amplitude depolarizations were observed. Excitation of mitral valve fibers elicited at a localized site did not propagate to other regions of the valve. Low-amplitude depolarizations were also observed in these other regions of the valve leaflet which demonstrated block. In some experiments three pairs of close bipolar electrodes were placed at widely separated regions of the valve leaflet; simultaneous stimulation of these regions resulted in conduction to the atrial wall (Fig. 3II). Stimulation through one pair of electrodes resulted in conduction block as described above. Stimulation through the first and second pairs of electrodes resulted in an increase in amplitude of the depolarization in the junctional region, still without conduction to the atrial wall. Stimulation through all three pairs of electrodes resulted in a further increase in amplitude of the junctional potentials, and the impulse was conducted to the atrial wall (Fig. 3III). The pathway of impulse spread from valve to atrial wall was verified by detailed microelectrode mapping in each experiment employing three pairs of bipolar electrodes.

When the muscle over the fibrous annulus was activated by impulses initiated by electrical stimulation of the valve leaflet, the characteristics of the action potentials recorded within this junctional...
or a prepotential which was not present when these fibers were excited by an impulse originating in the atrium. During mitral valve leaflet stimulation, junctional cells also had a markedly slowed rate of depolarization, and the time course of repolarization was sometimes prolonged. Furthermore, when activated by impulses originating in the mitral valve leaflet, the amplitude of action potentials recorded from junctional fibers varied in a graded manner, depending on the number of mitral valve regions excited, i.e., the number of stimulating sites (Fig. 4). Maximum action potential amplitude resulting from stimulation at three mitral valve sites rarely equaled the amplitude observed when the junctional fiber was excited by an impulse originating in the left atrial wall.

**SPONTANEOUS IMPULSE INITIATION IN THE MITRAL VALVE LEAFLET**

Muscle fibers in the mitral valve leaflet were capable of developing spontaneous depolarization during diastole (phase 4), which could result in automatic impulse initiation. Spontaneous activity was occasionally observed in preparations not subjected to any experimental interventions. Such spontaneous activity occurred immediately after the preparation was mounted in the tissue chamber and persisted for 10–30 minutes during superfusion with Tyrode's solution. Spontaneous activity always could be initiated in whole isolated valve leaflets (not attached to left atrium) and strips of valve leaflets by superfusion with catecholamine or application of tension causing stretch of the valve preparation.

Transmembrane action potentials recorded from mitral valve fibers exhibited some spontaneous decrease in membrane potential during phase 4 immediately after attaining maximum diastolic potential (Fig. 5, control). This decrease in membrane potential resembled an afterpotential, and the time course of repolarization was sometimes prolonged. Furthermore, when activated by impulses originating in the mitral valve leaflet, the amplitude of action potentials recorded from junctional fibers varied in a graded manner, depending on the number of mitral valve regions excited, i.e., the number of stimulating sites (Fig. 4). Maximum action potential amplitude resulting from stimulation at three mitral valve sites rarely equaled the amplitude observed when the junctional fiber was excited by an impulse originating in the left atrial wall.

**FIGURE 3**

Retrograde conduction from mitral valve leaflet (MV) to left atrial wall (At) during stimulation of multiple sites in the mitral valve leaflet. Bipolar stimulating electrodes are located on the mitral valve at S1, S2, and S3. The distance between each pair of stimulating electrodes is 5 mm. Transmembrane action potentials were recorded immediately adjacent to each pair of stimulating electrodes and at site Mj in the junctional area between mitral valve and left atrial wall. An extracellular electrogram (EG) was recorded from atrial muscle. IA and IB are records obtained during stimulation at S1 only. In IA stimulation at S1 initiated an action potential in a muscle fiber adjacent to the stimulating electrode (bottom trace), but conduction did not occur to a fiber located adjacent to S2 (middle trace, note low-amplitude deflection) or to the atrial wall as indicated by the lack of deflection in the atrial electrogram (top trace). Circulation also did not occur to sites adjacent to S3 (not shown). In IB stimulation at S1 again initiated an action potential in a different fiber adjacent to the stimulating electrode (bottom trace), but only a low amplitude deflection occurred in a fiber in the junctional region at Mj (middle trace). Conduction to the atrial wall did not occur (top trace) nor did conduction to other stimulating sites (not shown). In IIA, IIB, and IIC, the top trace is the atrial electrogram and the middle trace is the transmembrane potential recorded from the same single fiber in the junctional region at Mj. In IIA stimulation at S1 initiated an action potential in a fiber adjacent to the stimulating electrode (bottom trace), and only a low-amplitude deflection occurred at Mj without conduction to the left atrial wall (no deflection in EG). Conduction to other stimulating sites also did not occur (not shown). In IIB S1 and S2 were stimulated simultaneously. The bottom trace shows the transmembrane action potential at S1 only. Note that the amplitude of the response at Mj increased (middle trace), but conduction to the left atrial wall still did not occur (top trace). Conduction to S2 also did not occur (not shown). In IIC S1, S2, and S3 were stimulated simultaneously. The bottom trace shows the transmembrane potential at S1 only. The amplitude of the response at Mj increased further (middle trace), and conduction to the left atrial wall occurred as indicated by the deflection in the atrial electrogram (top trace).

Muscle fibers in the mitral valve leaflet were capable of developing spontaneous depolarization during diastole (phase 4), which could result in automatic impulse initiation. Spontaneous activity was occasionally observed in preparations not subjected to any experimental interventions. Such spontaneous activity occurred immediately after the preparation was mounted in the tissue chamber and persisted for 10–30 minutes during superfusion with Tyrode's solution. Spontaneous activity always could be initiated in whole isolated valve leaflets (not attached to left atrium) and strips of valve leaflets by superfusion with catecholamine or application of tension causing stretch of the valve preparation.

Transmembrane action potentials recorded from mitral valve fibers exhibited some spontaneous decrease in membrane potential during phase 4 immediately after attaining maximum diastolic potential (Fig. 5, control). This decrease in membrane potential resembled an afterpotential, since at long stimulation cycle lengths or after termination of electrical stimulation the decrease in membrane potential ceased when it reached −65 to −80 mV (before threshold potential was reached) and then membrane potential remained at a steady value (Fig. 5, control). The addition of either epinephrine or norepinephrine to the superfusate (final concentration 1 x 10⁻⁸ to 1 x 10⁻⁶ M) resulted in a marked increase in the slope of phase-4 depolarization. Spontaneous diastolic depolarization no longer terminated before threshold potential was reached but continued until spontaneous action potentials were generated in the absence of...
Comparison of action potential characteristics of a functional fiber during activation from the left atrial wall and from the mitral valve leaflet. All records are from a maintained impalement of the same single fiber. The topmost section of the figure demonstrates the transmembrane action potential recorded when the left atrial wall was stimulated: the bottom trace is an atrial electrogram recorded near the site of stimulation. A-E show action potential characteristics of the same fiber when the mitral valve leaflet was stimulated: the bottom trace is the electrogram recorded from the left atrial wall. In A only one mitral valve site was stimulated (Fig. 3). In B two mitral valve sites were stimulated, and in C, D, and E three sites were stimulated. Note the graded increase in action potential amplitude, the slow prepotential, the slowed rate of depolarization, and the altered configuration when this fiber is activated from the mitral valve (A-E).

For action potential amplitudes in A, B, and C conduction to the left atrial wall did not occur (no atrial electrogram deflection in bottom trace). In D and E conduction to the atrial wall occurred.

Catecholamine induced spontaneous activity in a mitral valve fiber. The preparation consists of the mitral valve leaflet only. Records are from maintained impalement of the same fiber throughout the experiment. The control record (top) shows the characteristics of the action potentials during electrical stimulation at a cycle length of 2000 msec. Note that some spontaneous depolarization occurred immediately after repolarization. This spontaneous depolarization appeared to be an after potential, since it ceased when membrane potential reached −65 mV (before threshold potential was reached). A and B show the response of this fiber to superfusion with Tyrode’s solution containing epinephrine (Epi) (1 × 10⁻⁷ M). During epinephrine intervention the mitral valve was not stimulated electrically. Note the increase in slope of spontaneous depolarization during diastole (phase 4) which gradually merged with the upstroke of the action potential. In A the fiber was firing spontaneously at a cycle length of 600 msec and in B the spontaneous cycle length was 500 msec.

Stretching the mitral valve leaflet 10% from resting length (see Methods) also resulted in spontaneous diastolic depolarization and automatic activity in the absence of electrical stimulation; this response was similar to that described for catecholamine (Fig. 6). In these experiments we were unable to maintain transmembrane recordings.
Spontaneous activity in mitral valve fibers induced by stretch. The preparation consists of the mitral valve leaflet only. Control records show a recording of transmembrane action potentials at resting length. Similar recordings were obtained for eight separate impalements. During control the preparation was electrically stimulated at a cycle length of 1100 msec. No spontaneous activity was observed when the stimulator was turned off. After the valve leaflet was stretched 10% above its initial length (see Methods), spontaneous depolarization occurred at a cycle length of 600 msec. Action potentials could not be recorded from the same fiber during the stretching procedure; the occurrence of spontaneous activity was documented in an additional eight fibers after stretching of the valve leaflet. Recordings from one such fiber are shown (bottom). We emphasize that this fiber is not the same one from which control action potentials were recorded. In the bottom part of the figure, the valve leaflet is not being electrically stimulated.

during the stretching procedure. Therefore, action potentials were recorded from eight to ten mitral valve muscle fibers prior to stretching and then from an additional eight to ten fibers after stretching had been accomplished. The rate of spontaneous action potential initiation after stretching was 80 ± 8/min in five experiments.

Acetylcholine suppressed spontaneous diastolic depolarization in fibers of the valve leaflet and eventually abolished the automatic firing of the mitral valve muscle (Fig. 7). The acetylcholine was added directly to the bath to give the desired concentration. Superfusion with Tyrode’s solution was not stopped. Initially, when automaticity was abolished, direct electrical stimulation of the valve leaflet could not elicit an action potential (Fig. 7B). Automaticity resulting from stretching the valve leaflet was completely abolished by acetylcholine (1 × 10⁻⁸M). Automaticity resulting from superfusion with epinephrine or norepinephrine (1 × 10⁻⁴ to 5 × 10⁻⁷M) also was abolished by acetylcholine (1 × 10⁻⁵M).

Activity arising spontaneously in the mitral valve leaflet as a result of superfusion with Tyrode’s solution containing catecholamine was capable of conducting into and activating left atrial muscle when it was left attached to the mitral valve leaflet. In these experiments, the sequence of activation was determined (see Methods) to localize the site of origin of electrical activity and the pathway of propagation. Fibers in the areas of earliest activation always exhibited typical pacemaker potentials, i.e., marked phase-4 depolarization gradually merging into a slow action potential upstroke (26). Determinations of the sequence of activation also enabled us to localize sites of conduction block when this phenomenon occurred. In addition, at the termination of each study, the valve leaflet was severed from the atrium, and we verified that only the valve, and not the left atrium, was spontaneously active under the experimental conditions. Isolated spontaneous impulses occasionally occurred in the anteromedial left atrial wall after the valve leaflet was severed from it, but these impulses were very infrequent and irregular.

During superfusion with catecholamine all impulses originating in the mitral valve leaflet at slow spontaneous rates (40-80/min) conducted into and activated the left atrial wall (Fig. 8). At more rapid rates of spontaneous impulse formation induced by catecholamines in the mitral valve leaflet (80-200/min), conduction to the left atrium often was impaired (Fig. 9); complete or partial conduction block often occurred. During complete conduction block, rapid spontaneously occurring action potentials were recorded in the valve leaflet in the absence of atrial activation (Fig. 9A). During partial block, every second, third, or fourth
FIGURE 8

Propagation of spontaneous activity in the isolated anterior mitral valve leaflet (MV) and left atrial wall (At) preparation superfused with Tyrode's solution containing epinephrine \((1 \times 10^{-5} \text{m})\). Left: Schematic diagram of the preparation. MJ designates the junctional area and EG the position of extracellular recording electrodes positioned on the left atrial wall. Numbers on diagram of the preparation indicate the time in msec from the extracellular electrogram deflection to the upstroke of a transmembrane action potential recorded at that site. Fifty such measurements were made, but only representative areas are included on the diagram for clarity. Note that all numbers are negative, indicating that these sites were activated earlier than the atrial site. The earliest activated region was in the mitral valve leaflet \((-175 \text{ msec})\), and the sequence of activation indicates that activity propagated from this area in the mitral valve across the junctional area and into the atrium. Right: Transmembrane action potentials recorded during the spontaneous rhythm from the region of earliest activity in the mitral valve (bottom trace) and from the junctional area immediately adjacent to the atrial wall (it was not possible to determine with certainty whether this recording was from the atrium or the junctional area) (middle trace). The atrial electrogram is shown in the top trace.

spontaneous valve action potential conducted into and depolarized the atrial wall muscle (Fig. 9D and E). Periods of complete block or partial block between the mitral valve leaflet and the left atrium often alternated with 1:1 conduction. This 1:1 conduction resulted in paroxysms of rapid atrial activity (up to 200/min) originating from the mitral valve leaflet and lasting several seconds to several hours (Fig. 9F).

We mapped the sequence of activation during conduction block and noted that the site of block to impulses originating in the valve leaflet occurred in the junctional area between the valve leaflet and the atrial wall. During conduction block of impulses from rapidly firing mitral valve fibers, low-amplitude depolarizations occurred in these junctional fibers; these low-amplitude depolarizations were synchronous with the spontaneous activity recorded in the valve leaflet (Fig. 9A, C, D, and E). Such low-amplitude depolarizations during spontaneous mitral valve leaflet activity were similar to the low-voltage potentials occurring in the junctional fibers during electrical stimulation of the valve leaflet.
associated with block of impulse conduction to the atrium. Conduction to the atrium of spontaneously arising impulses in the valve leaflet occurred when the amplitude of the junctional potentials increased; this increase sometimes occurred in a graded manner (Fig. 9). Possible mechanisms are considered in the discussion. A graded increase in action potential amplitude was also observed in junctional fibers during studies on electrical stimulation of the valve leaflet. Junctional transmembrane action potentials of large amplitude occurred when spontaneous impulses conducted from the mitral valve leaflet to the left atrial wall (Fig. 9C, D, E, and F). During conduction of catecholamine-induced spontaneous mitral valve impulses, transmembrane potentials of junctional cells showed a slow prepotential (Fig. 9C, D, E, and F) similar to that which occurred in the junctional region during electrical stimulation of the valve leaflet; activation of the same junctional fiber from the atrium resulted in a rapid upstroke of the action potential with no prepotential (Fig. 10). Also, during conduction of spontaneous impulses from valve leaflet to atrial wall, the total amplitude of depolarization of junctional fibers often was less than the total action potential amplitude of the same fibers when they were activated from the atrium (Fig. 10).

When there was a high degree of conduction block between the mitral valve and the atrial wall (3:1, 4:1, 5:1) of impulses arising from rapid spontaneous activity in the valve leaflet, action potentials could be elicited in the atrial wall independently by electrical stimulation (Fig. 11). When the stimulus cycle length was longer than the spontaneous cycle length of the valve muscle fibers, these atrial impulses did not propagate into the valve leaflet. It was possible that the fibers of the junctional region were refractory to excitation, since these fibers were being partially depolarized by the impulses arising in the valve leaflet. However, occasional impulses arising in valve leaflet muscle were capable of propagating into the atrium during atrial diastole (between electrically stimulated

![Figure 10](image1)

**Figure 10**
Alternate activation of junctional fiber from spontaneous mitral valve impulses and atrial stimulation. The top trace (AEG) is an atrial electrogram recorded from the left atrial wall. The middle trace is a transmembrane recording from a fiber in the junctional region (Mj), and the bottom trace is a recording from a fiber in the mitral valve (MV). The mitral valve leaflet is spontaneously active due to superfusion with Tyrode's solution containing $1 \times 10^{-4}$ M epinephrine. After every spontaneous impulse an electrical stimulus was applied to the atrial wall at different times in the basic cycle length. When the junctional fiber was activated by an impulse originating in the mitral valve leaflet (open arrow), it showed a slow initial depolarization which then became more rapid. This activity was conducted to the atrial wall. When the junctional fiber was activated by atrial stimulation (solid arrow) the slow prepotential was absent, and there was rapid depolarization and a marked increase in the total amplitude of the junctional action potential. These differences were not dependent on the time within the basic cycle length during which the atrium was electrically stimulated. The hump on the repolarization phase of the junctional fiber after atrial stimulation may be due to electrotonic interaction with mitral valve fibers.

![Figure 11](image2)

**Figure 11**
Interpolated extrasystoles arising in the mitral valve leaflet. The top trace in A and B is an atrial electrogram (AEG), the middle trace was recorded from the junctional region (Mj), and the bottom trace was recorded from a spontaneously depolarizing mitral valve fiber (MV). The preparation is being superfused with Tyrode's solution containing $(1 \times 10^{-4})$ M epinephrine. In A, the mitral valve leaflet was spontaneously active at a cycle length of 450 msec, and 3:1 conduction block existed between the valve leaflet and left atrial wall. In B, during the spontaneous activity in the valve leaflet, the atrial wall was stimulated at a cycle length of 1600 msec. This stimulated atrial activity depolarized the junctional fiber (not the same fiber as in A) but was not conducted into the mitral valve leaflet. Occasional impulses, however, were conducted from the valve leaflet to the atrium as interpolated atrial extrasystoles (arrow). Note the slow prepotential and the reduced amplitude of the junctional action potential.
Discussion

Except for the brief reference to single-cell electrical activity in the mitral valve by Cooper et al. (27) and our own previous report (22), this study represents the only documentation of transmembrane electrical properties in this anatomic area.

We clearly showed that the electrophysiological characteristics of left atrial muscle overlying the fibrous annulus (junctional region) and in the anterior mitral valve leaflet were quite dissimilar from those of atrial muscle in the anteromedial left atrial wall. In the junctional region, membrane potential and action potential amplitude were lower than they were in the left atrial wall; action potential configuration also changed. In the valve leaflet, the low-amplitude, slowly rising action potentials, the slow conduction, and the pacemaker activity resembled characteristics previously described for the sinus and AV nodes (26). We will consider several possible explanations for these unusual electrophysiological properties of the junctional region and the mitral valve leaflet, including (1) relation of the embryological origin of the valve leaflets to that of the AV node, (2) ultrastructural similarities of valve muscle to sinus or AV nodal muscle, and (3) ionic conductances underlying action potential genesis in the junctional region and the mitral valve leaflet which differ from those in atrial wall fibers.

The left AV valves are formed from projections of the endocardial cushions. The extremities of these projections, destined to form the valves, are initially connected with the ventricular wall by muscular trabeculae which become papillary muscles, and this ventricular muscle eventually invades the entire valve leaflet. Atrial muscle takes no part in the composition of the valve leaflets at this stage (28). The ventricular muscle is gradually entirely replaced by collagen, leaving a completely fibrous valve and chordae tendineae. The origin of the atrial muscle in the valve is unknown. The only explanation offered is that atrial muscle from the atrial wall spreads onto the surface of the valves in the later months of fetal life as the valves grow. This spread may result from increased infolding of the AV sulcus, which deepens with the increasing size of the heart (28). It is possible that this atrial tissue which spreads onto the surface of the valve during restructuring is derived from the AV ring, although this possibility is only our speculation. Detailed microelectrode investigations of the AV ring by Lieberman and Paes de Carvalho (29, 30) in chick embryo and by Hoffman and Cranefield (26) in the adult rabbit indicate that it has many electrophysiological characteristics similar to those of the AV node in these species and to those we demonstrated for the junctional region and the mitral valve leaflet.

The slow conduction and the pacemaker activity occurring in sinus and AV nodal tissue have also been attributed to certain structural features. The decrease in diameter of the atrial fibers at the atrial junction of the AV node, the profuse interconnections of such fibers (26), and the lack of specialized intercellular junctions may be responsible for slow conduction in the AV node (31, 32). The pacemaking properties of sinus and AV nodes have been attributed to cells which are structurally different from ordinary atrial muscle and are called P cells (31).

We did not attempt to compare the diameter of atrial fibers in the junctional area and the mitral valve leaflet with that of fibers in the atrial wall, and we cannot speculate on whether a difference in diameter is a factor in determining the alteration in action potential characteristics and conduction of this region. Intercalated disks containing specialized cell junctions are commonly found in the muscle of the mitral valve (22). Valve muscle has not been compared with the AV node with respect to the intracellular ultrastructure is identical for muscle in extent of fibers with direct membrane apposition, the valve and ordinary atrial muscle (21, 22). We did not see fibers resembling P cells in the mitral valve leaflet, although they may exist in this region. The only structural change which we did observe in the junctional region and the mitral valve leaflet was a significant decrease in the density of atrial muscle fibers (Fig. 1). In the atrial muscle overlying the fibrous annulus and in the proximal third of the valve, bundles of muscle fascicles are surrounded by connective tissue. In the remaining valve leaflet, individual, isolated cardiac muscle fibers exist surrounded completely by connective tissue (22). To our knowledge, this region is the only place in the heart where muscle cells exist in such isolation. Whether this anatomic arrangement is a determinant of the transmembrane action potential characteristics of these fibers requires further investigation, but these observations suggest an interesting possibility concerning the relation of
cell density and geometry to the characteristics of transmembrane action potentials (33, 34).

Another possible explanation for the different electrophysiological characteristics of the mitral valve region may be the presence of ionic conductances differing from those in ordinary atrial muscle. We do not, as yet, have any data on this possibility.

The phenomenon of automaticity in the region of the anterior mitral valve leaflet may be significant in relation to the genesis of certain atrial dysrhythmias. Spontaneous beating of the mitral valve leaflet of the beef heart has been observed in one instance by Erlanger (20). Automaticity has been demonstrated in specialized right atrial fibers (1-3). These fibers may be a site for initiation of ectopic impulses. However, the occurrence of automaticity in left atrial muscle has been doubtful, and usually it has been demonstrated under highly abnormal, unphysiological conditions (35).

Rothberger and Winterberg (13) in 1910 suggested that atrial extrasystoles and rhythms with negative P waves in lead I might originate in the left atrium. Since that time clinical reports of atrial rhythms, including single extrasystoles, persistent rhythms of moderate rate, and rapid tachyarrhythmias have been reported to originate in areas of the left atrium (14-19). The clinical occurrence of these left atrial rhythms is deemed to be rare on the basis of electrocardiographic criteria. However, the lack of consideration of the left atrium as a site of origin for clinically encountered atrial impulses has undoubtedly been caused, in part, by the scarcity of experimental evidence demonstrating an ability of left atrial cardiac muscle to generate impulses. It has been suggested that impulses originating in the lower interatrial septum and the adjacent right and left atrial areas may show an overlap in electrocardiographic patterns (36, 37). If this suggestion is true, atrial dysrhythmias originating in the left atrium may be more common than is currently believed and in some instances may be mistaken for dysrhythmias of right atrial origin.

Spontaneous impulse initiation was occasionally observed in valve muscle fibers immediately after the isolated preparation was mounted in the perfusion chamber. The reason for such spontaneous activity at this time is not known. Transient hypoxia or stretch during dissection of the preparation could be a factor. Automaticity occurred readily after intervention with catecholamine and tyramine. Since cardiac muscle of the mitral valve is well innervated with both sympathetic and parasympathetic nerves (38, 39), such automatic activity might be induced in situ by activation of sympathetic nerves to this region of the heart.

Acetylcholine in very low concentrations depressed automatic impulse formation in the mitral valve leaflet and simultaneously prevented direct electrical excitation of valve muscle fibers. Dysrhythmias arising in the mitral valve leaflet may thus be abolished by vagal stimulation. Activation of the vagus nerves to the heart is known to abolish many atrial dysrhythmias (40).

The prevention of electrical excitation of valve muscle fibers by acetylcholine could be a result either of a real loss of excitability of the valve fibers or of conduction block between stimulating and recording electrodes. Our previous study using electrical stimulation along the entire long axis of the valve leaflet suggests that both factors are involved (22). We can only speculate concerning the mechanism of such a loss in excitability and the occurrence of conduction block. If acetylcholine increases the potassium conductance of the valve muscle fiber cell membrane, as has been described for other atrial fibers (26), such an increase may be sufficient to overcome the "weak" depolarizing current of the valve fibers and thereby prevent excitation.

Automatic impulse formation also developed after stretching the mitral valve leaflet. This response is similar to the effects of stretch on Purkinje fibers of the ventricular conducting system (41). Stretch also accelerates the rate of depolarization of fibers in the sinus node and activates quiescent pacemaker cells (26, 42, 43). We do not know whether this response of mitral valve muscle to stretch has any physiological or pathological significance. Atrial dysrhythmias are a common sequelae of mitral valvular disease (44). The occurrence of deformation of the mitral valve leaflets after such pathological processes as rheumatic valvulitis may result in tension on mitral valve musculature. Dilatation of the mitral ring, commonly associated with the inflammation resulting from rheumatic heart disease, may also exert tension at the base of the valve leaflet. The effects of such processes on automaticity of the valve muscle is a subject of interest in relation to the genesis of the accompanying atrial dysrhythmias and worthy of future investigation.

Even though automatic impulse formation in the mitral valve leaflet usually occurred at a steady cycle length, the combination of such impulse generation and various degrees of block in the
junction could result in many different patterns of atrial activation. Slow spontaneous rates activated the atrial wall on a 1:1 basis (Fig. 8). If this event occurs in the in situ heart in the presence of sinus node depression, a steady ectopic rhythm might develop. At more rapid rates, spontaneous impulses arising in the valve leaflet showed variable degrees of conduction block in the junctional area. Instances of complete block alternating with 1:1 conduction (Fig. 9) occurring in the in situ heart might result in paroxysmal atrial tachycardias. Also, at rapid spontaneous rates with high-grade conduction block into the atrium, conducted impulses activating the atrium might produce single atrial extrasystoles and possibly resemble an atrial parasystolic focus (Fig. 11).

It is possible that conduction block between the valve and the atrium resulted from damage during the dissection or related hypoxia or stretch. However, since extreme care was taken during the dissection procedure to avoid the junctional region, alternative mechanisms may be related to the electrophysiological properties of this area. Some of our observations may permit a tentative description of these probable mechanisms. We showed that impulses originating in the atrium and propagating rapidly to the junctional region caused relatively rapid depolarization of junctional fibers and continuing propagation of the impulse for some distance into the valve leaflet. This process is to be expected, since impulse conduction from a region with a larger surface-volume ratio should be relatively easy into a region with a lower surface-volume ratio if the membrane properties are equal (45). In contrast, impulses initiated in the muscle fibers of the valve leaflet by focal stimulation propagated only slowly towards the atrium, and usually the propagating action potential decreased progressively in amplitude until it caused only a small, nonpropagated depolarization in some of the junctional fibers. However, if several areas of the valve were activated more or less simultaneously by applied stimuli, propagation through the junctional region to the atrium was more likely. These findings may result from both the geometric arrangement of the fibers in the valve and junctional region and from the voltage-time course of the action potential in muscle fibers of the valve which results from their focal activation. In the atrium, over the fibrous annulus at the base of the valve, there are many relatively large bundles of muscle fibers which have many lateral junctions. With increasing distance from the annulus, the density of muscle fibers decreases progressively. In the middle third of the anterior leaflet only a few individual fibers remain, and these fibers are separated from each other by dense accumulations of collagen (22). Lateral contacts between these fibers are sparse (unpublished observation). Under these conditions, stimuli applied through close bipolar electrodes to the middle region of the valve, unless excessively strong, probably excite only a relatively few muscle fibers. The impulse caused by such stimuli most likely will not spread laterally to the other fibers in the valve because of the sparse lateral connections. As this impulse spreads towards the junctional region, it may not be able to generate sufficient current to excite the junctional fibers because of relatively rapid branching and a large increase in the number of fibers and the membrane area (45). Stimulation at several sites in the valve, by exciting a larger number of fibers, may increase the likelihood that the junctional fibers will be excited because of both the increased magnitude of the excitatory current and the possibility of summation of excitatory inputs (46). When spontaneous activity arises in a sufficiently large number of valve fibers, successful propagation to the atrium will occur for the same reasons. It is highly unlikely that conduction from valve to atrium, resulting from summation of two or three electrical excitations, resulted from current spread from the stimulation electrodes to regions distal to the area of block. The stimulation sites were located 5-7 mm from the point where conduction block occurred, and the stimulation voltage was at threshold. Since the stimulating electrodes were bipolar, with an interpole space of less than 1 mm, current from these electrodes probably did not spread far from the sites of stimulation. In addition during conduction from valve to atrium, the sequence of activation indicated an orderly spread of the impulse across the junctional region; no evidence that excitation jumped the blocked gap was encountered.

Another factor which may be instrumental in the low margin of safety for conduction from mitral valve leaflet to atrium is the extremely slow conduction in the valve leaflet. Activation of the junctional area by impulses propagating from the valve (either electrically stimulated or spontaneously occurring) results in a slow prepotential, a slow depolarization, a reduction in action potential amplitude, and sometimes a conduction block. The slow prepotential may be a result of slow propagation of the wave of excitation from the
valve leaflet into the junctional region, thereby slowly depolarizing fibers in this area. Similarly, cells in the AN region of the AV node may show a prepotential when they are activated by an impulse slowly conducting retrograde through the N region; AN cells do not exhibit this prepotential when they are activated by a rapidly conducting impulse from the atrium (47). A slow prepotential has also been observed in depressed Purkinje fibers exhibiting very slow conduction (48). This slow depolarization of junctional fibers may result in a decreased upstroke velocity and amplitude of the transmembrane action potential due to inactivation of the inward carrier mechanism similar to that demonstrated by Dudel et al. (49) for Purkinje fibers during stimulation with a ramp-shaped wave form. When the amplitude and depolarization velocity are below a sufficient magnitude, junctional fibers are unable to excite atrial wall muscle and conduction block occurs.

References

Electrophysiological Properties of Cardiac Muscle in the Anterior Mitral Valve Leaflet and the Adjacent Atrium in the Dog: POSSIBLE IMPLICATIONS FOR THE GENESIS OF ATRIAL DYSRHYTHMIAS
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