Canine Mitral Complex

ULTRASTRUCTURE AND ELECTROMECHANICAL PROPERTIES

By John J. Fenoglio, Jr., Tuon Duc Pham, Andrew L. Wit, Arthur L. Bassett, and Bernard M. Wagner

ABSTRACT

The mitral complex is a functional entity composed of the annulus, valve leaflets, chordae, and papillary muscles. The mechanical properties of the complex are dependent on the unique structural relations of the collagen in the leaflets and chordae. In the chordae the collagen is arranged in avascular columns. These columns interdigitate between muscle fibers in the papillary muscles, and the collagen is anchored to the myofiber membrane by microfibrils. In the leaflet the chordae are continuous with the dense fibrous tissue, forming a sheet of collagen which merges with the annulus. Within the leaflet there are cardiac muscle fibers in direct continuity with left atrial muscle. Contraction of isolated valve preparations can be initiated by electrical stimulation and is preceded by a propagated depolarization. Action potentials from cells in the middle third of the leaflet have a slow upstroke velocity, prominent plateau, and a characteristic positive afterpotential. Valve muscle electromechanical properties are markedly altered by 1 X 10^-7 M acetylcholine; this concentration has little effect on working left atrial muscle. In preparations containing portions of the left atrium and valve leaflet, the excitation wave spreads into the leaflet after electrical stimulation of the atrial muscle. This suggests that the accompanying contractile event may occur in situ before the initiation of systole.

KEY WORDS

acetylcholine
mitral valve leaflet
action potential
comparative pathology
chordae tendineae
mitral excitation
papillary muscle
length-force relation
electrophysiology

The anatomical structures regulating the flow of blood at the mitral orifice have been termed the mitral complex (1). This is composed of the mitral annulus, valve leaflets, chordae tendineae, and papillary muscles.

Mitral valvular fibrosis in man and dog is well known clinically and pathologically. In the dog, the abnormal physiological function resulting in mitral insufficiency may be related to structural alterations in one or more regions of this complex. Although studies of the structural alterations in this disease have been made in both the canine and human heart (2-4), the interpretation of these structural changes is hampered by the lack of a systematic analysis. We have therefore undertaken to study the sequential changes in the ultrastructure of the canine mitral valve during the progressive stages of development of mitral fibrosis and the resultant mitral insufficiency.

This paper presents the ultrastructure of the normal mitral complex and the electromechanical properties of the cardiac muscle in the normal valve leaflet. Previous electron microscopic reports have not included detailed observations on the structural interrelations of the valve leaflets, annulus, chordae tendineae and papillary muscles (5-8). Such studies are a necessary prerequisite for any studies on...
pathological alterations leading to mitral fibrosis.

Methods

ULTRASTRUCTURAL STUDIES

Six healthy dogs of various ages on an ad libitum diet were anesthetized by pentobarbital sodium (30 mg/kg, iv), and the hearts were quickly removed. No significant pathological findings were found at autopsy.

The left ventricle was opened anteriorly while still beating. In four of the dogs, the anterior (septal) leaflet was severed circumferentially at the annulus and the papillary muscle was cut at its junction with the left ventricular wall. The entire anterior leaflet and papillary muscle was immediately placed in 3% phosphate-buffered glutaraldehyde (pH 7.4), and a ribbon of tissue not more than 0.5 cm thick was cut. This ribbon included valve leaflet, chordae tendineae, and the tip of the papillary muscle. After fixation for approximately 1 hour, the ribbon was oriented under a dissecting microscope in a pool of glutaraldehyde and cut into serial blocks. The valve-chordae junction and the chordae-papillary muscle junction were included in separate sets of blocks. These blocks were then returned to buffered glutaraldehyde. In the two remaining animals, sections were taken from the posterior mitral complex including the junctions of the valve leaflet and chordae and the chordae and papillary muscle. These sections were immediately cut into small blocks and fixed as described above.

After overnight glutaraldehyde fixation the blocks were rinsed in phosphate buffer (pH 7.4) and postfixed in 1% phosphate-buffered osmium tetroxide for 90 minutes at 4°C. The blocks were again rinsed in phosphate buffer and dehydrated through graded acetones. The embedding was done in Swiss Araldite. The valve and chordae blocks were embedded in flat silicone rubber embedding molds (9) after orientation under the dissecting microscope.

Sections were cut with a diamond knife on an LKB Ultrotome III. The sections were picked up on formvar- and carbon-coated grids (100 mesh), double stained with 1% uranyl acetate in 95% ethanol for 5 minutes and lead citrate (10) for 5 minutes, and examined in a Hitachi HU-11E electron microscope at 75 kv.

PHYSIOLOGICAL STUDIES

For physiological studies, 19 healthy dogs on an ad libitum diet were used. The animals were anesthetized as described above. The hearts were rapidly removed and dissected at room temperature in a modified Tyrode solution of the following composition (mmoles/liter): NaCl, 137; NaHCO3, 12; KCl, 4.0; MgCl2, 0.5; Na2HPO4, 1.8; CaCl2, 2.7; and dextrose, 5.5. The Tyrode solution was equilibrated with a gas mixture consisting of 95% O2-5% CO2. The left atrium was opened and the anterior leaflet of the mitral valve exposed. For mechanical studies, the leaflet was detached at least 2–3 mm below the level of the annulus. A 4-0 silk suture was tied around the chordae tendineae and the attachments to the papillary muscles were severed. A section approximately 7–10 mm wide was cut from the central portion of the leaflet. The preparation was then placed horizontally (atrial side upward) in a 10-ml water-jacketed muscle chamber. The chordae were tied to a hook-and-micrometer assembly in one end of the chamber; the other end of the valve leaflet was attached by two sutures to the tip of a rigidly mounted force transducer (Statham UC-2). The length of the mitral valve could be varied by movement of the micrometer assembly. Length was directly measured by a calibrated ocular micrometer. Each preparation was stimulated through silver wires arranged parallel to the long axis. Square-wave pulses of 10-msec duration were delivered at a voltage approximately 15% above threshold. Stimulation frequency was 30/min. The bath temperature was maintained at 30°C by the water jacketing and by constant superfusion (10 ml/min) with the modified Tyrode solution equilibrated with the 95% O2-5% CO2 mixture. The same gas mixture was also used to oxygenate the bath chamber directly. To study the effects of acetylcholine, the drug was added to the bath chamber after the inflow of the Tyrode solution was shut off; chamber temperature remained constant under such conditions.

Machine-pulled glass microelectrodes (15–30 meqohms resistance) were used for recording transmembrane potentials from cardiac muscle in the valve leaflet simultaneously with mechanical measurements. Microelectrodes were coupled to the input circuits of high-input impedance amplifiers with negative capacitance neutralization (Bioelectric NF1) by means of Ag-AgCl wires. Isometric force and transmembrane potentials were recorded on a dual beam oscilloscope (Tektronix RM565) and photographic records taken with a Grass kymographic camera or Polaroid oscilloscope camera.

In eight experiments, trabeculae (0.4–0.8 mm diameter) were isolated from the left atrium, mounted in the muscle chamber, and then studied in a manner identical to that described for the mitral valve preparations.

For studies on the pattern of excitation in the mitral valve leaflet, the anterior left atrial wall and part of the left atrial septum, along with the attached anterior mitral leaflet was removed and...
pinned to the wax-lined bottom of a 50-ml tissue bath. The left atrial muscle and the atrial surface of the valve leaflet faced upward. Care was taken to remove all ventricular muscle. The preparation was superfused at a rate of 20 ml/min with the modified oxygenated Tyrode solution at a temperature of 36°C. Bipolar silver wire stimulating electrodes placed on the atrial wall or septum 1–2 cm above the attachment to the valve leaflet were used to stimulate the preparation at a rate of 60/min. A surface electrogram was recorded from the left atrium adjacent to the stimulating electrodes using bipolar silver wire electrodes and an appropriate d-c amplifier. This atrial electrogram was used as a reference point and the spread of electrical activity from the stimulating electrodes over the anterior left atrial wall and septum and into the valve leaflet was mapped by using glass microelectrodes to record transmembrane action potentials at multiple sites in the atrium and leaflet (11). The time between the left atrial electrogram deflection and the upstroke of the transmembrane potential was calculated in milliseconds and plotted on a diagram of the preparation. In addition, to determine whether cardiac muscle in the anterior leaflet could be activated from the ventricle, similar experiments were done on a preparation consisting of the basal anterior left ventricular wall, ventricular septum, and the attached annulus and anterior valve leaflet. In these experiments, the stimulating electrodes were placed on the anterior ventricular wall and the spread of electrical activity was determined as described above.

Results

Ultrastructure of the Valve Leaflets

The body of the anterior leaflet of the mitral valve is divided into three zones on the basis of the presence of cardiac muscle. The proximal one-third (that portion closest to the annulus) is shown in Figure 1. The muscle of the leaflet is a direct extension of the cardiac muscle of the left atrium. Ultrastructurally (Fig. 2a), it is identical to the atrial myocardium (12), and atrial specific granules are present (13). Between the muscle fibers are scattered small nerves and vascular channels. Just beneath the ventricular endocardium are dense collagen bundles (throughout this paper called the fibrosa), which course toward the annulus. Occasionally, a strut of ventricular muscle extends into the undersurface of the leaflet. These muscle fibers are confined to the area of the annulus and are separated from the main muscle mass of the leaflet by the fibrosa.

In the middle third of the anterior leaflet, muscle fibers are progressively lost so that only individual fibers remain. These muscle fibers are separated from the surrounding stroma by a definite, although ill-defined, basal lamina (Fig. 2b). The individual muscle cells are surrounded by collagen, occasionally in dense bundles, fibroblasts, and scattered elastic fibers. These constituents are loosely arranged and distinctly separated from the fibrosa. Scattered nerves and vascular channels are again present between the muscle fibers. As the muscle fibers are lost, a distinct area of loose collagen (throughout this paper called the spongiosa) appears, and in the outer third of the leaflet no muscle remains.

In the posterior leaflet, the first two zones of the anterior leaflet are telescoped into one. The muscle layer ends abruptly in the

FIGURE 1

Close to the annulus of the anterior leaflet, the muscle (M) is grouped in distinct bundles and constitutes one-third to one-half the thickness of the valve leaflet. Scattered small vascular channels are readily visible in the center of the field. The lower one-third of the leaflet consists of dense fibrous tissue, the fibrosa (Fb). Close to the annulus, the two layers are often separated by loose fibrous tissue and adipose tissue (Ad). Hematoxylin-eosin stain.
420 FENOGLIO, PHAM, WIT, BASSETT, WAGNER

FIGURE 2

a: The cardiac muscle in the proximal third of the anterior leaflet (closest to the annulus) shows an orderly arrangement of muscle bundles, with distinct Z bands (Z) and broad A bands (A) bisected by distinct H bands (H). The mitochondria (Mc) are placed adjacent to sarcomeres, and atrial specific granules (arrow) are present. The sarcolemma (S) at the periphery is scalloped. The endothelium of a small capillary is seen in the upper corner. b: An individual cardiac muscle cell from the middle third of the anterior leaflet. The disarray of myofilaments and mitochondria is secondary to the plane of section. The sarcolemma (S) is distinct and a definite, although ill-defined basal lamina (arrow) is noted. The surrounding stroma consists of dense collagen bundles (C), fibroblasts (F), and elastic fibers (El) embedded in a mucopolysaccharide ground substance (Gs).

midportion of the leaflet and is immediately replaced by spongiosa. Ultrastructurally, the muscle is identical to that of the anterior leaflet, and small nerves and vascular channels are also present.

The outer portions of both leaflets are composed of spongiosa and fibrosa only. The spongiosa is a loose collection of random collagen fibers, scattered collagen bundles, and elastic fibers embedded in a mucopolysaccharide-protein ground substance. Within the loose collagen are scattered fibroblasts and Anitschkow cells. The fibrosa is a separate band of dense wavy collagen within which are entrapped fibroblasts. This band is especially prominent at points of chordae insertion into the valve leaflet (Fig. 3). In the smooth areas, the portions of the leaflet between points of chordae insertions, and toward the free edge of the leaflet, the fibrosa is more loosely arranged, but still distinguishable as a separate layer (Fig. 4a). In these areas, the junction between the spongiosa and fibrosa is indistinct, with an apparent merging of collagen bundles and ground substance of both layers. Here the collagen fibers overlap and en-
MITRAL ULTRASTRUCTURE AND PHYSIOLOGY

Electron micrograph of the ventricular surface of the leaflet near the insertion of a chorda tendinea. The endocardial cells are interconnected by junctions (J) lacking desmosomes. Mitochondria, smooth endoplasmic reticulum, and free ribosomes are haphazardly distributed through the cell. Along the basal plasma membrane are rows of micropinocytotic (Mp) vesicles, and the cells are separated from the underlying stroma by a distinct basal lamina (arrows). Just beneath the endocardial cells the stroma contains collagen fibers (C), elastic fibers (El), and fibroblasts (F). To the left is an unmyelinated nerve fiber (N). Beneath the loose stroma is a band of dense wavy collagen, the fibrosa (Fb), which is an extension of the core of the chorda tendinea.

mesh the fibroblasts. At high magnification, the collagen fibers appear to terminate on the plasma membrane of the fibroblast, although this may be due to the plane of section. At the true free edge, this distinction is further lost and the free edge is composed almost entirely of spongiosa. Although the outer portion of the leaflet contains no vascular channels, scattered nerves located just beneath the endocardium are present. Vascular spaces appear to be present only where muscle fibers are found.

The endocardium of both leaflets and that of the chordae tendineae are similar. The atrial endocardial cells are plump with large irregular nuclei and thick cytoplasmic processes, while the ventricular and chordal endocardial cells have flattened ovoid nuclei and attenuated cytoplasmic processes (Fig. 4a). The fine structure of an endocardial cell and the underlying stroma is shown in Figure 3. The underlying stroma is identical to the spongiosa and merges with it toward the free edge of the leaflet.

MECHANICAL AND ELECTRICAL ACTIVITY OF CARDIAC MUSCLE IN THE VALVE LEAFLET

The relation between length and force was obtained for eight preparations of the anterior mitral valve leaflet. Initial leaflet preparation length was considered to be that length at which total resting and developed force approached zero (Fig. 5). As length was...
increased, both total and resting force increased, first slowly and then more rapidly. At the greater lengths, there was marked stress relaxation, and the resting forces that are depicted in Figure 5 were obtained when 80-85% of the stress relaxation was complete (4-5 minutes after each increment in length). For all preparations, resting force was 5.28 ± 1.50 g (mean ± se) at a length 50% greater than initial length. Active force developed by the preparation varied as a function of length, increasing with length increments and eventually reaching a plateau level (Fig. 5). For all anterior leaflet preparations, the length increment necessary to obtain maximal active force was 43.5% ± 11.9% of the initial length (active force was measured after the major part of stress relaxation had occurred). At optimal length, the maximal active force developed by the preparations was 0.31 ± 0.07 g, range of 0.08-0.64 g.

For seven preparations, (developing 50-70% of maximal active force) recordings of transmembrane action potentials were obtained concomitantly with the force measurement (Fig. 6). Action potentials recorded from the middle third of the leaflet had low resting potentials (65 ± 5 mv), a slowly rising depolarization phase (phase 0; 5 ± 1 v/sec), and a prominent plateau (phase 2). A characteristic positive afterpotential also occurred; membrane potential was most negative immediately following repolarization.
Relation of force to length of a mitral valve preparation. Left: Resting force and total force are plotted as a function of change in length (force values were obtained after stress relaxation was 85% complete). Initial preparation length was 11 mm; stimulation rate 30/min; temperature 30°C. Right: Relation between active force developed during contraction and increase in preparation length. Note that active force increases and then reaches a maximum at a length approximately 50% greater than the initial length.

Electrical and mechanical activity of the isolated anterior leaflet of the canine mitral valve. Upper trace shows transmembrane action potential recorded from cardiac muscle in the middle third of the leaflet. Lower trace shows simultaneous recording of mechanical activity. Note that membrane depolarization preceded and appears to initiate the contractile event.
A: Effect of $1 \times 10^{-7}$M acetylcholine on electromechanical properties of isolated mitral valve anterior leaflet. Temperature 30°C; stimulation rate 30/min. Panel 1 shows control action potential and isometric contraction. Note the slowly rising upstroke of the action potential, the plateau phase, and the positive afterpotential. Panels 2-4 show the progressive effects of $1 \times 10^{-7}$M acetylcholine over a period of 1 minute after addition to the bath chamber. Note the marked effect of this acetylcholine concentration to completely suppress contraction. Initial hyperpolarization and shortening of the action potential is seen in panel 2; there is also an apparent fractionation of the transmembrane potential into an early phase of slow depolarization followed by a phase of more rapid depolarization. This fractionation is followed by a progressive decrease in action potential amplitude in panels 2 and 3, until in panel 4, only the initial slow depolarization remains. B: Effect of $1 \times 10^{-7}$M acetylcholine on electromechanical properties of isolated dog left atrial trabecula. Temperature 30°C; stimulation rate 30/min; muscle length and diameter, 4.3 and 0.8 mm, respectively. Left panel shows the control action potential and isometric contraction. Right panel shows the steady-state effects of $1 \times 10^{-7}$M acetylcholine in the same muscle (single-cell impalement was maintained). Note the acetylcholine induced hyperpolarization (increased resting potential). There was little change in action potential duration and only a very slight decrease in the peak active force developed in contraction during acetylcholine intervention. Compare with A.

Hyperpolarization, but little change in action potential duration was evident at the maximal steady-state effect of $1 \times 10^{-7}$M acetylcholine. The effects of this concentration of acetylcholine were consistently noted in all mitral leaflet and left atrial preparations studied. A tenfold greater concentration of acetylcholine ($1 \times 10^{-5}$M) had the same markedly depressant action on developed force and action potential characteristics in the leaflet as did the lower
concentration. Additionally, administration of 1X 10⁻¹⁰ M acetylcholine to the left atrial muscle always decreased developed force by 30-40% and considerably shortened action potential duration, but never decreased total action potential amplitude.

Our anatomical studies showed that valvular cardiac muscle is continuous with left atrial muscle. No continuity with ventricular muscle was ever observed. However, Priola et al. suggest that the valve is activated electrically from the ventricle (14). We therefore studied electrical activation of preparations consisting of the isolated basal anterior left ventricular wall, left ventricular septum, and attached annulus and anterior mitral leaflet by microelectrode techniques (see Methods). Electrical stimulation of the ventricular wall resulted in complete activation of all ventricular muscle up to the annulus, but the wave of excitation never propagated into the valve leaflet.

We also determined the spread of electrical activity in isolated preparations consisting of the anterior left atrial wall and septum and the anterior valve leaflet. Left atrial muscle was electrically stimulated and the spread of the propagated wave of excitation was determined by microelectrode mapping (see Methods). The spread of excitation through the left atrial muscle occurred rapidly and then progressed more slowly through the valve leaflet, demonstrating that the muscle of the anterior leaflet is in direct electrophysiological continuity with the left atrium under these experimental conditions (Fig. 8).

ULTRASTRUCTURE OF THE CHORDAE TENDINEAE

On cross section, the chordae tendineae consist of three distinct layers (Fig. 4B). The outer layer consists of single flattened endocardial cells. Beneath these cells and separated from them by a distinct basal lamina is an area of loosely meshed collagen, elastic fibers,
and scattered bundles of dense collagen. Nerves are infrequent but are found in this area. The central core consists of dense, wavy, closely packed bundles of collagen arranged parallel to the long axis of the chordae. Scattered fibroblasts are entrapped in the collagen, but no vascular channels were identified.

The chordae continue intact to their insertion into the ventricular aspect of the valve leaflet. At this point, the endocardial cells and the spongiosa are continuous with those of the ventricular aspect of the leaflet, and the dense collagen core divides. The bulk of the collagen is continuous with the fibrosa and courses towards the annulus. A portion of the central core, however, turns and courses toward the free edge of the leaflet, forming the fibrosa of the smooth areas (Fig. 4A) and the free edge. As the collagen fibers course toward the annulus they fan out to form a curtain of fibrous tissue. The collagen bundles crisscross and layer as they move toward the annulus. This arrangement is best seen in the midportion of the leaflet, where the collagen bundles appear to lie at right angles to one another. Figure 9 is a schematic drawing of the mitral valve illustrating the arrangement of the collagen in the leaflet.

At the tip of the papillary muscle (Fig. 10), the chordae divide into fingerlike processes which penetrate between cardiac muscle bundles. A portion of the collagen fans out to cover the tip of the papillary muscle, coursing between the muscle and the endocardial cells. At the junction of the chordae and the papillary muscle, the spongiosa broadens to form a cushion of loose collagen, within which are scattered elastic fibers and large lipid droplets. As the tongues of collagen penetrate deeper into the papillary muscle, they divide into smaller and smaller units, forming a branching anchoring system much like the roots of a tree. The smallest branches are composed of more loosely arranged collagen, although dense wavy collagen bundles are still present (Fig. 11a). Within the small branches are numerous small capillaries and nerve twigs.
FIGURE 11

a: As the branches of fibrous tissue penetrate deeper into the papillary muscle (M), the collagen is no longer as tightly packed, although dense wavy bands of collagen (C), are still present. Fibroblasts (F) are more numerous and small capillaries (Cp) are present. b: Electron micrograph from the papillary muscle demonstrating the ending of the collagen in the basal lamina of the muscle. The muscle cell (M) is limited by a distinct sarcolemma (S). The basal lamina (BL) is packed with numerous microfibrils (MF), which appear to enmesh the ends of the collagen fibers (C) ending in the basal lamina. In the muscle cell, groupings of myofilaments (MY) reach to the sarcolemma but do not appear to traverse it or enter the basal lamina.

Along the branches of fibrous tissue, the collagen fibers appear to terminate in the basal lamina of the muscle cells (Fig. 11b). The ends of the collagen fibers are enmeshed and anchored by a network of microfibrils. In other areas, the myofilaments of the muscle cell extend to the sarcolemma but do not appear to enter the basal lamina. The muscle fibers of the papillary muscle, even at its tip, are well oriented and ultrastructurally normal.

Discussion

The anatomical components of the mitral complex function as an integrated unit. Alterations of one of the components will eventually lead to structural and perhaps functional changes in the entire complex. An obvious example is the sequence of events following rupture of a single chorda tendinea (15). It is therefore necessary to understand the detailed structure and physiology of the normal complex to better appreciate disease processes of the same structures (1). This study has presented the ultrastructure and the electromechanical properties of the normal canine mitral valve complex as a prelude to further investigations on the sequential development of mitral fibrosis.

Our ultrastructural studies demonstrate the stability of the architectural arrangement of
the normal complex for performing its physiological function while withstanding the tremendous stress exerted on this system. This is particularly exemplified in the structural relation of the annulus, fibrosa of the valve leaflet, and collagen core of the chordae tendineae. These form a single unit of fibrous tissue extending in an unbroken sheet from the annulus to the papillary muscle. From anatomical considerations alone, stresses exerted on the mitral complex should be dissipated over the entire annulus because of this relation. This unit is further strengthened by the rootlike anchoring of the chordae tendineae into the papillary muscles. The division of the collagen into numerous small branches greatly increases the surface area of attachment and tends to further dissipate the stress forces. The weakest point in this entire system is the junction of the chordae tendineae with the papillary muscle. A cushion of spongiosa at this vital area may act as a shock-absorbing device.

Our studies also confirm that the collagen fibers from the chordae end in the basal lamina of the muscle cell (16-19). However, the possibility that this morphologic appearance is an artifact due to the plane of section cannot be totally dismissed. We were also able to show the presence of microfibrils which appear to fasten the collagen fibers into the basal lamina (16). However, microfibrils were not found above the basal lamina forming a cementing substance between the collagen fibers, as suggested by Hanak and Bock (16).

Both valve leaflets contain cardiac muscle in direct continuity with the muscle of the left atrium. The presence of cardiac muscle in the mitral valve leaflet has suggested to several investigators that the valve plays an active physiological role (5, 14, 20, 21). Erlanger in 1916 (22) and later Sonnenblick et al. (5) demonstrated that the anterior mitral valve leaflet possesses intrinsic contractility. The present report demonstrates that the development of force by the isolated mitral valve leaflet is preceded by electrical depolarization of the valvular cardiac muscle fibers. Sonnenblick et al. also demonstrated that acetylcholine has a negative inotropic effect on the mitral valve leaflet, thereby indicating that the valve contains atrial muscle (5). The negative inotropic effect of acetylcholine on isolated valve muscle was confirmed in our study. Additionally, we noted a marked difference in the electromechanical sensitivity of valvular and left atrial muscle to the same concentration of acetylcholine. The profound negative inotropic effect of 1 X 10^-4 M acetylcholine, a concentration much less than that used by Sonnenblick et al., on valve muscle was accompanied initially by hyperpolarization and decreased duration of action potential similar to the known effects of higher concentration of acetylcholine on atrial muscle (23, p. 55 and the present study). However, the further action of this relatively low concentration of acetylcholine to significantly decrease action potential amplitude and finally to abolish depolarization is unlike the usual effects on atrial muscle and, instead, resembles the actions of acetylcholine on atrial fibers at the junction of the atrioventricular node or on fibers in the upper nodal region (23, p. 139). It is also interesting that positive afterpotentials noted for muscle cells of the middle third of the leaflet are never seen in normal working atrial muscle fibers. Further characterization of the electrophysiological properties of single cells in the mitral valve leaflet may be necessary to explain our finding with respect to the sensitivity of valvular muscle to acetylcholine.

From in vivo studies, Priola et al. have suggested that the mitral valve is in electrophysiological continuity with the left ventricle rather than the left atrium (20). We were unable to demonstrate conduction from the anterior ventricular wall or ventricular septum into the anterior leaflet in vitro. Furthermore, the results of our electrophysiological investigations demonstrate that there is continuity...
from the left atrium into the anterior mitral valve leaflet. There is therefore no reason to believe that the valve cannot be activated from the left atrium in situ. However, this finding does not preclude the possibility that the valve can be activated from the ventricle in situ. This problem requires further study.

The functional significance of these results from studies on isolated preparations is uncertain. For contraction of the cardiac muscle in the mitral valve to function in preventing regurgitation of blood into the left atrium, it should be activated immediately before the initiation of ventricular systole. If the leaflet is activated from the inferior left atrium, a possibility which our data suggests, the resultant contraction could occur at a physiologically advantageous time in the cardiac cycle. Activation of the interior regions of the left atrium, which are in continuity with the mitral valve, occurs during the terminal part of left atrial activation (24, 25). At this time the excitation wave has already entered the AV node. Therefore, if the valve is activated from the left atrium in situ, valvular contraction might occur immediately before ventricular systole.

Acknowledgment

Sincere appreciation is extended to Drs. A. Hurvitz and L. Tilley of the Animal Medical Center, New York, who participated in the early phases of this study. Appreciation is also extended to Dr. R. Tashjian, Director, Animal Medical Center, for his continued support. The authors also wish to thank Mr. Martin Rotker for photographic assistance.

References


