Ultrastructure and Transmembrane Potentials of Cardiac Muscle in the Human Anterior Mitral Valve Leaflet

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SUMMARY We studied the ultrastructure and electrophysiology of an anterior mitral valve leaflet obtained from the heart of a recipient of a cardiac transplant. The anterior leaflet of this human mitral valve contained atrial muscle in direct continuity with the left atrial myocardium. The muscle fibers were 3–6 μ in diameter and contained atrial-specific granules. They were arranged in longitudinal bands of two or three muscle cells with intercalated discs at the cellular poles. These groups of cells were separated from one another by abundant loose connective tissue. The muscle fibers had maximum diastolic potentials of −61 ± 8 mV and action potentials with slow upstrokes (Vmax <15 V/sec) and low amplitudes (66 ± 6 mV) occurred during electrical stimulation of the valve. In the absence of electrical stimulation, valve muscle had phase 4 depolarization and initiated automatic impulses at a slow rate. The rate of impulse initiation was increased by epinephrine. During electrical stimulation, epinephrine also caused the appearance of delayed afterdepolarizations. Triggered sustained rhythmic activity could be elicited. Delayed afterdepolarizations and triggered activity were abolished by acetylcholine and verapamil. Our results indicate that cardiac muscle in the human mitral valve can initiate impulses and might be a site of origin of arrhythmias in the human heart.

ATRIAL MUSCLE EXTENDS into the leaflets of the mammalian mitral valve, including the valve of humans.1–4 We have previously reported that in the dog and in the monkey, the transmembrane action potentials of this valve muscle are quite different from ordinary atrial muscle.5, 6 although the ultrastructure of valve muscle is similar to ordinary atrial muscle.2 The transmembrane potentials of valve muscle are similar to atrioventricular (AV) nodal potentials.7 Furthermore, canine and simian valve muscle can initiate nondriven impulses in the presence of catecholamines, which led us to suggest that the valve may be a site of origin of some atrial arrhythmias.5, 6 Another study on the electrophysiologic characteristics of cardiac fibers in the rabbit mitral valve indicates that they are similar to cardiac fibers in the canine and simian valve.6 Because action potential characteristics sometimes vary from one species to another,7 we recorded action potentials from the cardiac muscle in the human mitral valve leaflet to determine whether they are the same as those in the valves of the other mammals described above and, in particular, whether nondriven impulses can be initiated in the human valve. Our results show that action potentials of normal human valve muscle also resemble AV nodal potentials and suggest that human mitral valve fibers can initiate both automatic and triggered impulses in the presence of catecholamines. Therefore, the mitral valve leaflet may be a source of ectopic atrial impulses in the human heart.

Methods

The Clinical Case

We obtained the anterior mitral valve leaflet from the heart of a recipient of a cardiac transplant. The patient was a 49-year-old woman who had an extensive myocardial infarct involving the anterior and lateral walls and the septum of the left ventricle. The patient had complete AV block and required ventricular pacing. Multiple recurrent ventricular premature depolarizations and ventricular tachycardia were treated first with digitalis and quinidine, but the arrhythmias persisted. Procainamide and diphenyldantoin were also not effective in controlling the arrhythmias. The cardiac index was 2.6 l/min/m² and the ejection fraction 25%. At the time of surgery the patient was on digitalis and quinidine.

The Electrophysiologic Study

The recipient’s heart was immediately placed in cooled, oxygenated, modified Tyrode’s solution upon removal from the thorax. The left atrium was then opened with an incision through the anterior wall and the septal leaflet of the mitral valve was dissected free at its origin at the AV ring. We could not obtain a preparation consisting of the valve and attached atrial wall as we did for the canine and simian studies because most of the atrial septum was not removed with the heart. The entire septal (anterior) leaflet measured approximately 35 mm long and 65 mm wide and was too large to be mounted in toto in our perfusion chamber. Therefore, we cut a strip 10 mm wide and 35 mm long from the base to the free edge in the center of the leaflet. This preparation was placed in a
5-ml tissue chamber, atrial surface facing upward, and held in place by a grid of chromic gut. The preparation was superfused at a constant rate of 15 ml/min with the modified Tyrode’s solution which was saturated with 95% O₂ + 5% CO₂. The temperature was maintained constant at 36 ± 0.5°C.

The valve leaflet was stimulated through bipolar electrodes (Teflon-coated silver wire) placed on its endocardial surface. The stimuli were rectangular pulses, 1.5–2 times threshold and 3 msec in duration. Membrane potentials were recorded through glass capillary microelectrodes filled with 3M KCl (tip resistances 10–20 MΩ) using techniques previously described in detail.6

We studied the effects of epinephrine, acetylcholine and verapamil on the valve action potentials. Epinephrine and acetylcholine were added directly to the bath from a syringe to give final concentrations of 0.2–2 μg/ml. The number of drops added to the bath determined the final concentration. Verapamil was added to the Tyrode’s reservoir perfusing the bath to give a final concentration of 0.5 μg/ml.

The Anatomic Study

Part of the valve which was not superfused in vitro was immediately fixed in 2% chilled, phosphate-buffered (pH 7.4) glutaraldehyde. At the end of the in vitro study, a strip of the leaflet 2 mm wide and 35 mm long from which action potentials were recorded was also fixed in 2% chilled glutaraldehyde and the remainder of the tissue was fixed in 10% neutral buffered formaldehyde.

Strips of the formaldehyde-fixed leaflet were embedded on edge in paraffin by standard techniques. Sections were cut at 4 μm and stained with hematoxylin-phloxine-safranin.

The glutaraldehyde-fixed tissue was trimmed under the dissecting microscope and the strips of the leaflet were serially blocked into blocks 2–3 mm long, 1.0 mm wide and 0.5 mm thick.7 The blocks were post-fixed in 1% phosphate-buffered (pH 7.4) osmium tetroxide, dehydrated in graded alcohols and embedded in Epon 812 in flat embedding molds. The blocks were oriented in the embedding molds to assure that sections were cut parallel to the long axis (AV ring to free edge) of the leaflet. Sections were cut with a diamond knife on a Sorvall MT2 ultramicrotome, double stained with 1% uranyl acetate and lead citrate, and examined in a Siemens Elmiskop 1A electron microscope at 80 kV.

Results

Histology and Ultrastructure of the Mitral Valve

The gross appearance of the mitral valve was normal. Both leaflets were thin, delicate and semitranslucent. The chordae tendineae were thin and inserted normally both into the valve leaflets and the papillary muscles. There was no evidence of fibrosis or calcification of the leaflets. The commissures were free and the fibrous annulus was not calcified.

The anterior leaflet had a distinct fibrosa along the ventricular aspect of the leaflet which extended from the fibrous annulus to the chordae tendineae (fig. 1). The free margin of the leaflet and the atrial surface of the leaflet consisted of loose connective tissue, the spongiosa, within which were embedded randomly distributed collagen and elastic fibers (fig. 1). Both the atrial and ventricular aspect of the leaflet were lined by plump endocardial cells. Just beneath the endocardial cells, and separating them from the valve substance, was a poorly defined layer of interrupted collagen and elastic fibers and occasional smooth muscle cells. This ill-defined layer of collagen and elastic fibers is the subendocardium. Within the spongiosa on the atrial aspect of the leaflet there was a well-defined band of cardiac muscle (fig. 1). This muscle was continuous with the left atrial myocardium at the fibrous annulus and extended from the annulus into the midportion of the leaflet (halfway between the annulus and the free margin) along the entire width of the leaflet. The muscle fibers were grouped in bundles, two to three fibers in thickness, and extended into the leaflet in a finger-like fashion. In the midportion of the leaflet, the individual muscle bundles were widely separated from one another by the spongiosa. Accompanying the muscle fibers were scattered vascular channels and nerve bundles. Vascular channels were not present in areas of the leaflet devoid of cardiac muscle.

The ultrastructure of the cardiac muscle in the leaflet was similar to normal atrial muscle. The cells were filled with myofilaments arranged in well-ordered sarcomeres (fig. 2). The sarcomeres were frequently in register across the cell and mitochondria were aligned between sarcomeres. The nuclei were centrally placed and ovoid. The perinuclear region contained a well-defined Golgi apparatus, numerous mitochondria and prominent atrial-specific granules, i.e., electron-dense granules found only in atrial cells with a deeply staining homogeneous central core, invested by a membrane.8 Intercalated discs, both end-to-end and side-to-side, were numerous and normal appearing within bundles of muscle cells. However, no junctions or cellular connections could be shown between different muscle bundles. As in normal atrial muscle cells, T tubules were not present in the muscle cells of the mitral valve. The valve muscle cells differed from normal atrial muscle cells only in their size: Normal human atrial muscle cells are 6–12 μ in diameter,9 while the muscle cells found in the anterior leaflet of the mitral valve were only 3–8 μ in diameter at the level of the nucleus.

Focal accumulations of abnormal Z-band-like material and focal thickening of Z bands were present in approximately 25% of the muscle cells in the mitral valve (fig. 2, inset). This was the only abnormality seen in these muscle cells. Similar Z-band characteristics have been reported in both normal and hypertrophied atrial muscle cells in numerous species, including man.10,11 The significance of these Z-band changes is unknown.

The fibrous tissue elements of the mitral valve were
ultrastructurally identical to fibrous tissue elements of the normal canine mitral valve.  

Electrophysiology of the Cardiac Muscle in the Valve Leaflet  
Characteristics of the Transmembrane Potentials  

We recorded transmembrane potentials at intervals of approximately 2–3 mm, progressing from the end of the valve which was originally attached to the annulus (base) toward its free margin. Action potentials were recorded from cardiac fibers over one-half of the distance from the base of the valve to the free edge. The preparation was being driven at a cycle length of 3800 msec during this time. Transmembrane action potential characteristics were similar to those we have described for the canine and simian valves (fig. 3). The action potential upstroke arose from a low diastolic potential (50.1 ± 5 mV) and had a slow foot. The upstroke velocity (V_max) was slow (11 ± 0.5 V/sec) and the total amplitude of the action potential was 66 ± 6 mV. The mean overshoot was 15 ± 4 mV. The repolarization phase was either slightly convex or concave. No distinct phase 1 or plateau was evident. The total action potential duration was 492 ± 10 msec. Repolarization was followed by an early afterhyperpolarization (fig. 3). Maximum diastolic potential occurred immediately after repolarization (61 ± 8 mV) and was more negative than the potential at which the upstroke was initiated. Diastolic potential then decreased slowly to less negative values. All values are mean ± SEM for 20 consecutive impalements. The characteristics of action potentials recorded from different regions in the valve were all similar with the exception of the appearance of a prominent notch on the upstroke of fibers toward the free edge, which was not apparent on the upstroke of fibers near the base of the valve (fig. 3). The apparent conduction velocity in the valve muscle determined from the time difference between activation of the fibers at the proximal and distal ends of the valve was 0.05 M/sec. At stimulus cycle lengths of less than 3800 msec, the notch on the upstroke of fibers toward the distal edge of the valve became more prominent. At stimulus cycle lengths of less than 800 msec, conduction block occurred between the proximal and distal regions of the valve.

In the absence of electrical stimulation, nondriven action potentials sometimes occurred in the cardiac muscle fibers of the valve at a slow rate of 10–15/min (fig. 4), but this nondriven activity was not always present. When we observed nondriven action potentials, the cycle length was irregular, causing the rate to increase and decrease suddenly. Spontaneous depolarization during phase 4 was evident. In 10 cells, during the initial diastolic period of 1000 msec, a precipitous decline in membrane potential of 9 ± 2
mV occurred (after the early afterhyperpolarization described above). During the rest of diastole, membrane potential declined only an additional 5–10 mV before the nondriven action potential occurred. In an additional five cells, no decrease in diastolic potential occurred during the last half of the diastolic period, before the initiation of the nondriven action potential.

**Effects of Epinephrine**

In the absence of electrical stimulation, we added epinephrine directly to the tissue chamber to give an estimated final concentration of 1.0 μg/ml. The slow, nondriven rhythm was present in the valve muscle fibers when the catecholamine was administered (fig. 4). Within 10 seconds after the addition of the catecholamine, the nondriven rate suddenly increased to 30–50 beats/min. This rate of activity continued for 4 minutes and then returned to control, presumably after the epinephrine was washed out of the bath. We repeated this procedure three times with the same results after each administration of the epinephrine.

The administration of epinephrine also caused the appearance of delayed afterdepolarizations in the valve fibers we studied (fig. 5). To determine whether afterdepolarizations were present, we stimulated the fiber for 10–20 impulses and then turned the stimulus off. When afterdepolarizations followed the last stimulated impulse, we measured their amplitude. Before exposing the fibers to epinephrine, afterdepolarizations were not evident when they were driven at cycle lengths of 2000–500 msec. After the administration of epinephrine to give the bath a final concentration estimated to be 0.5 μg/ml, the fibers were again driven at several rates. Automatic activity was not evident at this time in the absence of stimulation with this concentration of epinephrine. As shown in figure 5A, a small afterdepolarization of 3 mV occurred after stimulation at a rate of 35 beats/min and
Effects of epinephrine on the spontaneous rhythm of a cardiac fiber in the mitral valve leaflet. When these records were taken, the valve was not being stimulated and action potentials were occurring spontaneously. The spontaneous rhythm was irregular and occasionally short bursts of activity at a cycle length of 3500 msec occurred (A). More often the cycle length of nondriven activity was 20 seconds, as shown for the first two action potentials in B. Epinephrine was added to the bath at the arrow, causing spontaneous cycle length to decrease to a constant 2000 msec.
we are not sure because it occurs very late in diastole. The valve was then stimulated at a rate of 135 beats/min for 13 impulses and the stimulus turned off. At this time, nondriven action potentials occurred at a rate of 138 beats/min for 16 impulses before stopping suddenly. The first of this series of triggered impulses arose from the repolarizing portion of an afterdepolarization which followed the last driven impulse. The last of this series of nondriven impulses was followed by an afterdepolarization 10 mV in amplitude.

**Effects of Acetylcholine**

Figure 7 shows the effects of acetylcholine on mitral valve fibers. Acetylcholine was added to the bath to give a final concentration of about 0.2 μg/ml. The valve was driven at a rate of 20 beats/min. A small afterdepolarization is seen in the records shown in figure 7A because the valve had been exposed to epinephrine about 5 minutes earlier. The initial effect of the acetylcholine was to increase the level of the membrane potential during diastole. Maximum diastolic potential increased from −56 mV to −61 mV during the first five impulses after addition of the acetylcholine. The early afterhyperpolarization and the afterdepolarization were not abolished. The amplitude of the action potential increased from 68 mV to 73 mV. During this time there was no increase in overshoot of the action potential or in $V_{\text{max}}$ of phase 0. Overshoot remained 18 mV and $V_{\text{max}}$ was 12 V/sec before and after acetylcholine. Diastolic potential continued to increase after the fifth driven impulse, and then action potential amplitude declined rapidly until finally an action potential could not be elicited by the extracellular stimulus, though the stimulating electrodes were less than 5 mm away from the microelectrode and the intensity of the stimulus was increased (fig. 7A).

The same amount of acetylcholine was also added to the bath after epinephrine-induced, nondriven activity, as shown in figure 7B. Shortly after administration of the acetylcholine there was one prolonged cycle
Effects of acetylcholine on transmembrane potentials of a cardiac muscle fiber in the valve. In A, the fiber is driven at a rate of 20 beats/min and acetylcholine was added to the bath at the arrow. An increase in maximum diastolic potential and action potential amplitude occurred after acetylcholine. Eventually, an action potential could no longer be initiated, as shown on the right hand side of the record. In B, acetylcholine was added to the bath at the arrow during nondriven activity caused by epinephrine. Nondriven activity quickly ceased and resting potential increased markedly.

Effects of Verapamil

An action potential was recorded from a fiber toward the free margin of the valve while the preparation was stimulated at a cycle length of 2000 msec. Verapamil was added to the Tyrode's superfusate to give a final concentration of 0.5 mg/l. Verapamil caused a marked decrease in the amplitude of the action potential from 73 to 34 mV (fig. 8). The membrane potential at which the upstroke of the action potential was initiated remained approximately the same.

Discussion

The structure of the mitral valve leaflet that we studied appeared to be completely normal, though it was from a heart with extensive myocardial infarction. There was no evidence of any valvular disease. Furthermore, the ultrastructure of the cardiac muscle in the valve was almost identical to that of normal muscle in the mitral valve of the canine. The ultrastructure of the muscle fibers was also identical to that of ordinary atrial muscle. It lacked T tubules and contained atrial-
specific granules.14 No specialized or nodal-like cells were seen. Therefore, the action potentials that we recorded may be characteristic of normal valve muscle. These action potentials were quite similar to those we have described in the canine and simian valves6, 8, 9 and very different from the action potentials of normal working canine and human atrial myocardium.7, 15 Their low resting potential, slow rate of depolarization and low amplitude are similar to characteristics of transmembrane potentials in AV nodal cells and in the AV ring.7 Transmembrane potentials with these characteristics have also been recorded from atrial fibers in diseased human atrium16, 18 and sometimes also occur when cardiac tissue is not handled with care during dissection and mounting in the tissue bath. However, our observations suggest that it is unlikely that the transmembrane potentials of the human valve fibers we have described here are a result of pathology or poor handling.

When depressed action potentials are recorded from diseased human atrial myocardium, the ultrastructure of the tissue is usually highly abnormal (Pham TD, Mary-Rabine L, Rosen MR, Fenoglio JJ: unpublished observations); but as mentioned above, the structure of valve muscle was normal. When normal atrial action potentials are depressed as a result of a dissection procedure or the period of anoxia which may accompany it, acetylcholine will usually restore a nearly normal action potential by increasing maximum diastolic potential, thereby reactivating fast membrane channels.17 In the present study, 0.2 μg/ml of acetylcholine did increase membrane potential but not action potential overshoot or Vmax. Initially, there was no effect on the upstroke of the action potential and eventually action potential amplitude decreased until the fibers could not be excited by the extracellular stimulus. This is quite unlike the effects of comparable concentrations of acetylcholine on normal atrial fibers; action potential amplitude usually increases slightly.7, 17 Only much larger concentrations of acetylcholine decrease the amplitude of atrial action potentials. However, this response is identical to that observed with comparable concentrations of acetylcholine on canine valve fibers.5 This amount of acetylcholine also can decrease action potential amplitude of some sinus and AV nodal fibers.7, 18 That the upstroke velocity and action potential overshoot are decreased rather than increased when membrane potential is increased may occur because the inward current for the upstroke of valve action potentials is primarily the slow current. This is suggested not only by the slow rate of depolarization and low amplitude of the action potential, but also by the actions of verapamil which markedly decreased action potential amplitude. Verapamil has the same effect on the action potentials of cardiac fibers in the simian mitral valve.6 The mechanisms by which acetylcholine decreases action potential amplitude in mitral valve cells6 and in other fibers with slow-channel-dependent action potentials has been described elsewhere.19

Whether the drugs in the patient at the time the heart was removed (digitalis and quinidine) caused the low resting potential, action potential upstroke velocity and action potential amplitude that we observed should be considered. If they did, we would expect that at least some of the drugs would be washed out of the tissue after several hours of Tyrode’s superfusion and that resting potential, action potential amplitude and upstroke velocity would increase during this time. We did not observe this.

The valve cardiac muscle was spontaneously active at low rates during superfusion with oxygenated Tyrode’s solution. This rarely occurred in canine or simian valve muscle.5, 6 When exposed to catecholamines during this slow rhythm, the spontaneous rate increased as in the canine valve. This type of response to catecholamines does not generally occur in ordinary working atrial myocardium, but does occur in specific regions of the atria such as the crista terminalis or just outside the coronary sinus ostium.20, 21 Nondriven activity in response to catecholamines suggests that human valve muscle has electrophysiologic properties which are different from those of ordinary working atrial myocardium, as we have shown in dogs and in monkeys, although its ultrastructure is the same as that of working myocardium. The increase in spontaneous rate may have resulted from enhanced automaticity or from afterdepolarizations. In addition, nondriven activity after exposure to catecholamines can result from reentry, because conduction in the valve is very slow. We cannot eliminate this possibility. In any case, nondriven activity resulting from sympathetic influences on cardiac muscle in the valve might cause premature atrial depolarizations in the intact heart.5, 6 If this does occur, these atrial arrhythmias could be terminated by increasing vagal discharge because acetylcholine completely suppressed nondriven impulse initiation in valve muscle.

When we stimulated the mitral valve fibers in the presence of epinephrine at rates which were more rapid than those attained spontaneously, there were delayed afterdepolarizations of the kind we have described in simian valve fibers.6 The amplitudes of these afterdepolarizations increased when the rate of stimulation increased. After rapid stimulation, a rapid, nondriven rhythm ensued. These characteristics are identical to those of triggered, sustained rhythmic activity which were studied in detail in the simian mitral valve and the canine coronary sinus.8, 21 Triggered activity has also been demonstrated recently in diseased human atrial myocardium.22, 23 Triggered activity is caused by delayed afterdepolarizations which increase in amplitude with a decrease in drive cycle length and reach threshold at a critical drive cycle length. When a delayed afterdepolarization reaches threshold, a nondriven action potential is initiated and then the afterdepolarization of each subsequent action potential also reaches threshold, causing the sustained rhythmic activity.9 Nondriven activity induced by rapid stimulation can also be caused by reentry, and in the study on the simian valve, experiments were done to eliminate reentry as its cause.9 We did not definitely eliminate the
possibility that the rapid stimulation which induced nondriven activity in the human valve was in fact causing reentry. However, if triggering occurs in the human valve, it may cause atrial tachycardias.

References
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