An Intrinsic Neuromuscular Basis for
Mitral Valve Motion in the Dog

By Edmund H. Sonnenblick, M.D., Leonard M. Napolitano, M.D.,
Willard M. Daggen, M.D., and Theodore Cooper, M.D.

ABSTRACT

The anterior leaflet of the mitral valve of the dog contains blood vessels, nerve fibers, and cardiac muscle in addition to elastic fibers and collagen. When studied in a myograph, the electrically stimulated mitral valve actively developed tension and shortened. Active tension was found to be a function of initial length of the valve and was increased by norepinephrine and decreased by acetylcholine. The presence of neuronally releasable norepinephrine stores in the valve was indicated by responsiveness to tyramine. The negative inotropic response of the mitral valve to acetylcholine was consistent with an atrial origin of the tissue. Possible functional roles for mitral valve muscle and the potential significance of its neural control are discussed.

ADDITIONAL KEY WORDS

ventricular performance cardiac muscle mitral valve closure
acetylcholine tyramine norepinephrine

The valves of the heart are generally thought to be collagen-reinforced endothelial structures whose movement is passive in response to pressure gradients generated by the heart during rhythmic contractions. According to current concepts, the atrioventricular valves open passively during early diastole in the face of the thrust of blood from the atria, and are partially closed by atrial systole (1, 2). Tight closure of the valve then occurs with the onset of ventricular systole (3, 4). Shortening of the papillary muscles is also thought to contribute importantly to this valve motion (5), since the mitral valve does not float freely within the ventricular chamber, even during diastole, nor does it balloon into the atrium during systole (6). According to these views, the mitral valve is considered to be entirely inert. However, it has been shown that the mitral valve contains nerve fibers and blood vessels (7-9) and anatomical reference has been made to striated muscle within the valve of certain species (7, 9). Nevertheless, the possibility that active motion of the valve might also occur has not been considered previously. The present study was undertaken to assess the contractile capabilities of the intrinsic musculature of the mitral valve in vitro.

Methods

Mitral valves of 20 canine hearts were excised and fixed in 1% phosphate-buffered osmium tetroxide at pH 7.2. Prior to dehydration, the anterior (septal) and posterior (mural) leaflets distinctly distal to the atrioventricular ring were removed, and segments of each valve were dehydrated and embedded flat in plastic (Epon 812). Following polymerization of the plastic, selected regions of the leaflets were excised and suitably mounted for thin sectioning. By this process it was possible to examine the entire thickness of the valve in selected areas under either the light, or the electron, microscope. Sections 1μ thick were examined with phase contrast optics. Adjacent thin sections were picked up on uncovered grids, stained with...
uranyl nitrate followed by lead hydroxide, and viewed in a Phillips 200 electron microscope.

In order to study the contractile properties of the mitral valve, the septal leaflets of 11 dog mitral valves were detached 2 to 3 mm distal to the atrioventricular ring. A 0000 braided silk suture was tied so as to encompass chordae tendinae extending to both papillary muscles. A strip of the mitral valve about 5 mm wide extending from below the atrioventricular attachment to the chordae was placed in a myograph containing oxygenated Krebs solution at 30°C. The chordae were tied to a tension transducer (Statham G1-425) while the other end of the valve leaflet was placed in a spring-loaded clip. The length of the segment of mitral valve could be altered by changing the position of a calibrated Palmer Stand to which the strain gauge was attached. Following determination of the isometric length-tension relations, the valves were permitted to shorten isotonically with preloads of 1.0 to 1.5 g, and the effects of altering frequency of contraction and the addition of norepinephrine or tyramine, and acetylcholine were studied. The valve leaflet was stimulated through two platinum plates arranged parallel to the preparation by 10-msec pulses with a voltage approximately 20% above threshold. Preparations were stimulated at 30/min throughout, except when frequency-dependent phenomena were studied.

**Results**

**Morphological Observations.**—The translucent leaflet of the mitral valve represents an endothelium-covered matrix of collagen and elastic fibers that also contains cardiac muscle fibers, nonmyelinated nerves, and blood vessels. The vessels, nerve, and muscle extended toward the free margin of the valve leaflet for about two-thirds of its total length. These findings were consistent in all 20 mitral valves that were studied. Figure 1 is taken from a section 1 μ thick obtained from the middle third of the anterior (septal) leaflet of the mitral valve and photographically recorded with phase optics. The presence of cardiac muscle fibers (CMF) with typical intercalated discs is clearly demonstrated. These muscle elements appeared in the region of the valve's attachment to the atrioventricular ring as a compact layer of muscle several fibers thick. The cardiac muscle fibers were oriented primarily perpendicular to the atrioventricular ring and extended distally toward the free margins of the valve. Another much less prominent group of fibers was arranged parallel to the valve margin and atrioventricular ring. As this layer of muscle fibers proceeded toward the free margin of the leaflet, the muscle fibers became less distinct, with the fibers being separated by bands of connective tissue (C). The terminal free margin of the valve was composed primarily of endothelial cells arranged on a base of collagen and connective tissue and was devoid of cardiac muscle fibers, nerves or blood vessels.

In thin sections of the mitral valve examined in the electron microscope, typical cardiac muscle cells were seen as in Figure 2. In many instances a Schwann cell containing varying numbers of c-fibers was found close
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An electron micrograph of a thin section from the middle third of the septal leaflet of the mitral valve. Two nerves (N) containing a number of unmyelinated axons (AX) are found adjacent to a cardiac muscle fiber (CMF). Between the nerves and cardiac muscle are fibers of collagen (C) and elastic tissue (E). The cardiac muscle fiber in this preparation is contracted and the sarcolemma (Sar) has a scalloped appearance. The muscle is typical cardiac muscle with an abundance of mitochondria (M) and dense granules that are interpreted as glycogen. Photographed at × 16,000.

Mechanics of the Contracting Mitral Valve: Length-Tension Relations.—With the ends of the mitral valve between the chordae and the base held firmly in place, the relation between length and resting tension was determined in 7 anterior mitral valve leaflets. The starting length, L₀, was taken as that point at which both resting and developed tension approached zero (Fig. 3A). As length

to the cardiac muscle fibers. No intimate myoneural junctions were noted; however, the neural elements maintained the relationship typical of atrial and ventricular canine cardiac muscle.

Mechanics of the Contracting Mitral Valve: Length-Tension Relations.—With the
Relation between isometric tension and length of the mitral valve. A, resting tension (o) and the total tension following electrical stimulation (●) are shown as a function of increments (△) in length. Initial muscle length (L₀) was 11 mm; temperature, 30°C; frequency, 30/min. B, relation between actively developed tension—that developed over and above resting tension—and increase in length.

was increased, resting tension increased in an exponential manner, reaching 5.1 g ± 0.2 g (SE) at an extension 50% above L₀.

When stimulated electrically, the mitral valve leaflet contracted and developed tension that was a function of the extended length of the leaflet. Actively developed tension increased as valve length was extended, reaching a maximum with a valve extension 39% ±3% (SE) beyond L₀ (Fig. 3B). At the valve length at which actively developed tension was maximum (Lₘₐₓ), active tension averaged 0.66 g ± .13 g (SE) (range 0.25 to 1.12 g).

Relation between extent of shortening and frequency of contraction. Shortening at a frequency of 12 beat/min has been taken as 100% and the other results related to this. Brackets represent ± 1 se.
**FIGURE 6**

Response of mitral valve muscle to the addition of acetylcholine (1.0 μg/ml) to the bath. Extent of shortening is substantially reduced. ΔL = change in length.

The latency from electrical stimulus to the first evidence of force development averaged 35 msec ± 6 msec (se), while the absolute refractory period measured 210 msec ± 28 msec (se).

Shortening under isotonic conditions with preloads of 0.5 g to 1.5 g, the mitral valve leaflet contracted 0.1 to 0.7 mm, producing an average shortening of the mitral valve of 6%. With inotropic stimulation by norepinephrine (0.1 μg/ml), this shortening of the mitral valve was increased to as much as 10%.

**Force-Frequency Relations.**—The effects of changing frequency of stimulation between 12 and 90 contractions/min were studied in 4 preparations prior to administration of any drugs. A negative inotropic effect of increased frequency was observed in all instances, the extent of isotonic shortening decreasing slightly but progressively as frequency of contraction was increased (Fig. 4). The duration of contraction also decreased with increasing frequency, time from stimulation to peak shortening decreasing from an average of 165 msec at 12/min, to 148 msec at 50/min and 135 msec at 90/min. In two preparations, the force-frequency relation was not altered by administration of atropine following acetylcholine (1.0 μg/ml).

**Response to Tyramine and Norepinephrine.**—The response to tyramine (1.0 μg/ml) was studied in 4 mitral valve preparations. Tyramine increased the extent of shortening by an average 42% ± 5% (se). An example of this response is shown in Figure 5A. Similarly, in 5 mitral valve preparations, norepinephrine (0.1 μg/ml) augmented the extent of shortening by 48% ± 17% (se) (Fig. 5B).

Prior to receiving norepinephrine, three of these preparations had received tyramine, which was then removed by washing.

**Response to Acetylcholine.**—Acetylcholine was added to 6 mitral valve preparations. A negative inotropic response was consistently observed, with complete cessation of contraction following 30 μg/ml, and a 48% ± 8% (se) reduction in the extent of shortening following the addition of acetylcholine (1 μg/ml). An example of this latter response is shown in Figure 6.

The addition of atropine (1 to 4 μg/ml) reversed the effects of acetylcholine in all preparations, and in 4 of 6 instances the subsequent contractions were somewhat greater than the control before acetylcholine.

**Discussion**

In the present study, the existence of cardiac muscle fibers, nerves and small blood vessels in the mitral valve has been reaffirmed (7-9), and a potential function for these structures has been demonstrated. Substantial amounts of cardiac muscle in the mitral valve of the cat and man, as well as the dog, have been demonstrated. In this study, the valve muscle was shown to be contractile and capable of developing significant amounts of force and shortening. Except for the observation that the resting stiffness of the mitral valve is quite large, as might have been anticipated from the presence of substantial amounts of collagen, the length-tension curve is not unlike that generally observed with muscle. Further, this tissue responds appropriately to both adrenergic (norepinephrine) and cholinergic (acetylcholine)
stimulation with substantial changes in force and shortening.

Autonomic neural control of the contractile tissue in the mitral valve appears likely. While neural elements have been noted in the mitral valve by several investigators (8, 9), they have been thought to be sensory fibers only (8). However, in a previous study, it was suggested that both adrenergic and cholinergic nerves were present in the valve itself (9). In the present study, tyramine, which releases endogenous catecholamines from adrenergic nerves, has been shown to augment contraction in mitral valve preparations, indicating a potential function for these adrenergic nerves. The tissue is also very sensitive to acetylcholine, the cholinergic mediator. Thus a more active role for the nerves of the mitral valve is envisioned from the present findings, since the adrenergic and cholinergic nerve fibers may modulate the force of contraction of the mitral valve muscle.

Since the atrioventricular valve leaflets arise from the myocardial anlage in the embryo, it is not surprising to find that they contain cardiac muscle and neural elements. The absence of these elements in the aortic and pulmonic valves (9) is consonant with their extra-myocardial origin, namely from the truncus arteriosus. The fact that the myocardial muscle in the mitral valve exhibits a negative inotropic response to acetylcholine in amounts which have little effect on ventricular muscle also suggests that this tissue is primarily atrial in origin (11). This view is further substantiated by the profuse-ness of cholinesterase staining of neural structures in the mitral valve (9).

While it is clear that the mitral valve leaflets have a capacity to develop force and to shorten, the functional role of the mitral valve musculature in vivo remains a matter of speculation. The muscle cells are arranged primarily perpendicular to the atrioventricular ring, which would tend to shorten the valve during their contraction. Since these fibers appear to be atrial in origin, they are probably activated, like the atria in general, in late diastole and may serve to maintain the form of the valve during diastole. Such positioning of the leaflets might also aid in the early closure of the mitral valve. It is also conceivable that in shortening of the anterior mitral leaflet, this muscle might help to prevent excessive ballooning of the mitral valve into the atrium during early systole. Such a view is consistent with the findings of Rushmer (6) that the mitral valve in diastole remains relatively taut throughout the cardiac cycle and does not balloon into the atria with systole. Further, recent studies in our laboratory have indicated that the mitral inflow orifice decreases in area during systole. The ability to alter the size of the mitral leaflets might help to keep the size of the leaflet appropriate for the mitral orifice. Thus these observations indicate the need for further studies on the functional significance of the morphological components of the mitral valve in cardiac performance.

References
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Page from van Leeuwenhoek's first correct description of the systemic connection between arteries and veins. Malpighi had already seen the pulmonary capillary bed in the frog (1661). Figures 5 and 6A show a single file of red cells in capillary loops of the median caudal fin of the tadpole (life size in Figure 2 and magnified in Figures 3 and 4). Figure 10 shows the long capillary loops in the tail fin of a newly hatched fish (shown life size in Figures 7 and 8 and magnified in Figure 9). In the text, van Leeuwenhoek stated that he saw 34 distinct vascular loops in the tail fin of the tiny fish and commented that the number of such circuits in our own bodies must be incredible.
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