Excitability and Electrical Activity of Human Myocardial Strips from the Left Atrial Appendage in Cases of Rheumatic Mitral Stenosis

By R. T. VAN DAM, M.D, AND D. DIJKER, M.D.

Few observations have been published on the behavior of the human heart muscle in vitro. This paper deals with the excitability and electrical activity of muscle strips taken from left atrial appendages during mitral commissurotomy in four patients.

Methods

During commissurotomy, left atrial appendages were resected and divided into two more or less equal parts. One of these parts was sent to the pathology department for routine microscopy; the other was immediately transmitted, in 0.9 per cent NaCl at body warmth, to the experimental assembly in an adjoining laboratory. A muscle strip about 2 mm. in diameter and 12 mm. in length was prepared with its axis parallel to the main direction of the muscle fibers and mounted in an electrode-holder carrying stimulating and recording electrodes (fig. 1). During preparation, the muscle strip was immersed in 100 ml. of Krebs-Henseleit solution at 37 C, which was perfused with a mixture of 5 per cent carbon dioxide in oxygen and changed at one-hour intervals. After mounting the strip in the electrode holder, we waited one hour before the experiment was started.

Square-wave driving and testing pulses were delivered by a stimulator containing a variable delay mechanism and a counting system, allowing application of pulses of chosen strength and duration at every selected interval following every eighth regular beat. In the bath, one large silver plate was used for grounding; another, at the opposite side, was used as a peripheral electrode for the "unipolar" leads. The electrical responses were recorded by means of a two-channel cathode-ray oscillograph. Small time-pips were delivered every 200 msec., synchronously in both leads.

The four patients were as follows: (1) woman, 45 years; mitral stenosis, sinus rhythm. During operation the left atrial appendage was found to be of normal size. Postoperatively, atrial fibrillation existed for a few days. (2) Man, 25 years; mitral stenosis, atrial fibrillation, cerebral embolism three months prior to operation. During operation the left atrial appendage was found to be grossly dilated, containing no thrombus. (3) Woman, 23 years; mitral stenosis and slight aortic insufficiency, sinus rhythm. During operation the left atrial appendage was found to be slightly dilated; no postoperative complications. (4) Woman, 49 years; mitral stenosis, decompensation six months prior to operation, alternating periods of

Figure 1
Cross section through lower part of the electrode holder. (A) Lucite block. (B) Silver ring stimulating electrode. (C) Lucite cone immobilizing one end of the muscle strip in (A) and carrying linear stimulating-pin electrode (D) of platinum, 3 mm. in length and 1 mm. in diameter. (E) Ridge over which a silk thread runs, which immobilizes the other end of the muscle strip. This thread is fixed by means of a clamp in the upper end of (K). (H) Lucite block carrying seven platinum electrodes 0.1 mm. in diameter and 2 mm. long, which are introduced into the muscle strip by lowering (H) along the lucite cylinders (I) and (K). The distance between electrodes (1) and (7) is 5 mm. Electrodes (B) and (D) are used for delivering driving and testing pulses.
sinus rhythm, atrial flutter, and atrial fibrillation. During operation the left atrial appendage was found to be grossly dilated; no contractions were observed. Postoperatively, a period of nodal tachycardia with retrograde conduction supervened.

**Results**

**Inexcitable Strips**

In the muscle strips of cases 2 and 4, no distinct spontaneous electrical activity could be observed and none could be caused by applied current pulses. Only occasional spontaneous nonconducted discharges of an unknown nature were noticed, vaguely resembling monophasic potentials. Addition of epinephrine in concentrations up to 0.5 times $10^{-5}$ to the bath fluid did not cause any appreciable change in condition of the muscle strips. Consequently, they were judged to be inexcitable. In case 2 there was constant atrial fibrillation, and in case 4 paroxysmal atrial fibrillation was present. In case 4, no movements of the atrial appendage were observed after exposing the heart. In both cases, microscopic examination demonstrated hypertrophy of the muscle fibers, thickening of the endocardium, and gross increase in the amount of connective tissue between the muscle fibers.

**Excitable Strips**

In the two remaining strips, conducted responses could be elicited by applied current pulses. Occasional spontaneous conducted action potentials also occurred in both of them. A regular spontaneous rhythm, however, was not observed, even after the addition of epinephrine to the bath. In both of these patients, a sinus rhythm existed prior to operation and the left atrial appendages were of normal size, or only slightly dilated.

**Action Potentials**

The unipolar and bipolar action potentials recorded from these human atrial strips were characterized by numerous notches and spikes, which contributed to a more or less "toothbrush-like" appearance. The duration of these complexes was 15 to 30 msec. (fig. 2, A and

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*Figure 2*

"Unipolar" complexes between electrode (4) and the peripheral electrode, and "bipolar" complexes between electrodes (4) and (5), taken from driven isolated strips of human left atrial appendages. (A) Case no. 1. (B) Case no. 3.

*Figure 3*

Strength/duration curves for isolated strips of human left atrial appendage, case no. 1, under control conditions and after addition of strophanthin $10^{-8}$ to the bath fluid.
ELECTRICAL ACTIVITY OF HUMAN MYOCARDIUM

B). This suggests that the activation wave traveling along the strip is not sharply defined, but is dissociated into many small depolarization waves, probably conducted along different groups of muscle fibers.

Resting Excitability

Threshold current requirements were determined for square-wave pulses delivered at a rate of 60 per minute to the muscle strips. The resulting strength/duration curves do not follow the smooth course generally observed in tissues that are homogenous in excitable properties; they appear to consist of different parts (fig. 3), like the curves obtained by Rushton\(^1\) and Auersperg\(^2\) in tissues containing components with different excitable properties.

As can be seen from figure 4 (a through d), the bipolar and unipolar complexes recorded during application of just-threshold stimuli of various duration show great changes in form, retaining their multiple notching. This points to the presence in these strips of components which differ in response to excitation.

Figure 4
Unipolar complexes taken from isolated strips of human left atrial appendage driven at 60 pulses/min. by square-wave shocks of varying duration and just-threshold intensity.
Case no. 1.
Excitability Cycle

The time course of the threshold following application of driving pulses at a rate of 60 per minute was found to be consistent with the well-known pattern observed after cathodal stimulation of animal heart muscle. Some strength/interval curves exhibit irregularities which can be ascribed to the different rate of recovery of some of the components of these muscle strips (fig. 5). In the same experimental arrangement, a similar mechanism was demonstrated in isolated cat papillary muscles which had been slightly crushed.

Stimuli applied during the early, relatively refractory period gave rise to development of action potentials displaying a much larger degree of dissociation than complexes obtained by stimulation at a later interval in the excitability cycle. Figure 6 illustrates typical examples of this phenomenon, in which the duration of complexes elicited by stimulation during the refractory period amounted to approximately 40 msec., in contrast with some 10 msec. during diastole.

Effect of Strophanthin

Following the observations described in the previous sections, strophanthin was added to the bath fluid in a concentration of $10^{-8}$. In both experiments, this caused a definite shortening of the refractory period, with concomitant slight lowering of the diastolic threshold level (fig. 5). In regard to the strength/duration requirements, the effect of this concentration of strophanthin on the different components was contradictory. For pulses of a duration of less than 5 msec., a lowering of the threshold was observed, whereas the threshold for longer pulses was found to rise. At the same time, both strength/duration and strength/interval curves became smoother. Some of the irregularities in their course disappeared. The strength/duration curve illustrated in figure 3 ultimately showed two main parts, probably representing the excitabilities of two different components.

Microscopic Examination

Compared with the parts of the left atrial appendages not used in these experiments, the microscopic appearance of the strips was slightly changed after completion of the experiments. The muscle fibers showed some stretching, fragmentation, and segmentation; cross and longitudinal striation and the appearance of the nuclei were normal; there was minimal edema of the interstitial connective tissue.

Discussion

The results presented in this paper indicate a large degree of functional dissociation between the individual muscle fibers or between groups. For obvious reasons, strips of normal left atrial appendages could not be obtained for comparison. Epicardial records from normal humans and dog atria, however, do not show evidence of such a dissociation; rather, they represent one sharp intrinsic deflection.

In the normal dog, as van der Kooi et al. point out, atrial complexes showing a tooth-
brush-like appearance can be recorded from the sinus node only, not from other parts of the atria. Clearly, some disturbances must have been present in the muscle strips used. Two factors can be conceived to have been effective: first, the isolation of the atria by the surgeon and the insertion of the strips in the electrode holder; and second, the rheumatic disease present in these patients. However, in strips taken in the same fashion from the cat’s heart and the outer layers of the ventricular myocardium of the dog and subjected to the same experimental arrangement, such phenomena due to a functional dissociation were not observed. They could be demonstrated only in isolated cat papillary muscles which had been handled with less care than these human atrial strips. Although in these strips some microscopic alterations may have contributed to the functional dissociation, these changes were also present in the animal strips mentioned above.

In our opinion, the anatomic changes in the atria associated with the rheumatic disease have been the decisive factor. In all these muscle strips, evidence was found of hypertrophy of individual muscle fibers and of a marked degree of fibrosis. This may account for a structural division of the musculature into many different bundles, separated from each other by connective tissue.

One may speculate that this process of alteration of the atrial myocardium favors the establishment of arrhythmias, since it may give rise to a re-entry situation. However, in the two cases in which the atrial strips were found to be excitable, a sinus rhythm existed prior to operation. Microscopic examination, however, demonstrated the presence in both these strips of hypertrophic muscle fibers, fibrosis of the epicardium, Aschoff nodules in the subendocardial layer, and some degree of fibrosis between the muscle fibers.

Our material, although limited, suggests that the morphological changes developing in the left atrial appendages in cases of rheumatic fever continue for a long time to be compatible with a regular sinus rhythm.

The observation of an increase in functional dissociation during the refractory period renders some support to the theory that intrinsic or extrinsic stimuli acting during the refractory period may, in some conditions, facilitate the occurrence of fibrillation, since such an increased functional dissociation may give rise to a re-entry mechanism.

The observed effect of strophanthin on these muscle strips confirms the view, derived from
animal experiments, that digitalis-like substances shorten the refractory period. The lowering of the diastolic threshold level, however, is contrary to the animal findings. We can offer some explanation of this, since in these determinations 5-msec. testing pulses were employed, whereas the strength/duration curves indicate that in these experiments strophanthin caused the threshold for stimuli of a longer duration to rise.

Since, as Hoffman, Kao, and Suckling7 have demonstrated, the stimulating effect of a propagated activation corresponds to that of applied current pulses with a duration of approximately 15 msec., it seems probable that strophanthin, by increasing the diastolic threshold level for current pulses of this duration, impedes in some degree the propagation of an impulse in these strips of human atrial myocardium.

Summary

In isolated strips of human left atrium, obtained during commissurotomy for rheumatic mitral stenosis, recordings of the electrical action phenomena were made and determinations of the resting excitability and of the excitability cycle were carried out. Two of the four strips did not show any appreciable spontaneous electrical activity and were found to be inexcitable by applied current pulses. These strips were obtained from patients in whom permanent or temporary atrial fibrillation existed prior to operation, and in whom the left atrial appendage was grossly dilated. In the other two strips, obtained from patients with a sinus rhythm prior to operation and with left atrial appendages of approximately normal size, determinations of strength/duration and strength/interval requirements, together with the recorded complexes, demonstrated the occurrence of a functional dissociation between various elements in these muscle strips, which increased during the refractory period. This functional dissociation is thought to be related to the development of connective tissue between the muscle fibers, observed by microscopic examination, and due to the rheumatic process. Its significance in the genesis of atrial arrhythmias in cases of mitral stenosis is discussed. Strophanthin in a concentration of $10^{-8}$ was found to shorten the refractory period and to cause a lowering of the resting threshold for stimuli with a duration of less than 5 msec., and a rise in the threshold for larger stimuli.

Acknowledgment

The authors wish to express their sincere thanks to Prof. I. Boerema for his aid, and to Dr. J. F. Hampe for his examination and description of the microscopic preparations.

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Circ Res. 1961;9:509-935
doi: 10.1161/01.RES.9.3.509

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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