It has long been held that transmission of excitation from cell to cell in cardiac muscle is effected by local-circuit current, i.e., the myocardium is a syncytium. This idea arose on the basis of light microscopy and the all-or-none nature of cardiac contraction. Recent electron microscopy has shown that the myocardium is not a morphological syncytium; the intercalated discs are actually cell boundaries. If the resistance of the disc membranes is high, then local-current transmission is unlikely because the spread of current into the next cell would be greatly curtailed; the major possibility remaining would be that of chemical junctional transmission. Several lines of evidence have led to a questioning of the validity of the local-circuit current theory of transmission.

The work reported here provides additional evidence that the intercalated discs are of high resistance. A study was made of the ability of strips of parallel-fibered muscles to respond directly to current applied along the longitudinal axis of the muscle in the absence of propagation. It was found that responses to such direct stimulation continued during block of propagation in the short-celled cat papillary muscle, but not in the long-celled frog sartorius muscle.

**Methods**

Strips of parallel fibers, with the cells oriented parallel to the long direction of the strip, were obtained from papillary muscles of the right ventricle of the cat and from the frog sartorius (Rana pipiens). The papillary muscles, selected for uniform thickness throughout their length, were about 1 mm thick and 20 mm long when mounted under tension. Following experimentation, several of the papillary muscles were prepared for histological sectioning; examination revealed that the muscles contained only parallel fibers. The muscles were mounted within a chamber in air of high relative humidity to prevent drying. Air mounting did not affect performance of the muscles but did facilitate external recording of action potentials and did help to confine stimulating current to localized regions of the strips. Furthermore, such a system facilitates longitudinal orientation of electric fields with the long axis of the cells when the stimulating electrodes are located at the very ends of the strip. Even branching cardiac fibers will be almost longitudinal in orientation due to mounting under stretch. In the experiments reported here, it is assumed that the muscle cells, especially those located in the middle regions of the long, thin strips, were subjected to true longitudinal fields. External electrical recording, rather than intracellular, was preferred because it enabled monitoring of all cells beneath the recording electrodes. Periodically, bathing solution was pipetted onto the muscle. Composition (in mM) of the mammalian Ringer solution was 137 NaCl, 2.7 KCl, 1.8 CaCl₂, 1.0 MgCl₂, 11.9 NaHCO₃, 0.42 NaH₂PO₄, and that for frog Ringer was identical except for 100 NaCl and 8.5 NaHCO₃.

A pair of recording electrodes was located near the middle and a pair of stimulating electrodes was located near each end of the strip (fig. 1). Stimulating current was passed through one of the following three pairs of electrodes in the following sequence: S-1, one electrode located at each end of the strip; S-2, both electrodes located at the right end of the strip; S-3, both electrodes located at the left end. With this arrangement, propagation could be initiated at the ends of the strip (S-2 or S-3) or direct longitudinal fields could be applied to cells near the middle of the strip without propagation. Under conditions of blocked propagation produced by addition of isotonic sucrose solution, the muscle cells near the middle of the strip could be tested for direct response to longitudinal fields. The muscles were...
stimulated at an approximate rate of once every three seconds.

Isotonic contractions were recorded on the lower beam of a dual-beam cathode-ray oscilloscope; the upper beam recorded the action potentials. The stimulating and recording electrodes were reversible Ag-AgCl wick electrodes. The stimulating shocks were near threshold and consisted of rectangular constant-current pulses of brief duration (0.5 msec for cardiac and 0.1 msec for skeletal muscles) and of constant intensity; thresholds were determined by varying the shock duration. The stimulus parameters were the same for S-1, S-2 and S-3 stimulations. The cat papillary muscles were maintained at 30°C and the frog sartorius muscles at room temperature.

Results

CAT PAPILLARY MUSCLES

Electrical and mechanical responses to direct longitudinal field stimulation continued, without change in threshold, during block of propagation in the short-celled cat papillary muscle (fig. 2). Figure 2 A-C shows control responses to S-1 (A), S-2 (B) and S-3 (C) stimulation. Time and voltage calibrations represent 150 msec and 10 mv. Note the lack of propagation in A as evidenced by the fact that the electrical response emerges directly out of the shock artifact. Responses D-F resulted within three minutes following addition of isotonic sucrose solution. Markedly enhanced contractions were always observed to occur in cardiac muscle. The spikes following S-2 and S-3 stimulation were often transiently enhanced in magnitude due to a higher resistance between the two recording electrodes following sucrose replacement of extracellular ions (fig. 2 E, F). Photographs G-I were recorded within five minutes of additional application of the sucrose solution. Electrical and mechanical responses to S-1 stimulation (G) continued almost unchanged from those obtained during control bathing with Tyrode solution (A) and there was no increase in threshold. In contrast, electrical and mechanical responses to S-2 (H) and to S-3 (I) stimulations did not occur, denoting complete absence of propagation. Increase of shock duration ten-fold neither produced S-2 and S-3 responses nor altered the shape and magnitude of the S-1 responses; hence failure of propagation is not due to an increase of threshold. The slight contractions noted in H and I are due to direct stimulation and contraction at the ends of the muscle strips. Failure of propagation accompanied by unchanged behavior to S-1 stimulation could be maintained for periods exceeding 30 minutes. Excessive bathing with isotonic sucrose solution eventually led to failure of responses to S-1, S-2 and S-3 stimulations as expected; however, propagation always failed first. Recovery of all responses occurred within five minutes following addition of Tyrode solution. Several papillary muscles recovered after six successive additions of sucrose solution. Photographs J-L are a repeat of the conditions of G-I with another papillary muscle.

FROG SARTORIUS MUSCLES

In the long-celled frog sartorius muscle, responses to direct longitudinal field stimulation failed simultaneously with failure of propagation (fig. 3). Figure 3 A to C shows control responses to S-1 (A), S-2 (B) and
FIGURE 2
Cathode-ray oscillograph traces of electrical (upper beam) and mechanical (lower beam) responses from cat papillary muscle. Each row (A-C, D-F, G-I, J-L) represents S-1, S-2 and S-3 stimulation in succession. A-C: control responses; action potentials follow the brief shock artifacts. Spikes inverted between B and C because of propagation in opposite directions. D-F: within three minutes following addition of isotonic sucrose solution. G-I: within another five minutes of continued addition of sucrose solution. J-L: repeat of conditions of G-I with another muscle. Shock duration 0.5 msec and constant-current stimulation throughout. Horizontal time and vertical voltage calibrations in L represent 150 msec and 10 mv (upper channel), respectively. Backgrounds retouched for clarity.
FIGURE 3

Cathode-ray oscilloscope traces of electrical (upper beam) and mechanical (lower beam) responses from frog sartorius muscle. Each row represents S-1, S-2 and S-3 stimulation in succession. A-C: control responses. D-F: within 10 minutes following addition of isotonic sucrose solution. G-I: duration of stimulating pulses increased ten-fold showing more prominent shock artifacts. J-L: recovery of responses within a few minutes following addition of frog Ringer's solution. Shock durations all 0.1 msec, except in G-I where they are 1.0 msec; stimulating voltages constant throughout. Horizontal time and vertical voltage calibrations in F represent 20 msec and 20 mv (upper channel), respectively.

S-3 (C) stimulation. Time and voltage calibrations represent 20 msec and 20 mv. The contractile response to S-1 stimulation has a faster rate of rise and is larger compared to S-2 or S-3 because of a decreased propagation time which probably results either from stimulation of the cell membranes more centrally than with S-2 or S-3 stimulation or from excitation of motoneurons with S-1 stimulation. Photographs D-F were recorded within eight minutes following addition of isotonic sucrose solution. In contrast to cardiac muscle, no enhanced contractions were observed after the addition of sucrose solution. Failure of electrical and mechanical responses to S-1 stimulation (D) occurred almost simulta-
neously with failure of propagation to S-2 (E) and S-3 (F) stimulation. Increase of shock duration ten-fold, from 0.1 to 1.0 msec (note enlarged shock artifacts) neither produced a response to S-1 stimulation (G) nor produced propagated responses to S-2 (H) and S-3 (I) stimulation. Hence, failure of responses to S-1, to S-2 and to S-3 stimulation is not due to an increase in threshold. The larger contractions observed in G-I compared to D-F resulted from greater localized stimulation at the ends of the muscle strip. In all cases, propagation and direct stimulation by longitudinal electric fields failed simultaneously. Recovery of responses occurred within five minutes following addition of frog Ringer's solution (fig. 3 J-L).

Discussion

The experiments described in the present report clearly show a basic difference in behavior of long, narrow strips of cardiac and skeletal muscles. The cells are oriented parallel to the long axis of the strip in both instances. In the long-celled skeletal muscle, electrical and mechanical responses to longitudinal electric fields failed simultaneously with failure of propagation following addition of isotonic sucrose solution. Responses to S-1, S-2 and S-3 stimulations failed even when the stimulus duration was increased ten-fold. This result is expected because in the sartorius muscle the individual cells extend almost the entire length of the strip, and in a longitudinal electric field, excitation should only occur at the ends of the cells where current traverses the cell membrane (function of the space constant). This assumption was tested and found to be true. However, in cardiac muscle, addition of the sucrose solution produced failure of propagation following a ten-fold increase in stimulus duration. The latter responses continued without a change in threshold. This result would be expected only if the intercalated discs were high-resistance so that the functional cell lengths were short, allowing direct and simultaneous excitation of each cell along the middle region of the strip, without the necessity of propagation. The high-resistance transverse membranes "force" radial currents to flow within each cell because of the potential difference that would exist between either end of the cell and its respective adjacent extracellular point. Such potential differences would exist due to the voltage drops across the intercalated discs. Radial currents, wherever depolarizing, would trigger off each cell. The fact that the action potential responses of the middle of the strip appeared directly out of the brief shock artifacts further suggests that the underlying cells show a direct, simultaneous and nonpropagated response to longitudinal field stimulation.

Although most likely imperfect, the electric field applied in cardiac muscle is probably close to a true longitudinal field. Long, narrow strips of parallel-fiber papillary muscle suspended in air with stretching, and with the stimulating electrodes at the very ends of the strip, produce a reasonably good longitudinal field in the middle region of the strip. Even branching fibers would be subjected to an almost perfect longitudinal field. The fact that the responses to longitudinal field stimulation were almost identical in shape and threshold before and after block of propagation, further suggests that all cells subjected to the longitudinal field respond directly. The mechanism of the complete propagation block during addition of isotonic sucrose solution is unknown, but probably results from lowered extracellular Na concentration. Block of propagation by sucrose solution was selected because there was no change in threshold to longitudinal field stimulation; block of propagation with high Mg ++ concentration decreases excitability and uncouples contraction from excitation. Increased contractions, upon beginning bathing with isotonic sucrose solutions, were always observed in cardiac muscle but never in frog sartorius. The mechanism of this large increase in contraction is unknown but may be related to the [Ca ++ ]/[Na + ] 2 ratio. Because of binding, it is expected that extra-

Circulation Research, Volume XII, June 1963
cellular Ca\(^{++}\) would wash out more slowly than extracellular Na\(^{+}\), thereby increasing the \([\text{Ca}\(^{++}\)] /[\text{Na}\(^{+}\)]\) ratio and perhaps increasing the degree of coupling of contraction with membrane excitation.

Much evidence has been accumulating recently from morphological, histochemical and electrophysiological approaches which questions the validity of the local-circuit current theory of transmission of excitation between myocardial cells. Electron microscopy has shown that the intercalated discs are the boundaries of individual cells and synaptic-like vesicles have been observed at the intercalated discs. Bourne\(^2\) reported that a large concentration of alkaline phosphatase, suggestive of a high rate of energy production, is specifically localized at the intercalated discs of cardiac cells. Ito and Caillik\(^3\) have recently found that cholinesterase is localized at the intercalated discs, suggesting that acetylcholine plays some role at the discs. Acetylcholine reduces the spread of current to about one-half in atrial muscle.\(^14\) Schnitk postulated transverse electrical barriers in cardiac muscle because depolarization resulting from injury was confined to distances less than 1 mm; in contrast, depolarization from injury spread throughout the length of the fiber in skeletal muscle.

Previous results from our laboratory suggesting high-resistance, high-capacitance intercalated discs may be summarized as follows: a) During hypertonic perfusion of frog heart, electrically quiescent cells were often found adjacent to active ones, and individual cells often fired at a frequency lower than that of the electrocardiogram.\(^5\) It has been reported that under such conditions the cells are pulled apart at the intercalated discs and the space constant is reduced.\(^14\) This would argue in favor of high-resistance discs, at least under conditions of hypertonicity. b) The resistance between the interiors of two myocardial cells (electrodes about 0.5 mm apart) was double that of one cell (one electrode located intracellular, the other extracellular).\(^4\) c) The specific resistances of cardiac and smooth muscle strips were found to be relatively hypersensitive to interspace ion depletion when compared with skeletal muscle.\(^5\) d) The impedance of strips of cardiac and smooth muscles, but not of skeletal muscle, decreases with frequency, and this frequency dependence is accentuated upon raising the resistance of the extracellular fluid.\(^5\) e) Frog ventricular strips can be induced to exhibit unidirectional failure of propagation, in a predictable direction, upon passage of conditioning current pulses in the presence of high Mg\(^{++}\) concentrations.\(^7\) f) Frog ventricular cells can be induced to exhibit stopped rising phases when the strip is stimulated at one end and smooth rising phases when stimulated at the other end.\(^7\) Prepotentials have also been reported in atrial and ventricular cells during various treatments.\(^5\) g) Under conditions of impaired propagation, the cardiac action potential can be fractionated into two components, an initial fast spike and a graded slow wave corresponding to the plateau.\(^5\) Spike and slow wave components, having different \(Q_{01}\) values,\(^7\) have also been reported under the conditions of low temperature,\(^16\) procaine,\(^18\) acetylcholine or hypoxia,\(^17\) and hyperuricemia or overstretch.\(^19\) One may speculate that the intercalated disc is responsible for the prepotential and slow wave. Other investigators have postulated recently that a chemical mediator may be responsible for the plateau.\(^3\)\(^,\)\(^16\) The conductance changes responsible for the slow wave may be time dependent and not voltage dependent;\(^21\) this would agree with the finding that the slow wave is graded.\(^8\)

Space constant measurements have been cited to validate the syncytial concept.\(^14\)\(^,\)\(^22\) Woodbury and Grill\(^14\) have reported that efficient electrical transmission requires that the ratio of the cell radius (about 8 \(\mu m\)) to the space constant of the disk must be relatively large. Assuming that a gap of 80 \(\AA\) exists between adjacent cell membranes at the disks and that the surface area of the disks (about 2000 \(\mu m^2\)) is ten times the cross-sectional area of the cell (300 \(\mu m^2\)), they calculated that the
specific resistance of disk membrane could have a maximum value of only 12 ohm-cm². However, their experimental finding of a relatively short space constant of 130 μ in the direction parallel to the fibers of rat atrial trabeculae may be consistent with high-resistance intercalated discs in that this finding either requires that the disk resistance be much greater or that other less likely alternatives must be assumed. The fact that the propagation velocity in the parallel direction is faster than that in the transverse direction has also been cited as evidence for the syncytial concept. On the contrary, such differences in velocity would also be expected on the basis of the nonsynaptic hypothesis. Furthermore, temperature and strophanthin affected propagation in the parallel and transverse directions to different degrees.

Summary

Parallel-fibered strips of cardiac (cat papillary) and skeletal (frog sartorius) muscles were mounted in air and action potentials were externally recorded from the middle of the strip. Electric fields, oriented longitudinally with respect to the long axis of the cells, were applied. A pair of stimulating electrodes was located at each end of the strip to test for propagation. Under conditions of blocked propagation produced by addition of isotonic sucrose solution, the cells near the middle of the strip could be tested for direct response to longitudinal fields. In long-celled skeletal muscle, the sucrose solution produced simultaneously failure of electrical and mechanical responses to stimulation across the strip as well as failure of propagation; stimulation at ten times threshold failed to elicit responses. However, in short-celled cardiac muscle, the sucrose solution produced failure of propagation first; electrical and mechanical responses to longitudinal field stimulation continued without a change in threshold. From these experiments it may be concluded that transverse membranes of high resistance are present in cardiac muscle; thus, radial currents are forced to flow through each cell membrane leading to direct excitation.

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