Wave front analyses of impulses in tunicate heart

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KRIEBEL, MAHLON E. Wave front analyses of impulses in tunicate heart. Am. J. Physiol. 218(4): 1194-1200. 1970.—The U-shaped, tubular hearts of Ciona are composed of a single layer of cells joined by apical tight junctions. Pacemakers at each end alternate in periods of activity, reversing the direction of blood flow. Hearts are attached by a raphe along one edge to the pericardium. Impulses do not pass the raphe and impulse spread was not altered after hearts were isolated and opened by cutting along the raphe. Conduction velocities were determined by placing the opened myocardium over a Plexiglas plate containing suction electrode openings spaced 1 mm apart. Conduction velocity in the heart axis increased (or decreased depending on the direction of conduction) linearly from 1 mm/sec at the ends to 7 mm/sec in the middle half of the heart (10 C). Wave fronts propagating along the heart axis were found to be parallel to the cell axis (except at the ends and bend) in all directions of conduction. Conduction velocity was found to be greatest parallel to the cell axis (77 mm/sec at 65° to the heart axis) and least perpendicular to the cell axis (8.7 mm/sec). These results demonstrate that cell shape influences impulse spread in an otherwise uniform sheet of excitable tissue.

Conduction velocity

TUNICATES are protochordates with simple tubular, valveless hearts. There are pacemakers at each end of the heart which change the direction of peristalsis and blood flow every few minutes. Since these hearts are composed of a single layer of cells joined by apical tight junctions (zonula occludentes), this is an ideal tissue to study some general properties of the heart. It has already been demonstrated that action potentials are similar to those of mammalian hearts (8). In addition, an impulse is conducted from cell to cell, probably by local current flow, since transmission time from cell to cell is less than 0.3 msec at 10 C (8). Since current can be passed through heart tissue in a sucrose gap (11) and since electrotonic currents have been observed to spread across areas of conduction block (10), the heart can be considered as an electrical synecium.

In mammalian hearts, Draper and Mya-Tu (3) have demonstrated that the conduction velocity is greatest in the cell axis. However, vertebrate heart cells are usually associated in a complex array of branching cords and cells in adjacent cords may be electrically isolated (22, 23). Therefore, differences in conduction velocities in different directions may reflect tissue structure and not cell shape. The only morphological asymmetry in a sheet of tunicate heart is cell shape. The second interesting property of the tunicate heart is the observation that the resistance across the heart wall is so high that the junctions between cells must be so tight that there is no extracellular space for ionic currents (11).

The tunicate heart was found to be ideal for studies concerned with impulse spread through a tissue for various reasons: 1) an impulse spreads in two dimensions since the heart is only 10 μ thick, 2) the conduction velocity is low enough so that the temporal spread of an impulse can be analyzed in all directions from a point stimulus, 3) the conduction velocity is the same in both directions of conduction (9), and 4) the cell width to length ratio is about 1:15 (8, 11). It was the purpose of this investigation to study the temporal pattern of impulse propagation in order to determine how cell shape influences impulse spread in the tunicate heart.

METHODS

Hearts of adult Ciona intestinalis (from California) were removed and opened by cutting the heart wall on each side of the raphe which connects the heart to the pericardium. Hearts were 30-46 mm in length (from pacemaker to pacemaker) by 2.5-3.0 mm in diameter. A jet of seawater delivered from an eye dropper was used to flatten hearts (lumen surface down) to the bottom of a shallow bath filled with seawater at 10 C in a 1 mm interval (Fig. 1). This procedure slightly stretched the tissue so standardization among hearts was accomplished by allowing hearts to beat a minute or two and adjust to natural dimensions before securing their edges with hooks. Tension was the same as used in previous studies (8, 10, 11).

The bath contained a Plexiglas plate with a row of five holes (30-40 μ in diameter) spaced at 1.00 mm intervals (Fig. 1A). Each hole served as a suction electrode opening and was connected to its own hydraulic system made from a micrometer and a Clay-Adams tubing adapter (Fig. 1B).

Conduction velocity in the heart axis was determined by placing the long axis of the heart over the line of electrodes as shown in Fig. 1C. The conduction velocity for each millimeter of heart length was determined by moving the heart to new positions. The arrival times of a wave front at points perpendicular to the heart axis was determined by placing the heart axis perpendicular to the line of electrodes. In order to determine the conduction velocity in many directions, the middle portion of a heart arm was placed over the electrodes by lining the edge of the heart along a radial guide line (Fig. 1A) so that the end stimulating electrode started the impulse at the same place for each position.

Each recording electrode terminal (1, 2, 3, and 4) as shown in Fig. 1B was connected to one input terminal of a
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**C.**0. . . . . . . aIkleort 
Polyethylene tubing  
Sea water  
Wire  
Plastic tubing  

**FIG. 1.** Top and side views of recording system. A: top view of inscribed lines in Plexiglas plate containing line of electrodes. Holes are 1.00 mm apart and about 30 μ in diameter. Electrode labeled S was used for stimulation. Electrodes 1, 2, 3, and 4 were used for recording. Radial guide lines were inscribed into Plexiglas every 11.2°. In vector analyses, heart was rotated around central electrode and one edge of heart was positioned parallel to a guide line. B: schematic cross section through preparation chamber. Total inside bath diameter was about 6 cm. Each electrode opening was connected to its own hydraulic system. Briefest signals were obtained by “sucking” tissue into holes to a depth of about 30 μ. Steel plunger is 0.009 inch in diameter. C: schematic drawing showing a top view of bath with opened heart spread over 5 electrode openings. This heart placement would give conduction velocities in heart axis. Vector analyses were carried out by rotating heart around stimulating electrode in direction of arrow.  
Tektronix 122 preamplifier. The second terminal of the preamplifiers and the bath were grounded. Signals were displayed on a Tektronix 555 oscilloscope equipped with 3A74 amplifiers and were recorded on film with a Grass kymograph camera. Tektronix pulse and waveform generators were used to produce 8-msec-long supramaximal pulses which were applied at an end electrode in the line of five electrodes (S in Fig. 1A and B). Hearts were photographed with a Vickers 40X phase-contrast reflecting objective (NA 0.57) in order to determine the cell axis.

**RESULTS**

**A. Orientation of muscle cells with respect to heart axis.** At most, 0.5 mm of heart was left asymmetrically on one side of the raphe which altered the opened heart axis at most only 3°. The cell axis in relation to the long axis of the heart was found to gradually change at the ends and bend from 90° to 65° (range ± 8°) and remain at 65° for most of the middle region of each arm of the U-shaped heart (Fig. 2A). The apical (outer) membrane of each cell bulges around the nucleus and these bulges are located in rows oriented 0–30° to the heart axis which give the heart a corrugated appearance when viewed with a dissecting microscope (Fig. 2B and C). Each cell contains a single myofibril located near the lumen surface.

**B. Conduction velocity in heart axis.** The conduction velocity was not altered by removing the heart or by opening the heart along the raphe (8, 12). Thus, impulse spread in opened hearts is probably the same as in situ. Middle portions of heart arms were repeatedly positioned over the stationary suction electrode openings and the conduction velocity in the heart axis was found to be the same across the width of the heart. The conduction velocity was found to remain constant even when the width of the heart was reduced to about 250 μ.

Conduction times were determined for each millimeter length of large hearts and for impulse propagation along the heart axis in either direction. In 15- to 18-mm-long heart arms (one-half of the U-shaped heart), the conduction velocity increased linearly from about 1 mm/sec at the ends of the heart to about 6.5 mm/sec at a position 6–8 mm from the end of the heart, and then remained constant to the bend (Fig. 3). Except for the first millimeter or two at the

**FIG. 2. Ciona heart cells.** A: intact heart showing cell orientation in relation to heart axis. B: cross section of one heart arm 65° to heart axis. C: diagrams of a single cell. Apical view of cell shows widest dimensions. Lumen view shows only that portion of membrane which faces lumen. Apical nexus completely encircles each cell. Patch nexuses are also present, but they are not represented in diagrams.
ends of the heart, the conduction velocity was the same in both directions of conduction. In the first millimeter or two at the ends of the heart, the conduction velocity was usually less when the impulse started from the pacemaker closer to the recording electrodes; i.e., the conduction velocity was less during impulse acceleration than during impulse deceleration. In middle regions of heart arms successive impulses always had the same conduction velocities. However, towards the ends of hearts different conduction times were sometimes recorded (see discussion, Impulse acceleration and deceleration).

The conduction velocity around the bend of very large hearts (each arm 23 mm long) was found to depend upon the electrode placement. When the axis of the line of electrodes was placed across the inner edge of the bend close to the arms, the lowest apparent conduction velocity was recorded. This position would compare to position 14 in Fig. 4 and the lowest conduction velocity would be recorded between the two middle electrodes. At this recording position, the cell axis changes in relation to the line of electrodes, which explains the apparently low conduction velocity around the entire bend of the heart (cf. 8, 14). When the electrodes were placed on the outer edge of the bend region of the opened heart, the greatest apparent conduction velocity was recorded because the wave front reached the electrodes almost simultaneously (see wave front in bend region of diagram in Fig. 4). In Fig. 4 the impulse invaded all of the bend region before moving into the second arm of the heart. In extremely large hearts, the impulse moved into the second arm of the heart before the extreme outer edge of the bend region was invaded (see ref. 14 for complete analysis).

C. Arrival times of an impulse at points on imaginary lines perpendicular to heart axis. The curves delineating successive wave fronts were found to be parallel in both directions of conduction to the cell axis in the middle two-thirds of each heart arm (Fig. 4). At the ends of the heart, the curves representing successive fronts which started at the near pacemaker were usually closer together for the first millimeter or two than when impulses started at the pacemaker in the opposite arm of the heart. This is readily explained since the conduction velocity of an impulse increased until the wave of excitation extended across the width of the heart (see section D). At the bend, the curves representing wave fronts moving in opposite directions were mirror images.

D. Temporal vector analyses of impulses originating at a point stimulus. Conduction velocities of an impulse starting from a point stimulus were determined in different directions by rotating the middle regions of heart arms around a stimulating electrode. At the rotation position that gave the greatest conduction velocities, the wave front of an impulse starting from a pacemaker reached the four recording electrodes.

![Diagram of heart](image_url)

**FIG. 4.** Wave front analysis of an impulse moving along heart axis. Large, scale diagram shows wave front at successive 0.2-sec intervals in one heart arm during abvisceral contractions. Electrodes are indicated by circles and axis of line of recording electrodes are numbered. Axial placements of electrodes are 1 mm apart and correspond to electrode positions used to determine velocities in heart axis. Top row of records (each composed of 3 or 4 traces except position 14) shows arrival of abvisceral impulses and bottom row of records shows the arrival of advisceral contractions. Note that sequence of signals is reversed in two directions of conduction. Points in wave front curves and successive intervals were interpolated from spread of arrival times of wave front at each electrode axis (records) and velocities in heart axis as shown in Fig. 3. Reversal in direction of conduction was induced by locally heating the branchial pacemaker region. This procedure slightly warmed bath so that advisceral conduction velocities were a little greater than abvisceral velocities. Insert shows heart arm before it was opened along raphe and flattened with lumen surface down as shown in larger diagram.
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16.3

12.8

12.7

1
set

44
0
8

Oa
%
?ih
4%

Average conduction velocity between electrodes simultaneously (in Fig. 5 this occurred at an angle of 56° between the electrode axis and heart axis). When the hearts were rotated an additional 90°, the lowest conduction velocities were recorded for both impulses starting from a pacemaker and the stimulating electrode (Fig. 5 at 146°). The greatest conduction velocities of impulses starting from a point stimulus were recorded in the cell axis and the lowest velocities were recorded perpendicular to the cell axis (Table 1, Fig. 6).

The conduction velocities calculated with conduction times determined from electrode positions furthest from the stimulating electrode were greatest, indicating that the impulse increased in conduction velocity during the first millimeter or two of spread (Fig. 7, Table 1). In the direction of the greatest conduction velocity, the impulse velocity increased from 14 to 85 mm/sec. In the direction of the least conduction velocity, the impulse velocity increased from 4 to 9 mm/sec (Table 1). The results of one experiment (data from Fig. 5) are plotted in Fig. 7 to show the increase in conduction velocity as an impulse spreads from a stimulating electrode.

DISCUSSION

A. Evaluation of opened heart preparation and recording techniques. The fidelity of these data to normal impulse spread in hearts in situ can be established by considering the following arguments. In the middle of an arm, conduction velocity in the long axis of the heart was found to be constant throughout the width of the tissue for both natural and stimulated impulses. Since the conduction velocity remained the same after the middle portions of heart arms were cut down to a narrow 250-μ strip of tissue and since excitation does not pass the raphe (8), it can be concluded that conduction is normal along the edge of opened hearts. The slack condition of opened hearts corresponds to that of hearts in situ when they contain no blood. After diastolic filling the heart circumference increased up to 30% although the length of the middle two-thirds of each heart arm remained constant and the axial conduction velocity simultaneously.

TABLE 1. Conduction velocities obtained in vector analyses of an impulse spreading from a point stimulus

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Hearts</th>
<th>Length of Hearts, mm</th>
<th>Signals Used to Calculate Velocity</th>
<th>Greatest Conduction Velocity, mm/sec, and Degree of Rotation</th>
<th>Least Conduction Velocity, mm/sec, and Degree of Rotation</th>
<th>Conduction Velocity in Heart Axis, mm/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ciona</strong></td>
<td>4</td>
<td>36-46</td>
<td>S and 1</td>
<td>14 (±6) at 65° (±11)</td>
<td>4.2 (±2.0) at 155° (±11)</td>
<td>6.5 (±2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S and 4</td>
<td>24 (±10)</td>
<td></td>
<td>6.6 (±2.5)</td>
<td>8.6 (±2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 and 4</td>
<td>36 (±28)</td>
<td></td>
<td>8.7 (±1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 and 4</td>
<td>77 (±27)</td>
<td></td>
<td>8.7 (±1.8)</td>
<td>6.5 (±2.0)</td>
</tr>
<tr>
<td><strong>Chelyosoma</strong></td>
<td>1</td>
<td>24</td>
<td>S and 1</td>
<td>12.5 at 79°</td>
<td>4.0 at 169°</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S and 2</td>
<td>15</td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 and 2</td>
<td>25</td>
<td></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30-45</td>
<td>S and 2</td>
<td>12 (±2) at 70° (±6.0)</td>
<td>2.9 (±0.4) at 160° (±6.0)</td>
<td>5.5 (±2.0)</td>
</tr>
</tbody>
</table>

In rows S and 1 of the signal column, the average conduction velocities were calculated from the time intervals between the stimulus artifacts (S) and the signals at the first recording electrode, 1. In row S and 4, the average conduction velocities were calculated from the time intervals between the stimulus artifacts and the signals at the fourth, 4, recording electrode. In row 1 and 4, the average conduction velocities were calculated from the time intervals between the signals recorded by electrodes 1 and 4. Since the impulse reached its maximal velocity before it reached electrode 3, the maximal velocities were calculated from the time intervals between the signals at electrodes 3 and 4. Ranges are given in parentheses. *Values of Chelyosoma productum are given for comparison (see Kriebel (14) for complete analysis).
remained constant (8). It was demonstrated here that stretching the heart with two hooks in the cell axis decreased the conduction time between fixed electrodes equal to the increase in length. However, the error in estimating the position of wave fronts in situ is not 30% since the conduction velocity in the cell axis is at least 5 times that perpendicular to the cell axis and the cells are perpendicular to the heart axis at the ends and bend region and almost so in the remainder of the heart (see Fig. 2A). Thus, wave fronts spreading in the heart axis would be little influenced by differences in circumferences.

The maximal error introduced by variations in stretch (tension error) was less than 10%, which was determined by removing and repositioning hearts in the same configuration. The bend and end regions of hearts are flexed so that small folds developed in these regions when the hearts were opened. These folds increased the length of tissue between the electrodes up to 10% (folding error). Therefore, the conduction velocities determined with repeated heart placements at the ends and bend regions were reproducible to 20% (tension plus folding error). It is felt that the temporal wave front diagram in Fig. 4 closely represents that for in situ hearts.

B. Comparison of conduction velocity with vertebrate hearts.

It is more appropriate to compare the tunicate heart with vertebrate hearts than with invertebrate hearts for several reasons. The tunicate heart is myogenic and the beat is all-or-none; i.e., a compensatory pause follows an extrasystole so the heart cannot be tetanized (1, 12). In addition, tunicate heart cells have action potentials that are very similar to those of vertebrates with one to three phases of recovery (8) and the spread of excitation is by local current flow from cell to cell (9, 10, 14). The pacemaker regions of Ciona heart are located at each end of the U-shaped heart at the ostium of each blood vessel. The pacemaker is a ring of cells and the dominant pacemaker region may shift in position within the ring of cells (13).

There is, however, no structural similarity between the valveless, tubular tunicate heart and the chambered hearts of higher chordates. In many-chambered hearts the myocardium is nearly synchronously activated by a specialized conductile system (Purkinje fibers). This is in marked contrast to the tunicate heart where a wave of peristalsis com-

FIG. 6. Correlation between degrees of rotation and maximal and average conduction velocities. Maximal conduction velocities were calculated from time intervals between signals recorded by third and fourth recording electrodes. Average conduction velocities were calculated from time intervals between signals from first and fourth recording electrodes (Fig. 1A). Triangles and solid circles: data are from 18-mm-long Ciona heart arms (solid circles from Fig. 5). Circles: data are from a 12-mm-long Ciona heart arm.

FIG. 7. Time contour map of an impulse spreading from a point stimulus. Heart axis and width are indicated by unbroken lines. Curves represent wave front at successive 25-msec intervals starting from stimulating electrode(s). Positions of dots in curves have been calculated from data in Fig. 5. Conduction velocities of first contour interval were calculated from time intervals between stimulus artifacts and signals recorded by first recording electrode. Conduction velocities of second contour were calculated from time intervals between signals recorded at first and second electrodes. Velocities for third and fourth contour intervals were calculated from time intervals between second and third, and third and fourth electrodes, respectively. Note that velocity increased during first 50 msec. If these curves are smoothed by eye, they give average curves that were obtained from four preparations.
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fully occludes the lumen so that all of the blood is ejected (12). In a 30-mm-long heart the peristaltic constriction is about 1 mm long and filling occurs concurrently with ejection behind the wave of contraction. The most striking cardiovascular difference is that of the beat reversal. During the period of time (several minutes) that a heart beats in one direction, a pacemaker first accelerates in frequency, then maintains a constant frequency, then slows and finally stops and the opposite pacemaker starts the cycle. This is thus no retrograde conduction since blood appears to flow with equal facility in both directions (12). Conduction velocity was the same in the two directions of conduction which correlates with the cellular symmetry. In mammals, however, retrograde conduction is slower than forward atrioventricular (16, 20) and sinus-atrium (19) conduction.

Conduction velocity along the cell axis in the tunicate heart is about 77 mm/sec, which compares to that in the frog ventricle of 100 mm/sec (2) but is somewhat low when compared to that of 73,000 mm/sec in the mammalian ventricle (3, 18). However, the spread of excitation through the rabbit sinus is 20-60 mm/sec (19) and through the A-V node it is 20-50 mm/sec (6). The apparent conduction velocity through the chick A-V ring is only 3-5 mm/sec (15) and Irisawa et al. (7) measured a conduction velocity of only 10 mm/sec through the frog bulboventricular junction which they attributed mainly to the perpendicular orientation of the fibers in relation to impulse spread (see section D below).

C. Impulse acceleration and deceleration in ends of heart. Conduction velocity in the middle region of a heart arm in the cell axis near the origin of an impulse was about 14 mm/sec and perpendicular to the cell axis it was over 4 mm/sec (Table 1). Yet, at the ends of the heart, the maximal conduction velocity in the cell axis was only 2 mm/sec and perpendicular to the cell axis it was less than 1 mm/sec. These comparisons indicate that the deceleration observed as an impulse approached an end of the heart or acceleration as an impulse propagated out from its point of initiation were not due to the change in fiber orientation from 70° to the heart axis in the middle of the heart arms to 90° at the ends of the heart (cf. vertebrate hearts 4, 5, 6).

The lower conduction velocity in the ends of the tunicate heart could result in a lower safety margin for conduction. This could explain why waves of excitation were sometimes observed to die out and why local nonpropagated responses could be produced when stimulating with suction electrodes with tips of a small diameter (20 μ, cf. Sugi et al. 21), and for vertebrates (17)).

In middle regions of heart arms successive waves always had the same apparent conduction velocities. However, toward the ends of hearts different conduction times were sometimes recorded. Cell orientation does not influence impulse spread in the tissue at the ends of the heart as in the middle region of each heart arm (Fig. 4). The low conduction velocity and the nearly electrical isotropic nature of the tissue near the ends would permit impulses to take different pathways (14). These results demonstrate that differences in the relative position of the pacemaker to the recording electrode at each end of a heart and the deceleration and acceleration of the impulse in the two directions of contraction could give rise to apparent differences in conduction velocity in the two directions of conduction.

D. Effect of cell shape on impulse spread. Impulse spread from a point stimulus is about 5 times greater in the cell axis than perpendicular to the cell axis during the first millimeter of spread and then increases to a factor of 10 (Table 1). This increase in conduction velocity as an impulse spreads from a point source is easily appreciated by examining the contour lines representing successive wave fronts as demonstrated in Fig. 7. In this figure it can be seen that the impulse had reached maximal velocity in the cell axis by the time it had moved about 1.25 mm from the electrode; i.e., to the 50-msec contour line because the distance between the 50- and 75-msec contour lines is the same as between the 75- and 100-msec contour lines. Similarly, the impulse had reached maximal velocity during spread perpendicular to the cell axis within 0.25 mm because the distances between the 25- to 50-, 50- to 75-, and 75- to 100-msec time contour lines are the same. This explains how an impulse approaching an end of the heart sometimes had a greater conduction velocity than one starting near the electrodes.

The impulse gains velocity from the point of origin since the safety margin for conduction is very low near the stimulating electrode. In other words, the ratio of active current-generating membrane to adjacent inactivated membrane increases as the impulse spreads from the point of stimulus.

As the curvature of the wave front decreases, the front approaches that of one propagating along a very thin cable.

The internal resistance of the tunicate heart in a given direction is due to the series resistances of the cytoplasm and tight junctions, of which the latter is greater. The resistivity of tight junctions is 10^4 to 10^6 times greater than that of the cytoplasm (11). If the cell shape is envisioned as rectangular instead of elliptical, the internal resistance would be greater perpendicular to the cell axis since there would be more tight junctions per unit length than in the cell axis. Differences in conduction velocities in different directions indicate that the cell shape (and thus the position of the tight junctions) affects the distribution of current flow; i.e., the tissue is electrically anisotropic (cf. the rat atrium (24)).

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REFERENCES


3. Draper, M. H., and M. Mya-Tu. A comparison of the conduction


