Electrical Activity of Single Fibers of the Atrioventricular Node

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The mechanism of atrioventricular delay has been studied in isolated rabbit hearts. Multiple intracellular microelectrodes have been employed to obtain simultaneous records from single fibers of atrium, A-V node and His bundle. An appreciable delay in the transmission of excitation has been found only in the atrial portion of the A-V node. Action potentials recorded from single fibers in this area show a low resting potential, slow diastolic depolarization, slow upstroke and low amplitude. These action potentials frequently show one or more notches or steps on the rising phase. Action potentials recorded from fibers of the His bundle are similar in shape and amplitude to those of peripheral Purkinje fibers. Records obtained at several sites between the atrial portion of the node and the His bundle show a gradual transition in action potential shape. The mechanism of slow transmission across the A-V node is discussed in relation to the electrical activity of fibers at the atrial end of this structure.

The nature and cause of the delay in propagation of excitation from atrium to ventricle has long been the subject of speculation and investigation. Gaskell concluded that in the turtle heart atrioventricular delay results from slow conduction in the tissues forming the A-V ring. In mammalian hearts delay was at one time thought to be caused by slow conduction in the bundle of His. This possibility was ruled out by Hering and Erlanger. The latter concluded that A-V delay was attributable to a latency occurring at some point of transition of one type of tissue to another. Hering was convinced that most of the delay was localized in the atrial portion of the A-V node. The cause of the delay was variously ascribed by other workers to smaller fiber diameter, circuitous pathways or stimulation by the falling phase of the atrial action potential.

Recently the problem of A-V nodal transmission has been reinvestigated by transmembrane recording from single fibers in various parts of the atrium, A-V node and His bundle of the rabbit heart. Records obtained with plunge electrodes or by appropriately timed electrical stimuli applied to atrium and ventricle. Sher and Durrer, employing the former technic, have recorded low-voltage polyphasic electric complexes from the region of the A-V node similar to those obtained in electrograms from the sinoatrial region. These records are open to several interpretations. Moe has obtained data suggesting that A-V conduction takes place over a dual pathway, with rapidly and slowly conducting components.

Previous work has not provided a direct demonstration of slow conduction within the A-V node, or of the cause and precise localization of altered propagation. The present paper reports the results obtained by transmembrane recording from single fibers in various parts of the atrium, A-V node and His bundle of the rabbit heart. These results demonstrate that slow spread of the impulse near the atrial border of the A-V node causes the major part of normal A-V delay, and that in this area action potentials show consistent differences in rising phase and amplitude.

Methods

Rabbit hearts were perfused with a modified Tyrode solution equilibrated with a gas mixture of 5 per cent carbon dioxide in oxygen, and maintained at 35 to 36 C. In the early experiments coronary perfusion by aortic cannulation was
maintained throughout, and the region of the A-V node was approached through a window cut in the right atrial appendage. In subsequent studies the vena cavae, right atrium, interatrial septum, right atrioventricular ring and upper interventricular septum were freed from the remainder of the heart and placed in continuously flowing solution. The right atrium was then opened by an incision running through the anterior wall of the A-V ring, the anterior wall of the atrium and appendage and the superior vena cava. The atrium could then be opened widely and access given to all structures under investigation.

Records were obtained by means of glass capillary microelectrodes pulled from soft-glass tubing and filled with 3 M KCl. All records shown were obtained with Grass Model no. P-6 cathode followers and preamplifiers and were photographed either on moving film from a dual beam Dumont 322A oscilloscope or on stationary film from a switched-beam 12-inch long persistence oscilloscope. In all experiments 2 or 3 rigidly mounted microelectrodes were used simultaneously. Voltage calibration was achieved by applying a known voltage between the preparation and ground.

Rhythm was spontaneous except in certain experiments which will be noted.

**RESULTS**

**Location of Recording Sites.** It has been possible to map the spread of activity from its site of origin in the sinoatrial node to its arrival at the bundle of His by recording the transmembrane potentials of single fibers with 1 or 2 fixed microelectrodes and another microelectrode moved from one location to another. Two major criteria were employed in tracing the spread of the impulse. One was the anatomical location of the fiber with respect to clearly recognized landmarks such as those identified in figure 1. The second was the time of arrival of the impulse at a given location with respect to the time of activation of 1 or 2 fixed electrodes. Three reference points were employed for fixed electrodes in different experiments. One was a single pacemaker fiber on the endocardial surface of the sinoatrial node (see figs. 1 and

**Fig. 1 Left.** Schematic diagram of endocardial surface of right atrium. 1. Interventricular septum. 2. Right ventricular wall. 3. Valve leaflet. 4. Auricular appendage. 5. Crista terminalis. 6. Interventricular septum. 7. Superior vena cava. 8. Inferior vena cava orifice (postcava). 9. Ostium of the coronary sinus (left precava). 10. Region of the sino-atrial node. 11. A-V node. 12. Bundle of His. Dotted line roughly outlines extent of A-V node and His bundle. Atrial part of node from which typical action potentials are recorded is margin below ostium of coronary sinus. Area between this and narrow His bundle is area of transitional fibers.

**Fig. 2 Right.** Transmembrane action potentials recorded simultaneously with 2 microelectrodes. Lower trace in all records is obtained from single fiber of upper His bundle and serves as time reference. Upper trace shows action potentials recorded from A sinoatrial node; B, lower part of crista terminalis; C, atrial margin of A-V node; D, upper A-V node; E, Mid A-V node; F, lower A-V node; G, transitional fiber; H, His bundle. Voltage and time calibrations in G and H represent 10 msec. and 50 mv. respectively.
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Fig. 3 Left. Transmembrane action potentials of single fibers of A, atrium; B, upper node; C, D, E, mid node and lower node; F, upper His bundle. Upper trace represents line of zero potential and shows time calibration (dots) in intervals of 10 and 50 msec. Voltage calibration in A, from above down, shows —10, —50 and —100 mv. Overshoot in A is larger than commonly recorded.

Fig. 4 Right. Simultaneous records of transmembrane action potentials from fibers of atrium (top trace), A-V node (middle trace) and atrial portion of His bundle (bottom trace).

Such fibers were recognized by configuration and identifying characteristics described elsewhere; their distribution has been studied extensively and is the subject of separate reports. Another reference point was an atrial fiber mid-way in the crista terminalis (see figs. 1 and 3A). The third location for the fixed reference electrode was in a single fiber in the upper portion of the bundle of His (see figs. 1 and 3F). In early experiments the intra-atrial portion of the His bundle was located in the following manner: serial cuts were made in the upper part of the interventricular septum until A-V dissociation resulted. The His bundle thus located, it was possible to record action potentials typical of Purkinje fibers from just above the level of the cut. The bundle was then traced back into the atrium with the action potential shape characteristic of its component fibers being used as a criterion. The His bundle was employed most frequently as reference site because it was easier to keep a properly inserted electrode in this area for long periods.

Another indication of the recording site was the configuration of the action potentials. The earliest experiments showed that fibers in the vicinity of the upper end of the His bundle gave action potentials quite different in shape and amplitude from those of atrial muscle in either the right appendage, the interatrial septum, the crista terminalis or other areas. Action potential shape was thus evaluated in mapping the extent of the A-V node.

The Shape of the Action Potential at Various Sites. Figure 3 shows typical action potentials recorded from various sites identified in figure 1. It is likely that in many instances the amplitude of the action potential is reduced by movement due to contraction of the preparation, and in some cases movement artifacts are apparent. This possibility arises from the fact that the preparation was only partially immobilized to avoid damage from stretching. In figure 3A, the action potential recorded from an atrial fiber of the crista terminalis shows the usual configuration for this type of muscle. The important aspects of the record are the high rate of depolarization, the sharp peaked reversal, the rapid initial phase of repolarization and the constant level of resting potential during diastole. The action potential shown in figure 3A is of the type recorded from a sharply circumscribed region (fig. 1), identified as the atrial border of the A-V node. This action potential differs sharply from those of atrial muscle in that the rising velocity is low, the amplitude of reversal is decreased and the peak is rounded. Some slow diastolic depolarization is present and the maximum diastolic potential is low. There is a prominent foot on the rising phase of the action potential.
Furthermore, records from fibers of this type frequently show distinct steps on the rising phase (fig. 2, D and E). The duration of action potentials recorded at this site is not much greater than that of atrial fibers. Records similar to that shown in figure 3B differ from action potentials of sinoatrial fibers in the same atrium, in that both the action potential duration and rate of diastolic depolarization are greater in the S-A node (fig. 2). Figures 3C to E show a series of action potentials, recorded at different sites, which reveal a gradual transition, from the shape seen in the atrial border of the A-V node, to that recorded from fibers of the His bundle (fig. 3F). These records were obtained from the sites indicated in figure 1 and they show a progressive increase in rising velocity and amplitude, a decrease in the degree of slow diastolic depolarization, a disappearance of the step on the rising phase and an increased duration. Figure 3F, recorded from the atrial portion of the His bundle, shows an increased resting potential and rise velocity, a spiked reversal and a long plateau. At this location the resemblance to Purkinje fiber action potentials is clear. In all preparations the action potential shape recorded from each location was reasonably consistent with respect to the parameters described. Retrograde conduction was observed if the sinoatrial node was excised or arrested by acetylcholine. When the exploring microelectrode was moved at right angles to the direction of spread of the impulse through the A-V node and His bundle, some indication of the lateral extent of these structures could be obtained. In the region of the His bundle such lateral exploration usually revealed a rather abrupt transition from Purkinje fiber action potentials to those typical of atrial muscle. In the middle and head of the A-V node, on the other hand, such a sharp transition was not encountered. Although it was possible to delineate an area 2 to 3 times the width of the His bundle from which upper and midnodal action potentials could be obtained, the margin between this structure and atrial muscle could not be clearly outlined. Additional studies of this problem are reported elsewhere.

Sequence of Activation at Different Sites. Figure 2 shows records employed to map the spread of activity from the S-A node to the His bundle by the use of the criteria described. In this instance, the fixed reference electrode (lower trace) is located in a single fiber of the His bundle throughout. By comparing the latency between the upstroke of the action potentials recorded by the moving and fixed electrodes in each case, changes in the rate at which the impulse spreads can be noted. In figure 2A the movable electrode (top trace) records the action potential of a single fiber in the sinoatrial node; in figure 2B the action potential is recorded from a typical atrial fiber near the atrial margin of the A-V node. There is considerable delay between the upstrokes of the action potentials of the S-A node and atrium; this is due largely to slow spread of activity within the pacemaker fibers of the S-A node. In figure 2C the electrode was moved less than 1.0 mm. from the atrial site shown in B to a fiber quite near the atrial margin of the A-V node. At this location there is a marked change in the configuration of the action potential. Also, the conduction time from this site to the reference electrode is significantly less than that shown in 2B which suggests that the impulse spreads slowly in the tissues separating B and C. In figure 2D the electrode is moved approximately 0.2 mm. farther along into the A-V node for each record. These tracings show an additional decrease in rising velocity of the action potential and the appearance of slow diastolic depolarization. There is also the suggestion of a foot on the rising phase, and distinct steps can be seen on the upstroke. In each record there is a decrease in the latency separating the upstroke of the nodal action potential from that recorded at the reference site. In figure 2F the electrode is moved approximately 0.5 mm. toward the His bundle. The action potential again changes in configuration, and at this location latency between activity of the 2 recording sites has almost disappeared. In figure 2G the test
electrode is 1 mm. closer to the reference site. Here the action potential shows a transition to Purkinje fiber shape and latency between the 2 upstrokes is not apparent at the sweep velocity employed. In figure 2H, the test electrode is seen moved an additional 1 mm. towards the His bundle. The action potential is identical in shape to that recorded by the reference electrode, and depolarization is almost simultaneous at both locations. Figure 4 shows action potentials recorded simultaneously from 3 locations with 3 intracellular microelectrodes. In both A and B the top trace records the activity of an atrial fiber in the crista terminalis, and the bottom trace activity of a single fiber in the atrial portion of the His bundle. In figure 4A the middle trace shows an action potential recorded higher, and in 4B a somewhat lower in the A-V node. Records of this type emphasize the marked change in action potential configuration at different locations and also show the delay associated with spread of activity through A-V nodal fibers. These records show that slowing of the spread of activity from atrium to His bundle is largely localized in a narrow region and that action potentials recorded from fibers in this region have a consistent and characteristic shape.

This area is identified as the A-V node because of its anatomical site, because its fibers are in direct continuity with fibers of the His bundle below and atrium above, and because it is the site of most of the A-V delay. Measurements of Conduction Time. The apparent rate at which activity spreads through the A-V node has been studied in several experiments similar to that shown in figure 2 but with action potentials recorded at a much higher sweep speed. The results obtained from 2 experiments of this type are graphed in figure 5. In these studies the atrium was driven at a constant rate through a pair of surface electrodes to eliminate changes in latency due to shifts of the pacemaker site. When the exploring electrode was moved by steps of 1 mm. from atrium to His bundle (fig. 5A) or His bundle to atrium (fig. 5B) the records obtained suggest that almost all the A-V nodal delay is localized in a narrow zone extending the full width of the A-V node approximately 1 mm. across, and located near the junction of atrium with A-V node. On either side of this band conduction velocity of approximately 0.8 to 1 M./sec; within this area, however, the apparent velocity at which the impulse spreads falls to the surprisingly low value of 0.05 to 0.02 M./sec. In an attempt to localize the region of delay more closely action potentials were recorded at points separated by only 0.25 mm. These records revealed some irregularity in the spread of the wave of excitation along the path of the exploring electrode and suggest asynchronous activation of different fiber paths. This apparent asynchronous activation of closely adjacent fibers suggests that in each experiment the electrode may have been moved in a direction oblique to the actual spread of the impulse through the node. In all cases, unless movement of the electrode was perfectly parallel to the spread of the impulse, the apparent extent of the region of slowing would be increased and the calculated velocity of spread of activity would be errone-
ously high. Additional studies attempting to outline a three-dimensional map of activation of fibers in this area are now in progress.

**DISCUSSION**

**Electrophysiological Identification of the A-V Node.** One major question is whether or not the structure we have studied is in fact the A-V node. Identification of this particular group of fibers as nodal tissue has been based primarily on the following criteria: 1. These fibers show a close and consistent anatomical relationship to the upper end of the His bundle and other clearly defined landmarks. 2. A major portion of A-V delay elapses during passage of the impulse through these fibers. 3. These fibers are the site of block during failure of A-V transmission. 4. Action potentials recorded from these fibers are distinctive in shape, differing from records obtained from either the His bundle or atrial muscle and showing some resemblance to action potentials recorded from the sinoatrial node. 5. Only through this group of fibers, the site of the major part of A-V delay, can a continuous sequence of excitation from S-A node and atrium to the bundle of His be traced. Records obtained from closely adjacent areas show incongruous latencies with respect to the time of activation of the His bundle. Furthermore it has not been possible to trace any other paths which can be shown to activate the bundle of His. It appears justifiable, in consideration of these observations, to accept the premise that these fibers constitute the A-V node. Further support of this conclusion is given by studies of the effects of acetylcholine on transmission through these fibers.

Whether or not the area under study should be subdivided is a subject of additional studies. The upper margin of this fiber group is fairly sharply demarcated by the abrupt change both in the action potential shape and the rate at which activity spreads. Similarly, the upward extent of the His bundle can be outlined on the basis of an action potential shape similar to that of peripheral Purkinje fibers. On the other hand, it is difficult to decide whether the A-V node proper ends at the point at which the velocity of the action potential upstroke begins to increase (figs. 3D and 2F) or whether all tissue up to the area of typical Purkinje fiber action potentials should be classified as nodal.

Histologic studies of this preparation have been in progress for some time. The difficulty of histologic identification of a single fiber in which a microelectrode has been inserted is great. This is particularly true in an area, like the margin of the A-V node, where there are no sharp boundaries between atrium and nodal muscle. Also, there is little agreement regarding exact histologic criteria which might be employed to separate the several fiber types identified by their electrical activity. For these reasons histologic confirmation of our interpretations is not yet available.

**The Nature of A-V Delay.** A second major problem is the nature of the slow spread of activity through the A-V node. The 3 possibilities which have received serious attention in the past are normal conduction velocity through long paths, slow conduction, and delay occurring at the junction between two fiber types. One additional possibility which does not seem to have received attention in the past is that the nodal fibers might be wholly inexcitable and that transmission across the node might occur as the result of electrotonic spread. Although our observations on the normal node do not suggest that that is so, the question is discussed below.

The following observations made with transmembrane recording must be considered in relation to any explanation of the slow spread of the impulse in the node: 1. The major part of the delay occurs over a distance of approximately 1 mm. 2. If the spread of the impulse over that 1 mm. is regarded as conduction, then the conduction velocity may be as low as 0.05 to 0.02 M./sec. 3. The upstroke of the nodal action potential may be 30 msec. in duration. 4. The upstroke of the nodal action potential is often notched. 5. Nodal action potentials are of low amplitude and often do not show a prominent reversal.
These observations do not in themselves rule out any of the classic hypotheses, but the finding of action potentials showing the low amplitude and slow rise time which would be expected to be associated with slow propagation velocity does seem to make the assumption of long circuitous pathways unnecessary.

The possibility that A-V delay results from a long refractory period of nodal tissue is unlikely because of the short action potential duration recorded from the area in which conduction velocity is slow. The importance of a difference in the action potential duration of nodal and His bundle fibers in production of partial and complete A-V dissociation is under study.

Two possibilities must be considered under the general heading of slow conduction: "true" slow conduction, i.e., uniform propagation at a low velocity, and decremental conduction. On the whole it seems that conduction through the node is similar to decremental conduction in the following sense: As one moves a recording electrode from the atrium into the node, the recorded action potentials over the first 1 to 2 mm. diminish in amplitude and the rising velocity becomes slower and slower. Moreover, it seems that block of nodal transmission is associated with a diminution in amplitude of nodal potentials to the point where propagation fails even though the fiber is excitable. Decremental conduction could arise from any of several changes in the fiber properties, e.g., fiber diameter, threshold, membrane capacitance or resistance, or a low resting potential.

Even if the basic mechanisms of the slow spread of the action potential through the node is assumed to be slow conduction which perhaps is also decremental, the notched upstroke of the action potentials recorded from nodal fibers forces consideration of other mechanisms. At least 2 possible interpretations of notching of the upstroke may be advanced. These notches may result from delay in excitation caused by a sudden increase in fiber diameter. Multiple notches may result from the convergence of several small fibers into a single larger fiber, in which case block might arise from the inexcitability of 1 or 2 of these small fibers. On the other hand the possible role of the intercalated discs in cardiac fibers must be considered. There has been a long debate as to the significance of these discs, and students of electrophysiology of the heart have tended to ignore them on the ground that there are few if any observations of an electrical nature which would indicate that the intercalated discs play any role in conduction. If, however, the safety factor of conduction is low, as it undoubtedly is in the node, any additional increase in longitudinal resistance might play an exaggerated role and therefore account for the notched upstrokes.

The possibility that some nodal fibers are totally inexcitable and that transmission through part of the node results only from electronic spread is not likely. When the node is subjected to a concentration of acetylcholine high enough to produce total A-V dissociation, small potential changes are recorded from nodal fibers. When 2 such depolarizations coincide, the record of the transmembrane potential of the nodal fiber shows not just a summation of electrotonic potentials, but rather a larger depolarization which has the characteristics of an action potential. This observation, in addition to those mentioned, suggests that nodal fibers are in fact excitable.

The many mechanisms cited above are the subject of further study. It might be said that no theory of the nature of the slow spread can be adequately supported by experimental evidence. Subject to that reservation, we are inclined to regard the slow spread as the result of propagation which is probably decremental. The term decremental conduction is used here to imply that the action potential appears to diminish progressively in amplitude and rate of depolarization in such a way as to suggest that its efficacy as a stimulus to adjacent regions is continuously reduced. Such decremental conduction might result in fibers in which there were a progressive change along the length of the fiber either in threshold or
cable constants, or a progressive decrease in resting potential. It is evident that if the impulse is barely able to traverse the length of the nodal fiber and excite fibers of the His bundle, any small change in fiber properties would lead to block at this site. Indeed, this is presumably a frequent occurrence during partial and complete A-V dissociation. Whether or not part of the slow spread and low safety factor result from a low resting potential, which in turn decreases amplitude and rising velocity of the action potential, cannot be ascertained until additional studies are completed.

**Summary**

The nature of the spread of excitation through the A-V node in the rabbit heart has been studied by means of intracellular recording. Records obtained simultaneously from 2 or 3 intracellular microelectrodes show that fibers in the A-V node are characterized by a low resting potential, slow rate of depolarization and a tendency to show notched upstrokes. All of these characteristics are most prominent in cells in the atrial margin of the A-V node. The apparent conduction velocity in the node is so low (0.05 to 0.02 M./sec.) that theories other than true conduction are advanced to explain the transmission of excitation. It is considered possible that conduction in the node is decremental in nature.

**Sommario in Interlingua**

Le natura del propagacion de impulsos a transverso le nodo atrioventricular del corde de conillos esseva studiate per medio de registrations intracellular. Le registrationes obtenite simultaneemente ab 2 o 3 micro-electrodos intracellular monstra ne le fibras in le nodo atrioventricular es caracterisate per un basse potentiale de reposo, un lente dis-polarisation, e le tendentia de exhibir inden-tate bracios ascendente. Omne iste caracteristicas es le plus pronunziate in cellulas al margine atrial del nodo atrioventricular. Le apparente velocitate conductori in le nodo es si basse—0,05 a 0,02 M. per sec.—que il pare indicate cercar le explication del transmission de impulsos por theorias altere qui illo del ver conduction. Es considerate como possibile que le conduction in le nodo es de natura decremental.

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