"Unidirectional" Atrioventricular Conduction Studied by Microelectrodes

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The atrioventricular conduction has been called a unidirectional conduction because the orthograde conduction, i.e., the conduction from atrium to ventricle, occurs easily. The retrograde conduction, i.e., the conduction from ventricle to atrium, seems to occur only under exceptional cases, although the rarity of the retrograde conduction has been strongly questioned by some of the recent authors, especially on the basis of clinical experience with esophageal lead electrocardiograms.

The cause of the peculiarity of this unidirectional conduction has been explained in various ways. One group believes that either the nodal or atrial tissue is still refractory to stimuli when impulses are passing back. Another group believes that there is some other mechanism in the atrioventricular node in addition to the refractory period.

The purpose of this study is to decide experimentally, by applying the microelectrode technic to the isolated dog heart, whether or not the atrioventricular conduction is actually unidirectional, and if so, whether its cause can be ascribed solely to the refractory period.

Methods

After isolating the dog heart, the right atrium, interatrial septum, right atrioventricular ring, interventricular septum, and right ventricle were freed from the remainder of the heart. The right ventricle and the right atrium were opened by an incision running through the anterior wall of the atrioventricular ring and the anterior wall of the right atrium. The atrium and ventricle were then opened widely, and some of the parts adjacent to the incision were removed, care being taken not to injure the important parts of the special conducting system. In this way a flat, thin preparation was made suitable for mounting. (The details of how the muscle chamber was filled with about 180 ml. of oxygenated Tyrode solution, kept at 37°C, and how the perfusion system operated, have been described elsewhere.)

In one group of dogs, exposed hearts were perfused throughout with the oxygenated Tyrode solution, kept at 37°C, introduced through coronary arteries by aortic cannulation, and the regions of the A-V node and the bundle of His were approached through a window cut in the anterior wall of the right heart. The sinus node was removed in these cases also. The whole heart was immersed in a similar, but larger, muscle chamber filled with about 220 ml. of aerated Tyrode solution kept at the same temperature. The atrioventricular node and the bundle of His were, therefore, supplied with oxygen from coronary perfusion, and from oxygenated Tyrode solution entering the heart cavity through the window in its anterior wall.

A high oxygen content of the Tyrode solution, employed for perfusion of the whole heart as well as of the excised muscle preparation, was maintained by constant perfusion with 100 per cent oxygen during the experiment. (The composition of the Tyrode solution was modified in accordance with suggestions of Matsuda et al. It was as follows (m-mole/L.): NaCl 147, KCl 2.64, CaCl2 1.8, MgCl2 0.49, Na2PO4 1.5, Na2HPO4 4.5, and glucose 1 Gm. (pH 7.2). That oxygen lack did not occur was indicated by lack of action potential configuration change, characteristic of anoxia of nodal and other cardiac muscle. Only data obtained when the normal orthograde conduction from atrium to ventricle was occurring was used in compiling the following results and conclusions. Results with excised muscle preparations and with the whole heart were the same.

In all experiments, 2 rigidly mounted microelectrodes were employed simultaneously on different parts of the preparation. One microelectrode was inserted into the region of the atrioventricular node and another was inserted into an ordinary
ventricular muscle fiber or employed in searching for points of retrograde conduction block. In other cases one was employed in searching for these points of block, and the other was inserted into an ordinary ventricular muscle fiber. To identify the region of the atrioventricular node, previously reported experience9 was helpful. However, in this study, it did not matter whether the recording was from the atrioventricular node itself or from an atrial muscle fiber in its vicinity, because it is a recognized fact that nodal excitation can be conducted to the atrium, and atrial excitation to the atrioventricular node. It was repeatedly confirmed also that this held true in our preparations. It was also not important which ventricular muscle fibers were used for recording, because excitation of a part of the ventricular syncytium is conducted to the other parts in normal conditions, and this held true in our preparations. Our method of using glass capillary microelectrodes, the external tip diameter being about 0.5 μ, and the recording system employed, have also been reported elsewhere.50

Rhythm was either spontaneous or induced by electrical driving stimuli, depending upon the requirement of the experiment. Two pairs of stimulating electrodes were placed beforehand, one in the right atrium and the other in the right ventricle. In the majority of cases the atrial electrode was placed close to the orifice of the coronary sinus, and the ventricle electrode was placed in the center of the ventricular part of the preparation. They were so arranged that either of them could be used immediately only by turning a switch. Stimulation was produced by rectangular pulses of 1 to 3 msec. duration, triggered at a rate of 1 per second.

Histologic examination of the region adjoining the postero-inferior margin of the membranous portion of the interventricular septum was performed, to determine the origin of retrograde conduction block. When the characteristic behavior of the action potential was found, the microelectrode was gently cut near its tip with a pair of scissors, care being taken not to move the tip. After the tissues were fixed, imbedded in paraffin, and serially sectioned at 10 μ, they were stained by hematoxylin-eosin, by van Gieson stain, or by Masson's trichrome stain. The broken tip of the microelectrode was searched for, to locate structures involved in the characteristic reactions described later.

Results

Question as to Whether Retrograde Conduction Occurs Frequently When the Sinus Node is Removed

That retrograde conduction occurs only infrequently has been ascribed by many authors to the periodicity and duration of the refractory state of the nodal or atrial tissue. To evaluate this possible cause of block, one microelectrode was inserted in the region of the atrioventricular node, and another in the ordinary ventricular muscle in the excised preparations or whole hearts from which the sinus node was removed to slow rate of the heart.

When ventricular muscle was stimulated electrically, retrograde conduction was actually observed to occur frequently. This is shown in 1A by the finding that each nodal action potential (upper tracing) followed the ventricular action potential (lower tracing) with the same time interval. In this experiment, there remained the possibility that the electrical excitation of the ventricle might have stimulated the atrioventricular node directly. But the results, when the ventricle was prick-ed lightly with the tip of an injection needle, and when the ventricle became spontaneously active, were the same, as is shown in figure 1B and C.

However, in such experiments, it was just as frequently observed that for some reason retrograde conduction did not take place even though orthograde conduction still occurred. The question of the role of atrial refractoriness was tested in the following manner. In a number of cases in which retrograde conduction did not occur, the frequency of beat was very slow. Ventricular stimuli were placed at such intervals that nodal and atrial tissues certainly must have recovered from refractoriness by the time the ventricular impulses arrived. In spite of this, no retrograde conduction occurred, even though the tissues were shown to be capable of orthodromic conduction (fig. 2). Thus, refractoriness cannot be the sole cause for retrograde conduction block.

Site of Retrograde Conduction Block

When the stimulating electrodes were placed in the atrium, the atrioventricular node responded to electrical stimuli, usually by localized depolarization, when it did not respond by a full-sized action potential. The local response occurred under the influence of such drugs as procaine amide, or quinidine, and also in Wenckebach's phenomenon, as was
Figure 1

Retrograde conduction. In each photograph the upper tracing is the action potential obtained from the atrioventricular node, and the lower tracing is that from an ordinary ventricular muscle fiber. In A, electrical stimulation was applied to the ventricle; in B, the ventricle was pricked lightly with the tip of an injection needle; and in C, the ventricle was beating spontaneously, though periodically. Time marks appear at intervals of 100 msec. The vertical scale on the left side of each photograph denotes the zero level and -50 mV for the upper tracing and that on the right side denotes the zero level, -50 mV and -100 mV for the lower tracing.

reported elsewhere. When the stimulating electrodes were placed in the ventricle, the atrioventricular node was found to show no local response to electrical stimuli in the case of retrograde conduction block, as is shown in figure 2A. This led us to presume that the site of retrograde conduction block was not the atrioventricular node, but situated nearer to or on the ventricular part of the bundle of His. It was our presumption that by tracing the special conducting system downward to the ventricle, the site of such block could be found. It was found that all points on the atrial side of the line of attachment of the tricuspid valve showed more or less similar properties, as outlined above, i.e., no response to ventricular stimuli. Almost all the points beyond the line of attachment of the tricuspid valve, namely, the ordinary ventricular muscle fibers, responded by full-sized action potential to the stimuli applied in the ventricle. But one region in the ventricle did show peculiar features. This region was the site adjoining the posteroinferior margin of the membranous portion of the interventricular septum. This was proved later to be a part of the ventricular portion of the bundle of His.

Three types of cell potential were obtained from this region. The inferior part of the region responded to all the ventricular stimuli with full-sized action potentials, like the ordinary ventricular muscle fibers, but the shape and size of the action potential were different from the latter and showed more resemblance to those of the atrioventricular node, as shown in figure 3B. Namely, its rising velocity was low and it lacked a plateau.

The magnitudes of resting potential, of action potential, and of overshoot averaged 63, 64 and 1 mV respectively, and, thus, were much smaller than those of the ordinary ventricular muscle fiber. The average value of the duration of the action potential was 489 msec. Such a feature can be regarded as characteristic of the first type of cell.

A second type of cell, found in the right superior part of this membranous portion of the interventricular septum, showed no response to the ventricular stimuli, in spite of the fact that it was situated in the domain of the ventricle. In this, the tissue resembled the node, and the shape and size of the action po-
tentials recorded were also similar to those of the atrioventricular node. The average values of resting potential, action potential, and overshoot were 66, 69, and 3 mV respectively, and the average action potential duration was 610 msec. Furthermore, the action potential appeared in association with those of the atrioventricular node, as is shown in figure 3C. In this experiment the nodal action potential followed the action potential of the second type of cell. But it was more commonly observed in other experiments that the latter followed the former with a certain time lag. These findings gave us the impression that this tissue was a continuation of the atrioventricular node which crossed the tricuspid valve ring. This was our thought even before histological examination of the region.

From the features of the cell potential, it was judged that of the above-mentioned tissues, the first lay on the ventricular side of the blocking point of retrograde conduction. The second lay on the atrial side of the block. Thorough search in the mid portion of this region (figs. 3, 4) revealed fibers responding to the ventricular stimuli, mostly by localized depolarization, but, occasionally, by full-sized action potentials. These fibers were regarded as of the third type (figs. 3D, 4B and C). Thus, they were not readily excited from the ventricular side. When the stimulation was switched to the atrial side, they responded to each of the atrial excitations with full-sized action potential as is shown in figure 4A. In the case shown in this figure, there was no spontaneous activity in the atrioventricular node or in the atrium. In the other preparation, shown in figures 2 and 5, there was a spontaneous activity. As is shown in figures 2A and 5A, this point (figs. 3A and 4) responded to the electrical stimuli applied to the ventricle in most cases by localized depolarization only, but it responded to the excitation wave conducted from the atrioventricular node by full-sized action potentials. It is shown, in the lower tracings of figures 2A and 5A, that full-sized action potentials occur at this point, followed the action potentials of the atrioventricular node shown in the upper

![Figure 2](image-url)

Retrograde conduction block not ascribed to refractoriness of the atrioventricular node. A. Upper tracing from atrioventricular node, and lower tracing from fiber located near the postero-inferior margin of the membranous portion of the interventricular septum. Artifacts indicate time of stimulation of ventricle, but no effective responses to it occurred in either electrode (no responses in the upper tracing and only local responses in the lower tracing), even when the atrioventricular node recovered from refractoriness, and even though orthodromic impulses reached the ventricle from the atrium. B. Upper tracing from same point as lower tracing in A. Lower tracing is recorded from ventricular fiber near site of stimulation. Upper tracing shows that this specific point responded to almost all the ventricular stimuli by localized depolarization only. The first full-sized action potential of the upper tracing is of nodal origin, but the second one may be a response to the ventricular stimuli. The vertical scale on the left side of each photograph is for the upper tracing, and that on the right side is for the lower tracing. These are two kinds of vertical scales, one with markings for zero and -50 mV, and the other for zero, -50 mV, and -100 mV. Time marking denotes the same as in figure 1.
Three types of action potentials obtained from the special region in the domain of the ventricle. A. Photograph of the endocardial surface of the region. AS: Interatrial septum. AVN: Atrioventricular node. FO: Fossa ovalis. MP: Membranous portion of the interventricular septum. OCS: Orifice of the coronary sinus. Tr: Line of attachment of the tricuspid valve. VS: Interventricular septum. From points 1 and 2 in A, the first type of action potential shown in B was obtained. From point 3 the second type shown in C and from point 4 the third type shown in D were obtained. A microelectrode for obtaining cell potential from an ordinary ventricular muscle fiber was inserted at the point V, and stimulation electrodes were placed at the point St in this experiment. B. Action potentials obtained from points 1 and 2 in A when the ventricle was stimulated. C Upper tracing from atrioventricular node, and lower tracing from point 3 in A. Artifacts shown as downward pip from lower tracing indicated time of ventricular stimulation. In two instances retrograde conduction was started. This retrograde conduction was not from the ventricle, as realized by difference in time from ventricular stimulation. To the contrary, it showed that point 3 was a continuation to the atrioventricular node. The retrograde conduction from the ventricle must have been blocked in more distal points. D. Upper tracing from point 4 in A. Lower tracing from ventricular fiber (small amplification). Ventricular stimulations giving action potentials of lower tracing produced only local responses in the fibers at point 4 (third type). The fully developed action potentials of the upper tracing were of nodal origin. Time and amplification calibrations same as in figure 2. Horizontal marker in B denotes 1 second.
duced in the third type cell was usually about 10 mV, as is shown in figures 2 and 4, but this was not always so. In some preparations it was small as is shown in figure 3D or larger as in figure 5. Since it was rather rare to discover more than 1 point showing the third type of cell potential in 1 preparation, it could not be determined whether differences in potential magnitude definitely do exist. However, in 1 preparation the third type of cell potential was obtained from several adjacent points. In this case, the magnitude of the small potentials differed strikingly: smaller ones originating from the upper portion of the region (figs. 5A and B) and larger ones from the lower portion (fig. 5C). From these findings, it is concluded that, although we described the small potential as being localized depolarizations, the possibility of decremental conduction does exist.

All of the above results were obtainable in the whole hearts, as well as in the excised preparation. The pictures shown, however, were obtained from excised preparations.

**Histologic Examination of the Point of Retrograde Conduction Block**

When the third type of cell potential was obtained, the microelectrode was cut near its tip, and histologic examination was performed to determine the origin of this type of cell potential from the locus of the broken microelectrode tip. By this method, it was repeatedly found that the origin was the special junctional tissue of the bundle of His, located just above the bifurcating point of the right and left branches. One localization is shown in figure 6. As for the first and second types of cell potential, some histologic evidence was obtained to the effect that they originated from the special junctional tissue, distal and proximal to the origin of the third type of cell potential, but sufficient data have not been accumulated, as yet, in support of this claim.

**Discussion**

The finding of a third type of cell potential enables us to determine orthograde conduction, i.e., the conduction from atrium to ventricle passed this special point, without reduction in size of the action potential, but that retrograde conduction, i.e., the conduction from ventricle to atrium, was frequently blocked at this point in the ventricle. Histological examination showed cells giving the potentials to be situated in the ventricular portion of the bundle of His, just above the bifurcation.

Various electrophysiological properties of this point have resemblance in many respects to those of the atrioventricular node which we observed, and Hoffman and his associ...
Differences in magnitude of the small potential picked up from various points in area of retrograde propagation block. 

A. Upper tracing from atrio-

B. Lower tracing from point 2 of D. Retrograde conduction did not occur but ortho-

grade conduction did. B. Upper tracing from point 2 of D. Upper tracing from point 2 of D. Lower tracing from ventricular muscle. Ventricular stimulation caused small potentials at point 2, except when a greater degree of retrograde conduction occurred. The fifth complexes in upper tracing may be of retrograde conduction or of orthogenic conduction, such as the second. C. Upper tracing from point 3 of D, and lower from ven-

tricle. In the first, third, and last complexes, there seems to be evidence of orthogenic conduction. In lower tracing, the last ventricular action potential seems to be conducted from 3, judging from the interval between the preceding one. D. Schematic diagram of the membranous portion of the inter-

ventricular system (MP) and its vicinity (Tr: the line of attachment of the tricuspid valve). Time marking and vertical amplification scales as in figure 2.
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own rate, uninfluenced by the events in the ventricle. Our discovery of the point of retrograde conduction block in the ventricle furnishes a good explanation of this phenomenon. This particular tissue, blocking retrograde conduction, provides a kind of protecting mechanism. Retrograde conduction could contribute to disorganization of cardiac action.

It is presumed, however, that this special point, showing the small potentials of the third type during retrograde conduction block, would show full-sized action potentials when retrograde conduction is occurring. This presumption is supported by the finding that the tracing from this point occasionally showed full-sized action potentials resulting from some of the ventricular stimuli, as is shown in figure 4JB, and probably in figure 2B. When such retrograde conduction is occurring, it is difficult to differentiate fibers of the third type from those of the first type.

By what mechanism such a unidirectional conduction occurs at this point is now under investigation in our laboratory.

Summary

By applying microelectrode technic in isolated dog hearts, the nature of the so-called unidirectional conduction of the atrioventricular conduction was investigated. Retrograde conduction, i.e., conduction from the ventricle, occurred frequently in the isolated whole heart from which the sinus node was removed, or in a preparation composed of the interatrial septum, right atrium, right ventricle, interventricular septum, and atrioventricular conduction system. Retrograde conduction was blocked just as frequently. In such cases, this unidirectional block was found not to be ascribed solely to the refractory period of the atrioventricular node or of the atrial muscle fibers. The site of unidirectional block was found to be located in the domain of the ventricle, i.e., in the region adjacent to the posteroinferior margin of the membranous portion of the interventricular septum. This site is of a very limited extension. This point responded to ventricular stimuli, frequently by localized depolarization only, or in the manner of decremental conduction, whereas following atrial stimuli, it responded with full-sized action potential. By histologic examination, it was found that this point is situated in the ventricular portion of the bundle of His, just above the bifurcating point to the right and left branches.

Figure 6

Histologic examination of the point of retrograde conduction block. When the third type of cell potential was obtained, the microelectrode was cut near its tip, and histologic examination was made. Its broken tip was found to be located in the specific junctional tissue of the bundle of His just above the bifurcation of the right and left bundle branches. The black arrow in the top figure indicates the broken tip. The right bundle is not seen in this picture, but from other serial sections of the same preparation, the position was clarified to be just above the bifurcation. Microelectrode position is shown with more magnification in the bottom figure; Van Gieson stain. Magnification, ×25 in the top figure and ×90 in the bottom figure.
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Summario in Interlingua

Per medio de microelectrodos, le natura del si-appellate conduction atrioventricular 'unidirectional' esseva studiate in le isolate corde canin. Conduction retrograde, i.e. un conduction partiente ab le ventriculo, occurreva frequentemente in le isolate corde complete quando le nodo sinusale habeva essite eliminato ab illo e etiam in preparatos consistente de septo interatrial, atrio dextere, ventriculo dextere, septo interventricular, e sistema de conduction atrioventricular. Blocage del conduction retrograde occurreva con le mesme frequentia. In tal casos, le blocage unidirectional non esseva ascribibile exclusivemente al periodo refractori del nodo atrioventricular o del fibras atrio-muscular. Esseva constatate que le site del blocage unidirectional esseva in le region del ventriculo, i.e. in le region adiacente al margine posteroinferior del portion membranose del septo interventricular. Le extension de iste site es multo restricte. Iste puncto respondeva a stimulos ventricular, in multo casos solmente per dispolarisation localisate, o in lo maneira de un conduction decremental, durante que post stimulos atrial illo respondeva con un potential de magnitude complete. Le examine histologic revelava que iste puncto es situato in le portion ventricular del fascio de His, justo supra le puncto de bifurcation in le brancas dextere e sinistre.

References

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