Mechanism of Slow Conduction at the Bulbo-ventricular Junction

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Delay in atrioventricular conduction has been attributed mainly to slow conduction of excitation at the prenodal tissue of the A-V node. Electrophysiological studies of this prenodal tissue have revealed several common physiological properties, such as low amplitude and overshoot of action potential, low resting potential, low rate of depolarization and low conduction velocity which are specific to this tissue. These physiological characteristics of the A-V node are attributed to a special group of fibers having small diameter and profuse interconnections. If morphological and physiological experiments can be performed on a simple conduction system, a number of definite findings should be brought to light on the mechanism of conduction of excitation in such fibers.

It has been reported previously that the bulbus cordis can maintain its automaticity after isolation from the ventricle and is more sensitive to acetylcholine and adrenaline than the ventricle. It is known also that an appreciable amount of time is required for activity to spread from the ventricle to the bulbus cordis as in the case of atrioventricular conduction. Whether or not the mechanism of conduction between the former two cardiac tissues is similar to that in the A-V pathway has yet to be elucidated. This paper deals with experiments on conduction of excitation from the ventricle to the bulbus cordis, utilizing both physiological and histological methods. A preliminary report of this work has been published.

Methods

Frogs weighing from 80 to 150 g were pithed and the isolated heart was cleaned by removing the surrounding tissues under a dissection microscope. The specimen was then placed in Ringer's solution maintained at 24°C. Stimulation electrodes were placed on the designated sites of the apex and transmembrane potentials were recorded from various parts of the heart surface. The interval between the stimulus artifact and the onset of action potential was measured as the conduction time of action potentials. The heart muscles of the frog run in a complicated manner and excitation travels in three directions. Therefore, in measuring conduction velocity, it must be assumed that conduction is linear between the stimulation electrodes and the recording electrodes. The stimulus was delivered by a silver-silver chloride wire electrode 0.3 mm in diameter and insulated up to the tip. A biophysical electronic stimulator was used in delivering a square wave of 1 msec duration with variable intensity.

Glass ultramicroelectrodes filled with 3 M KCl were prepared by routine methods and transmembrane action potentials were recorded through a suspended microelectrode according to the method described by Woodbury and Brady. A large silver-silver chloride indifferent electrode was placed away from the specimen in the bath. A high input impedance negative capacity
amplifier was employed to record the membrane potential. Transmembrane action potentials were recorded with magnetic analogue tape using a PWM type data recorder and the data were analyzed with a high speed oscilloscope by reading the tape after the experiment. The record was displayed on Tektronix 502 dual beam oscilloscope and then photographed with a photokymograph camera.

For light microscopy, serial sections 5μ in thickness were prepared after Susa or Bouin fixation, and either hematoxylin-eosin or azan staining was employed.

For electron microscopy, the bulbus cordis was exposed and cold s-collidine-buffered 2.5% OsO4 at a pH of approximately 7.4 was dropped on the heart. After a few minutes for fixation in situ, the junctional region of the bulbus cordis and the ventricle were carefully dissected, cut longitudinally into bits, and immersed in the fixative for an additional two hours. The tissues were then dehydrated rapidly through a series of graded concentrations of ethyl alcohol and embedded in Epon. Sections were cut on a Porter Blum microtome and stained with lead by the method of Millonig. The stained sections were examined with Hitachi Hu-11 A electron microscope.

**Results**

**SITE OF LOW CONDUCTION VELOCITY BETWEEN THE VENTRICLE AND THE BULBUS CORDIS**

A narrow band of tissue 0.5 to 1.0 mm in width was observed from the ventral side between the ventricle and the bulbus cordis. There is a slight difference in light transmission between the major part of the bulbus cordis tissue and the bulbo-ventricular junctional tissue. This difference may be due to the presence of valve leaflets which are attached behind this boundary or to the fact that the wall of this junctional tissue is thinner than that of the bulbus cordis. Figure 1A shows a schematic drawing of the ventricle and the bulbus cordis. The junctional tissue 0.5 to 1.0 mm in width was observed from the ventral side, but could not be seen from the dorsal side in situ (fig. 1C). The values in the illustration indicate the conduction time measured at the ventricle, junctional tissue, and bulbus cordis. Figure 1B illustrates the relation between conduction time and dis-
FIGURE 2
Configuration of action potentials recorded from various parts of the heart. The four records at the left side illustrate the transmembrane action potential obtained from four different locations on the heart as shown. Stimulation artifacts are seen at the beginning of the potential tracing. Vertical bar: 50 ms; time reference: 200 msec.

A similar reduction in conduction velocity was observed on the dorsal side of the bulbo-ventricular junctional zone, but the conduction velocity here was slightly greater than on the ventral side (fig. 1C and D).

**CONFIGURATION OF ACTION POTENTIALS RECORDED AT VARIOUS SITES ON THE VENTRICLE AND THE BULBUS CORDIS**

The action potential of the junctional area showed a low rate of depolarization compared to other parts of the heart. The recorded resting potential of this junctional zone is about 50 mv, being smaller than that of the major part of the bulbus cordis which was, by measurement, about 70 mv. The duration of action potential in this junctional zone was almost equal to that of the bulbus cordis tissue, but was much longer than that of the ventricle (fig. 2). The characteristic transmembrane potentials recorded during forward propagation (upper tracing) and retrograde propagation (lower tracing) are illustrated in figure 3. During a single impalement, the ventricle was stimulated first, followed by the stimulation of the bulbus cordis. In the ventricle (fig. 3-1), the
time required for retrograde conduction appeared to be very much greater than that for forward conduction. On the other hand, in the bulbo-ventricular junctional tissue (fig. 3-2 and the bulbus cordis (fig. 3-3), retrograde conduction was much faster than forward conduction. However, careful examination of the junctional area revealed the existence of a group of cells along the conduction pathway which will fire with the same delay whether the stimulus is applied to the ventricle or the bulbus. These cells are located very close to the ventricular muscle, but almost all the junctional area possesses characteristics essentially identical to these shown in figure 3-2. When the electrode was inserted in the ventricle and the bulbus was stimulated, conduction of excitation took twice as much time as was required for excitation to travel from the ventricle to the bulbus. Thus, excitation from the ventricle to the bulbus cordis was conducted at low velocity within the bulbo-ventricular junctional area, but retrograde conduction was, apparently, much slower than forward conduction.

Plates on the right side (fig. 3-4, 3-5, 3-6) are recordings taken of the same specimen as those on the left side but after adding $10^{-6}$ acetylcholine to the bath. It should be noted that though the recordings on the right side were obtained from sites corresponding to those of the left side, the same cell was not necessarily impaled. Since the conduction

![Figure 3](image-url)

**FIGURE 3**

Relationship between forward and retrograde conduction. The upper tracing in each numbered section indicates the transmembrane action potentials recorded during forward conduction and the lower tracing represents retrograde conduction. 1 is recorded from the ventricle, 2 from the bulbo-ventricular junction, and 3 from the bulbus cordis. Tracings 4, 5, and 6 are recordings taken of the same specimen as those of 1, 2, and 3 but after adding $10^{-6}$ acetylcholine to the bath. Vertical bar: 50 mv; horizontal bar: 100 msec.
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tivity in the junctional area is extremely low, it can be assumed that even a negligible shift of the recording site in this area will introduce a considerable difference in both timing and configuration of the action potential. For instance, in the records on the left side, forward conduction from 2 to 3 was 33 msec slower than retrograde conduction between the same sites, but in the records on the right side, the former was 50 msec faster than the latter. This discrepancy may be ascribed to the fact that the recording electrode for the left records was inadvertently placed slightly proximal to the bulbar side and that for the right records slightly proximal to the ventricular side. Comparison of the absolute conduction time of excitation between the left and right tracings demonstrates a rather remarkable prolongation, indicating the effect of acetylcholine in lowering conduction velocity. A very noteworthy delay in retrograde conduction is seen in the lower tracing of record 4 and shortly after this record was taken retrograde conduction to the ventricle was blocked.

FIGURE 4
Action potentials from the junctional zone before and after adding cocaine. 1: recorded during forward conduction; 2: during retrograde conduction; 3: immediately after adding cocaine retrograde conduction became remarkably prolonged, and finally, in 4, spontaneous firing began at the junctional area. The 4 tracings were obtained from different cells. Vertical bar: 50 mv; time reference: 200 msec.

FIGURE 5
Wenckebach periodicity recorded from the bulbo-ventricular junction after the addition of cocaine. Tracings 1 to 5 show five consecutive transmembrane action potentials recorded from a single cell of the bulbo-ventricular junctional tissue and these five consecutive potentials were superimposed in 6. A clear step was seen at the initial phase of the action potential. Progressive delay in the rising phase was observed in these successive recordings, finally ending with a small slow potential. Vertical bar: 50 mv; time scale: 200 msec.
The configuration of the action potential in the bulbo-ventricular junctional zone changed remarkably after adding cocaine to the bath. Figure 4 illustrates the action potentials of the junctional muscle obtained with forward propagation (fig. 4-1) and with retrograde propagation (fig. 4-2). After adding cocaine, conduction time for retrograde propagation became remarkably prolonged as seen in figure 4-3, and a few minutes later both forward and retrograde conduction were blocked. A very small localized spontaneous contraction developed in a limited area of the bulbo-ventricular junctional zone. The transmembrane action potential shows a typical slow depolarization during the diastolic phase (fig. 4-4). From this area notched transmembrane potentials can also be recorded after the addition of cocaine. Figure 5 illustrates a series of action potentials in which a small slow potential is always followed by a rapid depolarization phase. If five successive tracings are superimposed so that small slow waves coincide, it is evident that the development of the spike phase is gradually delayed and finally the spike potential fails to develop (fig. 5-5). This is a phenomenon similar to Wenckebach's phenomenon in the mammalian atrio-ventricular node.

A remarkable coincidence of all these phenomena with the mammalian A-V node led us to make histological studies of the bulbo-ventricular junctional zone.

HISTOLOGICAL FINDINGS
At low magnification, the general structure of the frog ventricle somewhat resembles a hollow sponge enclosed in a thin bag (fig. 6).
On the other hand, muscle layers in the bulbus cordis are so tightly bound together that they are very difficult to dissect into individual fiber strands. Figure 7, a longitudinal section of the bulbus cordis and the ventricle, shows a remarkable difference in gross structure of these two tissues. In the junctional tissue, almost all fibers run in circular fashion so that the direction of the ventricular fibers change very suddenly from a longitudinal to a diagonal pattern. Moreover, it appears that the ventricle and the bulbus cordis are not attached together with a wide surface area around the whole circumference of the bulbus cordis, but rather that the area of contact is limited to a very small region (figs. 6 and 7).

Electron microscopic studies were done in order to elucidate the difference in fine structure of the muscle fibrils located between the ventricle and the bulbus cordis. Ventricular muscle fibers are arranged in large bundles subdivided by narrow clefts which contain collagenous filaments, nerve fibers, and capillaries. Within the small bundles, muscle fibers are closely packed around their long axes. Basement membrane material and scattered collagen filaments are found in the narrow interspaces. In many places, side-by-side close contact between neighboring cells
was noted, where for a considerable distance there is a separation of less than 200 Å between plasma membranes without intercalation of basement membrane material. The areas of dense attachment are situated frequently on the closely apposed plasma membranes (fig. 8). Very often the end of each fiber is branched and tapered. The end-to-end contact of adjacent muscle fibers, which is generally found in mammalian heart muscles, is rather rare. Ventricular muscle fibers are characterized by their high content of myofibrils which show regular striations. The scant cytoplasm which contains mitochondria and glycogen granules is found between myofibrils and beneath the surface plasma membrane. Vesicles of various size are found associated with the plasma membrane but the sarcotubular system is not so well developed as that of mammalian heart muscles.

At the bulbo-ventricular junctional area, specific cells, which are large in diameter and have pale cytoplasm, are frequently observed. Only a small number of myofibrils is seen at the periphery of the cells and the rest of the cytoplasm is free of contractile elements, and contains irregularly scattered filaments, about 70 Å in diameter, glycogen granules, mitochondria, and fat droplets. A large pale nucleus is situated in the center.
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of the cytoplasm. It was noted also that the myofibrils of these cells are very poorly organized (fig. 9).

It should be added that all cells in the bulbo-ventricular junctional area are not of this type. However, this type of cell is frequently found in the junctional area, but rarely seen in both the ventricle and the distal part of the bulbus cordis. The relation between adjacent cells at the junctional area is the same as that in the ventricle.

Discussion

The physiological findings obtained in the present experiment show a remarkable similarity to the conduction at the A-V node. Conduction velocity at the A-V node was calculated to be 0.05 m/sec according to Hoffman and more than 100 msec was required for impulses to propagate through 1 mm of bulbo-ventricular junctional tissue. This indicates the conduction velocity in this junction to be about 20% of that of mammalian A-V nodal tissue. The slow conduction in this junctional area can be explained if the junctional cells possess intrinsically a unique physiological property of low conduction velocity, but the following morphological possibilities should be considered also.

First, it has been clearly demonstrated that not all ventricular fibers are connected with muscle fibers of the bulbus cordis; rather these two cardiac compartments are mutually connected through a very limited number of myocardial fibers of the junctional tissue. It is probable that the difference in conduction velocity is related to this difference in structure. It has been demonstrated also that ventricular fibers, running longitudinally, change their direction at the bulbo-ventricular junctional zone to run in a circular fashion. Conduction of excitation along the fiber is said to be much faster than across it. These changes in fiber pattern appear to be one of the causes for slow conduction in this junctional zone.

Second, cells in the junctional area show physiologically a lower resting potential than ventricular cells and furthermore, in this area, difficulty is encountered with introduction of microelectrodes. This suggests that junctional cells may be smaller than ventricular cells, but it was found by electron microscope that the dimensions of myocardial fibers of the epicardial layer at the junctional area are not much different from those of ventricular tissue. It is therefore considered that smaller cell size is not the major factor for slow conduction in the bulbo-ventricular junction.

Third, since a special cell group has been discovered only in the junctional area, slow conduction might be attributable to the presence of these cells, but this third possibility is not supported by positive evidence. However, of these three possibilities, the first seems to be the major contributory factor for slow conduction.

This special cell group discovered in the junctional area closely resembles the cells found by Rhodin et al. in the impulse conducting system of the steer heart and also the cells found by Trautwein and Uchizono in the sino-atrial node of the rabbit heart. It is probable that these special cells in the junctional area are responsible for automaticity of this tissue.

The mechanism responsible for the difference between forward conduction and retrograde conduction may not be explained readily on the basis of the present histological findings alone. Conclusion should be deferred until more quantitative morphological observations of the junctional zone can be made. Thus, the combination of structural and physiological studies offers several suggestions concerning the delayed conduction in the bulbo-ventricular junction. It is hoped that these observations will contribute basically toward a better understanding of atrio-ventricular conduction.

Summary

Delay of conduction of excitation from the ventricle to the bulbus cordis in the frog heart occurred mainly in the narrow junctional area on the ventricular side of the bulbus cordis tissue. The action potential of
this tissue was different from that seen in the bulbus cordis and the ventricle, showing a low rate of depolarization and low resting potential. Several physiological phenomena which have been observed in this junctional tissue can also be seen in the A-V nodal tissue. Histological studies revealed a difference in the direction of fibers and the presence of special cells in the junctional tissue. The mechanism responsible for the low conduction velocity in this area can be attributed, in part, to the histological arrangement found in the junctional tissue.

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