Evaluation of Concept of Longitudinal Dissociation of His Bundle

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SUMMARY

In the isolated rabbit hearts the presence of an electrical longitudinal dissociation in the atrial portion of the His bundle was examined by the microelectrode method. Under the normal condition no marked difference was found in the duration of the action potential of the cells at the same transverse level of the His bundle. However, the possibility of separation to at least dual pathways of the His bundle was disclosed, when the arrival time of excitation was observed or when, in addition, the site of stimulation was changed or intracellular stimulation was applied.

By passing the current intracellularly into the His bundle the electrotonic potential was found to be large in a direction parallel to the running of the His bundle while it was found to be almost zero in a direction perpendicular to it. The longitudinal electrical separation was presumed, however, not to be complete under the normal condition, because the intracellular stimulation of one tract was found to be transmitted to the other tract and the spontaneous activity of one tract was found to be conducted to the other tract. A high potassium content or low sodium content did not increase the longitudinal separation markedly in most cases. But in 1 experiment a high potassium content increased it, showing a possibility that under abnormal conditions a complete longitudinal electrical dissociation may occur.

Additional Indexing Words:
A-V node Dual pathways Electrotonic potential High potassium solution Low sodium solution Microelectrode Predestination of conducted impulses Protection block Purkinje fibers Reciprocal beat and rhythm

There have been many studies to propose dual or multiple pathways of the A-V conduction system mainly in relation to the reciprocal beat or rhythm. Commonly they are postulated in the upper part of the A-V conduction system, namely in the A-V node, and a common pathway is supposed to be present in its lower part. Some clinical electrocardiograms can be best explained, however, by presuming that the longitudinal separation...
reaches closer to the ventricle. On the basis of the histologic finding of the His bundle that fibers form fascicles separated by collagenous sheaths, Sherf and James suggested the presence of an electrical longitudinal dissociation in the His bundle. Whether this concept is correct or not was examined by the microelectrode method on the His bundle above the atrioventricular ring.

**METHOD**

An atrial muscle preparation including the opening of the coronary sinus, the A-V node, the His bundle and the tricuspid A-V ring was isolated from the rabbit. The preparation was mounted in a muscle chamber filled with Tyrode's solution. The millimolar composition of Tyrode's solution was: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 4.6, NaHCO₃ 12.0, and glucose 5.5. Tyrode's solution perfusing the chamber was bubbled with 95% O₂-5% CO₂ and maintained at 33°C. In the first series of experiments a microelectrode was inserted successively into points in a line perpendicular to the atrial portion of the His bundle. For extracellular stimulation one pair of Ag-AgCl electrodes, insulated except for the tips, was employed and the rectangular pulses of 5 msec in duration and of twice the threshold intensity were applied. In the second series of experiments the atrial portion of the His bundle was isolated by making an incision both at its proximal end and its distal end. Two microelectrodes were inserted at the 2 points of the His bundle in a line perpendicular to it and the changes of action potential were observed. In the third series of experiments a current was passed through a point of the His bundle by one microelectrode and the electrotonic potential was recorded by another microelectrode.

In some experiments the potassium content of Tyrode's solution was increased to 2 or 4 times and in 1 experiment to 10 times the normal content or its sodium content was decreased to one-half or one-fifth of the normal content. In this case NaCl was replaced by isosmotic amount of sucrose. Our microelectrode method was reported previously.

Anatomically the His bundle seems to be usually classified into the penetrating and the branching portions. But in a portion continuing from the NH cells of the A-V node with an extent of about 1 mm in length, the action potential and the conduction velocity were identical with those of the His bundle mentioned above and definitely different from those of the NH cells. Only this portion was examined and is referred to the atrial portion of the His bundle in the text.

**RESULTS**

*Duration of action potentials of points at a transverse level of the His bundle*

Following the first series of the experimental design mentioned above, a microelectrode was inserted successively into as many points of the His bundle as possible in a line perpendicular to it from above to below. Ag-AgCl electrodes for stimulation were placed at the crista terminalis near the
inferior vena cava about 15 mm apart from the recording microelectrode. In order to have a rough estimate for refractoriness the duration of the action potential was examined. It was found in the 3 experiments that the duration of the action potential measured at 90% repolarization became slightly shorter and shorter as the microelectrode approached the A-V ring. But the difference between the longest and shortest duration was only several milliseconds (Fig. 1). The difference decreased as the stimulus frequency was increased. Such a small difference in repolarization in the normal condition does not seem to have a great significance in regard to excitation spread.

Following the second series of the experimental design, the atrial portion

![Diagram](image_url)

**Fig. 1.** Duration of action potential at various points at the same level of the His bundle.

The stimulating electrodes were placed at the crista terminalis near the inferior vena cava. A microelectrode was inserted successively into as many points of the atrial portion of the His bundle as possible in a line perpendicular to the His bundle from above to below. The duration of the action potential at such points is shown as the ordinate and the stimulus cycle length as the abscissa. At 500 msec of the stimulus cycle length the duration of action potential of A, B, C, and D measured at 90% repolarization is shown. At higher stimulus frequency only that of A and D is shown, since the difference in duration of the action potential is small.
Table I. Duration of Action Potential of Two Cells of the His Bundle in a Line Perpendicular to Its Running

a. Spontaneous Activity Occurred (msec)

<table>
<thead>
<tr>
<th>No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>165</td>
</tr>
<tr>
<td></td>
<td>112.5</td>
<td>115</td>
<td>115</td>
<td>185</td>
<td>190</td>
<td>140</td>
<td>172.5</td>
<td>175</td>
</tr>
</tbody>
</table>

b. Stimulated (msec)

<table>
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<th>10</th>
<th>11</th>
</tr>
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<tbody>
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</tr>
<tr>
<td></td>
<td>190</td>
<td>202</td>
<td>215</td>
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</tbody>
</table>

Fig. 2. Difference of arrival time of wave of excitation at various points of the His bundle. A recording microelectrode was inserted and stimulating electrodes were applied as in Fig. 1. The arrival time of “D”, measured by the steepest rise of the action potential, is several milliseconds earlier than that of “A”, “B”, and “C”. As calibration the vertical bar shows 100 mV and the horizontal bar shows 40 msec. Values show the latency in millisecond measured from the beginning of the stimulus artifact to the steepest rise of the action potential.
of the His bundle was isolated by giving cuts at its proximal and distal ends and 2 recording microelectrodes were inserted into the His bundle in a line perpendicular to its running. When a stimulation was not given, spontaneous activity appeared mostly from some point and a slow diastolic depolarization was observed more or less in many of the recordings. In such cases the duration of action potential of the 2 tracings was not always the same, when it was measured at 90% of repolarization from the top to the level of the take-off potential, as is shown in Table I, a. When stimulation was given, however, such duration became the same eventually as was observed in the first series of experiments (Table I, b).

Excitation arrival at points in a line perpendicular to the His bundle
Following the first series of the experimental design, the possibility of longitudinal separation of the His bundle in regard to depolarization was examined next. Namely, in similar experiments the arrival time of excitation was measured by the steepest rise of the action potential. In 6 experiments among 12 in which sufficient data were obtained all of the arrival times were almost the same. In the other 6 experiments, however, 2 series of different arrival times were obtained. The arrival times at points A and B, when stimulation was given by them, are shown in 1 with a slow sweep and in 2 with a fast sweep. Under the condition shown in 2, stimulation was changed to the right bundle branch and the result is shown in 3.

Calibration: 100 mV and 100 msec in 1, 100 mV and 10 msec in 2 and 3.
Values are the same as in Fig. 2.

Fig. 3. Check of the level of the insertion points of the microelectrodes. Two microelectrodes were inserted at 2 points of A and B, which are similar to A and D in Fig. 2, respectively. The stimulating electrodes were placed at the crista terminalis near the inferior vena cava. The action potentials at A and B, when stimulation was given by them, are shown in 1 with a slow sweep and in 2 with a fast sweep. Under the condition shown in 2, stimulation was changed to the right bundle branch and the result is shown in 3.

Calibration: 100 mV and 100 msec in 1, 100 mV and 10 msec in 2 and 3. Values are the same as in Fig. 2.
time were found, and the portion of the His bundle closer to the A-V ring showed an earlier arrival time. Here the difference was small and in the order of several milliseconds, but was definite. The result of the representative experiment is shown in Fig. 2. Since the distance between the points of insertion of the microelectrode was at most a few hundred microns, the difference could not be explained by the uniform conduction from one point to the other, considering the rapid conduction velocity along the His bundle fibers. In 2 experiments among these 6, possible insertion at different levels was ruled out, because the arrival time of such 2 points was found to be the same during the antidromic stimulation from the right bundle branch (Fig. 3).

In all the 3 experiments among the 12 in which all of the arrival times were the same it was found that by changing the site of stimulation to the atrial region close to the atroioventricular ring, keeping the distance from the recording microelectrode about the same, the difference of arrival time mentioned above became more conspicuous (Fig. 4).

Since the above-mentioned stimulation was done extracellularly by the Ag-AgCl electrodes, there was a possibility that the greater part of the proximal His bundle was stimulated together. Therefore, in the 2 experi-

![Fig. 4. Difference of arrival time of wave of excitation at various points of the His bundle depending upon sites of stimulation. At each of the 3 positions in a line perpendicular to the His bundle, recordings were made by the microelectrode, when stimulation was given by the electrodes placed at the crista terminalis near the inferior vena cava, St (1), and at the atrial region near the tricuspid valve, St (2), alternately. Note that, while in St (1) the difference of arrival time is small, in St (2) this amounts to about 10 msec between A and B or C.

Calibration: 100 mV and 30 msec. Values are the same as in Fig. 2.
Fig. 5. Difference of arrival time of wave of excitation at various points of the His bundle in the rabbit during extracellular stimulation and intracellular stimulation. The electrodes for extracellular stimulation, St (1), were placed at the crista terminalis near the inferior vena cava. For intracellular stimulation one microelectrode, St (2), was inserted into a portion of the His bundle distal to the sites of the recording microelectrode. At each of the 6 positions (A, B, C, D, E and F) in a line perpendicular to the His bundle, recordings were made by this recording microelectrode, stimulating extracellularly and intracellularly, alternately.

Note that, while in St (1) the difference of arrival time is small, in St (2) this amounts to several milliseconds between A or B and C, D, E or F. Calibration: 50 mV and 20 msec. Values are the same as in Fig. 2.

ments among the above-mentioned 12 experiments the stimulation was given intracellularly and antidromically by a microelectrode inserted into a portion of the His bundle distal to the sites of the recording microelectrode. Two different series of arrival time were also disclosed in these experiments (Fig. 5).

*Directional difference of electrotonic potential*

In order to know the degree of electrical separation between the muscle bundles of the His bundle, the third series of experiments was carried out. Namely, one microelectrode was inserted into a point of the His bundle and was used for passing the current of \(5 \times 10^{-7}\) amp in intensity and of 30 msec
Fig. 6. Directional difference of electrotonic potential in the His bundle of the rabbit. An incision was made between the A-V node (AVN) and the His bundle as shown by a broken line in the lower schematic figure. PE is a microelectrode used for passing the current. A and B are microelectrodes for recording at the same distance of about 50 μ from PE parallel and perpendicular to the running of the His bundle (HB) and the action potentials obtained are shown as the lower tracings in figures A and B, respectively. The upper tracings are the base lines and show the current passing. Note that the electrotonic potential of the current passing in the resting phase shown in the lower tracings is large in A, while it is zero in B.

in duration. First at point A close to and at a distance of about 50 μ from this point in a line parallel to the running of the His bundle another microelectrode was inserted, and next at point B close to and at about the same distance from this point in a line perpendicular to it. A representative result among the 4 similar experiments is shown in Fig. 6. It is noted that the electrotonic potential at point A was so large while that at point B was almost zero.

This result can be compared with similar experiments by us with the dog Purkinje fibers. In these experiments also the electrotonic potential in the parallel direction was larger than that in the perpendicular direction, but the difference was not so large as in the His bundle (Fig. 7).

Similarly a recording microelectrode was inserted into the His bundle of a rabbit heart at a distance of about 1.5 mm from the current-passing microelectrode, PE1 in a line parallel to its running. The electrotonic potential was
Fig. 7. Directional difference of electrotonic potential in the dog Purkinje fibers.

A square muscle preparation of about 1.5 x 1.5 cm² was isolated from the free wall of the right ventricle and the Purkinje fibers on its endocardial side were employed for inserting the microelectrodes. Microelectrode arrangement was similar as in Fig. 6, but the distance between the stimulating and recording microelectrodes was about 200 μ.

large as is shown in Fig. 8, but when the point of insertion of the microelectrode for passing the current was slightly shifted in the His bundle perpendicularly to its running, the electrotonic potential was almost zero. Similar results were observed in 3 other experiments.

**Directional difference of conduction velocity**

Following the second series of experimental design, 2 microelectrodes were inserted into the His bundle in a line parallel and perpendicular to its running and both microelectrodes were used for recording. Spontaneous activity appeared mostly from some point and the time difference of the steepest rise of both action potentials obtained was regarded as the conduction time between both cells. That in the perpendicular direction was extremely variable. Mostly it was several milliseconds, but was occasionally unexpectedly large. In 1 experiment among 5 it was as large as 57 msec between the cells which were about 200 μ apart (Fig. 9, Normal Tyrode).

Regarding the inter-microelectrode distance as the actual conducting distance, which is often less likely to be a reality, the conduction velocity was
Fig. 8. Difference of electronic potential depending upon the site of the microelectrode for passing the current.

Incision was made both at the proximal and the distal end of the His bundle (HB) above the A-V ring, as shown by the 2 broken lines in the schematic figure at the bottom left.

RE was the site of insertion of the microelectrode used for recording. PE was the site of the first insertion of the other microelectrode used for passing the current of $0.7 \times 10^{-7}$ amp in intensity and 30 msec in duration and about 1.5 mm apart from RE. The action potential thus obtained by the recording microelectrode is shown in the upper figure. The electrotonic potential by the current passing is seen in the diastolic phase and is fairly large. PE₂ was the site of insertion where the current-passing microelectrode was shifted very slightly. The action potential thus obtained is shown in the middle figure. The electrotonic potential is minimum.

The upper tracings in the upper and middle figures show the electrical current passing.

calculated in the direction parallel and perpendicular to the His bundle. In 6 measurements in the parallel direction the results were 1.6, 1.0, 1.2, 0.97, 0.95, and 0.92 m/sec, and in 6 measurements in the perpendicular direction they were 1.0, 0.13, 0.5, 0.02, 0.1, and 0.001 m/sec. The first 2 values in each direction were obtained successively from the same preparations. In these cases extracellular stimulation was applied near the proximal cut end of the preparation. The others were not obtained from the same preparations, and propagation from a spontaneous activity was measured.
**Possibility of longitudinal dissociation in high potassium or low sodium solution**

In a similar preparation isolated by both the proximal and distal cuts 2 recording microelectrodes were inserted into the His bundle in a line perpendicular to its running. It was confirmed that the electrotonic potential was minimal, when the current was passed from one of the microelectrode. In 4 experiments the potassium concentration of the Tyrode solution was made twice or several times the normal concentration and in 3 experiments the sodium concentration was reduced to one-half or one-fifth of the normal sodium content. In almost all the experiments the conduction time did not

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**Fig. 9. Effect of high potassium content on the electrical activity of the 2 cells of the His bundle.**

A, B, and C indicate the sites of insertion of the microelectrode in the His bundle. B was about 200 µ from A in the direction perpendicular to the His bundle. C was about 300 µ proximal to B.

The upper set of 2 tracings was obtained in the normal Tyrode’s solution. The middle and lower sets were obtained in a solution in which the potassium content was increased to 4 times the normal content of Tyrode’s solution.
change markedly, compared with that in the control Tyrode solution. In above-mentioned experiment, where the control showed an extremely large conduction time, an apparent dissociation occurred between the electrical activity of the 2 recorded cells in a high potassium solution (Fig. 9). If this is complete dissociation, this portion serves to pass a reciprocal beat. If this is incomplete dissociation, in addition to a similar separation in other parts the conduction time from B or C to A is so prolonged as to exceed the duration of the action potential and consequently the refractory period. Then the minimum requirement for reciprocal beat is almost filled.

**DISCUSSIONS**

The longitudinal dissociation of the A-V conduction system would be related clinically to the reciprocal rhythm and to predestination of the conducted impulses to the ventricle.

The most probable causes of reciprocal beats are 1) the presence of a sufficient difference of refractory period of cells at a transverse level, and 2) complete longitudinal electrical separation of a part or the whole of the A-V conduction system. Cases of incomplete separation will be discussed later.

The result shows only a minimal difference of the duration of action potential of cells at a transverse level of the atrial portion of the His bundle. Although their refractory period was not examined, this result negates the first mechanism of the reciprocal beat under a normal condition. Duration of action potential of the 2 cells became different, however, when a spontaneous activity occurred. Under abnormal conditions it is quite possible that the refractory period of some cells of the His bundle change focally. Then the reciprocal beats can occur by this mechanism, as has been proposed by Decherd and Ruskin.12)

As for the second mechanism, the result shows that the atrial portion of the His bundle is composed of at least 2 paths functionally. In one of the examples, the intracellular stimulation at 1 point of the His bundle disclosed the presence of 2 pathways with different excitation arrival time (Fig. 5), but the fact that this stimulation was conducted to all other points at the same transverse level of the His bundle shows also that the longitudinal electrical separation is incomplete. As an example is seen in the control state of Fig. 9, the spontaneous activity of 1 cell of the one path was conducted to the other path, which shows also that the longitudinal electrical separation is incomplete in the fully recovered fibers. A large conduction time in this case suggests that the functioning transverse interconnections between the presumed 2 paths of the His bundle are few. A marked detour may be necessary for the
impulses of the one path to reach the other path. Lack of alignment of successive transverse interconnections, resulting in zigzag transverse pathways, as demonstrated anatomically in the peripheral Purkinje strands, may be the reason. Or a marked slow conduction velocity due to a small diameter or higher resistance of the transverse interconnections must be presumed.

The finding that the transverse conduction takes several milliseconds means that one path can be vacant, i.e., unexcited for several millimeters, which amounts to be a considerable length of the main bundle of His, when an excitation wave proceeds in the other path in the longitudinal direction. This vacancy is probably filled in the more peripheral portion of the His bundle or bundle branches, as is shown by Lazzara, Yeh, and Samet. Even if asynchronicity due to presence of dual pathways is preserved, this amount would not mostly alter net excitation propagation, since the late-coming excitation reaches in the refractory period of the foregoing excitation, when both excitation waves meet. This situation allows, however, the following possibilities under special conditions: 1) The returning point of an atrial reciprocal beat is believed to be in the A-V node, but it can be also down in the level of the His bundle, when the dual or multiple pathways of the A-V node are present at the same time. 2) The transverse conduction can be delayed or blocked to a greater extent and the electrical longitudinal dissociation of the His bundle can be more marked or complete under some abnormal conditions. Although the result shows that the transverse interconnection is mostly strong in a solution of high potassium or low sodium content, our result (Fig. 9) shows a block almost sufficient to induce a reciprocal beat. Here a block of this transverse conduction occurred also in a fully recovered state in a high potassium solution. Anderson et al. and Myerburg et al. showed that the transverse interconnections do not function under some conditions of incompletely recovered fibers with strands of Purkinje fibers. Since our result shows that the longitudinal separation in the His bundle is more marked in the His bundle than in the peripheral Purkinje fibers, this inability of functioning may be more marked in the His bundle in a rapid rhythm. 3) When refractory period of 2 paths becomes different in some abnormal conditions, the incomplete longitudinal separation facilitates reciprocation. If, for instance, one path is refractory after exciting by a preceding orthograde sinus impulse and a retrograde impulse of a ventricular premature beat conducts in the other responsible path, it will have more chance to enter the former now responsible path, if the transverse conduction is slow, inducing a reciprocal beat. 4) The pacemaker in the His bundle can be protected from the A-V conduction. The slow diastolic depolarization of the pacemaker may protect it from the entry of the A-V conduction to some
extent, but this effect may be weak, as can be guessed from the fact that the sinus node activity is easily disturbed by atrial premature beats. If the pacemaker exists in a portion of one pathway only, it is more perfectly protected from the entry of the A-V conduction in the other pathways. Some examples of electrocardiograms suggest that the pacemaker of a nodal rhythm is protected from the excitation wave of the usual A-V conduction.\(^1\),\(^16\) Commonly this has been believed to occur in the A-V node, but this result shows that they can be equally considered to have occurred in the His bundle.

Lazzara et al\(^13\) showed little change distally by drastic transection of the right or left bundle branches. We did not examine the problem of predestination of the His bundle cells directly by such a method. However, since the intracellular stimulation of the atrial portion of the His bundle was conducted to all the other explored points of the His bundle at the same level within the above-mentioned delay, their results seem to be agreeable with ours.

**References**