An Experimental Study on the Conduction of Excitation in the A-V Nodal Region*

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Characteristics of the action potential and stimulus conduction in the A-V nodal region were studied electrophysiologically, especially in connection with the histological findings by means of ultramicroelectrode technique. The intracellular records in each case of normal sinus rhythm and A-V conduction disturbance were obtained. And situation of the tip of the microelectrode was ascertained by using immigration of cobalt ion. The specific muscle fibers were recognized in the vicinity of the A-V node. The action potentials of those fibers represented an intermediate type between the nodal type and the ordinary atrial muscle type. When the recording microelectrode was moved from the upper part of the node to the His' bundle, the shape of the action potential changed from the typical nodal type to the His' type, and the onset of the activation was gradually delayed. Disturbances of the A-V conduction were made using the effects of acetylcholine. And incomplete A-V block, complete A-V dissociation, and A-V dissociation with nodal or ventricular capture were observed and the boundary of characteristic changes of the action potential in all cases was found at the atrial margin of the A-V node which is netlike in structure.

It has been well known that the A–V node plays an important role on the transmission of stimulus between the atrium and the ventricle, and that the time lag between atrial contraction and ventricular contraction originates in the characteristics of stimulus conduction in the A–V nodal region.

In 1910, Hering indicated that the A–V conduction delay occurred in the specialized structure described by Tawara as the A–V node. Afterward, using the extracellular leading, Kooi et al., Pruitt and Essex, and Stucky and Hoffman found that the electrophysiological characteristics of this area differed from those of the other atrial muscle. After the introduction of the ultramicroelectrode technique by Ling and Gerard, Nastuk and Hodgkin, and others, the transmembrane action potential of the A-V node has been recorded by Matsuda et al., Hoffman et al., Sano et al., Miyauchi, and Tamai. On the other hand, the histological structure of the A–V node was studied by Tawara, Tandler, Mönckeberg, Oosawa, and James, and recently the ultraminuteness structure of this area has been clarified by electronmicroscopic study.

However, the study on the relation between the membrane action potential and the charac-

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teristic of the tissue in the A–V nodal region where that potential is obtained, seems to be scanty. In 1959, using the Tomita’s method 23, Sano et al. 24 found that the action potential obtained from the A–V nodal region originated essentially in the specific muscle fibers of the A–V node. Then in 1962, Kanazu 25 investigated the relation between the action potential and its tissue by using the special ultramicroelectrode which contained KCl and CoCl₂ solution. And he reported the form of the action potentials which were obtained from single fibers of the S–A node, the A–V node and the specific conducting pathways in the right atrium 26.

In this report, the feature of the membrane action potential and the stimulus conduction in the A–V nodal region were studied electrophysiologically and in relation to histological findings obtained by using the Kanazu’s method 25. And the relation between the stimulus conduction and its histological structure in this area was clarified in the cases of normal sinus rhythm, A–V block and A–V dissociation.

**Materials and Methods**

One hundred rabbits (2 to 3 Kg in weight) were used in this experiment. These animals were put to death with intravenous injection of about 10 ml of air. After removing the free wall of the ventricle, the right atrium was cut and opened along the right edge of the right appendage without injuring the S–A and A–V node and the main specific conducting systems 20 as shown in Fig. 1. These procedures were carried out in oxygenated Tyrode’s solution maintained at 10–15°C. The preparation was fixed on the paraffin bed in the lucite chamber in which oxygenated Tyrode’s solution was continuously perfused, and the right atrial septum was exposed (Fig. 2). When the temperature of Tyrode’s solution rose to 34°C and the preparation was controlled by normal sinus rhythm, the experiment was started. 3000 ml of Tyrode’s solution was prepared. And it contained NaCl 147 m M, KCl 2.67 m M, CaCl₂ 1.5 m M, MgCl₂ 0.49 m M, Glucose 5.6 m M, Na₂HPO₄ 13 m M, NaH₂PO₄ 4.8 m M and its pH was fixed at 7.3. Two ultramicroelectrodes, of which tips were less than 0.5 microns in diameter, were employed. One of which contained 3 m M KCl solution was used as a reference. The other was filled with solution which was made of 0.5 per cent CoCl₂ solution dissolved in 3 m M KCl. And then, it was inserted into the A–V nodal region. The resistances

![Diagram](image)

**Fig. 1.** After removing the free wall of the right ventricle, the right atrium was opened by an incision which extended from the right ventricular groove to the lower position of the sulcus terminalis.

![Diagram](image)

**Fig. 2.** The experimental apparatus.

![Diagram](image)

**Fig. 3.** The experimental processes.

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of these microelectrodes were found to be approximately 20 megohms.

In the experiments for studying the A–V conduction disturbance, acetylcholine ($5 \times 10^{-8}$g/ml) was added in perfusate. To observe and record the action potential, a dual beam oscilloscope and a two element penwriting electrocardiograph were used (Nihon Koden). In order to mark the position in which the microelectrode was inserted, 0.5 volt of a direct current was applied to the microelectrode for 3 seconds so as to make the electrophoretic immigration of the cobalt ion into the tissue. These tissues were sectioned serially and treated with α-nitroso β-naphthol reagent by OKAMOTO-SONODA’S method, and then they were examined after double staining by hematoxylin. These experimental processes were shown in Fig. 3.

Results

I. The relation between stimulus conduction and histological construction in the A–V nodal region.

In this experiment 40 rabbit atria were used, and the hearts were controlled by normal sinus rhythm. The A–V node has been recognized on the upper end of the A–V bundle between the orifice of the sinus coronarius and the septum membranaceum ventriculorum.

The intracellular recording by the micro-electrode containing CoCl$_2$ solution was performed in many points in this area. The other micro-electrode was maintained in a position
in the ordinary atrial muscle fibers in the vicinity of the A–V node. The membrane action potentials were recorded simultaneously through these two microelectrodes.

1) The vicinity of the A–V node.

Three different types of the action potentials were recorded from the vicinity of the A–V node.

The first type was characterized by a large amplitude in the resting and action potential, a steep upstroke in the depolarization phase, and a lack of plateau as shown in A-trace of Fig. 4.

The second type was characterized by a large resting and action potential, a steep depolarization upstroke, and a slight plateau as shown in B-trace of Fig. 4. The magnitude of the resting and action potentials of these two types were 60–84 mV and 60–95 mV respectively. The onset of activation of the stimulus was found to be simultaneous or slightly delayed (within 10 m/sec.) with that of the reference. The histological structure of the location of the tip of the microelectrodes of which these action potentials were obtained was shown in Fig. 5. In this tissue, the arrangement of the cells were well-balanced and the sizes of fibers were about the same. These features are usually found in the ordinary atrial muscle fibers.

The third type was steeper in the upstroke of the depolarization phase than that of the action potential of the A–V node, and was characterized by a slightly rounded peak of the upstroke, a lower resting potential (46–70 mV) and a lower action potential (50–72 mV) in comparison with those of the ordinary atrial muscle. These features represent that this type is an intermediate type between the nodal type and the ordinary atrial muscle type. And the plateau was not so clear as compared with that in the action potentials recorded from the Purkinje fibers. Moreover, the onset of the activation was earlier than that of the reference as shown in C-trace of Fig. 4 and B-trace of Fig. 15. These action potentials were recorded in 5 of 40 cases, and were obtained in some area slightly above the nodal region. Fig. 6 shows the histological structure of the location where the third type of the action potential was recorded. The arrow sign shows the point of immigration of

cobalt ion, which is the location of the tip of the microelectrode. The cobalt compound appears as a reddish-orange point in the tissue. In this portion the connected tissue was abundant and the nuclei were irregular and crowded. And these fibers formed one muscle bundle, and strands of muscle fibers looked like a cord and continued to the tissue of the A-V node.

2) The A-V node and the His' bundle.
   
Examples for the recording chart of the action potentials of single fibers at the A-V node were shown in Fig. 7, 9 and 11. Characteristics of the action potentials in the A-V node were as follows.
   a) Low resting potential (40–76 mV).
   b) Low action potential (35–72 mV).
   c) A gentle sloped upstroke in the depolarization phase.
   d) Appearances of a step or notch in the rising phase of the upstroke.
   e) Rounded peak of the upstroke.
   f) Slight and slow diastolic depolarization.

As the microelectrode was moved from the orifice of the sinus coronary towards the septum membrane ceum ventriculorum, the action potential characteristic to be the atrial muscle fiber and that to be the A-V node ap-

Fig. 7. The action potential was obtained from the location of the point A (Lower trace). This action potential shows the typical nodal type.

Fig. 8. The figure shows the histological finding in which the action potential in Fig. 7 was obtained. Arrow mark shows the point of immigration of cobalt ion, that is, the position of the tip of the microelectrode. In this tissue, the arrangement of cells is irregular and the nuclei are crowded. (10×10. 40×10)
Fig. 9. The action potential (B) was obtained from the location of the point B.

Fig. 10. The figure shows the histological finding of the middle part of the A–V node. Arrow mark shows the point at which the action potential (B) in Fig. 9 was obtained. In this tissue, the strands of the specific muscle fibers ramify profusely and unite with each other. (10×10, 40×10)

peared successively. The microelectrode was then moved from the upper parts to the lower parts in the A–V node, and there the action potentials were found to be of typical nodal type. When the microelectrode was moved further to the anterior lower part of the node, the action potential changed from the nodal type to the His' type.

The relation between the changes in the form of the action potentials in the A–V node and its histological structure were as follows.

i) The upper part of the node. Point A in Fig. 7 indicates the upper part in the A–V node. Fig. 8 shows the histological structure in this area. The location of the tip of the microelectrode was shown as a reddish-orange point (arrow mark). In this tissue, the arrangement of the cells is irregular but not predominantly so, and the nuclei are crowded. The action potential in this location appeared to have an upstroke with a gentle slope in the depolarization phase, a lower resting and action potentials, and a rounded peak of the upstroke (trace-A in Fig. 7). In a few cases, however, the upstroke in the depolarization phase was relatively steep (trace-B in Fig. 17).

ii) The middle part of the node. Point B in Fig. 9 indicates anatomically the middle part in the A–V node. Fig. 10 shows the his-

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Fig. 11. The action potential (C) was obtained from the location of the point C. The shape of the action potential shows the nodal type, but its upstroke is relatively steep.

Fig. 12. The figure shows the histological finding in the A-V node to some extent ventricular side. The action potential in Fig. 11 was recorded at the point of arrow mark in this tissue. In this tissue, the wire lath-like structure is still predominant, and connective tissues are still abundant. (10×10)

was characterized by a gentle sloped upstroke in the depolarization phase with a step at the beginning in the rising phase, a low resting and action potentials, and a slight and slow diastolic depolarization (trace-B in Fig. 9).

iii) The lower part of the node. Point C in Fig. 11 represents the lower part in the A-V node. Fig. 12 shows the histological structure in this area. The location of the tip of the microelectrode was indicated by a reddish-orange point (arrow mark). In this tissue, the wire-lath-like structure was still predominant, and the connective tissue was still abundant. The action potential in this area was characterized by a steeper upstroke in the depolarization phase and a larger resting and action potentials than those of the middle part of the node, but a slight step was observed at the beginning of the rising phase (trace-C in Fig. 11).

iv) The His' bundle. Point A of Fig. 13 indicates the anterior lower part of the A-V nodal region. Fig. 14 shows the histological structure in this area. In this tissue, the specific muscle fibers ran parallel with each other and also with the connective tissue beneath the endocardium. This histological structure was able to be identified with that of the peri-
Fig. 13. The action potential was obtained from the anterior lower part of the A-V nodal region (Point A). This action potential is characterized by a large magnitude, a steep upstroke, and a clear plateau.

Fig. 14. The figure shows the histological finding in which the action potential (A) in Fig. 13 was obtained. Arrow mark represents the location of the tip of the microelectrode. In this tissue, the specific muscle fibers run parallel to each other with connective tissue beneath the endocardium. (10 x 10)

phery of the His' bundle. The location of the tip of the microelectrode was indicated by a reddish-orange point (arrow mark). The action potential in this location was characterized by a steep upstroke in the depolarization phase, a large resting and action potentials, and a relatively pronounced plateau (trace-A of Fig. 13).  

3) Conduction delay in the A-V nodal region.

In order to investigate the conduction delay in the A-V nodal region, the author tried to map out the distribution of the activity in this region at a spacing of about 0.2 mm distance. The results obtained at these points were then compared with the reference.

Fig. 15 and Fig. 17 show the distribution of the activity in the A-V nodal region thus obtained. For the action potential in the A-V node, there have not yet been established any criterion to indicate the arrival of an impulse. Thus, the onset point and the point of the steepest upstroke in the depolarization phase were employed to evaluate the time difference from the reference.

The "numbers" in Fig. 15 and Fig. 17 represent the time differences from the reference which were defined with respect to the points of the onset of the activation, whereas the "numbers in parenthesis" correspond to those with the steepest points of the upstroke in the depolarization phase. Fig. 16 shows schematically the relation between the measured points.

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Fig. 15.  The figure shows the distribution of activity in the A-V node and its vicinity. "Numbers" show the time differences in millisecond of the onset of activation at various points from the reference record, whereas "Numbers in parenthesis" show those with the steepest points of the upstroke from the reference. At the B point in this area, the onset of activation was earlier than that of the reference. See text for discussion.

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Fig. 16.  The figure shows schematically the relation between the measured points and the changes of the shape of the action potentials in the case of Fig. 15. See text.
and the changes of the form of the action potentials for the data shown in Fig. 15.

As seen in these figures, the onset of the activation at the upper or atrial parts in the A-V node was slightly delayed as compared with the reference point, and its delay became remarkable at the middle and the lower parts of the node.

If the true arrival of the impulse would coincide with the onset of the activation, the conduction time through the A-V node for the data shown in Fig. 17 could be estimated to be about 40 m sec.

The conduction time was prolonged when the steepest point is adopted instead, but otherwise no general and definite tendency was found to exist. The action potentials having a much slower sloped upstream of the depolarization, that is, showing the low value of the maximum \( \frac{dv}{dt} \) of the upstream were recorded from the atrial or upper parts near the middle parts or the middle parts in the A-V node (point-C in Fig. 15 and 17).

If the steepest point of the upstream of the depolarization corresponds to the arrival of the impulse, the arrival of the impulse of the His’ bundle is to be earlier than that of the middle parts of the A-V node as shown in Fig. 15 and 17, which is not quite acceptable from the present physiological knowledge.

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Thus, the conduction velocity was estimated in this experiments by locating the onset point of the activation. While shifting the recording microelectrode from the upper or atrial part of the node to the His' bundle, it was generally noticed that the onset of the activation was gradually delayed indifferent to the changes of the form of the action potential. The increase in conduction time was observed throughout the A-V node, and the conduction velocity ranged 0.04–0.02 m/sec in the upper or atrial and the middle parts of the node.

II. The change of the action potential and the conduction disturbance by the effects of acetylcholine in the A-V nodal region.

As for the A-V conduction delay in normal sinus rhythm, the author observed that the onset of the activation was gradually delayed successively from the upper or atrial part of the node to the His' bundle. In order to obtain more detailed information on the location where the conduction delay occurs, the stimulus conduction delay in the A-V nodal region was studied experimentally by making disturbance in the A-V stimulus conduction.

In this experiment one microelectrode was stuck to the S-A node or its adjacent atrial muscle fiber as a contrast and the other which contained 3 mol KCl and 0.5 per cent CoCl₂ was inserted in the A-V node. For making disturbance in the A-V stimulus conduction, acetylcholine \(5 \times 10^{-8g}/ml\) was added in perfusate and 60 rabbit atria were used. The change in the action potential in the A-V node was recorded while moving the microelectrode in the A-V node area. Cobalt ion was penetrated through the microelectrode in the tissue which the transmission of sinus impulse was obstructed. The microelectrode technique and the histological procedure were the same as mentioned above.

After the application of acetylcholine, the action potential in the ordinary atrial muscle showed an acceleration in the repolarization phase and a shortening the duration. In the S-A node, the slope in the repolarization phase and the slow diastolic depolarization were decreased and the shortening in duration were observed. And in the A-V node, the action potential was decreased in amplitude and in duration, and also a very slow rising phase was generally observed (Fig. 18). These represent the typical effects of acetylcholine on the action potential of the A-V nodal fibers which are quite different of the other atrial muscle or the S-A nodal fibers, and acetylcholine produces the A-V conduction disturbance.

By adding acetylcholine in perfusate, there were 42 disturbances of the A-V stimulus conduction including incomplete A-V block and complete A-V dissociation, and 7 sinus bradycardias and 3 standstills and 8 cases which lower automaticity conducted conversely to the atrium. There existed three types of the disturbance of the A-V conduction in the above mentioned 42 cases.

Fig. 19 shows the process of change from normal conduction to incomplete A-V block in sinus rhythm \(2:1\) block. In this figure, the upper trace is the control record and the lower trace is that obtained at the middle part of the A-V node. These traces were obtained immediately (A), 4-minutes (B), and 7-minutes (C) after adding acetylcholine. Two kinds of waves appeared to exist among those obtained from the same fiber in the A-V node (B and C). Namely, the one had a gentle slope.
Fig. 19. The figure shows the process of change from normal conduction to incomplete A-V block (2:1 block). Each upper traces are the control record in the ordinary atrial muscle. Lower traces were obtained immediately (A), 4-minutes (B), and 7-minutes (C) after adding acetylcholine. Two kinds of waves are recorded from the same fiber in the A-V node. See text.

Fig. 20. The figure shows the action potentials of fibers near the S-A node and in the A-V node in the first type adding acetylcholine in perfusate. Each upper traces are recorded from the reference point near the S-A node. Lower traces, A, B, C and D are recorded from each point, A, B, C and D in the A-V node. In these traces, small wave, so-called hump, is seen. See text.
and was little, a hump, and the other had a greater wave and a conducting spike. In order to investigate the character of the two waves, the microelectrode was moved in the A–V node from the upper or atrial part to the His' bundle, and the changes of the waves were observed.

One example was shown in Fig. 20. The small wave was recorded from the middle part and the atrial part of the A–V node. And then, in the A–V node near the His' bundle this wave was found as a shake of basal line or more little or to be almost disappeared. The other was conducted to the His' bundle. Both action potentials were found to be synchronous with sinus activity. From the other atrial area, typical membrane action potentials of the ordinary atrial muscle fiber were recorded. Namely, the membrane action potentials in the A–V node area and also the other atrial area were synchronous, however, the characteristic changes were found between both of them. Immigration of cobalt ion was performed at the atrial margin of this area where the characteristic changes in the action potentials of fibers were found. Fig. 21 shows the histological structure in this area. A situation of the tip of the microelectrode could be ascertained as a reddish-orange spot in the tissue (arrow mark). Picture of the tissue in this area showed arborescent or netlike specific muscle fiber extended from the center of the

Fig. 21. The figure shows the histological finding at the atrial margin of the A–V node. Arrow mark shows the point of immigration of cobalt ion, namely in this portion, the characteristic changes in the action potentials of fibers were found. See text. (4 × 10)

Fig. 22. The figure shows the action potentials of fibers near the S–A node and in the A–V node after adding acetylcholine in perfusate. Trace from reference point is excited by sinus rhythm and trace from the A–V node by lower automaticity. See text.
A–V node into the ordinary atrial muscle. This feature accords with that of the tissue of the atrial end in the upper part of the node. The cobalt compound was found in the specific muscle fiber of the A–V node near the ordinary atrial muscle. This case was described as the first type.

In the second type, it was observed that the A–V node was excited by lower automaticity but the so-called hump could not be found. And other atrial parts were excited by sinus rhythm. As shown in Fig. 22, both of these rhythms were separated and no relation was seen. In the process of the separation of the rhythms by the effects of acetylcholine, two cases were observed. Namely, the one was found that the separation of the rhythms occurred directly between the atrium and the A–V node area, and the other was observed that, at first, the arrest of the activation of the nodal area was seen, and this area was excited by the onset of lower automaticity successively. At the boundary of both rhythms, the area which was activated by lower automaticity, immigration of cobalt ion was performed. The tissue which the situation of the tip of the microelectrode had presented, had specificity of the A–V node near the ordinary atrial muscle. The cobalt compound, in which was located the tip of the microelectrode was found in the specific muscle fiber of this area (Fig. 23).

![Fig. 23. The figure shows the histological finding of the A–V node and its vicinity. The cobalt compound is recognized at the atrial margin in the A–V node (Arrow mark). See text. (4 × 10)](image)

In the third type, the atrium was activated by the sinus rhythm and the A–V node was activated by the lower automaticity. But in the A–V node, the excitation from the sinus node was believed to be the so-called hump or to have influence on the curves of the action potential by the lower automaticity (Fig. 24). The so-called hump was recorded from the middle part to the atrial part of the A–V node and this curve turned into the shaking of basal line at the His bundle. And when the sinus activity was inferior to the lower automaticity, the influence of this hump appeared more clear into the form of the action potential in the A–V node (Fig. 26). Namely, the so-called

![Fig. 24. The figure shows the action potentials near the S–A node and at the A–V node in the third type of A–V conduction disturbance. Trace from reference point is excited by sinus impulse and trace from the A–V node by lower automaticity. But the excitation from the S–A node is seen as small wave in the A–V node. See text.](image)
hump gave a notch or a step on the action potential by the lower automaticity in the A–V node as seen in lower trace of B and D of Fig. 26, and at times, the action potential in the A–V node was occurred by the hump, and regular A–V nodal rhythm changed to irregular rhythm and arrhythmias (D in Fig. 26). In this case, immigration of cobalt ion was performed at the boundary of both rhythms, that is, at the area where the excitation by the lower pacemaker could be found. The spot of the cobalt compound, namely, the position of the tip of the microelectrode was found in the same tissue as shown in the first type, and this tissue showed the structure of the boundary of the atrial part in the A–V node (Fig. 25).

![Figure 25](image)

Fig. 25. The figure shows the histological finding at the atrial margin of the A–V node. The position of the tip of the microelectrode is found in the specific muscle fiber in this tissue (Arrow mark). See text. (4 × 10)

![Figure 26](image)

Fig. 26. Upper traces are recorded from the ordinary atrial muscle near the S–A node, lower traces (A, B, C and D) from the A–V node. The excitation from the S–A node is found as small wave in the action potential from the A–V node. See text for discussion.

Discussion

1. The relation between the spread of the activity and the histological structure in the A–V nodal region.

1) The vicinity of the A–V node.

The spread of the stimulus at the vicinity of the A–V node must be discussed as regards to the spread of the excitation from the S–A node to the A–V node. Since the S–A node was found by Keith and Flack and confirmed as the pacemaker of the heart beat, there were done many studies on the spread of the stimulus from the S–A node to the A–V node. And the existence of the specific conduction systems between the S–A node and the A–V node has been confirmed in various studies.

For making the existence of these specific conduction systems clear, it is necessary to confirm that the specific conducting fibers exist between the S–A node and the A–V node, and that the stimulus from the S–A node reach to the A–V node through these fibers.

In the present experiment, it was observed in some points slightly above the A–V node that the onset of the activation was earlier than that of the reference in 5 of 40 cases. The histological structure of this location represented the features of the specific muscle fibers and was resemble to that of the A–V nodal fibers. The action potential of this tissue was characterized by a lower magnitude than that of the ordinary atrial muscle fiber and by a steeper upstroke than that of the A–V nodal fiber. And it was thought that this action potential exhibited the feature of the intermediate type between the typical nodal type and the ordinary atrial muscle type.

In 1959, Oosawa found three specific conduction systems between the S–A node and the A–V node in the dog heart histologically. He indicated that one of these, the systema septo-angulares anterior, runs to the left along the Bachmann's bundle from the head of the S–A node, then continues to the middle part of the atrial septum and the right atrium on the aortic side, and then along the muscle bundle near the middle part of the atrial septum and the right atrial wall, and it des-
The author confirmed that the specific muscle fibers at the vicinity of the A–V node reached to the A–V node. And the location where the specific muscle fibers were found accorded anatomically to connection part with the A–V node of the systema septo-angulares anterior. Therefore, it was supposed that the action potentials of the A–V node may be mainly triggered by this system.

Kanazu recorded the action potential liking that of the Purkinje’s fiber from the specific muscle fibers of the systema septo-angulares anterior. And Wagner et al. recorded Purkinje-like action potential from specialized conducting fibers in the interatrial band (Buchmann’s bundle).

The particular action potentials obtained in the present study from the specific muscle fibers at the vicinity of the A–V node did not always showed the typical Purkinje-like type. The cause of this particular action potential which has not so predominant plateau, and which shows a lower magnitude than that of the Purkinje’s fiber, is not as yet certain. It was thought that this was due to the histological structure of this location near the A–V node, and that this location would be composed of the specific conduction fibers and the projection of A–V nodal fibers. On the other hand, the electrophysiological characteristic of the specific muscle fibers at the vicinity of the A–V node may be differed from that of the Purkinje’s fibers.

It has been known that in spite of prolongation of P–Q interval or appearance of intratrial block by cutting of the conduction systems in the right atrium, a conduction velocity along these systems was not so rapid as that along the Purkinje’s fibers. And this fact has made difficult to find out electrophysiologically the specific conduction fibers at the vicinity of the A–V node.

In the present experiment, the existence of specific conduction fibers was confirmed in 5 cases physiologically and histologically. But, in other cases, its existence could not be confirmed, and it would be caused by the reason of slower conduction velocity along above mentioned systems than that along the Purkinje’s fibers and by the reason of the difficulty in finding of these fibers technically. 2) The A–V node and the His’ bundle.

The action potential of single fiber of the A–V node has already been recorded by many investigators. It has been pointed out that the action potentials recorded from this area were characterized by a slow diastolic depolarization, a low resting and action potential, a gentle sloped upstroke of the depolarization with a step or notch at the beginning of the rising phase.

In the present experiment, the action potentials having these feature were obtained in the A–V nodal area. The tip of the microelectrode was examined by using immigration of cobalt ion. And it was confirmed that these particular action potentials were obtained actually from the specific muscle fibers of the A–V node as described by Sano et al. and Kanazu.

The A–V node was divided into three parts, upper (near the atrial ordinary muscle), middle, and lower (near the His’ bundle). And the relation between the feature of the tissue and the form of the action potential of single fiber at each part was clarified.

Namely, in the upper or atrial part of the node, the specific muscle fibers which were enclosed by many connective tissue ran irregular and arborescent but not so predominant. And a part of these specific muscle fibers extended to the atrial body as projection. The action potential in this location showed a gentle sloped upstroke of the depolarization and a lower magnitude, but some times, the upstroke was relatively steep.

In the middle part of the node, the ramiﬁcated specific muscle fibers crowded and united with each other like a wire-lath, and a part of those ran parallel in the lower parts near the His’ bundle.

And also, it was observed that the typical action potentials reported by Hoffman et al. as that of the atrial portion of the A–V node were obtained, in this experiment, from the middle parts and upper parts nearest to the middle parts of the node. Its action potential was characterized by the step at the
beginning of the upstroke in the depolarization phase and by the remarkable delay of
the spike. And in general, when the recording microelectrode was moved from this area to
the His' bundle, the shape of the action potential changed gradually from the typical
nodal type to the His' type.
As to the A-V conduction delay, Hoffman et al.\textsuperscript{125} said that an appreciable delay in the
transmission of excitation was found in the atrial portion of the A-V node.
Carvalho et al.\textsuperscript{40} mapped out the distribution of activity of the A-V node. They
divided the A-V node to three layers, such as an A-N layer, a N layer and a N-H layer,
and observed that the decremental conduction occurred at the lower part of the A-N layer
and at the N layer.
On the other hand, Matsuda et al.\textsuperscript{101,47,48} considered that the A-V conduction delay
would be due to the spike delay of the action potential occurring in the course of the
impulse conduction in a short distance at the proximal of the A-V node and the complicated
cytomatic of its tissue.
In the present experiment, the most remarkable delay of the spike of the action potential
was seen at the middle parts or upper parts nearest to the middle parts of the A-V node.
And the most remarkable ramificated structure of the specific muscle fibers was observed at
these area. Therefore, the author supports the opinion of Matsuda et al. that the spike delay
of the action potential would be due to the increase of ramification of fibers. But in the A-V
node, it was observed that the onset of the activation was gradually delayed from the
upper part to the His' bundle having no relation to the changes of the shape of the action
potential. Accordingly, the author's result was not agreed with the opinion of Matsuda et al.
that the A-V conduction delay would be due to the spike delay of the action potential.
The data obtained in this experiment suggests that the normal A-V conduction delay
would be due to the consecutive delay of the onset of the activation at the all area in the
A-V node between the atrial end of the upper parts and the lower parts nearest to the His' bundle. Thus, the result of the author was
similar to the resulrt of Carvalho et al.
Scher et al.\textsuperscript{49} reported that the conduction velocity of the atrio-A-V nodal junction was
about 0.05 m/sec. but in the A-V node its value was about 0.12 m/sec. But their experi-
ment was performed by using extracellular leading. Therefore, it is difficult to compare
the author's results with their results.
According to Hoffman et al.\textsuperscript{125,45} and Miyake\textsuperscript{47} the conduction velocity in the A-V
node were 0.05-0.02 m/sec, and 0.03-0.025 m/sec., respectively, at the atrial portion near
the junction of the atrium with the A-V node.
By the measurement of the author, its value was 0.04-0.02 m/sec. at the upper and middle
parts of the node. If the area described by Hoffman et al. as the atrial portion of the A-V
node is agree to the upper and middle parts of the node, their results coincide with that of
the author.
II. The change of the action potential and
the A-V conduction disturbance by the
effects of acetylcholine.
It has been known that the disturbance of
the A-V conduction was caused by the effects of acetylcholine.
In this experiment, study on the area which
the disturbance of the A-V stimulus conduc-
tion is caused, was performed using the effects
of acetylcholine on 60 rabbit atria. And, 42
disturbances of the A-V stimulus conduction
were made. These 42 cases were classified into
three types, the first, second and the third.
In the first and third type, two kinds of
curves, those were a greater wave and a small
wave (so-called hump), were recorded in the
same fiber. And the so-called hump was found
to be synchronous with sinus activity. It was
thought that the so-called hump was excited
by sinus automaticity.
The first type might be the second grade of
A-V block which could be found clinically.
In this type, the boundary of both rhythms
was in the reticulated area of the A-V node.
The greater wave with conductive spike con-
ducted to the His' bundle and the small wave
(so-called hump) declined gradually, disappear-
ed and did not conduct to the His' bundle as
Cranefield et al.\textsuperscript{81,12} noted.
It was thought that the second type was complete A–V dissociation. In this type, small waves could not be found and there were different rhythms between those of the ordinary atrial muscle and the A–V node. In these cases, the sinus excitation did not propagate to the A–V node by either specific or ordinary atrial muscle. Structures of the boundary of both rhythms in these cases were the same as shown in the first type.

The third type might correspond to the A–V dissociation with the nodal or the ventricular capture clinically. In this type, the ordinary atrial muscle was excited by sinus rhythm and sinus excitation was represented as small waves in the A–V node which had great influence upon automaticity of the A–V node. In these cases, the structure at the boundary of rhythms was the same as shown in the first and the second type.

Cranefield et al.\textsuperscript{56} said that the failure of impulse transmission was found in fibers of the atrial margin of the A–V node. But their experiments were not ascertainment histologically.

In this experiment, situation of the tip of the microelectrode was ascertainment by using the Kanazu's method.\textsuperscript{52} And it was testified that the boundary of the characteristic change of the action potential by the effects of acetylcholine was seen at the atrial margin of the A–V node with net-like structure.

From the results of the author's experiments, it is impossible to reach to the conclusion concerning the mechanism of the A–V conduction delay. But its main possible interpretation was suggested by this experiments.

To investigate the mechanism of the A–V conduction delay more clearly, it is necessary to learn two following problems: 1, the mechanism of the spread of impulse at the tissue of the A–V nodal area, and 2, the origin of particular action potential of single fiber of this area, particularly, a step or a notch or a slow rising upstroke in the depolarization phase.

Many investigators\textsuperscript{51–59} have been pointed out that the spread of impulse in the cardiac muscle was not syncytial electrophysiologically and histologically. And this fact show that the conduction of excitation in the cardiac muscle is not discontinuous, and the impulse is conducted from a cell to an another cell through the junctional membrane.

In this experiments, the spread of the excitation in the A–V nodal region was not discontinuous. And also, the small waves (so-called hump) by the effects of acetylcholine was declined gradually between the upper parts and the anterior lower parts in the A–V node, and was not conducted to the His' bundle. On the other hand, failure of transmission of sinus impulse was found at the atrial margin of the A–V node as seen in the second type of the A–V conduction disturbance. From these facts, it was thought that the decremental conduction occurred, and was limited in the A–V node.

As to the origin of the particular action potential of the A–V node, Cranefield and Hoffman\textsuperscript{45,51,52} thought from the results of the effects of acetylcholine on electrical activity of single fiber of the node that the excitatory process in nodal fibers consists of plural excitatory events, and these plural events were composed of one action potential in the normal conduction, but were separated by the effects of acetylcholine.

And Tamagaki\textsuperscript{56} supposed that a step or a notch at the beginning of the depolarization of the action potential in the A–V node would be due to the time difference of the arrival of impulses which was conducted through the plural conducting fibers.

Whereas, Goto et al.\textsuperscript{57–59}, Mashiba\textsuperscript{60}, and Hoshiko et al.\textsuperscript{61} considered that a step or a step-like prepotential would be due to the appearance of junctional membrane potential or junctional membrane response in the cardiac muscle fibers. And, they\textsuperscript{59,61} suggested that these step-like prepotential would be separated from the spike with the decline of safety-factor of impulse transmission among cells.

In this experiment, the increase of a step of the rising phase of the depolarization and the decrement of action potential in the A–V node were observed by the effects of acetylcholine (the first type of the A–V conduction disturbance). And at times, the waves caused by the decrement of the action potentials were seen.

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as a small wave (so-called hump). It was thought that this small wave (so-called hump) was independent step phase, others accorded to the small wave that the spike became extinguished leaving only a minute local depolarization as mentioned by MATSUDA et al. And also, in the third type of the A-V conduction disturbance, small waves (so-called hump) appears as very clear step or notch in the action potential by the lower automaticity, and incidently aroused its action potential, and became to the origin of arrhythmias of regular A-V nodal rhythm. It was thought that these small waves (so-called hump) would be due to the sinus excitation, and in normal conduction, those appeared as a step or a notch at the beginning of the depolarization of the action potential of the A-V node.

Therefore, from the data obtained in this experiments, the mechanism of normal A-V conduction delay was supposed as follows; sinus impulse reaches to the atrial side of the A-V node and produced the excitation of the nodal fibers, and its arrival time delay gradually to the HIs' bundle through the complicated net-like structure of fibers, accordingly, the onset of excitation delay gradually from the atrial side of the node to the HIs' bundle.

And also, remarkable delay of the spike of nodal action potential was seen in the process of impulse transmission in the A-V node. On the other hand, the action potential of the HIs' bundle characterized by the steep upstroke and its steep transition to the spike, and the discharge of a step at the rising phase of the depolarization represents that the A-V delay already finished.

But, the data obtained in the present experiments were limited only within 2 or 3 layers of the cells beneath the endocardial surface, and it was impossible to record the action potential of deeper layers of the A-V node. Of course, it may be supposed that there are many other factors which cause the A-V delay, excepting the data obtained in this experiments.

**SUMMARY AND CONCLUSION**

This experiments was performed with 100 rabbit atria to clarify the characteristics of the stimulus conduction at the A-V nodal region by using intracellular microelectrode technique. To find the location of the tip of the microelectrode in the tissue, the special microelectrode contained 0.5 per cent CoCl₂ in 3 mol KCl which was reported by KANZU was used, and the relation between the stimulus conduction and the histological structure at this area was clarified.

1) At some points in the upper part of the nodal region, the onset of the activation was earlier than that of the reference in 5 of 40 cases. In these cases, the location of the microelectrode tip was found in the specific muscle fibers connected with the A-V node. These specific muscle fibers were characterized by crowded and irregularly arranged nuclei, formed one muscle bundle and were surrounded by abundant connective tissue. And anatomical location in which the specific muscle fibers were found might accord with the connected parts to the A-V node of the system septo-angularis anterior which was reported by OOSAWA.

2) Histologically, the A-V node was divided into three parts as follows; the upper or atrial part, the middle part and the lower part near the HIs' bundle in the node. And generally, typical A-V nodal action potentials with a step at the beginning of the upstroke were obtained in the middle part and the upper or atrial part of the node. And also, the most remarkable delay of the spike was seen in the action potential at the middle part or the upper part nearest to the middle part of the node. The action potentials recorded from fibers of the lower part of the node nearest to the HIs' bundle were similar in shape to those of HIs' bundle. And a transition in shape of the action potential between the upper and middle parts of the node and the HIs' bundle was gradual. However, when the recording microelectrode was moved from the upper part to the HIs' bundle, the onset of the activation delayed gradually having no relation to the changes in shape of the action potential in normal sinus rhythm.

3) Moreover, the area where the conduction delay occurred was clarified by making disturbance of the A-V stimulus conduction
using the effects of acetylcholine. The disturbance of the A–V conduction could be divided into three types. The first type was considered to be the second degree of the A–V block, the second type to be complete A–V dissociation and the third type to be A–V dissociation with nodal or ventricular capture clinically. In the first and third type, two kinds of waves were recorded from the same fiber in the A–V node. Namely, the one is a large and conductive wave and the other is a small and non-conducting wave (so-called hump). This small wave (so-called hump) was found to be synchronous with sinus activity in the middle area and atrial side in the A–V node, and became smaller or disappeared in the His' bundle. In all cases, it was found that the boundary of characteristic change of the action potential was at the atrial margin of the A–V node with net-like structure of fibers.

From these facts, it was concluded that the specific muscle fibers existed at the vicinity of the A–V node, the impulse from the S–A node may be mainly conducted along these specific muscle fibers to the A–V node, decremental conduction was limited in the A–V node and the A–V conduction delay occurred continuously and gradually at all area in the A–V node with net-like structure of fibers.

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