Histologic Examination of the Origin of the Action Potential Characteristically Obtained from the Region Bordering the Atrioventricular Node

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In the region of the atrioventricular node a characteristic cell potential was obtained by microelectrode recording in dogs. The resting and action potentials obtained here were small and the latter had a step-like potential before the steepest rise. The site of origin was examined histologically by a special staining technic and was found to be from a cell of the atrioventricular node or a part of the special junctional tissue adjacent to it.

In the region of the atrioventricular node Matsuda and associates¹ and the present authors² found characteristic features in the resting and action potentials recorded by intracellular microelectrodes. The resting and action potentials obtained here were small and the action potential showed a step-like phase before the steepest rise which also had a lower rising velocity than that of the atrial or ventricular action potential. Shortly before these reports were published, Hoffman and associates³ described a slightly different feature of the action potential from this region. They found that the action potential from the atrioventricular was similar to that of the sinoatrial node in shape, having a low rising velocity. Although Hoffman and Matsuda ascribed the origin of such potentials to the atrioventricular node without histologic confirmation, nothing can be concluded definitely without histologic evidence, because it is anatomically a known fact that the atrioventricular node lies not in the subendocardial layer but under the atrial muscle layer in the human, dog, whale and other mammalian hearts. Therefore, it is even doubtful whether a microelectrode could reach the atrioventricular node without breaking.

In this research a study was made to locate histologically what layer the tip of the inserted microelectrode reached when the above-mentioned characteristic action potential was obtained.

METHODS

The hearts studied were isolated from dogs varying in age and weight. The inside of the right atrium of the heart was exposed and from its septal region a large muscle strip including the fossa ovalis, the coronary orifice and the septal cusp of the tricuspid valve was excised. The identification of the so-called Koch's triangle⁴ and preliminary anatomic examination of other dog hearts helped in locating the atrioventricular node. The microelectrode was inserted from the endocardial side.

In order to show what layer the tip of the microelectrode reached, a staining method which Tomita and associates⁵ applied to the fish retina was employed. The method consisted of ordinary glass capillary microelectrodes (with an external tip diameter of about 0.5 μ) was filled with 10 per cent ferricyanide solution instead of usual 3M KCl solution. Such microelectrodes had a slightly higher electrical resistance than those with 3M KCl, but enabled us to obtain resting and action potentials of the cell satisfactorily. A thin silver wire in the microelectrode, which was usually connected to the recording system, was now also connected to the negative pole of a 6 volt battery by a switch and 200 MΩ resistors. Its positive pole was connected to the ground for recording. After the desired resting and action potentials were obtained from the microelectrode inserted in the cardiac muscle fiber, the switch connected to the battery was switched on and a direct current was passed through the wire for 1.5 to 2 min. After the current was cut off, the microelectrode was withdrawn and a 2 per cent ferrous chloride solution was applied to the endocardial surface at the point of electrode insertion for about 2 min. In an ideal condition a blue ring was found on histologic examination, formed at the boundary between the ferricyanide driven out of the microelectrode...
Fig. 1. Difference of resting and action potential recorded from the region of the atrioventricular node. Number of each picture indicates the spot from which the photograph was taken. Numbers in the preparation correspond to those of the pictures. Marks on the top and bottom of the vertical bar on the left side of each action potential picture denote the zero level and the —50 mV level, respectively. Time marks, 100 msec. Pictures 3, 4 and 5 are the action potentials with a step. Pictures 1, 2 and 6 are those without a step and can be regarded as a transitional type. Picture 7 is the atrial cell action potential.

by passing the applied current and the ferrous chloride which had diffused down from the endocardial surface. This is a kind of Liesegang ring. Since the boundary was shown to be blue, the muscle preparation was stained by eosin only, avoiding the usual blue hematoxylin staining.

The details of the method of isolating dog hearts, of electrical stimulation, of the muscle chamber and of the perfusion and recording systems have been described elsewhere.

Results

Examination of the resting and action potential in the region where the atrioventricular node was assumed to be located revealed two different types of action potential (fig. 1).
Fig. 2. An example of the specific action potential with a step and its origin. The microelectrode was inserted at the black point and arrow in B. A is the picture of the action potential obtained then. Vertical bar, same as in figure 1. Horizontal bar, 100 msec. C is the histologic finding at the point of electrode insertion. (Upper picture, X 60; lower picture, X 160.) Under the atrial muscle layer (a) is seen the layer of the special junctional tissue (b). In this layer the broken tip of the microelectrode is seen remaining, arrow. Surrounding this there is the internal ring of the double blue ring. The external ring is more clearly identified.

One was the type which Matsuda and we reported. It had a step-like phase at the beginning of the rising limb, which gradually or suddenly was converted to the steepest rise. The steepest rise of this action potential was less steep and the magnitudes of the resting potential, action potential and overshoot were remarkably smaller than those obtained from the atrial or ventricular muscle fiber, having an average value (as obtained from the summary of 105 impalements in 25 dogs) of 53 mV., 58 mV. and 5 mV., respectively. The details of the characteristics of such cellular potential and the effects of some drugs on them have been reported elsewhere.2 The other type of action potential resembled that which Hoffman and his associates described and had no recognizable step-like part before the phase of steepest rise. Otherwise, its rising velocity was low, resembling that of potentials from the sinoatrial node. The magnitude of resting potential, action potential and overshoot of this second potential type was also small and about the same as the first type. Therefore, the only difference between the two types was whether or not there was a step-like potential at the beginning of the action potential and the latter (without a step-like phase) can be regarded as a transitional type between the former (with steps) and the atrial action potential.

In addition, it was found that the region of tissue from which the action potential with a step (a step-like potential) was obtained and that from which the action potential without a step was obtained, were adjacent to each other. The former portion was about 1 to 3 mm. in length and about 1 mm. in width, while the latter surrounded it and was but a few millimeters in width. The action potential without a step appeared as a transitional type in configuration and size between the action
potential with a step and the atrial action potential and was obtained from the transitional zone. Thus, the action potential with a step was presumed to be a typical one.

For this reason the experiment in which the action potential with a step was obtained while the microelectrode was inserted not so deeply or rather superficially was considered to be of great importance. It was found histologically that at such points the layer of the atrial muscle fibers was much thinner than expected and the action potential with a step originated actually from the atrioventricular node or at least from the special junctional tissue adjacent to it. Figure 2C shows the double blue ring formed. It was inferred that the tip of the microelectrode was located in and the action potential shown in figure 2A originated from a cell within the sphere of the inner ring of the double ring which are situated in the layer of the special junctional tissue. Such a presumption was supported by a rare incident in which the tip of the microelectrode was broken and left inside the inner ring, the fragments lying on top of each other. The step of the action potential in this case was so large that it fused into the steepest rise of the action potential, making the name "step" unsuitable. But this type belongs to the action potential with a step and not to the type resembling the action potential from the sino-atrial node according to our experience.

In the case shown in figure 3 the microelectrode was inserted more deeply and the action potential obtained was more characteristic of junctional tissue. Only a blue ring was formed here, which was found also in the layer of the special junctional tissue.

Although 23 preparations from the same number of dogs were examined histologically, none of them were shown clearly to originate from the ordinary atrial muscle fibers, although it was fairly difficult to make such a negative statement because of the infrequent successful formation of the blue ring. On the other hand, the action potential with a step having almost the same features was obtained on rare occasions from the ventricular side of the atrioventricular node, usually from the muscular portions close to the posterior and inferior margin of the membranous part of the interventricular septum through which are known to pass fibers of the bundle of His. Histologic examinations in such cases have not been successful as yet in definitely locating the origin of such potentials.

**DISCUSSION**

The step-like potential preceding the chief part of the action potential has now been proved to originate from the special junctional tissue in the region of the atrioventricular node. The fact that it is changed by various
conditions furnishes a good explanation for the well-known conduction delay in the atrioventricular conduction system, as was shown by examples of Wenckebach phenomenon and by the effects of noradrenaline, digitalis glycosides, quinidine and procaine amide reported previously. It was also shown that apparently this step-like potential corresponds to the localized depolarization occurring in these junctional fibers.

The existence of the step also reminds us of the phenomena recorded from synaptic structures between the nerve and the skeletal muscle. Whether or not such structure exists in the atrioventricular node is now under study in this laboratory.

It is interesting that a similar type of action potential could be obtained from the ventricular side of the atrioventricular nodal tissue. A phenomenon occasionally observed in this vicinity namely, that only premature beats have such a step-like potential, offers some suggestion as to the mechanism of the step.

**SUMMARY**

The origin of the special action potential obtained by the microelectrode in the region of the atrioventricular node was studied. It was found to be small in magnitude and to have a low rising velocity and a step-like potential before the steepest rise. The region of origin of this potential was examined histologically in dogs. The points which the tip of the microelectrode reached were stained by interaction of ferricyanide solution, discharged from the recording microelectrode by passing direct current through it, and ferrous chloride applied on the endocardial surface of the cardiac muscle strip. Thus, it was confirmed that the potential had originated from the cells in the atrioventricular node or at least in the special junctional tissue adjacent to it.

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**SUMMARIO IN INTERLINGUA**

Esseva studiate le origine del special potentia de action que es obtenite per le microelectrodo in le region del nodo atrioventricular. Esseva trovate che illo ha un basse magnitude, un basse velocitate ascendent, e un augmento progredente passo per passo ante le plus acute elevation. Le region de origine de iste potentia esseva examinate histologicamente in canes. La punctos attingite per le extremitate del microelectrodo esseva tincturate per le interaction de un solution de ferricyanuro (discargate per le microelectrodo registrante per le passage de un currente directe) e de chloruro ferrose que esseva applicate al superficie endocardial del banda de musculo cardiac. Assi il esseva confirmate che le potentia in question prendeva su origine ab le cellulas in le nodo atrioventricular o al minus in le special histos junctional que es adiacente a illo.

**REFERENCES**

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