Intracellular Electrogram of Embryonic Chick Heart

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The electrograms of both innervated and non-innervated embryonic chick hearts were reported by Fingl et al. (4), who used 3 day-old embryo as the youngest material. It has been reported (9), however, that the first contraction in the embryonic chick heart occurs at the 9- to 10-somite stage which is younger than 3-days of incubation, the electrogram of such preparations in earlier stages still remains to be elucidated.

The present communication reports our preliminary results concerning the electrogram of embryonic chick heart younger than 3 days of incubation.

MATERIALS and METHODS

About two hundred fertile eggs of white leghorns incubated for less than 96 hrs at 38° C were used. The hearts of 7 day-incubated embryos and 2 day-old chicks after hatching were also used for comparison. The embryo was excised together with "area pellucida" from the yolk and fixed with glass needles in a paraffin vessel containing warm Tyrode's solution (38° C).

The glass capillary electrodes (microelectrodes) were prepared manually or by using the KATSUKI-type puller (Narishige Sci. Inst. Lab.). They were filled with 3 molar KCl solution according to Tasaki's alcohol method (6). Although the external tip diameter and electrical resistance of each electrode were not measured precisely, it was estimated from the data of our similar work that they ranged between 0.5～1 microns and 30～50 megohms, respectively.

The electrode was placed on a micromanipulator with a half-rigid holder or with the flexibly mounting device of Woodbury et al. (7), and inserted into a heart cell under a binocular microscope. Potential difference between this exploring electrode and an indifferent one in the fluid bathing the preparation was amplified and recorded by a cathode-ray and/or inkwriting electromagnetic oscilloscope.

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RESULTS and DISCUSSION

1. Histological note

A brief histological survey was carried out to interpret the persent results. Figure 1 represents the photographs showing sagittal sections of embryos incubated for 77 hrs (A) and 52.5 hrs (B & C), in which the ventricle is indicated with the symbol v.

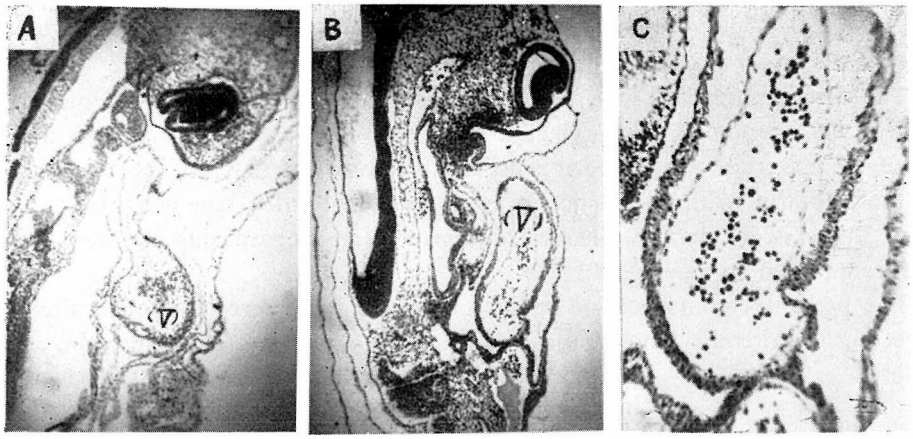

Fig. 1. Photographs showing sagittal section of chick embryos. (Symbol V shows ventricle.)
A: 77 hrs' incubation. Section through right optic vesicle.
B: 52. 5 hrs' incubation. Section through left optic vesicle.
C: Enlargement of Fig. B.

The size of the cardiac muscle cell can be estimated from the blood cells in the ventricular or atrial cavity. In Figs. 1-B and 1-C, the wall of the atrium can be seen inside the ventricular wall. It is notable that the muscle cells were very small and not yet organized fibrously, and that the developing cellular layer of the wall was very thin.

2. Electrogram of the ventricle

One of the electrograms obtained from the ventricle of a 72 hour-incubated chick embryo is illustrated in Fig. 2. The upper-left(A) shows a recording with a cathode-ray oscilloscope and the right figure (A'), which is continued to the lower one, is a simultaneous recording with an ink-writing electromagnetic apparatus. Upward deflection shows the positive change of potential at the exploring electrode.

It has been generally accepted that the cardiac pacemaker cell shows the characteristic "slow diastolic depolarization" in its action potential. This feature

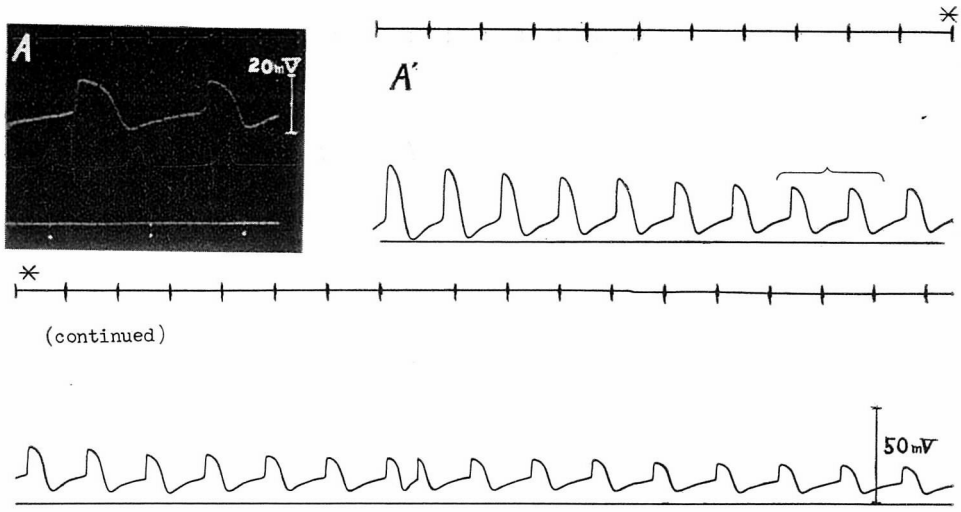

Fig. 2 Electrograms from the ventricle of 72 hr-incubated chick embryo.
A: Recording with cathode-ray oscilloscope. This figure was recorded simultaneously with the part indicated by the mark in Fig. A'.
B: Recording with inkwriting electromagnetic oscilloscope. Lower figure continues from the above.
Note the slow diastolic depolarization and the shape of action potential. Upward deflection indicates the positivity of the potential at the exploring electrode against the indifferent one (the same in the following Figs. 3 and 4). Time scale; 1 second.

was clearly observed in our experiments. And, other characteristics in configuration of the wellknown "intracellular action potential" of cardiac muscle cell, such as "systolic depolarization" (fast depolarization following the slow diastolic one), the "plateau" and the "relatively fast repolarization" were also identified in the recordings. The amplitude of action potential, however, decreased gradually after the successful insertion of the microelectrode.

It seems likely that the decrease of amplitude was due to the cell-injury which was caused by the movement of heart around the inserted electrode-tip. Because, when the electrode was pulled out and inserted into the another cell after the recording, an amplitude almost the same as at the beginning of the previous insertion could be observed. Therefore, the actual potential change would be greater than that of the first deflection in Fig. 2-A'; the value of which was 39 millivolts. Another interpretation of this decreasing amplitude will be discussed below.

Other recordings are shown in Fig. 3, where A was obtained from a preparation incubated for 92 hrs, and B, for 72 hrs. For comparisons, recordings of a 7 day-old embryo (C) and of a young chick 2 days after hatching (D) are illustrated in the lower half.

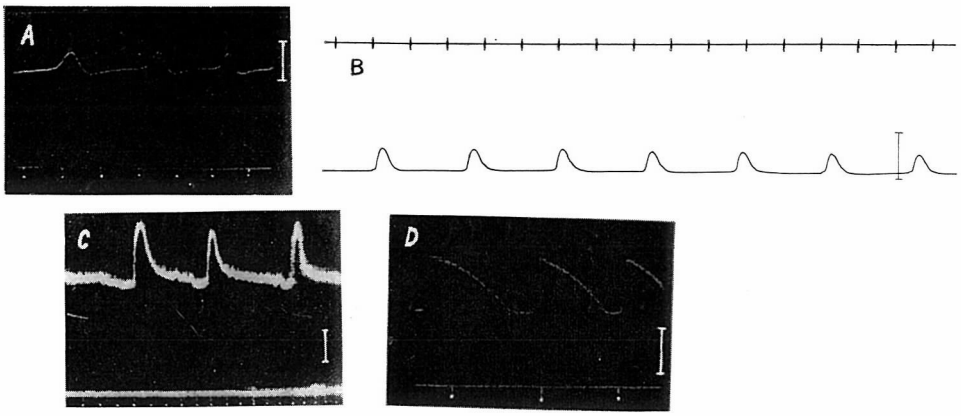

Fig. 3 Electrograms from the ventricle of chick heart.
Time of incubation: 92, 72 hrs and 7 days in A, B and C, respectively.
D: Recording from young chick 2 days after hatching.
Time scale: 0. 2 sec in D only; others, 1 sec.
Vertical bar: Potential calibration. 50 mV in D, 10 mV in others.

A slow diastolic depolarization was seen in Fig. 3-A, but not, in 3-C and 3-D. It was usually observed in the heart of embryos younger than 96 hrs. The absence of this depolarization in Fig. 3-B might be due to low sensitivity of CR amplifier used in that case. Another striking difference between the ventricular action potential of younger embryos (72～92 hrs of incubation, Fig. 3-A and 3-B) and that of older ones (7 days of incubation or after hatching, 3-C or 3-D) was the fast depolarization phase or the general shape of action potential.

For the interpretation of the pacemaker activity in the ventricular cell which was observed in our recordings of action potential (Figs. 2-A, 2-A' and 3-A), the following informations were taken into consideration. The slow diastolic depolarization and the following systolic one are the special features of the pacemaker cell in the mammalian or amphibian heart (2). That the adult ventricular cell has an ability to be a pacemaker has been well known since the Stannius' ligature-experiment.

According to the comparative embryology of the vertebrates (8), the heart of chick embryo incubated for 72 hours is tubular, having no valves. In this as well as in later stage, its automatic excitation is initiated in the sinus area and the impulse proceeds to the atrium and further to the ventricle (9). It is possible, however, that the conducting system of excitation might have not fully developed at this stage and that the anomaly of conduction would occur easily. The findings frequently observed in our experiment that the ventricle beats simultaneously with the atrium and that the frequency of heart-beat decreases gradually after the extirpation of the embryo, may support this possibility.

3. Electrograms of the sinus- and atrium-area.

In Fig. 4 (S_1, S_2, A_1 and A_2) are illustrated the records obtained from the sinus- or atrial area of the embryonic chick hearts incubated for 74, 54, 77 and 65 hours, respectively. For a comparison, the recording of an action potential from the atrium of young chick 2 days after hatching is illustrated in Fig. 4-A_3.

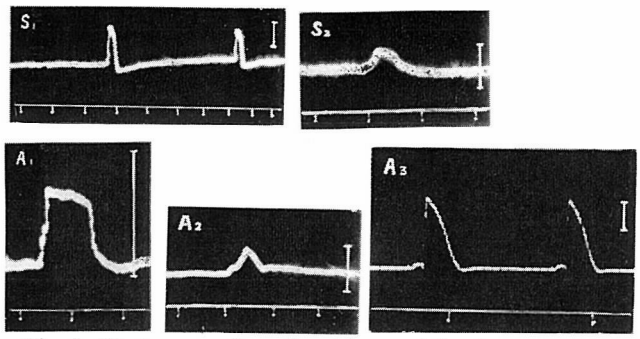

Fig. 4 Electrograms from the atrium and the sinus-area.
S_1 and S_2: Sinus-area; 74 and 54 hrs incubation, resp.
A_1 and A_2: Atrium; 77 and 65 hrs, resp.
A_3: Atrium of young chick 2 days after hatching.
Time scale: 0.5 sec in A_3, others, 1 sec.
Vertical bar: 20 mV in A_3, others, 10 mV.

The hump in the rising phase of action potential, which is seen in Figs. 4–S_2, 4–A_1 and 4–A_2, can be interpreted as follows. In the case where the potential recording was intracellular, the hump may be caused by the overlapping impulse conducted from the pacemaker to the cell, in which the microelectrode was inserted and a potential change owing to its automaticity occurred a little earlier than the arrival of the impulse. Another explanation may be possible that the impulse coming from one conducting pathway would sum-up to the already existing depolarization which was caused by the impulse from another pathway. The discussion presented by Hoffman and Cranefield (2) on the atrio-ventricular node of rabbit heart is consulted for the latter interpretation. Data are not sufficient, however, to draw out a conclusion from the present experiment.

Lastly, other experimental condition in which the electrode-tip was not placed intracellularly but extracellularly must be considered. If the recordings were taken intracellularly, the small amplitude of action potential as seen in Figs. 3–A, 3–B, or Fig. 4 (S_1, S_2, A_1, A_2), and the decreasing amplitude in Fig. 2–B might be interpreted to be the result of cell-injury around the inserted microelectrode, as discussed above. In the case of extracellular record, however, the following explanation might be possible: negative deflections immediately after the insertion of the microelectrode, if any, might be an injury potential and not

the membrane resting potential. It is possible that this negative deflection would continue for 10 min or longer if the injury were well demarcated and the injured area were a so-called "sink-area" for the local potential recording. Under these circumstances, the potential change recorded as an action potential would not be resulted from the injured cell groups at the exploring electrode-tip, but from the active cells in the vicinity. And, the polarity of exploring electrode would also be positive, as reported by Kawasaki (10) in our laboratory. The amplitude would be determined by the electrotonic spread of the action potentials of neighbouring cells and, therefore, would be less than that of intracellular recording. The "hump", in this case also, may be interpreted as an asynchronization of the activities of neighbouring cells. The possibility that the records in Fig. 4 were extracellular can not be denied.

SUMMARY

1. Electrograms of the exposed heart of chick embryo which was incubated for 54～96 hours, were recorded with the microelectrode technique. Embryo of 7 days' incubation and young chick 2 days after hatching served as the control.

2. The slow diastolic depolarization which has been accepted as the characteristic feature of the pacemaker cell in other vertebrate hearts, could be recorded from the ventricle, atrium or sinus-area. Other features such as systolic depolarization (rising phase of action potential), plateau-phase and terminal rapid repolarization were also observed in the present preparation.

3. The values of resting and action potential could not be measured precisely. It seemed likely that this unfavourable result was caused by the cell-injury around the inserted microelectrode-tip.

4. Certain characters of the action potential, such as the amplitude and the hump seen in the rising phase, were discussed from the view-points of intracellular as well as extracellular recordings.

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