ACTION OF CALCIUM ON ELECTRICAL AND MECHANICAL ACTIVITIES OF THE CULTURED CHICK EMBRYONIC HEART

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Electrophysiological characteristics of the cultured chick heart have been studied by several investigators\(^4\). Most of these studies demonstrated a difference in electrical properties between the cultured and non-cultured chick embryonic heart. For example, cultured cell clusters from the ventricle of the chick embryonic heart were insensitive to autonomic agents which usually produce a change in the membrane potential of intact cardiac muscle\(^1\). This difference may be explained by the hypothesis that nerve component was not existent in the cultured cells\(^1\). The cultured preparation also makes it possible to study the direct effect of chemical agents on the chick heart cell in absence of nerve component, connective tissue and blood vessels.

In recent years, it has been reported that calcium is essential at the molecular level in maintaining the normal structure and excitability of the cell membrane\(^6\). The effect of external calcium concentration upon the tension development of muscle fiber has been investigated by HAGIWARA et al.\(^5\). Considerable information has been accumulated from these studies on the relation between the electrophysiological characteristics and external calcium concentration. However, little work has been done by means of simultaneous recording of the electrical and mechanical activity of the cultured chick embryonic heart to observe the calcium effect.

The present study deals with the effects of calcium ion on the electrical and mechanical activities of cell clusters obtained from the ventricular part of the chick embryonic heart.

In addition, the electrical properties of the non-cultured embryonic heart were studied for comparison with those of the cultured one. The results presented in this paper clearly indicate the intricacy of using such a system for the experiments.

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MATERIALS AND METHODS

Under sterile conditions, the heart was dissected from a chick embryo of 5 days incubation, and the ventricular part was separated from the atria. The ventricle was minced into pieces of about 0.5 mm³ and were dispersed with 0.05% trypsin at 37°C. The dispersion was then centrifuged at 800 r.p.m. 3 min. After removing the supernatant, the residue was resuspended in Eagle's minimum essential medium containing 10% horse serum, and cultured for 5-10 days in a CO₂-incubator at 37°C. Finally, in all experiments, the culture medium was removed and replaced with Krebs-Ringer solution. The composition of the solution (in mM/l) was as follows: NaCl, 133.8; KCl, 4.56; CaCl₂, 3.3; MgCl₂, 0.2; NaH₂PO₄, 1.4; NaHCO₃, 15.8; Glucose, 8.2.

Non-cultured chick embryonic hearts which were removed from 10 days old chick embryos were also studied. Both cultured and non-cultured cells were immersed in Krebs-Ringer solution aerated with 95% O₂ and 5% CO₂ at 35°C during the experiment.

The action potentials were measured by means of an intracellular microelectrode with a resistances of 20-30 MΩ filled with 3 M KCl. The microelectrode was inserted into a cell cluster as close as possible to a wire to measure the mechanical activity. The potentials were then recorded with a preamplifier and dualbeam cathode ray oscilloscope. The method of bridge circuit for recording the mechanical activity was used. That is, a wire connected mechanically to the plate pin of a transducer (Toshiba 5734A) was touched on the surface of the cell cluster. The plate of this type of electrical tube was able to be moved according to the contraction of cell cluster. Change in the plate current continued as long as the force is applied. The current change was monitored on an oscilloscope through an amplifier. For electrical field stimulation, two silver wire electrodes coated with araldite, about 10 mm apart, were placed in the bath. The arrangements for stimulation, and electrical and mechanical recordings were schematically shown in Fig. 1.

![Schematic Diagram of Experimental Set up](image)

**Fig. 1.** Diagram of experimental arrangement for electrical and mechanical recording from cultured cells. The recording electrode is connected to the preamplifier. Field stimulation is applied with a pair of silver wire electrodes. Vacuum tube transducer (Toshiba 5734A) was mounted on cell cluster and the change is amplified through a bridge circuit.
RESULTS

It is well known that the duration of the action potential depends on its frequency. Therefore, spontaneously active cells were discarded and only quiescent cells were stimulated with the square wave pulse of 2 msec in duration. Typical action potentials (upper trace) and mechanical activities (lower trace) generated by repetitive stimulation were shown in Fig. 2. A slow contraction was regularly preceded by the electrical activity in normal Ringer solution (Ca\(^{++}\)=3.3 mM).

It has been reported that the action potential of the frog ventricle was shortened by the reduction of calcium concentration\(^{19}\). However, other investigator demonstrated that no change in the action potential of the frog ventricle was produced by a calcium free solution\(^{14}\). WEIDMAN\(^{15}\) also showed that four-fold increase or decrease of the calcium concentration produced no change in size or shape of the action potentials of calf and sheep purkinje fibers. On the whole, therefore, it seems that the influence of the reduction of calcium in the external medium on the cardiac cell membrane was variable from species to species and tissue to tissue. In the present experiments, duration of the action potential was determined by measuring the length of the action potential at the level of 70% of its peak height. When the normal Krebs-Ringer solution was replaced by calcium deficient solution (Ca\(^{++}\)=1.65 mM), a conspicuous change in shape of action potential was observed after 30 min., but the membrane potential fell a few millivolts during this time. Furthermore, when the calcium level was decreased to one-tenth of that of a normal solution, the duration of the action potential was more greatly prolonged than in one-half calcium concentration (Fig. 3). That is, the duration of action potentials in cultured cells increased by 35% in 1.65 mM and 80% in 0.33 mM calcium concentration, respectively. These results were different from the experimental observation of SPERELAKIS\(^{12}\) who showed that the lowering of calcium had only a little effect on the configuration of the action potential.

The electrical activity of intact embryonic hearts were used for comparison with the electrophysiological properties of cultured cells. The records of this activity are illustrated in Fig. 4. There were no obvious differences

![Fig. 2. Typical action potentials (rapid responses) and mechanical activities (slow responses) generated by repetitive stimulation (1/sec) from the same cultured chick cluster. The electrical activity was regularly followed by slow contraction.](image-url)
in configuration of electrical activity as a function of the age of the embryo. Similarly, the configuration of the action potential of the intact embryonic hearts was not so affected by a reduction in the calcium concentration as long as excitability was maintained. That is, the reduction of calcium level to one-tenth of normal value caused nothing but a slight prolongation of the duration of the action potential as shown in Fig. 4. This is in good agreement with the results obtained from dog auricle and ventricle.

**Fig. 3.** Effects of altering calcium concentration on cultured cells. In 1.65 and 0.33 mM calcium, the duration of action potential is markedly prolonged.

**Fig. 4.** Effects of calcium on intact embryonic hearts for comparison with electrophysiological properties of cultured cells. The reduction of calcium to one half of normal has little effect on the configuration of action potential, but in extreme calcium deficiency (Ca = 0.33 mM) duration of action potential is slightly prolonged.
Simultaneous changes in electrical and mechanical activities have also been observed by the alteration of calcium concentration. Decrement of calcium concentration had the well known effect of decreasing the strength of the cardiac contractions. This was not paralleled by any marked effect on the duration of the action potential (Figs. 5 and 6). The results of experiments concerning the effects of change in the calcium concentration on the electrical and mechanical activities of both cultured and non-cultured cells were summarized in Fig. 7. The effect of reduction of calcium level to one-tenth of normal concentration on the mechanical activity of cultured cells was not studied in this experiment. The results indicated that the electro-

![Graphs showing electrical and mechanical records](image)

**Fig. 5.** Simultaneous electrical and mechanical records from cultured cells during calcium deficiency (Ca=1.65 mM). The duration of action potential in cultured cells was increased by lowering calcium concentration, but mechanical activity was conversely reduced. g-Strophanthin restores mechanical activity in low calcium medium to normal level without affecting the action potential.

![Graphs showing electrical and mechanical records](image)

**Fig. 6.** The effects of external calcium on electrical and mechanical activities of intact cells for comparison with the electrophysiological properties of cultured cells. No detectable changes in the duration of action potential was produced by calcium of one-half normal, while the depressive effect on mechanical activity is very clear. The recovery process was also tested with the addition of g-strophanthin. The recovery from the suppression was smaller than that in cultured cells.
physiological properties of trypsin-dispersed cultured cells are different from those of intact hearts. The amplitude of the mechanical activities of both cultured and intact cells were depressed by the reduction of calcium concentration. Obviously, mechanical response of the intact cells were more sensitive to change, in the calcium concentration of the medium, than those of the cultured cells. When the extracellular calcium is decreased to 1.65 mM, the mechanical activity of cultured and intact cells decrease to about 30% and 60% of that in normal solution, respectively, i.e., the mean values of the amplitude were 9.2 mg in cultured and 17.8 mg in intact cells, while 12.9 mg and 46.5 mg in normal Krebs-Ringer solution. On the other hand, the decrease of external calcium caused a marked prolongation of the duration of the action potential in the cultured cells, while only a slight effect was observed in intact hearts. The fact that calcium deficiency had inverse effects on the electrical and mechanical activities in cultured and intact ventricles suggests a difference in the mechanisms underlying excitation and contraction in the cultured and intact cells. The difference may simply be due to difference in sensitivities of the two processes to calcium ions. Evidence for such different sensitivity has been obtained from another study of the recovery process in the cultured and intact cells (FIGS. 5 and 6). It has been reported by TRAUTWEIN et al. that an adequate dose of strophanthin restored the mechanical activity depressed in the calcium deficient solution to normal level. In the present experiment, the recovery process was tested with an addition of g-strophanthin in the calcium deficient Ringer. It was observed that g-strophanthin (10^-8 g/
ml) augmented selectively the mechanical activity of both cultured and intact cells decreased in the low calcium solution (1.65 mM), without affecting the configuration of action potential. Between them the effects are somewhat different. That is, an extent of recovery was smaller in intact cells than cultured ones.

DISCUSSION

The effects of variation in the external calcium concentration on electrical activity in materials of the same origin are rather inconsistent. The papers which have been mentioned in the introduction showed that various parts of the same heart differed markedly in their response to a change in the calcium concentration, so that the lack of uniformity in species is not so surprising.

It is generally accepted that the duration of the action potential in mammalian ventricle is increased by lowering the calcium concentration.

On the contrary, Hoffman and Suckling observed that the duration of the action potential of dog ventricle was only slightly prolonged in a low calcium medium. A relevant fact emerging from the present experiments was that the duration of action potential in cultured cells was prominently enlarged by low calcium concentration, whereas in intact cells it was only slightly affected.

The reasons for such a large difference in the excitation process may be explained that cultured cells were more simplified during its cultural procedure than intact cells. On the other hand, the mechanical activities of both cultured and non-cultured cells were decreased as the external calcium decreased. This might support an assumption that the influx of calcium ions through the membrane is an important factor in the development of contraction. In intact cells, however, mechanical activity became smaller than that of cultured ones. It may therefore be suggested that from the view of the excitation process, the degree of the enlargement of the action potential duration, caused by alteration of external calcium in cultured cells is prominently higher than that of intact cells. In contrast with the excitation process, the degree of suppression in contraction of cultured cells produced by calcium deficiency was lower than that of intact cells. The mechanical activities of both cultured and intact cells were augmented by the application of g-strophanthin. In careful comparison, the degree in cultured cell was slightly larger than that in intact cells. This effect may be the hypothesis that g-strophanthin presumably is available for increasing the concentration of intracellular free calcium.

Other experimental evidence supporting this idea has been reported by Lüllmann and Holland. Also, the differences between the effect of calci-
um on mechanical activities of both cultured and intact cells come to be questioned. The mode of action of calcium on the mechanical process cannot be explained satisfactorily from the present data, but there is one plausible possibility that density of the contraction system is less in the cultured cells than in the intact ones. Since only a small amount of calcium is required for contraction in the former, the response to calcium deficiency should be less marked. The application of this possibility may explain to a certain extent the known differences between the contracting system of cultured cells and that of intact cells in this experiment.

Furthermore, the reason why the effect of calcium on excitation and contraction process were not parallel to each other remains unknown, but various possibilities may be considered. Recently, Sperelakis and Lehmkuhl\textsuperscript{13} reported that various metabolic poisons in high concentration had no effect on the membrane potentials of cultured heart cells, and suggested that cultured cells are different in the following points: a) cytochrome oxidase activity, b) high energy phosphate pool, c) ATP-production. Other possibilities are differences in, development of the transverse tubular system (T-system) and in the number of probable sites of calcium-action between both cells. We presently have no data to exclude either of them, so that no definite conclusions can be drawn from the present results.

**SUMMARY**

By the methods of intracellular microelectrode technique and the bridge circuit for recording the mechanical activity, the effect of external calcium concentration of the cultured chick embryonic heart has been investigated. Trypsin-dispersed cells from the ventricle of 5 days old chick embryos were cultured. The cell clusters became attached to the bottom of cultured-dish. Then, results from trypsin-dispersed cells were compared with those of non-cultured intact embryonic hearts. Spontaneous cells were compared with those of non-cultured intact embryonic hearts. Spontaneous cells were discarded and only quiescent cells were driven by electrical field stimulation.

1. The reduction of calcium in the external medium strikingly prolonged the duration of action potential in cultured cells, while the amplitude of mechanical activity was prominently decreased.

2. In the intact embryonic hearts, the configuration of action potential is not significantly affected by the calcium deficient solution. On the other hand, the effect of calcium deficiency on mechanical activity is more dramatic than that in cultured cells. These results suggest that the inward movement of calcium ion through the membrane plays a significant role in the initiation of contraction.

3. The reduced mechanical activity of cultured and intact cells due to calci-
um deficiency returns to its normal level by the application of g-strophanthin, but the recovery rate was larger in cultured cells.

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REFERENCES