EFFECT OF ACETYLCHOLINE ON THE ELECTRICAL ACTIVITY OF CULTURED CHICK EMBRYONIC HEART

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The spontaneous mechanical activity of the cultured chick embryonic auricle is inhibited to a considerable extent by acetylcholine (ACh) (1), while the mechanical and electrical activities of cultured ventricle is practically unaffected by the drug (1, 2). The difference of sensitivity to ACh between the auricle and ventricle may be due to the presence or absence of cholinergic innervation, which may increase the sensitivity to ACh. On the other hand, many investigators suggest that ACh increases the permeability of the cell membrane to K^+ , and allows the resting potential to come near the K^+ -equivalent potential (3-5). It would be necessary to study the effect of ACh on the intracellular membrane potential of auricle, because the cultured cells were often completely excitation-contraction uncoupled (6). The cultured preparation enables one to study the effect of ACh on the cell membrane potential without interfering with the nerve component or neighbor cells-Jellinek *et al.* (7) demonstrated the absence of nerve tissue in the cultured cell clusters and Dahan and Gottlieb (8) observed no electrical interaction among conjoint cells.

METHODS

The atria were extracted under sterile conditions from about 30 embryos of age ranging from 3 to 10 days. The tissue was cut into pieces of about 0.5 mm³ and washed in Hank's solution, which contains no Mg^{++} and Ca^{++} at 37°C and then immersed in saline solution containing 0.05% trypsin. The cell suspension was centrifuged for 5 minutes at 1000 r.p.m. Then the cells were suspended in Eagle's essential medium (9) containing 10% horse serum. The cultures were incubated at 37°C for 2 to 10 days before being used.

For recording of intracellular membrane potential, a glass capillary microelectrode filled with 3 M KCl, resistance about 10 MΩ, was used. The electrode, which was connected to a high input impedance preamplifier (Nihon Kohden MZ-3B) was inserted into cells, using a micromanipulator. The output of the preamplifier was led to the amplifier of the oscilloscope (Nihon Kohden VC-7), and the display was photographed by a long recording camera. Occasionally, the cell clusters were driven through a pair of extracellular stimulating electrodes using a square wave generator (Nihon Kohden MSE-3). The bath temperature was kept at about 31°C during electrophysiological study. Acetyl-

choline chloride was dissolved in a saline solution and injected into the bath. Non-cultured whole auricle and ventricle of chick embryos were also investigated electrophysiologically to compare with the results from the cultured cells.

RESULTS

Intact atrial and ventricular cells

The non-cultured cells of the chick embryonic auricle beat spontaneously in Eagle's essential medium. The heart-rate varied widely between 20 and 240 beats/min. Sometimes, the cells had no spontaneous activity, but electrical field stimulation gave a synchronous contraction. Most of cells impaled were possessed of no pace-maker potential. In general, the magnitude of the resting and action potentials in older material showed somewhat lower values than that of younger ones (Table 1). The lower value in older muscle may be due to injury of the cell membrane, because with the older preparations, displacement of the electrode by the mechanical movement of muscle was more frequent. Therefore, it is not possible to show a significant correlation in the magnitude of the resting or action potentials as a function of age between 5 and 10 days.

TABLE 1. Average values of resting potential, amplitude of action potential, 50% action potential duration and rate of beating in intact and cultured (trypsin digested fragment) heart of chick embryos aged 5 and 10 days.

Preparation	Age of embryo	Resting potential	Action potential magnitude	Action potential duration	Rate of beating
	days	mV	mV	msec	Impulses/min
Intact auricle	5	64.2 (48-86)	87.9 (70-113)	40-100	60-180
Intact ventricle	5	72.4 (70-76)	100.8 (95-109)	160-200	60-120
Intact auricle	10	60.3 (48-79)	87.1 (74-104)	50-70	20-240
Intact ventricle	10	57.3 (49-60)	84.1 (60-103)	180-550	1-20
Trypsin digested fragment	5	48.8 (48-55)	63.5 (54-78)	230-360	1-30
Trypsin digested fragment	10	50.5 (44-60)	76.6 (60-90)	100-160	10-120
Trypsin non-digested fragment	5				
Trypsin non-digested fragment	10	57.3 (42-75)	82.0 (59-97)	100-290	10-120
Explant outgrowth	5	59.9 (45-75)	87.4 (60-115)	110-410	20-30
Explant outgrowth	10	53.3 (40-62)	84.5 (55-110)	100-200	10-120

The average resting potential of the atrial muscle of 5 and 10 days old embryos was 64.2 mV and 60.3 mV, respectively. The average action potential of 5 and 10 days old embryos was 87.9 and 87.1 mV, respectively. These values were higher than those previously observed (10). The differences between the previous and present data may be partially attributed to the difference of the experimental conditions, such as nutrition or temperature. The 50% duration of action potentials varied widely from 40 to 100 msec, depending on the heart-rate. Oscillatory potential was never observed in the non-cultured heart cells.

Non-cultured ventricle also had spontaneous activity. Atrio-ventricular block was commonly observed in the artificial nutrient medium and then the rate of beating of the

ventricle was slower than that of the auricle (Table 1). A pace-maker potential was not associated with an action potential recorded from the ventricular part. The plateau in the repolarization-phase was observed in most action potentials. The average resting potential of the non-cultured ventricle of the 5 and 10 days old chick embryos were 72.4 and 57.3 mV, and the average action potential were 100.8 and 84.1 mV, respectively. The 50% action potential duration of the ventricle ranged widely between 160 and 550 msec, depending on the heart-rate. The action potential of the ventricle was also elicited by electrical field stimulation.

Cultured atrial and ventricular cells

Most cell clusters showed spontaneous rhythmical contraction, which is usually synchronous. Sometimes, impalement of the electrode into the cell suddenly stopped the contraction. This quiescent stage of contraction after insertion of the electrode was frequently followed by an asynchronous contraction. In some cases, the asynchronous contraction of cell clusters spontaneously disappeared. In other cases, the asynchronous contraction changed to a synchronous contraction of the whole cluster. The asynchronous contraction was usually associated with oscillatory potentials (Fig. 1), and when the oscillatory potential reached a certain threshold, a spike potential was generated and then synchronous contraction occurred.

The resting potential and the amplitude of action potential were relatively low just after the impalement of the electrode. They were gradually increased and reached their maximum several minutes after the electrode-insertion. The upper and middle traces in Fig. 2 show the typical action potentials recorded from two cultured atrial cells of chick embryos after their resting potential and the amplitude of action potential reached maximum.

FIG. 1. Oscillatory potentials recorded from cultured embryonic auricle of chick aged 10 days. When oscillatory potentials reached a certain threshold, a spike potential was generated. Note that the amplitude of spike potentials was gradually increased.

FIG. 2. Typical action potentials recorded from cultured embryonic auricle (upper and middle traces) and ventricle (lower trace) of chick aged 10 days.

Fluctuation of the resting potential (subthreshold prepotential) was occasionally observed. Most of the action potentials recorded from the cultured auricle had a prepotential. The average amplitude of action potential of 5 and 10 days old embryos was 63.5 and 76.6 mV, respectively. The 50% duration of action potential observed in the present experiment varied between 100 and 360 msec, depending on the beat-rate and the presence or absence of plateau.

The lower trace of Fig. 2 shows typical action potentials recorded from the cultured ventricular cell of the chick embryo. Some of them showed a pace-maker potential, but never an oscillatory potential. The 50% duration of action potential varied widely depending on the beat-rate and the presence or absence of plateau. The average resting potential, amplitude of action potential, action potential duration (50%) and the rate of beating in the present experiment are summarized in Table 1.

Effects of ACh on the non-cultured auricular and ventricular cells

Generally, ACh (10^{-5} g/ml) had an inhibitory effect on the mechanical activity of the non-cultured auricle of chick embryos whose age ranged from 5 to 10 days old. The inhibitory effect was associated with hyperpolarization and shortening of the duration of the action potential, which was normally followed by the disappearance of action potentials. However, sometimes, the mechanical activity of the auricle was persistently resistant to ACh. In such cases, hyperpolarization and shortening of the action potential duration were still observed after administration of the drug (Fig. 3).

On the other hand, most of the non-cultured ventricles of chick embryos aged from 5 to 10 days showed insensitivity to ACh. That is, acetylcholine had almost no effect on the resting potential and the shape of the action potential of the noncultured ventricle.

Effect of Acetylcholine on Embryonic Chick Auricle

Control

10min. after Acetylcholine (10^{-5})

50mV

1 SEC

FIG. 3. Effect of acetylcholine (10^{-5} g/ml) on the resting potential and action-potential of the non-cultured embryonic auricle of chick aged 10 days. Note that acetylcholine produced hyperpolarization and shortening of action potential duration 10 minutes after the drug-administration.

Effect of ACh on the cultured auricular and ventricular cells

The mechanical activity of most auricular cells in embryos older than several days was depressed or stopped by ACh. The inhibitory action of ACh was usually associated with its hyperpolarizing action. Fig. 4 shows the action of ACh (10^{-5} g/ml) on the pacemaker potential in cultured chick auricle obtained from the embryo of 10 days. ACh acted firstly on the prepotential, decreasing its rate of rise, then, produced bradycardia.

Control

3min. after ACh (10^{-5})

10min after

Trypsin digested fragment

50mV

1 Sec

FIG. 4. Effect of acetylcholine (10^{-5} g/ml) on the resting potential and action potential of the cultured embryonic auricle of chick aged 10 days. Note that the rate of rise of prepotential was markedly decreased by the drug.

FIG. 5. Effect of acetylcholine (10^{-5} g/ml) on the resting potential and action potential of the cultured embryonic ventricle of chick aged 10 days. Note that the duration of action potential was markedly shortened by the drug.

Thereafter, the hyperpolarizing action of ACh became marked. It was difficult to determine the effect of ACh on the duration of the action potential, as the duration was largely influenced by the beat-rate. Mechanical and electrical activities of most cultured ventricular cells in embryos younger than 10 days were not affected by ACh. However, in a very few cases, ACh (10^{-5} g/ml) slowly depressed and finally stopped the rhythmical movement of cultured ventricular cells. The inhibitory effect of ACh was associated with hyperpolarization and shortening of the action potential duration. Thereafter, notched

TABLE 2. Comparison of the present experimental data with those of other investigators.

Source	Present experiment	Lehmkuhl <i>et al.</i>	Grill <i>et al.</i>	Fänge <i>et al.</i>
Preparation	Cultured auricle and ventricle	Cultured ventricle	Cultured ventricle	Cultured auricle and ventricle
Temperature	31°C	35°C	37°C	21°C
Age of embryo days	5-10	6-8	4	4-10
Resting potential (mV)	49.9 (44-60)	59.0 (40-84)	41.2	43.3
Action potential magnitude (mV)	72.0 (54-90)	71.2 (40-108)	57.0	59.8
Action potential duration (msec)	100-360	150-500	194	400 (200-1000)
Plateau	some	some	yes	usually
Rate of beating (impulses/min)	1-20	30-130	145	30
Positive after-potential (mV)	0-10 (estimated)	0-10	8 (estimated)	8-10
Oscillation	+	+	+	+
		Step in rising phase	Step in rising phase	

potential and disappearance of action potentials were observed (Fig. 5). Sometimes, the inhibitory effect of ACh on both auricle and ventricle of chick embryos was followed by a slight stimulating effect after prolonged exposure to the drug. During the stimulating period after ACh administration, hyperpolarization and shortening of the action potential were still observed.

DISCUSSION

Considering the difference of experimental conditions, the characteristics of electrical activity of cultured cells used in the present experiment were very similar to those previously observed by other investigators. Table 2 presents a comparison of the present data with those previously observed in cultured cells.

Fänge *et al.* (1) demonstrated that vagal innervation may increase the sensitivity of the cultured embryonic heart to ACh in the chick. This hypothesis was based on the finding that the mechanical activity of atrium was inhibited to a considerable extent by ACh, whereas the ventricle of embryos younger than 9 days were quite insensitive to the drug. The present experiment, which the effect of ACh on the intracellular membrane potential was dealt with, also supports their hypothesis. Namely, ACh produced hyperpolarization, shortening of the duration of the action potential and finally disappearance of action potential in the auricle of embryos older than 5 to 6 days, while the ventricle of embryos younger than 10 days were unreactive to ACh except in very few cases (Fig. 5).

Regarding the effect of ACh on the transmembrane potential of myocardium, many investigators have demonstrated its hyperpolarizing action associated with an acceleration of repolarization (5, 11). West (12) has also observed the depressive action of ACh on the rate of rise of the prepotential. They considered that these effect of ACh may be attributed to a specific increase of K^+ permeability of the cell membrane. Consequently, ACh may allow the resting potential to come near the K^+ equivalent potential of the cell membrane. The present experiment demonstrated that ACh produced a hyperpolarization, shortening of action potential duration and decreased rate of rise of prepotential in chicks older than several days. On the contrary, Fingel *et al.* (10) have observed a decrease of resting potential and a reduction of the amplitude of action potential associated with incomplete depolarization in intact chick hearts. As Hsu (13) has suggested, the chick embryonic hearts may have a wide-range of individual variation in sensitivity to ACh.

It was interesting that the inhibition of mechanical and electrical activity of the intact and cultured auricular cells produced by ACh, was sometimes followed by an increased frequency of contraction associated with hyperpolarization and shortening of action potential duration, because a biphasic response of the heart to ACh has been demonstrated by several authors (14–19). Armstrong (20) has reported that ACh acts via nerve elements, as the drug did not affect the non-innervated heart. However, it is likely that ACh acts directly on cardiac cells, because cultured cells, which have no nerve terminals, were affected by ACh. Therefore, the formation of receptors in cell membranes

is a more important factor for the response to ACh than the presence or absence of nerve terminals.

SUMMARY

ACh produced hyperpolarization, shortening of action potential duration and decreased rate of rise of prepotentials in both intact and cultured auricular cells of embryos older than several days, while the younger cells were usually insensitive to ACh. On the other hand, both intact and cultured ventricular cells were generally insensitive to ACh. However, ACh reacts very rarely on the ventricular cells of embryos older than 10 days, producing hyperpolarization and shortening of the duration of action potential, then finally disappearance of action potential associated with the cessation of spontaneous contraction.

The inhibitory effect of ACh was sometimes followed by an excitatory effect. During the later effect of ACh, hyperpolarization and shortening of action potentials were still observed.

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