

## Changes in Membrane Potentials and Mechanical Responses of Guinea Pig Atria During Anaphylaxis *in Vitro*\*1

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The anaphylactic reaction in the isolated atria of sensitized guinea pigs is principally to cause acceleration of cardiac activities as already reported. The present attempt was made to observe changes in transmembrane action potentials (AP) and mechanical responses of the atria during the anaphylaxis. Four types of changes in AP were found in spontaneously beating atria that is prolongation of AP at all points of the repolarization, prolongation of the duration with proceeding of repolarization, shortening of the duration at first half, and no change at all in AP pattern. However the change in electrically driven left atria was only a parallel prolongation of the duration to the control in repolarization phase. On the other hand, an increase in the rate shortend the duration of AP in the normal preparation electrically driven. The essential change of the contour of AP in the anaphylactic reaction is the prolongation of AP in repolarization phase. Histamine induced a similar reaction in electrical and mechanical responses to the anaphylactic reaction.

It has been reported by Y. NAKAZAWA<sup>5-8,9)</sup> and K. GREEFF<sup>1)</sup> that the anaphylactic reaction in the auricle of guinea pigs previously sensitized was acceleration of the cardiac movement, *i.e.* positive inotropic and chronotropic response. While a test of tissue anaphylaxis has been usually carried out with ileal and uterine segments, the anaphylactic reaction in the heart preparations occurs more dynamically and has more calculative factors than that with smooth muscle organs whose reaction in only muscle contraction. Both the cardiac muscle and innervation differ from smooth muscle organs, and have the considerable physiological specificity, so it is significant to analyse pharmacodynamically the mechanism of anaphylaxis provoked in the tissue.

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On the other hand, intracellular microelectrode technic brought by LING & GERALD was applied for cardiac muscle cell by HOFFMAN<sup>2)</sup> and others and electrophysiology of the heart has been greatly progressed. Transmembrane action potentials of single cell by means of an intracellular microelectrode provides an accurate and sensitive index of the electrical changes associated with activities of excitable tissue, and those have been applied for the research of pharmacodynamic actions of various drugs.

S. KATSH and J. M. MARSHALL<sup>6)</sup> reported a study about electrical and mechanical responses of uterine smooth muscle during anaphylaxis *in vitro*, in which a good correlation between membrane potential, rate of spike discharge and fluctuation in tension was observed. However, no report of transmembrane action potentials of the cardiac muscle cell following the anaphylactic provocation was uncovered. As the provocation is carried out after the auricle preparation used for the experiment were repeatedly washed by nutrient fluid, a site in which antigen interacts with the antibody series must be the cell.

There is a possibility that the interaction of antigen and plasma membrane might be as a trigger of dynamic phenomena of the heart anaphylaxis. Therefore, it is of our great interest of what electrical changes occurs at the transmembrane potentials and mechanical response of the cardiac muscle, and what correlation exists between the potentials and the tension of the muscle.

## MATERIALS AND METHODS

About one hundred male and female guinea pigs weighing 180 to 300g were used in the experiment. Antigenic sensitization of the guinea pig was passively carried out by intravenous injection of anti-egg-albumin rabbit serum, having  $1 \times 128$  to  $1 \times 256$  antibody title (0.3ml per 100g body weight), 24 hours prior to the experiment. The method of the sensitization are described in details elsewhere. The atrium was removed immediately from exsanguinated guinea pigs and placed in Ringer solution (NaCl, 0.9%; KCl, 0.042%; CaCl<sub>2</sub>, 0.024%; glucose, 0.1%; NaHCO<sub>3</sub>, 0.05%). And a right auricle of the atrium was pinned horizontally on the cork block in the bath and left one was joined to a unbonded strain gage transducer (Shinkoh UL-10-120) for recording the contractile tension of the atrium in connection with strain amplifier (Shinkoh DS6-P). This is sensitive enough to record a tension as small as 10mg and to respond lineally up to 130 c/s.

The bath was made by acryl resin and specially designed for the experiment in the light of the method of MATSUDA *et al*<sup>7)</sup>, total fluid content is 50 ml and bath temperature is 32°C. The nutrient fluid was actively circulated through a stream of bubbles of oxygen containing carbon dioxide at 5% level.

The ultramicroelectrode was made from a capillary tube 1 to 1.5 mm in diameter prepared from Telex glass (Toshiba) with aid of Andrews type puller and filled with 3 M KCl solution by means of ethanol-water-KCl replacements. The microelectrode of 15 to 55 M $\Omega$  was chosen for the experiment, and used tip of this as a suspension electrode.

The transmembrane action potential was led from a location near by top of the pinned right auricle. And this has been amplified by means of a conventional cathode follower input amplifier (Nihon Kodenshi MZ 3 A) and D.C. amplifier for observation of cathode ray oscilloscope and ink writing record. A dual beam oscilloscope was used to observe transmembrane potentials and contractile tension simultaneously and the tension beam was modulated at 5 or 10 msec interval for time orientation. The plateau of the tension originally indicates zero potential.

## RESULTS

### 1. Normal contour of transmembrane action potentials of proper atrial fibers of the guinea pig

Compared the contour of transmembrane action potentials of the proper atrial fibers with its mechanical activities, contraction of the fibers appeared at a gentle sloping point of the time course of spike potential, this is correspond to beginning of 'Phase 2'. Critical period of the tension in contraction was equal to one third or half in repolarization phase and muscle fibers reached relaxation state when the potential returned to the resting one (Fig. 1). Intracellular resting and action potentials of spontaneously beating atria of 70 cases of guinea pigs which survived in 32°C Ringer were found most frequently in -85 mV for diastolic and 104 mV for height of action potentials, so the overshoot was 19 mV. Total duration of the action potentials were varied from 80 msec to 150 msec. These values practically accord with higher bound of mammalian atria with reported by several investigators.

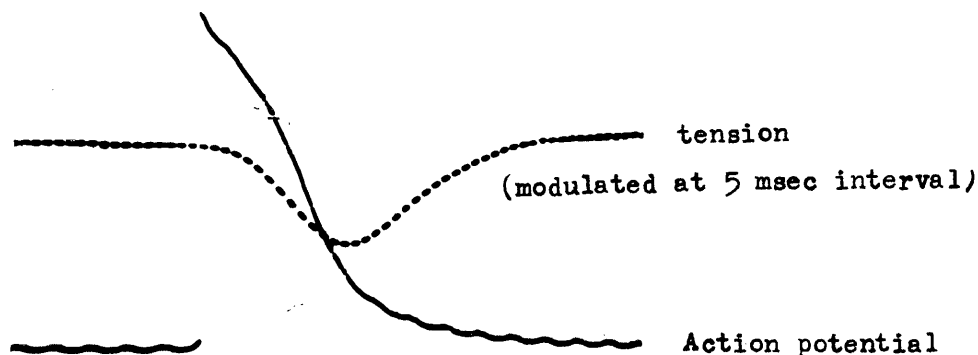


Fig. 1 Transmembrane action potential pattern and tension curve of the guinea pig atrium.

### 2. Modification of action potential pattern by anaphylactic reaction

At 20 to 50 sec after the auricle preparation was exposed to antigen (2mg% in the bath solution) an increase in the tension and the rate occurs, which continued 10 to 20 min and then returned to a state before the exposure. No reaction was observed in non-sensitized atria and nothing or very weak in the preparations once provoked by antigen exposure, that is desensitization.

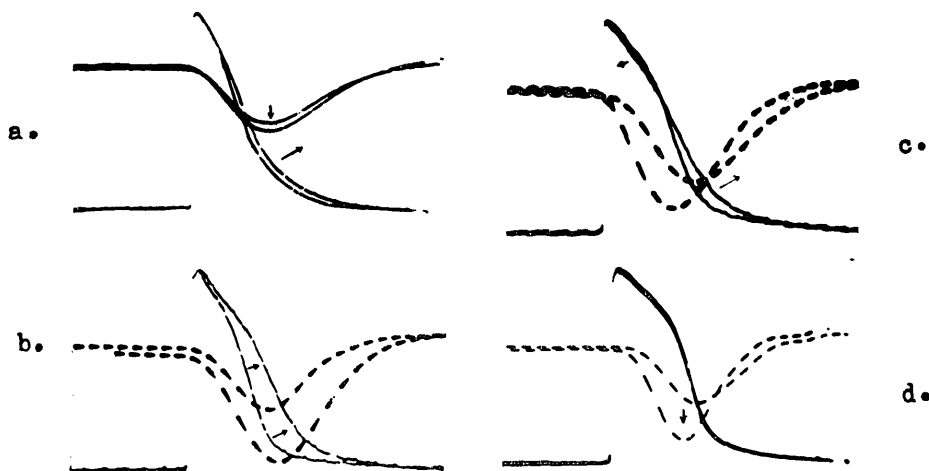


Fig. 2 Changes in transmembrane action potential during anaphylaxis. Arrows indicate direction of the change.

Change in transmembrane action potentials in spontaneously beating atria during anaphylaxis could be divided into four types of group as shown in Fig. 2, a) slight prolongation of action potential (APD) at almost all points of the repolarization phase (type a), b) prolonging APD with proceeding of repolarization (type b), c) prolongation of APD in the latter half of repolarization phase while it in the first half was shortened (type c), d) no change at all in AP pattern despite there were marked response in the mechanical activities (type d). However, in the electrically driven (2c/s) left atria only a parallel prolongation of the duration to the control in repolarization phase was constantly observed overall, this is likely to type a) of the change in spontaneously beating atria. Illustration in Fig.3 shows a relationship existed between tension change and duration change in 50% and 90% repolarized state.

### 3. Change of rate and contour of action potential during the heart anaphylaxis

A difference in the results between the spontaneously beating atria and the electrically driven left atria suggests a possibility that an increase the rate affects the contour of the action potential. In order to search a relation between the rate of frequency and contour of

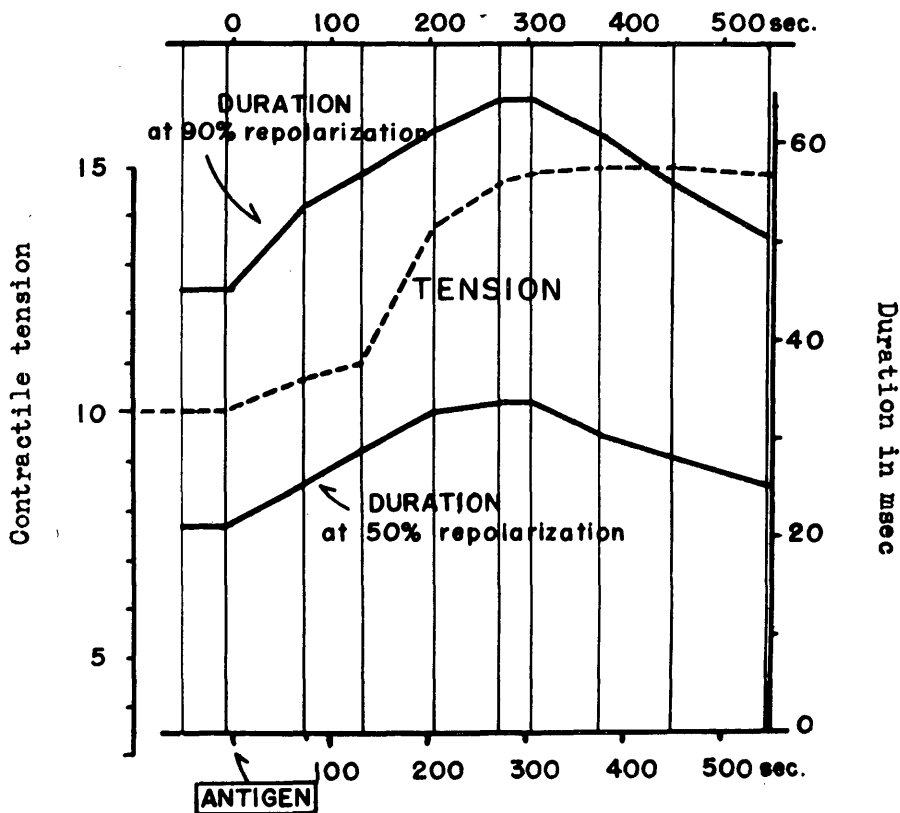


Fig. 3 Duration-Tension Curve after exposed to antigen.

action potentials, the left auricle not sensitized was electrically driven at various frequencies. When the impulse was changed from 1c/s to 3c/s the duration of action potential was shortened at almost all points of repolarization phase with increasing the rate. But it is observed in a small number of the cases that the shortening is remarkable in the start of repolarization phase. Such a change in the contour of action potential appeared at the same course of time required until a tension of the contraction reached the constant values after changing the rate.

4. Influences of some chemicals relating with anaphylaxis on action potential and mechanical responses.

a. Histamine hydrochloride (*Hist*)

When *Hist* ( $1\mu\text{g/ml}$ ) applied to the normal spontaneously beating atria, a similar reaction to the anaphylaxis occurred. The tension of contraction increased and the duration of repolarization of action potentials prolonged. But no change was observed in half cases.

b. Serotonin creatinine sulfate (*5HT*) and adrenaline hydrochloride (*Adr*)

Although *5HT* ( $2\mu\text{g/ml}$ ) and *Adr* ( $0.1\mu\text{g/ml}$ ) induced almost the

same effect as histamine on mechanical response an increase of the tension of contraction, no change in the contour of action potential was observed in any cases.

c. *Acetylcholine chloride (ACh)*

ACh (0.01 $\mu$ g/ml) reduced gradually contractile tension and the rate. The amplitude decreased as small as 20% before the drug application while the duration of action potential was markedly shortend.

5. *Influences of various drugs*

After the auricle preparations were treated by various drugs as follow: benzalkonium chloride (40 $\mu$ g/ml), methylen blue (5 $\mu$ g/ml), protamine hydrochloride (40 $\mu$ g/ml), heparine sodium (20U/ml), toluidine blue (40 $\mu$ g/ml), chicago blue (20 $\mu$ g/ml), nicotine tartrate (100 $\mu$ g/ml) the tissue was exposed to antigen. Some of these drugs might be possible to affect anaphylactic reactions, some of them release biogen amines. However the anaphylactic reaction in the preparation was provoked as well as in never treated preparation.

The contour of action potential was never changeable by the treatment of these drugs.

## DISCUSSION

The heart is a suitable organ for analysing pharamacodynamically the tissue anaphylaxis because the reaction is dynamic and the cardiac muscle has an electrophysiological constancy. Furthermore, as the cardiac muscle makes functionally a syncytium as a whole, action in all fibers synchronized each other. Therefore, the heart is also an excellent material to search the correlation between physiological function and transmembrane potential because the standard value and the contour of the action potential is constant, so that is usefull for quantitative analysis of the electrical change.

Katsh and Marshall<sup>19</sup> found in the experiments with ileal and uterine smooth muscle *in vitro* that the frequency of the spike discharge provoked by antigen exposure increased and the duration of spike discharge prolonged. As well known, the analysis of the contour of action potentials in smooth muscle is very difficult because the standard value of transmembrane potentials of smooth muscle is hardly determined.

When the sensitized auricle preparation with automaticity was exposed to antigen there appeared an increase in the contractility and the rate. However, it was very difficult to find out the certain effect on transmembrane potentials, *i. e.* there are four types of group (type a, b, c and d) in the contour of repolarization phase. On the other hand, it surely observed in the left auricle electrically driven that the change of action potentials during the anaphylactic reaction

was always prolongation of the duration at all points of repolarization phase.

HOLLANDER<sup>4)</sup>, HOFFMAN<sup>3)</sup> and TRAUTWEIN<sup>10)</sup> described that the accelerating effect in the heart rate influenced on the contour of action potentials from their experimental results in the hearts of the rat, the dog and the cat. Thus, the same influences might be given by the accelerating effect in the anaphylactic reaction. It is clear from our experiments that an increase of frequency in the left auricle electrically driven causes the shortening of the duration the action potential: the one appears at the all points of repolarization phase, and the other specially at the start.

When we analogize a relationship between the heart rate and the change in the contour of action potentials with the cases of the anaphylactic reaction, it is considered that the contour in itself in the anaphylactic reaction is modifies by an increase in the heart rate as a physiological variable. Thus, the essential change of the contour of action potentials in the anaphylactic reaction is the prolongation of duration of repolarization phase.

Illustration of Fig.3 clearly shown that the prolongation of APD is a transient change while the change in contractile tension lasts longer. The reason of the difference still remain in obscure whether the two phenomena are originally unconcerned each other, or transient change of APD to be a cause of increase of contractile tension, or anaphylactic manifestation itself takes different time course in each cell layers. But it is clear that the increasing period of contractile tension is in prolonging period of duration of action potentials.

Of some chemicals accepted as mediators to cause several symptoms in the anaphylaxis histamine played a similar reaction in action potentials and mechanical response to the anaphylactic reaction, serotonin and adrenaline induced no change in transmembrane potentials, and acetylcholine decreased markedly the rate, the contractile tension and the duration of action potentials on the contrary of the anaphylactic reaction. On the other hand no change was observed by application of various drugs such as benzalkonium chloride, protamine, heparine, methylen blue, tolidine blue and nicotine respectively having possibility to modify the anaphylaxis.

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