The mechanism of the anaphylactic contraction of smooth muscle have ever been discussed by several investigators. But the fundamental question have never been solved clearly; whether the anaphylactic contraction of smooth muscle can be produced by the direct effect of antigen-antibody reaction on the cell membrane and/or on the contractile elements in the cell or not? This is the most interesting and without dealing with it, anaphylactic contraction is hardly said to be completely studied. Some problems have recently been brought forward in the “chemical mediator theory”. Many substances are advocated as “chemical mediators”, acetylcholine, histamine, serotonin, bradykinin, slow reacting substance in anaphylaxis, plasmine, ATP and so on (1, 2). There is, however, no established view as to what is essential in anaphylaxis, and whether it is a single or plural. The chemical mediator theory seems to be supported only the fact that in anaphylaxis, endogenous chemical substances are increased in blood over the normal level, and that the symptom in anaphylaxis is resemble with that introduced by administration of such a substance.

On the other hand, the observations in the electrophysiological study have recently given me some important informations about a relation between excitability of cytoplasmic membrane and muscular contraction (3-8). In 1958, Evans et al. (9) reported that acetylcholine, histamine and serotonin could induce contraction of the smooth muscle even which cytoplasmic excitability was abolished by the “isotonic potassium-Ringer”. The present work was undertaken to elucidate this point in anaphylactic contraction in smooth muscle and for this purpose, changes in membrane potential and tension of the muscle were recorded during antigen-antibody reaction in the medium added potassium ion excessively.

MATERIALS AND METHODS

Experimental animals: Guinea-pigs, Hartley strain, weighing 350–400 g were kept in the standard animal stock room (22–24°C of the temperature and 60% of moisture) and were fed on the standard diet (GC-4, Oriental Yeast Co., Ltd., Tokyo) for 10 days or more. Only healthy males which showed body weight gain were used in the following experiments. Albino rabbits, weighing about 2 kg, were used in similar condition.

Sensitization: Rabbits were subcutaneously injected with each 2 ml of a 10% oval-
bumin saline solution twice a week, and intracutaneously with 0.1 ml on the foot pad once weekly for 4 consecutive weeks. At 2 weeks after the final injection, antibody titration was started with the ring-method, and when precipitation titer rose over 2,048 folds, the whole blood was drawn to be used as anti-ovalbumin serum (rabbit). The guinea-pigs were intravenously injected in an approximate dose of 4 ml/kg of the anti-ovalbumin serum and after 24 hours they were used as passively sensitized animals.

**Preparation of the taenia coli sample and recording:** Extirpation of taenia coli from guinea-pig was carried out by the method reported by Bülb-ring (10). The separated length was 20–30 mm in the sucrose-gap method, and 10–15 mm in the Magnus’ apparatus and the intracellular electrode method. The sucrose-gap method was based on the report of Stämpfli (11), who applied it for the estimation of membrane potential of the frog’s myelinated nerve fiber by the external electrodes. Taenia coli was dissected from the underlying tissue, and was extended to the length in situ. After mounting it in the apparatus, membrane potential, action potential and contractile tension were determined simultaneously. For the latter, the mechano-electronic transducer (RCA-5734) was applied, and all of the results were recorded on the ink-writing oscillograph. Through the tubes of the appliance, Ringer’s solution was continuously perfused at the rate of 7 ml/min, and in one tube it was kept at 33°C, and in the other at 18–20°C. In the intracellular electrode method, the taenia coli extirpated and mounted in a 7 ml water bath, were extended to the length in situ by means of a hand screw attached to the side of the bath; a rubber plate was placed beneath the sample, and microelectrodes were inserted, while 33°C Ringer’s solution was made to flow in the water bath at the rate of 7 ml/min. The microelectrodes were made of Pyrex glass tube 2 mm in external diameter and filled with 3 M-KCl; its resistance ranged 30–70 MΩ. The membrane potential was recorded on film.

The modification of Locke’s solution with the following composition was used; NaCl 154 mM, KCl 5.0 mM, CaCl₂ 1.8 mM, NaHCO₃ 3.6 mM, dextrose 5 mM. The increase of concentration of potassium ion in the medium above described was always compensated by the decrease of sodium ion so as to keep the physiological osmotic pressure (high potassium-Ringer).

Experiments with Magnus’ tube were performed at 33°C on the muscle which was depolarized by pretreatment with the medium containing an excess potassium ion for 5 minutes, and recorded on smoked paper.

**Immunological procedures:** The qualitative precipitin reaction was observed on agar plates by a modification of the Ouchterlony’s method. The plate was prepared by adding agar each 2% to 0.9% NaCl, 20 mM, 40 mM, 60 mM, 100 mM and 120 mM KCl. In the quantitative precipitin reaction, preliminary, the equivalence zone was determined by the two-drop method, and on the basis of this antigen-antibody ratio, the same volume of the egg albumin and the anti-egg albumin rabbit serum were mixed; after allowing to stand at 3–4°C for 2 days, the precipitate was washed well with saline solution, and after through removal of water, nitrogen content of the precipitate was estimated with the micro-Kjeldahl method.
Drugs used at present experiments are as followed: acetylcholine hydrochloride (ACh), histamine dihydrochloride (hist.), bradykinin acetate, BaCl₂ and egg albumin.

RESULTS

1) Anaphylactic contraction of taenia coli observed by the Magnus’ apparatus in the same way as Schultz-Dale reaction of intestinal preparation, was found to appear after a certain incubation period. And when the same amount of antigen was repeatedly administered, the muscle contraction was markedly diminished and incubation was prolonged. As a matter of course, in taenia coli from non-sensitized animals, the response was invisible after ovalbumin administration (Fig. 1). As shown in the same figure, the contraction provoked by 1% of ovalbumin was comparable with that by 10⁻⁸ g/ml of ACh, by 10⁻⁸ g/ml of hist., by 10⁻⁷ g/ml of bradykinin and 5 x 10⁻⁴ g/ml of BaCl₂.

Fig. 1. Anaphylactic contraction in ileum and taenia coli from guinea-pig sensitized passively (Magnus’ method). ACh: 10⁻⁵ g/ml of acetylcholine, Hist: 10⁻⁷ g/ml of histamine, Bradykinin: 10⁻⁷ g/ml of bradykinin, Ba: 5 x 10⁻⁴ g/ml of BaCl₂, EA: egg albumin.

Fig. 2. Spontaneous spike activity and tension in guinea-pig’s taenia coli (sucrose-gap method).
Fig. 3. Effect of egg albumin on taenia coli prepared from non-sensitized guinea-pigs (sucrose-gap method, Magnus' method). ACh: $10^{-6}$ g/ml of acetylcholine, EA: egg albumin.

Fig. 4. Anaphylactic contraction in taenia coli from passively sensitized guinea-pigs (sucrose-gap method and Magnus' method). For description see text. ACh: $10^{-6}$ g/ml of acetylcholine, EA: egg albumin.
2) Membrane potential of taenia coli from the nontreated guinea-pig as obtained by the sucrose-gap method was represented in Fig. 2. Herein can be seen periodical spike discharges, and concurrently with them the increases in tension. And with the disappearance of the spikes, the tension lowers abruptly. Moreover it can be observed that one increase in tension corresponds to one spike. When an 1% ovalbumin was added into medium, no effect was visible in membrane potential, spike frequency and tension (Fig. 3).

Effect of the ovalbumin on the taenia coli from passively sensitized guinea-pigs are shown in Fig. 4 (a) and (b). Fig. 4 (a) represents a sample, which did not show spikes before the adding the antigen, but which discharged them after gradual fall of membrane potential following the challenge; and in correspondence with this a transient increase of tension developed, which persisted nearly for 3 minutes. The lower part of Fig. 4 (a) represents changes after the second challenge. Here spike size is increased over that in the above curve, but comparable tension is not seen, and fall of membrane potential is smaller. Moreover, spike frequency is rather diminished in comparison with that before addition of antigen. In a sample having spike activity (Fig. 4b), antigen induced decrease in membrane potential, increase in spike frequency and spike size, and accompanying tonic contraction. And spike continued to be discharged nearly for 3 minutes. In all the cases, the fall of membrane potential occurred gradually after addition of antigen. The curves in the right lower parts of Fig. 4 (a) and (b) represent reactions, in the Magnus' tube, of the taenia coli which was obtained from the same animals as that used in the sucrose-gap method.

Effects of ACh, hist., bradykinin and barium ion as observed by the sucrose-gap method are shown in Fig. 5. Electro-physiological activities of ACh, hist. and bradykinin were manifested in decrease in membrane potential, increase in spike frequency and spike size and in corresponding tonic contraction immediately after their applications. Following

![Graphical representation of electrophysiological activities](image-url)
Fig. 6. Contractive action of antigen and drugs in medium including various concentrations of potassium (Magnus' method). For description see text. ACh: $10^{-6}$ g/ml of acetylcholine, Hist: $10^{-4}$ g/ml of histamine, Bradykinin: $10^{-7}$ g/ml of bradykinin, Ba: $5 \times 10^{-4}$ g/ml of BaCl₂, EA: egg albumin.
barium ion, however, membrane potential lowered scarcely, and continuous large spikes were followed by spikes, small in size but increased in frequency. And tension changed in proportion with frequency of discharge.

3) Fig. 6 show the result from Magnus' test by using the taenia coli for the guinea-pigs sensitized passively with ovalbumin, which was made to response with ACh, hist., bradykinin, barium ion and ovalbumin in the medium including various concentrations of potassium ion. When an 1% ovalbumin was applied on taenia coli in 10 mm potassium-Ringer, the anaphylactic contraction expected was found to be invisible, while ACh, hist., bradykinin and barium ion provoked the contraction (Fig. 6a). In the 20 mm potassium-Ringer, bradykinin failed to induce contraction, but ACh, hist. and barium ion induced it (Fig. 6b). In the 40 mm potassium-Ringer, the contractility was abolished against hist.,

| Conc. of K+ (mm) | BaCl2 | ACh. | Hist. | Bradykinin | Antigen |
|-----------------|-------|------|-------|------------|---------|
| 154             | +     | -    | -     | -          | -       |
| 60              | +     | +    | -     | -          | -       |
| 40              | +     | +    | -     | -          | -       |
| 30              | +     | +    | -     | -          | -       |
| 20              | +     | +    | -     | -          | -       |
| 10              | +     | +    | -     | +          | -       |
| 5               | +     | +    | -     | -          | +       |

but it remained against ACh and barium ion (Fig. 6d). In the 60 mm potassium-Ringer, ACh still exerted, but treatment with higher concentrated potassium-Ringer, finally abolished this effect (Fig. 6e, f). And barium ion still induced contraction in the muscle placed in 154 mm potassium-Ringer, in which NaCl was perfectly replaced with the same mol KCl (Fig. 6 f). These results are summarized in Table 1.

Membrane potentials of taenia coli, subjected to depolarizing influence of the high potassium-Ringer at various concentrations of potassium ion were determined by the intracellular electrodes. It was found that the potential decreased with increase

![Fig. 7. Relation between logarithm of external potassium concentration and membrane potential in guinea-pig’s taenia coli (intracellular electrode method).](image-url)
in the potassium ion concentration. As shown in Fig. 7, the potential was 53 mV in Ringer's solution, but it was decreased to 34 mV as the concentration of potassium ion was increased from 5 mM to 154 mM.

On the other hand, the muscle, which was extirpated from the guinea-pig sensitized passively and which did not response to ovalbumin in the 10 mM potassium-Ringer, was washed with the Ringer's solution to restore the normal response to ACh, hist., bradykinin and so on, but anaphylactic contraction was invisible in these washed sample after reapplication of antigen (Fig. 8 a). Furthermore, the response to ovalbumin could be observed in the muscle washed with Ringer's solution after pretreatment with 50 mM potassium-Ringer for 5 minutes (Fig. 8 b). The same results were obtained in the experiment by using 2% of ovalbumin solution, which was as strong as 10⁻⁶ g/ml of bradykinin in contracting activity, as shown Fig. 8(c).

**Fig. 8.** Effect of potassium ion on anaphylactic contraction (Magnus' method). For description see text. ACh: 10⁻⁶ g/ml of acetylcholine, EA: egg albumin.
4) Fig. 9 is photographs of the Ouchterlony's plate test carried out in agar containing various concentrations of potassium ion. Precipitations on agar plates containing 20 mm, 40 mm, 60 mm, 100 mm and 120 mm potassium ion are not different from that on the control consisted of physiological saline, all exhibiting two thin streaks. And the results from the quantitative precipitin test, given in Table 2, are showing that no influences of potassium ion concentrations in the medium can be found on the precipitate for antigen-antibody reaction.

**DISCUSSION**

It is widely known that the consecutive recording of the membrane potential is very difficult in smooth muscle by the intracellular electrodes, which is used for nerve and skeletal muscle, because the muscle fiber is generally fine, it being only 20-100 μ in taenia coli of the guinea-pig. In Stämpfli (11) found that absolute value of membrane potential could be measurable with extracellular electrodes, if potential fall at an injured or depolarised part during current flow was compensated, or if current flow was stopped by increasing the longitudinal resistance for the outer medium. The sucrose-gap method, which

| Dilution of antigen | Total N* in the precipitate (mg 20 ml) | pH of dilution |
|---------------------|--------------------------------------|---------------|
| 0.9% NaCl           | 4.51                                 | 6.81          |
| 10 mm KCl           | 4.76                                 | 6.60          |
| 30 mm KCl           | 4.76                                 | 6.65          |
| 50 mm KCl           | 4.78                                 | 6.73          |
| 100 mm KCl          | 4.81                                 | 6.89          |

N*: total nitrogen measured by micro-Kjeldahl method.
was originated by him and was applied for the smooth muscle by Burnstock et al. (12, 13), was considered to be suitable to study on the membrane potential and the spike activity of smooth muscle sample for prolonged period (Bülbring et al. (14)). Without an electrical stimulation, however, spikes of varying sizes which are obtained by this method, are dependent on the degree of intercellular potential interference. Moreover, the values of the resting potential must be considered to be obtainable in the high potassium-Ringer. Although Burnstock et al. (12) reported that resting potential was 60 mV with K₂SO₄ and 24 mV with KCl, it should be memorable that there was no agreement among the results by many investigators using the intracellular electrodes; 60 mV by Bülbring (10, 15), 51.5 mV by Holman (16), 55 mV by Kuriyama (17) and 53 mV by the present work. One of the factors, resulting this discrepancy, is suspected to ascribe that the resting potential in smooth muscle dose not consist of potential diffusion depending on intra- and extracellular concentration gradient only of potassium ion, but also other ions. According to Goodford et al. (18), the intracellular concentration of potassium ion ranges 98-119 mm and computation from this value gives 76-88 mV as resting potential, while membrane potential was never found to be 0 mV even in a 154 mm potassium-Ringer in the present experiment in which was found 60 mV or the less of resting potential in a normal Ringer's solution. Moreover, no relation was observed between membrane potential and concentration of potassium ion in a range below 40 mm of potassium ion. This finding is indirectly supported by Kuriyama (17, 19) who emphasizes roles of Na⁺ and Cl⁻ on smooth muscle membrane in taenia coli.

Bülbring et al. (20, 21), Burnstock (14) and Schild (22) reported that fall of membrane potential and increase in spike frequency produced by ACh or hist. might be attributed to increase in membrane permeability to ion. Likewise in the present experiment, action of ACh and hist. were very similar to each other and they resembled also action of bradykinin and antigen-antibody reaction. Furthermore, during antigen-antibody reaction, tension of muscle increase in association with excitatory phenomena of membrane. Similar results have recently been described by Katsh et al. (23) and Alonso-deFlorida et al. (24). On these grounds, it may be assumed that also anaphylactic contraction of taenia coli involves the increase in permeability to ion.

It was interest in the present experiment that contractions expected by application of ACh, hist., bradykinin and antigen were varying dependent on concentration of potassium ion in outer medium and especially, anaphylactic contraction seemed to be most sensitive for the potassium inhibition. But the contraction was easily produced by barium ion in 154 mm potassium-Ringer. This result is supported by Fujita (25) and Yukisada (26). Evans et al. (9) reported that ACh, hist. and serotonin induced contraction in the rat's uterus and guinea-pig's ileum still after depolarization in a high potassium-Ringer. This disagreement is considered to due to difference of the concentration of drugs tested between their and my experiment. On the other hand, it was evidenced by the Ouchterlony's test and the quantitative precipitin test that precipitation of ovalbumin against anti-ovalbumin rabbit serum was unaffected with concentration of potassium ion in the medium,
this result agreeing with that of Kleinschmidt et al. (27). Furthermore, the fact that anaphylactic contraction was provoked as strong as ever in the muscle which was, after pre-treatment with 50 mm potassium-Ringer, replaced to the normal Ringer's solution, could exclude the assumption that anaphylactic contraction was abolished because the antibody might be liberated from the muscle tissue by the procedure above mentioned. Consequently, it seems unreasonable that the anaphylactic contraction in the present experiment may have been produced by the effect of some chemical agents such as ACh, hist. or bradykinin, since anaphylactic response is invisible in 10 mm potassium-Ringer, in which the muscle is found responsive to these drugs. In either event, it is concluded from the present results that the presence of the normal membrane potential of muscle cell is essential to provocation of anaphylactic contraction and that anaphylactic response may be directly produced by antigen-antibody combination on the cell membrane.

SUMMARY

With the object of elucidating the mechanism of anaphylactic contraction, this was electrophysiologically compared with contractions by acetylcholine, histamine, bradykinin and barium ion by using taenia coli from sensitized guinea-pigs.

1. The electric phenomenon during anaphylaxis was similar to that provoked with acetylcholine, histamine or bradykinin.
2. In the muscle, depolarized by treatment with high potassium-Ringer, anaphylactic contraction was clearly different from contractions provoked with acetylcholine, histamine, bradykinin and barium ion.
3. In the muscle placed in 10 mm potassium-Ringer, anaphylactic contraction was not observed.
4. It was found that anaphylactic contraction was not provoked by direct action on the contractile elements in the muscle cell.
5. It is estimated that anaphylactic contraction is accompanied with increase of permeability of cytoplasmic membrane to ions.
6. It was concluded that the presence of the normal membrane potential is essential for anaphylactic contraction in guinea-pig's taenia coli. And it is supposed to deny a possibility that one or some substances among them play a role as chemical mediator in anaphylactic contraction.

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