# CHANGES IN THE MEMBRANE POTENTIAL AND TONE OF SMOOTH MUSCLE DURING ALLERGIC REACTIONS IN THE ABSENCE OF MAST CELLS

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The view is widely held that injury to the mast cells and their release of biologically active substances, subsequently acting on smooth-muscle structures, are a fundamental link in the development of the allergic reactions of smooth-muscle organs [10, 16]. Meanwhile, many investigations have yielded weighty evidence in support of the view that destruction of the mast cells and the release of biologically active substances from them are not essential for the development of the allergic reactions of smooth muscles [1, 2, 14]. In this connection, it is interesting to reproduce an allergic reaction in a smooth muscle containing no mast cells. A suitable object of such an experiment may be the longitudinal muscle of the large intestine of guinea pigs—the taenia coli, because mast cells in the alimentary tract are known to be localized in the submucosa, and no mast cells are present in the smooth muscle itself [17].

In the present investigation an anaphylactic reaction and a reaction to a soluble antigen—antibody complex, prepared in excess of antigen, were reproduced in the isolated smooth muscle of the guinea pig.

## EXPERIMENTAL METHOD

Experiments were carried out on male guinea pigs weighing 250-300 g. The animals were sensitized with three injections of crystalline ovalbumin, each consisting of 10 mg mixed with Freund's complete adjuvant, subcutaneously at intervals of 24 h. The experiments were carried out 4 weeks after the last sensitizing injection on segments of the taenia coli 1.5-2 cm long, separated by blunt dissection from the underlying layer of circular muscles and placed in a special chamber to allow simultaneous recording of the changes in the membrane potential by means of a sucrose bridge [7], and the tone of the smooth muscle. The nutrient fluid of Krebs [8], and a 10% solution of sucrose in bidistilled water were used in the experiments. The soluble antigen—antibody complex, prepared in the presence of a ten-fold excess of antigen, was obtained by mixing the appropriate quantities of ovalbumin and rabbit immune serum to ovalbumin.* The substances for testing, dissolved in Kreb's solution, were injected into the flow of the perfusion fluid bathing the tested segment of muscle. To determine the localization of the mast cells, the segments of large intestine were fixed for 4 days in an 8% solution of formaldehyde in 50% ethyl alcohol. The material was embedded in paraffin wax, and sections cut to a thickness of 10-30$\mu$ were stained in 1% toluidine.

## EXPERIMENTAL RESULTS AND DISCUSSION

It has been claimed that an increase in the tone of smooth muscle of Botzler's unitary type, during exposure to various pharmacological agents, is associated with a decrease in the membrane potential and an increase in the frequency or the appearance de novo of spike discharge [9]. It is interesting to examine the changes in the membrane potential and tone of the sensitized smooth muscle during the action of a specific antigen. The experiments were carried out in such a way that ovalbumin was added to the solution bathing the taenia coli not later than 40-50 min after its removal from the animal. This was because keeping the smooth-muscle organ in the nutrient fluid at 30-37° for a long time could bring about its partial desensitization [5, 15], possibly a result of elution of the fixed antibodies [15]. Since the taenia coli is a fairly thin bundle of muscle fibers and the possible number of fixed antibodies is not as large as, for example, in segments of the small intestine or uterus, this factor must receive careful attention.

*The complex was obtained and provided by Senior Laboratory Assistant A. A. Pol'ner.

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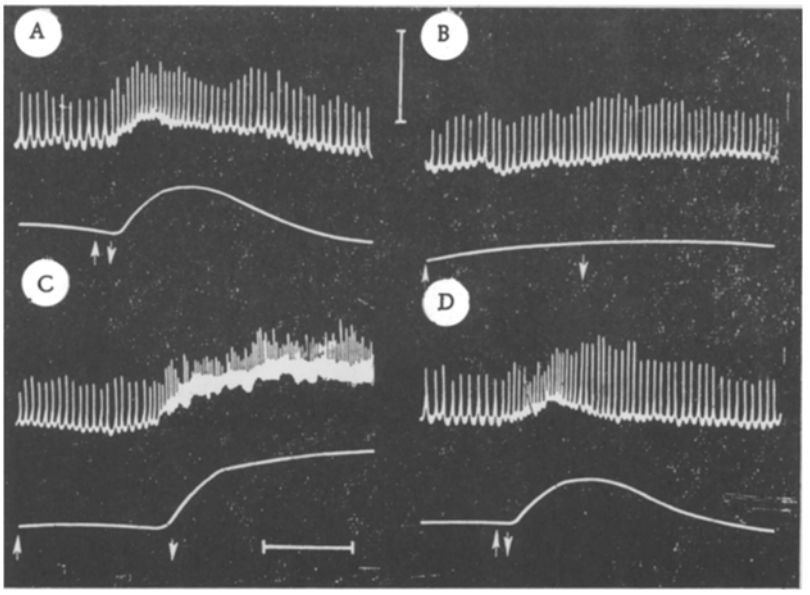


Fig. 1. Character of the electrical and mechanical activity of the taenia coli of a sensitized guinea pig during the action of acetylcholine ($0.5 \times 10^{-6}$; A and D) and ovalbumin (200 μg); B and C). Addition of the antigen was repeated 10 min after the first dose. Time marker 10 sec. The calibration signal corresponds to 5 mV and 5 g. The arrows denote the beginning and end of addition of the substances. Top curve—electrical activity, bottom curve—mechanical activity. Deflection of the beams upward corresponds to depolarization and to an increase in tone.

The anaphylactic reaction of the taenia coli of a sensitized guinea pig is shown in Fig. 1. The addition of 200 μg of crystalline ovalbumin led to a marked decrease in the membrane potential and to an increase in the frequency of the spontaneous action potentials. These changes in the electrical activity of the smooth muscle were accompanied by an increase in muscle tone. The latent period of the response varied from 15 to 30 sec.

Figure 1 also illustrates the action of acetylcholine on smooth muscle. Comparison of the character of the changes in the electrical and mechanical activity of the muscle during the action of acetylcholine and antigen showed that in both cases, the order of these changes was the same. In other words, the increase in tone of the muscle during the anaphylactic reaction was preceded by basically the same changes in the membrane potential and action potentials as during the response reactions evoked by various excitatory agents. The changes in the electrical and mechanical activity of the smooth muscle during the action of substances such as acetlycholine or histamine usually depend on the length of stay of these substances in the solution bathing the muscle. For instance, it is clear from Fig. 1 that acetylcholine, when added to the perfusion fluid, caused the changes in electrical and mechanical activity described above, and as the substance was washed out of the muscle, the original indices were restored. A different picture was observed in the anaphylactic reaction. Depolarization and the increase in the frequency of the spike discharges in this case continued for 2-3 min, and persisted after removal of the antigen from the fluid bathing the muscle. When a further addition of ovalbumin was made in the same dose, the smooth muscle ceased to react, reflecting the development of a state of desensitization of the muscle [3, 4, 11, 12].

The soluble antigen—antibody complex prepared in excess of antigen possesses high biological activity and during its action on isolated smooth-muscle organs it brings about changes resembling those in the anaphylactic reaction [6, 13, 18]. The changes in the electrical and mechanical activity of the taenia coli of an unsensitized guinea pig during the action of a soluble antigen—antibody complex, prepared in a ten-fold excess of antigen, are shown in Fig. 2. These changes, in their external characteristics, were similar to those described above during the anaphylactic reaction of the taenia coli. A soluble antigen—antibody complex prepared from equivalent proportions of immune serum and ovalbumin, ovalbumin, immune rabbit serum, and a mixture of normal rabbit serum with ovalbumin proved to be ineffective. The results of the special control experiments confirmed previous findings [17] that the mast cells in the large intestine are localized to the submucosa. The preparation of the taenia coli contained no mast cells.

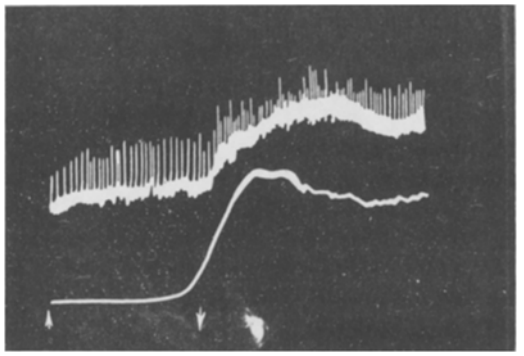

Fig. 2. Action of an antigen–antibody complex (1 mg protein) on the taenia coli of a normal guinea pig. Legend as in Fig. 1.

It may be concluded from the results of these experiments, first, that the anaphylactic reaction of the smooth muscle and its reaction to the antigen–antibody complex developed in the absence of mast cells. These cells are therefore not essential to the development of the allergic reactions of smooth-muscle structures. The results obtained are best explained by the concept of the direct action of the specific antigen on the smooth muscle [2].

The second conclusion from the results of these experiments is that the increase in tone of the smooth muscle during exposure to the action of the specific antigen or the antigen–antibody complex is preceded by a decrease in the membrane potential and an increase in the frequency of the spontaneous allergic potentials. The changes in the electrical and mechanical activity during allergic reactions, thus take place in the same order as in other states of excitation of smooth muscles. A special feature of the allergic reaction of the taenia coli, as of other smooth-muscle organs [12], is the long duration of the changes in the electrical and mechanical activity of the muscle, accompanying addition of the antigen. It is difficult at present to decide what is responsible for this long duration of the response reaction. It can only be suggested that in the case of action of the antigen the long duration of the smooth-muscle reaction is associated with the formation of an antigen–antibody complex on the surface of the reacting structures, where it exerts a prolonged excitatory action on the muscle. It has in fact been found that an antigen–antibody complex prepared in vitro, whose action on smooth-muscle organs of unsensitized animals is due to fixation on the cells [6], also leads to prolonged changes in the electrical and mechanical activity of an investigated smooth muscle.

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