Mechanism of the Schultz-Dale Reaction in the Denervated Diaphragmatic Muscle of the Guinea Pig

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ABSTRACT The mechanism of the contractions elicited by specific antigens in immunologically sensitized muscle tissue (Schultz-Dale responses) has been investigated on single fibers of denervated guinea pig hemidiaphragms. This preparation can be either actively or passively allergized, showing Schultz-Dale responses similar to those of visceral muscle. Specific antigens were applied with an electrically operated microtap to discrete areas of the cell surface while recording the electrical activity with intracellular microelectrodes. In this manner, a depolarizing action of the antigens on the muscle membrane was demonstrated. Brief applications of antigen gave rise to phasic potential changes (antigen potentials) similar to those elicited in the same fibers with acetylcholine-filled microtaps. However, antigen potentials occur only in denervated fibers sensitized to the specific antigen or closely related proteins; they are not seen in either innervated fibers of allergized animals or in denervated, nonallergized fibers. Repeated antigen application to the same area of the fiber causes a local irreversible desensitization. The antigen potentials are associated with a reduction in the resistance of the muscle membrane, similar to that caused by acetylcholine. It is concluded that besides causing the liberation of biogenic amines from the mast cells, antigens exert a direct action on the permeability of the muscle membrane; the molecules of antibody adsorbed to the cells appear to act as specific chemoreceptors for the antigen.

According to current theories (see, for example, Mongar and Schild, 1962) the Schultz-Dale reaction, as well as the visceral muscle contractions which occur during anaphylactic responses in vivo, is caused by compounds with strong stimulating actions on smooth muscle. Indeed, a number of biogenic amines are known to be released by the antigen-antibody reaction in tissues

allergized either by injecting the antigen into the intact animal or by exposing them, in vitro, to immunoglobulins.

However, a number of observations cannot be easily explained in this manner. Thus, (*a*) it is not always possible to establish a correlation, either qualitative (Austen and Humphrey, 1963) or quantitative (Paton, 1958; Boreus, 1961), between the released mediators and the pharmacological sensitivity of particular tissues or species; (*b*) the administration of pharmacological antagonists to known mediators often fails to block the anaphylactic response (Castillo, 1948; Mongar and Schild, 1962); (*c*) when the response to

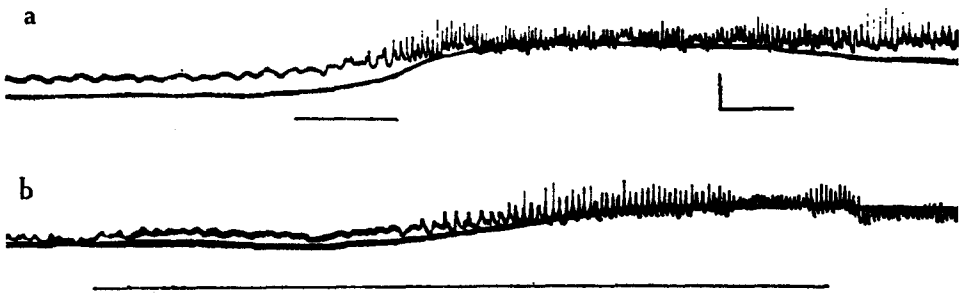

FIGURE 1. Responses of a piece of tenia coli from a guinea pig sensitized to ovalbumin, to histamine (10^{-6} w/v), record a, and to the homologous antigen (100 μg/ml), record b. Upper trace is the electrical activity recorded with the sucrose-gap technique; lower trace is the mechanical force. The horizontal lines indicate the period during which the stopcock controlling the flowing saline solution was switched over from normal Krebs' to the test solution. Calibrations for a and b, vertical, 10 mv and 1 g; horizontal, 10 sec.

some mediators has been reduced or abolished as a result of their repeated administration, application of the antigen still elicits strong mechanical responses (Schild, 1936); (*d*) the anaphylactic contraction of visceral muscle can be converted into a relaxation by partly desensitizing the tissue with a dose of antigen (Alonso-deFlorida and Córdoba, 1965); (*e*) finally, in tissues allergized to two or more different antigens the repeated administration of and desensitization to one of the proteins does not prevent contractile responses to the other antigens (Gijón, Contreras, Córdoba, and Alonso-deFlorida, 1967).

Separately, each of these phenomena could be accounted for by *ad hoc* hypotheses. Yet, a simpler and more general explanation is to assume that in addition to their effects on the mast cells and other possible stores of biogenic amines, antigens exert a direct influence on the ionic permeability of the surface membrane of sensitized muscle cells.

The present paper describes experiments performed to study the effects of the rapid application of antigenic proteins to discrete areas of the surface membrane of sensitized muscle cells. Our aim was to investigate the mech-

anism of the Schultz-Dale reaction with the aid of microtechniques similar to those used to study the action of acetylcholine on the end-plate receptors (see Katz, 1958).

Initial experiments in visceral muscle preparations were unsuccessful due both to the small size of smooth muscle cells and the failure to deliver protein electrophoretically through the tips of conventional microelectrodes (see below). However, the finding that the denervated diaphragmatic muscle of the guinea pig also exhibits Schultz-Dale responses (Alonso-deFlorida, del Castillo, González, and Sánchez, 1965) and the development of an electrically operated microtap which allows the application of proteins from micropipettes with an outside tip diameter of 5–10 μm (Bryant, del Castillo, García, Gijón, and Lee, 1967), provided both a preparation and a technique suitable for investigating the action of antigens on the cell surface membrane.

METHODS

(a) *Preparations* Most experiments were performed on muscle strips taken from denervated guinea pig hemidiaphragms. Hartley guinea pigs of both sexes weighing less than 300 g upon arrival at the laboratory were used. The left phrenic nerve was cut in the cervical region under pentobarbital sodium anesthesia. 2–4 wk after denervation, and at least 7–10 days after the injection of the antigens, the animals were killed and the left hemidiaphragm was removed and cut into strips about 5 mm wide. The fascia was removed under microscopic control exerting the utmost care to avoid excessive damage to the underlying muscle fibers. Strips taken from the right innervated hemidiaphragms of both sensitized and nonsensitized animals and from the denervated hemidiaphragms of nonsensitized guinea pigs were used as controls. A few experiments were carried out also on the tenia coli and tracheal rings of guinea pigs.

(b) *Sensitization* The animals were sensitized with a single subcutaneous injection of 10 mg of protein in complete Freund adjuvant given from 1–7 days after denervation. The following antigens have been used: ovalbumin, human serum albumin, ferritin, ferritin conjugated with pyridoxal, and lactic acid dehydrogenase.

(c) *Electrical Recording and Polarization* The electrical activity of the muscle fibers was recorded with conventional intracellular microelectrodes filled with 3 M KCl, connected via a high input impedance preamplifier (Bioelectric Instruments, Inc., Hastings-on-Hudson, N.Y.) to a Tektronix, Type 502 cathode ray oscilloscope. The sucrose-gap method (see Stämpfli, 1954; Burnstock and Straub, 1958) was employed in the experiments on the guinea pig tenia coli. A plastic three-way stopcock allowed switching over from normal Krebs' solution to one of the test solutions in less than 1 sec.

(d) *Application of Antigenic Proteins* In some experiments the antigen was added to the bath in final concentrations of 30–100 μg/ml (see Alonso-deFlorida et al., 1965). In others, the proteins were applied directly to the cell surface with the aid of a microtap (see Bryant et al., 1967) which was opened either by manually turning the potentiometer controlling the energizing current or with an automatic switch.

(e) *Solutions* The Krebs solution employed had the following composition (mM): Na^+, 142.90; K^+, 5.88; Ca^{++}, 1.26; Mg^{++}, 1.18; Cl^-, 125.22; HCO_3^-, 24.90; $SO_4^=$, 1.18; and $H_2PO_4^-$, 1.18. A mixture of 98% O_2 + 2% CO_2 was bubbled through the solution, which was maintained at a temperature of 39°C.

(f) *Kymograph Recording* Strips of muscle from the same hemidiaphragms used for the experiments with the microtap were placed in an organ bath and attached to a myograph writing on a smoked drum. The condition of the preparations was tested by the administration of histamine and acetylcholine, whereas their sensitivity was determined by recording the contraction elicited by the specific antigen at concentrations of 30 or 100 μg/ml.

RESULTS

The Anaphylactic Response in the Tenia Coli

Changes in the electrical activity of uterine muscle taken from allergized guinea pigs following exposure to homologous antigens were first described by Katsh and Marshall (1959). Working with intracellular electrodes, they showed that a depolarization and bursts of action potentials, accompanied by strong contractions, appear about 2 min after adding the protein to the bath.

In order to obtain a continuous picture of the electrical and mechanical events caused by the antigen, experiments were performed on the tenia coli of sensitized guinea pigs, using the sucrose-gap technique. One such test is shown in Fig. 1. After recording the activity of the tissue for several minutes, the stopcock controlling the flowing solution was switched over from normal Krebs' solution to one containing histamine (10^{-6} w/v). This was followed by depolarization, enhanced electrical activity, and shortening of the muscle.

After the initial conditions had been restored by washing with normal Krebs' solution, the stopcock was switched over to a solution containing the homologous antigen (100 μg/ml) and left in this position. The anaphylactic reaction (see Fig. 1 b) took place in about 30 sec as opposed to a latency of only about 10 sec for the effects of histamine.

Successive applications of the antigen resulted in progressively increasing latencies and decreasing amplitudes of the responses until the tissue became desensitized. In many preparations this occurred after the fourth or fifth exposure to the protein, although when working with pieces of tissue immersed in a conventional bath only two or, at most, three responses could be observed. This difference may be related to the fact that in an organ bath the muscle is exposed to the antigen for significantly longer periods of time at each test.

Also, it was noted that after the reaction to the antigen had subsided and the muscle was surrounded again by normal Krebs' solution, waves of electrical and mechanical activity, similar to those accompanying the initial response, appeared spontaneously in some preparations.

Attempts at Localized Application of Antigens to Smooth Muscle

Direct application of antigens to small areas of sensitized tenia coli and tracheal ring preparations, while recording their electrical activity, met with only partial success. Yet, these experiments gave us an insight into the potential usefulness of micropipettes for the application of proteins. In addition, they showed that exposure of small areas of the tissue to relatively high concentrations of antigen gave rise to local Schultz-Dale responses with latencies much shorter than those previously reported.

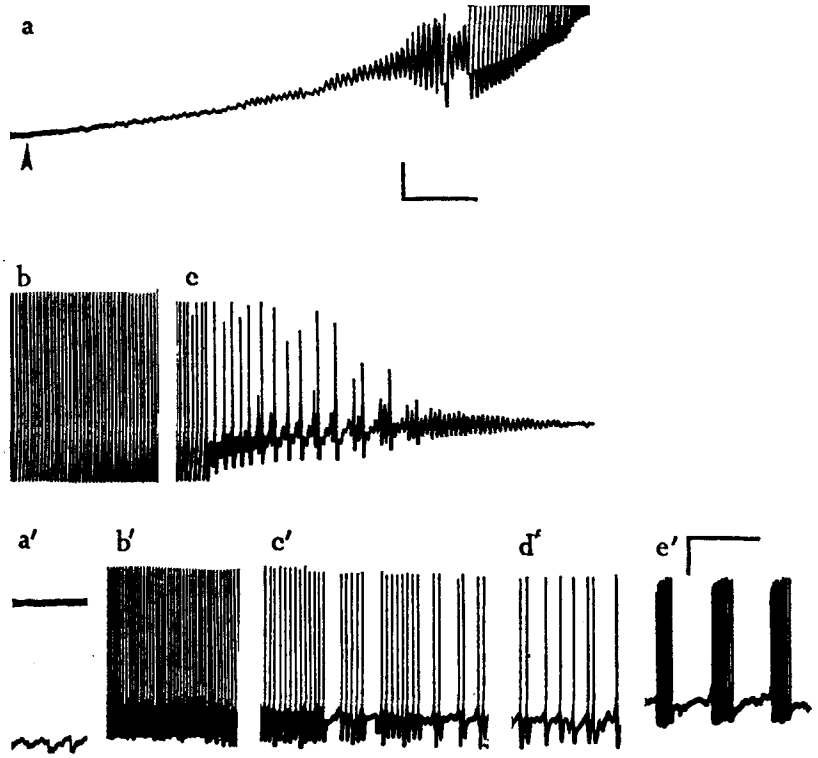

FIGURE 2. Records a, b and c and a′, b′, c′, d′, and e′, show two anaphylactic responses intracellularly recorded from single fibers of two strips of denervated diaphragm from guinea pigs sensitized to ovalbumin. Antigen was added to the bath (100 μg/ml) about half a minute before record a, which begins with a membrane potential of about 80 mv. The arrow marks the sudden development of a depolarization. Between a and b the microelectrode was more firmly inserted into the fiber (see text). The response seen in the lower records was elicited by antigen added to the bath (100 μg/ml) between records a′ and b′. A rhythmic discharge of action potentials, shown in b′, c′, d′, and e′, suddenly started in about 30 sec and continued for approximately 45 sec. Calibrations in a (valid also for b and c), 10 mv and 2 sec. Calibrations in e′, valid for all lower records, 20 mv and 1 sec. The spikes have been retouched.

Ordinary intracellular microelectrodes, with an OD at the tip of less than 1 μm, proved to be totally inadequate to deliver protein; no evidence of spontaneous diffusion of antigen from such pipettes was ever obtained. Attempts to apply the protein electrophoretically were also unsuccessful, even when the pH of the solution was adjusted to extreme values to increase the net electrical charge per molecule. Large potential changes could be elicited on the impaled smooth muscle cells when working with such solutions, but they proved to be only the result of transient changes in the extracellular pH.

However, by using micropipettes with tips of about 5 μm (OD) filled with protein solutions at concentrations of 1–10 mg/ml, circumscribed Schultz-Dale reactions could be elicited repeatedly with latencies shorter than 1 sec. Indeed, local contractions were produced whenever the tips of such pipettes, from which protein was diffusing freely, were brought close to the surface of the muscle.

Electrical recording with microelectrodes at the site of protein application revealed depolarizations and bursts of action potentials. Yet, it was difficult to separate genuine depolarizations from mechanical artifacts.

Anaphylactic Responses Recorded from Single Denervated, Diaphragmatic Muscle Fibers

Electrical recordings of Schultz-Dale responses in strips of denervated, allergized, diaphragmatic muscle were obtained with intracellular microelectrodes following the addition of antigen to the bath. In some instances (see upper records of Fig. 2) the first change observed was a depolarization. This led to undamped oscillations of the membrane potential which, upon reaching a threshold level, caused the firing of spikes. However, part of the depolarization recorded in this particular instance seems to have been a result of the contraction rather than its cause. Indeed, between records a and b the microelectrode was more firmly inserted into the cell thus increasing the recorded membrane potential by about 15 mv. The spike discharge continued for 25 sec at the frequency shown in b (about 20/sec).

In other instances, as in the experiment shown in the lower records of Fig. 2, firing began suddenly without a noticeable depolarization. The action potentials were probably generated at distant regions of the fiber, while at the site of the recording electrode the depolarization developed more slowly (the further decrement in membrane potential seen in record e′ was due to a dislodgment of the microelectrode). Occasionally, small hyperpolarizations were observed before the action potentials, but they may have been the result of a deeper insertion of the micropipette caused by the contraction of adjacent areas of the preparation.

The same effects were observed working with muscle fibers which were fibrillating before the addition of antigen. The frequencies of discharge of the

action potentials suddenly increased and returned to the initial level after periods of time comparable to those observed in fibers which were initially silent.

Depolarizing Action of Homologous Antigens on Denervated, Allergized, Diaphragmatic Muscle Fibers

The nature of the effects of the antigen on the resting potential was definitely established in experiments in which the tips of protein-filled pipettes (5 μm OD) were brought close to the preparation near the point of insertion of a recording microelectrode. A depolarization was observed whenever the diffusing protein reached the surface of the muscle fiber. In the record shown in Fig. 3, from a preparation sensitized to lactic acid dehydrogenase, the tip of a

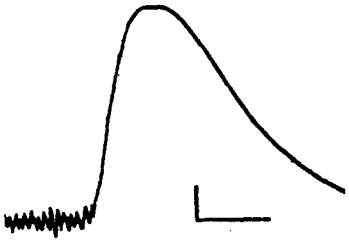

FIGURE 3. Depolarizing effect of lactic acid dehydrogenase on a fiber of a denervated hemidiaphragm taken from a guinea pig sensitized to this protein. A micropipette, ~5 μm O.D. at the tip, filled with a solution of the enzyme (4 mg/ml) was brought close to the cell surface and rapidly withdrawn as soon as the membrane potential (~50 mv) was seen to decrease. Calibrations, 5 mv and 2 sec.

pipette filled with a solution of the enzyme was brought to the vicinity of the fiber and rapidly withdrawn as soon as the resting potential began to decrease. This effect was extremely consistent and reproducible.

"Antigen Potentials" Elicited with the Microtap

With the help of microtaps filled with solutions of antigens brief membrane depolarizations could easily be elicited (Fig. 4). These potential changes, which will be referred to as antigen potentials, were studied in a large number of preparations. The results of these experiments can be summarized as follows.

EFFECT OF DISTANCE BETWEEN MUSCLE FIBER AND ANTIGEN SOURCE The amplitude of the antigen potentials increased as the distance between the tap and the surface of the cell was decreased. At the same time their rising phase became faster, as can be seen by comparing records a and b of Fig. 4. At times, such a relationship did not hold when the tap was very close to the surface of the cell. In Fig. 4, for example, the amplitude of potential c was not larger than that of b, though the microtap was moved 10 μm towards the fiber between b and c (as well as between a and b). As will be shown below,

such a reduction in the amplitude of antigen potentials is caused by the desensitization always observed when the protein is applied repeatedly to the same site on the muscle fiber surface.

PROTEIN SPECIFICITY Potentials similar to those shown in Fig. 4 have been produced by all the antigens employed whenever the preparations were taken from animals sensitized to them or to closely related proteins. Indeed,

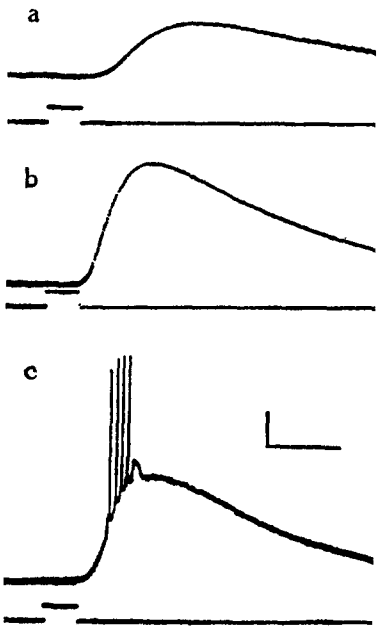

FIGURE 4. Antigen potentials elicited by applying ovalbumin to a denervated, allergized, diaphragmatic muscle fiber. The upper trace in each record shows the intracellularly recorded membrane potential; the lower trace shows the current pulses used to activate the microtap coil. The microtap was moved towards the cell surface 10 μm between both a and b and b and c. Calibrations, 10 mv and 1 sec.

cross-reactions described with regard to the Schultz-Dale reaction in isolated smooth muscle preparations have been observed regularly in our experiments. An example of such interaction is given in Fig. 8 which shows antigen potentials elicited in the same preparation by ovalbumin (record b) and human serum albumin (record a).

LACK OF CONTAMINANTS At the beginning of this work, the possibility was considered that the potential changes elicited by the antigens were due to ions or other contaminants of small molecular weight present in commercial protein preparations. To eliminate this contingency, the experiments were repeated using protein solutions which had been dialyzed against 0.9% NaCl for 24 hr. This procedure, however, had no effect on the observed depolarizations.

SENSITIZATION AND DENERVATION No depolarizations were ever observed when proteins were applied to diaphragmatic muscle fibers, either normal or denervated, taken from nonallergized animals. Likewise, no potential changes

were seen following the application of the various proteins employed to fibers of intact, innervated hemidiaphragms of allergized guinea pigs.

SENSITIVITY AND DISTRIBUTION OF ANTIGEN-SENSITIVE SITES The sensitivity of different preparations to the depolarizing action of the antigens was seen to vary greatly. In some instances, particularly when working with muscle taken from large guinea pigs, we failed to elicit any responses at all. Strips from the same hemidiaphragms suspended in an organ bath reacted to the antigen with extremely weak contractions.

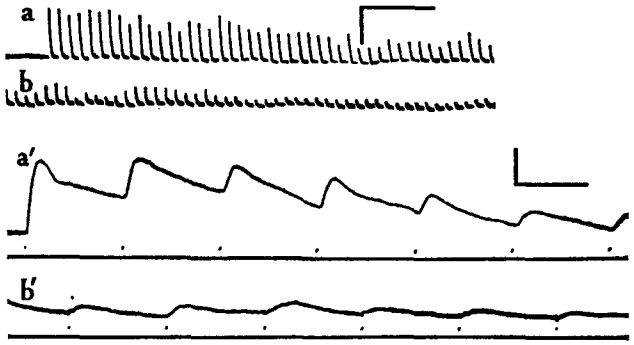

FIGURE 5. Gradual decrease in the amplitude of successive antigen potentials (ovalbumin) elicited at the same site at the surface of a denervated, allergized muscle fiber. In a′ and b′ summation of consecutive potentials took place causing a more rapid desensitization. The fluctuations in the upper records were due, probably, to movements caused by fibrillatory activity. Calibrations, in a and b; 5 mv and 2 min. Calibrations in a′ and b′, 5 mv and 6 sec.

Apart from possible changes in the amount and distribution of antibodies, the diminishing sensitivity observed when the guinea pigs grew older seems to be related to the quantity and consistency of the connective tissue of the diaphragm. This proved to be a barrier to the diffusion of protein, since antigen potentials could not be elicited, even in highly sensitive preparations, through a moderately dense connective tissue layer. Moreover, the increased thickness of the fascia was responsible for widespread cell damage during the dissection of preparations taken from older animals.

Contrariwise, the sensitivity of preparations taken from young, small animals killed from 10–14 days after the injection of protein was very high and seemingly uniform throughout the exposed regions of individual muscle fibers. It is, therefore, unlikely that the sensitivity to antigen is limited to certain areas of the muscle fiber, such as the end-plate region. Large mechanical responses were recorded using diaphragmatic strips taken from the same animals.

DESENSITIZATION A characteristic feature of the Schultz-Dale reaction in visceral muscle is the fact that exposure to antigen leads to a rapid desensiti-

zation. Indeed, such a reaction can be elicited only two or, at most, three consecutive times in one preparation. The same is observed when working with strips of denervated guinea pig diaphragm placed in an organ bath (see Alonso-deFlorida et al., 1965).

Nevertheless, when using the microtap we observed that antigen potentials could be elicited many times at the same site on the surface of a muscle cell. As shown in Fig. 5, it is possible, in some instances, to see almost 100 consecutive potentials. Furthermore, although antigen potentials add up if elicited in rapid succession, the resulting depolarization is largest at the peak of the first or the second potential, since the very rapid decline in amplitude usually offsets its electrotonic summation (see Figs. 5 and 6).

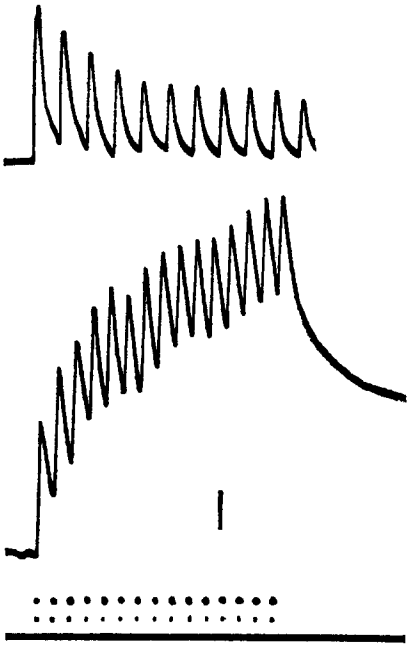

FIGURE 6. Lower record, repetitive histamine potentials elicited with the help of a microtap; their amplitude decreases only slowly. Upper record, antigen potentials (ferritin conjugated with pyridoxal) showing a very rapid desensitization. Vertical calibration, 2 mv for the lower record and 5 mv for the upper one; intervals between pulses, 8 sec in both records. The lower trace in the lower record shows the current pulses applied to the microtap.

One may conclude from these observations that the antigen potentials elicited with a microtap on denervated diaphragmatic muscle fibers undergo a progressive, irreversible desensitization, just as the anaphylactic contractions of visceral tissue in a bath do. The differences between the apparent rate of both processes reflect, probably, the very brief periods during which the cell surface is exposed to the antigen in the microtap experiments.

The desensitization of our preparations could also be shown by adding a single dose of the homologous antigen (100 μg/ml) to the bath. After the contraction caused by the protein had subsided, no antigen potentials could be elicited at all.

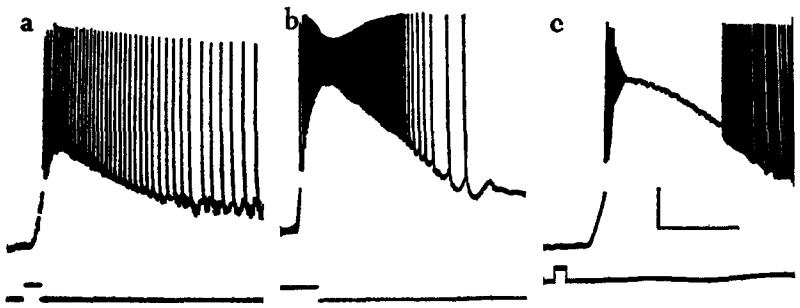

FIGURE 7. Spike firing associated with antigen potentials. In fiber a the membrane potential was increased from about 40 to 70 mv by the injection of inward membrane current before the application of ovalbumin with a microtap. The resulting action potentials did not reach the zero base line. Record b was taken from another fiber in the same preparation which had a resting potential of about 65 mv with no artificial polarization. The amplitude of the spikes markedly decreases at the peak of the antigen potential. In record c, elicited in another preparation by lactic acid dehydrogenase, a complete inactivation of the spike-generating mechanism is seen to occur. Calibrations, vertical, 10 mv for all records; horizontal, 1 sec for a and 2 sec for b and c.

When the antigen potentials elicited at a particular spot are greatly diminished or abolished, a small shift in the position of the microtap along the axis of the fiber is usually accompanied by the reappearance of the depolarizations. The potentials also come back, though with a slower time course, if the duration of the pulses applied to the microtap is increased. These observations suggest that the reduction in the size of the antigen potentials is due to the

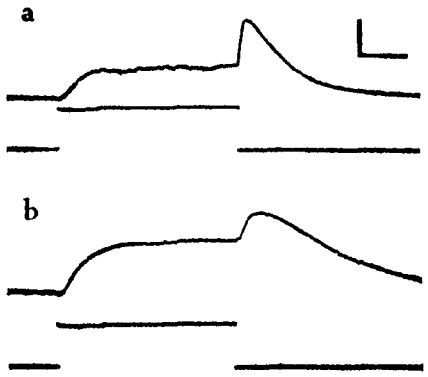

FIGURE 8. Biphasic antigen potentials (see text). This figure illustrates also an immunological cross-reaction; both records are from a preparation sensitized to human serum albumin (HSA). The potential in a was elicited by the application of HSA, but that in b was produced by ovalbumin. In both records two separate depolarizations are seen; the first one is probably distorted by the suction of a small volume of external solution. The second one is due to the ejection of protein when the inner micropipette bounces back at the end of the pulse. Calibrations for both a and b, 10 mv and 1 sec.

inactivation, or permanent binding, of the immunological receptors at a given region of the cell surface. By shifting the position of the microtap new receptors are reached by the protein. Similarly, when the duration of the pulses is increased more protein diffuses reaching distant, still available receptors. As a result, the antigen potentials reappear with a slower time course due to the longer paths for diffusion.

ANTIGEN POTENTIALS AND ACTION POTENTIALS Like other denervated muscle preparations (see, for example, Li, Shy, and Wells, 1957) strips of denervated guinea pig diaphragm exhibit a marked fibrillatory activity. Intracellular recordings from single fibers, in both allergized and nonallergized preparations, revealed a considerable instability of the membrane potential, as well as a remarkably low degree of accommodation. Rhythmic firing of action potentials was seen to occur during long periods of time at a rate determined by the level of membrane potential.

The effect of the antigen potentials on the firing of spikes appears to be no different from that of the depolarizations caused by an externally applied current. In damaged fibers with a membrane potential lower than 50 mv, large antigen potentials, leading to an almost complete depolarization of the membrane, could be elicited without action potentials being fired. Yet, if the membrane potential is restored by flowing an inward membrane current, the antigen potentials trigger the firing of action potentials just as they do in normally polarized fibers. Also, during the largest depolarizations induced by the antigen the spike-generating mechanisms may become completely but temporarily inactivated. These effects are illustrated in Fig. 7.

Biphasic Antigen Potentials and Other Artifacts

As emphasized by Bryant et al. (1967) the satisfactory operation of the microtap entails a tight sealing of the outer pipette and the immediate diffusion of the compound being tested when the tap opens. Control experiments with acetylcholine (ACh)-filled microtaps on normal frog muscle showed that these two conditions are not always achieved.

Often, an imperfect matching of the concentric pipettes caused ACh to leak from the closed microtap, giving rise to a small depolarization of the muscle fiber. In some instances, apparent membrane hyperpolarizations were observed when the microtap was opened by brief current pulses. However, these were the result of a transient stoppage of the leak by a piston-like action of the inner pipette. Such "hyperpolarizations" were often followed by large depolarizations due to the ejection of ACh solution when the inner pipette bounced back.

Similar observations have been made while working with antigens. Protein leaks from the closed microtap can develop suddenly during an experiment from either the suction of particles or the formation of a precipitate between

the two pipettes. A combination of leaks and syringe effects can produce antigen potentials with complex shapes. For example, in addition to the response elicited by the protein diffusing while the microtap is open, a second and larger depolarization can occur when the tap closes (see Fig. 8).

If a small leak of protein causing a steady depolarization coexists with a piston-like action of the inner pipette, the antigen potential can begin with a transient hyperpolarization. For example, the shape of the upper record of Fig. 9 can be explained as follows: (*a*) when the microtap opens, a hyperpolarization is produced by the transient suction of external solution which stops the

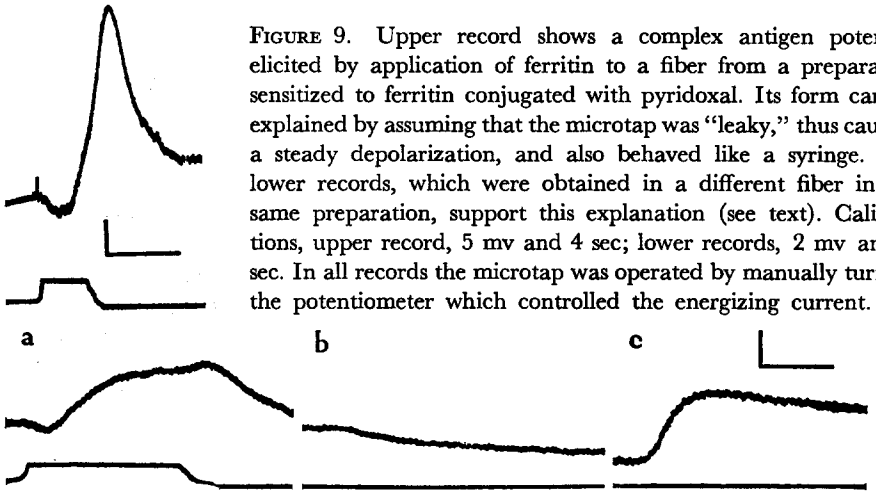

FIGURE 9. Upper record shows a complex antigen potential elicited by application of ferritin to a fiber from a preparation sensitized to ferritin conjugated with pyridoxal. Its form can be explained by assuming that the microtap was "leaky," thus causing a steady depolarization, and also behaved like a syringe. The lower records, which were obtained in a different fiber in the same preparation, support this explanation (see text). Calibrations, upper record, 5 mv and 4 sec; lower records, 2 mv and 2 sec. In all records the microtap was operated by manually turning the potentiometer which controlled the energizing current.

depolarization produced by a steady leak of protein; (*b*) while the microtap is open, such a hyperpolarization is converted into a depolarization by the free diffusion of protein; (*c*) finally, the rate of depolarization is seen to increase suddenly when the microtap closes as a result of the ejection of a volume of protein solution. This explanation is substantiated by the lower records of the same figure. Record a, taken at a faster sweep speed, is similar to the upper record showing the same components. At the end of record a the microtap was left in the "closed" position, but between a and b its tip was withdrawn from the fiber surface with the consequent repolarization of the membrane. Finally, in record c the closed microtap was brought momentarily back to the vicinity of the fiber and a new depolarization, similar to that illustrated in Fig. 3, was observed.

The Membrane Change Responsible for the Antigen Potentials

It has been shown that the depolarization elicited by ACh acting on the muscle receptors is due to the establishment of a shunt, or short-circuit, across the cell surface (Fatt and Katz, 1951; del Castillo and Katz, 1954). Such a

change can be measured by injecting pulses of inward membrane current into the fiber with an intracellular micropipette while recording the resulting potential changes. If the strength of the current pulses remains constant, the amplitude of the anelectrotonic potentials will be proportional to the effective resistance of the fiber, which decreases following the application of ACh.

This is illustrated in record a of Fig. 10. The ACh potential, with a peak amplitude of about 16 mv, was accompanied by a decrease in the effective resistance to approximately one-third of the initial value. Moreover, the

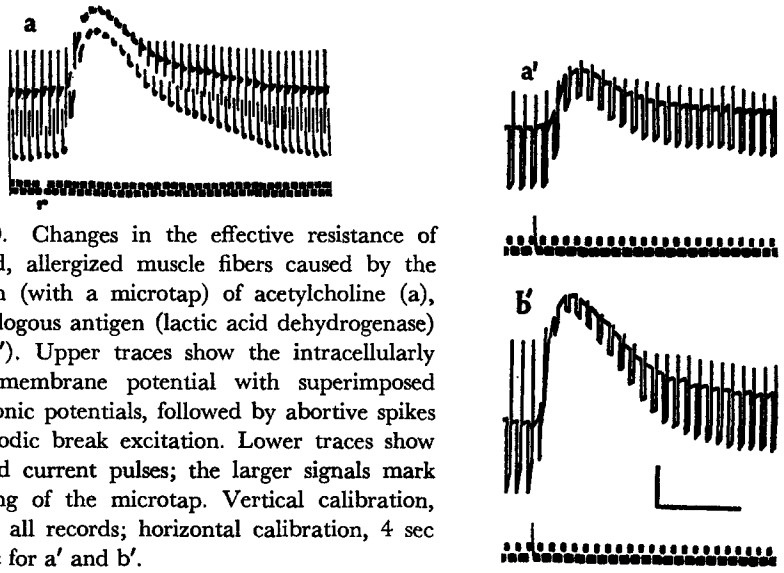

FIGURE 10. Changes in the effective resistance of denervated, allergized muscle fibers caused by the application (with a microtap) of acetylcholine (a), and homologous antigen (lactic acid dehydrogenase) (a′ and b′). Upper traces show the intracellularly recorded membrane potential with superimposed anelectrotonic potentials, followed by abortive spikes due to anodic break excitation. Lower traces show the applied current pulses; the larger signals mark the opening of the microtap. Vertical calibration, 10 mv for all records; horizontal calibration, 4 sec for a, 2 sec for a′ and b′.

shape of the anelectrotonic potentials was seen to change due to the decreased time constant of the muscle membrane.

Similar observations have been made in experiments in which the effective resistance of allergized muscle fibers was monitored during the production of antigen potentials, as shown in records a′ and b′ of Fig. 10. Between a′ and b′ the microtap was moved closer to the surface of the muscle fiber as shown by the faster rise and increased amplitude of the antigen potential. In a′, the effective resistance of the fiber decreased to about one-half the initial value and a much larger decrement is seen in b′.

ACh and Histamine Potentials

As described by Alonso-deFlorida et al. (1965) strips of denervated diaphragm, taken from either allergized or nonallergized guinea pigs, are not only sensitive to ACh but also to histamine. The effects of these drugs on the membrane potential, as delivered both with the microtap and iontophoret-

ically, have been compared during the present experiments. Some of these observations will be briefly described here.

When applied with the microtap to any point on the surface of the denervated muscle fibers, both drugs give rise to a depolarization (see Fig. 6). The shape and time course of the resulting ACh and histamine potentials resemble those elicited by ACh-filled microtaps on the end-plate region of normal, innervated frog muscle fibers (Bryant et al., 1967).

However, when these compounds were applied electrophoretically from micropipettes with an OD at the tip about 1 μm a difference became apparent. Whereas ACh potentials could be elicited consistently at almost any point on the fiber surface, histamine potentials were observed only occasionally. This is particularly puzzling since: (*a*) the molecular weights of both drugs differ only by about 1%, (*b*) their pharmacological activity on denervated diaphragm preparations is almost identical (see Fig. 2 of Alonso-deFlorida et al. 1965), and (*c*) as indicated, the results of their application with the microtap were very similar.

Such disagreement may be related to the nature and distribution of the receptors for both substances. One could assume, for instance, that the receptors for histamine are sparingly and irregularly scattered on the surface of the muscle fiber. Therefore, the probability of being activated in sufficient numbers to give rise to a detectable depolarization would be much less when using a micropipette than a microtap.

DISCUSSION

The idea that antigens may act directly on the surface of sensitized cells is by no means new. A "membrane hypothesis" of anaphylaxis was proposed by Doerr in 1929. This theory received strong support from Kallos' observations (1938) showing that the latency between the addition of an homologous antigen to the bath and the onset of the smooth muscle contraction is relatively constant and independent of the molecular weight of the protein. Such latencies were the same for ovalbumin (mol wt = 34,000) and for *Helix* hemocyanin (mol wt = 6,680,000) suggesting a direct action of the antigen on the surface of the cells rather than its diffusion into the cytoplasm. More recently, the possibility of an interaction of the antigen with the muscle membrane has been discussed by Schild (1956) and Paton (1958).

The observation that the rapid and direct application of specific antigen to the surface of allergized, denervated skeletal muscle cells causes an immediate depolarization can be regarded as evidence for the membrane hypothesis. Yet, it could be argued that the antigen potentials are produced by chemical mediators released in the tissue under the influence of the applied antigen.

These two possible mechanisms are illustrated in Fig. 11 (see legend). Both are equivalent so far as the final result is concerned: the membrane depolari-

zation. But one should expect the time course of the potentials, as well as their dependence on the position of the microtap, to be different in A and B. In A, the shape of the antigen potentials should resemble that of simple diffusion curves; depending mainly upon the amount of protein delivered and the distance between the microtap and the surface of the cell (see del Castillo and Katz, 1955). In B, however, the time course of the depolarization would be determined not only by the above factors but should also depend very critically upon the number and position of the tissular stores of biogenic amines and the rate of operation of the mechanisms whereby the concentration of protein reaching those stores gives rise to a mediator output.

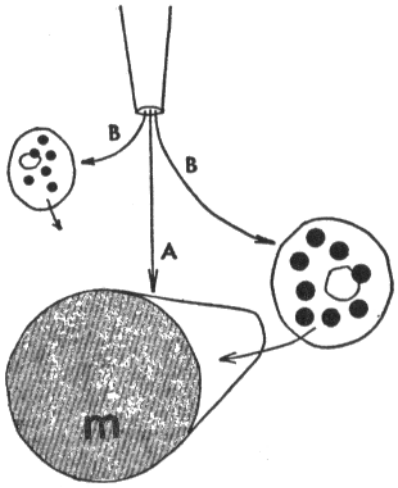

FIGURE 11. Two possible ways in which the antigen released from a microtap could depolarize a denervated, allergized muscle fiber (*m*). In A the protein acts directly upon the cell surface membrane, whereas in B the depolarization is due to the chemical mediators released from the mast cells under the influence of the antigen.

The fast depolarizing effect of proteins on allergized preparations and the smooth time course of the recorded antigen potentials strongly support the hypothesis of a direct action of the protein on the allergized muscle membrane. The biphasic form of some potentials and other departures from simple "diffusion curve" shapes can be explained by a defective performance of the microtap. Potentials with similar forms have also been observed, it must be emphasized, working with ACh-filled microtaps on the end-plate region of normal frog muscle.

Moreover, the experiments reported in this paper suggest that the molecules of immunoglobulin incorporated into the denervated diaphragmatic muscle play the role of cellular chemoreceptors specific for the homologous antigens, a possibility first envisaged by Dale in his Croonian Lecture on the biological significance of anaphylaxis (1920) in which he pointed out that one may regard antibodies as a particular kind of "receptive substance" which we can have in a test tube separated from the cells.

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