The Membrane Components of Crustacean Neuromuscular Systems

I. *Immunity of different electrogenic components to tetrodotoxin and saxitoxin*

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ABSTRACT Axon spikes in crayfish and lobster neuromuscular preparations were blocked by tetrodotoxin or saxitoxin (concentration 10^{-9} to 10^{-8} g/ml). Responses evoked in the excitatory synaptic membrane by ionophoretically applied glutamate, or in the inhibitory by GABA were unaffected by concentrations of the poisons up to 10^{-5} g/ml. These confirm other findings that the poisons do not affect electrically inexcitable membrane components. "Miniature" p.s.p.'s, which indicate local secretory activity in the presynaptic terminals were unaffected by the poisons. Electrical stimuli applied to the axon terminals elicited localized p.s.p.'s after spike electrogenesis of the axons was blocked. Thus, persistence of secretory activity may be linked to persistence of depolarizing K activation in the axons. Spikes induced in the muscle fibers by procaine were not affected by the poisons. In correlation with other data this finding indicates that the depolarizing electrogenic element, which does not depend upon Na activation in the normally gradedly responsive muscles, differs chemically from the Na activation component which is present in the conductile membrane of various cells. Three other varieties of electrically excitable response which are present in crayfish muscle fibers (hyperpolarizing Cl activation, depolarizing K inactivation, and K activation) were, likewise, immune to the toxin.

INTRODUCTION

Pharmacological data were among the evidence which led to the view that the over-all electrophysiological manifestations of different excitable membranes are composites of a number of independently assorting electrogenic processes (Grundfest, 1961 *a*, *b*). Subsequently available data (cf. Grundfest, 1963, 1966 *a*, *b*) have reenforced this view, that the excitable membrane is a

heterogeneous system in which the various components are distinguished by changes in conductances for different ions and by differences in the characteristics of their reactivity to stimuli, to changes in membrane potential, and to pharmacological agents.

The membrane of crustacean muscle fibers is particularly richly endowed in the variety of its electrogenic components. The fibers of the walking leg muscles of crayfish and lobster that were used in the present study are each innervated by an excitatory and an inhibitory motor axon (Boistel and Fatt, 1958; Grundfest et al., 1959). Thus, the fibers have two electrogenically and pharmacologically distinctive components of electrically inexcitable, postsynaptic membrane (Grundfest, 1957 *a*, 1961 *b*). In response to the excitatory postsynaptic potentials (e.p.s.p.'s) or to depolarization by an intracellularly applied current the fibers normally develop only a relatively small graded depolarizing electrogenesis (Fatt and Ginsborg, 1958; Werman and Grundfest, 1961), as is also the case in many other arthropod muscles (Cerf et al., 1959; Hagiwara et al., 1964). However, spikes can be elicited after treating the cells with a variety of agents (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Reuben and Grundfest, 1960 *b*; Belton and Grundfest, 1961 *a*; Werman and Grundfest, 1961; Werman et al., 1961; Hagiwara and Naka, 1964). Three other electrically excitable electrogenic processes are also readily evidenced in crayfish muscle fibers, but they manifest themselves primarily in the changes which they evoke in the membrane conductance rather than as changes in the membrane potential (Grundfest, 1961 *a*). These responses reflect changes in the membrane permeability for Cl and for K. They include hyperpolarizing Cl activation (Reuben et al., 1962), depolarizing K inactivation, and K activation which is initiated at higher levels of depolarization than the K inactivation (Reuben and Gainer, 1962).

The present study contributes further evidence for the conclusion (cf. Grundfest, 1961 *b*, 1964) that the marine poisons, tetrodotoxin (Tsuda et al., 1964) and saxitoxin (mussel poison, Schantz, 1960), do not affect electrically inexcitable components, nor the activity of presynaptic terminals which generate "miniature" p.s.p.'s. Spike electrogenesis of the muscle fibers and all the other electrogenic processes of the electrically excitable membrane component are also immune to the toxins. The significance of these findings for interpretations of membrane structures is discussed. A preliminary account of this work has been published (Ozeki and Grundfest, 1965).

METHODS

Most of the experiments to be described were done on crayfish of three genera (*Cambarus*, *Orconectes*, and *Procambarus*) which were obtained from dealers in California, Louisiana, and Wisconsin. Crayfish muscle fibers exhibit marked species differences with respect to various electrophysiological properties (Girardier, 1965; Reuben,

1965; Girardier, Reuben, and Grundfest, to be published), but none was observed with respect to the measurements employed in the present work. Lobster neuromuscular preparations were used to take advantage for specific purposes of the larger neurally evoked as well as spontaneous miniature p.s.p.'s (Grundfest et al., 1959; Grundfest and Reuben, 1961). Crayfish muscle fibers, however, exhibit a larger repertoire of electrically excitable electrogenic processes. Furthermore, one aspect of this study called for the application of glutamate to elicit depolarizing electrogenesis of the excitatory synapses (Takeuchi and Takeuchi, 1964). Lobster fibers proved to be about tenfold less sensitive to glutamate than fibers of homologous crayfish muscles. The development of maximal responses in the lobster fibers accordingly required applying high currents to the ionophoresis electrode.

Several nerve-muscle preparations of the first and second walking legs were used, but mainly those of the abductor of the carpopodite and the main flexor of the meropodite (Wiersma, 1961). The methods of dissection and precautions to avoid damage to the preparations have been described earlier (Grundfest et al., 1959; Girardier et al., 1963). The standard bathing media were those of Cole (1941) for lobster and of van Harreveld (1936) for crayfish preparations. The experiments were done in New York and at Woods Hole at room temperature, which ranged between 19 and 22° C. Membrane potentials were recorded intracellularly from the superficial fibers of the muscle with one or several microelectrodes filled with 3 M KCl. Polarizing currents were applied intracellularly with an electrode filled with 2 M K citrate. Ionophoretic currents were delivered through an external microelectrode filled with 1 M L(+) Na glutamate. To hold back the leakage of glutamate a steady outward current was applied in most experiments. Presence or absence of this braking current only shifted the dose response curve (Figs. 1 and 2). Axons were stimulated through a pair of fine Teflon-insulated silver wires. The electrophysiological equipment was standard for the laboratory.

RESULTS

Effects on Axons Spike electrogenesis of lobster axons is blocked by saxitoxin (Reuben and Grundfest, 1960 *a*) and by tetrodotoxin (Narahashi et al., 1964). Applications of 10^{-9} g/ml tetrodotoxin blocked spike electrogenesis in crayfish axons. Saxitoxin appeared to be somewhat less effective, but 10^{-8} g/ml of this agent always caused block. Saxitoxin also is somewhat less effective than is tetrodotoxin in eliminating Na activation of eel electroplaques (Nakamura et al., 1965 *b*).

Excitatory Synapses Since the poisons block spike electrogenesis of the axon, pharmacological activation of the synaptic membrane was resorted to by ionophoretic applications of glutamate. For the reason given above, crayfish neuromuscular preparations were used. The ionophoretically ejected glutamate must be applied to rather localized regions of the muscle fiber in order to obtain optimal effects (Takeuchi and Takeuchi, 1964). After some practice the ramifications of the excitatory axon on the surface of the muscle

fibers could be visualized and these proved to be the regions which were reactive to stimulation with glutamate. The responses were recorded within 25 to 100 μ of the site of the ionophoresis electrode outside the fiber.

In crustacean muscle fibers as in cat sympathetic ganglia (Pappano and Volle, 1966) e.p.s.p.'s are abolished when the NaCl of the bathing medium is replaced with LiCl, whereas spike electrogenesis in the axons is maintained (unpublished data). Depolarization by ionophoretic applications of glutamate to the muscle fibers is then also abolished. Glutamate no longer elicits the conductance increase which causes the electrogenesis or the normal e.p.s.p.

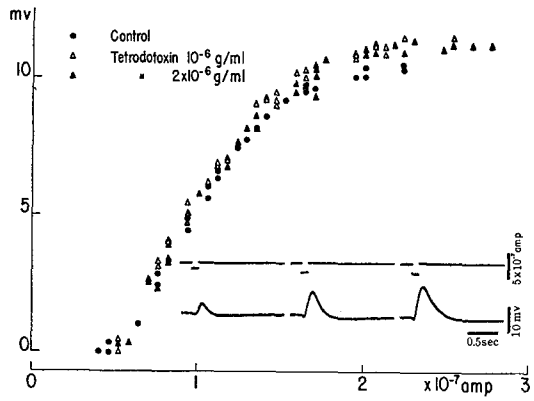

FIGURE 1. Depolarizations evoked by different amounts of ionophoretically applied L-glutamate. Abscissa, current through the ionophoresis electrode. Ordinate, peak depolarization. Sample records for three different ionophoretic currents are shown in the insets. Application of tetrodotoxin had no effect on the dose-response curve. Slight displacements of the ionophoresis electrode during changes of the solution probably account for the small deviations in the three dose-response curves.

These findings have a number of theoretical implications that will be discussed elsewhere. However, it is pertinent to note here that they provide confirmatory evidence that glutamate in concentrations which are adequate for maximal activation of the excitatory synapses has no effect on the electrically excitable components in the membrane of crayfish muscle fibers.

The records of Fig. 1 show the depolarizations elicited at different strengths of the ionophoresis current. In this experiment the maximum depolarization was ca. 11 mv, and it ranged between 10 and more than 40 mv in other fibers. The graph of Fig. 1 shows the complete relation between ionophoretic currents and depolarization in the fiber before treating the preparation with tetrodotoxin (circles) and upon exposure to two concentrations of the latter (triangles). At a concentration more than 1000-fold greater than was needed to block spike electrogenesis in the axons the toxin had no effect upon the

glutamate-evoked depolarizations. In other experiments doses of tetrodotoxin up to 2×10^{-5} g/ml were used, also without effect.

In the experiment of Fig. 2 similar data were obtained with saxitoxin. A concentration of 5×10^{-5} g/ml applied for almost 1 hr was without effect. In this experiment a braking current was not applied to the ionophoresis electrode whereas in that of Fig. 1 a braking current was used. The difference is reflected in the threshold values for the minimally effective ionophoresis current, ca. 10^{-8} amp in Fig. 2 and ca. 5×10^{-8} amp in Fig. 1. However, the spread between minimally and maximally effective currents was about the same, the peak depolarization being attained when the ionophoresis

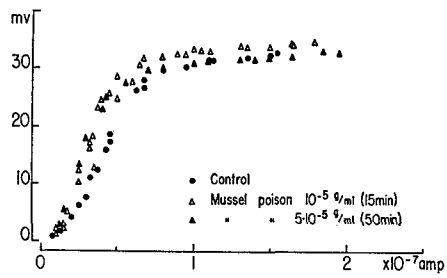

FIGURE 2. Dose-response curves in a fiber before and after applying saxitoxin. In this experiment no braking current was applied to hold back the diffusion of glutamate from the microelectrode. The lower concentration of mussel poison was allowed to act for 15 min before measurements were made. The filled triangles show data obtained 50 min after applying 5×10^{-5} g/ml of the poison.

current was increased about tenfold. An equally narrow dosage (or dynamic) range is observed with respect to the effect of increasing concentrations of γ-aminobutyric acid (GABA) on the membrane resistance of crayfish muscle fibers (Girardier, Reuben, and Grundfest, unpublished; cf. Grundfest, 1963, Fig. 28). The scatter of the data in Fig. 2 is somewhat greater than in Fig. 1, perhaps because the absence of a braking current may have permitted some leakage of glutamate from the ionophoresis microelectrode.

Inhibitory Synapses Applications of GABA, in concentrations which activate the inhibitory synapses maximally, decrease the membrane resistance of arthropod muscle fibers (Boistel and Fatt, 1958; Grundfest et al., 1959; Usherwood and Grundfest, 1965). In the crayfish muscle fiber the emf of the Cl battery, which is involved in the inhibitory electrogenesis, is usually depolarizing with respect to the resting potential. The addition of GABA thus also causes marked depolarization of the fibers. Both effects are shown in Figs. 3 and 4. In the experiment of Fig. 3 tetrodotoxin was applied after GABA and at a concentration of 10^{-5} g/ml did not neutralize the

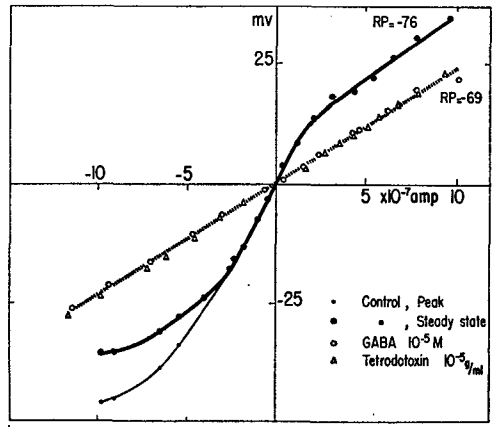

FIGURE 3. Failure of tetrodotoxin to block activity of inhibitory synaptic membrane. Abscissa, intracellularly applied currents. Ordinate, change in membrane potential. Origin is the resting potential (−76 mv). The nonlinear current-voltage relation (continuous line) indicates that ionic changes are induced in the electrically excitable (non-synaptic) membrane components by the applied currents as described in the text. On applying GABA the inhibitory synaptic membrane was activated and as a consequence the conductance increased. The effective resistance decreased more than three-fold, as indicated by the smaller slope of the broken line. The fiber also depolarized (to −69 mv) as a result of the increase in Cl permeability of the synaptic membrane. Neither of these changes was affected by adding tetrodotoxin.

effects of the synapse activator agent. In the preparation of Fig. 4, the same concentration of tetrodotoxin was applied before the GABA. The poison did not modify the current-voltage relation, nor did it prevent the effects of GABA on that relation or the depolarization which this activator of the post-

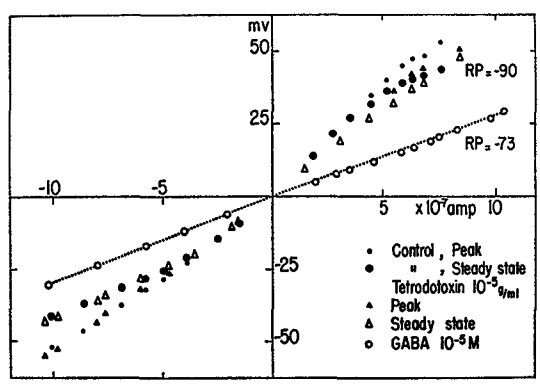

FIGURE 4. Further evidence of the ineffectiveness of tetrodotoxin. Symbols and ordinates are like those of Fig. 3. Applying tetrodotoxin to the control preparation did not change the *I-E* relation nor the resting potential (−90 mv). On applying GABA the membrane conductance increased and the fiber depolarized (to −73 mv), although tetrodotoxin was present.

synaptic (inhibitory) component induced in the muscle fiber. In contrast are the effects of picrotoxin, which is an inactivator of arthropod inhibitory synapses (Boistel and Fatt, 1958; Grundfest et al., 1959; Usherwood and Grundfest, 1965). This drug reverses the effects of GABA with respect to both the decrease in resistance and to the change in membrane potential.

Conductance Changes of Electrically Excitable Components The data of Figs. 3 and 4 also illustrate the complex *I-E* relation of the muscle fibers and Fig. 4 provides evidence that none of the components which enter into determining that relation is affected by 10^{-5} g/ml tetrodotoxin. These components will be analyzed in the next paper (Ozeki et al., 1966), but as already noted, they comprise three reactive elements as well as one passive "leakage" (Hodgkin and Huxley, 1952) conductance pathway. The conductance increases due to hyperpolarizing Cl activation and depolarizing K activation are most clearly

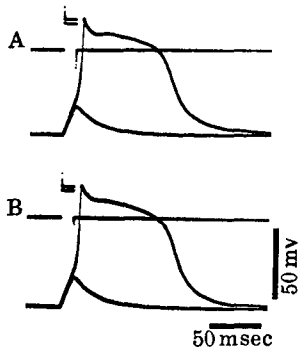

FIGURE 5. Failure of tetrodotoxin to eliminate the procaine-induced spike of crayfish muscle fiber. Two superimposed sweeps are shown in each record. In one the intracellularly applied stimulating current (monitored on upper traces) was insufficient to excite the spike. In the second sweep the current was increased sufficiently to cause a response. *A*, control. *B*, after applying 10^{-5} g/ml tetrodotoxin.

delineated in the curvatures of the *I-E* relation, but the presence of depolarizing K inactivation is also shown in Fig. 4.

Spike Electrogenesis The graded response which is normally evoked in these muscle fibers by depolarizing stimuli is unsuitable as a test object for the evaluation of the action of drugs. However, the spikes with which fibers respond after treatment with procaine (Fig. 5 *A*) are propagated without decrement and thus, as all-or-none events, provide a clear-cut end point. Spikes of the crayfish muscle fiber are not eliminated by 10^{-5} g/ml tetrodotoxin (Fig. 5 *B*) or by saxitoxin.

The foregoing data demonstrate that none of the electrogenic components of the crayfish muscle fibers is affected by tetrodotoxin or saxitoxin, whereas the spikes of the axons are readily blocked. Further comments will be reserved for the Discussion.

Presynaptic Terminals The membrane of the presynaptic terminals which is involved in transmission of activity to crustacean muscle fibers differs at

least in some respects from the conductile component of the axons (Grundfest and Reuben, 1961; Grundfest, 1961 *b*; Dudel, 1965). Even after spikes of the axon were abolished by saxitoxin, spontaneous miniature p.s.p.'s were recorded in lobster muscle fibers (Reuben and Grundfest, 1960 *a*). In the present work persistence of this activity was also observed in both crayfish and lobster muscle fibers after treatment of the preparations with tetrodo-

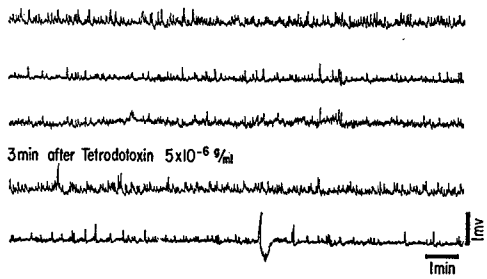

FIGURE 6. Persistence of transmissional activity in nerve terminals after application of tetrodotoxin. Lobster, abductor muscle of carpopodite, intracellular recording, and ink writer registration from two muscle fibers. In one fiber (upper traces of each set) there was a high frequency of spontaneous miniature p.s.p.'s. The two lower traces of the control were simultaneous recordings from two sites in another fiber. Only one of the registrations after tetrodotoxin is shown.

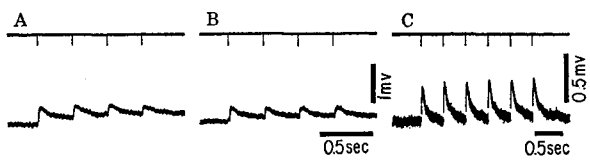

FIGURE 7. Further data on persistence of transmissional activity. Lobster preparation as in Fig. 6. Stimulating electrodes were applied to a fine branch of the exciter axon. A train of four stimuli, each 10 μsec in duration, evoked depolarizations in the muscle fiber (*A*). There appeared to be no antidromic propagation into the main trunk of the axon. *B*, responses recorded after application of tetrodotoxin (3×10^{-7} g/ml). *C*, Records at higher amplification and slower sweep speed. Upper traces in each record monitor the stimulating currents applied to the axon branch.

toxin (Fig. 6). No effect of the poison upon either the frequency or the amplitudes of the spontaneous potentials was evident in the records. Small hyperpolarizations, indicative of activation of the inhibitory synaptic membrane, were observed in the lobster preparations. The i.p.s.p.'s of crayfish muscle fibers normally tend to be depolarizing (Figs. 3 and 4) and thus require the use of special methods to distinguish them from miniature e.p.s.p.'s. Their occurrence in the crayfish preparation was not examined in this study.

Persistence of the spontaneous p.s.p.'s after applying the marine poisons

suggests that transmitter release from the nerve terminals (Fatt and Katz, 1952) still occurs after spike electrogenesis of the axon is blocked. When the nerve terminals of the poisoned axons are stimulated by a pair of fine, closely spaced electrodes, small p.s.p.'s can still be generated in the muscle fiber (Fig. 7). They are localized to the region of the exciter nerve terminals. Similar observations have been reported for frog neuromuscular junctions (Katz and Miledi, 1965). K activation is not abolished by the poisons in the axons of lobster (Narahashi et al., 1964), squid (Nakamura et al., 1965 *a*), and frog (Hille, 1966). It is therefore tempting to suppose that K activation is set up in the nerve terminals by the electrical stimulus and that the increased membrane permeability for K is associated with release of transmitter agent from the terminals.

DISCUSSION

The significance of these findings derives from the large variety of electrogenic processes that are to be observed in the crustacean neuromuscular systems. The data thus permit comparisons with a considerable body of physiological and pharmacological observations on other cells. Neither tetrodotoxin nor mussel poison has any effect on the various electrogenic components of crayfish muscle fibers, although both poisons applied in concentrations of 10^{-9} to 10^{-8} g/ml eliminate spike electrogenesis of the axons in the same preparations. Spontaneous miniature p.s.p.'s are not eliminated, however.

The present findings add support to the generalization that tetrodotoxin and saxitoxin are without effect on electrically inexcitable components of electrogenic membranes (Grundfest, 1961 *b*, 1964). Neither variety of synaptic activity of the crayfish fibers was blocked by the poisons (Figs. 1 to 4). Tetrodotoxin is without effect on the cholinoceptive membrane of frog muscle fibers (Furukawa et al., 1959; Cheymol et al., 1961) and eel electroplaques (Higman and Bartels, 1962), while the spike electrogenesis is eliminated in both the muscle fibers and electroplaques by selective block of Na activation (Narahashi et al., 1960; Nakajima et al., 1962; Nakamura et al., 1965 *b*). Lobster muscle fibers which were exposed to saxitoxin continued to exhibit spontaneous miniature potentials, signifying continuing responsiveness of and activity in both the excitatory and inhibitory synaptic membranes (Reuben and Grundfest, 1960 *a*). The conductance increase which results from the activation of the inhibitory synapses by GABA also remained unaffected (Grundfest et al., 1959), although saxitoxin, like tetrodotoxin (Narahashi et al., 1964), eliminated spike electrogenesis of the axons. Confirmatory evidence has also been obtained in a number of receptor neurons in which the depolarizing generator potentials of the electrically inexcitable input components were unaffected by tetrodotoxin, whereas spike electrogenesis was eliminated (Loewenstein et al., 1963; Nakajima, 1964; Ozeki and Sato, 1965;

Wolbarsht and Hanson, 1965; and personal communications from Drs. Bauman and Fuortes, 1964).

Spontaneously occurring transmissional activity of the presynaptic terminals persists after the spike electrogenesis of the axons is eliminated (Fig. 6) as had already been observed earlier (Reuben and Grundfest, 1960 *a*). Furthermore, localized p.s.p.'s can still be elicited in the muscle fibers by electrically stimulating the axon terminals (Fig. 7). The responses thus indicate that the terminals are electrically excitable, as they also are in frog motor axons (Katz and Miledi, 1965). However, electrical excitability is not synonymous with spike electrogenesis (Grundfest, 1961 *a*). The data of Figs. 3 and 4 as well as the data of the subsequent paper (Ozeki et al., 1966) provide further confirmation, showing that crayfish muscle fibers possess three varieties of electrically excitable responses which do not involve spike electrogenesis.

In fact, stimulation of the finer ramifications of lobster or crayfish axons fails to generate antidromic invasion of other terminals of the same axon (Fig. 7 *A*), although spike electrogenesis can be elicited somewhat more distally (Ozeki, Freeman, and Grundfest, data to be published). General electrophysiological considerations also raise the possibility that the transmissional (secretory) activity of nerve terminals may not and need not be accompanied by spike electrogenesis at these sites (Grundfest, 1957 *b*). Crustacean muscle fibers are densely innervated and, if spikes were generated at the terminals, intracellular recordings from the muscle fibers might be expected to detect some of that electrogenesis. Electroplaques of *Electrophorus* and of the marine electric fishes are perhaps even more densely innervated, and in these cells also no trace of presynaptic electrogenic activity is observed with intracellular recordings (Grundfest, 1957 *b*; Grundfest and Bennett, 1961). External recordings from the fine terminals of crayfish motor axons indicate that the axon spike in fact does not invade the terminals (Dudel, 1965).

Thus, it is possible then that the terminals do not generate a spike and yet are excited into transmissional activity by electrical stimuli, including spikes of the axons. The transmissional, presumably secretory, activity obviously does not depend upon spike electrogenesis, since it can proceed in the poisoned preparations (Figs. 6 and 7). An electrically excitable component which can remain after poisoning is the increased conductance due to K activation and, as already noted, this might be the trigger for releasing the transmitter agent or K itself might in fact be a transmitter agent. In order to initiate K activation depolarization of only some 10 to 30 mv is required in most cells. This depolarization could result from electrotonic spread of spike depolarization, without the need for actual invasion of the terminals by the spikes. The electrical signs of a secretory activity which would be triggered by K activation might well be negligible. Amphibian slow muscle

fibers respond to electrical stimuli with K activation (Burke and Ginsborg, 1956; Belton and Grundfest, 1961 *b*) and the electrogenesis associated with the conductance increase is usually only a small increase in the inside-negative polarization. Rajid electroplaques respond to electrical stimuli with a marked conductance increase which is due to Cl activation (Cohen et al., 1961; Grundfest et al., 1962; Hille et al., 1965), but the electrogenesis is a depolarization of only a few millivolts.

Relevant in this connection are recent studies on neuromuscular transmission in frog (Braun et al., 1966; Braun and Schmidt, 1966). Unlike the presynaptic nerve terminals of crayfish axons, those of frog apparently possess a spike-generating membrane component. The action potential recorded outside the fine terminals decreases on repetitive stimulation of the nerve. After a single conditioning stimulus a test response is depressed for 30 to 60 msec. The depression lasts for several hundred milliseconds after prolonged repetitive conditioning stimulation. The transmissional effectiveness of the test volley, however, is increased during this time. The degree of the long lasting facilitation of the end plate potential increases with the number and frequency of the conditioning stimuli. In the chick ciliary ganglion, also, effectiveness of synaptic transmission does not seem to be correlated with changes in amplitude of the presynaptic spike (Martin and Pilar, 1964). These findings are consistent with the demonstration that the spike electrogenesis of the axon is not directly involved in the transmissional activity of the nerve terminals. However, they are also not necessarily in conflict with data that indicate a correlation between amplitude of the presynaptic spike and transmissional effectiveness (Hagiwara and Tasaki, 1958; Takeuchi and Takeuchi, 1962; Hubbard and Schmidt, 1963; Dudel, 1965). Spike electrogenesis, whether occurring in the nerve terminals, or spreading depolarization into the latter electrotonically, is to be regarded as the trigger for eliciting K activation which, in the view proposed here, is the direct functional correlate of transmissional activity at the terminals. Diminution of repetitively evoked spikes in the terminals of the frog axons may indicate that K activation, which is a graded and a long lasting response of the membrane, is large relative to the Na activation in the terminals, as it is in some cells (Grundfest, 1961 *a*). Thus, while the electrogenic response which is functionally correlated with the secretory activity of the nerve terminals is increasing, the overt electrical response, the spike, would tend to diminish in amplitude. Since both effects are due to the rise of K conductance, the time courses of the depression of spike electrogenesis and of facilitation of the postsynaptic response would be roughly the same.

Neither depolarizing K activation nor depolarizing K inactivation is affected by the marine toxins in other cells (Narahashi et al., 1960, 1964; Nakajima et al., 1962; Nakamura et al., 1965 *a*, *b*; Hille, 1966). Thus, it is

not surprising that these two electrogenic processes of the crayfish muscle fibers are not blocked by the poisons (Fig. 4). The immunity of the hyperpolarizing Cl activation is a new finding. The same electrogenic process probably also occurs in barnacle muscle fibers (Hagiwara et al., 1964) and perhaps also in molluscan neurons (Tauc and Kandel, 1964), but to our knowledge its sensitivity to tetrodotoxin in these preparations has not been tested. The depolarizing Cl activation which occurs in the skate electroplaques is not affected by tetrodotoxin (Hille et al., 1965).

The failure of the toxins to block spike electrogenesis in the muscle fibers (Fig. 5) was not unexpected. Earlier but less systematic observations (unpublished) indicated that the spikes evoked in procaine-treated muscle fibers of lobster and of several insect forms were not blocked by the poisons. It was at first suspected that the immunity might have been conferred by the pretreatment with procaine. This became unlikely, however, since rats or mice were not immunized against saxitoxin by injections of procaine (personal communication from Dr. E. J. Schantz, March, 1961). Spikes evoked in the muscle fibers by other means also were unaffected. It seemed likely therefore that the immunity of arthropod spike electrogenesis to the marine toxins might be ascribable to differences in the ionic mechanisms which lead to spike electrogenesis. While the presence of Na is normally required for producing spikes in axons and many other cells, arthropod muscle fibers can produce spikes when Na is absent from the medium. The electrogenesis is presumably due to changes in permeability for the divalent alkaline earth cations, Ca, and/or Mg (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Reuben et al., 1960; Werman and Grundfest, 1961; Werman et al., 1961; Belton and Grundfest, 1961 *a*, 1962; Hagiwara et al., 1964; Hagiwara and Naka, 1964). The evidence for the participation of Ca in the spike electrogenesis of barnacle muscle fibers is particularly clear (Hagiwara and Naka, 1964; Hagiwara et al., 1964) and these fibers also are immune to tetrodotoxin (personal communication from Drs. Hagiwara and Nakajima). Neurons which produce spikes in the absence of Na from the bathing medium have been observed in ganglia of molluscs (Gerasimov, 1964). Other neurons, however, require the presence of Na. Spike electrogenesis of the latter neurons is blocked by tetrodotoxin, but the responses of the former cells are immune to the poison (Meves, 1966).

These various findings thus lead to the conclusion that the membrane structures which mediate the process of depolarizing Na activation must differ in some specific chemical aspect from the components of membrane structure which mediate all the other electrogenic processes that are immune to the toxins. It also follows that there must be some chemical structure in common among the Na activation components in all the cells in which the toxins block spike electrogenesis by eliminating Na activation. Further im-

plications of the foregoing data, regarding the hetrogeneity of the cell membrane, will be discussed in the subsequent paper (Ozeki et al., 1966).

The work in this laboratory is supported in part by Public Health Service Grants NB 03728-03 NB 03270-04, and 5TI NB 5328-03 from the National Institute of Neurological Diseases and Blindness; by a grant from the National Science Foundation (GB 2940), and one from the Muscular Dystrophy Associations of America.
Dr. Freeman is a Trainee Fellow in Neurology on Training Grant 5TI NB 5328.
Received for publication 28 May 1965.

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