The Membrane Components of Crustacean Neuromuscular Systems

II. *Analysis of interactions among the electrogenic components*

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ABSTRACT The present work provides additional evidence for the occurrence of a number of electrogenic components in crustacean and particularly crayfish muscle fibers. It demonstrates that these different components are independent. One or another can be eliminated or modified by various procedures without affecting the rest. However, interactions can occur between the different components. The coupling may be effected by the changes in membrane potential which result from changes in ionic permeabilities or by changes in the membrane conductance. Some interactions of such couplings which can perturb electrophysiological measurements are described and analyzed.

INTRODUCTION

The membrane of crayfish muscle fibers exhibits a particularly large variety of electrogenic processes (cf. Reuben and Gainer, 1962; Ozeki et al., 1966). The last mentioned work showed that none of these components is affected by the marine toxins which eliminate Na activation of conductile activity in the axons. The present work details further evidence that the different electrically excitable processes of the muscle fibers, like the two electrically inexcitable electrogenic activities of the excitatory and inhibitory synaptic membrane components, have distinctive electrophysiological and pharmacological properties. A system with such a variety of independent electrogenic components may give rise to complex overt physiological properties and a number of such complexities have been analyzed in studies from this laboratory (Reuben and Gainer, 1962; Girardier et al., 1963; Reuben et al., 1964; Girardier, Reuben, and Grundfest, to be published). The present study de-

scribes some of the interactions which occur among the electrogenic components of crayfish muscle.

The coupling of independently assorting electrogenic processes of a cell can arise by way of changes in the membrane potential or by way of changes in the conductance of the cell membrane. The first type is exemplified in the excitatory effects of the depolarizing (excitatory) p.s.p.'s; by the regenerative action of the depolarizing electrically excitable electrogenesis which is induced either by e.p.s.p.'s or by electrical stimuli; and by the pulse-shaping effects of the same electrogenesis in eliciting K activation and/or inactivation. The "poising" or "clamping" action by inhibitory synaptic electrogenesis on e.p.s.p.'s exemplifies coupling through conductance changes. Since crayfish muscle fibers possess a number of electrically excitable components which cause marked changes in conductance, they provide an opportunity for analysis of an interplay of both types of coupling.

METHODS

The preparations and the experimental techniques were essentially like those described in the previous paper (Ozeki et al., 1966). Some data were also obtained (cf. Fig. 5) on homologous muscles of lobster. In some of the experiments the impermeant anion, propionate, was substituted for the Cl of the standard media (van Harreveld, 1936; Cole, 1941), or the K level was reduced. In other experiments Cs replaced K. Details are given in connection with the specific instances.

RESULTS

Effect of Changes in Membrane Potential on the Responses to Glutamate The depolarizations evoked by applications of glutamate are believed to be responses to activation of the excitatory postsynaptic component (Takeuchi and Takeuchi, 1964). The magnitude of the depolarization evoked by a constant dose of glutamate (or of ionophoretic current) thus ought to vary when the membrane potential is changed by an intracellularly applied current. The changes in the response should be linearly related to the membrane potential as predicted from the theory of electrically inexcitable electrogenesis (Grundfest, 1957, 1961 *a*, *b*). However, as is shown in Fig. 1 (inset records and filled circles) the effect on the depolarizing response of changes in membrane potential deviated widely from the expected relation. The glutamate-evoked depolarization decreased markedly when the muscle fiber membrane was hyperpolarized, whereas the e.p.s.p.'s of many cells are enhanced by hyperpolarization (cf. Grundfest, 1961 *b*). For small depolarizations of the fiber the glutamate-evoked response increased, but when the membrane was depolarized by 10 mv or more, the response diminished.

The changes induced by the depolarizing currents are similar to those which were observed in the e.p.s.p.'s of crayfish muscle fibers bathed in high Ca media (Reuben and Gainer, 1962). These "anomalous" effects had been

observed earlier by Dudel and Kuffler (1960). They were accounted for (Reuben and Gainer, 1962) in terms of the conductance changes which result from the operation of the depolarizing inactivation and activation processes of the electrically excitable membrane components of crayfish muscle fibers. This interpretation is confirmed and extended by the data of Fig. 1. The open circles and broken line describe the changes in effective resistance which result from the changes in membrane potential of the fiber. The resistance ordinate is in relative units, the effective resistance of the unpolarized fiber being scaled at 3.5 units. With a hyperpolarization of about 15 mv the resistance decreased to about half. It was approximately doubled when the

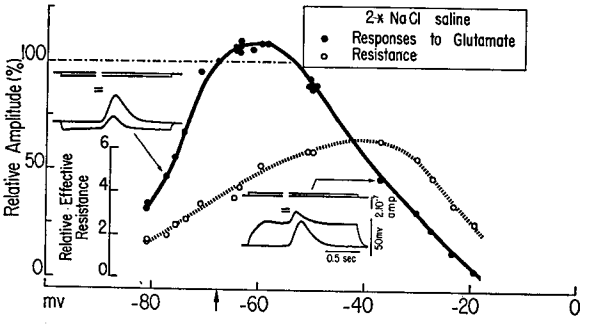

FIGURE 1. Changes in the amplitudes of glutamate-induced depolarizations with changes in membrane polarization. Inset records show the depolarizations evoked by the ionophoretically applied glutamate at the resting potential and while the fiber was being hyperpolarized (left) or depolarized (right) by an intracellularly applied current. The preparation was in a hyperosmotic medium (2× the normal NaCl) in order to eliminate contractile activity that might be evoked normally by the large (ca. 40 mv) depolarization. Arrow shows the resting potential. Filled circles and heavy line, relative changes in amplitude of the depolarizations. Open circles and dotted line, relative changes in effective resistance during the intracellularly applied currents which changed the membrane potential to the values indicated on the abscissa. Further discussion in text.

membrane had been depolarized by about 30 mv, but the resistance declined steeply from this peak value as the membrane was depolarized further.

Effects of Abolishing Hyperpolarizing Cl Activation The decrease in resistance which is evoked in the crayfish fibers even by small hyperpolarizations is due to hyperpolarizing Cl activation. The evidence for this conclusion rests in part upon the finding that the time-variant nonlinearity of the *I-E* relation in the hyperpolarizing quadrant is abolished or diminished by eliminating most of the diffusible intracellular Cl (Reuben et al., 1962; cf. Grundfest, 1963, Fig. 24). The preparation of the experiment of Fig. 2 was soaked for 20 hr in a bathing medium in which all the Cl had been substituted with propionate. The effective resistance of the fiber which had been thereby de-

pleted of Cl remained constant within the limits of measurement, while the cell membrane was hyperpolarized by more than 60 mv (open circles, broken line). In the same fiber the depolarizations which were evoked by applications of glutamate were now augmented (filled circles).

In the Cl-free medium the muscle fibers tend to develop spikes when the membrane is depolarized by about 15 mv (Girardier et al., 1963). This perturbation precluded extension of the measurements of Fig. 2 to a wide range of depolarizations. However, a second means is available for blocking the conductance increase due to hyperpolarizing Cl activation (Reuben et al., 1962). Picrotoxin, which is a potent blockader of the inhibitory synapses of arthropod muscle (Boistel and Fatt, 1958; Grundfest et al., 1959; Usherwood

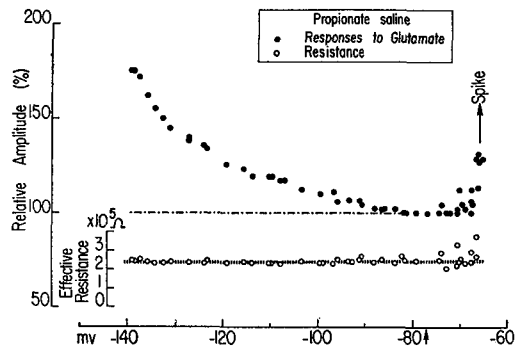

FIGURE 2. Changes in depolarizations evoked by ionophoretic applications of glutamate during changes in membrane potential. Experiment like that of Fig. 1, except that the preparation had been kept for 20 hr in a Cl-free medium, substituting the impermeant anion propionate for the Cl and with the normal Na level. In this case the effective resistance remained constant on hyperpolarizing the fiber. The depolarization due to glutamate applications increased with hyperpolarization of the fiber. Appearance of spikes on depolarizing the fiber prevented observations with stronger depolarizations.

and Grundfest, 1965) also blocks or diminishes Cl permeability of the nonsynaptic membrane components, but the effect requires much higher concentrations of picrotoxin and a longer exposure time. The preparation used in the experiment of Fig. 3 had been treated with 10^{-3} g/ml picrotoxin. The effective resistance of a tested muscle fiber remained almost constant with hyperpolarization of the membrane by as much as 60 mv (open circles, broken line). The depolarizations evoked by glutamate in the same fiber (filled circles, solid line) also increased with increasing hyperpolarization, as in Fig. 2. In the experiment of Fig. 3 the relation was nearly linear over the full range of hyperpolarization and an extension of the line to the abscissa intersects the latter at −5 mv. This is a reasonable magnitude for the reversal potential of the e.p.s.p. (Fatt and Katz, 1951; Grundfest, 1961 *b*).

In the experiments illustrated by Fig. 3, the measurements could be ex-

tended into the range of considerable membrane depolarization. As was already noted in connection with Fig. 1, the effective resistance at first increased with increasing depolarizations. The resistance reached a peak for a depolarization of about 30 mv, but then decreased sharply on further depolarization. These changes in the conductance of the membrane are reflected in the changes in the glutamate-evoked depolarizations. The decrease in the response that should have been caused by an increase in outward current was apparently compensated for by the increased resistance. The decrease in resistance with stronger depolarizations acted on the response in the same

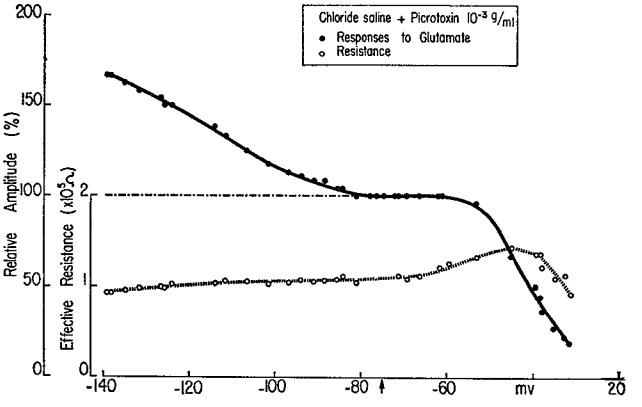

FIGURE 3. Changes in responses to glutamate with changes in membrane potential of muscle fiber treated with 10^{-3} g/ml picrotoxin. Otherwise the experiment is like those of Figs. 1 and 2. The drug blocked Cl activation and the effective resistance (open circles) remained nearly constant for hyperpolarization by more than 60 mv. The amplitude of the glutamate-evoked response increased with hyperpolarization, but the effects of depolarizations were complex because of the complexity of underlying conductance changes. Small depolarizations caused an increase in resistance which is indicative of K inactivation. K activation developed with stronger depolarization. Further description in text.

direction as did the changes in membrane potential and the glutamate-evoked depolarization thus decreased steeply. The fall in the response led the fall in resistance, as it also did in the experiment of Fig. 1, and in the data of Reuben and Gainer (1962, Fig. 2). The intercept of this portion of the curve on the abscissa is at about −25 mv in Fig. 3 and about −20 mv in Fig. 1.

Effects of Changes in Membrane Potential on the Neurally Evoked e.p.s.p.'s The effects on the neurally evoked e.p.s.p.'s of changing the membrane potential are rather different (Fig. 4) from those which were observed with the glutamate-evoked depolarizations. For the experiment of Fig. 4 two intracellular recording electrodes were used which were respectively close to (R_1) and

farther away (R_2) from the site at which currents were applied through a third microelectrode (I). The change in membrane potential produced by a given current was larger at recording site R_1 than at R_2, the decrease representing a length constant (λ) of 2.4 mm. The changes in the e.p.s.p.'s with membrane potential appeared to be linear, but closer examination shows considerable deviations when the changes in membrane potential were large. Furthermore, the electrophysiological conditions in the experiments of Figs. 1 and 4 are not comparable. The response to ionophoretic applications of glutamate was evoked in sharply localized regions of the membrane, close to the site at which the depolarizations were recorded and the polarizing currents were injected. The e.p.s.p.'s were elicited simultaneously at the numer-

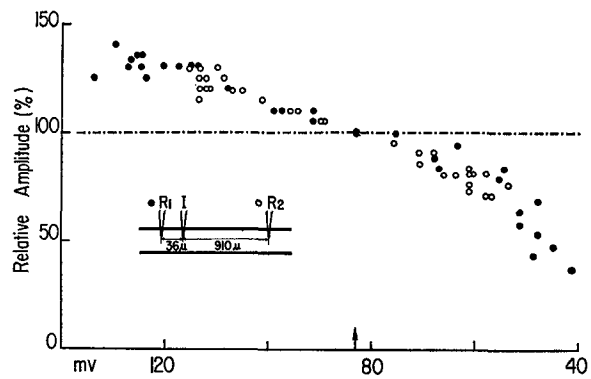

FIGURE 4. Changes in e.p.s.p.'s with changes in membrane potential recorded simultaneously at two sites (R_1 and R_2, inset diagram) 36 μ and 910 μ distant respectively from and straddling the site of the polarizing electrode (I). Abscissa, the membrane potential at each site. The maximum inward current caused about 20 mv more hyperpolarization at R_1 than at R_2. Arrow shows the resting potential. Further discussion in text.

ous sites where the muscle fiber is innervated and the polarization of the membrane with currents injected through an intracellular microelectrode was far from uniform.

Further Evidence for the Independence of Activation and Inactivation Processes

The data of Fig. 3 show clearly that virtual abolition of the hyperpolarizing Cl activation did not alter significantly the processes of depolarizing inactivation and activation of the electrically excitable membrane. They thus contribute evidence for ascribing the two responses to the depolarizing stimuli to changes in membrane permeability for K. Further evidence on this score is given in Fig. 5. Pharmacological K inactivation by Cs has been demonstrated in eel electroplaques (Nakamura et al., 1965) and in lobster muscle fibers (Gainer, Reuben, and Grundfest, to be published).

In the two experiments shown in Fig. 5 homologous lobster and crayfish

muscles were exposed to salines in which the KCl, 15 mM/liter in *Homarus* saline and 5 mM/liter in the crayfish medium, was replaced with CsCl. The *I-E* relation in both preparations was changed within as little as 15 min after exposure to the Cs salt. The effective resistance of the lobster fibers[1] increased for hyperpolarizing currents and for weak depolarizations, but as the depolarization by the applied current increased above about 5 mv the effective resistance after the Cs treatment decreased essentially to the same low value as that in the muscle fiber prior to Cs treatment. The change in the *I-E* relation on replacing K with Cs was not due to the mere removal of K. In the

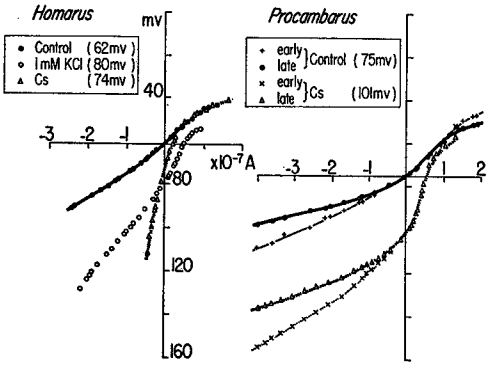

FIGURE 5. Different effects of Cs on the *I-E* relations of fibers in homologous muscles (abductor of the carpopodite) in lobster and crayfish. The lobster fiber was first studied with the preparation bathed in the control saline, then in a medium containing only 1 mM/liter KCl, and finally after 2 hr exposure to a medium in which the KCl was replaced with 15 mM/liter CsCl. The crayfish preparation was studied first in the normal medium (5 mM/liter KCl) and then 2 hr after replacement of the KCl with CsCl. The resting potentials (inside negative) are given in the inserts. Note that the time-variant increase in conductance of the crayfish fiber which is due to hyperpolarizing Cl activation persists in the presence of CsCl, although pharmacological K inactivation is denoted by the steepened slope in the depolarizing direction. Further details in text.

experiment shown the preparation was first exposed to a low K medium before introducing CsCl. The decrease in K in the medium caused a marked hyperpolarization (circles) and some increase in resistance. On introducing 15 mM/liter CsCl the membrane depolarized from −80 mv to −74 mv whereas the resistance increased very markedly (triangles). The marked change in emf which resulted from removal of K also is denoted by the fact that for depolarizing currents which caused K activation the membrane potential was more negative than during K activation in the control condition or after Cs treatment.

The changes produced in the *I-E* relation of the crayfish muscle fibers by

[1] Slope resistance ($\delta E/\delta I$) was measured since the system has several conductance branches in parallel and exhibits different values of resting potentials.

the Cs treatment were markedly different. The whole curve was displaced on the voltage axis because of the marked hyperpolarization (ca. 25 mv) which resulted from the change in the bathing medium. The effective resistance increased, but only in the depolarizing quadrant. Hyperpolarizing currents caused the same time-variant nonlinearity as was observed prior to treatment with Cs. Thus, while Cs produced pharmacological K inactivation, it did not affect hyperpolarizing Cl activation. Stronger depolarization also evoked K activation in the crayfish muscle fibers and the conductance increase was so large that it swamped both the hyperpolarization induced by the change in the bathing medium and the increase in resistance caused by the pharmacological inactivation. As in the case of the lobster muscle fiber, therefore, with strong depolarization the *I-E* relation after treatment with

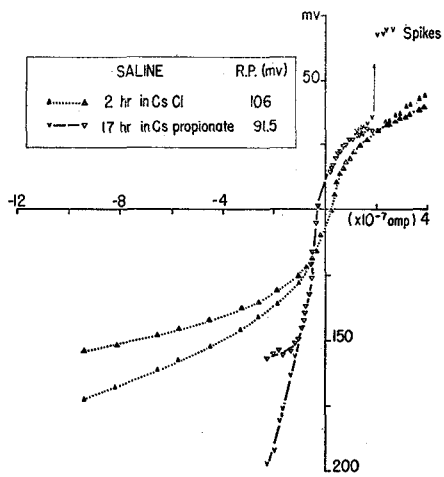

FIGURE 6. Effect of removing intracellular Cl on *I-E* relation of Cs-treated crayfish muscle fiber. The low threshold time-variant hyperpolarizing Cl activation is eliminated and the relation becomes nearly linear in the hyperpolarizing quadrant, with the steep slope indicating pharmacological K inactivation. A delayed conductance increase is still present, but is manifested only at larger hyperpolarizations. Further description in text. The abscissa of this graph and that of Fig. 7 are set at −100 mv.

Cs came to be nearly coincident with that obtained before this treatment. The inability of Cs to block depolarizing K activation in lobster muscle fibers had already been noted in earlier work from the laboratory (cf. Grundfest, 1961 *b*, Fig. 13).

As was already shown in connection with Fig. 2, removal of diffusible intracellular Cl by soaking the muscle in a Cl-free (propionate) medium eliminates the Cl activation which is evoked by low threshold hyperpolarizations. This hyperpolarizing Cl activation is also eliminated in Cs-treated preparations which are exposed to the Cl-free medium (Fig. 6). The high membrane resistance due to pharmacological K inactivation is now evidenced at membrane potentials as large as −200 mv. A delayed increase in conductance still does occur, but only when the membrane potential is strongly negative (cf. Grundfest, 1963, Fig. 23). For depolarizing currents the curvature of the *I-E* relation which indicates K activation is still evident. However, the spike

electrogenesis which occurs in the Cl-free media prevented extending the observations to membrane potentials less negative than about −70 mv.

Picrotoxin was used to diminish the Cl permeability in the experiment of Fig. 7. Diminution of the low threshold hyperpolarizing Cl activation (cf. also Fig. 3) is again evidenced. It was also possible to follow the *I-E* relation into the range of depolarization to about −50 mv. The depolarizing K activation was unaffected by the presence or absence of picrotoxin and the *I-E* relation after Cs treatment was nearly coincident with that of the control measurements, although the initial membrane potentials differed by 25 mv

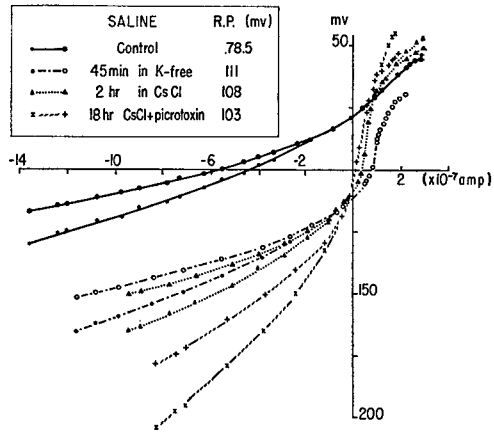

FIGURE 7. Changes in *I-E* relations evoked in a crayfish muscle fiber by changes in ionic and pharmacological conditions. Removing K or replacing K with Cs caused similar changes in membrane potential, but the *I-E* relation differed markedly. Picrotoxin diminished the conductance increase which was produced by hyperpolarizing Cl activation but did not change the curvature of the *I-E* relation in the depolarizing quadrant. Further description in text.

or more. In contrast, when the muscle fiber was in a K-free medium the *I-E* relation deviated markedly (circles) although the resting potential (−111 mv) was nearly the same as under the conditions of Cs treatment.

DISCUSSION

In an attempt to verify the view (Takeuchi and Takeuchi, 1964) that the depolarizations elicited by glutamate are responses of the excitatory synaptic membrane, we have observed and analyzed some illuminating consequences of the interactions between electrically excitable and electrically inexcitable components of the crayfish muscle fibers (Figs. 1 to 4). The current-voltage relation of crayfish muscle fibers (Figs. 5 to 7) is markedly nonlinear (Reuben and Gainer, 1962). The presence of several nonlinear portions in the *I-E* relation indicates that the electrically excitable membrane component under-

goes a number of reactions in response to changes in the membrane potential (Grundfest, 1961 *a*, 1963, 1966). The membrane develops a time-variant decrease in resistance when the fibers are hyperpolarized by some 15 mv or more. This has been shown to be due to hyperpolarizing Cl activation (Reuben et al., 1962), and the present data have confirmed that evidence, since removal of diffusible Cl from the cell eliminated the decrease in resistance which is normally evoked by hyperpolarizing currents (Fig. 2).

The normally occurring decrease in resistance by hyperpolarizing Cl activation as well as the consequent introduction of a component of the Cl battery (E_{Cl}) obscured (Fig. 1) the increases which are to be expected if the glutamate-evoked depolarizations are responses of electrically inexcitable membrane (Grundfest, 1957). When the effects of the electrically excitable Cl activation were eliminated, the depolarizations evoked by glutamate increased with hyperpolarization of the membrane (Figs. 2 and 3). The same distortion by the effect of hyperpolarizing Cl activation must also operate in the case of e.p.s.p.'s, but was not clearly evidenced (Fig. 4). The difference is due to the different electrophysiological conditions. The response to glutamate must be evoked by applying the agent to sharply localized regions, the sites of innervation of the muscle fibers (Takeuchi and Takeuchi, 1964). The experiments of Figs. 1 to 3 were done with the intracellular polarizing and recording microelectrodes close to the site of the extracellular ionophoresis electrode, and the nonlinearities of the electrically excitable membrane therefore were optimally effective. The innervation of the muscle fiber, however, is multiterminal and thus the e.p.s.p.'s originate at widely distributed sites which are differently affected by the polarizing current. The effects of such anatomical complexity on the responses of e.p.s.p.'s during intracellular applied currents have been noted in the diffusely innervated muscle fibers of frog (Burke and Ginsborg, 1956) and grasshopper (Cerf et al., 1959), and might perhaps play a role in other cases (cf. Smith et al., 1965).

Bennett et al. (1966) have analyzed the distortions of the recorded potentials which are caused in the distributed p.s.p.'s of crustacean muscle fibers as a result of the cable properties of the cells.

The responses to application of glutamate also were not affected in a straightforward manner by depolarizing changes in the membrane potential (Fig. 1). However, the resistance measurements of Fig. 1 confirmed the earlier finding (Reuben and Gainer, 1962) that the resistance at first increases as the membrane is depolarized, by the development of depolarizing K inactivation (Grundfest, 1961 *a*). With further depolarization the muscle fiber develops what is presumably K activation and the resistance falls. A late component of depolarizing K activation is also observed in cardiac Purkinje fibers (Deck and Trautwein, 1964) and in electroplaques of some Gymnotid electric fishes (Bennett and Grundfest, 1959; cf. Grundfest, 1963, Fig. 23). As

in the crayfish muscle fibers this activation process of these cells is also superimposed on an inactivation process which develops at smaller depolarizations of the membrane.

The foregoing, reasonably satisfactory analysis of the modifications in the *I-E* relation of the electrically inexcitable components which are caused by the changes in conductance of electrically excitable membrane components was made possible by the relatively simple experimental conditions and by the availability of a considerable armamentarium of ionic and pharmacological agents. It has been noted above, that the various activation and inactivation processes also are found in other cells. When they are present they may cause similar perturbations in the overt electrophysiological behavior of the electrically inexcitable components. The possible effects of such perturbations on generator potentials have been discussed by one of us (Grundfest, 1964).

The data of Figs. 3 and 7 confirm an earlier finding (Reuben et al., 1962) that picrotoxin blocks hyperpolarizing Cl activation. Thus, this drug acts on both electrically inexcitable and electrically excitable membrane, but for the latter system a larger concentration of the drug is required and its action is much slower. GABA which increases the Cl permeability of inhibitory synapses in arthropods, also increases it in the nonsynaptic membrane of crayfish muscles (Reuben et al., 1962). Both GABA and picrotoxin also have effects on the presynaptic nerve terminals of lobster axons that are consistent with similar actions on the Cl permeability of the terminals (Grundfest and Reuben, 1961). Furthermore, both GABA and picrotoxin change the resistance of lobster axons, the former decreasing and the latter increasing the resistance (Freeman et al., data to be published). Since lobster axons (unlike those of squid) are permeable to Cl (Freeman et al., 1966), it is likely that the effects of the drugs on the resistance are due to changes in permeability of the axon membrane to Cl. Electrophysiological data support this conclusion (Freeman et al., data to be published).

Thus, GABA and picrotoxin appear to act on the Cl-permselective components of the cell membrane, at least in crustacean muscle fibers and axons, no matter whether this component is that of electrically excitable or electrically inexcitable membrane structures. Presumably, therefore, these components have some common structural chemical feature. Electrically excitable Cl activation appears to be absent from the insect muscle fibers which have inhibitory synapses that are sensitive to GABA and picrotoxin (Usherwood and Grundfest, 1965). However, hyperpolarizing Cl activation appears to be present in giant muscle fibers of barnacles (Hagiwara et al., 1964) and some molluscan neurons (Tauc and Kandel, 1964) and it would be worthwhile to test the effects of the drugs in these preparations. The specificity of the effects, which appear to be confined to the Cl-permselective components

suggests that, whether electrically excitable or not, these presumably positively charged membrane sites (Girardier et al., 1963), have some common structural feature, at least in crustacean muscle fibers and axons.

When CsCl replaced KCl in the bathing medium hyperpolarizing Cl activation was not affected although other electrophysiological conditions of the crayfish muscle fibers were altered (Figs. 5 to 7). The curvatures in the *I-E* relations which characterize the time-variant Cl activation process in the control saline and in the CsCl medium were essentially parallel, but displaced along the voltage axis. Cl activation may introduce a twofold perturbation in the *I-E* relation, as has also been noted in the depolarizing Cl activation of Rajid electroplaques (cf. Grundfest, 1963, Fig. 14). The increased conductance of the electrically excitable Cl component causes a curvature of the *I-E* line toward the abscissa, but the conductance change may also alter the relative contribution of the Cl battery (E_{Cl}) to the membrane potential, if E_{Cl} is markedly different from the resting potential at zero current. In fact, the parallel curves of Fig. 5 are spaced about 40 mv apart, whereas the difference in resting potential is only about 25 mv. Thus, it is conceivable that E_{Cl} may be considerably more negative than −101 mv, perhaps because in the K-free medium KCl is lost from the muscle fibers. In lobster muscle fibers kept in a K-free medium E_{Cl} becomes very markedly inside-negative, approaching values of about −125 mv (Grundfest and Reuben, 1961). However, the data now available on the crayfish fibers are insufficient to carry the analysis further. Another matter which requires additional study is the fact that the time-variant curvatures in the *I-E* relations which denote hyperpolarizing Cl activation and depolarizing K activation were initiated at about the same values of applied current in the control and after Cs treatment of the crayfish muscles. During K activation the *I-E* relations in the control and in the experimental conditions became coincident, indicating that the contribution of the K battery (E_K) was important in setting the level of the curves. This is also indicated by the marked shift of the *I-E* relation for the depolarizing quadrant when the preparations were bathed in a K-free medium (Fig. 7).

Low threshold hyperpolarizing Cl activation is absent or insignificant in lobster muscle fibers. Thus, on treatment of the fibers with Cs only an increase in resistance which is due to pharmacological K inactivation is evidenced in the hyperpolarizing quadrant. Although the resting potentials of the lobster and crayfish fibers differed both in the control and in the experimental conditions, the curvature of the *I-E* relation which denotes K activation occurred at about the same values of membrane depolarization. As in the crayfish fibers, the *I–E* relation in the control condition and after treatment with Cs became nearly coincident.

The fact that the conductance increase due to hyperpolarizing Cl activa-

tion could be eliminated or diminished in the presence of Cs (Figs. 6 and 7) emphasizes the independence of the processes of hyperpolarizing Cl activation and of pharmacological K inactivation. Regenerative responses due to hyperpolarizing K inactivation like those which normally are observed in lobster muscle fibers (Reuben et al., 1961) can also be obtained in crayfish muscle fibers if the effects of Cl activation are diminished (cf. Grundfest, 1963, Fig. 23). In the Cs-treated preparations they do not occur, however, even when the Cl conductance is eliminated (Figs. 6 and 7). This finding indicates that the pharmacological K inactivation produced by the Cs also eliminates those channels which would have responded with hyperpolarizing K inactivation. A similar relation between pharmacological and hyperpolarizing K inactivation has also been observed in lobster muscle fibers (cf. Reuben et al., 1961, Fig. 5). As in the lobster, so also in the crayfish (Figs. 6 and 7), a secondary increase in conductance is still present which is manifested only with large hyperpolarizations.

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