The Ionic Mechanisms of Hyperpolarizing Responses in Lobster Muscle Fibers

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ABSTRACT Lobster muscle fibers develop hyperpolarizing responses when subjected to sufficiently strong hyperpolarizing currents. In contrast to axons of frog, toad, and squid, the muscle fibers produce their responses without the need for prior depolarization in high external K^+. Responses begin at a threshold polarization (50 to 70 mv), the potential reaching 150 to 200 mv hyperpolarization while the current remains constant. The increased polarization develops at first slowly, then becomes rapid. It usually subsides from its peak spontaneously, falling temporarily to a potential less hyperpolarized than at threshold for the response. As long as current is applied there can be oscillatory behavior with sequential rise and subsidence of the polarization, repeating a number of times. Withdrawal of current leads to rapid return of the potential to the resting level and a small, brief depolarization. Associated with the latter, but of longer duration, is an increased conductance whose magnitude and duration increase with the antecedent current. Hyperpolarizing responses of lobster muscle fibers are due to increased membrane resistance caused by hyperpolarizing K inactivation. The oscillatory characteristic of the response is due to a delayed superimposed and prolonged increase in membrane permeability, probably for Na^+ and for either K^+ or Cl^-. The hyperpolarizing responses of other tissues also appear to result from hyperpolarizing K inactivation, on which is superimposed an increased conductance for some other ion or ions.

INTRODUCTION

A number of workers have reported "hyperpolarizing responses" in various types of cells. They are manifested as a non-linear increase in membrane polarization when an applied hyperpolarizing current is raised above some threshold value. The hyperpolarization may diminish while the current remains constant and may then increase again. These changes in potential

give rise to one or more negative-going, spike-like pulses of considerable amplitude which, however, differ from the spikes of conductile activity not only in sign but also in time scale. Also, unlike the spike, the hyperpolarizing response is terminated when the current is withdrawn, although a small depolarization, or in some cells a hyperpolarization, may persist for a considerable time.

Hyperpolarizing responses were first observed by Lorente de Nó (34) in frog nerves, but were firmly established by their discovery independently by a number of investigators in single frog axons (37, 45), squid axons (43), Gymnotid electroplaques (4, 5), and lobster muscle fibers (22, 23, 41, 42). They have been studied in some detail recently in squid axons (36, 47), nodes of frog (35, 46), and toad (47) axons, dog cardiac muscle (7), *Noctiluca* (6) and *Raia* electroplaques (8, 9).

In the axons and in cardiac muscle, hyperpolarizing responses are elicited only when the cells are first depolarized by high external K^+. The hyperpolarizing responses of *Noctiluca* develop in the absence of a recorded resting potential (6). In electroplaques of Gymnotids and of *Raia* and in lobster muscle fibers they occur without prior depolarization in cells which have inside-negative resting potentials of some 60 to 90 mv. Thus, the view that hyperpolarizing responses denote a return from a "stable" depolarized state (44) cannot apply to the electroplaques and muscle fibers.

Lobster muscle fibers offer especially favorable material for analysis of the nature of hyperpolarizing responses. A considerable amount of data is available on the properties of the electrically excitable membrane in which the hyperpolarizing responses presumably arise (38, 41, 48) as well as on the electrically inexcitable membranes of the inhibitory and excitatory synapses (21, 22, 39, 40). Of particular value is the fact that the membrane of the inhibitory synapses becomes a very effective Cl electrode during activity (22). The preparations also have the advantage that a number of fibers in the same muscle may be examined simultaneously or consecutively. Concurrent studies of the electrically excitable membrane of crayfish muscle fibers (13, 14) were also helpful, since they provide comparative data on muscle fibers which do not exhibit hyperpolarizing responses when bathed in their normal medium.

METHODS

The extensor (stretcher) muscle of the propodite of the walking legs of *H. americanus* was used, prepared as described previously (22). Currents were applied intracellularly through KCl-filled micropipettes. The amplitudes of these stimuli were usually monitored on one trace of a cathode ray oscillograph. The changes in membrane potential were simultaneously recorded with another KCl-filled microelectrode, coupled to a second trace of the oscillograph through a neutralized-capacity amplifier

(2). However, in a number of experiments the data were recorded with a two-channel ink-writing oscillograph, to register long term events.

The standard Ringer's solution used was that developed by Cole (10). However, in experiments in which addition of Ba^{++}, Ca^{++}, or Sr^{++} was required the $SO_4^{=}$ was replaced with acetate or Cl^-. Acetate or NO_3^- was used to replace Cl^- in some experiments and choline was used to substitute for Na^+ in others.

RESULTS

Characteristics of the Hyperpolarizing Responses

FORM OF THE RESPONSE The responses occurred in many, but not all muscle fibers of a given preparation, and were most readily obtained in fresh preparations. They developed when the membrane was hyperpolarized by 50 to 70 mv in different preparations. Before this threshold value was attained (Fig. 1*A*), the polarization produced by an inward current applied through an intracellular microelectrode (*i.e.* with the latter negative) had the form expected for an electrotonic change in membrane potential (31, 34), the polarization rising and falling with a time constant ranging from 30 to 60 msec. in different fibers. At threshold for the response (*B*, *C*) the polarization did not stay at the plateau value that might have been predicted from the current-voltage relation for smaller currents, but continued to increase, slowly at first, and then more rapidly. The duration of the initial slow phase varied considerably with liminal applied currents (*B*, *C*, *E*) so that the latency of the onset of the large change in membrane potential was variable. In successive trials the latency might undergo progressive shortening or lengthening, although the applied current remained constant (*E*). Stronger currents, however, always tended to initiate the pulse earlier (*B-D*). This sharp rise in membrane polarization attained a maximum that was dependent to some degree on the applied current (*B*, *D*), and which was somewhat greater if the peak was attained early (*E*).

Usually, soon after the peak polarization was attained, and although the current was still being applied, there began a decline, at first slow, then more rapid (*B-D*), which produced the characteristic spike-like appearance of the hyperpolarizing response. The membrane potential temporarily became considerably less negative than it had been at the threshold for the hyperpolarizing response, but the negativity then began to increase slowly. If the initial pulse had developed early (*D*) or if the current was applied for a long enough time, a second spike-like pulse developed and in fresh preparations the oscillation was frequently repeated a number of times (Fig. 2).

The hyperpolarizing responses were quite labile in their characteristics. The pulses varied in duration, and as will be shown below, the durations could be modified. Sometimes the hyperpolarizing response appeared as a

peak followed by a plateau at a slightly less negative value of the potential (Fig. 1*E*). Variations in the form appeared "spontaneously" and could also be induced by repeated hyperpolarization of the fiber, particularly

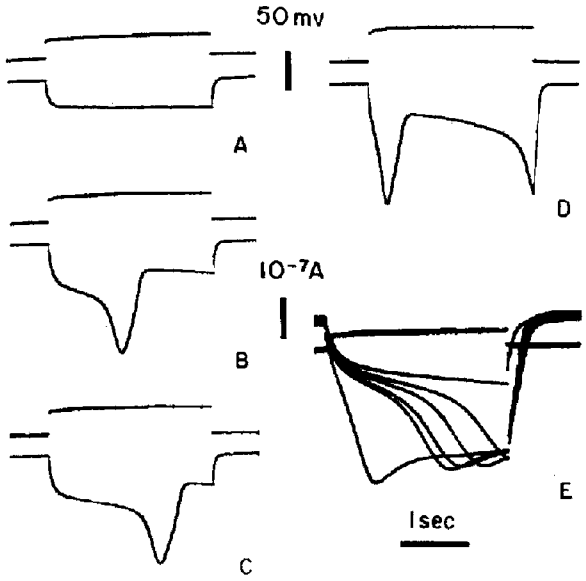

FIGURE 1. Varieties of hyperpolarizing responses. *A–D*, records from one fiber. Current monitoring on upper trace, upward deflection for inward current. *A*, subthreshold current. *B*, *C*, responses to the same liminal current. The pulse phase arose with different latencies out of the gradually increasing hyperpolarization. The pulses subsided at first slowly, then more rapidly into relative depolarization. A second rise in polarization was developing in *B* when the current was withdrawn. The membrane returned to its resting potential. *D*, early onset of the pulse phase with a stronger current. The amplitude was slightly larger. The duration was briefer than with weak currents. The membrane potential became more negative again and another pulse developed as the current was withdrawn. Note that the return to the base line was rapid and without an inflection (*cf.* Fig. 8). *E*, another fiber of the same preparation, six superimposed sequences, showing variation of the effects of a too frequent application of a current (at about 20 sec. intervals). The first response was a large hyperpolarization, the peak subsiding to a smaller plateau. Subsequent responses developed progressively later, due to more gradual onset of the slow increase in membrane potential. In the last of the responses the rise was so slow that a pulse phase did not develop. There was a slight depolarizing shift in the resting potential of the fiber denoted by the thickness of the superimposed terminal portions of the voltage traces.

on applying currents more frequently than 1/min. These effects probably arose from long lasting changes in the membrane properties that will be described below. However, the pattern of responses evoked by a constant inward current was remarkably reproducible in many experiments.

ABOLITION OF A HYPERPOLARIZING RESPONSE BY BRIEF DEPOLARIZING CURRENTS Fig. 2 shows an example of the pattern of an oscillatory sequence of three hyperpolarizing pulses, evoked by a long lasting current. Weak, brief depolarizing currents applied during the sequence were without effect (*B*). Stronger depolarizations delayed (*C*), and still stronger ones abolished (*D*) the next response of the train, but abolition of the response did not eliminate the process which led to production of a subsequent hyperpolarizing pulse.

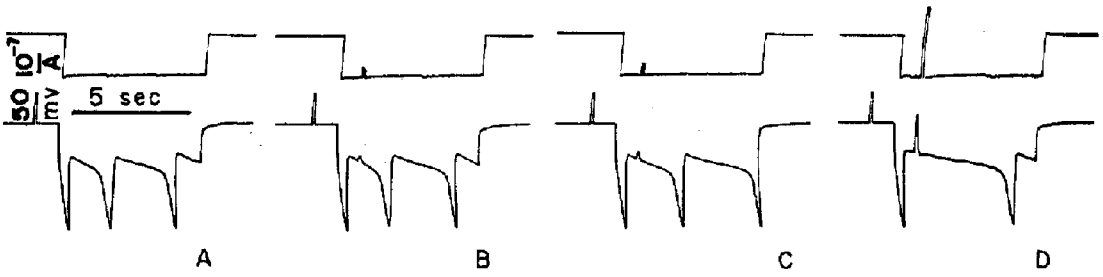

FIGURE 2. Modification of a train of oscillatory hyperpolarizing responses by a brief depolarizing pulse. Pen recorder traces, upper monitoring the current, inward currents down. Weak pulses had little effect. Stronger stimuli delayed or blocked production of the second of the train of three hyperpolarizing pulses.

THRESHOLD AND REFRACTORINESS When muscle fibers were hyperpolarized somewhat less than the amount necessary to evoke a hyperpolarizing response, a small brief additional current could evoke the response (Fig. 3*C*). The peak of the hyperpolarization during the pulse phase of the latter was almost as large as when the pulse was initiated by a long lasting supra threshold hyperpolarizing current (*D-F*). Record *C* shows with particular clarity the diminished membrane polarization with which the pulse phase of the response terminates and the subsequent gradual rise of the polarization.

The fiber developed a graded "abortive" hyperpolarizing response with a slightly weaker current pulse (*B*). Smaller responses also occurred when strong brief currents were applied after hyperpolarizing responses had already been evoked (*E*, *F*), and the fiber was relatively refractory (*D-F*).

The Nature of the Membrane Events In Hyperpolarizing Responses

The data of the foregoing section have shown clearly that the hyperpolarizing responses of lobster muscle fibers represent manifestations of voltage-induced reactions of the membrane. They appear to involve some regenerative process, since a weak pulse can trigger a hyperpolarizing response (Fig. 3). The nature of the membrane changes that are initiated by hyperpolarizing currents is explored in the experiments of this section.

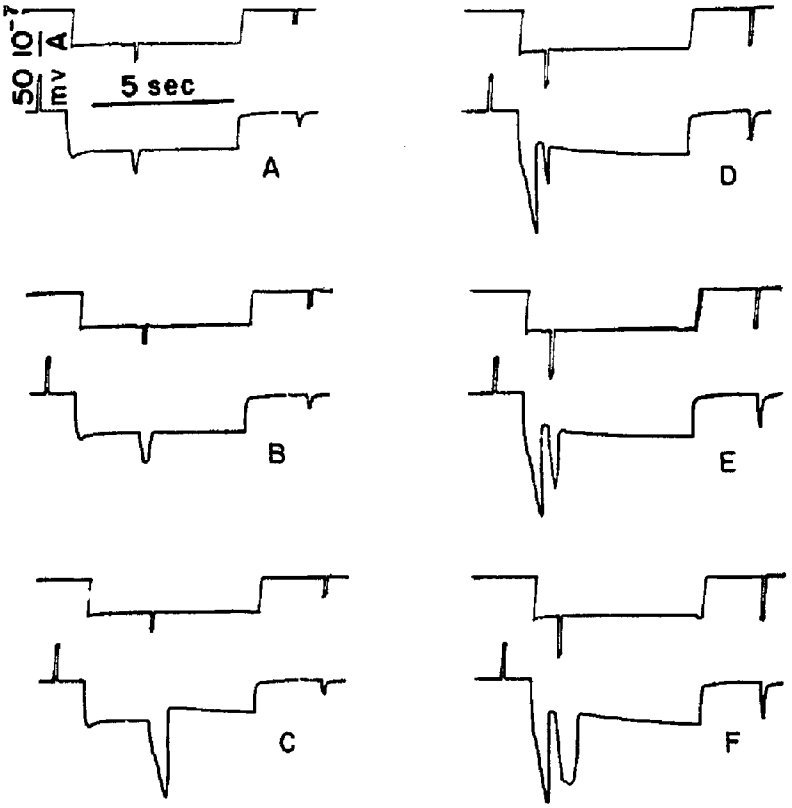

FIGURE 3. Gradation of hyperpolarizing responses, at threshold (*left*) and after a previous response (*right*). Pen-recorder traces, upper monitoring the current, inward current down. The initial deflection on the voltage traces is a 50 mv calibrating pulse. *Left*, Three sequences with a long lasting current pulse slightly subliminal for a hyperpolarizing response. A brief pulse of constant duration, but slightly increasing amplitudes was injected during the large current. The effects of the same brief pulses in isolation are shown at the ends of the records. The durations and amplitudes of their effects during the background hyperpolarization were markedly greater, and a full fledged hyperpolarizing response developed in the lowest set of records. Note the relative depolarization and gradual rise in hyperpolarization when the pulse of the hyperpolarizing response terminated. *Right*, The same fiber. A hyperpolarizing response developed early on applying stronger hyperpolarizing currents. The brief pulses were also stronger and were increased slightly in successive records (downward). The amplitudes and durations of the potentials which they evoked during the long lasting current were disproportionately larger, particularly in the middle and lowest records, in comparison with their effects after the long lasting current was withdrawn.

CURRENT-VOLTAGE RELATION DURING HYPERPOLARIZATION The relation between applied current and the maximum hyperpolarization that it caused is shown in Fig. 4 for three muscle fibers of a single preparation. At low values of hyperpolarizing currents the polarization increased nearly

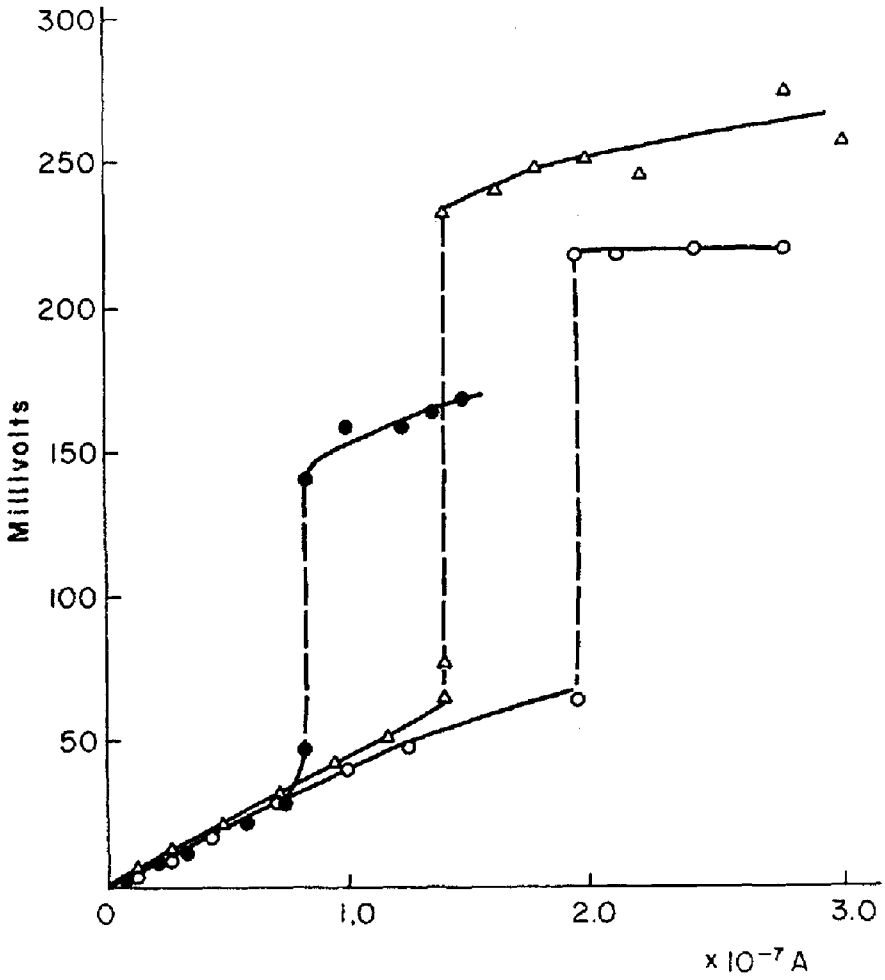

FIGURE 4. Current-voltage relation during hyperpolarizing responses. Three fibers of one preparation; *abcissa*, inward current; *ordinate*, hyperpolarization. The fibers had nearly the same initial effective resistance, but differed in threshold for the hyperpolarizing responses. The occurrence of the latter is shown by the abrupt rise in hyperpolarization (*broken lines*). The peak hyperpolarization subsequently changed relatively little with increasing current. The slopes of the upper lines varied considerably.

identically in all three fibers, the slope of the line indicating an effective resistance of about 4×10^5 ohms. At currents producing hyperpolarization between 50 and 70 mv in different fibers the potential rose steeply between three- and fourfold. However, further increase in the current caused relatively little increase in polarization, indicating that the dynamic (or "slope") resistance of the muscle fiber had fallen. The slope of this portion of the current-voltage relation varied considerably from fiber to fiber in different ex-

periments, and could also be modified experimentally. When currents were applied at intervals less than 1 min. apart (open circles) the line tended to become parallel to the current axis, signifying a very high dynamic membrane conductance.

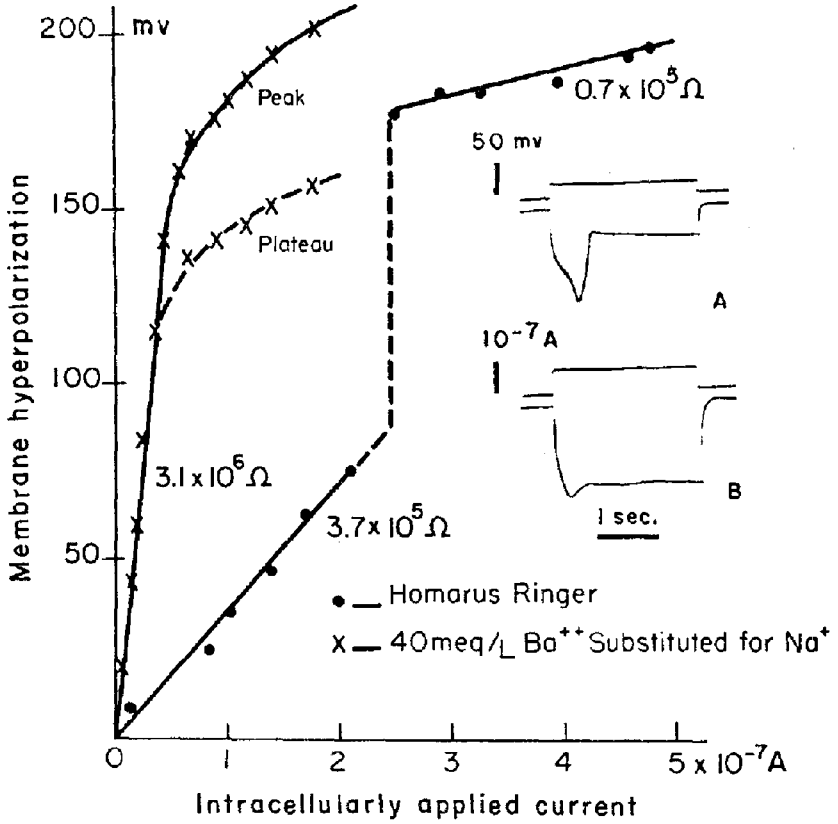

FIGURE 5. Abolition of the hyperpolarizing response by alkali-earth ions. The two graphs are voltage-current curves (as in Fig. 4) for a single fiber before and after treatment with Ba^{++}. *Inset records*, potentials for a fiber from another experiment before (*A*) and after (*B*) applying 4x Ca^{++}. Despite a current twice as large in *B* as in *A*, the peak polarization was about the same.

EFFECTS OF IONS Substitution of $NaNO_3$, Na acetate, or choline chloride for NaCl did not prevent the hyperpolarizing responses and did not alter their thresholds. Hyperpolarizing responses are also obtained in preparations in which all Cl^- is replaced by pyroglutamate (12). Somewhat increased external K^+ increased the threshold hyperpolarization required to elicit the responses. On doubling external K^+ there was no hyperpolarizing response to applied hyperpolarization of up to 100 mv. Removing K^+ from the bathing medium partially or completely blocked the response.

Addition of Ca^{++}, Ba^{++}, or Sr^{++}, the two latter in very low concentrations, resulted in disappearance of the responses (Fig. 5). That the hyperpolarizing responses are eliminated when the muscle fibers are exposed to Ba^{++} or Ca^{++} is of considerable significance, since the membrane resistance of lobster muscle fiber is increased markedly by Ba^{++} and Sr^{++} and to a lesser degree by Ca^{++} (22, 48). The same peak polarization that was attained during the hyperpolarizing response in the untreated fiber was caused by a much smaller current after treating the preparation with 40 meq/liter Ba^{++}, and this value was then attained along a smooth curve. However, for stronger hyperpolarizing currents, the increase in polarization declined and the peak that was attained initially decreased when the current was maintained. This change in slope appears to be similar to that of "anodal breakdown" frequently encountered in other cells (3, 24, 32). It is noteworthy that the "breakdown" in the lobster muscle fiber occurred at about the same value of membrane potential as did the flattened upper portion of the current-voltage curve of the hyperpolarizing responses.

INTERACTION OF E.P.S.P.'S AND HYPERPOLARIZING RESPONSES Since the excitatory postsynaptic potential (e.p.s.p.) is generated in an electrically inexcitable membrane component (17, 18) the conductance of this membrane component and its electrogenic properties should be unaffected by the changes in membrane potential during hyperpolarizing responses (*cf.* reference 20). The change in amplitude of the e.p.s.p. at a given membrane potential therefore indicates the relative effectiveness of "short-circuiting" of the membrane generator (11) by the electrically inexcitable membrane. A train of testing e.p.s.p.'s was evoked during hyperpolarization of the membrane to various degrees by applied currents (Fig. 6). For increasing hyperpolarization of the membrane up to about 50 mv the amplitude of the e.p.s.p.'s rose progressively in a linear manner. When the applied current was large enough to initiate a hyperpolarizing response the e.p.s.p.'s also increased linearly with the increasing membrane polarization, but the slope of the relation was about two- to threefold steeper. This change indicates that the resistance which the synaptic membrane was short-circuiting had increased, so that the short-circuit was more effective. The increase in dynamic resistance which is indicated by the increased slope occurred abruptly.

RESISTANCE DURING HYPERPOLARIZING RESPONSES The sum of the foregoing experiments indicates that the hyperpolarizing responses were accompanied by and, perhaps, resulted from an increase in membrane resistance. Measurement of the resistance change by testing pulses applied during the hyperpolarizing response involves difficulties, however, because the response develops at a threshold hyperpolarization. The currents that must be applied

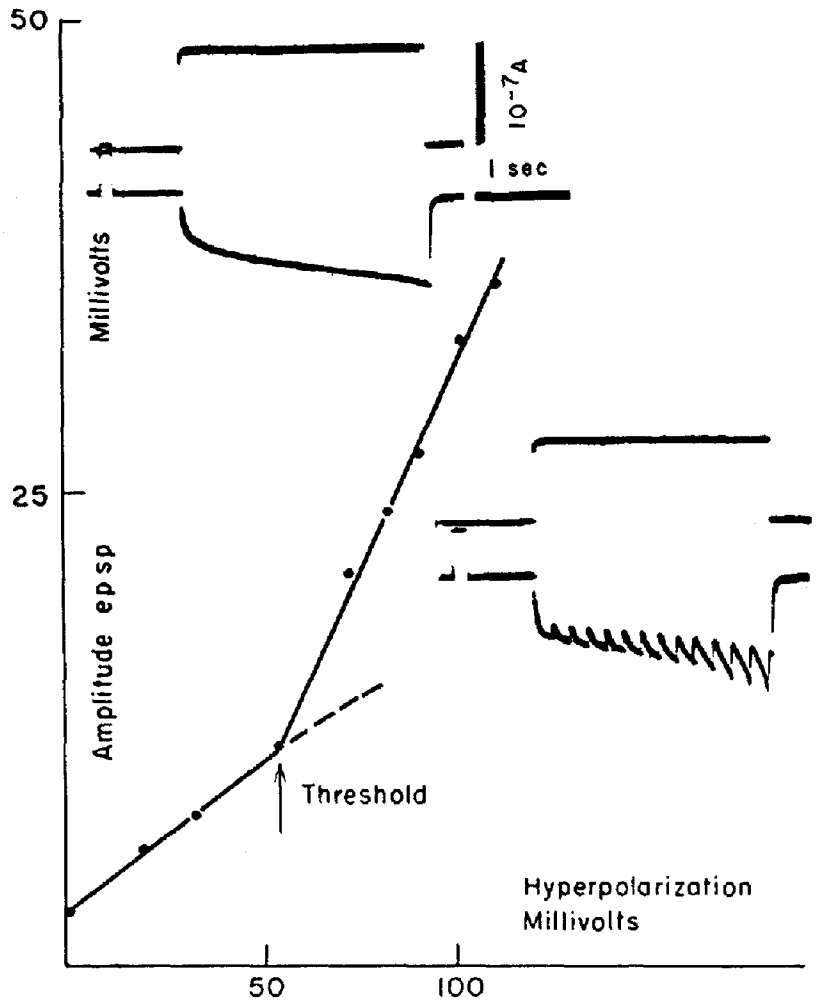

FIGURE 6. Abrupt change in membrane properties as measured by amplitudes of excitatory postsynaptic potentials (e.p.s.p.'s). Inset records show the type of records on which the graph is based. *Upper left,* The gradually increasing membrane potential to a constant hyperpolarizing current. Calibration on voltage trace is 50 mv. *Lower right,* the excitatory axon was stimulated at 5/sec. during application of the hyperpolarizing current to the muscle fiber. Note the increase in amplitude of the e.p.s.p.'s and the marked prolongation of their falling phases. *Graph,* the increase in amplitude of the e.p.s.p.'s with increasing membrane hyperpolarization took a different slope when at a threshold of 55 mv hyperpolarization hyperpolarizing responses were initiated.

for the usual type of measurement with depolarizing (Fig. 6) or hyperpolarizing (Fig. 7) pulses therefore tend respectively to suppress or to evoke hyperpolarizing responses. The increased amplitudes of the testing hyperpolarizing pulses (Fig. 7*B*) during the response, nevertheless, do indicate an

increase in membrane resistance of the muscle fibers as the membrane polarization increased.

Quantitative data for the resistance during the peak of the hyperpolarizing response were obtained by evoking the response with a brief strong pulse of hyperpolarizing current (Fig. 8). When the current pulse was terminated at the peak of the hyperpolarizing response, the membrane polarization declined exponentially, at first with a time constant of 390 msec. When the membrane polarization fell to about 75 mv the time constant changed to that of the unpolarized cell, 45 msec. A series of experiments of this type, on muscle fibers of another preparation, yielded average values of 315 msec.

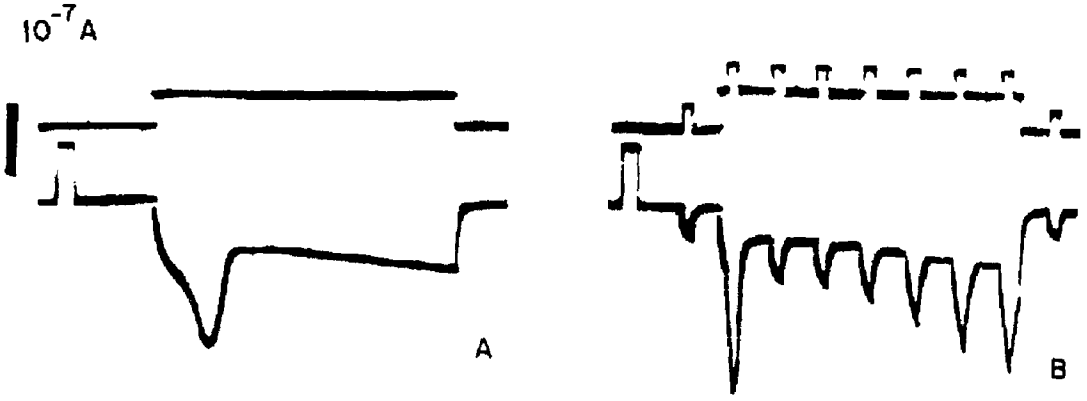

FIGURE 7. Distortion of a hyperpolarizing response by brief hyperpolarizing pulses. *A*, hyperpolarizing response. *B*, effect of a train of superimposed brief hyperpolarizing pulses. Calibrating pulses on voltage traces, 50 mv and 100 msec.

and 45 msec. for the time constants of the two phases, indicating a sevenfold greater effective resistance immediately after the peak of the hyperpolarization. It will be recalled (Fig. 5) that Ba^{++} increased the resistance of the muscle fiber to about the same degree as did the brief hyperpolarizing pulse.

ELECTRODE PROPERTIES OF THE MEMBRANE DURING THE HYPERPOLARIZING RESPONSE A change of the membrane from a K electrode to a Cl electrode should signify an increase in membrane conductance for the latter ion relative to the condition of the resting membrane (18, 26). Since the membrane resistance increased markedly with the development of hyperpolarizing responses, the change in electrode properties (if this occurred) would necessarily be due to a relative increase in Cl conductance resulting from marked decrease in K conductance (as occurs in frog muscle fibers under some conditions; *cf.* references 1 and 19). Since the equilibrium potentials for K^+ and Cl^- are near the resting potential in lobster muscle fibers (22) this change

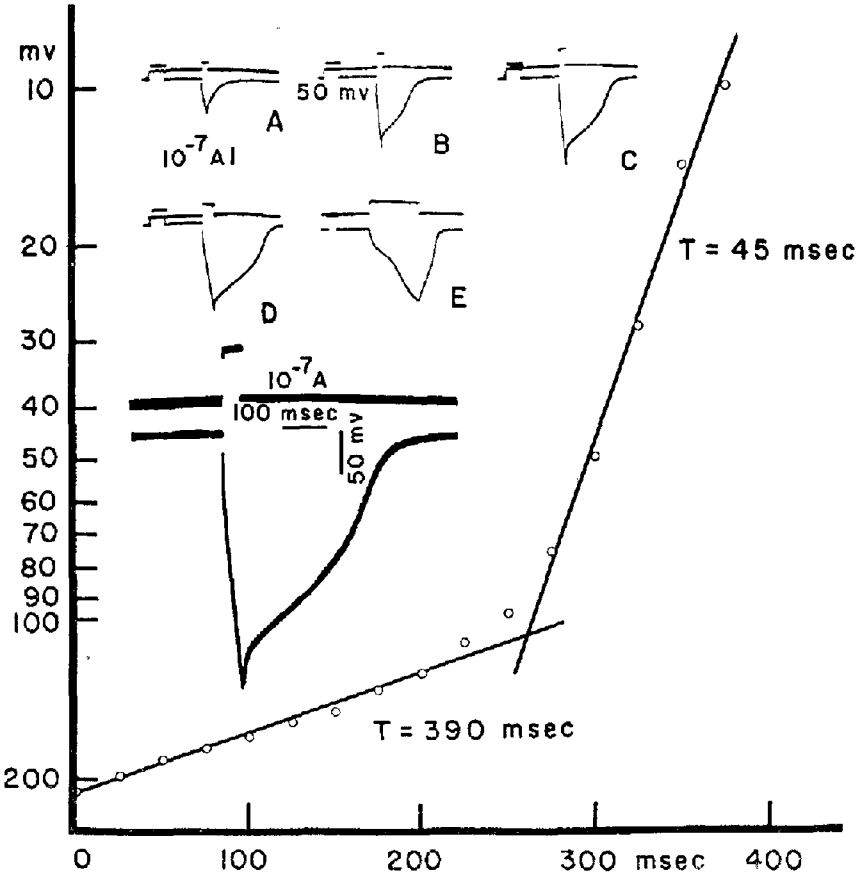

FIGURE 8. Change in time constant of the membrane during subsidence of hyperpolarizing responses. *Upper inset records*, magnitude of hyperpolarizing currents on upper (current monitor) trace. Each voltage trace below carries a 100 msec. and 50 mv calibrating pulse. Note that record *E* was made at half the sweep speed of *A–D*. *A–C*, 30 msec. pulses of increasing intensity. *D*, *E*, 60 and 600 msec. pulses respectively at same intensity as in *B* (0.75×10^{-7} A). *Lower inset trace*, enlarged record of *C* above. *Graph*, a semilogarithmic plot of the membrane voltage at various times after the end of the 30 msec. pulse. The initial sharp change in potential due to subsidence of the capacitative artifact was neglected. The time constant of the subsequent decay of membrane potential was almost nine times as large as the time constant of the terminal phase. The value of the latter (45 msec.) was also that found in the resting muscle fiber. The resting potential is taken as the final value for both decay curves.

in electrode properties should not markedly alter the membrane potential, when the preparation is in its standard medium.

The membrane does become a very effective Cl electrode during activity of inhibitory synapses in lobster muscle fibers (13, 14, 22). When a train of fused inhibitory postsynaptic potentials (i.p.s.p.'s) was evoked in different

phases of the hyperpolarizing responses (Fig. 9) the membrane potential fell rapidly to a value about 12 mv more negative than the resting potential. This may be considered as representing the sum of the equilibrium potential for Cl^- and the small voltage drop caused by the applied hyperpolarizing

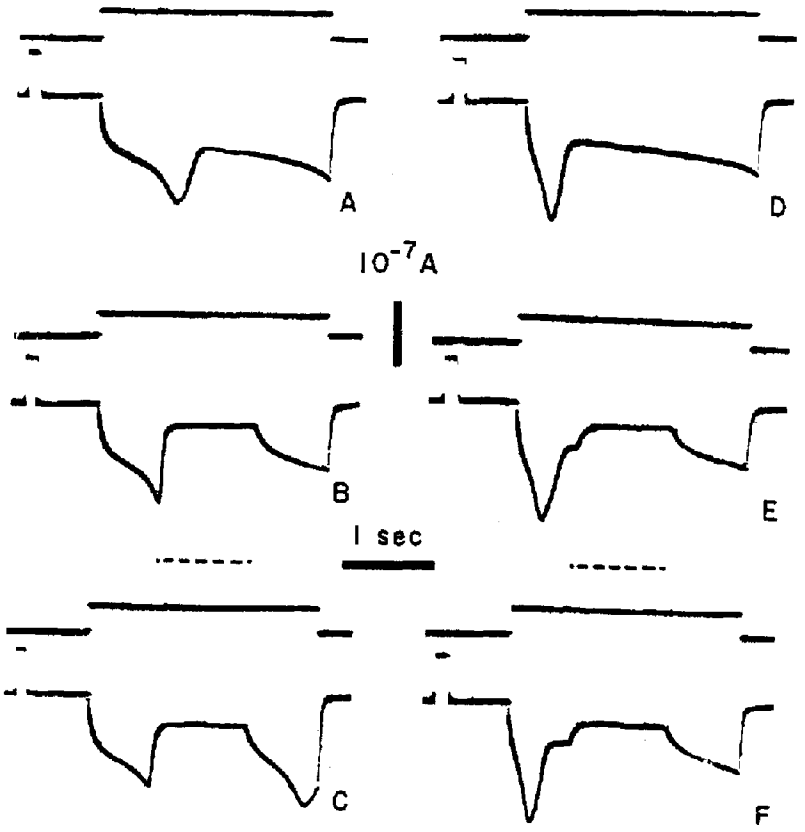

FIGURE 9. Effect of inhibitory p.s.p.'s on membrane potential during hyperpolarizing responses. The same muscle fiber exhibited two types of hyperpolarizing responses, with long latency (*A–C*) and with short (*D–E*). *B*, *C* and *E*, *F*, stimulation of the inhibitory axon reset the membrane potential to the same value independently of the potential that prevailed during the hyperpolarizing response. Voltage calibrating pulses of 50 mv at beginning of each trace. The broken lines indicate stimulation of the inhibitory axon at 100/sec.

current flowing through a membrane of greatly diminished resistance. When the synaptic activity was terminated the fiber again developed the characteristic pattern of the hyperpolarizing response, the polarization rising again slowly and developing (record *C*) a pulse phase. Thus, while the membrane conductance for K^+ had fallen during a hyperpolarizing response, this change apparently did not lead to conversion of the membrane to a Cl electrode.

Subsidence of Hyperpolarizing Pulses

MASKED CONDUCTANCE CHANGES If the hyperpolarizing response is, indeed, due to an increase in membrane resistance, the diminished membrane polarization which gives the hyperpolarizing response its pulse-like component accordingly must represent a decrease in resistance during continued application of a constant current. The subsequent rise of potential and the oscillation in the latter indicate further alterations in the resistance. Other data also indicate that there are changes in resistance even during development of the hyperpolarizing responses. Thus, the resistance is not maintained as a seven- or eightfold increase during the pulse. This is shown by the peak value of potential attained by the hyperpolarizing response (Figs. 3, 4, and 5). At the threshold current it rose only some three- to fivefold, and for higher currents the increments of hyperpolarization became very small (Figs. 4 and 5). Thus, in addition to causing an increase in resistance the hyperpolarizing currents themselves effect another change, to lower the resistance. This change seems related to the anodal breakdown which occurs in the high resistance Ba^{++}-treated muscle fibers (Fig. 5).

INCREASED AFTER-CONDUCTANCE Further data on the additional effects of hyperpolarizing currents are presented in Fig. 10 with recordings over long periods of time after the hyperpolarizing responses. During the application of a hyperpolarizing current the pen-writer chart speed was increased 50-fold. Brief, testing hyperpolarizing pulses were applied during the phase of slow speed recording to indicate the change in membrane conductance caused by the hyperpolarizing response. On the occurrence of a hyperpolarizing response (*B*) there was a small after-depolarization and a temporary decrease in the amplitudes of the testing pulses. The after-depolarization increased with stronger hyperpolarizing currents, the testing pulses decreased further, and the conductance increase, that this change denoted, lasted longer. The duration of the spike-like phase of the hyperpolarizing response decreased, but the amplitude of the peak and the level of the subsequent plateau phase did not increase in proportion to the current.

The data of Fig. 10, like those of Figs. 4 and 5, also suggest that the conductance increase, which is clearly seen after the hyperpolarizing current is terminated, already occurs during the application of the current. That effect could be responsible for shortening of the hyperpolarizing pulse, for its diminished peak values, and for the plateau phase. These secondary conductance changes appear to increase with the strength of the hyperpolarizing current and are relatively independent of the duration of the latter. Indeed, as is seen in records *G* to *I* of Fig. 10, the conductance after the hyperpolarizing pulse may diminish again as is indicated by the rise of hyperpolarization

when the current is applied for longer times. The increase in after-conductance was approximately the same after the application of a constant current for different durations (*G* to *I*).

INTERACTION OF HYPERPOLARIZING RESPONSES WITH APPLIED BRIEF PULSES The complex effects of the hyperpolarizing currents on the membrane conductance are further manifested by the seemingly anomalous re-

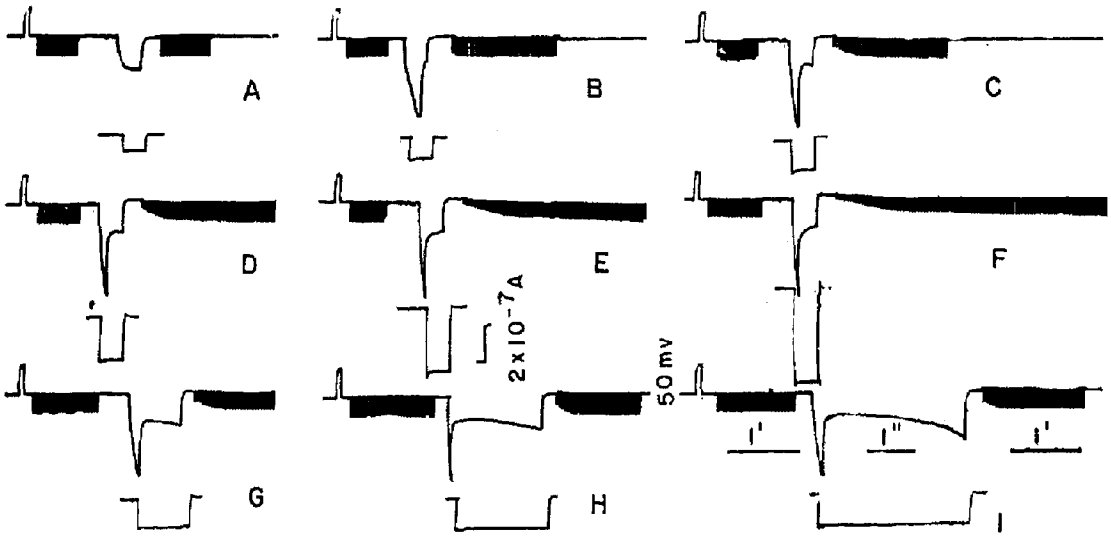

FIGURE 10. Long lasting conductance increase after hyperpolarizing currents. Pen-writer registration of membrane potential in a single muscle fiber. Each voltage trace recorded at two chart speeds. The initial fast phase carried a 50 mv calibrating pulse. The subsequent slow phase shows the deflections produced by a train of brief hyperpolarizing pulses. During the second fast phase a hyperpolarizing current was applied. Current monitoring trace is shown below the voltage record. After the current was terminated the chart speed was reduced, and the train of testing hyperpolarizing pulses was again applied. The decrease in their amplitude indicates the magnitude and time course of the increase in membrane conductance. Two upper rows show records in which the duration of the hyperpolarizing current was constant, but the amplitude was increased successively. Note the increase and prolongation of the after-conductance change. In the records of the lowest row the current was constant, but its duration was increased. There was little additional effect on the conductance change with increasing durations of current.

sults of applying brief hyperpolarizing or depolarizing pulses during a hyperpolarizing response (Fig. 11). An additional hyperpolarizing pulse, when made strong enough, could "abolish" the response (*C*). The membrane polarization diminished much more after the "abolition" than it did when the hyperpolarizing response terminated "spontaneously" (*A*, *D*). Depolarizing pulses applied during the hyperpolarizing response could either abolish the response (*E*) or could initiate a new peak of hyperpolarization (*F*).

DISCUSSION

The data presented above show clearly that at some threshold value of hyperpolarization of lobster muscle fibers there occurs a marked resistance increase. This change is adequate to account for the non-linear rise in membrane polarization to increasing applied currents. However, the complex form of the

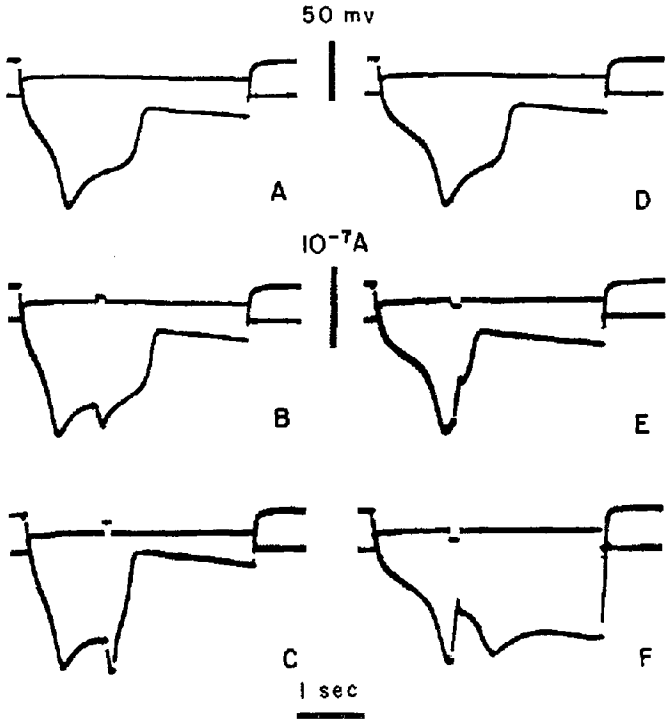

FIGURE 11. Modification of hyperpolarizing responses by brief pulses of either sign. A strong hyperpolarizing pulse (*C*) and a moderate depolarization (*E*) caused an earlier onset of increased conductance with apparent abolition of the hyperpolarizing response. A stronger depolarizing pulse (*F*) decreased the conductance and reinstituted a hyperpolarizing response.

hyperpolarizing response makes it obvious that the change induced by the inward current involves more than a transition from a low resistance "state" to a high value. Additional effects are indicated by the spontaneous subsidence of the hyperpolarization from its peak value, usually to a membrane potential which represents hyperpolarization less than that initially produced by the applied current. The gradual increase in membrane potential, both during the initiation of the hyperpolarizing response by a threshold current, and in the development of subsequent responses after the first during long

applied currents, bespeak of further time- and potential-dependent effects. The totality indicates the occurrence of conductance changes which involve both increase as well as decrease in permeability, the former probably to several ion species.

THE ROLE OF K^+ The initial resistance increase is shown by the data of Figs. 3 to 8. The peak change is a seven- to eightfold increase (Fig. 8), like that produced by alkali-earth ions (Fig. 5), and the increased resistance caused by the alkali-earth ions eliminates the hyperpolarizing response. The increase in resistance of arthropod muscle fibers caused by alkali-earth or onium ions is due to a marked decrease of K conductance of the membrane (48). It is reasonable therefore to regard the hyperpolarizing responses as also due to a decrease of K conductance by the applied current, a phenomenon that is also observed in other contexts and that has been termed hyperpolarizing K inactivation (19). There seems hardly any possibility that some other ion is involved in the increase of resistance, not only because of the magnitude of that change, but also because substitution of Cl^- and of Na^+ did not prevent the hyperpolarizing responses nor affect their threshold.

That K conductance changes are implicated, however, is further indicated by the effects of removal or of addition of K^+. Removal of K^+ from the bathing medium increases the membrane resistance of lobster muscle fibers, and this in itself would tend to operate against development of a hyperpolarizing response, or to diminish its manifestation. Increased K^+ in the medium lowers the membrane resistance markedly chiefly by increasing K conductance (29). The hyperpolarizing currents apparently could not overcome that action, even when the membrane was hyperpolarized by 100 mv. Larger hyperpolarization might have initiated K inactivation, but would have required currents larger than could be delivered through the microelectrode.

ADDITIONAL FACTORS If the increased polarization is due to a resistance increase, then the spontaneous termination of the large hyperpolarization accordingly denotes that the membrane resistance becomes lower again, while the current is maintained. The membrane potential during this plateau becomes temporarily even less negative than it was at the beginning of the pulse phase (Figs. 1 and 3). Furthermore, after the hyperpolarizing current is withdrawn the membrane conductance is higher than in the resting fiber (Fig. 10). Since the membrane also becomes slightly depolarized it seems likely that the increased after-conductance and probably also the increased conductance during the plateau of the hyperpolarizing responses (Fig. 5) are both due in part to increased Na permeability. This might result from diminution of Na inactivation (30) during the hyperpolarization. Another ion of repolarizing electrogenesis, and therefore either Cl^- or K^+, must also be in-

volved, for the conductance increase after the current is turned off may be large in relation to the depolarization (Fig. 10). If Na^+ alone were involved the change in potential probably would have been much greater and might have led to anode break responses.

MEMBRANE PROCESSES The kinetics of the changes in membrane conductance can only be inferred at the present time. The gradual and sometimes very prolonged rise in membrane potential which initiates the hyperpolarizing response indicates that the permeability to K^+ decreases at first slowly, once a threshold hyperpolarization is attained, but that at some new value of the potential the rate of change is greatly augmented. This regenerative behavior and the subsequent subsidence of the pulse phase lead to potentials which bear a striking resemblance to various types of oscillatory potentials during prolonged spikes (19) or "upside-down" responses of frog muscle (1). The possibility that the same general properties are inherent in the behavior of populations of "ion valves" (15, 16) has been discussed elsewhere (19).

It seems likely that the increased membrane conductance develops with a delay relative to the initial decrease in conductance. However, the delay probably is shortened with strong hyperpolarizing currents. The peak hyperpolarizations produced by sustained applied currents are never as large as might be expected from the data with brief pulses (Fig. 8). Furthermore, the decay of hyperpolarization after a pulse lasting 600 msec. (*E*) was significantly more rapid than that following briefer pulses (*C*, *D*). A delayed increased conductance is also indicated from the diminished rate of increase of the peak hyperpolarization with increasing applied currents (Figs. 4, 5, and 10). The latter effect may be identified with the "breakdown" observed in the Ba^{++}-treated fibers (Fig. 5) and in other tissues, as noted above. However, it seems unnecessary to apply that term in the present case, since the decreased resistance is obviously only a temporary state. Return to the high resistance condition can be initiated by applied hyperpolarizing pulses (Figs. 3 and 7) or it may occur spontaneously during applied currents (Figs. 1 and 2).

The time course of the hyperpolarizing response, both at its beginning and its termination represents two "steps" (47), although these steps are not necessarily homologous. The beginning of the hyperpolarizing response is at first a slow increase in membrane resistance (Figs. 1 and 6) with a subsequent more rapid transition to a maximal value. The termination of the pulse (Fig. 8) is at first a slow and then a more rapid return to the resting potential. The sequence of changes may be repeated several times (Fig. 2), ending with some depolarization and heightened conductance (Fig. 10).

HYPERPOLARIZING RESPONSES IN OTHER CELLS The processes which

appear to be responsible for the hyperpolarizing responses of lobster muscle fibers are probably also present in other cells (19). The requirement that axons and cardiac muscle fibers, unlike lobster muscle fibers, must first be depolarized in order to exhibit a hyperpolarizing response can be ascribed to differences in the resting K permeability of various cells. Frog axons in "good" condition have a high resting resistance and are insensitive to increased external K^+ (45). The membrane resistance is lowered on soaking in high K^+, but hyperpolarization reduces K sensitivity and reinstitutes a high resistance (45, Figs. 4 and 7). Thus, K inactivation by the applied current accounts for the hyperpolarizing response (19).

Secondary effects which, as in lobster muscle fibers, cause increased conductance even during the application of a hyperpolarizing current and lead to changes in membrane potential when the current is terminated have been described in toad and squid axons (47). Termination of the hyperpolarizing current leaves the toad axon somewhat hyperpolarized, but the available data are insufficient for analysis of this effect. However, in a Na-rich medium the hyperpolarization may be cut short or abolished, with onset of a brief depolarization that may attain 50 mv. The depolarization thus may be regarded as an anodal break response, which in the depolarized axon is probably graded (33).

In squid axons the hyperpolarizing response terminates with depolarization, as in lobster muscle fibers. Tasaki (47) has noted the similarity of this depolarization to that following hyperpolarization of a TEA-treated axon. The major difference is that the depolarization of the latter develops into a prolonged spike. The spike can be accounted for (19) by specific effects of TEA, pharmacological K inactivation, and block of Na inactivation (48). The depolarizing overshoot at the end of the hyperpolarizing current thus can be regenerative and causes the prolonged spike. A prolonged "spike," but probably involving regenerative Cl activation occurs under appropriate electrochemical conditions in Rajid electroplaques (8, 19).[1]

As in lobster muscle fibers, the depolarizing overshoot observed in the depolarized squid axons when the hyperpolarizing current is terminated is brief (47). However, the conductance increase which is already evidenced during the hyperpolarizing current persists for a much longer time than does the change in membrane potential. The overshoot in the potential is probably associated with increased Na conductance, and is terminated as the K con-

[1] Recent work on Rajid electroplaques (19*a*) has confirmed that an electrically excitable Cl activation is responsible for "spike" evoked in preparations bathed in a Cl-free medium not only by depolarizing stimuli, but also for those produced at the termination of strong hyperpolarizing currents. It seems likely that anode-break responses in general are manifestations of the increased conductance induced by and outlasting the hyperpolarizing current. Under normal electrochemical conditions of excitable cells this increase is for Na^+.

ductance is also increased under the combined influence of the high external K^+ and of the depolarizing overshoot itself (19).

The hyperpolarizing responses of various electroplaques have not yet been analyzed in adequate experimental detail. Study of the responses in Rajid electroplaques should provide strategic data, since these cells, though they have an electrically excitable Cl activation (8, 19) lack the Na activation component that gives rise to the spike in conductile tissues (18). The responses occur in Cl-free media (9) and therefore are probably caused by hyperpolarizing K inactivation. Also potentially important is the fact that crayfish muscle fibers do not normally develop a hyperpolarizing response (14). These fibers have a low resting K conductance (13) and the effects of hyperpolarizing currents are like those seen in lobster muscle fibers treated with alkali-earth ions (Fig. 5). However, under some experimental conditions crayfish muscle fibers develop a slow increase of membrane negativity, like that which initiates the pulse phase of the hyperpolarizing responses in lobster muscle fibers (Fig. 6), and may also develop the pulse phase (14).

HYPERPOLARIZING RESPONSES AND ELECTRODE PROPERTIES OF THE MEMBRANE On the basis of the above data it seems possible to formulate some specifications for the conditions under which hyperpolarizing responses may develop. The membrane must be relatively permeable to K^+; moderate inward currents should produce hyperpolarizing K inactivation; anomalous rectification (*i.e.*, increased conductance for inward currents) if it occurs, should appear only with currents that are much larger than are required for K inactivation (19).

The foregoing specifications ascribe the hyperpolarizing response primarily to a change in K permeability. However, Hodgkin's explanation (*cf.* reference 45) that the hyperpolarizing response of frog nodes represents a shift of the membrane from a K electrode to a Cl electrode is partially correct (19). The membrane of electrogenic cells is a complex electrode for K^+ and Cl^-, and sometimes also for Na^+ (13, 14, 27, 28), but changes in permeability for K^+ and/or Cl^- affect the potential relatively little under steady-state ionic conditions, while the membrane resistance may be markedly changed.

Thus, hyperpolarizing responses are to be regarded as caused by the larger IR drop across an increased membrane resistance when an inward current initiates hyperpolarizing K inactivation. Various manifestations of hyperpolarizing, depolarizing, and pharmacological K inactivation are now known, and some have been subjected to analysis (19). A second dynamic factor, which is manifested by increased membrane conductance and which gives hyperpolarizing responses their pulse-like character, has thus far been explored only in preliminary work. This change appears to involve other ions, besides K^+, and thus may be regarded as altering the electrode properties of

the membrane. To the extent that Na conductance may change there may be a large change in membrane potential. However, the depolarization associated with the change of the membrane toward a Na electrode may be countered by increased permeability for K^+ and/or Cl^-. Thus the change in membrane potential may be small, or even in a hyperpolarizing direction.

Viewed in these terms, the electromotive forces postulated by the ionic theory (25, 26, 30) can account for a considerable number of apparent discrepant "anomalous" phenomena (19), including the hyperpolarizing response.

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