Analysis of Depolarizing and Hyperpolarizing Inactivation Responses in Gymnotid Electroplaques

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ABSTRACT. In electroplaques of several gymnotid fishes hyperpolarizing or depolarizing currents can evoke all-or-none responses that are due to increase in membrane resistance as much as 10- to 12-fold. During a response the emf of the membrane shifts little, if at all, when the cell either is at its normal resting potential, or is depolarized by increasing external K, and in the case of depolarizing responses when either Cl or an impermeant anion is present. Thus, the increase in resistance is due mainly, or perhaps entirely, to decrease in K permeability, termed depolarizing or hyperpolarizing K inactivation, respectively. In voltage clamp measurements the current-voltage relation shows a negative resistance region. This characteristic accounts for the all-or-none initiation and termination of the responses demonstrable in current clamp experiments. Depolarizing inactivation is initiated and reversed too rapidly to measure with present techniques in cells in high K. Both time courses are slowed in cells studied in normal Ringer's. Once established, the high resistance state is maintained as long as an outward current is applied. Hyperpolarizing inactivation occurs in normal Ringer's or with moderate excess K. Its onset is more rapid with stronger stimuli. During prolonged currents it is not maintained; i.e., there is a secondary increase in conductance. Hyperpolarizing inactivation responses exhibit a long refractory period, presumably because of persistence of this secondary increase in conductance.

INTRODUCTION

In response to depolarizing or hyperpolarizing currents excitable cells may exhibit nonlinear voltage changes which have a threshold both for their onset and abolition but which are distinct from the phenomena of spike electrogenesis. These responses involve increase in membrane resistance rather than decrease, as during spikes, and there is little change in membrane emf. Thus, they have been characterized as due to inactivation processes (Grundfest, 1957, 1961, 1963, 1966) in contrast with the activation processes by which conductance is increased (Hodgkin and Huxley, 1952). These "inactivation responses" can be elicited only during extrinsic polarization of the cell and they inevitably terminate when the polarizing current is withdrawn. In some cases

they may be obtained in isosmotic K solutions when there are none of the ionic disequilibria that provide the energy for action potentials. Inactivation responses must involve an ion for which the cell has an appreciable permeability in the resting state and therefore one which is not normally involved in providing the inward current flow that takes place during spike electrogenesis. Thus, in most inactivation responses the change would be in potassium permeability (Grundfest, 1957, 1960).

The analysis of hyperpolarizing responses in lobster muscle fibers (Reuben et al., 1961) demonstrated that they result from an increase in membrane resistance. In these cells hyperpolarizing currents exceeding a certain threshold cause repetitive hyperpolarizing excursions which may involve an increase in resistance to about 10 times the resting value. The muscle fibers are cylinders which are several space constants long and isopotentiality is not maintained over their entire length when currents are applied through a single intracellular electrode. Satisfactory measurements under voltage clamp conditions could not be carried out, thus preventing a full analysis of the phenomenon. Depolarizing K inactivation of eel electroplaques has recently been studied in detail (Nakamura et al., 1965 *b*).

The present work took advantage of the unpublished finding of inactivation responses made during an earlier study of electroplaques of *Gymnotus carapo* (Bennett and Grundfest, 1959 *a*). Electroplaques of some of the weakly electric gymnotid fishes exhibit both depolarizing and hyperpolarizing inactivation responses (Fig. 1), and they are well suited to application of the voltage clamp technique. Both kinds of inactivation responses were studied under constant current (current clamp) as well as under voltage clamp conditions. Each type of data demonstrates the occurrence of K inactivation as a response of the electrically excitable membrane component of the electroplaques to depolarizing and hyperpolarizing currents. Both techniques also demonstrate the existence of negative slope regions in the steady-state voltage current characteristics. It is this property which accounts for the threshold phenomena and regenerative all-or-none responses. Preliminary reports on this work have appeared (Bennett and Grundfest, 1962 *a*, 1962 *b*).

METHODS

The electroplaques studied in the course of this work were from the electric organs of 3 weakly electric gymnotid relatives of the electric eel; *Gymnotus carapo*, *Steatogenys* sp., and a species of *Hypopomus*, the electric organ of which develops a diphasic discharge (Bennett, 1961, 1962; Bennett and Grundfest, 1959 *a*). The electroplaques from the caudal regions of all these species generate diphasic external potentials, although there are minor differences in form. The results obtained with all 3 fishes were essentially identical.

Small pieces of the tail of the fish were normally used. They were bathed in saline and were dissected sufficiently to expose the electroplaques. In some experiments the

cells were studied in the intact fish, after spinal section was performed to eliminate the continuously emitted discharges of the electric organ. Routinely a cell was impaled with two KCl-filled microelectrodes. Current was applied through one electrode and the membrane potential was recorded with the other. The microelectrodes were shielded from one another by an interposed grounded foil. The interelectrode capacitive coupling was sufficiently small so that cross-compensation (Tomita, 1962) proved unnecessary.

In voltage clamp experiments the voltage-monitoring electrode controlled the current applied to the cell through the second intracellular electrode. With electronic circuitry similar to that employed by other workers (Hodgkin et al., 1952; Moore and Cole, 1963) this technique was adequate to clamp the cells during the inactivation responses (see Discussion). In a number of experiments the cell was clamped to a linearly changing or "ramp" voltage. The ramp is termed "increasing" when the voltage change (of either sign) was toward a maximum value and "decreasing" when the initial voltage was maximal and decreased with time. As long as the rate of change of the voltage was slow compared with the rate of change of the characteristics of the membrane, this method allowed presentation of the steady-state current voltage (*I-E*) characteristic during a single sweep of the cathode ray oscilloscope.

Stimulating and recording equipment were standard for the laboratory. The normal bathing medium was *Electrophorus* saline (Altamirano et al., 1953). Media which were made high in potassium were isosmotic, sodium being diminished correspondingly. In some experiments acetate was substituted for chloride. Isosmotic potassium solutions were Ca-free and unbuffered.

RESULTS

A. *Depolarizing Inactivation*

CURRENT CLAMP MEASUREMENTS When spike electrogenesis was abolished in electroplaques that were depolarized by bathing in high K solutions, the cells remained capable of responding in the manner shown in Fig. 1*A* to *C* to the application of outward currents. Responses due to hyperpolarizing inactivation (*D*) will be described below. The depolarizing inactivation responses appeared in an all-or-none manner when the current was at a certain threshold value, and they represented a marked increase in internal positivity (*A*). The responses developed at shorter latency with stronger stimulation (*B*). Although these responses are similar in appearance to prolonged action potentials that are observed in many tissues (Grundfest, 1961), they differed in requiring continued outward current, and in terminating immediately at the end of the polarizing stimuli which evoked them. If the stimulating current was terminated at any time during a response, including on its rising phase, the potential immediately returned toward the base line (*C*). When the current was turned off during a response, the return of the potential to the resting level had two phases. The first phase was slower and, as will be shown below, is ascribable to the persistence of the higher resistance which had caused the response. The

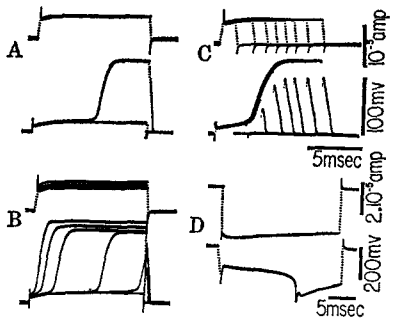

FIGURE 1. Inactivation responses of an electroplaque of *Gymnotus carapo*. The preparation was bathed in a saline solution containing 50 mM/liter KCl. Spike electrogenesis was thereby eliminated. In these and all subsequent figures upper traces are of the currents, upward deflections representing outward flow, and lower traces are of voltage, upward deflections representing increased internal positivity. Rising and falling phases which were not reproduced well in the photographs are indicated by broken lines.

A, two superimposed sweeps with threshold current. A response occurred during one of the sweeps. The change in membrane potential with the effective stimulus was all-or-none, but it terminated when the current pulse ended.

B, six superimposed sweeps, the current increasing successively. An inactivation response began almost at the end of the current pulse during the second sweep but was cut short as the current terminated. In the subsequent sweeps the response arose earlier. The plateau level of the response also increased, as a result of the increased *IR* drop across the elevated resistance of the membrane.

C, eight superimposed sweeps, the duration of the applied current being made successively longer. The two shorter pulses terminated before a response began. The next two terminated before the plateau was reached. Note that the fall of the membrane potential at the end of the longer pulses was slightly slower at first and became more rapid in its terminal phase. This indicates that the membrane capacity was at first discharging through a higher resistance.

D, the same electroplaque was capable of responding to hyperpolarizing currents, but a high curent was required because the cell was in a medium with elevated K. Although the current did not remain constant and diminished gradually due to electrode polarization, the hyperpolarization of the membrane increased, slowly at first and then more rapidly. The subsequent changes in membrane potential indicate a complex of changes in membrane permeability. However, the membrane remained strongly hyperpolarized until the current was terminated. Voltage and current calibrations for *A-C* are the same, time calibrations for *A*, *B*, and *D* are the same.

second, faster phase signaled the return of the resistance to its normal, lower value. The transition from the slower to the faster fall was quite abrupt, indicating that the change from high to low resistance was rapid. The rapidity of the change was demonstrated directly with voltage clamp measurements which will be described below.

That these depolarizing responses resulted from a regenerative process which involved a threshold phenomenon is seen in Fig. 2. In this experiment subthreshold outward currents lasting about 18 msec were applied. On these

were superimposed 2 msec pulses, also of outward current. By carefully adjusting the amplitude of the brief pulse, a value could be found at which the pulse either caused only a brief additional polarization, or the potential rose to a stable, larger value, at which it remained until the longer lasting current was terminated. A response which was initiated by a brief outward pulse could also be abolished by a brief inward pulse (*B*, *D*). The threshold potentials for initiating and terminating a response by the brief pulses were identical for a

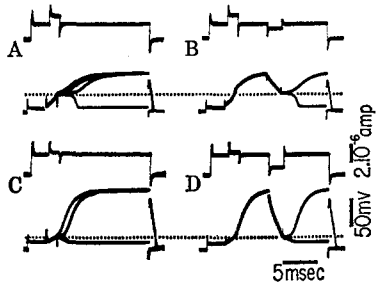

FIGURE 2. Threshold phenomena in depolarizing inactivation responses. An electroplaque from *G. carapo* which was bathed in an isosmotic KCl medium. Several superimposed sweeps in each record of short pulses superimposed on long lasting subthreshold pulses. *A*, *C*, a brief outward pulse was adjusted so that it either initiated or failed to initiate a response. Once initiated the response lasted for the entire duration of the long lasting pulse. The threshold potential (dotted lines) was lower with a stronger long lasting pulse (*C*). The potential could remain poised at threshold for a variable period. *B*,*D*, a suprathreshold brief pulse initiated a response shortly after the onset of the subthreshold long lasting pulse. A brief pulse of inward current was applied during the response and adjusted so that it either terminated or failed to terminate the response. For a given strength of the long lasting pulse, initiation and termination occurred at the same membrane potential (dotted lines). With the larger long lasting pulses the responses were larger and more rapidly rising.

given level of maintained polarization (dotted lines), but lower for larger polarizations.

The complete data from the experiment of Fig. 2 are shown in graphic form in Fig. 3. As the current was increased slightly above 5×10^{-6} amp, the steady-state polarization exhibited a sharp discontinuity, rising from about 10 mv to almost 120 mv (open circles). The higher values of voltage for a given current represent the occurrence of inactivation responses. These values and also the potentials obtained when the responses were evoked by brief pulses superimposed on a steady current (crosses, cf. Fig. 2) fall about a straight line which passes through the origin, which is the initial potential of the cell. The steeper slope of this line indicates that the resistance increased during the depolarizing inactivation responses, and the fact that the line intersects the origin indicates that there was little or no change in the emf of the membrane. The

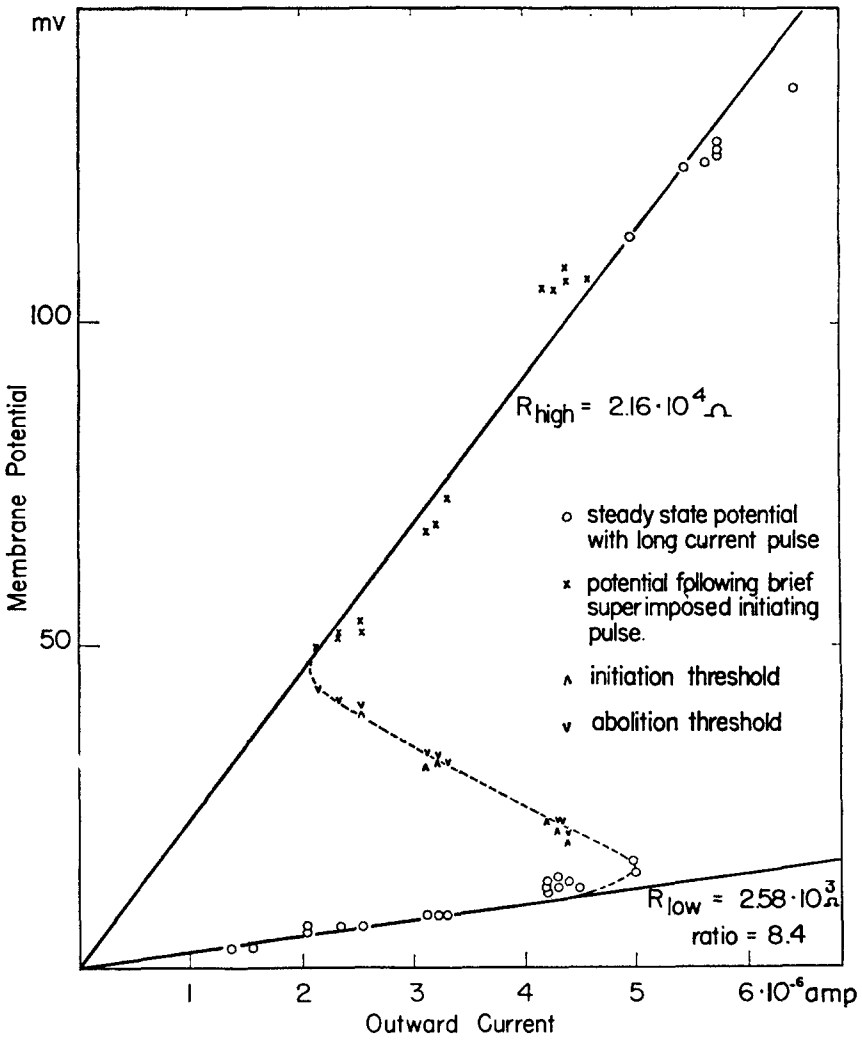

FIGURE 3. The current-voltage characteristic of the electroplaque of Fig. 2. Open circles represent the steady potentials during the long lasting pulses of constant current. They fell on a line whose slope indicated a resistance of 2.58×10^3 ohms as long as the currents did not exceed about 5×10^{-6} amp. For slightly larger currents all the values fell about another line of steeper slope, indicating that the resistance had increased about eightfold to 2.16×10^4 ohms. When the steady current was in the range between 2 and 5×10^{-6} amp brief pulses were applied to trigger inactivation responses (as in Fig. 2*A* and *C*). The steady potentials during these responses (crosses) also fell about the high resistance line. The threshold potentials at which the brief pulses initiated the inactivation responses (inverted V symbols) were inversely related to the steady applied currents and fell along a line of negative slope connecting the lines of positive slope. The potential at which brief inward pulses terminated the inactivation responses as in Fig. 2*B* and *D* (V symbols) also fell on the line of negative slope, which represents a position of unstable equilibrium between the low and high resistance states. Note that the two lines of positive slope extrapolate to the origin which was the membrane potential in the absence of applied current.

ratio of the high resistance to the low was 8.4. The nonlinearity may be regarded as a "rectification," like that which occurs in response to depolarizing currents during "anomalous rectification" of frog muscle fibers (Katz, 1949; Adrian and Freygang, 1962; Adrian, 1964).

The threshold potentials for initiating and abolishing the inactivation responses (Fig. 2) are also plotted in Fig. 3. The values fall along a line of negative slope which connects the low and high resistance branches of the current-voltage relation. This line represents a condition of unstable equilibrium. Small deviations of the induced voltage tend to shift the membrane potential to one of the stable points; an increase causes transition to the high resistance branch; a decrease, transition to the low resistance branch.

VOLTAGE CLAMP MEASUREMENTS The data of Figs. 1 to 3 were extended by employing the voltage clamp technique using both step and ramp voltage changes that increased internal positivity. In the lower traces of Fig. 4*A* is shown a sequence of four voltage steps of increasing amplitude taken from different oscilloscope sweeps. The current (upper traces) was greater for the second step than for the first. However, it fell substantially for the two subsequent larger voltage steps. Fig. 4*B* shows the changes in current (upper trace) when the membrane was clamped to an increasing ramp voltage, which changed at the rate of 12 v per sec (lower trace). At first, the current increased as the voltage increased. However, at about 20 mv from the initial membrane potential the current began to decrease. It reached a minimum value at about 45 mv and then began to increase again, but at a slower rate than initially. Before the end of the ramp voltage an additional positive step polarization of ca. 30 mv was superimposed. The current rose only by a small amount (3×10^{-6} amp), indicating that the membrane resistance was well above the resting value. Comparison of the large changes in current during the two relatively small voltage steps illustrated in Fig. 4*A* with the small change in current for the larger voltage step in Fig. 4*B* shows particularly clearly that the resistance was increased at the greater internal positivity.

When the cell was polarized so that it passed from its low to its high resistance state, the time required for the resistance to increase was too short to be resolved with the clamping circuitry which was employed. In the third and fourth voltage steps of Fig. 4*A* the current reached its steady-state value in less than the 0.5 msec required for oscillations of the clamping system to be damped out. The rapidity of the inactivation response was also indicated by the fact that currents in responses to the ramp voltage of Fig. 4*B* which lasted only 10 msec, were identical with those induced by much slower ramp voltages. Capacitative currents whose magnitudes are a function of the rate of rise of a ramp voltage were negligible, because the time constant of gymnotid electroplaques is very small (Bennett and Grundfest, 1959 *a*; Grundfest, 1957).

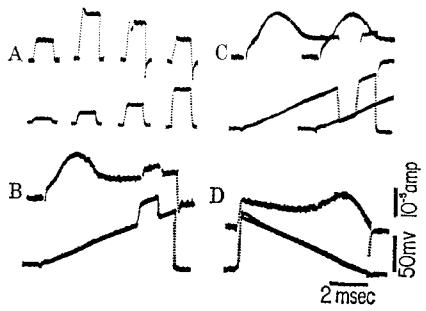

FIGURE 4. Voltage clamp measurements, electroplaque of *Hypopomus* sp. bathed in an isosmotic K acetate saline.

A, four voltage steps of increasing amplitude. The current increased with the second step but decreased with the next two, indicating the onset of depolarizing inactivation.

B, the cell was clamped to a ramp voltage increasing at 12 v/sec. The outward current increased rapidly at first, reached a peak at a clamping voltage of about 20 mv, and then declined to a minimum from which it increased again but at a slower rate. Near the end of the ramp voltage a step of 30 mv depolarization was superimposed. The resulting increase in current was considerably smaller than for the first two voltage steps in *A* which represent smaller depolarizations.

C, two records of clamping with increasing ramp voltages superimposed photographically. The first sweep is a ramp voltage as in *B*, but with a 50 mv step change of voltage in the opposite (repolarizing) direction. The current, which had decreased as depolarization increased, rose rapidly to a new peak value during the repolarization. The second trace is shifted on the time scale so that the ramp voltage applied to the membrane coincides with the voltage during the repolarizing step of the first sweep. The corresponding portions of the current traces are also coincident, indicating that the onset of and recovery from depolarizing inactivation were both rapid compared to the resolution of the clamping system.

D, photographically superimposed records of clamping with increasing and decreasing ramp voltages. In one of the sweeps the ramp voltage rose abruptly to its maximum, and then decreased at 12 v/sec. The changes in current were thus in the reverse direction from those seen in *B* and *C*. Superimposed is a second trace with a rising ramp voltage (as in *B* and *C*), but inverted photographically left to right. The coincidence of the current traces indicates that the processes involved in the inactivation responses were rapidly reversible. The two voltage traces did not quite superimpose at the largest voltage, due to failure of the clamping circuit to follow large rapid transient displacements.

When the membrane potential was changed in the reverse direction, so that the resistance passed from the high to the low value the time required for the change was also too short to be measurable in the present experiments. This is shown by two different methods in Fig. 4*C* and *D*. In Fig. 4*C* an oppositely directed pulse was superimposed near the end of a ramp voltage like that in 4*B*. The pulse caused a return of the cell to the potential where the maximum current was required. The change to this new current value occurred without a measurable delay. When the voltage during this step was photographically superimposed on the rising phase of another ramp voltage, the currents were

also superimposed. The rapid return from the high resistance state to that of low resistance was also demonstrated by using decreasing ramp voltages; that is, by suddenly imposing a large polarization which then decreased linearly to zero. The time course of the current change was virtually identical with that for an increasing ramp voltage. In Fig. 4*D* the comparison is made by photographically superimposing oscilloscope sweeps made with decreasing and increasing ramp voltages, the latter being inverted right to left.

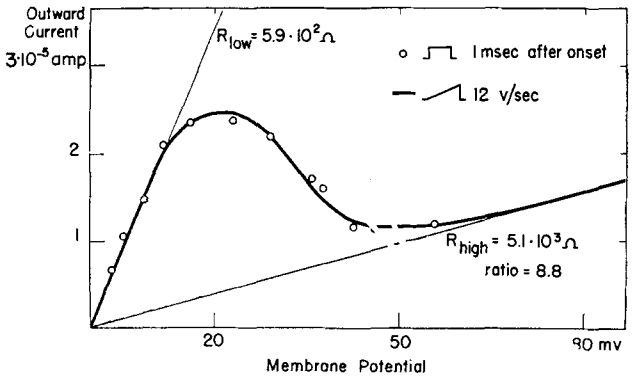

FIGURE 5. Voltage-current characteristic, same electroplaque as in Fig. 4. The origin is the initial level of membrane potential, the abscissa representing increments toward inside positivity. The heavy line is for data with a ramp voltage as in Fig. 4*B*, *C*, and *D*, but transcribed to voltage current coordinates from the voltage time and current time values. The thin lines are the limiting slopes which represent the low and high states of membrane resistance. The change in resistance was a nearly ninefold increase when the membrane polarization exceeded about 60 mv. The circles are the measurements with voltage steps (as in Fig. 4*A*). A region of negative slope occurred in the transition from the low to the high resistance state, and under constant current conditions (as in Fig. 3) would have given rise to depolarizing inactivation responses like those of Figs. 1 and 2.

The rapid changes in conductance under voltage clamp conditions are to be contrasted with the slow changes that are observed under current clamp near threshold. The slow development of the inactivation response is presumably due to an interplay between the kinetics of the regenerative change in resistance and of the process of charging the membrane capacity when the membrane is near a point of unstable equilibrium. The slow rise of spike electrogenesis at threshold in "space-clamped" squid giant axons (cf. Hodgkin et al., 1952; Figs. 8 and 9) is probably an equivalent condition. A slow rise can also be produced in a bistable electronic circuit when the loop gain is adjusted to a suitable value (cf. Grundfest, 1957, Fig. 30).

The voltage-current relations obtained in the experiment illustrated in Fig. 4 are shown in graphical form in Fig. 5. The data for the ramp voltage clamp measurements are represented by the heavy line. The open circles represent

the steady-state values of the currents obtained with step voltage changes. The two types of data in Fig. 5 are superimposable and form a curve which is bounded by two lines with positive slopes. Both lines pass through the value of the initial membrane potential, the origin of the graph, indicating that essentially no change occurred in the membrane emf. The curve connecting the limiting lines has a negative slope and the voltage-current relation in Fig. 5 is thus similar to the relation obtained with current clamp measurements in Fig. 3. At low levels of polarization the slope represented a resistance of 5.9×10^2 ohms, which increased to 5.1×10^3 ohms for high levels of polarization.

IONIC REQUIREMENTS FOR DEPOLARIZING INACTIVATION RESPONSES The inactivation responses shown in Figs. 1, 2, and 4 were all recorded from cells which were bathed in solutions high in potassium. Spike electrogenesis was blocked when the potassium was elevated to about 25 meq/liter. Thus, inactivation responses can occur independently of spike electrogenesis. In the experiment of Fig. 1 the bathing medium contained 50 mM/liter KCl while in the experiment of Figs. 2 and 3 the bathing solution was isosmotic KCl. The amount of polarization required to produce the inactivation responses appeared independent of the resting potential. The responses in relatively low concentrations of potassium, where a resting potential of about 50 mv remained (Fig. 1), were indistinguishable from those in isosmotic K solutions (Figs. 2 and 4). The responses appear not to involve an anion. In the experiment of Figs. 4 and 5 they were elicited in a cell which was immersed in an isosmotic potassium acetate saline. This finding and the fact that there was no change in the membrane emf (Figs. 3 and 5) indicate that the responses were due to changes in permeability to K. Depolarizing inactivation in eel electroplaques (Nakamura et al., 1965 *b*) and anomalous rectification in frog muscle (Nakajima et al., 1962; Adrian, 1964) also are due to changes in K permeability which are independent of the resting potential.

The occurrence of a negative slope characteristic was not dependent upon the presence of excess potassium in the bathing medium, since such a characteristic could also be demonstrated in preparations which were bathed in normal *Electrophorus* Ringer (Fig. 6). Following the application of large and long lasting depolarizing currents, the cells became incapable of generating spikes. The cells could still develop inactivation responses, however (*A*). A brief pulse of outward current superimposed on a longer lasting pulse could initiate a response at some threshold value and such responses also exhibited a threshold for termination by oppositely directed brief pulses (*B*). The responses did not outlast the currents evoking them. Thus, these results are analogous to those shown in Fig. 2. However, the time course of the responses in Fig. 6*A* and *B* was much slower and the form of the transient voltage changes was different. Thus, the brief outward pulses in *A* and *B* which were

about 0.1 sec in duration apparently caused a slow increase in resistance. The sudden change in potential at the onset of the brief polarization was smaller than that at the end, and the apparent increase in resistance was sufficient to account for the amount by which the potential just after the brief pulse exceeded that before it. The opposite changes occurred during the brief inward

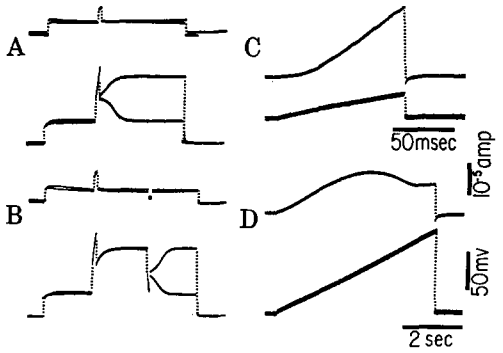

FIGURE 6. Depolarizing inactivation in an electroplaque bathed in normal saline. Preparation from *Hypopomus* sp. *A–B*, current clamp data. Two superimposed sweeps in each record. *A*, a brief pulse of outward current, added to a longer lasting subthreshold current, elicited an inactivation response which is indicated by the slow change in potential during the pulse and by the greater change at the end of the pulse than at the beginning. The potential immediately after the pulse was at threshold for the inactivation response. In one sweep the potential then subsided gradually but in the other it rose, also slowly, to a new level, appropriate for the applied steady current. *B*, the brief pulses were stronger and suprathreshold for the inactivation responses. A brief inward current pulse was threshold for abolition of the inactivation response, which subsided in one sweep but rose again to its full value in the other. The fall of the potential at the end of the long lasting current pulse was rapid in all cases. *C–D*, clamping experiments with ramp voltages rising at the rate of about 0.3 v/sec in *C*, and 20 mv/sec in *D*. During the more rapid depolarization (*C*) the resistance of the cell decreased when the membrane was depolarized by about 10 mv and the slope of the outward current flow increased, indicating the occurrence of K activation. When the pulse terminated the resistance was 1.3×10^3 ohms. The slowly increasing depolarization (*D*) eliminated K activation. The resistance initially was 3.8×10^3 ohms, but it increased as the membrane was depolarized by about 70 mv. At the end of the ramp voltage when the potential of the cell was changed by about 120 mv the resistance was 1.1×10^4 ohms. Time calibrations the same for *A*, *B*, and *D*.

terminating pulse in *B* and had about the same time course. Moreover, the resistance can be seen to be greater during the response since the initial change produced by the inward pulse in *B* was proportionally greater than that produced by the larger outward pulse. The difference in resistance is just enough to account for the magnitude of the response.

Inactivation responses in normal saline were also studied by voltage clamp techniques. When the membrane was clamped to a rapidly rising ramp voltage

(0.3 v/sec), the current increased slowly at first but then rapidly as the threshhold for "delayed rectification" (Hodgkin et al., 1949) was reached (Fig. 6). This second phase which is probably due to depolarizing K activation (Hodgkin and Huxley, 1952), though linear, extrapolated back to a potential some 10 mv depolarized from the resting value. The intercept is also positive to the resting potential in squid giant axons (Hodgkin and Huxley, 1952) and *Onchidium* neurons (Hagiwara and Saito, 1959), although the equilibrium potential for K is negative to the resting potential. As in these cells, the observed value of the intercept in the electroplaques indicates that K conductance was still increasing during the later part of the ramp voltage. More rapidly increasing ramps than in *C* evoked an early inward current phase during which the cell was not adequately clamped. In the same cell with a much more slowly rising ramp voltage (20 mv/sec, *D*) the current first increased at a more or less steady rate and then decreased. Thus, it displayed a negative slope region, after which it increased again approximately linearly. The slope of the last rise was less than that for the initial depolarization, indicating that a new, increased value of membrane resistance had been reached.

The results of Fig. 6 suggest that depolarizations by applied currents and by high K solutions may not be completely equivalent, since the time course of the responses in the former condition is considerably slower, both during their initiation and termination. Further evidence is given in Fig. 7, from an experiment in which the preparation was bathed in a medium enriched with 50 mM/liter K. Depolarizing 1/sec pulses were given in the presence or absence of a maintained hyperpolarizing current (upper trace). The middle trace shows the membrane potential and the lower serves as a reference line. When the cell was maintained near its normal resting potential by applying a hyperpolarizing current, it could respond to a brief depolarizing stimulus with a spikelike pulse that was followed by a long lasting depolarizing after-potential (*0*). The cell immediately depolarized when the hyperpolarizing current was turned off and the capacity to generate the initial pulse was lost within 1 sec (*1*). The depolarizing inactivation response was not fully developed, however, until several more seconds had elapsed (*3, 4*). When the hyperpolarizing current was reapplied (between records 9 and 10), the pulse response recovered within about 2 sec, while the depolarizing after-potential did not recover its full amplitude for several seconds longer.

A definitive analysis of the changes in potential observed in Fig. 7 in terms of ionic phenomena will require further study, but a reasonable account can be deduced. It seems likely that the repolarized cell (*0*) responded to the depolarizing current with both Na and K activation. Both would have occurred during the brief spike, which was followed by a steady voltage during the remainder of the depolarizing pulse. Indicating maintained K conductance, the resistance during this phase was low, since the potential change at the end of

the pulse was smaller than the change when a much smaller pulse was applied after the hyperpolarizing current was terminated (*1*). The peak of the after-potential in *0* was slightly negative to the resting potential in the absence of hyperpolarizing current (*1–9*) and its slow decline indicates the slow return of the conductance to the resting level.

While the resistance was already increased in *1* over that seen during the pulse in the first record (*0*), some increase in K conductance may have persisted for several seconds. This increase could have shunted the component of

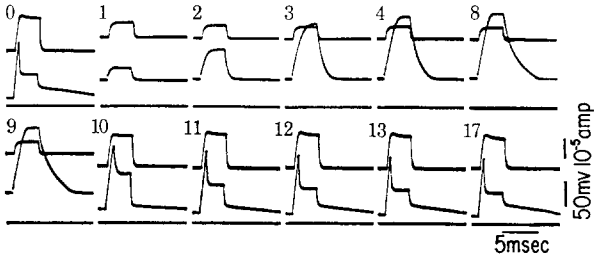

FIGURE 7. Changes in responses under different conditions of membrane potential. Preparation from *G. carapo*, bathed in 50 meq/liter K. 0, the electroplaque was depolarized about 25 mv by the excess K, but its original resting potential was restored by an applied inward current. The lowest trace in this and subsequent records is a reference line for the upper traces. A strong pulse of outward current caused a response which was composed of a spike of about 70 mv amplitude, followed by a plateau of depolarization. When the stimulus terminated, the potential fell to a value close to that of the resting potential in the absence of the hyperpolarizing current. This after-potential declined slowly. The polarizing current was removed for records *1–9*, the numbers signifying the time, in seconds, after the current was withdrawn. The changes in membrane potential produced by currents of constant amplitude gradually increased to a peak value. Within 1 sec after a polarizing current was reapplied (*10*) the spike reappeared, but was somewhat longer lasting than before. It became brief again in the subsequent records. The plateau during the depolarizing current gradually decreased in amplitude while the after-potential increased (*10–17*). Further description in text.

increased membrane resistance, delaying the appearance of the inactivation response. Na inactivation was eliminated rapidly after the cell was repolarized (*10*). Reappearance of K activation, however, required some time (*10* to *17*). This is shown by the longer lasting spike in record *10*, by the gradual decrease, in the successive records, of the plateau after the spike, and by the slow reappearance of the after-potential.

B. *Hyperpolarizing Inactivation*

CURRENT CLAMP MEASUREMENTS Application of large hyperpolarizing currents resulted in changes in membrane potential (Fig. 8) which were similar to the hyperpolarizing responses of lobster muscle fibers (Reuben et al.,

1961), except that oscillations did not occur even when prolonged currents were applied. Like the depolarizing responses described above, the changes in membrane potential were due to a change in membrane resistance rather than to a change in membrane emf. The responses were terminated as soon as the applied current was withdrawn. Tests with brief pulses superimposed during a response showed that the resistance was indeed increased and by an amount sufficient to account for the voltage change during the response (*D*). Stronger stimuli evoked the responses at shorter latency (*A–C*). Correspondingly the brief pulses (*D*) accelerated or delayed the response depending on their direction.

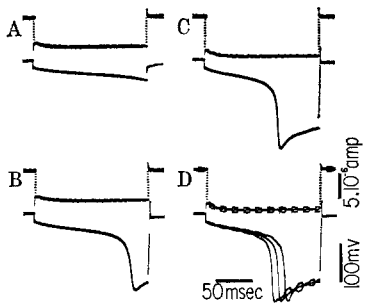

FIGURE 8. Hyperpolarizing inactivation responses in an electroplaque from *Steatogenys* sp. Preparation bathed in normal saline. *A*, the inward current was subthreshold for a response. The hyperpolarization of the membrane increased slowly throughout the pulse. *B–C*, with larger inward currents hyperpolarizing responses were evoked which were earlier with stronger stimuli. The large hyperpolarization during the response decreased slowly while the current was maintained. The response terminated rapidly when the current was withdrawn and was followed by anode break excitation. *D*, three superimposed sweeps, with a sequence of brief depolarizing current pulses added in one sweep and hyperpolarizing pulses added in another. The onset of the response was speeded by the hyperpolarizing pulses and delayed by the depolarizing. Note increase in the potential produced by the test pulses as the hyperpolarizing response developed and the marked increase during the response.

Hyperpolarizing inactivation responses were frequently followed by spikes which presumably were anode break responses similar to those in other tissues (Fig. 8*B–D*; cf. also Ooyama and Wright, 1961). Following hyperpolarizations which were subthreshold for the inactivation responses the membrane often remained slightly hyperpolarized for some time. A similar effect is seen in frog muscle fibers (Adrian and Freygang, 1962), in which a hyperpolarizing inactivation response does not develop, however. The inactivation responses to the hyperpolarizing currents of the electroplaques had a distinct threshold, as indicated by the criterion of their all-or-none initiation by a brief pulse superimposed on a prolonged subthreshold current (Fig. 9*A*). Responses initiated in this manner could also be abolished in all-or-none fashion by brief, op-

positely directed pulses (*B*, *C*). Hyperpolarizing inactivation responses were not maintained indefinitely, unlike the depolarizing inactivation responses (Figs. 1 and 2). Tests with superimposed pulses indicate that the membrane resistance was decreasing during the response, approximately enough to account for the decrease in the amplitude of the voltage (Fig. 8*D*). Two possibilities are indicated by the "spontaneous" diminution or termination of the responses. Either the inactivation process is reversible, or an additional ionic process comes into play. The latter has been observed in the hyperpolarizing responses of lobster muscle fibers which are terminated with a phase of marked increase in conductance (Reuben et al., 1961). As is also the case in lobster

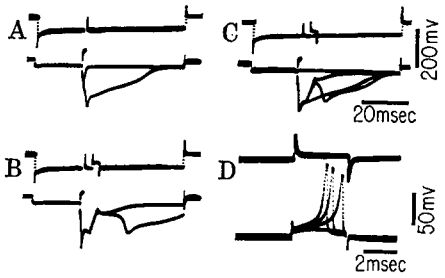

FIGURE 9. Threshold phenomena in hyperpolarizing responses of an electroplaque from *G. carapo*. *A*, two superimposed sweeps. A brief hyperpolarizing pulse superimposed on a longer lasting subthreshold pulse evoked a response during one trace. The hyperpolarizing response was brief. *B*, two superimposed sweeps. A stronger brief hyperpolarizing pulse evoked a response during each sweep. It was followed by a depolarizing pulse which terminated the response in one sweep. *C*, four superimposed sweeps. One was with only the long lasting hyperpolarizing current. In three, a hyperpolarizing response was evoked by an added brief hyperpolarizing pulse. In two of these a depolarizing pulse was also added and the hyperpolarizing response was abolished in one. *D*, four superimposed sweeps showing that the cell was still capable of developing spikes after the responses of *A–C*.

muscle, the hyperpolarizing inactivation responses of the electroplaques exhibited a marked refractory period, which lasted approximately 30 sec.

The hyperpolarizing inactivation responses occurred independently of the presence (Fig. 9*D*) or absence of the normal mechanism of spike electrogenesis. Thus, they could be elicited in cells which had been depolarized with an amount of excess potassium which was sufficient to block spike electrogenesis (Fig. 1*D*). However, it was more difficult to evoke hyperpolarizing inactivation responses in cells depolarized with excess potassium, because their resistance was lower, and it was then difficult to apply sufficiently high currents to hyperpolarize the cells beyond the threshold value for the response.

VOLTAGE CLAMP MEASUREMENTS When small hyperpolarizing step voltage pulses of 5 msec duration were applied under voltage clamp conditions (Figs. 10 and 11), the current was constant during the pulse and its amplitude

increased linearly with the voltage. With larger voltage steps the current decreased gradually throughout the application of the clamping pulse to a value smaller than with smaller clamping voltages (Fig. 10*A*, *B*). With still larger clamping voltages (*C*), an initial surge of current was followed by a rapid decrease which took place in less than 1 msec, after which the current remained constant during the remainder of the pulse. On increasing the clamping voltage still further (*D*) the previous sequence was followed by a later increase in current, so that at the end of the pulse the current might be several times

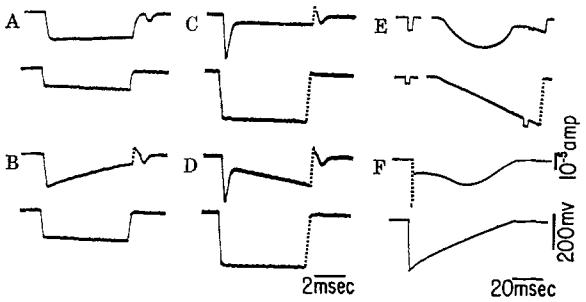

FIGURE 10. Measurements with hyperpolarizing voltage clamps on an electroplaque of *Hypopomus*. *A–D*, with 5 msec step voltages of different amplitudes. *E*, *F*, decreasing and increasing ramp voltages lasting about 65 msec and changing at the rate of 4.4 v/sec. *A*, the inward current decreased slightly during the applied voltage. A brief pulse of inward current after the step was terminated probably represents an anode break response. It is also seen, arising somewhat earlier, in *B* to *D*. *B*, the decrease in current during the larger voltage step was more marked. *C*, a still larger voltage step caused a rapid decrease in the current to a steady level. *D*, further increase in the voltage caused a gradual increase in outward current following the early minimum. *E*, as the hyperpolarizing ramp voltage increased, the inward current reached a peak, then decreased, and then increased again, but at a lower rate. Near the end of the ramp voltage an additional hyperpolarizing step was applied. It caused only a small increase in current compared with the effect of the same voltage step applied at the resting potential as shown in the inserted portion of a sweep to the left of the ramp. *F*, during a ramp voltage of decreasing hyperpolarization the relation between voltage and current differed from that in record *E*. The initial current surge is indicated by the dotted line. Further description in text.

greater than at the time when the minimum value was attained. The entire sequence is of the type which is called for by an inactivation response which is all-or-none and which "spontaneously" terminates. There is a negative slope region in the voltage-current relation in the transition from low to high resistance and a secondary increase in conductance. In Fig. 10*A–D* are also seen brief inward currents after termination of the clamping pulses. They are probably the result of anode break responses in an inadequately clamped region of the cell.

The negative slope characteristic was also demonstrated by clamping the cell to a ramp voltage (Fig. 10*E*, *F*). The changes described on the current

trace are analogous to those shown in Fig. 4*B*. As the hyperpolarizing ramp voltage increased, the current increased. The latter then decreased in the negative slope region, and finally increased again (Fig. 10*E*), but the slope of the increase in current with voltage was smaller for the larger voltages, indicating that the resistance had increased. The increase in resistance may also be seen by comparison of the current during a brief hyperpolarizing pulse which was superimposed on the ramp voltage in the high resistance region with the same voltage step in the unpolarized cell (*E*, left). The current was much greater in the latter.

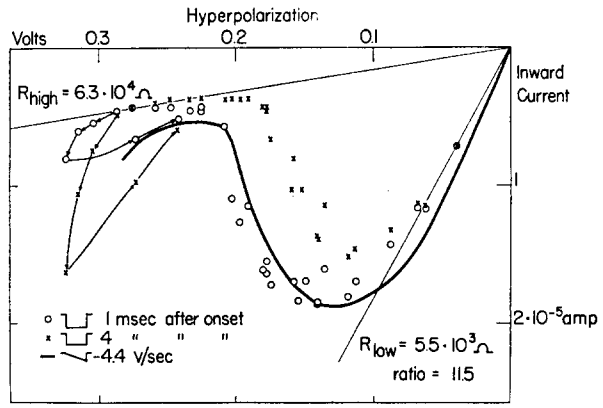

FIGURE 11. Current-voltage relations in hyperpolarizing inactivation. Same experiment as in Fig. 10. The heavy line represents ramp voltage measurements as in Fig. 10*E*, but transferred from the voltage time and current time values to voltage current coordinates. The circles show the currents at 1 msec while the crosses represent the currents measured at 4 msec. Further discussion in text.

The data from the experiment of Fig. 10 are shown with greater detail in graphical form in Fig. 11. The voltage-current relation obtained from the ramp voltage clamp (Fig. 10*E*) is shown as the heavy line. The open circles and crosses show the currents during step voltages, respectively 1 msec and 4 msec after onset of the clamping pulses. In the range between about 90 mv and 250 mv hyperpolarization the current decreased with time during the clamping pulses, since at a given level of hyperpolarization the crosses lie above the circles.

The time required for development of the hyperpolarizing inactivation process is considerably longer than that for depolarizing inactivation. Thus, at the lower voltages the current decreased throughout the clamping pulse (10*A*, *B*). The time is a function of the degree of hyperpolarization. At the voltage of Fig. 10*B* the minimum of the current had not been reached after 5 msec of hyperpolarization. At the voltage levels of records *C* and *D* the mini-

mum was attained in about 0.7 msec, although during an appreciable fraction of this time the voltage was below the final value. It is also evident from the foregoing data that the inactivation process is not simply dependent on the total current which flows across the excitable membrane. Considerably more current flowed during the 5 msec period in *B* than in the 0.7 msec period in *C*, before the maximum degree of inactivation was reached.

The time course of the return to normal values of resistance following a clamping pulse like that in Fig. 10*C*, where no secondary increase in conductance occurred, has not been systematically studied. The time constant of the process of reversal from inactivation is, however, several milliseconds. Recovery is markedly slower than the return to normal conductance with depolarizing inactivation responses (Fig. 4). The slowness of the return to normal conductance can be seen in qualitative fashion from Fig. 10*F*, an experiment in which a decreasing hyperpolarizing ramp voltage was given; i.e., the cell was first strongly hyperpolarized and then the hyperpolarization was decreased linearly with time. The current during the initial part of the decreasing ramp voltage was about the same as at the corresponding points of the increasing ramp voltage in *E*, indicating that the hyperpolarizing inactivation had occurred during the initial current surge. The current then increased slightly rather than decreased, indicating that secondary conductance increases were occurring in response to the hyperpolarization. However, during the last part of the decreasing ramp the current was smaller than in *E*, probably due to slow reversal of the inactivation process.

When the hyperpolarizing pulses exceeded about 280 mv, the secondary increase in conductance developed during the pulses used for the measurements of Figs. 10 and 11. This is shown in Fig. 11 by the reversal of the relative positions of the circles (early currents) and crosses (later currents), with the later currents now exceeding the earlier values. The arrows connecting the points on the left side of Fig. 11 show the order of taking successive measurements at 1 sec intervals with the large hyperpolarizing clamping pulses. As the amplitudes of the voltage pulses increased the currents also increased, deviating considerably from the slope of the high resistance state. The last three measurements in the series connected by arrows in Fig. 11 were made with pulses of successively decreasing amplitude. The currents were larger than for the corresponding earlier measurements, indicating that at least several seconds were required for full recovery after strong hyperpolarizations evoked the secondary increase. Subsequent measurements with still smaller pulses fell close to the original data indicating that the membrane had undergone complete recovery. The slowness of the recovery from the secondary increase explains the long refractory period after responses were produced by constant current stimulation, particularly since the increase is likely to have been considerably greater. In the experiment of Fig. 11, the negative slope region of the voltage-current

characteristic developed at a hyperpolarization of about 120 mv, with a current slightly less than 2×10^{-5} amp. The resistance then increased about 11.5 times. During application of about 2×10^{-5} amp in current clamp measurements, therefore, the electroplaques would have developed hyperpolarizing responses in which the voltage would have tended to increase from a threshold of about 120 mv to a maximum value exceeding 1 v. So large a change in potential during hyperpolarizing responses did not occur (Fig. 8), however, due to the onset of the secondary increase in conductance.

The magnitudes and time courses of these secondary changes have not been investigated enough to permit quantitative statements, for the reason that recovery times after large hyperpolarizing pulses are inconveniently long, requiring waiting periods of the order of 1 min between measurements. Frequent application of these currents also leads to irreversible loss of spike electrogenesis, suggesting damage to the membrane. However, the data of Figs. 10 and 11 give some indications as to the complexity of the changes. Thus, in the next to the last pulse, connected by arrows, in Fig. 11, not only was the maximum resistance lower than for an earlier pulse of the same voltage, but the current increased during the pulse rather than remained constant. It appears that activation of the secondary increase in conductance decreased the voltage required to produce it in the subsequent pulses. Probably, also, a lower threshold voltage would have been observed if longer pulses had been used, for with the ramp voltage the secondary increase began at a lower level than with the pulses.

There is a possible discrepancy in Fig. 11 between the results of the ramp voltage clamp and the (earlier) pulse measurements. As the ramp voltage was increasing rather slowly the values should have fallen closer to those for 4 msec after onset of the pulse clamps (crosses), than to those for 1 msec after onset (open circles). In fact they fell nearer the latter. However, early during application of the ramp voltage the resistance was lower than the value observed with the prior measurements with voltage steps, and the minimum current at a hyperpolarization of about 220 mv was greater. These differences cannot be explained quantitatively by assuming a small leak in the membrane and it is probable that the characteristics of the cell had become somewhat altered as a result of the previous hyperpolarizations. It may be noted as well, that the hyperpolarization with the ramp voltage attained a value of 100 mv only after about 25 msec had elapsed, and the observed differences in current may have been due in part to slow changes in the conductance processes.

The ionic characteristics of the secondary conductance increase have not been studied adequately as yet. After a hyperpolarizing current was terminated there was usually a small remnant of hyperpolarization, whether or not there was a response. Whether the secondary conductance increase is nonspecific,

involves an increased permeability for K, or represents hyperpolarizing Cl activation (Grundfest, 1961, 1963, 1966) cannot be determined at this time.

DISCUSSION

Adequacy of the Voltage Clamp

A criterion to determine whether a spherical cell can be voltage-clamped has been derived by W. Rall and K. S. Cole (personal communication). The current electrode is assumed to be at the center of the cell and the voltage is measured between one electrode just inside the cell membrane and another just outside it. The maximum allowable radius (a in centimeters) is a function in the absolute value of the minimum negative resistance (r_m in Ω cm^2) and the resistivities of the intracellular and extracellular media (r_i and r_e respectively, in Ω cm), according to the relation:

$$a < \frac{r_m}{r_e/2 + r_i}$$

The electroplaques studied in the present work were drum-shaped (Bennett and Grundfest, 1959 *a*; Bennett, 1962), with a radius and thickness of about 0.02 cm. The minimum negative resistances measured were about 1 K ohm. Assuming internal and external resistivities of 100 and 50 Ω cm respectively and considering the cells as approximately spherical, the maximum allowable radius calculated from the above expression is 0.04 cm. Thus, there was a moderate theoretical margin of safety for voltage-clamping the cells. It is also probable that if voltage-clamping fails the clamping current is not constant. A simple criterion of adequate voltage-clamping is thus provided if the membrane current remains stable when the potential is held in a region of negative resistance of the voltage-current relation. This criterion was satisfied in the measurements of depolarizing inactivation (e.g. Figs. 4 and 5).

However, if the current is not stable this may be due to changes in membrane properties as well as to failure of clamping. During clamping with hyperpolarizing pulses (Figs. 10 and 11) the membrane current did not remain constant when the potential was held in the negative resistance region. The large differences in the current were not reflected in changes of the membrane potential (Fig. 10*A* to *D*), and, since the first criterion described above was satisfied, the changes in the current observed in Figs. 10 and 11 were no doubt due to changes in membrane properties.

The termination of hyperpolarizing clamping pulses was frequently followed by a surge of inward current. This probably represented membrane activity which results in anode break spikes of cells that are not voltage-clamped (Fig. 8*A*–D). During spike electrogenesis of the electroplaques the negative resist-

ance is apparently too low to allow adequate clamping with current applied through an intracellular microelectrode (cf. Nakamura et al., 1965 *b*).

Nature of Inactivation Processes

The voltage and current clamp measurements of the present work show that the conductance of the electroplaques decreases as much as 10- or 12-fold under the action of either depolarization or hyperpolarization. Both types of stimuli may produce a conductance decrease in the same cell. These changes in conductance are aspects of the nonlinear current-voltage (*I–E*) relations of electrically excitable membrane components and are to be regarded as responses to the electrical stimuli fully as much as the responses which lead to spike electrogenesis (Grundfest, 1961). The inactivation responses of gymnotid electroplaques can occur in cells which are at their usual resting potential (cf. also, Nakamura et al., 1965 *b*) as is likewise true for a number of other cells (cf. below).

The nonlinear processes which cause spike electrogenesis belong to a family of activation responses in which the membrane conductance increases for specific ions under the influence of hyperpolarizing as well as depolarizing stimuli (Grundfest, 1961, 1963, 1966). In analogy with the Na inactivation process which is a response to depolarization (Hodgkin and Huxley, 1952), the changes in membrane properties which lead to decreased conductance have been termed inactivation processes (Grundfest, 1961). In this sense inactivation can be produced by pharmacological agents as well as by changes in membrane potential. The Na-permselective electrically excitable channels are normally closed. They open to permit Na flow down its electrochemical gradient and subsequently close as a consequence of depolarization. During action potentials most if not all the Na channels become inactivated following activation (Hodgkin and Huxley, 1952). However, pharmacologically evoked Na inactivation (as for example, by tetrodotoxin) presumably is not preceded by activation and does not affect the resting membrane conductance (Narahashi et al., 1964; Nakamura et al, 1965 *a*, *b*). It is manifested by the block of spike electrogenesis and of the activation response to normally adequate stimuli. During slow depolarization electrically evoked conversion of the Na channels to the inactivated state might occur directly without an intermediate stage of activation. This change also would not affect the resting membrane conductance.

Thus, two situations which differ in respect to electrical measurements are subsumed under the term, inactivation: (*a*) block of open channels, i.e. a decrease in resting conductance, and (*b*) prevention of opening of channels, i.e. block of activation, which is not accompanied by a change in resting conductance. Because these two processes may have the same molecular mechanism,

e.g. adsorption of a divalent cation at a reactive site, it is reasonable to class them both as inactivation.

Occurrence of Depolarizing K Inactivation

This process appears to accompany spike electrogenesis in a number of cells. The clearest evidence is that on eel electroplaques, which lack the process of depolarizing K activation (Nakamura et al., 1965 *b*). Substitution of the K of the medium by Cs or Rb causes pharmacological K inactivation but Na activation and spike electrogenesis are not affected. The steady-state *I–E* relation during voltage clamp becomes linear over the whole range of changes in membrane potential, with the resistance now at the "high" level which had previously been attained only during maximum depolarizing K inactivation (cf. Nakamura et al., 1965 *b* Figs. 11 and 12).

In electroplaques of the weakly electric gymnotids, *Sternopygus* and *Eigenmannia*, the resistance during the peak of the spike is about 2 times higher than in the resting cell, while it is low during the rising and falling phases of the spike (Bennett and Grundfest, 1959 *b*; Bennett, 1961). These changes indicate the occurrence of K inactivation during the interval that the membrane becomes strongly positive with respect to the resting potential. Depolarizing K inactivation has been recently demonstrated more directly in electroplaques of *Eigenmannia* (L. Goldman and Grundfest, unpublished data).

Depolarizing K inactivation was postulated to occur in cardiac muscle (Grundfest, 1957) to account for the increased resistance during the plateau of the spike (Weidmann, 1951). This process has been observed recently by a number of workers, in some cases with voltage clamp measurements (Carmeliet, 1961; Hall et al., 1963; Deck et al., 1964; Deck and Trautwein, 1964; Hecht and Hutter, 1965). Depolarizing K inactivation has also been observed recently in frog axons (Meissner, 1965). In this case the conductance increases for depolarizations up to about 100 mv, because of K activation (Dodge and Frankenhaeuser, 1958; Hille, 1966). When the membrane is further depolarized the resistance returns approximately to the high value that it has in the resting state. This transition has a threshold at which an all-or-none change in potential of about 80 mv develops under constant current depolarization. Depolarizing K activation of amphibian axons is abolished by tetraethylammonium chloride (Lüttgau, 1960; Grundfest, 1961; Hille, 1966) and the depolarizing inactivation response of frog axons is then also eliminated (cf. Meissner, 1965, Fig. 5).

Anomalous rectification of frog muscle fibers (Katz, 1949; Nakajima et al., 1962; Adrian and Freygang, 1962; Adrian, 1964) involves 2 types of responses by the K channels, hyperpolarizing activation and depolarizing inactivation (Grundfest, 1961, 1966). On substituting Rb for the K of the bathing medium,

anomalous rectification is eliminated and the steady-state *I–E* relation becomes linear (cf. Adrian, 1964, Figs. 9 and 10) as it does in eel electroplaques (Nakamura et al., 1965 *b*). The membrane resistance is raised by pharmacological K inactivation to the high value which in K-rich solution is attained only during depolarizing stimuli. Thus, with respect to anomalous rectification pharmacological K inactivation by Rb not only closes off channels which are normally open to K and which respond to depolarizing stimuli with closure, but also prevents K channels which are normally closed from responding by opening (or activation) to hyperpolarizing stimuli.

Depolarizing K inactivation also occurs in crayfish muscle fibers (Reuben and Gainer, 1962; Ozeki et al., 1966). What appear to be depolarizing inactivation responses have been observed in a ciliated protozoan and are described as "disproportionately large potential variations, which resembled graded responses in poorly excitable tissues" (Tasaki and Kamiya, 1964, p. 369). Rather complex types of depolarizing K inactivation appear in supramedullary neurons of puffer (Nakajima and Kusano, 1963, 1966; Nakajima 1966) and in squid axons (Ehrenstein and Gilbert, 1964). The changes in conductance may undergo waxing and waning which indicate at least two inactivation processes with different rates.

In the present work also there is an indication that depolarizing inactivation may include several processes operating at different rates. Only a single, rapidly occurring inactivation process appears to occur in cells which had been depolarized by elevating the K of the medium (Figs. 1 to 5). However, the interplays of activation and inactivation which were described in connection with Figs. 6 and 7 indicate that a second, more slowly developing inactivation comes into play. The slower process may be restored to cells which have been depolarized by K. If the cells are now maintained polarized to near their normal resting potential the slower K inactivation is again evidenced (Fig. 7). Thus there seem to be several K inactivation processes which are more or less dependent on the absolute value of the membrane potential. Only the rapid K inactivation, which is independent of the steady-state membrane potential, occurs in eel electroplaques (Nakamura et al., 1965 *b*).

Occurrence of Hyperpolarizing Responses

Hyperpolarizing K inactivation occurs under normal ionic conditions in a number of cells and it has been studied in this laboratory in lobster muscle fibers (Reuben et al., 1961), rajid electroplaques (Cohen et al., 1961; Grundfest et al., 1962; Grundfest, 1962), eel electroplaques (Nakamura et al., 1965 *b*; Nakamura and Grundfest, 1965, and unpublished data), and insect muscle fibers (Kusano, Belton, and Grundfest, unpublished data), as well as in the present work. In crayfish muscle fibers the inactivation process is normally

masked by the simultaneous occurrence of hyperpolarizing Cl activation (Reuben et al., 1962), but can be demonstrated when the conductance increase due to the Cl activation is eliminated (cf. Grundfest, 1963, Fig. 23).

Hyperpolarizing responses of lobster muscle fibers are eliminated by alkaline-earth or onium ions (Reuben et al., 1961). These substances increase the resting resistance about tenfold, (Grundfest et al., 1959) or to the same degree as during the hyperpolarizing inactivation, indicating that normally open K channels which are closed by the hyperpolarizing stimuli are also closed by the pharmacological agents. The pharmacological inactivation does not affect the secondary conductance increase which occurs during the hyperpolarizing responses (Reuben et al., 1961) and which appears to be due to hyperpolarizing Cl activation (Grundfest, 1961, 1963, 1966). The conductance processes for depolarizing electrogenesis are not eliminated by the pharmacological agents. In fact, the normally graded responses are converted to all-or-none spikes, at least in part a result of the K inactivation (Werman and Grundfest, 1961). A delayed conductance increase occurs in lobster muscle fibers (Reuben et al., 1961) and in some other cells which have been studied in this laboratory, but appears to be absent in eel electroplaques. The hyperpolarizing responses of the electroplaques studied in the present work are also composed of a complex of processes, with the increase in membrane resistance followed by a decrease.

A response involving increased resistance to applied currents has been reported in frog skin and toad bladder (Finkelstein, 1964). In this complex tissue, however, it is not clear whether the inactivation is due to outward or inward flow of current across cell membranes. Hyperpolarizing responses occur in frog and squid axons, but only after the fibers are depolarized by increasing the KCl of the medium (Mueller, 1958; Stämpfli, 1958; Lügau, 1960; Segal, 1958; Tasaki, 1959; Moore, 1959). Voltage clamp measurements on frog axons (Müller-Mohnssen and Balk, 1965) confirm the analysis given by one of us (Grundfest, 1961; cf. also, Stämpfli, 1963). The K depolarization causes an increase in K permeability due to maintained K activation. Hyperpolarization returns the K permeability to its original low value when the membrane potential is negative to the threshold for depolarizing K activation. This analysis is confirmed by pharmacological data (cf. Schmidt, 1964, Fig. 37). When TEA is applied to an axon that is maintained in 40 mM/liter KCl, the membrane conductance is decreased to about the same value as that of the resting axon in 2.5 mM/liter KCl. As a result of the pharmacological K inactivation hyperpolarizing inactivation responses are abolished, just as are those due to depolarizing K inactivation as described above. Thus, the hyperpolarizing response of frog axons is identical in principle with that of the electroplaques or of other cells studied in this laboratory. Only the initial conditions (or "resistance states") of the different cells are different. It remains to

be noted that increased resistance in response to applied currents has been observed in artificial bimolecular films (Mueller et al., 1962), across capillary tubes which form the junction between two saline media (Kobatake and Fujita, 1964), and across porous barriers between two saline media (Teorell, 1962). These changes is resistance can be accompanied under appropriate conditions by oscillatory changes in "membrane" potential. As is the rule in systems which exhibit bistable or oscillatory phenomena, these devices possess "negative-resistance" characteristics. For further discussion of the negative resistance characteristics of excitable membranes, cf. Grundfest (1966).

POSSIBLE FUNCTIONAL SIGNIFICANCE OF K INACTIVATION A considerable degree of utility may be ascribed to the interplays between depolarizing activation and inactivation in some cells. In eel electroplaques K inactivation in the innervated face increases the voltage change produced by Na activation and thereby increases the unidirectional flow of current. This is clearly advantageous for the functioning of an electric organ which is used for offensive and defensive purposes. K inactivation also tends to prolong the spike duration since the membrane capacity recharges through an increased resistance. Furthermore, the ionic movements which are very considerable in eel electroplaques are decreased (Nakamura et al., 1965 b).

In the weakly electric fishes studied in the present work both faces of the electroplaques generate spikes (Bennett and Grundfest, 1959 *a*; Bennett, 1961). The inactivation responses must also develop in both faces, since they produce large changes in resistance. If the threshold for rapid depolarizing K inactivation were high, this process would occur only in membrane that was generating a spike, and would tend to minimize eddy currents.

No obvious utility can be ascribed to hyperpolarizing inactivation, although it occurs in many cells. The reactivity of excitable membranes to hyperpolarization may be an accompaniment of different properties which are of functional significance or a random evolutionary event. In either case there is a potentiality for responding to depolarization or hyperpolarization, and with activation or inactivation. The functional requirements would appear to have weighted responsiveness of electrically excitable membrane chiefly in the direction of the depolarizing activation processes which are involved in spike electrogenesis and thus are important for conductile activity.

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