

Potassium Inactivation and Impedance Changes during Spike Electrogenesis in Eel Electroplaques

FRANCISCO RUIZ-MANRESA, ADOLFO C. RUARTE,
TOBIAS L. SCHWARTZ, and HARRY GRUNDFEST

From the Laboratory of Neurophysiology, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York 10032, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Ruarte's present address is Centro de Investigaciones Neurológicas, Instituto T. di Tella, Gallo 1330, Buenos Aires, Argentina. Dr. Schwartz' present address is Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268.

ABSTRACT Various degrees of pharmacological K inactivation were induced by Cs or Ba in isolated single electroplaques of the electric eel. The resulting changes in K conductance give rise to very different steady-state current-voltage characteristics. They also induce differences in ion dynamics during spike electrogenesis. The dynamic changes were studied by AC bridge methods, registering the changes in impedance in synchrony with the neurally or directly evoked spikes. While spike electrogenesis was virtually unaffected by addition of Cs or Ba, the patterns of impedance changes were very different. The various patterns are accounted for by the changes in the respective current-voltage characteristics. The data constitute new evidence for regarding the electrically excitable component of the reactive membrane as a heterogeneous electrochemical system with separate and independently reactive channels that in the electroplaques are permselective for Na and K, respectively.

INTRODUCTION

Spike electrogenesis of eel electroplaques is associated with a sequence of ionic events (Nakamura et al., 1965 *b*; Morlock et al., 1968) that differs markedly from the sequence in squid axons (Hodgkin and Huxley, 1952) or many other electrically excitable cells (Grundfest, 1966). As in squid axons, the depolarizing electrogenesis of the electroplaques is caused by a transient phase of Na activation. The inward Na current is blocked by tetrodotoxin (TTX) in both cells (Nakamura et al., 1965 *a, b*). In the squid axon, however, there is a repolarizing electrogenesis due to K activation while in the electroplaque the electrically excitable K channels are normally open but become closed by depolarizing K inactivation (Grundfest, 1957 *a*). The different

kinetics of spike electrogenesis in the axon and electroplaque give rise to different sequences of impedance changes when the cell is inserted into a Wheatstone bridge (Cole and Curtis, 1939; Morlock et al., 1968).

The K channels can also be closed by pharmacological K inactivation (Grundfest, 1961, 1966), and in eel electroplaques this can be achieved with Cs or Rb (Nakamura et al., 1965 *b*) or, as will be shown in the present work, even more readily with Ba. Furthermore, K inactivation may be complete or only restricted, depending upon the experimental conditions. The different states of the K conductance channels should lead to different patterns of impedance change during spike electrogenesis and the data might provide some additional insight into the nature and properties of the permselectivity of excitable membranes. Further analysis of the behavior of so extreme a variant of electrically excitable, spike-generating membrane as that of the eel electroplaque might thus produce some useful evaluations of the various theoretical explanations of the ionic mechanism of bioelectrogenesis. The experiments to be described were done with these purposes in mind.

METHODS

Single electroplaques were dissected from slices of the Sachs organ and mounted in a chamber somewhat modified from that described in earlier work (Nakamura et al., 1965 *b*; Morlock et al., 1968). The cell and chamber formed one arm of an AC Wheatstone bridge which was driven by 10 K Hz sine waves. Details of the preparation and instrumentation will be found in the cited references. Spikes were evoked by indirect stimulation (Altamirano et al., 1955) exciting the nerve terminals on the innervated and reactive caudal membrane by passing a current inward across this surface, or by direct stimulation, outward current being applied to depolarize the electrically excitable membrane. The spikes were recorded differentially against ground by a pair of microelectrodes that straddled the reactive membrane. The spike and the impedance changes that accompany it could be photographed simultaneously (Morlock et al., 1968), but for better photographic results they were photographed superimposed on a single frame during successive sweeps. Experiments were done at room temperature which ranged between 21° and 24°C.

In each experiment control registrations were made with the cell bathed in an *Electrophorus* saline of the following composition (in mM): NaCl 195; KCl 5; CaCl₂ 3; MgCl₂ 1.5; glucose 10; phosphate buffer 1.15 (pH 7.2). The solution bathing the caudal surface of the cell was then replaced with another in which CsCl or BaCl₂ was present. The salt was added either to the bathing medium, making the latter slightly hyperosmotic, or the solution was kept isosmotic by removal of KCl or NaCl stoichiometrically. No differences in the electrophysiological data were observed with the different procedures. Changes in the steady-state current-voltage relations, such as are seen in Figs. 1, 5, and 6, were complete within about 30 sec.

The current-voltage measurements were made either from analysis of a sequence of potentials resulting from different applied currents and then plotting the curves, as in Fig. 1, or by superimposing a series of X-Y deflections on the oscilloscope face and photographing the ensemble, as in Figs. 4 and 5.

RESULTS

Figs. 1-3 are taken from one experiment in which the electroplaque was exposed to 5 mM CsCl. The steady-state characteristics of the cell are shown in Fig. 1 A for the control (solid circles) and after applying Cs (open circles). The corresponding values of the membrane (chord) resistance, as a function of the membrane potential, were obtained from the data of A and are plotted in B. In the control this cell had unusually low resistance (about 1 ohm cm^2) which increased on depolarization to a maximum of about 12 ohm cm^2 . As already reported (Nakamura et al., 1965 *b*; Morlock et al., 1968), the change

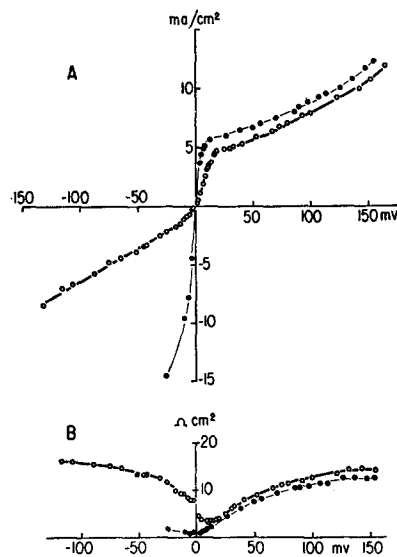


FIGURE 1. Steady-state characteristics of an eel electroplaque in control saline (solid circles) and on addition of 5 mM Cs (open circles). A, current-voltage relations. B, chord resistance of the membrane as a function of the voltage. Calculated from the data in A. Further description in text.

is ascribed to depolarizing K inactivation. In low concentrations, Cs inactivated only part of the K conductance system. The resting resistance of the cell increased to 8 ohm cm^2 . When hyperpolarizing or depolarizing currents were applied the resistance increased to a maximum of about 14 ohm cm^2 . However, for small depolarizations the resistance first decreased to a minimum of about 3 ohm cm^2 when the cell was depolarized by some 10 mv . The resistance then increased as the depolarization increased.

The changes in the steady-state characteristic induced by Cs were essentially without effect on the initial inward current (Nakamura et al., 1965; Nakamura and Grundfest, 1965, and unpublished data). This is also seen in the records of Fig. 2, where the amplitude of the neurally evoked spikes was not altered by the addition of Cs. However, the impedance changes that were registered in synchrony with the spikes were markedly altered by the presence of Cs. For the records of Fig. 2 A and D the bridge was nulled. In the control (A) the impedance changes seen during the spike were like those

described by Morlock et al. (1968). The initial elevation on the impedance trace is indicative of the increased conductance that gives rise to the spike electrogenesis. The return to the resting level while the spike is still near its peak denotes the moment in time when Na inactivation reduces this high conductance just to the level where it compensates for the closure of the K channels by the depolarizing K inactivation that is engendered by the spike itself. The subsequent elevation on the impedance trace denotes the un-

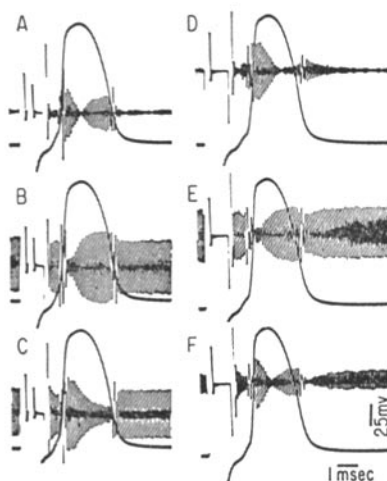


FIGURE 2. Impedance changes during neurally evoked spikes. Same cell as in Fig. 1. A to C, control saline; D to F, Cs added. The bridge output was nulled for A and D. Note the difference in timing of the second elevation in the two registrations. In B and E the bridge was unbalanced so that a decrease in output denotes a decrease in resistance. Note that in B the output corresponding to the second elevation of A was out of phase with that for the first elevation, while in E it was in phase. The difference in phase relations also obtains in C and F when the bridge was unbalanced in the opposite sense.

masking of the high resistance state as the conductance of the Na channels diminished to the low fully inactivated level. Only the conductance of the leak channels now remained to dissipate the charge on the membrane capacity. The high resistance state terminates abruptly when the spike amplitude falls to below about 40 mv.

For the control records in B the bridge was unbalanced so that the decreased output during the rising phase of the spike represents an increase in conductance. The falling phase of the spike was then correlated with an increased bridge output, confirming that the two elevations in A are in opposite phase. In C the imbalance was reversed, so that the rising phase of the spike coincided with an increase in bridge output. The falling phase then was correlated with a decreased output.

After applying Cs the sequence of impedance changes was very different. When the bridge had been nulled at the outset (D), the initial elevation was longer lasting and the impedance trace returned to the base line well after the peak of the spike. The second elevation developed later, reaching its peak when the spike had fallen to about 40 mv, and it persisted until the spike had terminated. In E and F the impedance registrations were made with the bridge unbalanced in opposite directions, as in B and C. In both records the late elevation was in phase with the early one, indicating that after applying Cs the impedance change during the falling phase was a decrease in resistance relative to the resting value. Thus, the second impedance change not only developed later, but also was altered in nature, although the spikes were not greatly affected by Cs.

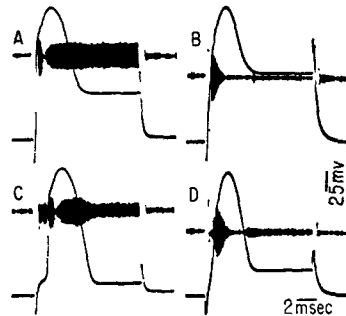


FIGURE 3. Impedance changes during spikes evoked directly, by currents lasting about 10 msec. A and C, control saline; B and D, after adding Cs. Same cell as in Figs. 1 and 2. A and B, strong currents which caused depolarizations of 65 and 80 mv, respectively, during the plateau phase after the spike. C and D, weaker stimulating currents were applied. The plateau depolarizations were 15 mv and 30 mv, respectively. Further description in text.

For the records of Fig. 3 the cell was stimulated directly, with currents lasting about 10 msec. The bridge was nulled for all these registrations. A and C present data for the control and B and D were obtained after applying Cs. The impedance changes depended not only upon the presence or absence of Cs, but also on the amplitude of the stimulating current. The currents were large for A and B, as may be seen from the levels of maintained depolarization after the spike had terminated. The plateau represents a depolarization of 65 mv in A and of 80 mv in B. The impedance change in A is like that shown for the same condition by Morlock et al. (1968). After the brief initial elevation that signals the increased conductance of spike electrogenesis, the bridge output rose to a value that remained steady while the current was applied. This impedance change reflects the high resistance state that was induced by and maintained during the large depolarization. When the cell was exposed to Cs, however, the impedance trace exhibited only the initial elevation. Once this had terminated the bridge output remained essentially null (B).

For the control recording in C the applied current was barely threshold. The spike developed after a latency lasting almost 2 msec. During the latency the membrane was depolarized by about 15 mv, and the same level was

attained during the plateau after the spike. During the initial depolarization the impedance trace registered an output which increased briefly during the rising phase of the spike. During the falling phase of the spike and the subsequent plateau the output exhibited two levels. At first it was as high as in the second elevation in record A. It then fell to the same level as during the latency before the spike had been evoked. This level persisted as long as the cell was depolarized by the applied current. For D the current was somewhat larger, so that the spike developed with little latency and the plateau after the spike was a depolarization of 30 mv. The transient initial output of the bridge was followed by a rather prolonged period during which the bridge was nearly in balance, but during the plateau there was a small, persistent imbalance representing an increased conductance relative to the resting level.

The various impedance registrations in Figs. 2 and 3 are readily explained by the steady-state measurements of Fig. 1. The resting resistance was at a minimum in the control. Depolarization induced a rise in resistance, but during the spike this was masked transiently by the increased conductance due to Na activation. However, the high resistance state was disclosed by the increasing Na inactivation. In the condition of Fig. 3 A the cell was maintained in the high resistance state after the spike had terminated, since the applied current depolarized the cell strongly. During the neurally evoked spikes (Fig. 2 A-C) this state persisted only while the potential of the spike was above about 40 mv. Thereafter, the increase in conductance acted regeneratively to return the membrane conductance to its resting state (Morlock et al., 1968).

In the condition of Fig. 3 C the depolarization by about 15 mv before a spike was evoked induced a rise in the resistance from about 1 ohm cm^2 to about 3 ohm cm^2 . The rise in bridge output was almost instantaneous, confirming the conclusion (Nakamura et al., 1965 *b*; Morlock et al., 1968) that depolarizing K inactivation develops rapidly, within at most 100–200 μsec . The next elevation, which coincided with the rising phase of the spike, denoted the increased conductance of the latter. The depolarizing spike electrogenesis then induced the high resistance state which caused the large subsequent elevation in bridge output. When the spike ended and the potential returned to a steady depolarization of about 15 mv, the output fell to the same level as during the latency before the spike.

The changes that were induced by Cs stem from the fact that the resting resistance of the cell had increased to about 8 ohm cm^2 . Thus, when the bridge was nulled for the neurally evoked spike (Fig. 2 D), the initial elevation exhibited the full time course of the Na activation. After Na inactivation had terminated the high conductance the membrane resistance became somewhat higher than during the resting state, but the nonlinear properties of the bridge configuration (Morlock et al., 1968) precluded a faithful reflection of this

increase. However, as the membrane potential fell back toward the resting value the steady-state resistance decreased. When the depolarization decreased below about 40 mv, the resistance became lower than in the resting state and it remained lower until the membrane was repolarized.

When a large current was applied so as to maintain the cell depolarized by about 80 mv (Fig. 3 B), the resistance during this plateau was about the same as in the resting state for which the bridge had been nulled. Thus, only the initial elevation is observed in the impedance trace of Fig. 3 B. However, when the cell was kept depolarized by only about 30 mv (Fig. 3 D), the resistance during the plateau was lower than in the resting cell. The late and maintained elevation in the impedance trace reflects this and represents a decrease of impedance relative to the resting state, as in Fig. 2 D.

Figs. 4–6 are from an experiment on another cell that was treated with 5 mM Cs (Fig. 4) and then with 2.5 mM Ba (Fig. 5). Direct oscillographic registrations of the steady-state characteristics are shown for the control (Fig. 5 A), as well as for the two experimental conditions. In these presentations the spikes evoked by the applied currents are also registered as excursions to the right of the steady-state points. The resting potential was -84 mv in the control, and -82 mv in Cs and Ba. The spikes were also essentially unaffected in the course of the experiment.

The resting resistance of this cell was 5 ohm cm^2 in the control and during depolarization it increased to about 8 ohm cm^2 . In Cs the resting resistance increased also to about 8 ohm cm^2 and remained essentially at this value in the hyperpolarizing quadrant. For small depolarizations the resistance decreased to about 5 ohm cm^2 , but with larger depolarizations there was a rise in resistance again to a maximum of about 8 ohm cm^2 . In 2.5 mM Ba the characteristic became linear over the entire range of applied currents and the high resistance (9 ohm cm^2) denotes that the K channels are fully inactivated. The impedance registrations with the cell in Cs and Ba showed striking differences that are, however, predictable from the respective steady-state characteristics. During spikes evoked neurally, or by brief direct stimuli in the presence of Cs (Fig. 4 B and C), the impedance traces showed the two elevations that were described and analyzed in connection with the records of Fig. 2 D–F. In Fig. 4 D a depolarizing current lasting 12 msec but too weak to evoke a spike was applied and the depolarization at the plateau was 14 mv. The resistance decreased from its resting value in Cs, and the bridge imbalance thus represents a decrease in impedance from the level for which the bridge was nulled. A slightly stronger stimulus was applied in E and the plateau after the spike was a depolarization of 27 mv. The resistance during the plateau still was lower than that of the resting cell and again the bridge imbalance denotes a decrease in resistance relative to that of the resting cell. When a strong stimulus was applied so that the plateau after the spike was

115 mv, full K inactivation was induced and only the initial elevation was seen on the impedance trace.

The very different effects of Ba on the impedance changes (Fig. 5 C-E) also reflect the change which this agent induced in the steady-state characteristic (Fig. 5 B). Only the initial change in impedance associated with the

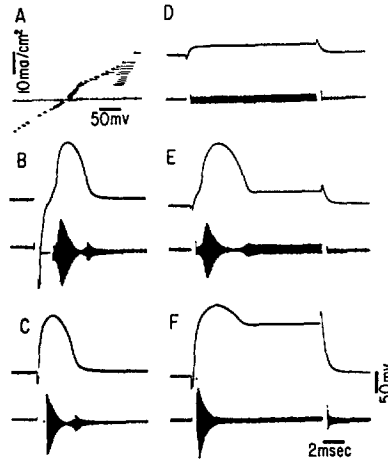


FIGURE 4. Another cell, measurements after addition of 5 mM CsCl. A, current-voltage relations elicited by a series of applied pulses, registered by multiple exposure. The data of this recording are replotted as the middle graph in Fig. 6. The brief deflections to the right of the steady-state line are the registrations of the spikes evoked by the suprathreshold currents measuring the I-V characteristic. B, impedance changes during the neurally evoked spike are like those seen in Fig. 2 D. C, spike evoked directly by a brief but strong depolarizing current. The earliest part of the impedance registration was lost by blocking of the bridge channel, but the remainder clearly resembles that in B. D to F, pulses of increasing strength, all lasting about 12 msec. D, the weak current caused a depolarization of only 14 mv, below threshold for the spike. The conductance during the depolarization was above that of the resting level, as predicted from the characteristic in A. E, stimulus was now above threshold, causing a depolarization of 27 mv during the plateau. This depolarization was also associated with an increased conductance which became manifest as the spike declined from the large depolarizations that had induced K inactivation. F, the strong stimulus caused a depolarization of 115 mv after the spike. K inactivation was maintained during the plateau, and only the initial conductance increase is seen in the impedance registration.

rising phase was observed no matter how the spike was evoked. The low concentration of Ba had induced complete pharmacological K inactivation over the full range of the characteristic. Thus, there was only one electrically excitable component remaining in the spike electrogenesis, that of the Na channels.

The records for the characteristics of this cell under the three different experimental conditions (Figs. 4 A, 5 A and B) are replotted in Fig. 6 so as

to provide comparative data on the effects of the agents on various membrane properties. The measurements are tabulated in Table I. The leak conductance (G_L) was measured from the slope of the characteristic at large depolarizations and from the identical slope which developed in the hyperpolarizing quadrant on addition of Cs or Ba. The maximum steady-state conductance ($G_L + G_K$) was measured from the slope of the characteristics for small depolarizations. It was almost identical in the control and in Cs, but, of course, in the Ba the slope yielded only G_L . G_K was determined as the difference between the high and low slope values. The peaks of the spikes in the records fall on a line which is designated $G_L + G_{Na}$ and represent the

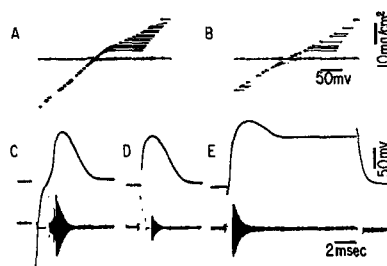


FIGURE 5. Effects of Ba (2.5 mM). Same cell as in Fig. 4. A and B, current-voltage relations in the control saline (A), and after adding Ba (B). Note the conversion of the curved steady-state characteristic in A into a straight line by Ba, indicating complete pharmacological K inactivation. The slope is identical with that of the strongly depolarized region in A. Spike electrogenesis was unchanged and the deflections to the right of the steady-state lines denote their amplitudes at different values of membrane voltage. C to E, responses evoked in the presence of Ba. Only the initial elevations developed in all three records during responses evoked neurally (C) by a brief direct stimulus (D) and by a current lasting 12 msec which depolarized the cell by 130 mv (E). The strong currents for the direct stimuli transiently blocked the bridge registrations and the early parts of the initial elevations are lost.

conductance during the peak of the spike. In these measurements G_{Na} is not necessarily the maximum Na conductance. The G_{Na} term could be determined by subtraction. The potential at which the two lines cross is the "reversal potential" for the spike and approximates E_{Na} . For all three conditions the measurements of most of the parameters (Table I) were close. The values fall within the range of the previous measurements by Nakamura et al. (1965 *b*) and Morlock et al. (1968). However, E_{Na} appeared to decrease progressively in the course of the experiment. The value for G_{Na} was higher in Cs. That E_{Na} should decrease is not unexpected. The influx of Na during each spike must be rather large (Nakamura et al., 1965 *b*) and, if the presumed Na pump activity decreases with deterioration of the cell, a considerable amount of Na may accumulate intracellularly. The effect of Cs on G_{Na} in this cell is not explained by the available data.

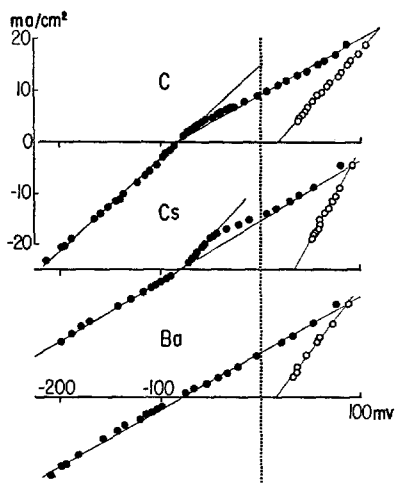


FIGURE 6. Characteristics of the same cell as in Figs. 4 and 5 replotted from the records of Figs. 4 A (Cs), 5 A (C), 4 A (Cs), and 5 B (Ba). Open circles, peaks of the spikes; solid circles, steady-state values. Dotted vertical line is absolute zero. The region of lowest slope which in C is seen only for large depolarizations also occurs in the hyperpolarizing quadrant in Cs and throughout the steady state in Ba. This slope represents the leak conductance, G_L . Note that in Ba the I-V relation is linear over the range, -200 to 100 mv. The higher slope for small depolarizations in Cs and in C for the hyperpolarizing quadrant, as well, is $G_L + G_K$. The slope of the lines connecting the peaks of the spikes is $G_L + G_{Na}$. The crossing between this line and that for G_L is the reversal potential for the spike, approximating the sodium potential, E_{Na} . Further description in text and in Table I.

TABLE I
SUMMARY OF THE PARAMETERS OF AN ELECTROPLAQUE
IN THE CONTROL MEDIUM, IN Cs, AND IN Ba

	Control	5 mM Cs	2.5 mM Ba	Average
G_L , mho/cm ²	0.12	0.12	0.11	0.12
$G_L + G_K$, mho/cm ²	0.20	0.22	—*	0.21
G_K , mho/cm ²	0.08	0.10	—*	0.09
$G_L + G_{Na}$, mho/cm ²	0.24	0.37	0.26	0.29
G_{Na} , mho/cm ²	0.12	0.25	0.15	0.17
RP, mv	-84	-82	-82	-83
Threshold, mv	26	28	32	29
E_{Na} , mv	+111	+85	+78	+91

* Omitted from the average. G_K is 0 in the presence of Ba and $G_L + G_K = G_L$.

DISCUSSION

Pharmacological K Inactivation Cs and Rb induce pharmacological inactivation in eel electroplaques (Nakamura et al., 1965 *b*) and, as shown in

the present work (Fig. 5), Ba also induces this state. Raising Ca to 30 mM or 10 times the normal level is without effect (Nakamura and Grundfest, 1965, and unpublished data). Cs also induces K inactivation in crustacean muscle fibers (Gainer et al., 1967; Ozeki et al., 1966). Rb induces K inactivation in muscles of frog (Adrian, 1964) and lobster (unpublished data). Ba has a wider range of action. In addition to causing K inactivation in various arthropod muscles it can also contribute to the depolarizing electrogenesis (Fatt and Ginsborg, 1958; Reuben et al., 1961; Werman and Grundfest, 1961; Werman et al., 1961). In eel electroplaques it also blocks the electrically excitable Na channel, but more slowly than it affects the K channels. Tetraethylammonium, which induces K inactivation in very many cells, is without effect in eel electroplaques (unpublished data).

The changes in membrane resistance induced by low concentrations of Cs or Rb—an increase for inward currents and a decrease for outward—suggest that the pharmacological inactivation by these cations may result from obstruction of the reactive K permselective channels. Inward current would drive the Cs or Rb into the channels, while outward current would drive them out and make the channels available for K efflux. Complete block of the channels by Ba might be explained by the larger electrostatic charge of these divalent cations. However, it seems fruitless to speculate further upon the mechanism of the pharmacological inactivation in the absence of data regarding the molecular structure of the excitable membrane and the changes that the latter might undergo during excitation. It is noteworthy that Sr, Ca, and Mn, divalent cations which have larger or smaller ionic radii than Ba, do not induce pharmacological K inactivation (unpublished data).

Impedance Changes and the Form of the Spike Even under normal conditions the impedance changes of eel electroplaques are unusual if compared with similar data on squid or amphibian axons. They become even more unusual when the ion dynamics during spike electrogenesis of the electroplaque are altered by pharmacological K inactivation. Nevertheless, these changes are readily predictable from the changes that the agents induce in the steady-state characteristics. Despite these changes there is little effect on the form of the spike and none on its amplitude. These findings are in accord with the conclusion (Morlock et al., 1968) that the inward Na current of the electrogenesis is returned in the main across the passive "leak" resistance. The Na current calculated from a combination of impedance and phase plane data lasts about 2.5 msec (Morlock et al., 1968). In the present work the impedance data indicate that the conductance increase lasted about 1.5 msec. However, the bridge is highly nonlinear in the configuration used for these measurements (Morlock et al., 1968) and 1.5 msec is therefore the shortest duration. Furthermore, the spikes of the electroplaques vary considerably in form and duration and these variations undoubtedly reflect differences in dynamics of ion movements from cell to cell.

Theoretical Implications In the present work as well as in earlier studies (Nakamura et al., 1965 *b*; Nakamura and Grundfest, 1965, and unpublished data) elimination of K conductance channels did not affect spike electrogenesis. On the other hand, the elimination of Na activation by TTX, saxitoxin, or procaine or by substitution of impermeant cations for Na blocks the inward Na current and spike electrogenesis, but does not alter the steady-state characteristic which is determined by the parallel conductance pathways for leak (G_L) and K (G_K) channels. The present data reinforce the conclusion that the reactive and permselective Na and K channels are independent entities. The K channels could be closed completely or partially without affecting spike electrogenesis. Still more significant in this connection is the finding that when the K channels were in part closed by Cs they could be reopened by small depolarizations. In other words, the membrane could exhibit depolarizing K activation as well as inactivation. The activation developed at very low membrane depolarizations and thus could occur before as well as simultaneously with the Na activation. Also important for theoretical concepts is the finding that K inactivation can precede the onset of Na activation.

According to the Hodgkin and Huxley model (1952), spike electrogenesis is due to the interplay of two activation processes, for Na and K, respectively. These processes might be mediated by separately reactive and permselective sets of channels (Grundfest, 1957 *b*, 1961), or, as Mullins (1956, 1959, 1968) has suggested, by a single system of channels that react with sequentially variable permselectivity, first for Na and then for K. In eel electroplaques there is only one activation process under normal conditions. Furthermore, the reactive K channels, which normally in the electroplaque undergo inactivation, do not exhibit this change in an obligatory sequence. During the normal synaptic excitation of the electroplaques the K inactivation either occurs simultaneously with, or follows close upon Na activation, but under various experimental conditions the K inactivation can be permanent, it may precede the Na activation, or the latter can itself induce some K activation. The membrane conductance can vary a great deal in the resting cell (Fig. 1 and Table I), depending upon the proportion of the reactive K channels that are open. Nevertheless, spike electrogenesis and the Na conductance system are not markedly altered by the large changes in K conductance. Thus, the present evidence strongly favors the existence of two reactive channel systems. There is no reason to believe that the duality of the channel mechanisms does not apply to other excitable cells in which spike electrogenesis is evoked by depolarizing Na activation.

In other cells more than two channel systems might be involved in spike electrogenesis (Grundfest, 1961, 1969). This appears to be the case with some of the cells that generate spikes by influx of both Na and Ca (Geduldig and

Junge, 1968; Hagiwara and Nakajima, 1966; Rougier et al., 1969). However, under appropriate experimental conditions it is also possible to generate spikes by an increased conductance for a single ion species that normally induces repolarizing, rather than depolarizing electrogenesis. The K spikes of *Tenebrio* muscle fibers (Belton and Grundfest, 1962) or the Cl spikes of *Rajid* electroplaques (cf. Grundfest, 1967) provide such examples.

The present data also bear on another theoretical question, whether the electrogenic cell membrane is to be regarded as a heterogeneous or a homogeneous electrochemical system. The differences between the two models have been discussed by Finkelstein and Mauro (1963), who noted that the "divided equivalent circuit" which constitutes the Hodgkin and Huxley model implies a heterogeneous membrane since the model assumes independently reacting ionic conductances with fixed, but different ionic batteries. An alternative model has been proposed by Tasaki (1968) which is based on Teorell's (1953) development of the homogeneous membrane system as envisaged by Nernst and Planck (MacInnes, 1961). In Tasaki's model the ion fluxes are not separated in time and space. Instead, there is "interdiffusion" and the electrogenesis is described (Tasaki, 1968, p. 56) by a single time-variant conductance $(g)_t$ and by a single, also time-variant, emf $(E)_t$. This formulation is strictly in accord with a homogeneous regime (Finkelstein and Mauro, 1963).

The findings of the present work appear to differ decisively from the homogeneous interdiffusion model. The membrane conductance during the spike of the electroplaque increases only transiently. Subsequently, there may be a marked and well-defined decrease from the conductance of the resting cell, or the conductance may increase, depending on experimental conditions. Nevertheless, the membrane potential varies along an essentially fixed course during the spike electrogenesis. The potential of the spike electrogenesis is insensitive to the changes in K conductance mainly because the K channels contribute very little to the shaping of the spike of the electroplaques (Morlock et al., 1968). There is, indeed, interdiffusion, in the sense that the outward current is carried by the leak channels, which form a high conductance system in the electroplaques (Morlock et al., 1968). This system of channels is electrogenically unreactive and its conductance is fixed.

Work in this laboratory is supported in part by funds from the Muscular Dystrophy Associations of America, Inc.; by Public Health Service Research Grants (NB 03728, NB 03270) and Training Grant (NB 5328) from the National Institute of Neurological Diseases and Stroke; and from the National Science Foundation (GB 6988X).

Dr. Ruiz is a Fellow of the Universidad Central de Venezuela.

Dr. Ruarte was a Fellow of the National Council for Scientific and Technical Research in Argentina.

Dr. Schwartz was a Trainee Fellow in Neurophysiology (Grant NB 5328).

Received for publication 28 July 1969.

REFERENCES

- ADRIAN, R. H. 1964. The rubidium and potassium permeability of frog muscle membrane. *J. Physiol. (London)*. 175:134.
- ALTAMIRANO, M., C. W. COATES, and H. GRUNDFEST. 1955. Mechanisms of direct and neural excitability in electroplaques of electric eel. *J. Gen. Physiol.* 38:319.
- BELTON, P., and H. GRUNDFEST. 1962. Potassium activation and K spikes in muscle fibers of the mealworm larva (*Tenebrio molitor*). *Amer. J. Physiol.* 203:588.
- COLE, K. S., and H. J. CURTIS. 1939. Electric impedance of the squid giant axon during activity. *J. Gen. Physiol.* 22:649.
- FATT, P., and B. L. GINSBORG. 1958. The production of regenerative responses in crayfish muscle fibres by the action of calcium, strontium and barium. *J. Physiol. (London)*. 140:59P.
- FINKELSTEIN, A., and A. MAURO. 1963. Equivalent circuits as related to ionic systems. *Biophys. J.* 3:215.
- GAINER, H., J. P. REUBEN, and H. GRUNDFEST. 1967. The augmentation of postsynaptic potentials in crustacean muscle fibers by cesium. A presynaptic mechanism. *Comp. Biochem. Physiol.* 20:877.
- GEDULDIG, D., and D. JUNGE. 1968. Sodium and calcium components of action potentials in one *Aplysia* giant neurone. *J. Physiol. (London)*. 199:347.
- GRUNDFEST, H. 1957 a. The mechanisms of discharge of the electric organ in relation to general and comparative electrophysiology. *Progr. Biophys. Mol. Biol.* 7:1.
- GRUNDFEST, H. 1957 b. Excitation triggers in post-junctional cells. In *Physiological Triggers*. T. H. Bullock, editor. American Physiological Society, Washington, D. C. P. 119.
- GRUNDFEST, H. 1961. Ionic mechanisms in electrogenesis. *Ann. N. Y. Acad. Sci.* 94:405.
- GRUNDFEST, H. 1966. Comparative electrobiology of excitable membranes. *Adv. Comp. Physiol. Biochem.* 2:1.
- GRUNDFEST, H. 1967. Some comparative biological aspects of membrane permeability control. *Fed. Proc.* 27:1613.
- GRUNDFEST, H. 1969. The excitable electrogenic membrane as a heterogeneous electrochemical system: New evidence. *Israel J. Med. Sci.* In press.
- HAGIWARA, S., and S. NAKAJIMA. 1966. Differences in Na and Ca spikes as examined by application of tetrodotoxin, procaine, and manganese ions. *J. Gen. Physiol.* 49:793.
- HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (London)*. 117:500.
- MACINNES, D. A. 1961. *The Principles of Electrochemistry*. Dover Press, New York. 2nd edition.
- MORLOCK, N. L., D. A. BENAMY, and H. GRUNDFEST. 1968. Analysis of spike electrogenesis of eel electroplaques with phase plane and impedance measurements. *J. Gen. Physiol.* 52:22.
- MULLINS, L. J. 1956. Structure of the nerve cell membrane. In *Molecular Structure and Function of Neurons*. R. G. Grenell and L. J. Mullins, editors. American Institute of Biological Sciences, Washington, D. C. P. 123.
- MULLINS, L. J. 1959. An analysis of conductance changes in squid axons. *J. Gen. Physiol.* 42:1013.
- MULLINS, L. J. 1968. A single channel of a dual channel mechanism for nerve excitation. *J. Gen. Physiol.* 52:550.
- NAKAMURA, Y., and H. GRUNDFEST. 1965. Different effects of K and Rb on electrically excitable membrane of eel electroplaques. Abstracts 23rd International Congress Physiological Society, Tokyo. Abstr. 167.
- NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965 a. The action of tetrodotoxin on electrogenic components of squid giant axons. *J. Gen. Physiol.* 48:985.
- NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965 b. Analysis of spike electrogenesis and depolarizing K inactivation in electroplaques of *Electrophorus electricus*, L. *J. Gen. Physiol.* 49:321.

- OZEKI, M., A. R. FREEMAN, and H. GRUNDFEST. 1966. The membrane components of crustacean neuromuscular systems. II. Analysis of interactions among the electrogenic components. *J. Gen. Physiol.* **49**:1335.
- REUBEN, J. P., R. WERMAN, and H. GRUNDFEST. 1961. The ionic mechanisms of hyperpolarizing responses in lobster muscle fibers. *J. Gen. Physiol.* **45**:243.
- ROUGIER, O., G. VASSORT, D. GARNIER, Y. M. GARGOUIL, and E. CORABOEUF. 1969. Existence and role of a slow inward current during frog atrial action potential. *Arch. gesamte Physiol. Menschen Tiere (Pfluegers)*. **308**:91.
- TASAKI, L. 1968. Nerve Excitation. A Macromolecular Approach. C. C. Thomas Publisher, Springfield, Illinois.
- TEORELL, T. 1953. Transport processes and electrical phenomena in ionic membranes. *Progr. Biophys.* **3**:305.
- WERMAN, R., and H. GRUNDFEST. 1961. Graded and all-or-none electrogenesis in arthropod muscle. II. The effect of alkali-earth and onium ions on lobster muscle fibers. *J. Gen. Physiol.* **44**:997.
- WERMAN, R., F. V. MCCANN, and H. GRUNDFEST. 1961. Graded and all-or-none electrogenesis in arthropod muscle. I. The effects of alkali-earth cations on the neuromuscular system of *Romalea microptera*. *J. Gen. Physiol.* **44**:979.