Synaptic Electrogenesis in Eel Electroplaques

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ABSTRACT Whether evoked by neural or by chemical stimulation, the synaptic membrane of eel electroplaques contributes a depolarizing electrogenesis that is due to an increased conductance for Na and K. The reversal potential (E_S) is the same for the two modes of synaptic activation. It is inside-positive by about 30–60 mv, or about midway between the emf's of the ionic batteries for Na (E_{Na}) and K(E_K). The total conductance contributed by synaptic activity (G_S) varied over a fivefold range, but the individual ionic branches, $G_{S_{Na}}$ and G_{S_K}, change nearly equally so that the ratio $G_{S_{Na}}:G_{S_K}$ is near unity. G_{S_K} increases independently of the presence or absence of Na in the bathing medium, and independently of the presence or absence of the electrically excitable G_K channels. When activated, the synaptic membrane appears to be slightly permeable to Ca and Mg. When the membrane is depolarized into inside positivity the conductance of the synaptic components decreases and approaches zero for large inside-positive values. Thus, the synaptic components become electrically excitable when the potential across the membrane becomes inside-positive, responding as do the nonsynaptic components, with depolarizing inactivation.

INTRODUCTION

The electrogenically reactive membrane of the caudal surface of an eel electroplaque is densely innervated by axons from a number of spinal roots. Stimulation of these axons evokes large depolarizing excitatory postsynaptic potentials (EPSP's) which, in turn, elicit the neurally evoked spikes of the nonsynaptic electrically excitable component of the membrane (Altamirano, Coates, and Grundfest, 1955). The conductance change that causes the synaptic electrogenesis was studied in the present work under various ionic conditions which permitted identification of the ionic batteries that are responsible for the EPSP. As in frog muscle, the EPSP of the electroplaque results from an increased conductance for both Na and K. Consequently, the reversal potential of the EPSP's is the lumped equivalent (Thevenin) emf of a system

that comprises two ionic emf's, E_K and E_{Na}, and the conductances of the respective synaptic batteries, G_{S_K} and $G_{S_{Na}}$.

The synaptic generators operate in conjunction with the nonlinear reactive components of the nonsynaptic membrane, the electrically excitable conductance channels for K (G_K) and Na (G_{Na}), respectively, as well as the nonreactive elements, the membrane capacity (C_m) and the leak channels (G_L) which have been analyzed in previous work (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970). Under the experimental conditions of the present work these different components could be evaluated separately. That possibility has disclosed a new finding, that when the synaptic component is depolarized strongly it, too, develops a nonlinear characteristic; i.e., it becomes electrically excitable.

METHODS

Single electroplaques were prepared and mounted as described in previous work (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970). The main experimental data were measurements of the current-voltage characteristic with and without synaptic activity. The membrane potential was perturbed by pulses of current lasting about 20 msec, which were long enough to establish the steady-state characteristic of the cell. In some experiments a neurally evoked synaptic activation was superimposed on the steady-state potential. In the other experiments a synapse activator agent (acetylcholine, carbamylcholine, butyrylcholine, or tetramethylammonium) was added to induce a change in the characteristics. Examples of these data are shown in Figs. 3, 4, 7, and 8. Experiments were also done (Fig. 2) with the cell inserted into an AC Wheatstone bridge (Morlock et al., 1968; Ruiz-Manresa et al., 1970). The details of the experimental techniques and of the equipment are given in the references cited above.

At the beginning of each experiment the cell was bathed in a standard eel saline that contained (in mM/liter) NaCl 195; KCl 5; $CaCl_2$ 3; $MgCl_2$ 1.5; glucose 10; phosphate buffer 1.15 (pH 7.2) (Ruiz-Manresa et al., 1970). In some experiments the Na was replaced isosmotically with K or with Ca or Mg. Neither of the divalent cations alters the steady-state characteristic of the membrane, nor do they support spike electrogenesis (Ruiz-Manresa, 1970). Low concentrations of CsCl or $BaCl_2$ were added when it was desired to eliminate the electrically excitable component of the K conductance (G_K) by pharmacological K inactivation. In these cases the steady-state characteristic became linear, either over part of the range of membrane potentials or it became completely linear over a range of about ± 200 mv from the resting potential (Nakamura et al., 1965; Ruiz-Manresa et al., 1970).

RESULTS

Spikes are evoked by EPSP's or by applied currents that depolarize the electroplaque some 30–40 mv from its resting potential (Altamirano, Coates, and Grundfest, 1955; Nakamura et al., 1965). Thus, the range of measurements of the characteristic of the synaptically activated cell is limited for

membrane potentials that are positive to the resting potential. Spike electrogenesis can be blocked by inducing the synaptic activity while the cell is hyperpolarized by an applied current. The range of these measurements is also limited, however, by the onset of hyperpolarizing K inactivation, which like the depolarizing K inactivation, introduces a nonlinearity into the characteristic when the cell is hyperpolarized by some 75–100 mv (Nakamura and Grundfest, 1965, and unpublished data; cf. Grundfest, 1969, Fig. 25; Ruiz-Manresa, 1970). This nonlinearity could be eliminated by inducing pharmacological K inactivation.

Form of the EPSP's Fig. 1 A shows an EPSP that was evoked by a brief inward (hyperpolarizing) current with the cell in the standard saline and the membrane at the resting potential. The EPSP, which appeared after a characteristic synaptic delay, was kept subthreshold for a spike. A larger stimulus could be applied to the nerve terminals without generating a spike when the neural volley was evoked while the electroplaque was hyperpolarized by an applied current (Fig. 1 B). In this record the initial rapid hyperpolarization was followed by a slower increase of the negativity while the current remained constant, as in other hyperpolarizing inactivation responses (Reuben et al., 1961; Bennett and Grundfest, 1966). However, the steady-state characteristic can be linearized, as already noted, over the range of ±200 mv from the resting potential by pharmacological K inactivation, upon addition of Ba (Ruiz-Manresa et al., 1970; Ruiz-Manresa, 1970). This was done prior to registration of the EPSP in Fig. 1 C. The inward current pulse now immediately induced the large hyperpolarization that is indicative of the high resistance state. The current was so large that it itself stimulated the nerve terminals and a large EPSP was evoked after the synaptic delay.

The EPSP is depressed and blocked by *d*-tubocurarine (*d*TC). While the electrically excitable activity is also abolished by *d*TC the latter effect develops much more slowly (Altamirano, Coates, Grundfest, and Nachmansohn, 1955). The rapidity of the action of *d*TC on the EPSP is shown in the nine superimposed traces in Fig. 1 D, recorded on the same cell as in Fig. 1 C. The registrations were begun 5 sec after applying *d*TC (30 μg/ml) to the innervated surface. By this time the EPSP had been reduced from its maximum (Fig. 1 C) by about 70%. The subsequent traces show the further progressive decrease. The EPSP was abolished within <1 min after the *d*TC was applied.

The peaks of the EPSP's in Fig. 1 B and C were kept negative to the resting potential by the hyperpolarizing currents. Neither these responses, nor the depolarizations that were evoked (near the end of each sweep) by brief applied currents, evoked anode break responses such as are sometimes seen following strong hyperpolarization of the electroplaques (Fig. 2 D). Thus,

Na activation of the electrically excitable membrane was absent in the EPSP's. Nevertheless, the EPSP's lasted much longer than did the approximately equal depolarizations that were induced by the applied currents. The decay of the latter indicates a time constant of 300–400 μsec, which is consistent with the low resting conductance of the cells after K inactivation.

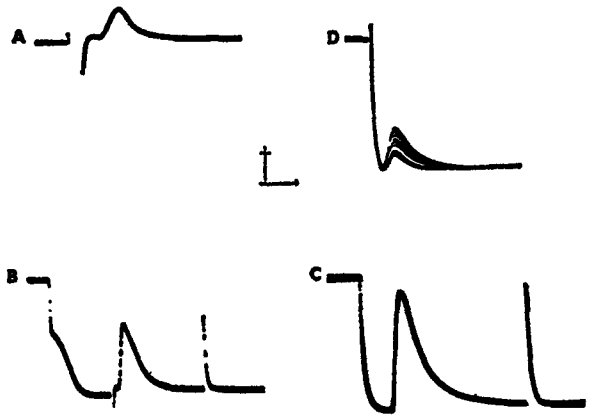

FIGURE 1. EPSP's in eel electroplaques. A and B, cell bathed in standard saline. In A the EPSP was evoked with the cell at its resting potential. The response was kept subthreshold for evoking spikes. In B, the EPSP was evoked after the membrane had been hyperpolarized by an applied current. The latter, an inward current, at first caused a rapid shift in the potential and then there was a slow further increase in the negativity, which was due to the onset of hyperpolarizing K inactivation. Near the end of the record a brief outward current was applied to cause a depolarization of about the same magnitude as that of the EPSP. Note its rapid subsidence. C, D, the slow increase in hyperpolarization was absent because 2.5 mM Ba was added to the saline, causing pharmacological K inactivation. The EPSP's were evoked by the long lasting current. In C a brief depolarization was also induced by an applied pulse. Like that in B this depolarization decayed rapidly whereas the EPSP was much longer lasting. Prior to the records in D (nine superimposed traces registered at 1/sec) the cell was exposed to *d*TC (30 μg/ml). The first response of the sequence, 5 sec after applying the drug, was depressed to about 30% of the amplitude of the control in C. The last EPSP seen in this sequence was only about 10% of the control. The calibrations are 25 mv, 1 msec for A; 50 mV, 5 msec for B and C; and 50 mv, 10 msec for D.

The decay of maximal EPSP's lasts some 10–15 msec in different cells. Thus, the postsynaptic membrane remains activated for a considerable time after a neural volley had caused the onset of transmitter release. Since the transmitter is probably acetylcholine and is probably short-lived, it seems likely that there is continued release of the transmitter after the nerve impulse had subsided.

Conductance Change during the EPSP The electrogenesis of the EPSP is caused by an increase in conductance. The magnitude of the latter and the

nature of its ionic components will be detailed below. The experiments to be described in this section show that the change is, in fact, an increase of conductance and that the increase persists throughout the duration of the EPSP, subsiding as the latter returns toward the resting potential.

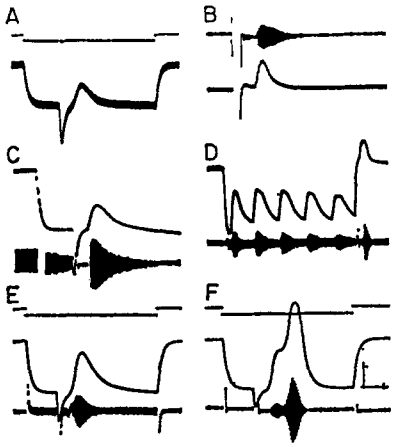

FIGURE 2. Conductance changes during EPSP's. A, cell in the presence of 2.5 mM Ba. Upper trace monitors the hyperpolarizing current during which the EPSP was evoked (membrane potential on lower trace). The record is of a number of superimposed sweeps, with very weak brief hyperpolarizing pulses applied continuously at a frequency of about 10/sec. The thickness of the trace shows the amplitudes of the hyperpolarizations evoked by these test pulses. The trace became thinner during the rising phase of the superimposed EPSP's and during part of the falling phase, denoting that the conductance of the membrane had increased during the EPSP. B–F, cells arranged for impedance registrations. B and C, cells in standard saline. The EPSP in B was evoked at the resting potential and was kept subthreshold for a spike. The small response was associated with an output denoting that the impedance had decreased. Note that the duration of the impedance changes is similar to that of the electrogenesis. C, the EPSP was evoked during a hyperpolarization of the cell. The changes in bridge output preceding that correlated with the EPSP are described in the text. D–F, cells in saline containing 2.5 mM Ba. D, the hyperpolarizing current itself stimulated the nerve terminals giving rise to repetitive EPSP's. The changes in form of these potentials probably reflect asynchronous activity in the different nerve terminals. Each EPSP was associated with a conductance increase. When the current was terminated the cell produced a small anode break spike. The form and magnitude of the impedance change accompanying this response are characteristic for spike electrogenesis in Ba-treated cells. E, F, records from one cell with only an EPSP evoked in E and with a spike following the EPSP in F. The bridge output during the spike was about three times larger than during the EPSP. Calibrations (in F) represent 25 mv, 1 msec for B; 50 mv, 2 msec for all other records.

In the experiment of Fig. 2 A the cell had been treated with Ba. A hyperpolarizing current was applied during each of the sweeps that are superimposed in the record and another brief but strong pulse elicited an EPSP. A train of brief and small hyperpolarizing currents was applied continuously

during the registration and the width of the trace indicates the hyperpolarization induced by these test pulses. The trace became markedly thinner during the rising phase and the early falling phase of the EPSP, indicating that the electrogenesis was associated with an increased conductance. The maximum conductance of the cell increased about twofold during the EPSP. This increase and its time course are demonstrated by the AC Wheatstone bridge measurements. The EPSP of Fig. 2 B was evoked with the cell at its resting potential and therefore the response was kept small so as to prevent spike electrogenesis. The small EPSP is associated with a change in the bridge output which represents a decrease in the impedance. For the records of Fig. 2 C the bridge was originally nulled when hyperpolarizing K inactivation had reduced the membrane conductance to that of the G_L channels alone. The slow onset of the hyperpolarizing K inactivation (Fig. 1 B) is reflected in the gradual decline of the bridge output when an inward current was applied (Fig. 2 C). The brief but strong current required to stimulate the nerve terminals and elicit an EPSP triggered the transition to full K inactivation as it does in other regenerative inactivation responses (Reuben et al., 1961; Nakamura et al., 1965; Bennett and Grundfest, 1966; Grundfest, 1966 *a*, *b*, 1969). When the EPSP was evoked the bridge became unbalanced again, but returned toward null output as the EPSP subsided.

Occasionally, and particularly in the presence of Ba, the nerve terminals are excited repetitively by a maintained hyperpolarizing current. An example is shown in Fig. 2 D. Each elevation during the hyperpolarizing current is associated with a decrease in impedance. The bridge output followed the changes in duration of the EPSP's as the latter became prolonged, presumably by asynchrony in the excitation of different nerve terminals. When the polarizing current was terminated the cell developed an anode break response of somewhat briefer duration than the EPSP's. The form of the impedance change accompanying this electrically excitable response is markedly different from that associated with the EPSP's and resembles the change accompanying a spike (Fig. 2 F).

A comparison of the impedance changes accompanying the EPSP and the spikes is shown in Fig. 2 E and F, on a cell that had been treated with Ba. Only an EPSP was evoked in Fig. 2 E and the accompanying change in impedance is similar to the changes seen in Fig. 2 B–D. In the record of Fig. 2 F the EPSP evoked a spike. The maximum change in bridge output induced by the spike was three to four times larger than that induced by the EPSP, indicating that the conductance increase which results from the electrically excitable Na activation is considerably larger than the conductance increase which causes the synaptic electrogenesis. The time course of the impedance changes during the two responses is also markedly different. The inward currents in Fig. 2 E and F caused a symmetrical rise and fall of the

hyperpolarization of the cell membrane. Their time course reflects the time constant of the membrane. A rather similar time course is seen late during the falling phase of the spike (Fig. 2 F) as is to be expected when Na inactivation is complete and the spike decays passively across the resistance, G_L, and the membrane capacity. The time course of the falling phase of the EPSP (Fig. 2 E) is much slower as was already noted in connection with Fig. 1 B and C.

The "Reversal" Potential (E_S) and the Conductance Increase (G_S) during the EPSP Experiments like those shown in Fig. 3 generated information regarding the over-all conductance increase associated with the EPSP and also regarding the Thevenin equivalent emf of the ionic batteries of the synaptic generators. Pulses of current lasting about 20 msec and varying in intensity were applied to examine the I-E characteristics of the cell. The open circles denote the membrane potentials attained at the end of these pulses and represent the steady-state characteristic. The solid circles plot the peaks of the spikes evoked by the depolarizing currents that were suprathreshold. In addition to the 20 msec pulses, brief currents were applied to evoke the EPSP's and the indirect spikes. The peaks of these are denoted by the + and × symbols, respectively.

In Fig. 3 A are measurements that were made while the cell was bathed in the standard saline. In Fig. 3 B the measurements were repeated after Ba had been applied to eliminate the nonlinearities of the steady-state characteristic which are introduced by the occurrence of K inactivation. In both sets of measurements the peaks of the spikes fall on a straight line which crosses the steady-state characteristic at about 175 mv and represents the reversal potential of the Na battery, E_{Na} (Ruiz-Manresa et al., 1970). The peaks of the EPSP's also fall on a straight line which extrapolates to cross the linear steady-state characteristic at about 65 mv inside-positive. This juncture (E_S) should determine the reversal potential of the EPSP (Grundfest, 1961 *a*). The conductance line for the synaptically activated membrane represents the sum of the synaptic component itself (G_S) and that of the resting membrane. In the experiment of Fig. 3 B the resting conductance is G_L (ca. 0.26 mho/cm²). The total conductance ($G_L + G_S$) is 0.46 mho/cm² and G_S is 0.20 mho/cm².

The "Reversal" Potential of the Chemically Activated Synaptic Membrane The experiments for these measurements were similar to those described in the preceding section, except that the synaptic membrane was excited by applying a synapse activator agent. Since the cells were maximally depolarized by the latter, spike electrogenesis was abolished. E_{Na} was determined during control measurements prior to applying the agent and the subsequent measurements were done fairly rapidly so as to avoid the possibility that the sus-

tained depolarization might have altered the distribution of ions across the membrane. Effects that may be ascribed to such changes have been observed and will be described at another time.

Fig. 4 A shows an experiment on an electroplaque which exhibited a

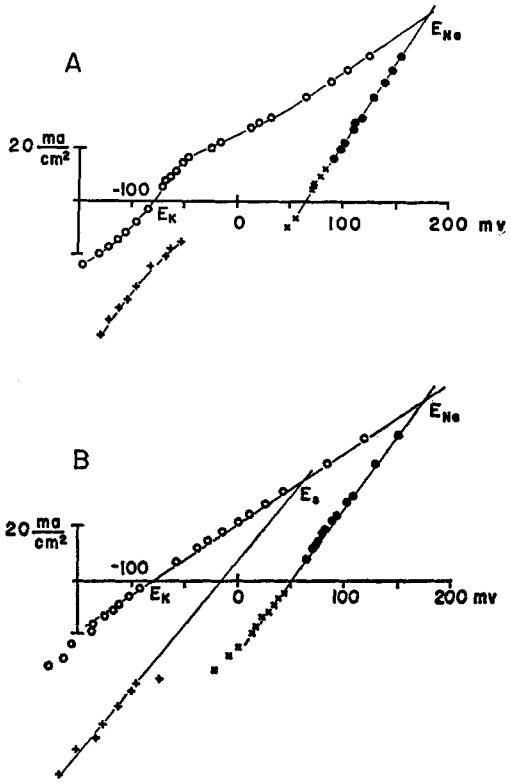

FIGURE 3. I-E characteristics of nonsynaptic and synaptic components of an electroplaque. A, cell in standard saline; B, after adding 2.5 mM $BaCl_2$. The steady-state characteristic (open circles) was nonlinear in A, but became linear in B when Ba induced pharmacological K inactivation. The resting potential (E_K) was not changed, nor was E_{Na}, the value at which the steady-state characteristic crosses the line formed by the peaks of the directly evoked spikes (solid circles). EPSP's were evoked with the membrane held negative to the resting potential. The peaks (+) fall about straight lines which cross the steady-state characteristic at E_S, the theoretical reversal potential for the EPSP. Note that this value (ca. 65 mv) is strongly positive in eel electroplaques. When the EPSP's evoked spikes their peaks (×) fell on or close to the line generated by the peaks of the directly evoked spikes.

markedly nonlinear steady-state characteristic. The resting conductance was rather high so that closure of the G_K channels by either depolarizing or hyperpolarizing K inactivation changed the characteristic substantially. When carbamylcholine was applied the cell depolarized to about −15 mv

and the steady-state characteristic was curved. A straight line could be drawn through the measurements made with hyperpolarizing currents, but this straight line crosses the conductance lines for either condition of the steady-state characteristic and therefore the value of E_S is indefinite.

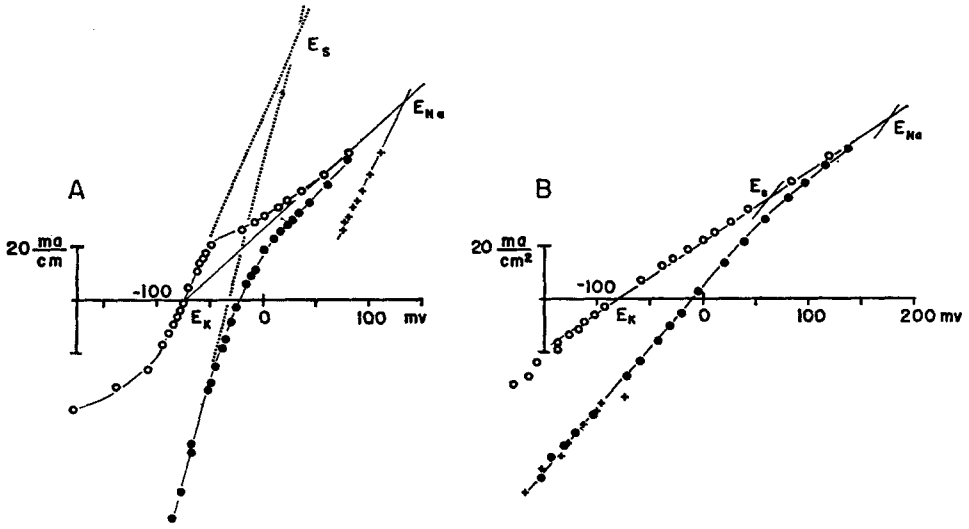

FIGURE 4. I-E characteristics of electroplaques before and after activation of the synaptic component by carbamylcholine. A, the control, with the cell in the standard saline, showed a markedly nonlinear characteristic in the steady state (open circles). The straight dotted line through E_K represents the combined conductance for $G_L + G_K$. The straight continuous line for large depolarization represents G_L alone, when G_K was abolished by depolarizing K inactivation. The curvature in the lower quadrant represents a partial closure of the G_K channels by hyperpolarizing K inactivation. The peaks of the spikes evoked by depolarizing currents are plotted as + and fall on a line which crosses the G_L characteristic at E_{Na}, about 140 mv in this cell. On applying carbamylcholine the cell depolarized to about −20 mv. The characteristic which shifted to that plotted by solid circles was nonlinear and for larger depolarizations approached the G_L characteristic of the control. Further description in text. B, same cell as in Fig. 3 B. The open circles plot the steady-state characteristic (G_L) which was the same as in Fig. 3 B. Carbamylcholine caused the shift in the characteristic plotted by the solid circles. The peaks of the synaptic potentials obtained in Fig. 3 B are included (+) and fall on the same line which extrapolates to cross the G_L characteristic at about 65 mv. This value (E_S) is the same as was obtained in Fig. 3 B. Note, however, that for strong depolarizing currents the solid circles tend to lie close to or on the G_L line. Further description in text.

However, when the steady-state characteristic is linearized by applying Ba, measurement of the effects of the synapse activator drug also becomes simplified. The experiment shown in Fig. 4 B was done on the same cell as the one in Fig. 3. In fact, the peak values of the EPSP's recorded in Fig. 3 B are also shown in Fig. 4 B (+ symbols). The change in the characteristic on

adding carbamylcholine (solid circles) was the same as that induced by neural activation of the synaptic membrane, except that the measurements could now be made for a larger range of depolarizing currents. The characteristic became somewhat curved for large depolarizations, and at the limit appeared to approach the characteristic of the G_L channels. The straight line portion of the characteristic extrapolates to about 60 mv for E_S, quite similar to the value obtained with neural stimulation (Fig. 3 B). Thus, it may be concluded that E_S is the same when the synaptic membrane is activated chemically or neurally.

Effects of Other Synapse Activators Results similar to those shown in Fig. 4 were also obtained with acetylcholine, butyrylcholine, and tetramethylammonium. The concentrations of these agents required to induce maximal depolarization varied with the dose-response relations of the respective systems, and will be described elsewhere. No secondary effects of the drugs were observed even at the highest dosages employed (ca. 10^{-3}M) when the time allowed for ionic redistribution was minimized.

Electrical Inactivation of the Synaptic Membranes The curvature in the characteristic of the membrane with the synapses activated which is clear-cut in Fig. 4 B is also seen in Fig. 4 A and has been found in all similar experiments. For strongly positive values of the membrane potential the characteristic line for the synaptically activated membrane tends to approach the characteristic line for G_L. This finding implies that the conductance channels of the synaptic membrane, which have been opened by a neural transmitter or its mimetic, become closed by strong depolarization; i.e., in this range of the membrane potential the synaptic channels become electrically excitable, responding as do the K channels of the nonsynaptic membrane, with depolarizing inactivation.

The conclusion that the synaptic membrane becomes inactivated by strong depolarization was tested in two types of experiments. In that of Fig. 5 the cell was treated with Ba. With the membrane at its resting potential (A, B) outward and inward pulses of current (monitored on the upper traces) caused symmetrical changes in potential, since the steady-state characteristic is linear in the Ba-treated cell. Carbamylcholine was then applied, causing the cell to depolarize. The outward current (C) now caused a larger change in E_M than did the inward current (D). The difference in membrane resistance which this indicates becomes even more marked in the tests of Fig. 5 E. When the cell was repolarized to its original resting potential by a steady applied inward current the test pulse of outward current caused a much smaller depolarization than did the same pulse delivered to the depolarized cell. It is evident on comparing the test pulses in E that the conductance of the strongly depolarized membrane is very near to that of G_L channels alone (A).

Fig. 6 provides another variety of evidence regarding the effect of strong depolarization on the conductances of the synaptic membrane. In this case the depolarization was that of the spike itself and the synaptic membrane was activated by a neural volley. The electroplaque was mounted in a chamber which divided the excitable membrane into three compartments

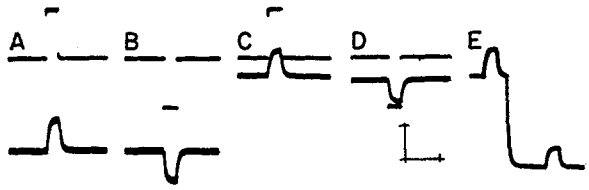

FIGURE 5. Further verification of the nonlinear conductance change in the synaptic membrane. A, B, depolarizing and hyperpolarizing pulses were applied with the cell at the resting potential (−75 mv) and bathed in saline containing 2.5 mM $BaCl_2$. C, D, similar test pulses which were applied after the cell was depolarized to −15 mv with carbamylcholine induced potentials that were almost as large as in A and B. E, a pair of depolarizing current pulses were applied first to the depolarized cell and then during repolarization to the original resting potential. The second potential was considerably smaller, as would be predicted from the characteristics in Fig. 4 A and B. Calibration, 30 mv and 1 msec. The upper trace monitors the current in A to D and also indicates the zero potential reference.

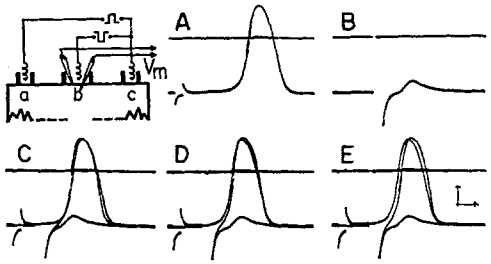

FIGURE 6. Further evidence of depolarizing inactivation of the synaptic membrane. Diagram shows the experimental arrangement. A, spike recorded in the central compartment (*b*) after propagation from the end compartment (*a*) where it originated. B, subthreshold EPSP evoked within the central compartment. C-E, each record shows three superimposed traces; of the spike alone; of the EPSP alone; and of the two timed so as to occur nearly simultaneously in the central compartment. Calibration, 25 mv, 1 msec. Further description in text.

interconnected by narrow channels. A pair of electrodes in the outer chambers (*a* and *c*) passed current into and out of the membrane, evoking a spike at the cathodal site. This direct spike propagated into the central compartment (*b*) and was recorded there with a pair of microelectrodes that straddled the membrane. The propagated spike is seen in Fig. 6 A. Auxiliary stimulating electrodes passed a current inward across the membrane in the central compartment to evoke the subthreshold EPSP seen in B. Records C-E each

show three superimposed traces; of the direct spikes alone, of the EPSP alone, and of both together. When the EPSP was evoked as the spike was invading the central compartment (C) the invasion was facilitated (Altamirano, Coates, and Grundfest, 1955). However, there was little if any change in the form of the spike. When the stimulus to evoke the EPSP was delayed (D, E) the hyperpolarization caused by the inward current slowed invasion by the propagating spike. Aside from this effect the spikes were not altered by the superimposed excitation of the nerve terminals.

The "reversal" potential of the EPSP is substantially negative relative to E_{Na} (Figs. 3 and 4). It might be expected, therefore, that a conductance increase caused by the active synaptic membrane would cause some "short-circuiting" of the spike, as in the case of frog muscle (Fatt and Katz, 1951). Absence of such an effect supports the conclusion that the conductance of the synaptic membrane becomes small or negligible when the electroplaque is strongly depolarized.

The Ionic Components of the Synaptic Electrogenesis The "reversal potential," E_S, is approximately midway between E_{Na} and E_K (Figs. 3 and 4). It therefore seems likely that E_S is the Thevenin emf of two conductance systems, one for Na ($G_{S_{Na}}$) and the other for K (G_{S_K}). The experiments illustrated in Figs. 7 and 8 were designed to investigate this aspect.

The electroplaques of Fig. 7 were bathed in isosmotic KCl which replaced all the other salts of the standard bathing medium. In this medium the resting potential becomes close to zero (-15 mv in the cell of Fig. 7 A), the conductance becomes very high (0.65 mho/cm² in Fig. 7 A) except for large applied depolarizing currents. Depolarizing K inactivation then ensues and the membrane conductance falls to G_L, 0.22 mho/cm² in Fig. 7 A. The shift is regenerative through a negative slope region which is a forbidden range in current clamp experiments (Nakamura et al., 1965). The solid circles in Fig. 7 A represent measurements made before and the open circles measurements made after applying carbamylcholine. Absence of an effect of the agent on the high conductance branch of the characteristic might be ascribed to a masking of the expected conductance increase by the already high conductance of the cell before addition of carbamylcholine. However, this masking should not occur during strong depolarization when the non-synaptic conductance is only that of G_L. Absence of a change in conductance for this branch in the presence of the synapse activator is similar to the effects seen in Fig. 4.

The cell of Fig. 7 B, which was also bathed in the isosmotic KCl medium, was treated with Ba sufficient to cause pharmacological K inactivation (Ruiz-Manresa, 1970). The steady-state characteristic now became nearly linear over a range $> \pm 100$ mv, and the conductance, G_L, became 0.19 mho/cm².

The resting potential remained low (ca. −5 mv in this experiment), confirming the conclusion of earlier work (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970) that E_K is the emf for the G_L channels.

When carbamylcholine was introduced (open circles) the characteristic shifted essentially by rotation of the lower branch about the resting potential. The conductance increased to 0.46 mho/cm², signifying that the conductance of the synaptic membrane (G_S) now was 0.27 mho/cm². Absence of further depolarization on adding the activator agent is to be expected, if K is the only available permeant cation and the resting potential is already at E_K.

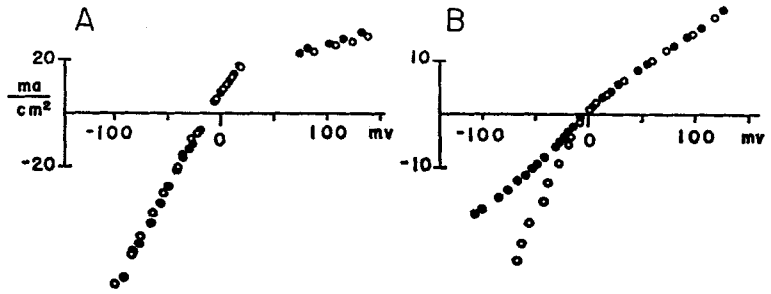

FIGURE 7. Electroplaques bathed in isosmotic KCl saline. A, cell without Ba. Solid circles before, open circles after applying carbamylcholine. The left branch of the characteristic shows the high conductance typical of cells bathed in high K_o, and a regenerative shift of the characteristic to the low conductance state G_L. The drug did not appear to affect the characteristic. B, another cell, after applying Ba. The characteristic now became almost linear (solid circles) with the conductance decreased nearly to that of the G_L channels alone. Open circles show the change induced by carbamylcholine. There was a conductance increase only in the lower left quadrant, but there was no shift in the resting potential, which was at E_K.

However, the branch of the characteristic for more positive membrane potentials was unaffected by addition of carbamylcholine. Clearly, the conductance of this branch of the characteristic was as low in the presence of the drug as in the control.

The data of Fig. 7 B demonstrated that the conductance of the synaptic membrane can be increased by a synapse activator agent even in the absence of Na_o and with the membrane depolarized by the presence of isosmotic K. Thus, an increase in K conductance is certainly one element in the synaptic electrogenesis. The role of Na was tested in experiments like those of Fig. 8, in which the only salt was that of the divalent cation Ca (left) or Mg (right). Since K_o was zero for these cells the cells hyperpolarized to about −120 mv in Ca and −100 mv in Mg and the characteristic (solid circles) became linear (Nakamura and Grundfest, 1965, and unpublished data; Ruiz-Manresa, 1970) over the range −200 to 100 mv. This conductance, which represents G_L, was 0.12 mho/cm² in Ca and 0.085 mho/cm²

in Mg. When carbamylcholine was applied the cells depolarized (open circles). The conductance increased by 0.08 mho/cm² (Ca) and 0.11 mho/cm² (Mg), but when the cells were depolarized by the applied current their conductance diminished and approached that of G_L. Thus, as in the experiments of the previous sections depolarization of the membrane tended to eliminate the channels that had been opened by activation of the synaptic membrane.

The depolarization produced by carbamylcholine indicates that the conductance of the synaptic membrane had increased with respect to some cation other than K. It is possible, though unlikely that E_{Na} was more positive than E_K in the absence of Na_o. While the cell membrane at rest is effectively

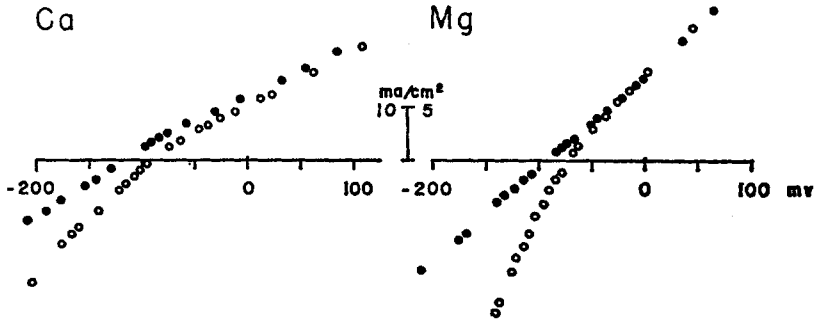

FIGURE 8. Conductance changes induced by carbamylcholine in cells bathed in full $CaCl_2$ (left) and $MgCl_2$ (right). The depolarizations (ca. 30–25 mv) caused by carbamylcholine (open circles) indicate that E_S must be positive relative to E_K, which was strongly inside-negative because of the absence of K_o. Extrapolation of the characteristic indicates that E_S was approximately −60 mv. The high conductance seen in the lower quadrant decreased on depolarization of the cells.

impermeable to the divalent cations that were present in the two experiments of Fig. 8, it is also possible that when the synaptic membrane is activated it may become slightly permeable to Ca and Mg. A similar situation has been found with respect to the permeability of the inhibitory synaptic membrane of lobster muscle fibers for some large anions (Motokizawa et al., 1969). Even though the conductances for these ions might be very small the electrochemical gradient would be large and the emf's of the cationic batteries (E_C^{++}) would be strongly inside-positive. The emf of the synaptic generator would then be the Thevenin equivalent of an inside-negative E_K with a large G_K and of a strongly positive E_C^{++} with a very small G_C^{++}. Extrapolations of the lines formed by the open circles in the lower quadrants of Fig. 8 (left) and (right) cross the steady-state characteristic at about −60 mv (Ca) and −75 mv (Mg).

The Equivalent Circuit of the Synaptic Generator The experiments of the preceding sections demonstrated that on becoming activated, either by the neural mechanism or by chemical agents, the synaptic membrane changes its conductance to K (G_{s_K}). With an increase in the latter the emf of the K battery (E_K) contributes to the synaptic electrogenesis. The reversal potential of the synaptic generator (E_S) is strongly inside-positive, by as much as 50 mv or more, under normal ionic conditions. E_S decreases when all the Na_o is replaced by Ca or Mg. Hence E_{Na} is also a factor in the synaptic electrogenesis. The absolute value of E_{Na} is considerably greater than that of E_K and this can account for the strongly inside-positive value of E_S.

These conclusions can be symbolized in the equivalent circuit of Fig. 9.

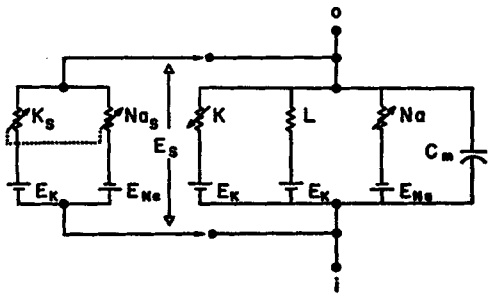

FIGURE 9. Equivalent circuit of the electroplaque. Right branch, the three parallel ionic channels of the nonsynaptic membrane symbolized as resistances of the nonreactive leak channels (L) and of the electrically excitable channels for K (arrow pointing down indicates inactivation) and Na (arrow indicates activation). The emf's of the respective ionic batteries are E_K for L and K and E_{Na} for Na. C_m is the membrane capacity. External and internal surfaces of the caudal membrane are 0 and i, respectively. On the left are the two ionic channels of the synaptic battery (K_S and Na_S) with the emf's E_K and E_{Na}, respectively. The synaptic branch does not contribute an emf to the membrane, i.e. the conductances G_{S_K} and $G_{S_{Na}}$ are zero, until the synaptic membrane is activated by neural or chemical stimuli. The net result then is a switching in of a synaptic electrogenesis E_S, which is the Thevenin equivalent emf resulting from the finite conductances, G_{S_K} and $G_{S_{Na}}$. The two arrows are connected with a broken line to indicate that the changes in G_{S_K} and $G_{S_{Na}}$ probably occur simultaneously. Both synaptic channels become inactivated when the membrane is depolarized strongly.

The section on the right represents the equivalent circuit of the nonsynaptic membrane that has emerged from earlier work (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970). On the left is the complex which forms the synaptic generator. Block of synaptic activity by *d*-tubocurarine does not alter the resting potential of the electroplaques. The Thevenin potential of the generator (E_S) is large. It must be assumed therefore that the resting synaptic membrane makes no contribution to the emf of the cell, which is E_K. For this reason the synaptic generator is shown as switchable, in the circuit only when the synaptic membrane is activated.

The individual components of the synaptic generator thus are the K battery (E_K) with a peak conductance, G_{S_K}, and the Na battery (E_{Na}) with a peak conductance $G_{S_{Na}}$. The two conductances together make up the Thevenin equivalent conductance:

$$G_S = G_{S_K} + G_{S_{Na}} \quad (1)$$

which can be determined from experiments like those of Figs. 3 and 4. The Thevenin equivalent emf is:

$$E_S = \frac{E_K G_{S_K}}{G_S} + E_{Na} \frac{G_{S_{Na}}}{G_S} \quad (2)$$

The values of G_S, E_K, E_{Na}, and E_S are all accessible from experimental data. Therefore, G_{S_K} and $G_{S_{Na}}$ can be obtained by appropriate substitutions in the above equation.

TABLE I

E_K	E_{Na}	E_S	G_S	G_{S_K}	$G_{S_{Na}}$	$G_{S_{Na}}/G_{S_K}$
mv	*mv*	*mv*	*mho/cm²*	*mho/cm²*	*mho/cm²*	
−80	175	55	0.21	0.10	0.11	1.1
−85	197	33	0.12	0.07	0.05	0.69
−85	125	37	0.30	0.13	0.17	1.30
−80	97	−7	0.24	0.12	0.12	1.0
−80	100	37	0.19	0.07	0.12	1.71
−75	130	35	0.60	0.28	0.32	1.14

More than 30 electroplaques were analyzed in this manner. The range of variation in the different parameters is exemplified by the data on the six cells in Table I and the two cells of Figs. 3 and 4. The resting potential (E_K) varied relatively little from cell to cell, ranging between −75 and −85 mv in our total sample. E_{Na} varied considerably from 97 to 197 mv (average ca. 150 mv). A large variation in E_{Na} was also observed in earlier work (Nakamura et al., 1965; Morlock et al., 1968). The total synaptic conductance (G_S) also varied widely, over a fivefold range. However, the conductances of the two ionic components, $G_{S_{Na}}$ and G_{S_K}, tended to vary together, so that the ratio $G_{S_{Na}}:G_{S_K}$ ranged only between 0.69 and 1.71. Thus, the variation in the emf of the synaptic battery (E_S) was mainly dependent upon the magnitude of E_{Na}. In all the cells tested, except one (where it was −7 mv), E_S was substantially positive at ca. 30 mv or more.

DISCUSSION

The Synaptic Transmitters The neurally evoked EPSP and the depolarizing electrogenesis that is evoked by the synapse activator agents, acetyl-

choline, carbamylcholine, butyrylcholine, and tetramethylammonium, are associated with a similar increase in conductance (Fig. 4 B). The reversal potential, E_S, is the same for both types of synaptic activation. Thus, these electrophysiological measurements confirm the earlier conclusion that the synaptic activation in the eel electroplaque is cholinergic. The decay of the EPSP from its peak is much slower than the decay of a passive change in the membrane potential (Fig. 1) and the impedance data (Fig. 2) demonstrate that the conductance of the membrane is high during the falling phase of the EPSP. Thus, the duration of the EPSP represents persistent activation of the receptors of the postsynaptic membrane by the transmitter. This finding is particularly striking because of the high concentration of acetylcholinesterase in the electric organs of the eel. It is more than 100 times greater than in mammalian skeletal muscle and about 1,000 times greater than in frog skeletal muscle (Nachmansohn, 1953).

A slow decay of the synaptic potential as compared with the time constant of the membrane has been observed in neurons of *Aplysia* (Fessard and Tauc, 1957), supramedullary neurons of puffer (Hagiwara and Saito, 1959), lobster cardiac ganglion cells (Hagiwara et al., 1959), sympathetic ganglion cells of frog (Nishi and Koketsu, 1960), electroplaques of marine electric fishes (Grundfest and Bennett, 1961), and chick ciliary ganglion cells (Martin and Pilar, 1963). In neuromuscular junctions of the frog the action of the transmitter disappears rapidly and the decay of the EPSP is mainly determined by the membrane time constant (Fatt and Katz, 1951; Boyd and Martin, 1956).

The difference between the cholinergic systems of frog muscle and eel electroplaques, particularly in the face of the discrepancy in their cholinesterase content cannot be resolved with the available data. Assuming that the transmitter is identical, and presumably it is acetylcholine in both cases, the difference in the duration of transmitter action might be caused by a much larger or prolonged release of acetylcholine at the electroplaque, by a mismatch in the topology of the esterase and acetylcholine, or by a persistence of the activated state of the synaptic receptors even after the transmitter had been hydrolyzed by the enzyme. It is noteworthy in this connection that the neurally mediated excitation of the eel electroplaques as well as of *Torpedo* is blocked after several hundred repetitions at relatively low rates of stimulation (du Bois-Reymond, 1881; Grundfest and Bennett, 1961). The block appears to be due to exhaustion of the transmitter since the electroplaques of the eel continue to respond to direct stimulation at high frequencies. The EPSP's of the strongly electric marine electric fishes are in general maximal depolarizations (Grundfest and Bennett, 1961). It is also likely that a single volley into all the axons that innervate an electroplaque likewise generates a maximal EPSP, since the neurally evoked spike then occurs simultaneously everywhere along the very large surface of the cell

(Altamirano, Coates, and Grundfest, 1955). Thus, it is likely that the total amount of transmitter released during each neural volley is very high in the eel as compared with the amount released at the frog end plate.

The Driving Force (E_S) of the Synaptic Battery The Thevenin emf of the postsynaptic electrogenesis is generally somewhat negative to reference zero in most cells. In some a reversal of sign can be demonstrated at a given range of membrane potential, as is demanded by the theory of electrically inexcitable electrogenesis (Grundfest, 1957, 1961 *a*, 1961 *b*). This does not occur in eel electroplaques (Fig. 6, and Altamirano, Coates, and Grundfest, 1955) and E_S was estimated by extrapolation and in all but one of our measurements on some 30 cells E_S was estimated to be ca. 30 mv or more. The large positive value for E_S in the eel electroplaques is the consequence of the large positive value of E_{Na}. A positive value for E_S, approaching E_{Na} in value, has been reported for the EPSP of the squid giant axon (Gage and Moore, 1969; Miledi, 1969). Since E_{Na} in this cell is much smaller than it is in eel electroplaques (ca. 60 mv; Hodgkin and Huxley, 1952) the contribution of $G_{S_{Na}}$ to the synaptic electrogenesis must be predominant in the squid axon and the conductance increase for other ions may be small or negligible.

E_{Na} and Its Variation The very high value for E_{Na} obtained in the present work confirms the previous finding (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970) that this value is in general on the order of 100 mv. In the present work, in fact, the average was close to 150 mv. The Na in the bathing medium was 190 mM and application of the Nernst formula would indicate that the intracellular Na concentration was of the order of 0.5 mM. Since the inward Na current during a spike is of the order of 20–70 ma/cm² there must be considerable influx of Na (Nakamura et al., 1965). The cells are able to sustain repetitive activity at frequencies close to 100/sec for a considerable time (Altamirano, Coates, and Grundfest, 1955). Thus it is very likely that the electroplaques must be endowed with a highly effective Na pump (Nakamura et al., 1965).

In a considerable number of eels that were delivered to the laboratory all the electroplaques were depolarized. When the resting potential was of the order of −70 mv the directly excited cells generated only graded responses. When the resting potentials were −60 mv or less the cells did not respond to the strongest direct stimuli. These animals were, of course, discarded for the work of the present study. However, their frequent occurrence provides a possible clue to the variation of E_{Na} observed in the spike-generating cells.

Although we had no means of controlling the conditions obtaining in the capture, transport, and maintenance of the animals prior to their arrival in the laboratory we suspect that the eels with depolarized electroplaques may

have suffered insults to their metabolic activity. Presumably the impaired metabolism reduced the supply of energy necessary to operate the Na pump of the electric organs. It should be noted that the electric eel can survive removal of all or most of its electric organs, which are situated behind the anus. Thus, malfunction of the electric organs need not be fatal to the fish, at least in captivity. Smaller degrees of such insults might have caused various gradations in the effectiveness of the pump, leading to variations in intracellular Na and causing, in turn, the observed variation in E_{Na}.

The Two Conductance Components of the Synaptic Electrogenesis E_S lies about midway between the emf's for the two ionic batteries E_{Na} and E_K. The two conductance channel systems appear to be independent (Figs. 7 and 8). When all the Na is replaced with K (Fig. 7 B) there is still an increase in conductance on applying a synapse activator drug. The increase is smaller than the maximal G_S and is approximately equal to G_{S_K}, as is shown in the measurements of Figs. 3, 4, and Table I. E_S is thus equal to E_K. When part of the Na is replaced with Ca or Mg there is little or no change in E_{Na} and E_S (unpublished observations). When all the Na is replaced with either Ca or Mg there is an increase in conductance on addition of a synapse activator (Fig. 8). This may be due in large measure to G_{S_K}. However, there is a depolarizing electrogenesis when no current is applied to the membrane, indicating that E_S is positive to E_K. In fact the value of E_S, ca. -50 mv when the resting potential is > -100 mv, indicates that the emf of the other ionic battery contributing to E_S must be close to reference zero or inside-positive. It is possible that some Na may still have been present in the vicinity of the membrane, but it is unlikely that the concentration gradient could be in the direction of a positive emf. A more likely explanation is that the synaptic membrane is somewhat permeable to the divalent cations. The emf of the Ca or Mg battery would be even more inside-positive than E_{Na} and though G_{Ca_S} or G_{Mg_S} would be small the contribution of the emf's to E_S could be appreciable.

In the normal synaptic electrogenesis of the electroplaques the net inward synaptic current, as estimated from the reversal point of the EPSP, is on the order of 50 ma/cm². Since G_{S_K} and $G_{S_{Na}}$ are approximately equal while the driving forces for the two ions are about 130 mv for K ($E_S - E_K$) and about 230 mv for Na ($E_K - E_{Na}$), the total inward Na current must be between 75 ma/cm² and 100 ma/cm², or of the same magnitude as the inward current during the spike (Nakamura et al., 1965). Synaptic currents of such magnitude occur in the torpedine electroplaques, in which the strong discharge is generated by the postsynaptic potential alone (Bennett et al., 1961; Bennett and Grundfest, 1961 *a*; Grundfest and Bennett, 1961).

Although G_{K_S} and G_{Na_S} are nearly equal for the individual electroplaques

measured, the variation in G_S was very considerable, fivefold in the samples of Table I. The density of the synaptic innervation is not uniform over the surface of the electroplaque, being largest at the lateral border of the cell and decreasing toward the mesial edge (Altamirano, Coates, and Grundfest, 1955). The measurements of the present experiments were restricted to small areas of the membrane in order to insure adequate space clamp conditions (Nakamura et al., 1965; Morlock et al., 1968; Ruiz-Manresa et al., 1970). Thus, it is likely that the variation in G_S resulted from random variations in the density of the innervation at the site of measurements and consequently of the area of the synaptic membrane. The current densities estimated above are average for the entire area of the window in the chamber. The total area of the synaptically active patches must be smaller than this, and the density of the synaptic current would be higher still if the true area were known and could be corrected for.

The Nonlinearity in the Synaptic Characteristic Electrically inexcitable processes must exhibit a linear characteristic (Grundfest, 1957, 1961 *a*, *b*; Grundfest and Bennett, 1961). Linearity has been observed for a range of about twice the reversal potential in the electrically inexcitable electroplaques of *Torpedo* (Bennett et al., 1961), *Narcine* (Bennett and Grundfest, 1961 *a*), and *Astroscopus* (Bennett and Grundfest, 1961 *b*). The eel electroplaques, however, exhibit a nonlinear region in the characteristic of the electrogenesis induced by synapse activator agents when the membrane is polarized to inside positivity. When the membrane becomes inside-positive the conductance increase that is induced by the agent is blocked almost completely and the characteristic then approaches the linear relation for G_L. This implies that the synaptic membrane becomes inactivated when strongly depolarized even though the synapse activator agent is present.

The direction of the depolarizing current is such as to favor displacement of the positively charged activator molecules away from the receptor sites. This iontophoretic effect should decrease receptor occupancy and would produce a decrease in the conductance. However, an increase in the concentration of the activator agent should then increase the conductance since the occupancy of receptor sites would be increased. No change was observed in the nonlinear characteristic when the drug concentration was increased by about fivefold and this explanation seems unlikely.

Another possibility is that the strong electric field modified the affinity of the receptor molecules for the drug. Kordaš (1969) has reported that the time course of the synaptic potential in the frog neuromuscular junction varies with the membrane voltage, and has suggested that the attachment of the mediator with the receptor is stronger in the hyperpolarized membrane. A third possibility is that the very strong electrostatic force has a

direct effect on the gates of the Na and K channels like the effects that can be produced by weaker electrical forces on nonsynaptic electrically excitable membrane. In the case of the eel electroplaques the electrical excitability of the synaptic membrane would have a high threshold and would cause closing of the gates (depolarizing inactivation) for both Na and K. This response is in the same sense as is that of the two reactive nonsynaptic channels, G_{Na} and G_K, of the electroplaques. It would be of interest to compare the behavior to strong depolarization of the synaptic channels in other cells in which the normal electrically excitable response is a persistent increase in conductance that is due to depolarizing K activation of the nonsynaptic membrane.

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