ELECTRICAL ACTIVITY IN ELECTRIC TISSUE

III. MODIFICATIONS OF ELECTRICAL ACTIVITY BY ACETYLCHOLINE AND RELATED COMPOUNDS*

by

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INTRODUCTION

The electroplax of most electric organs, with the probable exception of those of *Malopterurus*, have evolved from striated muscle cells[1,2]. Therefore, some compounds with strong action upon end plates have been tested by different investigators as to their effects upon electric tissue [2–7]; however, the results reported have been incomplete and sometimes contradictory. A reinvestigation appears promising due to the development of new techniques and preparations, using microelectrodes inserted into single innervated electroplax[7–9], and in the light of information accumulated about the biochemical system with which these compounds react[10–11]. Moreover, a re-evaluation of the effects of certain compounds upon the synapses and excitable membranes in terms of the electroplaque seems of interest, particularly because in this case the action is limited to the modification of electrical phenomena, whereas in striated muscles direct action upon the contractile mechanism may interfere with the study of the electrical response. Furthermore, quaternary ammonium compounds, like curare or prostigmine, which apparently do not affect the nerve or muscle fiber membrane, exert a powerful action on the propagated action potential of the electroplaque.

The action upon the electrical potentials of the electroplaque caused by some compounds known to be related with the acetylcholine system, has been analyzed in a preceding paper[12] in relation to differences of chemical structure and special chemical effects. The electrical components recorded with intracellular electrodes are as follows: the resting potential; the prefatory response evoked only by a neural stimulus, identified as a postsynaptic potential; the local graded potential which may be initiated either by neural or direct stimulation and which has the properties of a local response; and finally, the all-or-nothing spike. Whereas the various aspects of the electrical potentials are

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discussed in a separate paper, here are described the modifications which have been observed on exposure to the special compounds under study.

METHODS: The same methods were used as in the two preceding papers (I, II).

RESULTS

A. Effects upon excitability to neural stimulation.

In the single layered preparation, each nerve usually supplies 5 to 7 cells[8]. A single maximal nerve volley produces spikes in a few of the electroplax, in the others only a post-synaptic potential appears. A second test stimulus to the same nerve delivered within the facilitation interval caused by the first (conditioning) stimulus may now discharge spikes in all or nearly all the cells supplied by the given nerve. Therefore this kind of preparation is particularly suitable for studying whether or not the effectiveness of neural stimulation is altered in the presence of small concentrations of eserine or prostigmine. Two aspects have been examined in these experiments: (i) the action of the drugs on the effectiveness of neural stimulation of the cell and (ii) their influence on the prolonged facilitation caused by a neural volley.

Alteration of the effectiveness of neural stimulation is illustrated in the curves of Fig. 1 which represent the height of the response caused by a weak neural volley (triangles and lowest curve); by a maximal volley (squares and middle curve); and by the weak volley facilitated by a preceding maximal stimulus (circles, upper curve). Ten minutes after the measurements were begun, eserine (25 μg/ml) was added to the Ringer's solution of the bath. After another 10 minute period, this was replaced with the standard Ringer's solution.

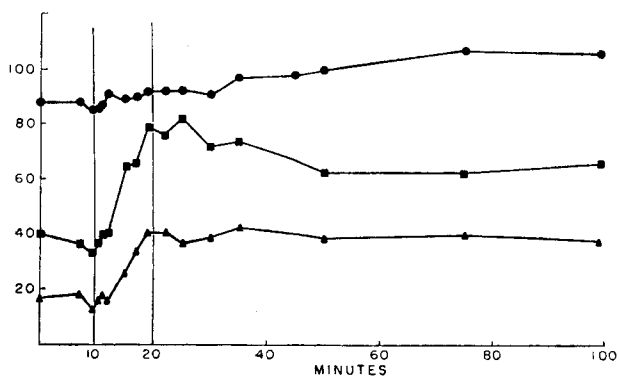

Fig. 1. Potentiation produced by eserine. Ordinate: absolute height of the response in arbitrary units. Abscissa: time in minutes. ■ response to the conditioning stimulus (maximal); ▲ response to the test stimulus (submaximal) in isolation; ● response to the test stimulus when delivered 27 msec after the conditioning. The eserine (25 μg/ml) was added to the Ringer solution between the vertical lines.

Immediately after application of eserine, the response produced by either neural volley began to increase. In each case the potential doubled within the 10 minute period and the effect persisted subsequently with only small diminution for more than 120 min. The development of increased neural effectiveness follows the same time course in both curves. This indicates that within the relatively small number of cells involved, the population was rather homogeneous with respect to the effect of the drug. Prostigmine has the same action.

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The increase in the effectiveness of neural stimulation by eserine or prostigmine might be due to an increase in the effectiveness of the neural impulses or by decrease of the threshold of the effector cell. The former change, whether due to enhancement of the number of active nerve terminals or of the effectiveness of each point of junctional transmission may be expected to increase the magnitude of the postsynaptic potential. However, in none of the numerous experiments with intracellular recording electrodes was the postsynaptic potential increased or prolonged by the drugs. On the contrary, the only effect observed was the decrease of the potential when the drugs were applied in rather high concentrations. Although recording with external electrodes does not permit accurate determination of the magnitude of the postsynaptic potential, the duration of potential is measured with rather high accuracy because the response represents the summated activity in a number of cells. In more than 60 experiments of this type, no significant change occurred in the duration of the postsynaptic potential when eserine or prostigmine were applied in concentrations ranging from 25 μg/ml to 5 mg/ml.

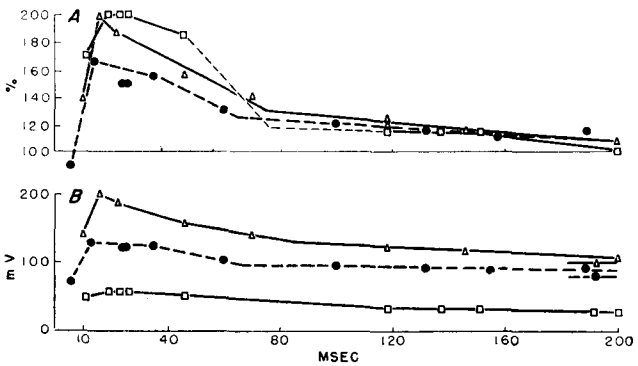

Fig. 2. Action of eserine upon the time course of facilitation. Abscissa: time in msec between the two stimuli. Ordinates: in A the second response is expressed as percentage of the testing response in isolation. The absolute height of the test response is plotted in B; the horizontal lines under the curves indicate the amplitude of the testing response in isolation. The testing stimulus is submaximal. ● control curve; △ 12 min after the addition of 50 μm/ml of eserine; □ 76 min after eserine.

Attempts to test the possibility that eserine or prostigmine increased the excitability of the cell were also made by examining their action on the threshold of the electroplaque to direct stimulation. No significant change was found. However, the procedure used, applying a stimulus to the outside of the cell by means of electrodes insulated to their tips which straddled a given cell is not very sensitive. The fraction penetrating the cell is probably a small part only of the total applied current, the major portion flowing through external shunting tissues. Small, but perhaps decisive changes in threshold therefore might not have been detected.

The drugs do not increase the number of cells discharged by the weak but facilitated testing volley (Fig. 1, upper curve). This is to be expected if facilitation had caused discharge of all the cells accessible to the testing stimulus. The subsequent small rise of the height of the facilitated response may be due to change in recording conditions or to involvement of a previously irresponsive electroplaque. At any rate, the change occurred late and was not observed in most experiments.

Eserine or prostigmine therefore are capable of enhancing the excitatory effects of a neural volley. This potentiation persists for a very long time. However, higher concen-

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trations of the drugs (100 μg/ml or more) cause depression of the response after a brief initial enhancement. The higher the concentration the shorter is this initial period and the more profound the subsequent depression.

The effect of the drugs on the time course of facilitation was studied as described earlier[8], by paired stimuli delivered to the nerve at separations ranging from a few msec to more than 1 sec. Two variants of these experiments are illustrated in Fig. 2 (the first stimulus maximal, the second weak) and Fig. 3 (both stimuli of the pair maximal). The data are shown in terms of the recorded potentials (Figs. 2B and 3B) reflecting the number of responding cells; and as percent facilitation (Figs. 2A and 3A). The data for the control experiments are represented by the filled circles; the triangles represent the data after addition of 50 μg/ml eserine had caused enhancement of the number of cells discharged by the testing volley; the squares represent the results when prolonged action of the drug had caused depression of the response.

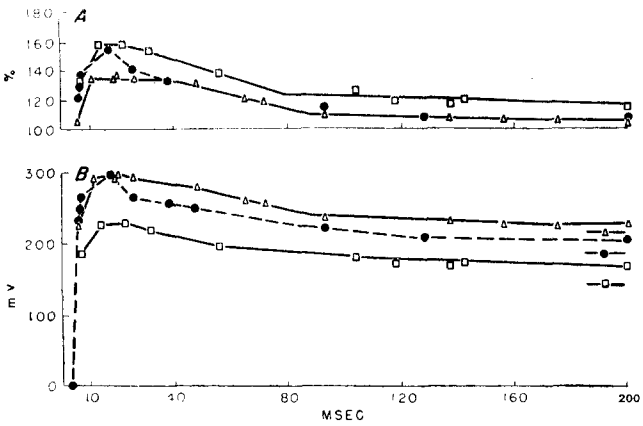

Fig. 3. Action of eserine upon the time course of facilitation. Same experiment as illustrated in Fig. 2, but with a maximal test stimulus. Ordinates and abscissas: same as Fig. 2. ● control curve; △ 18 min after the addition of 50 μg/ml of eserine to the Ringer solution; □ 78 min after eserine.

When the testing stimulus is weak (Fig. 2) the enhancement of the testing reponse produced by eserine also causes a larger number of facilitated cells to discharge (Fig. 2A), but the duration of the first phase of the curve, which represents the period of increased number of discharged cells[8] is not markedly altered. The later phase during which the postsynaptic potential alone is facilitated is not affected at all. The conclusion that the differences seen in the facilitation curves of Fig. 2 are without significance, is supported by the data of Fig. 3. Both stimuli were maximal and therefore the maximal number of cells available for discharge were activated at peak of facilitation in the normal preparation. Treatment with eserine therefore could not cause an increase in the number of cells discharging in the early course of facilitation (Fig. 3B). Since the level of the isolated testing response had increased, the relative amount of facilitation apparently decreased (Fig. 3A). In their subsequent time course, however, the facilitation curves become identical as in the experiment of Fig. 2. Neither eserine, nor prostigmine were found to alter the time course of the processes underlying facilitation in more than 60 experiments of this type.

The facilitation curves after the drug had depressed neural excitation (squares, Figs. 2 and 3) are instructive because they illustrate the need for caution in interpreting

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the significance of results from experiments of this type. After 76 minutes of action of eserine, the response to a weak testing stimulus had decreased from the initial value by about 70% (Fig. 2B). Percentage of facilitation (Fig. 2A) in the first phase of the process had apparently risen and decline of this phase had been apparently slowed. The late phase of facilitation of the postsynaptic potential was unchanged. However, both phases apparently were increased and prolonged when the testing stimulus was maximal. This contradiction, and the discrepancy with the results obtained in the earlier stage of eserine action are ascribable to the significance of such measurements when made in a synaptic system. The neural stimulus to the cell is not amenable to variation and the facilitation curves were therefore obtained by measuring the increase of the response rather than by determining the threshold of stimulation for a constant amplitude of response. The facilitation curves (Figs. 2A and 3A) may therefore be seriously falsified by several factors such as amplitude of the unconditioned testing response, which enters as a constant divisor, or shifts in the relative proportion of discharged and excited cells.

B. Effects on the potentials of the cell.

The results described in this section were obtained by recording with one or two intracellular electrodes.

Effects on resting potential: The average resting potential fo the normal cell recorded across the innervated membrane is 73 mv[13]. The drugs tested (Table I) fall into two groups with respect to their effects on this potential[12]. One group comprises those compounds which act even in small concentration to produce depolarization. When these compounds are applied to the cell in high concentration, depolarization may be complete, the resting potential falling to zero. The threshold concentrations for depolarization are not yet accurately determined but approximate values, derived from experiments with a range of concentrations are also shown in the Table. Determination of the threshold value is especially complex in the cases of acetylcholine (ACh) and dimethylaminoethyl acetate (DMEA) since both substances are hydrolyzed by acetylcholinesterase and require addition of eserine[12].

TABLE I

SUMMARY OF COMPOUNDS TESTED, INDICATING MINIMAL ACTIVE AND MAXIMAL CONCENTRATION USED

	Concentration (μg ml)	
	minim. active	*maxim. used*
Depolarizing compounds		
Acetylcholine*	5	2000
Dimethylaminoethyl acetate*	50	10000
Carbamylcholine	10	100
Prostigmine	50	5000
Decamethonium	10	100
Non-depolarizing compounds		
Procaine	200	5000
d-Tubocurarine	50	5000
Tertiary analogue of prostigmine	1000	3000
Eserine	25	5000
DFP	100	5000

* In presence of eserine (25 μg/ml).

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The second group includes those substances which do not cause depolarization even when applied in high concentrations and for times up to 3 hours or more. Occasionally, in some experiments with eserine, *d*-tubocurarine and procaine, the resting potential increased by a few mv, but the change was of the same order of magnitude as the error of measurements. Although the non-depolarizing substances do not cause change in the resting potential they are capable of antagonizing the action of the depolarizing compounds. Thus, whereas carbamylcholine causes profound depolarization, antecedent treatment of the cell with all the non-depolarizing compounds except DFP protects the electroplaque against depolarization on subsequent exposure to a depolarizing concentration of carbamylcholine (Fig. 6)[12].

Both groups of substances affect the electrical signs of activity and eventually eliminate these, but as will be described below, the modes of action are different. One substance studied, DFP, appears to fall into an intermediate category. In low concentrations this substance causes block of activity long before an eventual depolarization develops. When high concentrations of the drug are applied, depolarization sets in rapidly but only after activity is blocked. Since the blocking action of DFP may thus be separated from its depolarizing action, this compound is included in the group of non-depolarizing substances, but its special properties should be stressed.

Effects of the depolarizing compounds on the response: When these substances are used in low concentration, there is a progressive decrease in the spike and its eventual disappearance (Fig. 4). The initial change in the spike amplitude occurs with little or no change in resting potential (B). The duration of the depressed spike is shortened considerably (B, B‴). At a later stage both the resting potential and spike decrease (C), but as ascertained in other experiments with simultaneous recording at 2 loci in the cell, propagation still takes place when the spike is as small as 60 mv. When first the spike (D) and then local responsiveness to direct stimulation are lost (E, F), the postsynaptic potential can still be produced by a weak neural volley (E′, F′) and stronger neural excitation may still cause electrical activity in the tempo of the spike (E″, E‴). In time, though its latency is not measurably affected, the postsynaptic potential is also depressed, (F′), but is still considerable with stronger neural stimulation (F″) and may still cause a small local response of the cell (F‴). Postsynaptic potentials may be elicited when the resting potential has fallen to 10 or 20 mv. Repetitive stimulation of the nerve at this stage causes facilitation of the postsynaptic potential.

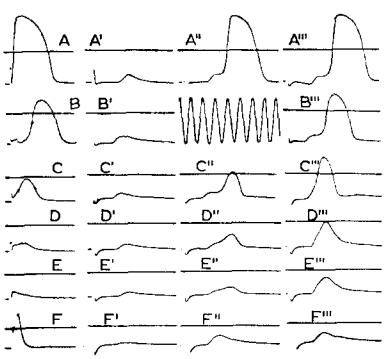

Fig. 4. Effect of acetylcholine. 1st column: direct stimulation; 2nd, 3rd and 4th column: indirect stimulation of increasing strength. Calibration: 1000 cycles and 100 mv. A–A‴: control. B–B‴: 114 min after the initiation of the experiment. Eserine (25 μg/ml) was added from 0 to 78 min and acetylcholine (1 μg/ml) from 20 to 78 min; no effects were observed. At 78 min the bathing solution was changed to Ringer with 5 μg/ml of acetylcholine. C–C‴: 123 min; D–D‴: 142 min; E–E‴: 153 min; F–F‴: 156 min

Effects of the non-depolarizing compounds. All the substances which do not depolarize the cell (Table I) cause rapid and nearly selective depression of the postsynaptic potential (Figs. 5 and 6). The cell soon becomes irresponsive to neural excitation (Fig. 5C′), but a large response can still be elicited by direct stimulation of the cell (C to E) and for a long time thereafter (5C and D) this is propagated.

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An early sign of depression of the effectiveness of the neural volley is manifested by prolongation of the refractory period (Fig. 7). After treatment of the cell with a non-depolarizing drug, the neural volley in order to activate the cell must fall later in the refractoriness caused by a conditioning direct stimulus. In the neurally evoked response of the normal cell (D) the postsynaptic potential was large and the spike developed out of it with very short latency. In the cell treated with the drug a small postsynaptic potential is clearly demarcated from the delayed spike. Before transmission is blocked, the neurally evoked response can be produced by repetitive neural stimulation in the almost complete absence of a postsynaptic potential (Figs. 5 and 6) which may become as small as 2–4 mv or less. Spikes are never obtained in the normal cell with postsynaptic potentials of this amplitude (Fig. 6A).

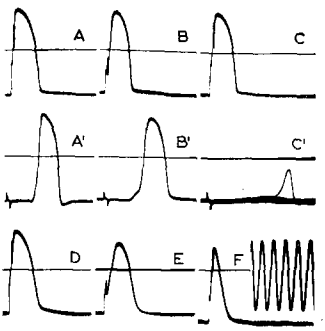

Fig. 5. Action of *d*-tubocurarine. Same experiment as Fig. 7. A, B, C, D, E and F: direct stimulation. A′, B′ and C′: nerve stimulation. Cal.: 1000 cycles and 100 mv. A, A′: control. B, B′: 3 min after addition of *d*-tubocurarine (5 mg/ml). C, C′: 5 min nerve stimulation at 50/sec. Last spike(?) obtained in this experiment by nerve stimulation. D: 46 min. E: 110 min. F: 220 min.

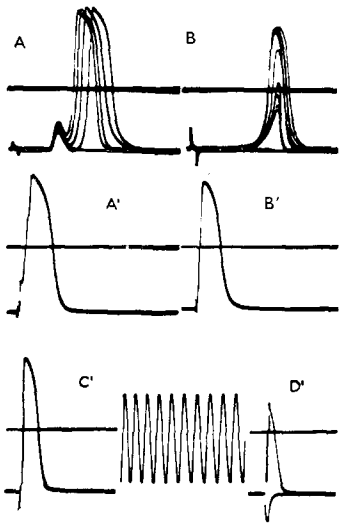

Fig. 6. Action of procaine. A and B: neural stimulation. A′ to D′: direct excitation. A, A′: control. No spikes were produced in this electroplax by a single nerve volley: A represents a multiple exposure during stimulation at 10/sec. B–B′: 10 min after addition of 1000 μg/ml of procaine. In B the nerve was stimulated at 50/sec. C′: 27 min. D′: 113 min after procaine. 10 μg/ml of carbamylcholine were added to the Ringer 74 min before this last record was taken. No depolarization is observed.

When higher concentration of the non-depolarizing substances is used an additional phenomenon develops. The cell, of course, no longer excitable by a neural volley, responds to direct stimulation by developing a potential which is graded, rather than all-or-nothing, but which can reach a maximum amplitude, with strong stimuli, as high as the all-or-nothing spike of the normal cell (Figs. 8–9). This response is not propagated, as may be seen in records A–D of Fig. 8. As the stimulus strength is increased further, however, a response develops at the distal recording electrode, rising slowly (record E) as did the small response at the proximal electrode (records A to C) and also decreasing abruptly. With still stronger stimulation this distal response also grows, develops an overshoot and approaches the amplitude of the normal all-or-nothing response (J).

The delay between the responses at the two recording sites is a curious phenomenon, which creates a resemblance to normal propagation, but which cannot involve this process. The first sign of the distal response occurs without any change in the proximal (D and E), and therefore cannot have been caused by this. The amplitudes of the

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responses at successive loci between the proximal and distal recording sites probably grow with increasing stimulation, and each site upstream contributes some local circuit current for excitation of the next loci downstream. These summate with the instantaneous electrotonic effects of the spreading stimulus. At each successive site downstream, however, there must occur a slowed rise such as is seen in the proximal responses of records A to D. These delays will be accentuated at a distant recording locus, but will become shorter with stronger stimuli (records E to J).

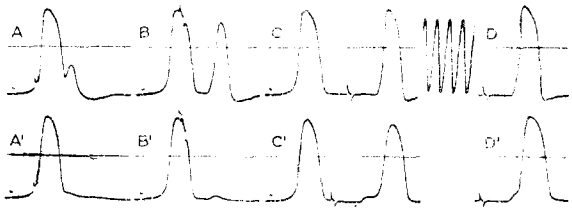

Fig. 7. Action of *d*-tubocurarine. A, B and C: refractory period determined with successive nerve volleys. D: response to the test stimulus in isolation. A', B', C' and D': same as before but 3 min after the addition of *d*-tubocurarine (5 mg/ml) to the bathing solution. Cal.: 1000 cycles, 100 mv.

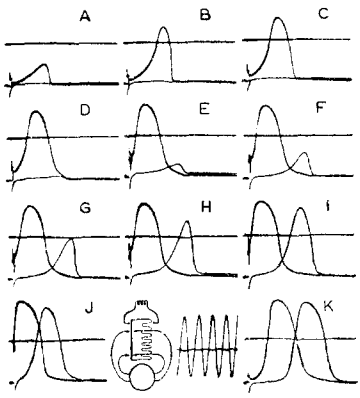

Fig. 8. Action of eserine. Electrical activity of a directly stimulated electroplaque recorded with two intracellular electrodes as shown in the diagram. Distance between stimulating electrode and nearest micropipette: 1 mm. Distance between recording micropipettes: 1.86 mm. Cal.: 1000 cycles, 100 mv. A to J: stimulation with increasing strength, after the addition of 1 mg/ml of eserine. K: control.

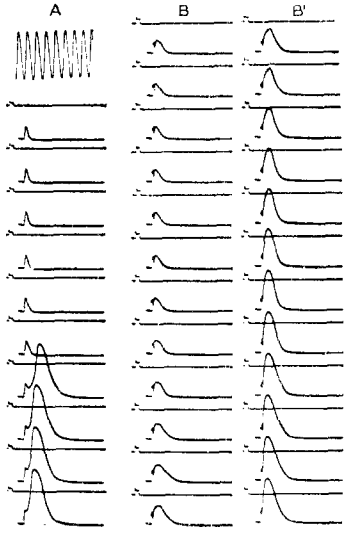

Fig. 9. Action of eserine. Continuous recording of the electrical activity of an electroplaque directly stimulated at 5/sec. Column A: control (see text); Columns B and C: 88 min after addition to the Ringer of 500 μg/ml of eserine.

The forms of the potentials consequent to the action of the drugs initially are rather different from those of the spike (record K), but eventually they take on the appearance of the spike. Their durations and time relations, particularly in comparison with the very short duration of the stimulus, preclude the possibility that these potentials are caused by artifacts ascribable to the strong stimuli. However, a demonstration that the potentials are responses of the cell is afforded in Fig. 9. The cell directly excited with weak repetitive stimuli exhibits latent addition which lasts somewhat more than 200 msec (8).

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The terminal portion of such a train of stimulation of a normal cell at 5/sec is shown (from above down) in column A of the Figure. The upper (longer) trace of each pair is the reference zero line and carries a monitoring signal of the strength of the stimulus. The lower trace of the pair is connected with the internal recording electrode and the distance between the traces measures the resting potential. The first 6 of these pairs of traces show the electrotonic potentials caused by the stimulus and a small, growing local response denoted by the broadening of the deflection. Out of this there suddenly develops a spike which is maintained thereafter. Column B and its continuation, B', depict the sequence of events after the cell had been treated with eserine. The stimulation was somewhat stronger and the first stimulus of the sequence (upper record) caused a recognizable local response. Subsequent growth of this was at first slow but then accelerated and at the end of the train (lowest record in B') the response had grown through a sequence of gradations to develop an overshoot and to reach 80% of the spike amplitude. In many experiments with cells treated with non-depolarizing drugs the maximum response reached amplitudes of 120 to 140 mv. The threshold direct stimulus necessary to produce a spike in the normal cell causes only a small response of the non-propagated variety after treatment of the cell with a non-depolarizing compound, but the strength of stimulus necessary to produce this small response does not change for a long time. However, to elicit a response of a magnitude similar to that of the normal spike, stimulating currents 3 to 5 times greater than this are needed.

When cells are exposed to very high concentrations of the non-depolarizing substances, the sequence of events described in the foregoing is accelerated. Block of synaptic transmission and disappearance of the propagated response develop very rapidly and may coincide, but none of the compounds, except DFP, as noted above, cause depolarization at any time.

DISCUSSION

A. The excitable properties of the electric organ in response to the compounds tested do not appear to differ fundamentally from those of other excitable tissues, particularly striated muscle. These similarities and differences with respect to the various compounds tested are analyzed in the following.

Depolarizing substances

Acetylcholine (ACh). It is well known that ACh produces contraction or contracture of normal or denervated striated muscles of birds, amphibia or mammals, and that this effect is produced by a selective action upon the end plate region[14,15,16]; amounts a thousand times higher are needed for stimulating other points. Similarly, the synapses of sympathetic or parasympathetic ganglia may be excited by this compound[17]. Excitation of electroplax has not been obtained in the electric organ of the electric eel, even when close intra-arterial injections have been performed[18] (and ALTAMIRANO, unpublished). This apparent difference may, however, depend on anatomical differences. Stimulation of striated muscles or of sympathetic ganglia of mammals requires rapid intra-arterial injection at very close range. The structural conditions of the electric tissue of the electric eel prevent rapid diffusion to the electroplax of a drug injected into the circulatory system[18]. Furthermore, in other electric organs with slight differences in circulation, electrogenic activity has been produced by injections of ACh[6].

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The stimulation caused by ACh has been ascribed to its depolarizing action upon the end plate[12,13,14,15,19]. A decrease of the resting potential is observed in the electroplaque. It is not possible to ascertain whether the depolarization takes place only in the postsynaptic region or also in the surrounding membrane; however, the latter is affected in a critical way, since the response to direct stimulation can be blocked before significant change of the postsynaptic potential appears. The block in striated muscles produced by high amounts of ACh[15,20] may be due to a similar process and not to inhibition of synaptic transmission as is usually believed. ACh blocks direct stimulation or the contraction produced by a successive injection of the same compound in denervated muscles[21]. At the incidence of block of synaptic transmission to frog muscle the e.p.p. still remains approximately twice as large as that when the block is caused by curare. Not only the end plate but also the contiguous membrane is believed to be affected[20].

The permeability of excitable tissues to quaternary ammonium salts is very small, except at synaptic regions. ACh has practically no effect on the membrane of striated muscle[15] and does not readily penetrate into the inerior of the squid axon[22]. The same is true for curare and prostigmine. Since all these compounds affect the activity of the electroplaque, it is obvious that electroplaques lack the penetration barriers which prevent the action of these drugs in other cells. An alternative explanation may be that, due to the enormous number of synapses at the innervated face of the electroplaque, the barrier in that cell resembles a "sieve" permitting these compounds to reach the active membrane of the electroplaque. Another factor may be the non-innervated side at which no spikes are produced. Thus, this structure may differ from that at the innervated side and the penetration may take place, at least partially, through this side.

Carbamylcholine in contrast to acetylcholine is not split by cholinesterase. Its actions are similar.

Decamethonium (C_{10}). Stimulation of the electroplaque has not been observed with C_{10}, although it provokes contraction of striated muscle fibers[23]. This difference may also be attributed to the structural factors discussed in relation to acetylcholine. Otherwise the effects of C_{10} upon the electroplaque parallel those on muscle. Thus, C_{10} may block "synaptic transmission" when the e.p.p. is still three and a half times as high as after block by curare[23,24]. It depolarizes the end plate region and the surrounding muscle membrane is also affected. It is, therefore, probable that the impulse elicited by nerve stimulation is blocked in the membrane beyond the end plate region.

Prostigmine. Most considerations analyzed below with regard to eserine apply to the pharmacological action of prostigmine. Such action has not been described in muscle fibers[19], but rapid injections of prostigmine produce contraction or contracture of denervated muscles[25] and it is generally accepted that these effects are produced by depolarization of the membrane, at least in the end plate region. Prostigmine does not affect direct excitability of curarized frog sartorius muscles[26] and this indicates that the muscle fiber, like the nerve fiber, is not readily permeable to this compound.

Non-depolarizing substances

Eserine, in low concentrations, potentiates neural stimulation of the electroplaque, as in ganglionic or neuromuscular synapses[14,17,27,28]. While these effects are obtained with indirect stimulation, direct excitability remains unchanged. Concentrations which at first potentiate, may eventually depress the effector cell. At high concentrations only

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depression ensues. The refractory period of the electroplaque is, as in striated muscle, prolonged by eserine.

Facilitation tested by two successive stimuli is not prolonged by eserine. Similar lack of effect has been found in ganglionic[29] or neuromuscular synapses[14,28]. Therefore, the long lasting facilitatory process observed in many synapses may not depend upon the liberation of ACh. Furthermore, since facilitation does not coincide, at least in the electroplaque, with any measurable electrical activity of the effector cell, some as yet unknown chemical processes may underlie facilitation. A plausible alternative explanation may be that this process takes place at the nerve terminal[30].

In striated muscle eserine increases and prolongs the end plate potential[14,27]. This effect has not been observed in electroplaque, nor in the superior cervical ganglion[31].

Eserine blocks conduction in the nerve fiber without depolarization[32] as in the electroplaque. The initial action upon the nerve consists in a decrease of the conduction velocity, but since this compound does not increase the response time for the postsynaptic potential of the electroplaque it appears likely that the synapses are blocked before there is any significant effect upon the presynaptic fibers.

Curare. Curare blocks synaptic transmission of striated muscle or autonomic ganglia; this action has been attributed to the inhibition of the postsynaptic potential[33,34]. The effects upon the electroplaque are similar. However, in this tissue the depression of the postsynaptic potential may be nearly complete, but with repetitive stimulation transmission may still be obtained. The same is true for all the compounds that do not depolarize the electroplaque. Repetitive stimulation may decrease the threshold of the electroplaque to such a low level, that extremely small postsynaptic potentials are still able to set up a spike.

Curare antagonizes the depolarizing action of acetylcholine in striated muscle[15]. A similar antagonism has been obtained in electroplax between various non-depolarizing compounds and carbamylcholine. The postsynaptic potential of the electroplaque as that of striated muscle is not delayed by curare. Curare does not depolarize the electroplaque under the conditions used, as is generally accepted for neuromuscular junctions[15,19], although depolarization has been described[35,36].

Curare does not act on the muscle membrane[37], yet in the electric organ it affects direct excitability as do other non depolarizing compounds. Permeability barriers in the muscle membrane appear to be responsible for this difference, however; curare may also affect the muscle membrane under certain conditions[38].

Procaine. Procaine blocks transmission in synapses of electroplax, ganglia or striated muscle and it decreases or eventually suppresses the postsynaptic potential[28,39]. Concentrations adequate to produce this block do not modify the response elicited by direct stimulation either in denervated or curarized striated muscle or electroplaque. In high concentrations procaine in all these tissues also blocks the electrical activity produced by direct stimulation[39]. Block of propagation along nerve fibers or across synapses in muscle[40,41] and in the electroplaque occurs without depolarization.

DFP. Although DFP causes depolarization of the electroplaque, as well as block of postsynaptic response, it has been grouped with the non-depolarizing compounds, because the depolarizing action sets in long after the block is developed. Synaptic transmission is blocked more rapidly by DFP than is the activity of nerve fibers[27]. The action on nerve occurs without depolarization[32]. However, these measurements may not have been carried sufficiently long times to observe a later depolarization if this also takes place in nerve.

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B. The depolarizing compounds decrease the spike amplitude and shorten its duration, with the rising phase remaining unchanged or little affected. The spike amplitude may decrease by 60 to 70% before propagation is blocked and, at this time, the resting potential has fallen about 20 mv. According to the theory of HODGKIN AND HUXLEY[42] a decrease and shortening of the spike would be caused either by increase of what they refer to as "sodium inactivation" or by increased and earlier potassium outflow. A steady depolarization of 30 mv increases "inactivation" sufficiently so as to abolish the inward sodium current and excitability of the squid giant axon[43].

NACHMANSOHN has proposed[10,44] that intracellular release of acetylcholine from a bound form during passage of a stimulating current or of that produced by the local circuit of activity is responsible for excitation. It might, therefore, appear surprising that application of the substance blocks direct excitability of the electroplaque. However, even in those tissues in which rapid injection of acetylcholine has an excitatory effect, prolonged action of this subtsance causes depression of activity. Increase of "inactivation" and consequent decrease of sodium influx caused directly by the compound, or secondarily by its depolarizing action, or by both effects in combination may, therefore, account for the blocking action.

The non-depolarizing compounds exert an effect on the electroplaque which resembles the response of the cell in refractoriness. The directly elicited response becomes non-propagative, it exhibits gradations up to the height of the normal, all-or-nothing spike, and responses of intermediate amplitude are of shorter duration than the spike. HODGKIN AND HUXLEY[42] explain refractoriness as due to increased "sodium inactivation" and outward potassium current. Late in the action of these non-depolarizing compounds, when the maximal response height becomes decreased and the duration shortened, enhancement of either or both processes may occur. However, the ability of the electroplaque exposed to the non-depolarizing substances to develop in early stages of their action a response with an amplitude equal to that of the spike, and with about the same duration, indicates that neither "inactivation" nor outward potassium current are enhanced in the initial action of the compound.

SUMMARY

1. The electrical activity of isolated electroplax of electric eel stimulated directly or by nerve volleys has been recorded.

2. Experiments performed with extracellular electrodes show that small concentrations of eserine or prostigmine may potentiate the nerve stimulus without modifying significantly the prolonged facilitation which follows a nerve volley.

3. The effect produced by a number of substances upon the resting potential, postsynaptic potential, local response elicited by direct excitation and spikes evoked by nerve or direct stimulation, has been studied by means of intracellular electrodes.

4. The following nitrogen compounds decrease the resting potential: acetylcholine and its tertiary analogue, dimethylaminoethylacetate, carbamylcholine, prostigmine and decamethonium.

5. The resting potential can be reduced to zero, but no reversion, *i.e.* internal positivity of the electroplaque during rest, has been observed.

6. The reduction of the resting potential to about 70% of the normal value by the action of the depolarizing substances, coincides with the block of the spike elicited by direct or nerve stimulation. At this moment the postsynaptic potential may be very little modified. Small postsynaptic potentials which increase in size with repetitive nerve stimulation can be observed even after the resting potential has been reduced to about 10 mv.

7. Depolarizing substances do not change the threshold or the rising phase of the spike, except just before this latter disappears.

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8. The following nitrogen compounds do not decrease the resting potential: eserine, procaine, the tertiary analogue of prostigmine, and *d*-tubocurarine.

9. The non-depolarizing substances reduce the size of the postsynaptic potential, which eventually disappears, and block synaptic transmission. At this time the directly elicited spike may be unchanged. However, with adequate concentrations only non-propagated local responses, which reach 100 to 140 mv, can be elicited. Eventually, even this type of activity may be inhibited.

10. The latter compounds may not change the threshold of the minimal response of the cell, but in order to evoke the maximal action potential it is necessary to utilize stimulus at least 3 to 5 times as strong as the control. In appropriate conditions they markedly prolong the raising phase of the local response.

11. DFP, similar to the non-depolarizing compounds, blocks the synaptic transmission or the propagation of the spike along the electroplaque membrane without reduction of the resting potential, although it may eventually depolarize.

12. Evidence is discussed that no fundamental differences have been found between the effects upon striated muscles and those upon the electroplax of the electric eel of the compounds studied.

RÉSUMÉ

1. L'activité électrique de l'électroplaque isolée de la cellule électrique, excitée directement ou par voie nerveuse, a été enregistrée.

2. Des expériences réalisées avec des électrodes extracellulaires montrent que de faibles concentrations d'ésérine ou de prostigmine peuvent potentialiser le stimulus nerveux sans modifier de façon significative la facilitation prolongée qui suit une décharge nerveuse.

3. L'action d'un certain nombre de substances sur le potentiel de repos, le potentiel postsynaptique, la réponse locale provoquée par excitation directe et les ondes de variations de potentiel suscitées par stimulation nerveuse ou directe, a été étudiée au moyen d'électrodes intracellulaires.

4. Les composés azotés suivants diminuent le potentiel de repos: acétylcholine et son homologue tertiaire, acétate de diméthylaminoéthyle, carbamylcholine, prostigmine et décaméthonium.

5. Le potentiel de repos peut être annulé, mais son inversion, c'est-à-dire la positivité interne de l'électroplaque pendant le repos n'a pas été observée.

6. La réduction du potentiel de repos à une valeur égale à 70% de la valeur normale par l'action de substances dépolarisantes, coïncide avec le blocage de l'onde provoqué par stimulation directe ou nerveuse. A ce moment le potentiel postsynaptique peut être très peu modifié. Des faibles potentiels postsynaptiques dont la valeur augmente par des stimulations nerveuses répétées peuvent être observés même après que le potentiel a été réduit à environ 10 mv.

7. Les substances dépolarisantes ne modifient pas le seuil ou la phase croissante de l'onde, si ce n'est au moment où cette dernière disparaît.

8. Les composés azotés suivants ne diminuent pas le potentiel de repos: ésérine, procaïne, l'analogue tertiaire de la prostigmine et la *d*-tubocurarine.

9. Les substances non dépolarisantes diminuent la valeur du potentiel postsynaptique, qui peut même disparaître, et bloquent la transmission synaptique. A ce moment l'onde provoquée directement peut être inchangée. Cependant, pour des concentrations convenables, seules des réponses locales non transmises, qui atteignent 100 à 140 mv, peuvent être obtenues. Il peut même arriver que ce type d'activité soit lui aussi inhibé.

10. Les derniers composés peuvent ne pas changer le seuil de la réponse minimum de la cellule, mais, pour obtenir le potentiel d'action maximum, il est nécessaire d'utiliser un stimulus de 3 à 5 fois plus fort que le témoin. Dans des conditions convenables, ils prolongent sensiblement la phase croissante de la réponse locale.

11. Le DFP, de même que les corps non dépolarisants, bloque la transmission synaptique ou la propagation de l'onde le long de la membrane de l'électroplaque sans diminuer le potentiel d'action, quoiqu'il puisse provoquer éventuellement une dépolarisation.

12. Il ne semble pas, en conclusion, que des différences fondamentales aient été mises en évidence entre l'action des corps étudiés sur les muscles striés et leur action sur l'électroplaque de la cellule électrique.

ZUSAMMENFASSUNG

1. Es wurde die elektrische Aktivität an direkt oder vom Nerven aus gereizten elektrischen Zellen (electroplax) des elektrischen Aals registriert.

2. Mit extrazellulär liegenden Elektroden durchgeführte Versuche zeigen, dass Eserin oder Prostigmin, in kleinen Konzentrationen, den Nervenreiz verstärken können, ohne die verlängerte Erregbarkeitsteigerung, die einer Nervenerregung folgt, deutlich zu verändern.

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3. Mit intrazellulären Elektroden wurde die Wirkung, die eine Reihe von Substanzen auf das Ruhepotential, das postsynaptische Potential, die lokale Antwort ausgelöst durch direkte Erregung und auf die durch Nerven- oder direkte Reizung ausgelöste Aktionsströme, ausüben, untersucht.

4. Folgende Stickstoffverbindungen setzen das Ruhepotential herab: Azetylcholin und sein tertiäre Analog, Dimethylaminäthylacetat, Carbamylcholin, Prostigmin und Dekamethonium.

5. Das Ruhepotential kann bis auf null erniedrigt werden. Eine Umkehr, d.h. eine Positivierung des Inneren der elektrischen Zellen während der Ruhe, konnte nicht beobachtet werden.

6. Die Erniedrigung des Ruhepotentials um etwa 70% des normalen Wertes durch die Wirkung der depolarisierenden Substanzen stimmt überein mit dem Block des durch direkte oder indirekte Reizung ausgelösten Aktionsstromes. Zur gleichen Zeit kann das postsynaptische Potential wenig verändert werden. Selbst wenn das Potential auf 10 mV gesenkt worden ist, können keine postsynaptische Potentiale, deren Grösse bei wiederholter Nervenreizung zunimmt, beobachtet werden.

7. Die depolarisierenden Substanzen verändern die Schwelle und den Anstieg des Aktionsstromes nicht. Nur kurz vor dem Verschwinden des Aktionsstromes treten Veränderungen auf.

8. Folgende Stickstoffverbindungen verändern das Ruhepotential nicht: Eserin, Procain, die tertiären Analogen des Prostigmin und *d*-Tubocurarin.

9. Die nicht depolarisierenden Substanzen reduzieren die Grösse des postsynaptischen Potentials, das manchmal verschwindet und blockieren die Übertragung in der Synapse. Der durch direkte Reizung ausgelöste Aktionsstrom kann zur gleichen Zeit unverändert sein. Jedoch können mit geeigneten Konzentrationen nur nichtfortgeleitete lokale Antworten, die 100 bis 140 mV erreichen ausgelöst werden. Manchmal wird sogar diese Art der Erregung unterdrückt.

10. Diese Verbindungen ändern nicht die Schwelle für die schwächste Erregung der Zelle, aber um den maximalen Aktionsstrom auszulösen, ist es notwendig, den Reiz um das 3 bis 5 fache gegenüber den Kontrollen zu verstärken. Unter geeigneten Bedingungen verlängern sie merklich die Anstiegsphase der lokalen Antwort.

11. DFP blockiert ähnlich den nicht depolarisierenden Verbindungen die Übertragung in der Synapse oder die Ausbreitung des Aktionsstromes über die Membran der elektrischen Zelle ohne das Ruhepotential herabzusetzen, obgleich es manchmal depolarisiert.

12. In der Diskussion wird gezeigt, dass keine grundlegenden Unterschiede zwischen den Wirkungen dieser Substanzen auf den quergestreiften Muskel und auf die elektrischen Zellen des elektrischen Aals gefunden worden sind.

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