Conductance Increases Produced by Bath Application of Cholinergic Agonists to *Electrophorus* Electroplaques

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ABSTRACT When solutions containing agonists are applied to the innervated face of an *Electrophorus* electroplaque, the membrane's conductance increases. The agonist-induced conductance is increased at more negative membrane potentials. The "instantaneous" current-voltage curve for agonist-induced currents is linear and shows a reversal potential near zero mV; chord conductances, calculated on the basis of this reversal potential, change *e*-fold for every 62-mV change in potential when the conductance is small. Conductance depends nonlinearly on small agonist concentrations; at all potentials, the dose-response curve has a Hill coefficient of 1.45 for decamethonium (Deca) and 1.90 for carbamylcholine (Carb). With agonist concentrations greater than 10^{-4} M Carb or 10^{-5} M Deca, the conductance rises to a peak 0.5–1.5 min after introduction of agonist, then declines with time; this effect resembles the "desensitization" reported for myoneural junctions. Elapid α-toxin, tubocurarine, and desensitization reduce the conductance without changing the effects of potential; the apparent dissociation constant for tubocurarine is 2×10^{-7} M. By contrast, procaine effects a greater fractional inhibition of the conductance at high negative potentials.

INTRODUCTION

The electric organs of *Electrophorus electricus* constitute a major source material for biochemical studies on purified cholinergic receptor protein (Olsen et al., 1972; Klett et al., 1973. In isolated electroplaques from the organ of Sachs, moreover, the response to cholinergic agonists can be followed by electrophysiological methods. Physiological and biochemical data have not been easily comparable, however, because experiments on purified receptors employ known, steady concentrations of agonist, whereas neurally released and iontophoretically applied agonists have unknown, time-variant concentrations.

We have therefore investigated the physiological response of the electroplaque during bath application of agonists. Ruiz-Manresa and Grundfest (1971) showed that such treatment increases the conductance of the innervated membrane, and we have employed this increase as a measure of activation of acetylcholine receptors. At present we describe only the "steady-state" dependence of conductance on potential, except where the "instantaneous" *I-V* curve helps to reveal the reversal potential for agonist-induced currents.

We find that dose-response relations for the *conductance* differ markedly from those measured with membrane *depolarizations*, because bath-applied agonists produce a distinctive and rather puzzling effect which can be described as a "shift" in the membrane's current-voltage relation.

The agonist-induced conductance decreases when the membrane is depolarized (Ruiz-Manresa and Grundfest, 1971). We have wondered whether the structure responsible for this voltage sensitivity also governs other receptor characteristics. We have therefore studied the effects of voltage on the form of the dose-response curve for agonists, on desensitized versus normal receptors, on the inhibition produced by curare, and on that produced by a local anesthetic. Only the effects of the local anesthetic depend strongly on voltage. Preliminary reports of the data have been published (Lester and Changeux, 1973, 1974).

METHODS

The isolated *Electrophorus* electroplaque was studied (Schoffeniels and Nachmansohn, 1957; Nakamura et al., 1965; Ruiz-Manresa and Grundfest, 1971). About one-fifth of the surface of the caudal, innervated face was exposed to a pool of Ringer's solution ("pool A") through a window in a Mylar sheet. The entire noninnervated face was exposed to another pool, called B. The temperature was 20–22°C, unless noted.

Solutions

The Ringer's solution contained NaCl, 160 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 2 mM; Na phosphate buffer, 1.5 mM (pH 7.4). Pool A usually contained tetrodotoxin (10^{-7} M) to eliminate Na^+ activation.

The resting membrane has a nonlinear K conductance that resembles anomalous rectification in skeletal muscle fibers. Therefore, pool A also contained $BaCl_2$ (3 mM) at all times to maintain the membrane in a nearly linear, low-conductance state (Ruiz-Manresa et al., 1970).

Drugs applied in pool A include carbamylcholine chloride (Carb), decamethonium iodide and bromide (Deca), tubocurarine chloride (dTC), procaine hydrochloride, and α-toxin from *Naja nigricollis* (gift of Dr. P. Boquet, Institut Pasteur). Ouabain (1 mM) was sometimes added to pool B and did not affect the agonist-induced currents.

Electrical Arrangements

Two micropipette tips straddled the innervated membrane; signals were led from these electrodes to a differential amplifier which measured the transmembrane potential

(inside minus outside). Currents were applied across the cell between two chlorided silver plates in pools A and B. A complete current-voltage (I-V) curve of the membrane was generated in about 700 ms. For most experiments, we employed feedback control of V; the command voltage was usually a cycle composed of continuous linear ramps. One such cycle is called a "ramp-clamp" trial. The voltage-clamp circuit was disconnected from the preparation between trials, so that usually no current was applied. A few experiments were performed with a controlled-current circuit.

The circuit corrected V for the series resistance of the solution between the microelectrode tips (Hodgkin et al., 1952; Nakamura et al., 1965). Assuming (*a*) equal conductivity (63 Ω-cm) for the intracellular and extracellular solutions, and (*b*) a typical electrode separation of 150 μm, the calculated series resistance was 1 Ω-cm². Since the membrane's slope resistance sometimes decreased to this value, the correction for series resistance was important.

Slope conductance dI/dV was obtained electronically, by dividing dI/dt by dV/dt. This procedure provided a more sensitive measure of membrane properties than did recordings of *current* alone: agonists often produced small but measurable changes in slope conductance under conditions (lower agonist concentration, more positive membrane potential) where agonist-induced currents were undetectable.

Complicating Factors

ERRORS IN CURRENT-FLOW MEASUREMENTS If currents flow extracellularly around the Vaseline seals, measurements of small conductances would be distorted. However, for cells which completely filled the window, the action potential had the same amplitude when recorded (*a*) with the microelectrodes in their usual position, straddling the innervated face, or (*b*) with extracellular electrodes in pools A and B. From this observation we estimate that the external leakage path had at least 100 times the resistance of the innervated face at rest and contributed negligible errors.

SPATIAL UNIFORMITY The microelectrode pair does not accurately record transmembrane potentials which originate more than one cable length from the recording point. Assuming a theoretical planar electroplaque 20 μm thick, with an internal resistivity of 63 Ω-cm and a membrane resistance of 2 Ω-cm² (a typical value during exposure to high agonist concentration), the DC cable length was only 82 μm. We therefore wondered whether the results remained valid for all regions exposed by the window, which was elliptical with axes of about 1 and 3.5 mm. A Carb application (10^{-4} M) was repeated while the microelectrode pair was in several different positions and as close as 100 μm to one edge of the window. Voltage-clamp currents agreed to within 20% over the series.

Evaluation of the Ramp-Clamp Technique

This method gives an accurate description of membrane properties which attain their steady-state values faster than the rate of voltage sweep (0.8 mV/ms in most cases) and which show no further dependence on time alone for an interval corresponding to the length of the cycle (about 700 ms). To ascertain that these conditions were met, preliminary experiments were performed with "step-clamps" to a constant potential lasting 1 s. At 20–22°C, currents changed within 2 ms and remained constant during the step, with the following two exceptions.

Firstly, Ba does not linearize completely the membrane conductance for K (see Ruiz-Manresa et al., 1970). When the membrane was depolarized to potentials more positive than −40 mV, a small outward current (less than 1 mA/cm²) appeared. This current subsequently decreased to zero with a time constant of about 50 ms; it therefore caused little difficulty in our measurements during depolarization either by currents or by agonists.

Secondly, during clamps to high negative potentials in the presence of high agonist concentrations, currents often decreased slowly with time. Thus during the approach to a negative extremum of V, the current was larger than during the return. If the clamp was maintained for a second or third cycle, the later I-V curves superimposed on the first one. Curiously, this "hysteresis" did not change markedly when the speed of voltage sweep was increased by a factor of 4. In the presence of agonists only, the hysteresis never exceeded 10%. The effect may reach 15% with the addition of procaine.

RESULTS

Properties of the Membrane of the Innervated Face in the Absence of Agonists

The isolated electroplaque has a resting potential of −80 to −90 mV. Under our experimental conditions (notably the presence of tetrodotoxin and of Ba^{++}), the innervated face of a cell displayed a linear I-V relation in the absence of agonist (Fig. 1). The resting conductance was 41 ± 3 mmho/cm²

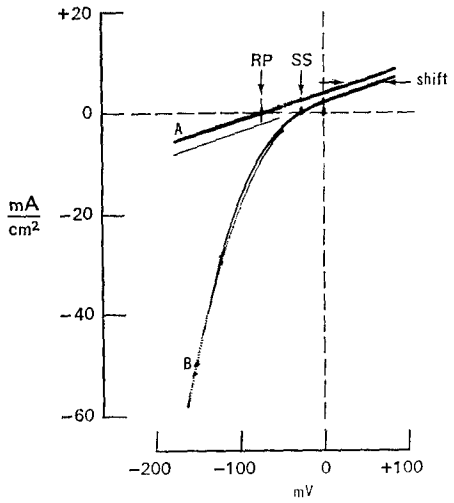

FIGURE 1. Traces from an X-Y scope showing I-V relations with the ramp-clamp technique. Two trials: A, in control solution; B, 1 min after flushing chamber with 10^{-4} M Carb. RP, resting potential in control solution; SS, zero-current membrane potential in presence of Carb. Continuous thin line, extrapolated from Carb trace at positive potentials, is used as base line to measure current induced by Carb (see text). Shift is pointed out on voltage axis.

(mean ± SEM, range 24–64), a somewhat lower value than that (110 mmho/cm^2) reported by Ruiz-Manresa et al. (1970).

Shift in the I-V Curve Caused by Nicotinic Agonists

Upon bath application of agonists to the innervated face, the membrane potential moved toward zero (Schoffeniels and Nachmansohn, 1957; Higman et al., 1963). This depolarization seems to arise from a combination of two mechanisms. One of these, a reversible increase in the conductance of the membrane (Fig. 1; Changeux et al., 1970; Ruiz-Manresa and Grundfest, 1971), resembles the effect of agonist on most postsynaptic membranes. The present work deals mainly with this conductance.

As previously described, agonists cause conductance increases only at negative potentials (Ruiz-Manresa and Grundfest, 1971). For positive potentials the *I-V* curves show equal slope conductance in the presence and absence of agonist (Fig. 1). This situation reveals the second effect of the agonist, which can be described as a reversible shift of the *I-V* curve in the direction of positive membrane potentials or outward currents.

We shall arbitrarily describe the shift as a voltage (Fig. 1). We observed its onset by measuring the *I-V* curve every 10 s. When pool A was flushed with solution containing agonist, the shift developed about as rapidly as the conductance; however, at high agonist concentrations, the *shift* showed little subsequent decline even though the *conductance* underwent desentization (see below). If the agonist was washed out the original *I-V* plot reappeared in a few minutes. The shift increased in size with Carb concentration (Fig. 2).

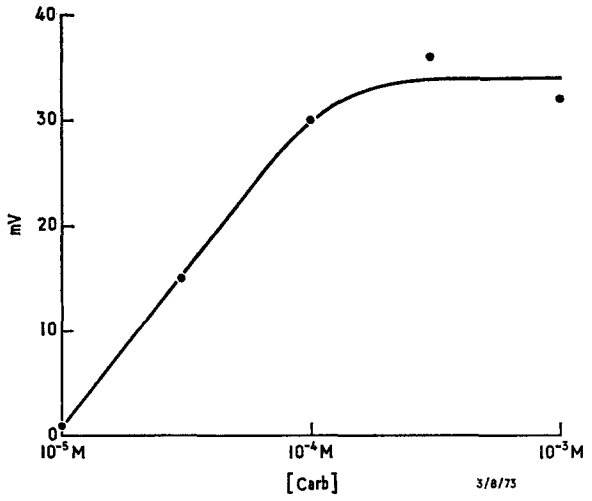

FIGURE 2. Variation of the shift with Carb concentration for one electroplaque. Measurements were made, as in Fig. 1, at time of the peak conductance increase after addition of Carb (see Fig. 5).

The currents which flowed during our rather long ramp-clamps did not contribute to the shift since varying the duration of the clamp, the peak-to-peak voltage, or the total number of trials had no effect on the shift. Furthermore, the shift appeared with equal magnitude in *I-V* curves taken with a series of step-clamps 10 ms in duration.

Reversal Potentials and Measurement of Agonist-Induced Currents

The shift complicates measurement of agonist-induced currents because steady-state *I-V* curves (Fig. 1) do not yield a reversal potential which can be taken as the point of zero agonist-induced current. The reversal potential was nonetheless found with instantaneous *I-V* measurements. In the presence of agonist, the membrane potential was held at −150 mV for 6 ms, then jumped to various other potentials. The detailed results will be described elsewhere; in brief, the voltage-clamp currents relaxed along an exponential time-course to the steady-state value appropriate to the new voltage. The time constant was several milliseconds. Upon extrapolation to the time of the jump, the data formed linear instantaneous *I-V* curves. The reversal potential, at the intersection of the instantaneous and steady-state curves, was 12 mV in the experiment of Fig. 3 and in other cells ranged from zero to +20 mV.

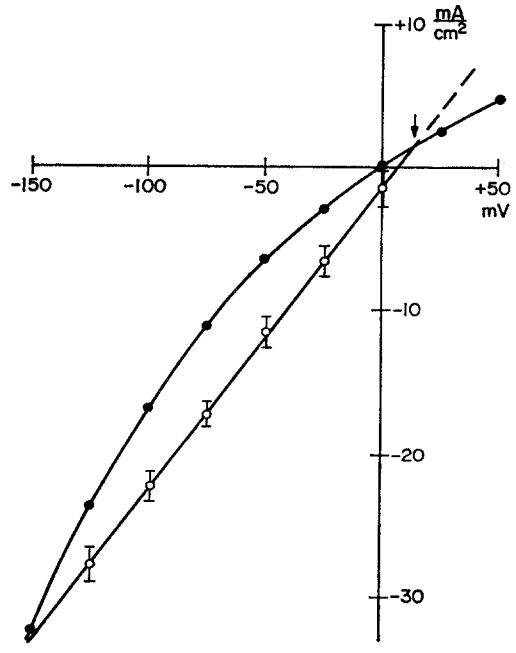

FIGURE 3. *I-V* curves in presence of 5×10^{-5} M Carb. ●, steady state; ○, instantaneous. Error bars give uncertainty of extrapolation to time of the voltage jump. Arrow points to reversal potential. See text. Temperature 15°.

At V more positive than -10 mV, the steady-state agonist-induced currents are very small, and only the resting conductance contributes to the I-V curves. Therefore, in practice the zero level for agonist-induced currents could be found by extrapolating the linear, low-conductance part of the I-V curve (upper right quadrant) to negative potentials (see light line in Fig. 1). *Indeed, if the reversal potential were found at any voltage more positive than about -10 mV, the agonist-induced currents would have essentially the same amplitude.*

Calculated *chord conductances*, on the other hand, are sensitive to possible errors in measuring the reversal potential. The unknown mechanism causing the shift may have distorted our values of the reversal potential. Though this seems unlikely, to eliminate the uncertainty we present results in the form of agonist-induced *currents and slope conductances* rather than chord conductances. Interpretations in terms of chord conductances are given in the Discussion.

Time-Course of Conductance Changes during Bath Application of Agonists

The conductance undergoes a sequence of changes with time during bath application of cholinergic agonists. These events may be analyzed more completely with slope conductances (Fig. 4) than with currents because we could measure the former property with greater sensitivity (see Methods).

When pool A was flushed with the solution containing agonist, the conductance first rose to a peak, then fell to a plateau. With increased agonist concentrations, the rising and falling phases shortened, the peak amplitude increased, and the value of the plateau decreased. These data confirm earlier observations that the electroplaque's response declines after prolonged exposure to high agonist concentrations (Changeux et al., 1970; Larmie and Webb, 1973 *a*, *b*; see also Higman et al., 1964).

This phenomenon presents several similarities with "desensitization" observed at the myoneural junction: it occurred in the electroplaque in isotonic KCl, K acetate, or K_2SO_4 (compare Katz and Thesleff, 1957; Manthey, 1970), and also in solutions with either 80 or 100% of Na replaced by Tris (cf. Manthey, 1966). At 3×10^{-4} M Carb, desensitization in the electroplaque followed an exponential time-course with a half-time of 46 s, in close agreement with Manthey's (1966) measurements on the myoneural junction (half-time of 40 s with the same Carb and Ca concentrations). However, it must be noted that our solutions contained Ba^{++}, which accelerates desensitization at the myoneural junction (Magazanik and Vyskočil, 1970).

In Fig. 4, for a given Carb concentration, the several curves showing time dependence of the conductance differ among themselves only by a scaling factor. This is equivalent to the statement that desensitization has no effect on voltage sensitivity of the agonist-induced conductance. In fact, there are minor changes in voltage sensitivity during desensitization, but we have not carefully studied these effects.

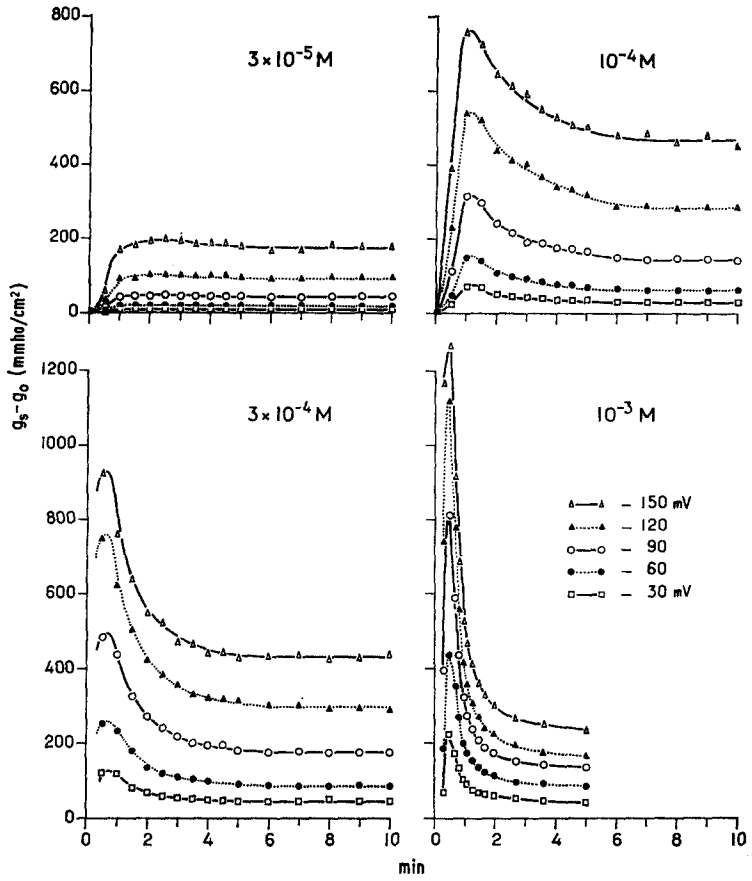

FIGURE 4. Equipotential plots of Carb-induced slope conductance ($g_s - g_o$) vs. time. Pool A was flushed with the indicated Carb solution beginning at time zero. Ramp-clamp trials (Fig. 1) were made at indicated times over the next 10 min. The times of occurrence of the peak conductance are assigned from other experiments in which this point was carefully determined. Data at 10^{-3} M are from a separate cell.

Desensitization may have occurred while agonist was still accumulating near the receptors. Although concentrations in the bulk solution changed with a half-time of 8.5 s (dye dilution), we have no direct measurement of the agonist concentration in the vicinity of the receptor site. Thus we cannot estimate, for Carb concentrations greater than about 10^{-4} M, the steady-state conductances which would occur in the hypothetical absence of desensitization. Similar considerations apply to Deca at concentrations greater than 3×10^{-6} M. The uncertainty may reach a factor of 2 at a concentration of 10^{-3} M Carb. In subsequent sections, we describe the conductances at their peak with time.

Variation of Agonist-Induced Currents with Membrane Potential and with Agonist Concentration

The peak agonist-induced currents and slope conductances increased with agonist concentration. In addition, at a given concentration of agonist, the conductance decreased with more positive membrane potentials (equimolar curves in Fig. 5).

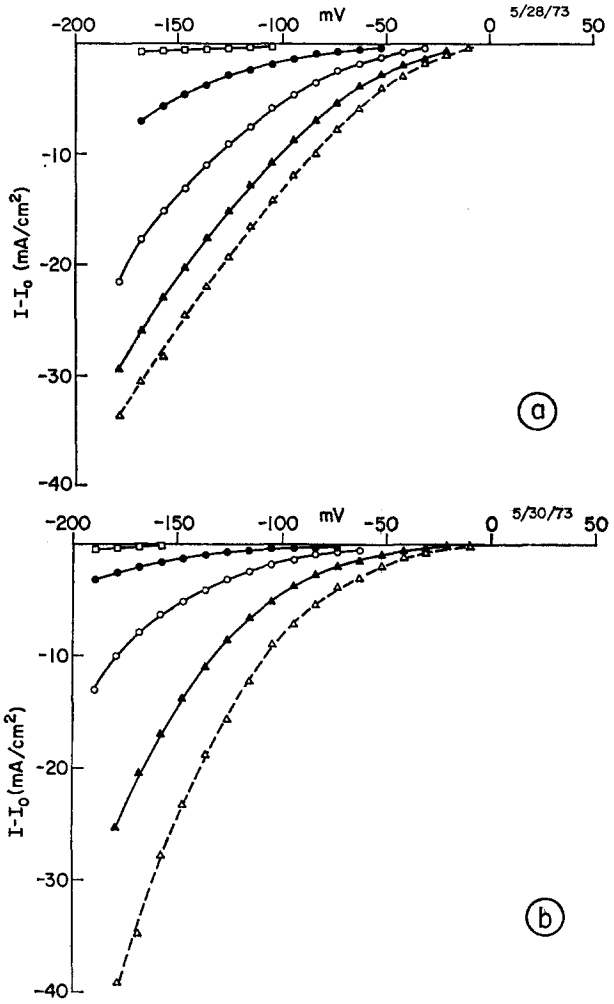

FIGURE 5. Equimolar plots of agonist-induced current vs. potential. Data for the peak of conductance with time (see Fig. 4). (A) Carb concentrations: △, 10^{-3} M; ▲, 3×10^{-4} M; ○, 10^{-4} M; ●, 3×10^{-5} M; □, 10^{-5} M. (B) Deca on another cell. Average values from two exposures to each concentration: △, 3×10^{-5} M; ▲, 10^{-5} M; ○, 3×10^{-6} M; ●, 10^{-6} M; □, 3×10^{-7} M. The data for 10^{-3} M Carb and 3×10^{-5} Deca are connected by a dashed line to emphasize that they represent peak rather than steady-state measurements (see text).

When the response was small, either because the agonist concentration was small or the potential very positive, the effect of voltage did not depend on the nature of the agonist or its concentration. This point is shown more precisely by comparing small slope conductances in an experiment where the signal-to-noise ratio was improved with averaging techniques (Fig. 6).

However, at more negative potentials, conductances at high agonist concentrations no longer scale up linearly from those at lower concentrations. In Fig. 5 *a* the largest slope conductances level off at an upper limit of about 0.3 mho/cm^2.

EFFECT OF *Naja nigricollis* α-TOXIN. The leveling off might conceivably result from artifacts associated with the measurement of large conductances. To eliminate this possibility, we exposed seven electroplaques to *N. nigricollis* α-toxin (4–10 × 10^{-8} M) for various periods (5–15 min). The α-toxin decreased the response to Carb with very low reversibility; the blockade increased with α-toxin concentration and with the time of exposure. In any one electroplaque, however, an exposure to α-toxin reduced agonist-induced currents and slope conductances by an equal factor at *all* potentials and at *all* agonist concentrations.

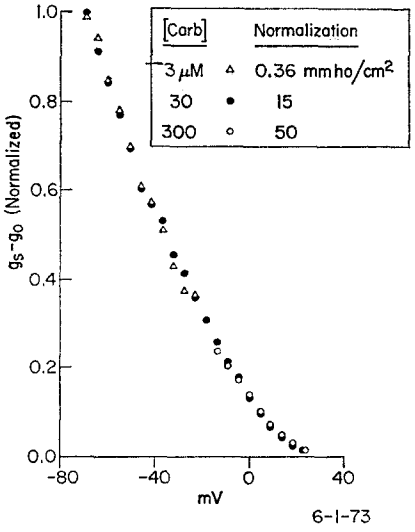

FIGURE 6

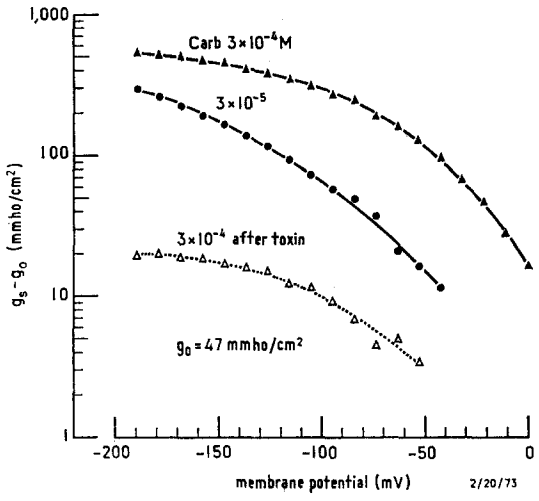

FIGURE 7

FIGURE 6. Equimolar plots of small Carb-induced slope conductances ($g_s - g_o$) versus potential, for one cell. △, 3 × 10^{-6} M; ●, 3 × 10^{-5} M; ○, 3 × 10^{-4} M. Data are expressed as a fraction of the following values: △, 0.36 mmho/cm^2; ●, 15 mmho/cm^2; ○, 50 mmho/cm^2. For △, ●, a computer was used to average dI/dt during 20 consecutive ramp-clamp trials; for ○, only one trial was used.

FIGURE 7. Effect of *Naja nigricollis* toxin on Carb-induced slope conductance ($g_s - g_o$). Equimolar plot; note log scale on vertical axis. Electroplaque was tested before and after a 15-min incubation in 10^{-7} M toxin.

Fig. 7 illustrates clearly that the leveling off is caused by the combination of high Carb concentration and high negative potential, rather than by the presence of high conductance. After α-toxin treatment, which reduced the agonist-induced slope conductance by a factor of 25, the conductance still leveled off at high negative potentials in the presence of 3×10^{-4} M Carb.

EFFECT OF CURARE. Tubocurarine (dTC) decreased the conductance induced by agonists; tubocurarine blockade could be overcome by high agonist concentrations, as expected for a competitive antagonist. The apparent dissociation constant K_I for dTC was studied as a function of potential in one electroplaque (Table I). The "dose-ratio" method (Jenkinson, 1960) was used

TABLE I

DOSE RATIOS IN AN EXPERIMENT WITH TUBOCURARINE (dTC) AND DECA

	([Deca']/[Deca]) − 1	
V	dTC, 4×10^{-7} M	dTC, 1.2×10^{-6} M
mV		
−63	1.4	4.1
−84	1.7	6.7
−116	1.7	6.8
−147	1.7	5.2
Averages	1.6	5.7

Equimolar curves of slope conductance vs. V were taken for Deca alone and for Deca plus tubocurarine. [Deca] and [Deca'] are concentrations giving equal conductances with and without dTC. Then K_I = [dTC]/([Deca']/[Deca] − 1).

to determine K_I even though this method's assumptions, viz., that both dTC and the agonist bind noncooperatively, may be unjustified in the present case (see below). The data show that K_I does not vary significantly with potential in the measurable range and equals 2.3×10^{-7} M, in agreement with the value obtained by other techniques (Higman et al., 1963; Kasai and Changeux, 1971 *a*).

EFFECT OF PROCAINE. Unlike desensitization or exposure to α-toxin or to curare, exposure to procaine produced effects which varied strongly with potential (Fig. 8). Procaine inhibited the Deca currents most effectively at high negative potentials. This inhibition was nearly independent of Deca concentration: over the explored range at −170 mV, the Deca currents varied more than 50-fold, but the percentage inhibition by procaine varied less than twofold.

The Hill Coefficient Does Not Vary with Membrane Potential

Conductances were measured by varying V at constant agonist concentration, but results can also be plotted as a function of agonist concentration at a given

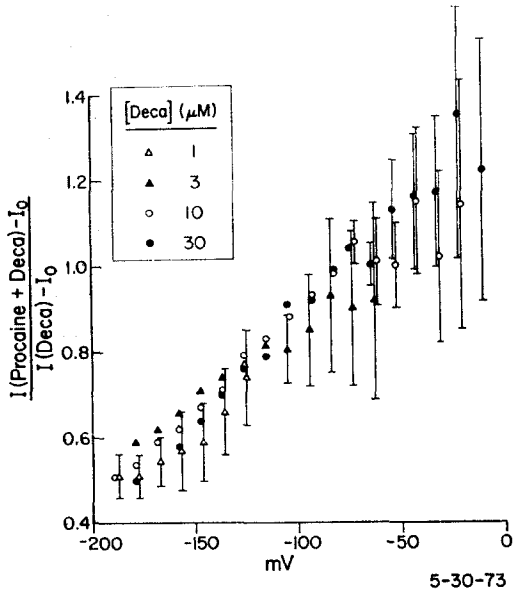

FIGURE 8

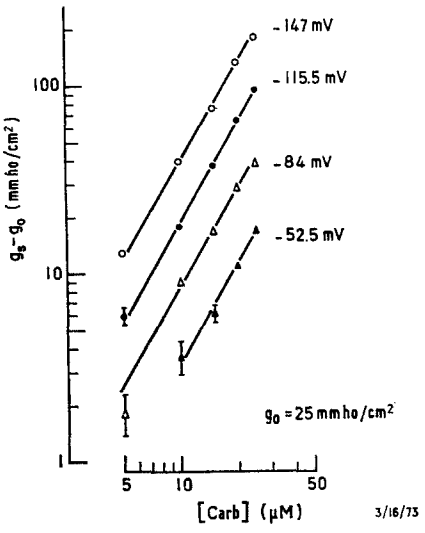

FIGURE 9

FIGURE 8. Interaction between effects of potential and of procaine at various Deca concentrations. The current induced by Deca alone is I (Deca) $- I_o$. The current for the same concentration of Deca, in the presence of 3×10^{-4} M procaine, is I (Deca + procaine) $- I_o$. The ratio of these two currents is plotted as a function of membrane potential to show how voltage affects the inhibition of the Deca currents by procaine. Deca concentrations: △, 3×10^{-5} M; ▲, 10^{-5} M; ○, 3×10^{-6} M; ●, 10^{-6} M. Standard errors are shown where they exceed size of the symbols. A control series in the absence of procaine preceded and followed the procaine series and showed complete reversibility of the procaine effect. The two control series for this electroplaque yielded the data in Fig. 5 *b*. Procaine had no effect on resting currents (I_o) in absence of agonist.

FIGURE 9. Equipotential dose-response curves for the increase in slope conductance ($g_s - g_o$) produced by small Carb concentrations. Data are averaged from an ascending and a descending series of concentrations. For this cell the slope of the lines on double-logarithmic coordinates is 1.8.

potential. As expected from data in Fig. 6, the shape of such *dose-response curves* (Fig. 9) does not depend on voltage at low agonist concentrations. Furthermore, in a double-logarithmic plot, the dose-response curves give straight lines at low agonist concentrations. The slope, the Hill coefficient, is greater than unity, equaling 1.90 ± 0.08 (mean ± SEM, seven cells) for Carb and 1.45 ± 0.14 for Deca (five cells).

DISCUSSION

Our goal is to study the effect of agonists under conditions where the reaction between agonist and receptor is approximately at thermodynamic equilibrium. To this end, we measured increases in membrane conductances

during bath application of agonists to the innervated face of an isolated electroplaque. At high agonist concentration, however, our results are distorted to an unknown extent by the presence of desensitization.

For assurance that nicotinic acetylcholine receptors do in fact produce the observed effects, we note that curare, elapid α-toxins, and positive membrane potentials also abolish the neurally evoked conductance increase which underlies the postsynaptic potential (Ruiz-Manresa and Grundfest, 1971).

Organization of Innervated Face

Nerve terminals establish synaptic contacts with only about 1% of this face's membrane area (Luft, 1958; Bourgeois et al., 1972). The membrane contains binding sites for *Naja nigricollis* α-toxins both between the synapses and at the synapses, in approximately equal total numbers (Bourgeois et al., 1972). Therefore the agonist-induced conductances probably include contributions from both the synaptic and extrasynaptic receptor populations, but we do not know whether the two receptor types have the same properties.

Depolarization and Shift in I-V Curve

During bath application of agonist, the membrane depolarizes initially. One would expect desensitization to cause a subsequent repolarization even in the continued presence of agonist. This effect is observed (Larmie and Webb, 1973 *a*, *b*), but for a given agonist concentration the repolarization occurs several times more slowly than the desensitization of the conductance. The repolarization is delayed because the *I-V* curve seems to shift toward positive potentials and because the shift outlasts the agonist-induced conductance. Consequently, the dose-response relations for the depolarization (Higman et al., 1963; Changeux and Podleski, 1968) and for the shift (Fig. 2) resemble each other closely. On the other hand, the apparent dissociation constants for *conductance* (see below) exceed by at least 10-fold the values for depolarization (Changeux and Podleski, 1968).

We have made several unsuccessful efforts to explain the shift. Ouabain fails to depolarize the resting cell; thus there is no electrogenic pump. Experiments with Cl^--free solutions rule out a change in ϵ_{Cl} (cf. Karlin, 1967; Jenkinson and Terrar, 1973). Direct measurements of internal K concentration suggest no change in ϵ_K (Blumenthal and Changeux, 1970). If we postulate a K-sensitive membrane in a special compartment which exchanges slowly with the bulk solution, we cannot explain another fact: a brief exposure to both agonist and ouabain produces a *permanent* depolarization (at least 4 h). Thus, at present we can decide neither whether the shift is a direct consequence of agonist acting on the acetylcholine receptor, nor whether it occurs when agonist is present for only about a millisecond, as during normal synaptic transmission.

Chord Conductances

In experiments with both synaptic stimulation and bath application of agonists to electroplaques, Ruiz-Manresa and Grundfest (1971) observed linear *I-V* relations at very negative potentials; they extrapolated these results to yield a reversal potential of +65 mV. Our experiments reveal that such a situation is a special case occurring only at high concentrations of agonist.

The instantaneous reversal potential (V_o) for the agonist-induced voltage-clamp currents (I) ranged from 0 to +20 mV. Chord conductances g_c were calculated from the *I-V* plots:

$$g_c(V) = \frac{I}{V - V_o}. \tag{1}$$

In addition, since slope conductance g_s is measured with greater sensitivity than is current, the chord conductances were extended to more positive potentials with the formula

$$g_c(V) = \frac{1}{V - V_o} \int_{V_o}^{V} g_s(v)\, dv. \tag{2}$$

Calculated chord conductances (Fig. 10 and Table II) increase at more negative potentials; within a certain range of potentials and of Carb and Deca

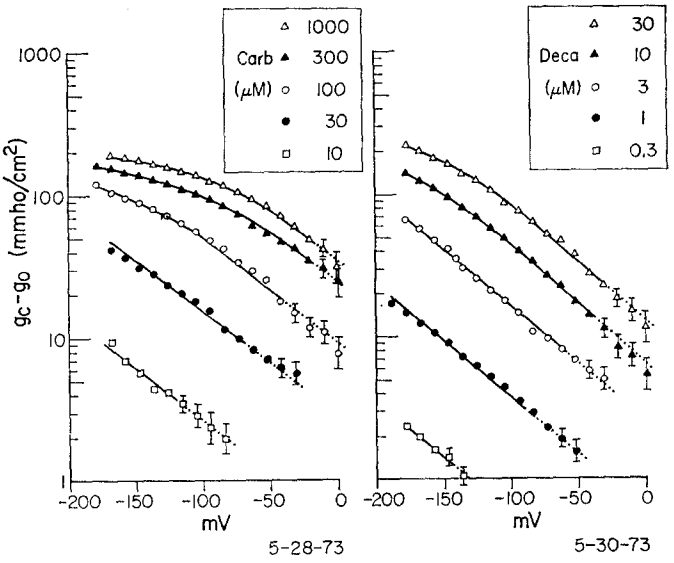

FIGURE 10. Agonist-induced chord conductances ($g_c - g_o$) from the data in Fig. 5. (A) Carb; (B) Deca. Equimolar plots on a logarithmic scale for conductance. Smooth regions of the lines are calculated from currents (Eq. 1); dotted regions, from slope conductances (Eq. 2).

TABLE II

CHARACTERISTICS OF CHORD CONDUCTANCES INDUCED BY TWO DIFFERENT AGONISTS

Agonist	*e*-fold change in $g_c - g_o$*	Largest $g_c - g_o$ measured‡
	mV	*mmho/cm²*
Carb	63.0±1.5 (12)	340±60 (10)
Deca	60.1±1.5 (5)	130±20 (4)

* Small agonist concentrations.

‡ Large agonist concentrations, −150 mV.

Values are mean ± SEM; number of cells in parentheses.

concentrations, a 62-mV change of potential causes an *e*-fold change of chord conductance. However, like slope conductance, chord conductances level off in the region of high negative potentials and high agonist concentrations, particularly in the case of Carb. Observed chord conductances seem to approach an upper limit of about 0.3 mho/cm².

DOSE-RESPONSE RELATIONS FOR CHORD CONDUCTANCE An equipotential dose-response curve for chord conductance and one for current have the same shape at a given potential (Eq. 1). Apparent dissociation constants for the two measures are therefore equal. Because of desensitization only a lower limit on the apparent dissociation constant can be given; at potentials more positive than −100 mV, the dissociation constant is at least 3×10^{-4} M for Carb and 10^{-5} M for Deca.

Small agonist-induced slope conductances may be expressed as the product of two factors which depend *separately* on potential and on agonist concentration (Figs. 6 and 9). Eq. 2 then shows that Hill coefficients observed with slope conductances (1.45 for Deca, 1.9 for Carb) also apply to chord substances.

Possible Nature of the Acetylcholine Receptor

In other recent experiments with bath application of agonist, we have jumped the voltage from one level to another while monitoring the voltage-clamp currents. By measuring postsynaptic currents, we have also studied the decay of conductance associated with a sudden decrease of acetylcholine concentration. In combination with the present results, these data give both the *steady state* of conductance for a given voltage and agonist concentrations, and the *kinetics* of the transition from one steady state to another. The kinetic results suggest a formal description: a first-order rate constant governs transitions from nonconducting to conducting forms of the agonist-sensitive membrane. This rate constant depends upon the agonist, increases with increasing agonist concentration, and varies little if any with potential. The reverse transition is governed by a first-order constant which depends upon the nature of the

agonist, does not depend upon agonist concentration, and varies exponentially with the potential. This description accounts for several features of the present steady-state measurements, including the "leveling off" at high negative potentials (Sheridan and Lester, in preparation).

Unfortunately, the formal description yields little information on the *molecular* nature of the acetylcholine receptor. We shall therefore interpret the present data within the framework of a molecular model which motivates much of the recent biochemical work on the receptor protein.

We consider that the receptor has two functionally distinct areas. The cholinergic *protomer* has been defined as the elementary unit which carries a single acetylcholine binding site (Changeux et al., 1970). The receptor also carries or shares an *ionophore*, or Na-K channel, which controls the passage of Na and K through the membrane. The data suggest that the ionophore has a linear *I-V* curve and a reversal potential near zero mV. *The agonist-induced chord conductance thus measures the number of active ionophores.*

Presence of an agonist must somehow increase the probability that the ionophore be in a conducting state. More specifically, it has been postulated that binding of the agonist to the protomer increases the likelihood of an active state of the protomer; this active state in turn favors the conducting state of the ionophore (Changeux et al., 1970).

EFFECTS OF POTENTIAL The electric field could affect the agonist-protomer interaction (as suggested by Kordas, 1972 *b*) or the transitions of the protomer. However, certain facts render this interpretation unlikely. Firstly, for both a monoquaternary ligand (Carb) and a bisquaternary one (Deca), the conductance changes *e*-fold per 62 mV of membrane potential. Secondly, membrane potential has no effect on the apparent binding constant for curare, which is thought to compete with agonists at the receptor binding site (Jenkinson, 1960; Weber and Changeux, 1974 *b*). Thirdly, Hill coefficients, which may measure cooperative interactions among binding sites, do not change with potential. It is still possible that the potential affects the functioning of the ionophore, conceivably because some element of the ionophore presents a dipole moment (Magleby and Stevens, 1972 *a*, *b*).

The underlying mechanism of this voltage dependence seems identical to the one which affects the time-course of end-plate currents and of end-plate "noise" (Kordas, 1969, 1972 *a*, *b*; Magleby and Stevens, 1972 *a*, *b*; Anderson and Stevens, 1973). Our kinetic experiments, which will be reported elsewhere, support this conclusion. The voltage-dependent inhibition of conductance by procaine may arise from the same process as the voltage-dependent modification of end-plate current waveforms by this drug (Kordas, 1970). The interaction between procaine and the receptor protein probably occurs at a site distinct from the agonist binding site (Weber and Changeux, 1973 *c*).

SIGMOID SHAPE OF DOSE-RESPONSE CURVES The Hill coefficient exceeds unity and differs for different agonists. These facts suggest the presence of cooperative, homotropic interactions between the conformational states of neighboring cholinergic protomers. The cooperative binding of acetylcholine to membrane fragments from *Torpedo* electric organs (Weber and Changeux, 1973 *a*, *b*) supports this interpretation. The smaller Hill coefficient for Deca than for Carb would be expected if Deca binds "nonexclusively" to both active and inactive states of the protomer (Rubin and Changeux, 1966).

APPARENT DISSOCIATION CONSTANTS AND MAXIMUM CONDUCTANCES The present data yield lower limits for agonist dissociation constants; these values are in order of magnitude greater than those found with excitable microsacs or with purified receptors (Meunier and Changeux, 1973). Meunier and Changeux (1973) have commented on possible causes for such discrepancies. The smaller maximum conductances for Deca than for Carb could arise either from nonexclusive binding of Deca or from a lower conductance of the ionophore (cf. Colquhoun et al., 1975).

Steady-State Flux Associated with a Single Receptor Site

Biochemical and electrophysiological measurements may be combined to estimate the net ionic flux which is controlled by a single protomer in the active state. Neglecting desensitization for the moment, we assume that all the receptors go to this state for the highest Carb concentrations. At normal resting potential (-85 mV), the maximum observed agonist-induced currents are about 15 mA/cm^2.

There is about one ^{3}[H]α-toxin binding site per agonist binding site (Weber and Changeux, 1973 *b*; Meunier and Changeux, 1973). Several studies show that the eel electroplaque has on the order of 10^{12} toxin binding sites/cm^2 of window area (Karlin et al., 1970; Bourgeois et al., 1972). Therefore at -85 mV the net flux is about 90,000 ions/s for each active protomer.

COMPARISON WITH NOISE DATA At the myoneural junction the effect of carbachol can be described as arising from elementary events lasting 3×10^{-4} s (Katz and Miledi, 1972) and involving a net flux of 5,000 ions (Anderson and Stevens, 1973). In eel electroplaques the elementary event is very similar (R. E. Sheridan and H. A. Lester, unpublished results). If the structure producing the elementary event were in its conducting state at all times, it could therefore transport 1.5×10^7 ions/s. This number is some two orders of magnitude larger than the flux we have measured for a single protomer in the active state.

The discrepancy just calculated remains unchanged (*a*) if one uses another membrane potential for the calculations, because the voltage probably has similar effects both on the steady-state conductance and on the duration of the

elementary event (Anderson and Stevens, 1973); (*b*) if one uses Deca rather than Carb, because Deca exhibits both smaller maximum conductance and a shorter elementary event (Katz and Miledi, 1973; Sheridan and Lester, unpublished observations).

Several factors could contribute to this discrepancy:

(*a*) We have no assurance that all the protomers are in the active state at our largest Carb concentrations. If, for instance, the Carb dissociation constant is about 10^{-2} M, then desensitization, which appears at Carb concentrations greater than 10^{-4} M, allows us to measure only about 1% of the maximum response.

(*b*) The elementary noise event could result, not from a single ionophore, but from the concerted action of several receptor oligomers in a "domain."

(*c*) We do not yet know the number of protomers per ionophore. If the ionophore is the "hole" observed in electron micrographs of purified receptor protein from *Electrophorus* and of receptor-rich membranes from *Torpedo* (Cartaud et al., 1973; Nickel and Potter, 1973), then this number is three or more.

(*d*) Perhaps the ionophore can be in its conducting state for only a small fraction of the time. Thus, an upper limit of 18 elementary events per second for each ionophore would give the maximum flux of 90,000 ions in a second. This frequency might be constrained by (*a*) the microscopic "on" and "off" rates for binding to the agonist site, (*b*) the kinetics of conformational transitions of the protomer, or (*c*) possible autonomous fluctuations of the ionophore itself (Kasai and Changeux, 1971 *b*).

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REFERENCES

ANDERSON, C. R., and C. F. STEVENS. 1973. Voltage clamp analysis of acetylcholine induced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol. (Lond.).* **235**:655.

BLUMENTHAL, R., and J. P. CHANGEUX. 1970. About the changes of internal ionic concentration in the isolated electroplax during chemical excitation. *Biochim. Biophys. Acta.* **219**:398.

BOURGEOIS, J. P., A. RYTER, A. MENEZ, P. FROMAGEOT, P. BOQUET, and J.-P. CHANGEUX. 1972. Localization of the cholinergic receptor protein in *Electrophorus* electroplax by high resolution autoradiography. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **25**:127.

CARTAUD, J., E. L. BENEDETTI, J. B. COHEN, J. C. MEUNIER, and J.-P. CHANGEUX. 1973. Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **33**:109.

CHANGEUX, J.-P., and T. R. PODLESKI. 1968. On the excitability and cooperativity of the electroplax membrane. *Proc. Natl. Acad. Sci. U.S.A.* **59**:944.

CHANGEUX, J.-P., T. R. PODLESKI, M. KASAI, and R. BLUMENTHAL. 1970. Some molecular aspects of membrane excitation studied with the eel electroplax. *In* Excitatory Synaptic Mechanisms. P. Andersen and J. Jansen, editors. Universitets Forlaget, Oslo. 123.

COLQUHOUN, D., V. E. DIONNE, J. H. STEINBACH, and C. F. STEVENS. 1975. Conductance of channels opened by acetylcholine-like drugs in muscle end-plate. *Nature* (*Lond.*). **253:** 204.

HIGMAN, H., T. R. PODLESKI, and E. BARTELS. 1963. Apparent dissociation constants between carbamylcholine, D-tubocurarine and the receptor. *Biochim. Biophys. Acta.* **75:**187.

HIGMAN, H., T. R. PODLESKI, and E. BARTELS. 1964. Correlation of membrane potential and potassium flux in the electroplax of *Electrophorus electricus*. *Biochim. Biophys. Acta.* **79:**138.

HODGKIN, A. L., A. F. HUXLEY, and B. KATZ. 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol.* (*Lond.*). **116:**424.

JENKINSON, D. H. 1960. The antagonism between D-tubocurarine and substances which depolarize end-plate. *J. Physiol.* (*Lond.*). **152:**309.

JENKINSON, D. H., and D. A. TERRAR. 1973. Influence of chloride ions on change in membrane potential during prolonged application of carbachol to skeletal muscle. *Br. J. Pharmacol.* **47:**363.

KARLIN, A. 1967. Permeability and internal concentration of ions during depolarization of the electroplax. *Proc. Natl. Acad. Sci. U.S.A.* **58:**1162.

KARLIN, A., J. PRIVES, W. DEAL, and M. WINNIK. 1970. Counting acetylcholine receptors in the electroplax. *In* Ciba Foundation Symposium on Molecular Properties of Drug Receptors. R. Porter and M. O'Connor, editors. J. & A. Churchill, Ltd., London.

KASAI, M., and J.-P. CHANGEUX. 1971 *a*. *In vitro* excitation of purified membrane fragments by cholinergic agonists. I. Pharmacological properties of the excitable membrane fragments. *J. Membr. Biol.* **6:**1.

KASAI, M., and J.-P. CHANGEUX. 1971 *b*. *In vitro* excitation of purified membrane fragments by cholinergic agonists. II. The permeability change caused by cholinergic agonists. *J. Membr. Biol.* **6:**24.

KATZ, B., and R. MILEDI. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol.* (*Lond.*). **224:**665.

KATZ, B., and R. MILEDI. 1973. The characteristics of "end-plate noise" produced by different depolarizing drugs. *J. Physiol.* (*Lond.*). **230:**707.

KATZ, B., and S. THESLEFF. 1957. A study of the "desensitization" produced by acetylcholine at the motor end-plate. *J. Physiol.* (*Lond.*). **138:**63.

KLETT, R. P., B. W. FULPIUS, D. COOPER, M. SMITH, E. REICH, and L. D. POSSANI. 1973. The acetylcholine receptor. I. Purification and characterization of a macromolecule isolated from *Electrophorus electricus*. *J. Biol. Chem.* **248:**6851.

KORDAS, M. 1969. The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. *J. Physiol.* (*Lond.*). **204:**493.

KORDAS, M. 1970. The effect of procaine on neuromuscular transmission. *J. Physiol.* (*Lond.*). **209:**689.

KORDAS, M. 1972 *a*. An attempt at an analysis of the factors determining the time course of the end-plate current. I. The effects of prostigmine and of the ratio of Mg^{2+} to Ca^{2+}. *J. Physiol.* (*Lond.*). **224:**317.

KORDAS, M. 1972 *b*. An attempt at an analysis of the factors determining the time course of the end-plate potential. II. Temperature. *J. Physiol.* (*Lond.*). **224:**333.

LARMIE, E. T., and G. D. WEBB. 1973 *a*. Desensitization in the electroplax. *J. Gen. Physiol.* **61:** 263.

LARMIE, E. T., and G. D. WEBB. 1973 *b*. Transmembrane potential and resistance changes during desensitization in the electroplax. *Fed. Proc.* **32:**333. (Abstr.).

LESTER, H. A., and J.-P. CHANGEUX. 1973. Conductance of single acetylcholine receptors in electroplaques from *Electrophorus electricus*. *Society for Neuroscience Annual Meeting* 1973, Abstr. 231.

LESTER, H. A., and J.-P. CHANGEUX. 1974. Voltage effects on the activation of acetylcholine receptors as measured by chord conductance. *Fed. Proc.* **33:**1471.

LUFT, H. 1958. The fine structure of electric tissue. *Exp. Cell. Res.* **5** (Suppl.)**:**168.

MAGAZANIK, L. G., and F. VYSKOČIL. 1970. Dependence of acetylcholine desensitization on the membrane potential of frog muscle fibre and on the ionic changes in the medium. *J. Physiol. (Lond.)*. **210**:507.

MAGLEBY, K. L., and C. F. STEVENS. 1972 *a*. The effect of voltage on the time course of end-plate currents. *J. Physiol. (Lond.)*. **223**:151.

MAGLEBY, K. L., and C. F. STEVENS. 1972 *b*. A quantitative description of end-plate currents. *J. Physiol. (Lond.)*. **223**:173.

MANTHEY, A. A. 1966. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. *J. Gen. Physiol.* **49**:963.

MANTHEY, A. A. 1970. Further studies of the effect of calcium on the time course of action of carbamylcholine at the neuromuscular junction. *J. Gen. Physiol.* **56**:407.

MEUNIER, J.-C., and J.-P. CHANGEUX. 1973. Comparison between the affinities for reversible cholinergic ligands of a purified and membrane bound state of the acetylcholine-receptor protein from *Electrophorus*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **32**:143.

NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965. Analysis of spike electrogenesis and depolarizing K inactivation in electroplaques of *Electrophorus electricus*. *J. Gen. Physiol.* **49**:321.

NICKEL, E., and L. T. POTTER. 1973. Ultrastructure of isolated membranes of *Torpedo* electric tissue. *Brain Res.* **57**:508.

OLSEN, R., J.-C. MEUNIER, and J.-P. CHANGEUX. 1972. Progress in purification of the cholinergic receptor protein from *Electrophorus electricus* by affinity chromatography. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **28**:96.

RUBIN, M. M., and J.-P. CHANGEUX. 1966. On the nature of allosteric transitions: implications of non-exclusive ligand binding. *J. Mol. Biol.* **21**:265.

RUIZ-MANRESA, F., and H. GRUNDFEST. 1971. Synaptic electrogenesis in eel electroplaques. *J. Gen. Physiol.* **57**:71.

RUIZ-MANRESA, F., A. C. RUARTE, T. L. SCHWARTZ, and H. GRUNDFEST. 1970. Potassium inactivation and impedance changes during spike electrogenesis in eel electroplaques. *J. Gen. Physiol.* **55**:33.

SCHOFFENIELS, E., and D. NACHMANSOHN. 1957. An isolated single electroplax preparation. I. New data on the effect of acetylcholine and related compounds. *Biochim. Biophys. Acta.* **26**:1.

WEBER, M., and J.-P. CHANGEUX. 1974 *a*. Binding of *Naja nigricollis* [^{3}H]α-toxin to membrane fragments from *Electrophorus* and *Torepedo* electric organs. I. Binding of the tritiated α-neurotoxin in the absence of effector. *Mol. Pharmacol.* **10**:1.

WEBER, M., and J.-P. CHANGEUX. 1974 *b*. Binding of *Naja nigricollis* [^{3}H]α-toxin to membrane fragments from *Electrophorus* and *Torpedo* electric organs. II. Effect of cholinergic agonists and antagonists on the binding of the tritiated α-neurotoxin. *Mol. Pharmacol.* **10**:15.

WEBER, M., and J.-P. CHANGEUX. 1974 *c*. Binding of *Naja nigricollis* [^{3}H]α-toxin to membrane fragments from *Electrophorus* and *Torpedo* electric organs. III. Effects of local anaesthetics on the binding of the tritiated α-neurotoxin. *Mol. Pharmacol.* **10**:35.