The Effects of External Ca^{++} and Mg^{++} on the Voltage Sensitivity of Desensitization in *Electrophorus* Electroplaques

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ABSTRACT Desensitization onset was studied in voltage-clamped *Electrophorus* electroplaques during prolonged exposure to bath-applied carbamylcholine. The time-course of desensitization was described by a first-order rate constant k_{obs}, which increased exponentially with membrane hyperpolarization from −20 to −90 mV. When Ca^{++} was increased from 2 to 10 mM, the voltage sensitivity of k_{obs} decreased; k_{obs} decreased for voltages more negative than −40 mV, and increased slightly at voltages more positive than −40 mV. 10 mM Mg^{++} had a less pronounced effect and the voltage sensitivity of k_{obs} was unchanged. The equilibrium level of desensitization, estimated from the carbamylcholine-dependent conductance which remained after desensitization was apparently complete, also increased with hyperpolarization.

INTRODUCTION

The application of acetylcholine or other depolarizing drugs to the electric eel nerve-electroplaque junction induces a rapid increase in the ionic conductance of the postsynaptic membrane, which then slowly declines despite the continued presence of agonist (Larmie and Webb, 1973; Lester et al., 1975). This decrease in permeability marks the onset of desensitization which has been described for the frog neuromuscular junction (Fatt, 1950; Thesleff, 1955; Katz and Thesleff, 1957), and occurs in the electroplaque at rates comparable to those observed in the frog when agonist is iontophoretically (del Castillo and Webb, 1977) or bath-applied (Lester, et al., 1975). At the frog neuromuscular junction the rate of onset of desensitization is affected by agonist type and concentration, extracellular ion concentrations and membrane voltage (Thesleff, 1955; Katz and Thesleff, 1957; Manthey, 1966, 1970; Rang and Ritter, 1970; Nastuk and Parsons, 1970; Magazanik and Vyskocil, 1970, 1975; Parsons et al., 1974; Adams, 1975).

We have examined the effects of extracellular Ca^{++} and Mg^{++} on the rate of desensitization onset in voltage-clamped *Electrophorus* electroplaques at several membrane voltages. Our objective was to describe aspects of the mechanism for the voltage and cation sensitivity of the desensitization process.

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Some of our preliminary data have been published in an abstract (Pallotta and Webb, 1977).

METHODS

General

Single electroplaques were dissected from the Sachs organ of *Electrophorus electricus* (Schoffeniels, 1957) and mounted in a two-compartment Lucite chamber (Fig. 1). A cell was held between a nylon mesh and mylar sheet, with its innervated membrane uppermost. A 3.5 mm^2 rectangular "window" in the mylar sheet restricted the area of

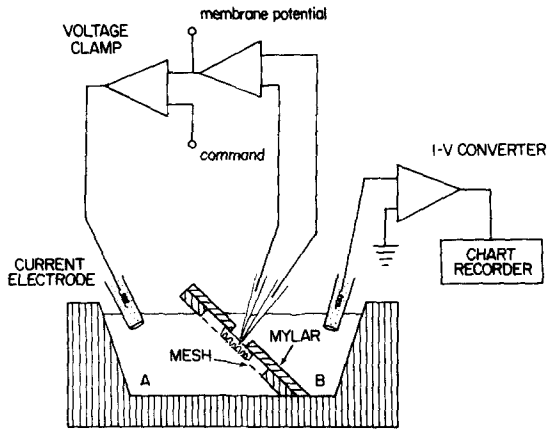

FIGURE 1. Simplified schematic of the voltage clamp and mounted electroplaque cell. The cell was held between a nylon mesh and a 1 × 3.5-mm window in a mylar sheet with the innervated face in contact with the solution in pool B. Agonist was bath-applied into this pool after the innervated membrane of the cell was clamped to the voltage of interest. Microelectrodes monitored the membrane potential, and current from the voltage clamp passed between the two extracellular Ag-AgCl coils (embedded in agar) and through the cell. This current, which varied with the permeability characteristics of the innervated membrane, was converted to a voltage and its time course recorded with a chart recorder.

innervated membrane that was exposed to the solutions in the adjacent chamber, and through which current could pass. Hyperpolarizing stimuli, delivered through extracellular Ag-AgCl pellet electrodes, evoked action potentials indirectly by stimulating transmitter release from the many nerve terminals that remain on the electroplaque after dissection (Podleski, 1962). Direct action potentials were elicited by briefly depolarizing the innervated face sufficiently to trigger an action potential in the electrically excitable membrane (about 98% of the total innervated face membrane, the other 2% comprising synaptic membrane; Bourgeois et al., 1972). The average resting potential of a healthy cell was about −84 mV, and cells that had resting potentials less negative than −75 mV or did not fire both direct and indirect action potentials were not used.

Electroplaques were voltage clamped using a modification of a circuit designed for

Electrophorus electroplaques by Dr. George Katz and Mr. Sidney Steinberg[1] of Columbia University; the circuit employed feedback principles described by Katz and Schwartz, (1974). The potential across the innervated membrane was monitored by two KCl-filled glass microelectrodes (tip resistance 5–15 MΩ) which straddled the membrane. In order to minimize interelectrode separation, the intracellular electrode was retracted as far as possible while still allowing a stable impalement. The extracellular electrode was placed as close to the point of entry of the other electrode as practical, with its tip almost in contact with the innervated membrane. From this initial position near the innervated membrane, a cell could be impaled by advancing the extracellular electrode about 30 μm. Approximately this distance separated the tips of the intra- and extracellular microelectrodes during an experiment and gave rise to a small resistance in series with the innervated membrane. Cells were voltage clamped by passing current between two large extracellular Ag-AgCl coils embedded in short agar bridges, one in each chamber compartment, so that the current passed through the entire cell. The area of innervated membrane through which current could pass was limited by the window. The clamp was capable of changing the potential of the low resistance (3–6 $\Omega \cdot cm^2$), high capacitance (15–50 $\mu F/cm^2$), innervated membrane to 90% of its final value within 150 μs. During an experiment the membrane potential deviated from the command potential by < 1 mV. The desensitization process(es) described in this paper occurred with a time constant(s) of several seconds and allowed the use of a chart recorder (frequency response 3 Hz full scale) to record the time-course of the agonist-induced synaptic current.

The composition of *Electrophorus* physiological saline was (millimolar): NaCl, 188; KCl, 5; $MgCl_2$, 2; $CaCl_2$, 2; glucose, 5; NaH_2PO_4, 0.3; Na_2HPO_4, 1.2; (pH 7.4) (Webb et al., 1973). Solutions of up to 10 mM Ca^{++} or Mg^{++} were obtained by adding either $CaCl_2$ or $MgCl_2$ to the saline hypertonically. No precipitate formed in these solutions at the temperature at which our experiments were performed (20–23°C). Osmotic differences among solutions were not responsible for the observed effects inasmuch as experiments that were performed in normal saline and in saline made isosmotic to the 10 mM $CaCl_2$ (or $MgCl_2$) saline by the addition of sucrose yielded identical results.

"Activation" was achieved by bath application of 0.27 mM carbamylcholine (CCh) (Sigma Chemical Co., St. Louis, Mo.). With this concentration, reproducible results could be obtained over a wide voltage range without cell deterioration during the experiment. Solutions were changed by rapidly flushing 13 ml of the new solution through the 1.5 ml compartment on the innervated side of the cell. Dye-dilution measurements indicated that by this method the solution change was complete (99%) in the 5–8 s required to add the new solution.

Data Analysis

Fig. 2 illustrates a record obtained during a desensitization experiment. The cell was initially clamped to its resting potential (−82 mV). When the cell potential was changed (over several seconds) to −60 mV, a small outward "holding current" appeared; this current defined the zero agonist-induced current. This changed to a large inward synaptic current when CCh was applied and the synaptic channels opened. Several seconds after the start of the CCh exposure, current began to decrease despite the continued presence of the agonist. This decrease reflected the declining ability of chemical agonists to activate the synaptic channels. For purposes of discussion, the peak synaptic current (I_0) provided one index of maximum cell

[1] Personal communication.

activation, and the time required for the current to reach I_0 was called "time-to-peak current." After reaching its peak the current decayed towards the "plateau current," I_∞, with an exponential time-course characterized by rate constant k_{obs}. Another measure of peak activation, considered more reliable than I_0, was the peak current with respect to the "final zero" shown in Fig. 2. As described below, this peak current ($A_2R^*_0$) was not sensitive to the base-line shifts that occurred early in the experiment because of changes in intracellular ion concentrations. All states or conformations of the ACh-receptor which were unresponsive to agonist, and nonconducting would be classed as "desensitized" by this method.

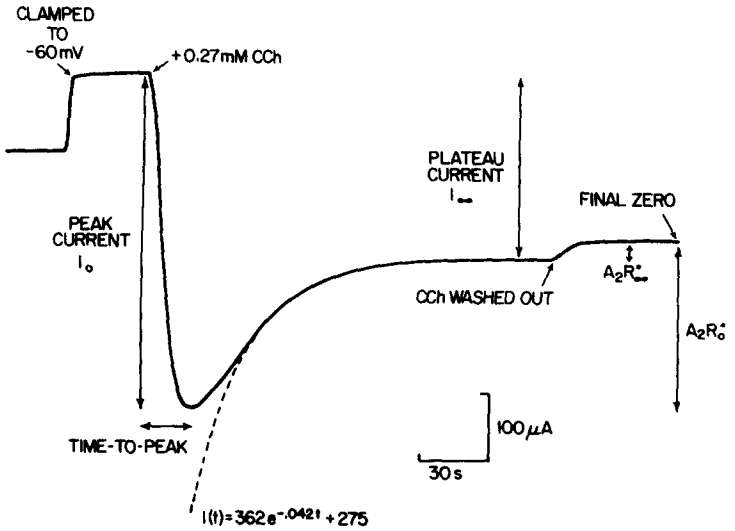

FIGURE 2. Analysis of a sample current record. Time-to-peak current, peak synaptic current I_0, and plateau current I_∞ are indicated on this tracing of a current record from a cell voltage clamped to −60 mV and activated with 0.27 mM carbamylcholine (CCh). After the current leveled off at I_∞ the CCh was washed out with physiological saline. This "washout current" $A_2R^*_\infty$ showed that, even though desensitization was apparently complete, there was still a population of open channels. $A_2R^*_0$ was the peak current relative to the final zero. The time-course of the current after it peaked was described by a single exponential plus a constant (Eq. 2). The broken line is the computer-generated exponential fit to the latter portion of the current record and demonstrates that the current decay, or desensitization onset phase, was exponential over most of its time-course. In this example, $k_{obs} = 0.042\ s^{-1}$ and $I_\infty = 275\ \mu A$.

Factors That Might Produce Systematic Errors

Measurement artifacts might be introduced by at least three sources in the experimental system employed. These were (*a*) changes in intracellular ion concentrations due to the large induced currents, (*b*) the shunt and series conductances arising from current pathways around the cell and the small separation between the tips of the microelectrodes, and (*c*) the gelatinous material covering the innervated face of the electroplaque and which could slow the rate at which agonist reached the receptors. These problems are discussed in more detail below, where we argue that errors from

these sources were minimized by calculating k_{obs} from the latter part of the current record.

Intracellular Ion Concentration

Lester (1978) has shown that depolarizations in the electroplaque that occur during bath-application of agonist measure primarily the change in intracellular K^+ concentration. It is possible then that the time-course and magnitude of desensitization as shown in Fig. 2 were distorted by a simultaneous change in E_K. However, non-voltage-clamped electroplaque cells exposed to 0.27 mM CCh depolarize to a steady level within 30 s,[2] so that the E_K shift was completed well before that phase of the current record from which the rate of desensitization was estimated. In addition, since the reversal potential for postsynaptic currents does not change during bath application of agonist (Lester, 1978), changes in intracellular ions would not be expected to contribute to the apparent rate of desensitization. The E_K shift was, however, responsible for the current which remained after the removal of agonist (Fig. 2). Possible artifacts due to slow changes in E_K were evaluated with a computer simulation in which it was assumed that the onet of desensitization and the E_K shift occur simultaneously. It was found that changes in E_K during the onset of desensitization would introduce only small errors in the apparent rate of desensitization. Thus, changes in intracellular ion concentrations during prolonged application of agonist would not be expected to affect the conclusions reached in this study.

As an experimental check of the conclusion that the current measurements were proportional to the membrane conductance, brief hyperpolarizing voltage pulses were applied to the electroplaque periodically throughout several experiments. The time-course of the changes in membrane conductance, calculated from the magnitudes of the constant-voltage pulses and resulting currents, was identical to the time-course of the current record (Fig. 2).

Shunt and Series Conductances

During an experiment, voltage-clamp current passed not only through the electroplaque, but also through the shunt conductance for current flow around the cell (Schoffeniels, 1957; Lester et al., 1975). Since the shunt conductance was probably very small (Schoffeniels, 1957) and time- and current-independent, the relation between k_{obs} and the actual time-course of the synaptic conductance was not affected.

The series conductance affected the precision with which the voltages across the synaptic conductance could be controlled. Since the separation between the tips of the intracellular and extracellular voltage-sensing microelectrodes was about 30 μm and saline resistivity was 55 Ω·cm, the large currents during activation produced a voltage drop across the saline and cytoplasm which was not expressed across the membrane. We calculated that the series conductance caused the actual voltage drop across the membrane to be from 5 to 8% less than the voltage drop measured by the microelectrodes when the membrane was maximally activated. k_{obs} was, however, calculated from the latter part of the current record when desensitization was well under way. Since the current was then considerably less than maximal, the uncertainty in the actual membrane voltage was less than 5%. Thus, we did not compensate for the series resistance electronically.

The Unstirred Layer

The relatively flat innervated surface of the isolated electroplaque is covered with a 40–75-μm-thick layer of gelatinous material, which normal dissection procedures

[2] Our observation.

cannot remove. Agonist must diffuse through this gel layer before it can bind at the synaptic membrane to induce a conductance change. Diffusion through this layer appears to have been responsible for the delay between the presence of agonist in the bath and peak current, which at −60 mV averaged 25 s with 0.27 mM CCh. Since the agonist concentration went from zero to its final concentration in the chamber in 5–8 s, the remainder of the delay was attributed to the diffusion time through the gel, which probably constituted more of an unstirred layer than an actual barrier to diffusion, as the mobilities of ions are about the same in the gel as in saline (Altamirano, 1961).

The effects of this unstirred layer on the observed kinetics of desensitization were assessed by assuming (*a*) that the time-course of agonist diffusion through the gel was described by equations treating diffusion through a plane sheet of known thickness (Crank, 1956; Cuthbert and Dunant, 1970, (*b*) that the time-to-peak current was entirely determined by the relatively slow diffusion of agonist through the unstirred layer, since the binding of agonist to the receptor and channel opening are very rapid, and (*c*) that the activation-desensitization sequence in electroplaque was described by the following sequential model:

$$A + R \xrightarrow{k_{diff}} AR^* \underset{k_4}{\overset{k_3}{\rightleftharpoons}} AD, \tag{1}$$

where A is agonist, R the ACh receptor, AR^* the open channel conformation of the agonist-receptor complex, and AD the nonconducting and agonist-insensitive desensitized state. Rate constant k_{diff} is a function of the diffusion coefficient of CCh (Dionne, 1976) and the thickness of the unstirred layer. Initially, values for the rate constants were selected such that with an assumed agonist concentration of 0.27 mM, the calculated time-course of AR^* closely resembled current records such as the one shown in Fig. 2. The rate constants were then varied singly, and the effect upon the time-course of AR^* evaluated by analyzing these records in exactly the same manner as actual current records for determination of k_{obs}. From this procedure it was found that peak current, time-to-peak current, and k_{obs} varied substantially with the assumed unstirred layer thickness. Though the magnitudes of these errors were dependent also on the values assumed for k_3 and k_4, part of our experimental variation might have been due to small, unavoidable variations in gel thickness. Another consequence of this analysis was that at low agonist concentrations or relatively thick gel layers, activation would become so slow that the onset of desensitization was obscured by the continuing activation. Thus, the gel layer sets a lower limit to the agonist concentration at which the observed rates of desensitization accurately reflect the underlying kinetics. At the concentration of CCh (0.27 mM) we used, calculations showed that the thickness of the unstirred layer had very little effect on the value of k_{obs} until the thickness exceeded 75 μm. The gel layer was usually <70 μm thick, thus allowing us to determine k_{obs} using the bath application technique.

One way to reduce the effective thickness of the unstirred layer is to use iontophoretic application of the activating agent. del Castillo and Webb (1977) found that when this technique was used with the electroplaque, activation occurred within milliseconds, and desensitization half-times as short as 0.6 s were seen. In our experiments, we observed slower rates of desensitization than del Castillo and Webb, undoubtedly because much higher concentrations of CCh were rapidly achieved with the iontophoretic technique. Since the time-course of the CCh concentration and the exact concentration reached are difficult to determine with the iontophoretic technique, we chose bath application for the present study.

RESULTS

The Time-Course of Desensitization Is Exponential

The effect under investigation is illustrated in Fig. 2. After the application of agonist, the synaptic current first increased and then slowly decreased even though the agonist concentration remained constant. The decay of the inward synaptic current, which represents the desensitization onset phase, was exponential over most of its time-course and described by the following equation:

$$I(t) = He^{-k_{obs}t} + I_{\infty}, \tag{2}$$

where $I(t)$ is the time-course of the clamp current after peak, H is a constant, I_{∞} is the "plateau current," and k_{obs} is the observed rate constant of the desensitization process. The values of these constants were obtained from the current record using a nonlinear least-squares exponential fitting program. The latter two-thirds to one-half of the current record was used to estimate k_{obs} in order to minimize the effects of (*a*) changes in intracellular ion concentrations, (*b*) any voltage deviation due to the series conductance, and (*c*) the slowing of activation by agonist diffusion (see Methods). The onset of desensitization before the completion of activation was responsible for the nonexponential decay of the current for the first 10–20 s after peak current. After that, the time-course of the actual current was always closely described by an exponential as shown in Fig. 2, where the fitted and observed lines superimpose.

The Rate of Desensitization Onset Is Voltage Sensitive

The rate of desensitization onset in electroplaque was increased by hyperpolarization. The data in Fig. 3 demonstrate the sensitivity of the rate constant k_{obs} to membrane voltage, V. Under all experimental conditions employed for this study, the following equation could be used to describe the relation between k_{obs} and voltage:

$$k_{obs}(V) = k(0)e^{-SV}, \tag{3}$$

where $k(0)$ is the zero voltage rate of desensitization, and S is the voltage sensitivity coefficient. These constants were calculated from a regression analysis of the data and are listed in Table I. Experiments could not be performed at positive potentials because at these voltages the electroplaque was unresponsive to agonist, as has been observed by others (Ruiz-Manresa and Grundfest, 1971; Lester et al., 1975).

Mg^{++} Decreases k_{obs} at All Voltages Studied

The rate of desensitization was decreased by increasing extracellular Mg^{++} from 2 mM (control) to 10 mM, and this effect was observed at all voltages studied (Fig. 3). A fit of Eq. 3 to the data yielded a $k(0)$ which was decreased from the control value, but the voltage sensitivity coefficient S was virtually unchanged from control (Table I).

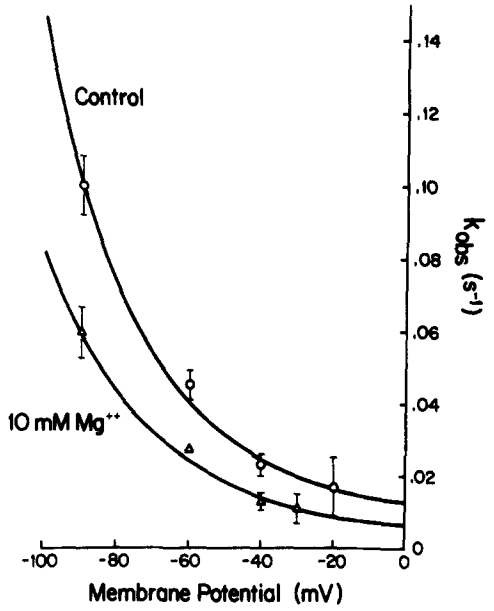

FIGURE 3. The rate constant for desensitization onset k_{obs} as a function of membrane potential and extracellular Mg^{++}. Control cells (○) maintained in physiological saline (which contains 2 mM Mg^{++}) were activated with bath-applied 0.27 mM CCh just after the cell was clamped to the voltage of interest. The 10 mM Mg^{++} data (△) were obtained from cells equilibrated for 45 min in high Mg^{++} saline prior to activation. Each point in the figure represents the mean ±SE of four to seven experiments. Data shown were pooled from eight different eels. Solid lines: comparison of model (Eq. 4) predictions with experiment. Lines show fits to Eq. 5 with the following parameter values: $\delta_0 = 0.007$ s^{-1}, $D = 0.035$ mV^{-1}, $\psi = 0.002$ s^{-1}, $\rho + \sigma = 0.005$ s^{-1}, $[A] = 0.27$ mM, $\omega[C^{++}]/\tau = 0.10$ (2 mM Mg^{++}) or 1.80 (10 mM Mg^{++}). From Sheridan and Lester (1977), $k_c(0) = 1.8$ ms^{-1}, $k_0 = 10^7$ M^{-1} s^{-1}, $K_d = 180$ μM, $B = 0.012$ mV^{-1}. The free parameters used to fit the data were found with the aid of a PDP-11/34 computer (Digital Equipment Corp., Marlboro, Mass.) which displayed plots of k_{obs} vs. voltage derived from Eq. 5. These variables were input continuously as voltages sampled from potentiometers by analog-to-digital converters and adjusted until the best fit by eye was obtained.

Ca^{++} Decreases k_{obs}

Ca^{++} decreased the rate of desensitization in electroplaque over most of the voltage range studied. Fig. 4 shows k_{obs} as a function of voltage and extracellular Ca^{++} concentration. The reduction by Ca^{++} of the onset rate was greatest at −90 mV, and decreased progressively with voltage until it was negligible at −40 mV. The decrease in k_{obs} by Ca^{++} depended on the extracellular Ca^{++} concentration and was more marked with 10 mM Ca^{++} than with 10 mM Mg^{++} at −90 mV.

The Ca^{++} and Mg^{++} Effects Do Not Require an Equilibration Period to Develop

The length of time required for the Ca^{++} and Mg^{++} effects to develop might suggest possible mechanisms by which these ions affected desensitization. The data presented so far were obtained from cells which were equilibrated for 45 min in the Ca^{++} or Mg^{++} solution of interest prior to voltage clamping and activation, and another series of experiments was performed to determine the effects of only brief exposures to altered Ca^{++} or Mg^{++} concentrations. Fig. 5 shows the results of experiments in which the extracellular Ca^{++} concentration was changed at the time of activation. Cells which were clamped to −90 mV in control solution but activated in 10 mM Ca^{++} with 0.27 mM CCh (B) desensitized as slowly as cells which were equilibrated in 10 mM Ca^{++} for the full 45 min prior to activation (D). Cells activated with a 2 mM Ca^{++}, 0.27 mM CCh solution after a 45 min equilibration in 10 mM Ca^{++} (C) desensitized as quickly as controls (A) which had been exposed only to 2 mM Ca^{++} throughout. These results implied that the kinetics of the action of calcium

TABLE I

COEFFICIENTS DERIVED FROM EQ. 3 DESCRIBING MEAN VOLTAGE SENSITIVITIES AND ZERO VOLTAGE RATES OF DESENSITIZATION ONSET

Treatment	Voltage sensitivity, S	Zero voltage rate, $k(0)$	n
	mean ± SEM, mV^{-1}	*mean ± SEM*, s^{-1}	
Control	0.030 ± 0.005	0.007 ± 0.001	27
10 mM Mg^{++}	0.031 ± 0.004	0.004 ± 0.001	16
5 mM Ca^{++}	0.016 ± 0.003	0.013 ± 0.001	23
10 mM Ca^{++}	0.012 ± 0.002	0.015 ± 0.001	16

were rapid on the time scale of the desensitization kinetics even at −90 mV, a voltage at which desensitization was very fast. Similar results were obtained from three cells activated in the presence of 10 mM Mg^{++} after an equilibration in physiological saline.

The Equilibrium Level Of Desensitization Increases with Hyperpolarization

The equilibrium level of desensitization was estimated from the CCh-dependent conductance which remained after desensitization was apparently complete. As illustrated in Fig. 2, the removal of agonist resulted in a small decrease in inward current. This "washout current" $A_2R^*_\infty$ indicated that an equilibrium was established between the desensitized, the open channel, and the unactivated receptor populations. With the removal of agonist, the current returned to a steady-state value, which, because of the change in intracellular K^+ concentration, was different from the holding current at which the experiment was begun.

Table II lists mean washout currents $A_2R^*_\infty$, peak currents $A_2R^*_0$, and the ratio $A_2R^*_\infty/A_2R^*_0$ for control, 10 mM Ca^{++}, and 10 mM Mg^{++} treatments at several voltages; $A_2R^*_0$, which is the peak current measured relative to the "final zero" (Fig. 2), was used for peak current rather than I_0 because I_0 is larger than the "true" peak activation current as it includes a shift from the

"holding" current due to a shift in the K^+ equilibrium potential (Lester, 1978). A washout current that was small relative to peak current implied that at equilibrium most of the receptor population was desensitized. It is seen from the table that the equilibrium level of desensitization increased with hyperpolarization for all three treatment groups since the ratio of the washout to peak currents decreased with increasing voltage (Table II).

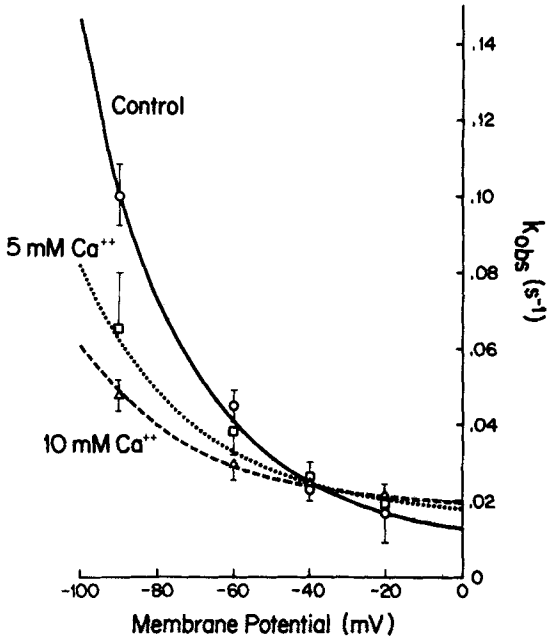

FIGURE 4. k_{obs} as a function of membrane potential and extracellular Ca^{++}. Electroplaque cells were voltage clamped and activated with 0.27 mM CCh after a 45-min equilibration period in physiological saline which contains 2 mM Ca^{++} (○), or in the same saline with added $CaCl_2$ (5 mM, □; 10 mM, △). Each point represents the mean ± SE of four to seven experiments. Data shown were pooled from eight different eels. Lines: comparison of model (Eq. 4) predictions with experiment. Parameter values were the same as those in Fig. 3 except that $\psi = 0.02\ s^{-1}$, $\rho + \sigma = 0.006\ s^{-1}$, and $\omega[C^{++}]/\tau = 0.1$ (2 mM Ca^{++}) or 2.0 (5 mM) or 4.8 (10 mM).

Ca^{++} and Mg^{++} Do Not Significantly Affect Peak Current

The rate of desensitization is a function of agonist concentration at the frog neuromuscular junction (Katz and Thesleff, 1957; Adams, 1975), and at the *Electrophorus* electroplaque (Lester et al., 1975). The Ca^{++} and Mg^{++} concentrations used here did not affect activation (which is a function of agonist concentration and type, desensitization rate, and thickness of the unstirred layer), as no significant differences between the control, 10 mM Ca^{++}, and 10 mM Mg^{++} groups were found when peak currents were measured relative to either the initial or the final "zero" ($A_2R\delta^*$, Table II). Thus, a decrease in the

"effective" agonist concentration by the high cation concentrations could not account for the results.

DISCUSSION

Desensitization in the electroplaque, characterized here by the rate of decay of agonist-induced current in the continued presence of agonist, changes with membrane voltage, extracellular Ca^{++}, and Mg^{++}. When the membrane is hyperpolarized, desensitization develops more quickly (Figs. 3 and 4), and the steady-state level of desensitization is increased (Table II). Divalent cations

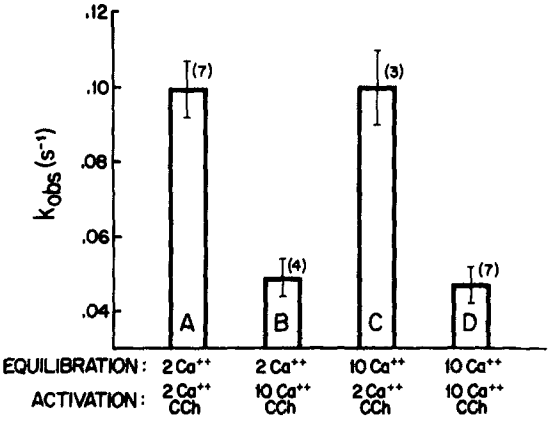

FIGURE 5. The effect of calcium on k_{obs} does not require an equilibration period to develop. The rate of desensitization onset was measured in cells which were activated with 0.27 mM CCh in the presence of either 2 mM or 10 mM Ca^{++}, after an equilibration period (45 min) with either the same (A, D) or a different (B, C) Ca^{++} concentration. Note that the rate of desensitization is a function of the concentration of Ca^{++} present at the time of activation and that even long equilibration periods had no residual effect. All experiments shown in this figure were performed at −90 mV to maximize the Ca^{++} effect. The error bars indicate the SE of the mean of (n) experiments.

alter both the voltage-sensitive and -insensitive components of the desensitization rate (Table I and Figs. 3 and 4). Possible mechanisms that might account for the properties of desensitization are presented below, and a kinetic model is presented which describes the data.

Mechanisms Suggested by the Voltage Sensitivity of the Rate of Desensitization

CONFORMATIONAL CHANGE The exponential relation observed between voltage and the rate of desensitization is consistent with the idea that desensitization involves a conformational change of the receptor. Magleby and Stevens (1972) were able to account for the exponential relation between the endplate current decay rate and voltage by assuming that the open and closed conformational states of the ACh-receptor have different free energies

and the rate of transition from one state to the other depends on membrane voltage. A similar mechanism could account for the voltage sensitivity of the onset rate of desensitization such that the desensitizing transition is voltage sensitive. Additional evidence for a conformational change mechanism is that the affinity of membrane-bound ACh-receptors for cholinergic agonists increases after prior exposure to agonist (Weber et al., 1975; Colquhoun and Rang, 1976; Weiland et al., 1976). These authors suggested that agonists promote a conversion of the receptor to a state with an enhanced agonist affinity, as was postulated by Katz and Thesleff (1957).

CURRENT SENSITIVITY Another possibility is that the observed voltage sensitivity of the rate of desensitization results from an underlying current sensitive process. Since the peak-current vs. voltage relations were approximately linear over the voltage range employed here (Table II), the effects of

TABLE II
WASHOUT AND PEAK CURRENTS*

Treatment	Membrane voltage	Washout current, $A_2R^*_\infty$	Peak current, $A_2R^*_0$	Washout/peak	n
	mV				
Control	−90	0.63 ± 0.17	8.77 ± 2.86	0.07 ± 0.01	3
	−60	0.94 ± 0.08	8.23 ± 1.08	0.11 ± 0.01	2
	−40	0.83 ± 0.08	4.20 ± 0.43	0.21 ± 0.03	5
	−20	0.71 ± 0.08	2.91 ± 0.71	0.27 ± 0.05	3
10 mM Ca^{++}	−90	1.20 ± 0.28	10.94 ± 2.43	0.11 ± 0.02	3
	−60	1.08 ± 0.06	5.08 ± 0.77	0.23 ± 0.05	3
	−40	0.74 ± 0.08	4.31 ± 0.40	0.18 ± 0.02	4
	−20	0.66 ± 0.08	3.34 ± 0.51	0.22 ± 0.06	3
10 mM Mg^{++}	−90	0.66 ± 0.31	7.57 ± 2.54	0.08 ± 0.02	3
	−60	0.77 ± 0.11	6.28 ± 0.51	0.12 ± 0.02	3
	−40	0.66 ± 0.08	3.94 ± 0.23	0.16 ± 0.01	2
	−30	0.63 ± 0.11	1.86 ± 0.43	0.34 ± 0.03	3

* Currents are expressed as mA/cm^2 of window area and were measured with respect to the final zero shown in Fig. 2. Values are mean ± SEM of n experiments.

current could not be separated from those of voltage. Thus, the existence of a current-sensitive mechanism cannot be ruled out.

CATION BLOCKADE OF THE OPENED POSTSYNAPTIC CHANNEL Voltage-sensitive cation blockade of the postsynaptic channel seems unlikely to account for much of the data. From this hypothesis one would expect the rate of desensitization to increase with the cation concentration, contrary to the results presented here in which both Ca^{++} and Mg^{++} slowed the rate. In addition, this hypothesis could not easily account for the effects of Ca^{++} on the voltage sensitivity of the rate.

AGONIST BLOCKADE OF THE OPENED POSTSYNAPTIC CHANNEL The voltage sensitivity of the rate of desensitization could be accounted for by allowing the binding of agonist molecules within the opened synaptic channel to block

the flow of ions through the channel (Adams, 1975). In simplest form, however, this model would require that the blocked receptor molecules assume different properties from nonblocked receptors in order to account for (*a*) the time-course of changes in the intrinsic fluorescence of membrane-bound ACh receptors (Bonner et al., 1976) and (*b*) the fact that the recovery from desensitization is much less sensitive to voltage than is the onset of desensitization at the neuromuscular junction (Scubon-Mulieri and Parsons, 1978).

Effects of Cations on the Voltage Sensitivity of the Rate of Desensitization

INTERACTIONS WITH THE RECEPTOR DIPOLE Cations could decrease the voltage sensitivity of the rate of desensitization by binding to the presumed receptor dipole and neutralizing part of the charge responsible for the dipole moment. It should be noted, however, that the calcium and voltage effects on the rate of desensitization might reflect effects upon any dipolar receptor groups, including those groups which do not constitute the ion channel but which also undergo a change of conformation during desensitization.

SURFACE CHARGE EFFECTS. The effects of calcium on the voltage sensitivity of the rate of desensitization are probably not due to alterations in the local electric field of the receptor. Modification of the field would shift the rate-voltage relation (Figs. 3 and 4) along the voltage axis with no effect upon the voltage sensitivity. Since Ca^{++} did change the voltage sensitivity, mechanisms in which Ca^{++} affects desensitization by binding to the membrane (Frankenhaeuser and Hodgkin, 1957) or by screening a possible membrane surface charge (McLaughlin et al., 1971) are unlikely. These mechanisms cannot be ruled out in the case of Mg^{++}, however, since this ion did not alter the voltage sensitivity.

Modeling the Data

Based upon the considerations outlined in the previous section, the most reasonable mechanism to account for the data would involve a voltage-sensitive conformational change of the receptor from the open channel state to the desensitized state. The cation effects would result from an interaction between the ions and any dipolar groups on the receptor that take part in coincident conformational changes. These mechanisms are consistent with the kinetic model below, which could satisfactorily account for the effects of Ca^{++}, Mg^{++}, and membrane voltage on the rate of desensitization. In addition, the model could quantitatively predict the magnitude of the steady-state level of desensitization.

In this model (Eq. 4), which is an extension of a model proposed for activation of electroplaque acetylcholine receptors (Sheridan and Lester, 1977), the formation of desensitized receptors (A_2D or $C - A_2D$) occurs after the channels have opened (state A_2R^*). This assumption follows from the observations that antagonists such as curare and α-toxin, which bind to the receptor but do not open the channel, do not cause any desensitization (Jenkinson, 1960; Weber et al., 1975; Weiland et al., 1977).

Two populations of desensitizing receptors are proposed. In the absence of cations, the open-channel configuration becomes nonconducting (desensitized)

with a voltage-sensitive rate $\delta(V)$, defined by $\delta(V) = \delta_0 e^{-DV}$, where δ_0 is the zero-voltage rate of desensitization, D the voltage sensitivity coefficient, and V membrane voltage. When cations (C^{++}) are present, a fraction of the open channels desensitize with a slower, voltage-insensitive rate ψ. k_c and k_o are the single-channel closing and (bimolecular) opening rate constants, and K_d the dissociation constant for the binding of the first agonist molecule A to the receptor R.

$$\begin{array}{l} 2A + R \overset{K_d}{\longleftrightarrow} A + AR \underset{k_c}{\overset{k_o}{\longleftrightarrow}} A_2R^* \overset{\delta}{\underset{\rho}{\longleftrightarrow}} A_2D \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \omega[C^{++}] \Big\downarrow \tau \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad C\text{-}A_2R \overset{\psi}{\underset{\sigma}{\longrightarrow}} C\text{-}A_2D \end{array} \tag{4}$$

The predictions of this model, shown as the continuous lines in Figs. 3 and 4, were calculated from the following equation, which describes the rate constant for desensitization k_{obs} as a function of the membrane voltage. It was derived from scheme 4 by assuming that channel opening and closing rates (millisecond time scale) are much faster than the desensitization rate (tens of seconds):

$$k_{obs}(V) = \frac{\delta(V) + \psi\dfrac{\omega[C^{++}]}{\tau}}{1 + \dfrac{\omega[C^{++}]}{\tau} + \dfrac{k_c(V)}{k_o}\left(\dfrac{1}{[A]} + \dfrac{K_d}{[A]^2}\right)} + \rho + \sigma. \tag{5}$$

Values of K_d, k_o, and k_c were from the literature (Sheridan and Lester, 1977), with $k_c(V) = k_c(0)e^{BV}$, where $k_c(0)$ is the zero-voltage rate of channel closure, B is the voltage sensitivity coefficient, and V the membrane voltage. The fit to the data obtained from this model did not require either the binding of cations or the rate of desensitization of the cation-bound state to be voltage sensitive (see legends, Figs. 3 and 4), and could describe the different effects of Ca^{++} and Mg^{++} on the desensitization voltage sensitivity.

An interesting property of this model, similar to that of the cyclic model of Katz and Thesleff (1957), is that factors which affect the probability of channel opening or channel lifetime will influence the rate of desensitization. This follows from the observation that activation occurs quickly relative to the onset of desensitization, so that the affinity of the receptor for agonist (K_d), and channel opening and closing rates (k_o and $k_c(V)$) appear in Eq. 5. It is possible then, that the variation of desensitization rate with different agonists (Adams, 1975) might arise, in part, from the different single channel properties associated with each agonist (Katz and Miledi, 1972; Colquhoun et al., 1975; Sheridan and Lester, 1977).

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