ROLE OF VOLTAGE-SENSITIVE RECEPTORS IN NICOTINIC TRANSMISSION

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ABSTRACT This paper compares the conductance induced by bath-applied acetylcholine (ACh) and by the same transmitter released from nerve terminals at *Electrophorus* electroplaques. For the former case, dose-response relations are characterized by the maximal agonist-induced conductance, $r\gamma$ (130 mmho/cm^2), and by the concentration which induces half this conductance; this concentration is termed K_{app} and equals 50 μM at −85 mV. For the latter case, neurally evoked postsynaptic currents (PSCs) are characterized by the peak conductance during strongly facilitated release, g_{PSC}, and by the rate constant for decay, α. Since g_{PSC} roughly equals $r\gamma$, it is concluded that the PSC activates nearly all available receptor channels. These and other data agree with recent estimates that during the growth phase of the quantal response, (*a*) the ACh concentration is at least several hundred micromolar; and (*b*) most nearby channels are activated. However both α and K_{app} increase during depolarization, at a rate of about *e*-fold per 86 mV. These observations on voltage sensitivity suggest that a suprathreshold synaptic event is rapidly terminated because the action potential abruptly releases ACh molecules from receptors.

INTRODUCTION

This paper examines two questions about the activation of nicotinic acetylcholine (ACh) receptors by quanta of transmitter released from the presynaptic nerve. The first problem concerns the growth phase of the miniature postsynaptic current (MPSC). By studying the interaction between MPSCs and the effect of iontophoretically applied ACh, Hartzell et al. (1975) concluded that more than half of the nearby receptors are activated by a quantum of ACh. From the correlation between amplitude and rise time of miniature endplate potentials, Negrete et al. (1972) also reasoned that most nearby receptors are activated. A similar conclusion is reached in this study from comparisons of the response to neurally released and bath-applied ACh. These findings imply that the local ACh concentration exceeds the value required for half-maximal receptor activation under equilibrium conditions; i.e., the concentration at the midpoint of the dose-conductance relation. We call this value K_{app}.

With improved techniques of micro-iontophoretic mapping (Kuffler and Yoshikami,

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1975*a*) and new measures of the ACh in a single quantum (Fletcher and Forrester, 1975; Kuffler and Yoshikami, 1975*b*), it was estimated that the ACh concentration in the synaptic cleft is about 300 μM at the peak of the MPSC. A lower limit of 300 μM was also given by Fertuck and Salpeter (1976) on the basis of the cleft's geometry. As for K_{app}, *Electrophorus* electroplaques showed half-maximal conductances when voltage-clamped to −90 mV and exposed to about 50 μM ACh (Sheridan and Lester, 1975, 1977). Dreyer and Peper (1975) obtained similar values at frog endplates. Thus the estimated values of cleft concentration seem capable of activating a substantial proportion of receptors during nicotinic transmission. In this paper we present dose-response curves that allow further, albeit still qualitative, discussion of these ideas.

The second point concerns alterations in this picture during depolarizations. At certain nerve-muscle and nerve-electroplaque synapses, the postsynaptic membrane potential influences the ACh receptors. When the cell is depolarized, the ACh-receptor channels have a briefer lifetime. Thus depolarization results in briefer neurally evoked postsynaptic currents (PSCs) and in smaller conductances during steadily applied ACh. This voltage sensitivity has been exploited in experiments that enhance our knowledge of gating processes at the channel (Gage and Armstrong, 1968; Ruiz-Manresa and Grundfest, 1971; Magleby and Stevens, 1972*a*,*b*; Kordas, 1972*a*,*b*; Gage and McBurney, 1975; Dionne and Stevens, 1975; Lester et al., 1975; Neher and Sakmann, 1975; Sheridan and Lester, 1975, 1977). However, little information is available on the physiological role of voltage sensitivity during the PSC.

In this paper we evaluate voltage sensitivity in the context of the fact that the pulse of free ACh in the synaptic cleft lasts for only a few hundred microseconds—much briefer than the PSC itself (Magleby and Stevens, 1972*a*,*b*; Anderson and Stevens, 1973). Thus during the falling phase of the PSC, most transmitter in the cleft is bound to ACh receptors. Voltage sensitivity, we suggest, plays a role during the large depolarization that occurs when a regenerative impulse has been evoked by the PSC. We hypothesize that the action potential is able to drive the bound ACh molecules off the receptors, allowing (*a*) channels to close rapidly and (*b*) transmitter to be removed from the cleft by diffusion and by enzymic hydrolysis. This concept has already been briefly mentioned (Sheridan, 1976; Lester, 1977).

METHODS

Single electroplaques from the organ of Sachs of *Electrophorus electricus* were employed. The observations on neurally evoked PSCs and on bath-applied ACh were made at 15°C during experiments described in the paper by Sheridan and Lester (1977), which should be consulted for details.

For extracellular recording of MPSCs, we employed the methods of del Castillo et al. (1972). A single row of cells (one cell deep by three or four cells across) was dissected free. The connective tissue was mostly removed from one of the cells, and the preparation was pinned (innervated side up) to a transparent layer of silicone rubber in a glass dish. To facilitate penetration through the remaining connective tissue, a beveled 3 MΩ KCl electrode (resistance 2–5 Ω) was used for recording. With the circuit of Lettvin et al. (1958), we checked that the electrode rise time was sufficiently short to resolve the growth phase of MPSCs. Often the

advancing electrode penetrated into the cytoplasm without detecting extracellular signals; however, in some positions MPSCs were recorded for several minutes. The MPSCs were digitized and stored for later analysis. The MPSCs were recorded at 23°C.

RESULTS

Neurally Evoked Postsynaptic Conductance

The neurally evoked PSCs of an electroplaque are shown in Fig. 1 *a* of Sheridan and Lester (1977). For the experiments described here, the strength and duration of the stimulus pulse were adjusted to give the largest PSC. Moreover, the voltage-clamp episodes were repeated at 3/s in order to facilitate transmitter release and to provide the largest possible PSCs (see Sheridan and Lester, 1977).

Ba^{++} (3 mM) was present for all the PSC and voltage-jump measurements reported in this paper. During exposure to Ba^{++}, the PSC amplitude increased slowly, sometimes by a factor of two, over a time-course of an hour or so. Data were taken when the PSC amplitude again stabilized. We have not carefully studied the reason for the amplitude changes; they probably arise from alterations of the presynaptic action potential, since repetitive firing in the electromotor nerve terminals also increases in Ba^{++} (Ruiz-Manresa and Grundfest, 1971). Barium did not affect PSC kinetics or their voltage dependence.

A plot of peak synaptic current vs. voltage is linear at potentials more negative than about −30 mV (see Fig. 1 of Sheridan and Lester, 1977). Thus the slope of this line is the maximal synaptic conductance, which we term g_{PSC}; values for three cells are listed in Table I.

Dose-Conductance Relations with Bath-Applied Agonist

After the PSC measurements, the cells were treated with methanesulfonyl fluoride to inhibit acetylcholinesterase and were washed several times (Sheridan and Lester, 1977). Acetylcholine, at various concentrations, was applied in the solution bathing the innervated face. During each application, voltage-jump relaxations were measured at

TABLE I

NEURALLY EVOKED POSTSYNAPTIC CONDUCTANCE COMPARED WITH MAXIMUM AVAILABLE CONDUCTANCE

Cell	g_{PSC}	$r\gamma$	Ratio: $g_{PSC}/r\gamma$
	mmho/cm^2		
33-13	210	180	1.2
33-43	90	80	1.1
33-52	140	120	1.1

several voltages. After a voltage step, the currents relax to a new equilibrium value. Unlike the PSC, the steady-state conductance shows a nonlinear current-voltage relation: the agonist-induced conductance increases with increasingly negative potential (Lester et al., 1975; Sheridan and Lester, 1975, 1977).

In this study we sought to characterize the dose-response relation for ACh. One important parameter is the conductance, $r\gamma$, which would result from simultaneous activation of all the receptor channels (r channels per unit area, each with a conductance γ); a second value of interest is K_{app}, the ACh concentration that activates half the maximal conductance under steady-state conditions. Very large [ACh] would presumably give complete activation if applied rapidly enough to avoid desensitization. But with bath application of concentrations higher than about 20–200 μM (depending on the cell), desensitization occurs while ACh is still equilibrating near the receptors. A large fraction of the receptor pool could be activated by a brief step to more negative voltages, without appreciable desensitization; but the membrane frequently suffers dielectric breakdown when this step goes beyond −175 mV. Therefore, to compute $r\gamma$, we sought to calculate the conductance which would have been measured in the absence of desensitization.

Both K_{app} and $r\gamma$ can be estimated from a study of voltage-jump relaxations and of equilibrium conductances. From such an analysis, Sheridan and Lester (1977) concluded that two molecules of agonist must bind to the receptor to open the channel; the second binding is voltage-sensitive and may also be rate-limiting. For simplicity in the present analysis we have used a slightly different version of this sequential model: the first, rapid binding is assumed to be voltage-sensitive as well and is assumed to have the same dissociation constant as the second binding. In this version the dose-conductance relation (Eq. 14 of Sheridan and Lester, 1977) becomes

$$g = r\gamma\{[\mathrm{ACh}]/([\mathrm{ACh}] + 0.414\,K_{app})\}^2 \qquad (1)$$

The two versions of the sequential model yield quite similar (±20%) values for $r\gamma$ and for K_{app}. The version used here gives a slightly better description of the currents at the expense of a slightly poorer description of the relaxation time constants. The advantage of the present version for these studies is that the dose-conductance relation (Eq. 1), when plotted on double-logarithmic coordinates, has a constant shape despite changes in K_{app} and $r\gamma$. Thus trends in these quantities are easily revealed. Fig. 1 *a* presents the data for one cell along with best fits to Eq. 1; for each curve the cross gives K_{app} and $r\gamma/2$. K_{app} and $r\gamma$ are also revealed by modified double-reciprocal plots (Fig. 1 *b*). Clearly K_{app} increases with depolarization.

Sheridan and Lester (1977) have shown how K_{app} is also estimated from the kinetics of voltage-jump relaxations. In brief, channel opening rates must equal closing rates at K_{app}, since at this concentration half the receptor channels are open in the steady state (Stevens, 1972; Colquhoun and Hawkes, 1977; Sheridan and Lester, 1977). Voltage-jump relaxations yield a time constant whose reciprocal is assumed to equal the sum of a concentration-dependent opening rate and a concentration-independent

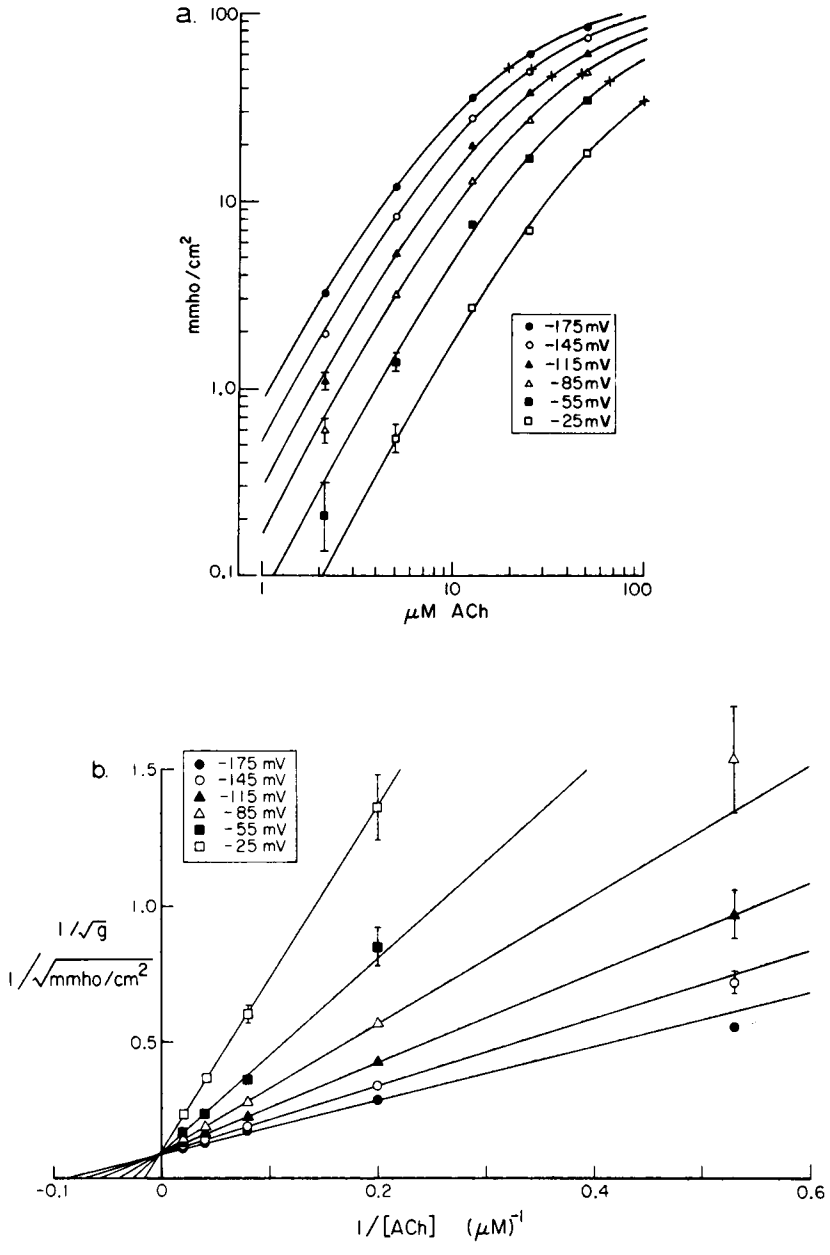

FIGURE 1 *a*. Equipotential dose-response curves for an electroplaque. Agonist-induced conductance (g), expressed as mmho/cm^2 of window area, was calculated by assuming that the agonist-induced currents reverse at +10 mV. Experimental uncertainty is shown where it exceeds size of the symbols; but points at −25 mV (□) have an additional ±20% uncertainty owing to uncertainty of the reversal potential. Lines are drawn according to Eq. 1; for each curve the cross gives K_{app} and $r\gamma/2$. *b*. Same data with $1/\sqrt{g}$ plotted against $1/[ACh]$. Straight lines are drawn according to Eq. 1. Vertical and horizontal intercepts correspond respectively to $1/\sqrt{r\gamma}$ and $-2.42/[K_{app}]$.

closing rate. On this basis the kinetic data show that opening and closing rates equal each other at 15–25 μM ACh and -175 mV (Sheridan and Lester, 1977). Furthermore, the point of equality shifts toward higher [ACh] with depolarization, like the crosses in Fig. 1*a*. This trend is shown for carbachol, another agonist, in Fig. 6 of Sheridan and Lester (1977). Thus the kinetic data provide further support to the values of K_{app} given in Fig. 1.

As for $r\gamma$, we note that at high [ACh] and at high negative potentials, agonist-induced conductances approach the theoretical maximum. Thus at least under these conditions one need not make large extrapolations to estimate $r\gamma$. In contrast to the clearly discernible trend in K_{app}, $r\gamma$ varies less than 20% at voltages more negative than -25 mV.

An additional fact reinforces our conviction that $r\gamma$ is accurately estimated by our measurements without appreciable distortion by desensitization. In this laboratory we have recently improved the method of photochemical "agonist concentration jumps" (Lester and Chang, 1977) to the point where the concentration of 3,3′-*bis*-[α-(trimethylammonium)methyl]azobenzene (Bis-Q), a photoisomerizable agonist, can be increased from 0.3 times K_{app} to 3 K_{app} in 0.5 ms, presumably rapid enough to avoid desensitization. Within a few milliseconds after such a jump, the agonist-induced conductance increases from less than 20% of $r\gamma$ to near maximal values. The conductances thus measured are near the present values for $r\gamma$ (M. M. Nass and H. A. Lester, unpublished). Of course we do not know whether γ for Bis-Q and for ACh are equal, but differences greater than twofold are unlikely (Colquhoun et al., 1975; Dreyer et al., 1976).

There is probably a decrease in $r\gamma$ at more positive voltages; we have discussed this trend, the technical difficulties in quantifying it, and the fact that it may arise from ion shifts secondary to the prolonged agonist-induced conductance (Sheridan and Lester, 1977). Another complication with some cells is that repeated applications of the same agonist concentration give progressively decreasing currents (Koblin and Lester, unpublished). The latter effect was small or absent for the cells in Table I.

Dose-Conductance Data Compared with PSC Data

g_{PSC} IS CLOSE TO $r\gamma$ In Table I, $r\gamma$ is compared with the peak synaptic conductance produced by presynaptic stimulation, g_{PSC}. The two figures are similar for each cell.

K_{APP} HAS THE SAME VOLTAGE SENSITIVITY AS α Postsynaptic currents have an exponential decay phase characterized by the rate constant α. At both nerve-muscle and nerve-electroplaque synapses, α depends exponentially on voltage (Magleby and Stevens, 1972*a*; Gage and McBurney, 1975; Sheridan and Lester, 1975, 1977). Fig. 2 shows that K_{app} also depends exponentially on voltage and to a similar extent as α. Table II gathers the data for three cells and shows that there are no detectable differences between the voltage sensitivities of K_{app} and of α.

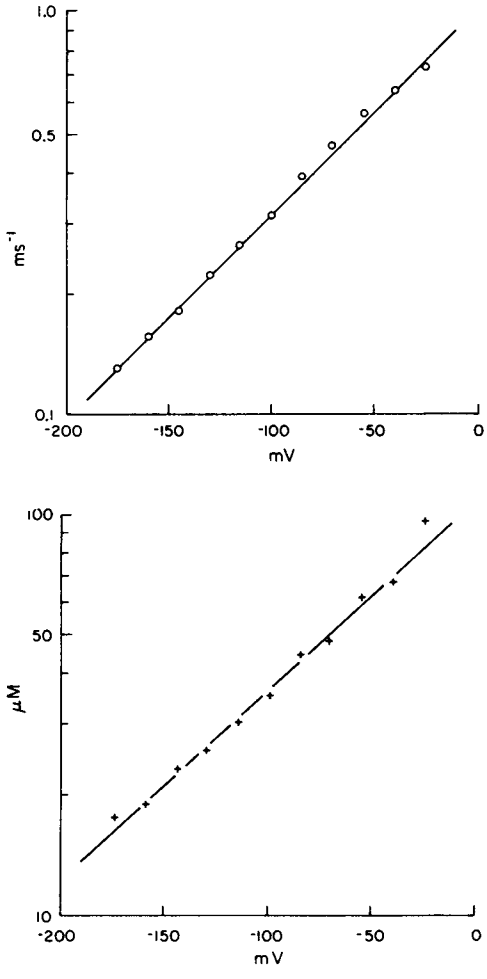

FIGURE 2 Voltage dependence of two types of ACh response. Upper graph, PSC decay rate, from traces like Fig. 1 *a* and *b* of Sheridan and Lester (1977). Line corresponds to an *e*-fold change for 84 mV. Lower graph shows K_{app}, from equipotential dose-conductance data like those of Fig. 2. Line corresponds to an *e*-fold change for 91 mV.

Miniature Postsynaptic Currents

Externally recorded MPSCs, shown in Fig. 3, have a fast rising phase followed by a slower decay phase. The MPSC growth phase can be characterized by the time required to increase from 20% to 80% of the maximum amplitude (Gage and McBurney, 1975). This "growth time" was 160 ± 55 μs (34 measurements ± SD; range of 50–320 μs) at 23°C. This value agrees well with the growth time of miniature endplate currents at the toad neuromuscular junction (Gage and McBurney, 1975). The decay phase of the MPSCs was exponential and had a rate constant of about 1 ms^{-1}, in

TABLE II

VOLTAGE SENSITIVITY OF THE DOSE-CONDUCTANCE RELATION COMPARED WITH THAT OF THE PSC

Cell	K_{app} at −85 mV	mV for e-Fold Change	
		K_{app}	α
	μM		
33–13	50	86	74
33–43	52	87	94
33–52	48	91	84
Mean ± SD	50 ± 2	88 ± 3	84 ± 10

Based on data like those of Fig. 2.

agreement with the PSC measurements of Sheridan and Lester (1975) at 23°C and at −90 mV.

DISCUSSION

We shall evaluate the present results in the light of related work on nerve-muscle synapses of vertebrate twitch muscle. The microphysiology of nerve-electroplaque transmission in *Electrophorus* shows many quantitative similarities with these other nicotinic synapses (see for example Steinbach and Stevens, 1976; Sheridan and Lester, 1977). The present study has disclosed that these similarities extend to the detailed time course of MPSCs. We shall therefore assume that transmitter movement, binding, and hydrolysis are quantitatively similar in the synaptic cleft of all these junctions.

K_{app} versus Cleft Concentration at the Resting Potential

Recent studies allow estimates of the ACh concentration in the synaptic cleft during nicotinic transmission. From micro-iontophoretic studies and a determination that a quantum contains roughly 10,000 molecules of ACh, Kuffler and Yoshikami (1975*a*,*b*) estimated a peak transmitter concentration of 0.3 mM. From studies of

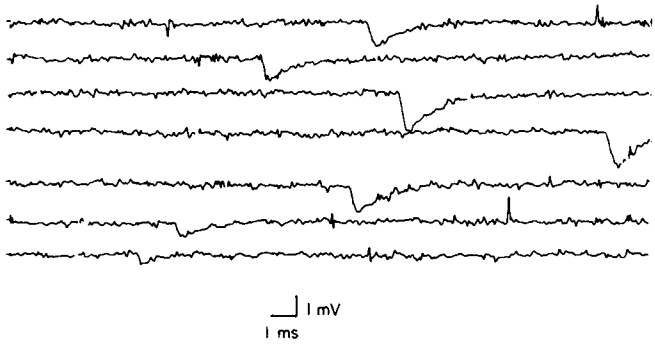

FIGURE 3 Typical spontaneous MPSCs recorded externally from an electroplaque.

the synaptic cleft's geometry, Fertuck and Salpeter (1976) estimated concentrations in excess of 0.3 mM. These estimates substantially exceed our value for K_{app}, 50 μM at the resting potential. At this potential any [ACh] in excess of 50 μM would eventually activate more than half the channels if maintained for several milliseconds. Thus at first glance one might conclude that nicotinic transmission occurs with a vast excess of transmitter.

In fact, the high cleft concentration apparently underlies the *speed* of transmission. Hartzell et al. (1975) estimated that a single quantum of ACh activates more than half of the channels near the point of release; even higher fractional activation was suggested by Negrete et al. (1972). This activation occurs within a time (the MPSC growth phase) several fold shorter than the channel duration (roughly 3 ms at 15°C and 1 ms at 22°C). Therefore during the growth phase, channels are opening at a rate that substantially exceeds the closing rate. It may be inferred that [ACh] is at least several times K_{app} during the growth phase, a conclusion quite consistent with the values for cleft concentration estimated by Kuffler and Yoshikami (1975*a*,*b*) and by Fertuck and Salpeter (1976). Elsewhere, we have treated these points quantitatively and have estimated that [ACh] equals about 700 μM during the MPSC growth phase (Lester et al., 1976).

An Alternative Way to Conclude That the MPSC Involves Substantial Local Activation

The argument just given provides a rationale for the high cleft concentration during the MPSC; however, the reasoning rests heavily on the estimates that most nearby receptors are activated during a quantal event (Negrete et al., 1972; Hartzell et al., 1975). As the evidence on this point is somewhat indirect, we note here that a similar estimate can be made by comparing the present data on PSCs and on conductances induced by bath-applied ACh.

THE PSC INVOLVES SUBSTANTIAL GLOBAL ACTIVATION In the present experiments, conditions were adjusted to obtain the largest possible number of released quanta. The ratio $g_{PSC}/r\gamma$ indicates the fraction of available receptor channels activated at the peak of the PSC. The ratio is close to one and it can be stated that most of the channels are activated during the PSC rising phase. It should be noted that extrasynaptic receptors could contribute to the conductance induced by bath-applied ACh (Bourgeois et al., 1972). A correction for this effect would increase the ratios of $g_{PSC}/r\gamma$ listed in Table I. On the other hand, this ratio would be decreased by desensitization or by any irreversible decreases in r or γ which accompany bath application of drugs.

The conclusion that our PSCs activated nearly all available receptor channels may seem surprising on two grounds. Firstly, the number of channels activated during a normal frog PSC is one-tenth the number of binding sites for α-toxins (Porter et al., 1973; Matthews-Bellinger and Salpeter, 1976, 1978; Steinbach and Stevens, 1976). However, this discrepancy probably results from inactive release sites. Again we emphasize that in our experiments, release was deliberately facilitated with high-frequency

stimulation; there appear to be few inactive release sites during strong facilitation (Wernig, 1975). Possibly the presence of Ba also lengthened the presynaptic action potential, further enhancing release.

Secondly, $r\gamma$ (about 130 mmho per square centimeter of window area in our chamber) is much smaller than the conductance obtained when frog single-channel conductances are multiplied by the estimated number of α-toxin binding sites exposed per square centimeter of window area (see Lester et al., 1975). However, the channel conductance in *Electrophorus* might differ from that in the frog. Perhaps more importantly, it should be pointed out that estimates of receptor density derive principally from a careful autoradiographic study which found about 33,000 binding sites per square micrometer of *postsynaptic membrane* (that is, membrane which actually faces electromotor nerve terminals) (Bourgeois et al., 1972). Postsynaptic membrane comprises a small fraction of the innervated face; and one must employ a correction factor of nearly 100 to estimate the number of receptors exposed per square centimeter of window area in our chamber. This factor deserves more attention.

INDEPENDENCE OF QUANTAL EVENTS Hartzell et al. (1975) found that there is neither cooperativity nor occlusion between the effects of simultaneously released quanta at endplates when the esterase is intact. We therefore view the maximally facilitated PSC as an ensemble of almost independent quantal events which sum to activate a large proportion of the channel population at the synapse. One infers that each quantum activates most of the nearby receptor population. This is the conclusion reached by Hartzell et al. (1975) on the basis of iontophoretic studies.

It would be desirable to specify more precisely what is meant by "the nearby receptor population." For frog endplates the "synaptic unit" (Heuser and Reese, 1973) is clearly defined. Each unit is centered at a presynaptic "active zone" (Couteaux and Pécot-Dechavassine, 1970), presumably the site of transmitter release, and extends longitudinally halfway to the next active zone on each side. At frog endplates each active zone is opposite a postsynaptic junctional fold. Synaptic units occur every 0.7 μm along the synapse (Peper et al., 1974); and if the postsynaptic membrane has a transverse width of 1.5 μm, each synaptic unit contains about 1 μm^2 of postsynaptic membrane. When esterase is active, we suggest that each MPSC is approximately confined to the synaptic unit in which it was released. During facilitated release each synaptic unit contributes one or two MPSCs in response to a presynaptic impulse and a focal external electrode records events from several synaptic units (Wernig, 1975, 1976). If there are 20,000 α-toxin binding sites/μm^2 of membrane (Matthews-Bellinger and Salpeter, 1976) and four α-toxin binding sites per channel (Raftery et al., 1975), there would be 5,000 channels/μm^2 of membrane, a figure which agrees with the maximum density of membrane-associated particles in freeze-fracture studies (Peper et al., 1974; Heuser et al., 1974). An MPSC involving about 2,000 channels (Anderson and Stevens, 1973) would therefore activate 40% of the channels in a synaptic unit. These ideas agree fairly well with the suggestion (Hartzell et al., 1975) that a quantal event activates between 50 and 75% of nearby channels.

The Effect of Depolarization

Membrane depolarization results in an increase of K_{app}, primarily through an increased channel closing rate. We may ask whether this voltage sensitivity plays a role in shaping synaptic events. Any channels permeable to K^+ in the innervated membrane would tend to shunt the Na currents during the impulse (see for instance Bennett, 1970). But because ACh receptor channels close with depolarization, they are inactivated by the impulse which they initially triggered. Thus voltage-sensitivity improves the impedance match with the external medium. It is not yet possible to determine the absolute magnitude of this effect, since with bath-applied agonists one observes an instantaneous rectification of the agonist-induced conductance. This rectification may not be present in vivo but under our experimental conditions it vitiates measurements at positive voltages (Sheridan and Lester, 1977). If, however, at positive potentials K_{app} continues to increase by e-fold for each 86 mV, and if in vivo impulses are represented by the largest ones we have measured in our chamber (195 mV), then K_{app} would increase by 10-fold, to about 500 μM, at the peak of the impulse. This shift would suffice to place [ACh] within the initial, upward-curved region of the dose-response relation during most of the PSC decay phase, and possibly even during the growth phase. The effect would be to amplify further the voltage-controlled inactivation. In this connection we have reproduced the observation by Ruiz-Manresa and Grundfest (1971) that the PSC does not appreciably shunt the impulse (see Fig. 4 in Changeux, 1975). This mechanism seems more important for *Electrophorus* electroplaques than for muscle fibers (del Castillo and Katz, 1954).

A related effect seems to play a role at all voltage-sensitive nicotinic synapses. We do not yet know the rate-limiting step in opening and closing of the ACh receptor channel or the specific effect of voltage on the receptor protein. Nonetheless, a given depolarization directly or indirectly decreases agonist-receptor binding to the same extent that it decreases the channel duration. This can be concluded because when acetylcholinesterase is inhibited, PSC decays reflect the buffering of ACh diffusion by multiple binding to receptors; yet the decay rates retain their original exponential dependence on voltage (Magleby and Stevens, 1972*a*,*b*; Gage and McBurney, 1975; Colquhoun, 1975; Sheridan and Lester, 1977). The conclusion is strengthened by our observation that the dose-conductance relation (measured by K_{app}) has the same voltage sensitivity as the channel lifetime α. Thus it is very likely that as the channel closes, one or both agonist molecules leave the receptor. Consequently during normal transmission with active esterase, one effect of the action potential is to release ACh from the receptors, freeing it for elimination from the cleft by hydrolysis or by diffusion. The voltage-sensitive binding affinity presumably arises because depolarization increases the free energy of the agonist-receptor complex (Magleby and Stevens, 1972*b*; Kordas, 1972*b*; Gage and McBurney, 1975; Sheridan and Lester, 1977). This energy is drawn from the Na and K gradients set up by the sodium pump.

In summary, because of voltage sensitivity, the occurrence of an impulse rapidly

terminates the effect of an ACh molecule already bound to a receptor and decreases the effect of subsequent binding if it occurs. These nicotinic synapses seem remarkably well specialized to transmit brief signals.

Our interpretation of voltage sensitivity is consistent with its absence in the nicotinic receptors of two cells which do not have Na impulses, skate electroplaques (Sheridan, 1976) and frog slow muscle fibers (Dionne and Parsons, 1977). Voltage sensitivity would be counterproductive in these cases. Skate electroplaques generate their discharge with the PSC; if channels closed more rapidly with depolarization, the discharge currents would be weaker. Likewise in frog slow muscle, the contracture is controlled by the ACh-induced depolarization; this depolarization would tend to oppose itself if receptors were voltage-sensitive, as in twitch fibers.

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