Acetylcholine receptor site density affects the rising phase of miniature endplate currents

(α-bungarotoxin/voltage clamp/acetylcholine diffusion/neuromuscular junction/binding kinetics)

BRUCE R. LAND*, EDWIN E. SALPETER†, AND MIRIAM MARK SALPETER‡

*Section of Neurobiology and Behavior, Division of Biological Science, and †Department of Physics, Cornell University, Ithaca, New York 14853

Contributed by Edwin E. Salpeter, March 24, 1980

ABSTRACT The relationship between acetylcholine receptor (AcChoR) site density (σ) and the rising phase of the miniature endplate current was determined in esterase-inactivated lizard intercostal neuromuscular junctions. The currents were recorded by using a voltage clamp. The receptor site density was determined by electron microscope autoradiography after labeling with 125I-labeled α-bungarotoxin in normal endplates and in those partially inactivated with nonradioactive α-bungarotoxin. We found that as σ is decreased the rise time is increased and the amplitude is decreased. These results are compatible with a previously stated "saturating disk" model, which suggests that a quantum of acetylcholine (AcCho) acts on a small postsynaptic area at saturating concentration. We conclude that in the normal neuromuscular junction the most likely number of AcCho molecules needed to open an ion channel is 2, and that the 20–80% rise time of <100 μsec is influenced both by the σ-dependent factors such as diffusion and binding of AcCho to AcChoR and by the σ-independent time delays such as the conformation change time to open the ion channels. From our data we calculate the lower limits to the forward rate constant of AcCho binding to AcChoR ≥ 3 × 10^10 M^-1 sec^-1, and the diffusion constant for AcCho in the cell 4 × 10^8 cm^2 sec^-1.

A unique molecular organization of acetylcholine receptors (AcChoRs) and acetylcholinesterase has been found in all neuromuscular junctions (nmjs) of vertebrate twitch muscles examined. This organization consists of a high and stable AcChoR site density σ of ≈20,000 sites/μm^2 of surface area at the top of the postjunctional membrane (1–4) and a uniform distribution of acetylcholinesterase of ≈2500 sites per μm^2 of surface area along the entire postjunctional folded membrane (5–8). In the present paper we attempt to determine some physiological consequences of this organization by studying the effect of decreasing σ on the rise time and amplitude of miniature endplate currents (mepcs) in esterase-inactivated nmjs. We found that, as σ is decreased, the rise time increases and the amplitude decreases. From these data we suggest some physiological parameters which control the action of acetylcholine (AcCho) in the cell.

METHODS

Muscle Preparation. We used lizard (Anolis carolinensis) intercostal muscle. This muscle has compact endplates that are much smaller than the electrical length constant, and thus may be uniformly clamped. The muscle is thin (one to two fiber layers thick), allowing easy visualization of the endplates with Hoffman modulation optics. The physiological studies were performed on endplates pretreated with diisopropylfluorophosphate (iPr2P-F) (1 mM for 20 min), which effectively inactivates esterases. The AcChoRs were either left intact or were partially inactivated with α-bungarotoxin (BTX) (9) (40 nM) for 20 or 40 min. The muscle was thoroughly washed (30 min) to remove excess iPr2P-F and BTX. The mepcs were measured in fibers that had been voltage clamped at −100 mV (23°C), using 5–6 MΩ electrodes. The clamp output was passed through a two-pole Butterworth lowpass filter with 4-kHz cut-off to remove high-frequency noise. Approximately two hundred mepcs were obtained from each fiber. After the physiological recordings, the muscles were incubated in 125I-labeled BTX (125IBTX) (500 nM) for 2 hr, as described for the frog (4), and prepared for electron microscope autoradiography by the flat substrate procedure of Salpeter and Bachmann (10, 11), using Ilford L4 emulsion and D-19 development (2 min at 20°C). In order to determine that there was no loss of BTX during the 6 hr before fixation while the physiological studies were performed, control preparations were labeled with 125IBTX (500 nM) immediately after the incubation with the nonradioactive BTX.

Improving Resolution of mepc Rise Time Data. For these studies we needed an undistorted record of the average mepc rise time. To obtain this, several procedures were followed: (i) We drained the dish during recording in order to lower electrode capacitance to ground (100–200 μm of liquid was left over the cells); we calculate that the small fluid layer increased the total resistance by less than 10%. (ii) We penetrated the cell within one fiber diameter of the mmj. (iii) We used a 4-kHz filter and digitized the current waveform at 40,000 samples per sec. (iv) We injected a 1-mV command voltage to the clamp and measured the actual voltage rise time prior to recording the mepcs. (v) Cells were used only if the clamped voltage change was less than 5% of the unclamped miniature endplate potential amplitude. These procedures allowed us to obtain a fast (20 μsec) clamp rise time (20–80%) and to minimize distortion of the mepc waveform but left us with a noisy signal. We therefore used averaging of the traces to resolve the signal. Because mepcs are spontaneous random events, the individual traces were aligned before averaging, using a two-step procedure. First each raw trace was smoothed by using a 16-point running average, which lengthens the rise time artifically but leaves the amplitude and "half-time point" (the time when half of the peak

Abbreviations: mepc, miniature endplate current; A, mepc amplitude; t_r, 20–80% rise time of a mepc; AcCho, acetylcholine; AcChoR, acetylcholine receptor; σ, AcChoR binding sites per μm^2; BTX, α-bungarotoxin; nmj, neuromuscular junction; iPr2P-F, diisopropylfluorophosphate.

† To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1794 solely to indicate this fact.
amplitude is reached) relatively unaltered. The smoothed traces were used only to provide the “half-time point” onto which the original unsmoothed mepc were then aligned and computer averaged. We thus obtained an improved signal-to-noise ratio without major distortion of the rising phase of the mepc (top three traces, Fig. 1). By contrast, a single mepc after 1-kHz filtering also has low noise but is broadened appreciably (bottom trace, Fig. 1).

RESULTS

Fig. 2 shows an autoradiogram of a lizard intercostal nmj treated with $^{125}$I-BTX. Electron microscope autoradiographic

![Fig. 2. Electron microscope autoradiogram of lizard endplate labeled with $^{125}$I-BTX. A, axon; M, muscle; pjm, postjunctional fold. (Ilford L4 emulsion; D-19 development; $\times$24,000.)](image)

Table 1. The mepc amplitude ($A$) and 20–80% rise time ($t_r$) for various AcChoR (BTX)-binding site densities ($\sigma$) in esterase-inactivated endplates

<table>
<thead>
<tr>
<th>Condition</th>
<th>$A$, nA</th>
<th>$t_r$, usec</th>
<th>$\sigma$, sites/$\mu$m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPr$_2$P-F only</td>
<td>7.5 ± 1.3</td>
<td>91 ± 16</td>
<td>18,000 ± 4,000</td>
</tr>
<tr>
<td>iPr$_2$P-F + 20-min BTX</td>
<td>4.0 ± 0.7</td>
<td>160 ± 25</td>
<td>8,300 ± 1,800</td>
</tr>
<tr>
<td>iPr$_2$P-F + 40-min BTX</td>
<td>2.5 ± 0.5</td>
<td>196 ± 25</td>
<td>6,900 ± 2,000</td>
</tr>
</tbody>
</table>

Error limits as explained for Fig. 3. Where indicated, BTX (40 nM) was present for 20 or 40 min.

analysis indicated that the mean AcChoR site density is approximately 18,000 sites/$\mu$m$^2$ of specialized dense membrane at the top of the junctional folds and thus similar to values for mouse and frog. Furthermore, using previously described techniques (5–8), we have recently found that there are $\approx$5000 acetylcholinesterase sites per $\mu$m$^2$ of postjunctional membrane in the lizard nmj (unpublished). The molecular organization of the lizard intercostal nmj is thus essentially the same as that of the mouse and frog muscles studied to date.

Table 1 summarizes both the autoradiographic and physiological data. Note that the average $t_r$ (defined as the 20–80% rise time) for normal nmjs (treated with iPr$_2$P-F only) is $\approx100 \mu$sec. This value is in line with that obtained by Gage and McBurney (13), and with the values used in mathematical modeling of the mepc (14). It is, however, considerably faster than the value often reported in the literature (15, 16), perhaps due to the procedures described above for obtaining a better time resolution. Table 1 also shows that the $t_r$ increased when the muscle was incubated with BTX. [A similar observation was made by Katz and Miledi when using curare (17).]

Fig. 3 gives the relationship between $t_r$ and $\sigma$ for averaged values obtained from several muscles after three different inactivation conditions, and Fig. 4 gives the relationship between mepc amplitude ($A$) and $\sigma$. We see that $t_r$ increases and $A$ decreases as $\sigma$ is decreased by BTX inactivation. The stated errors in Table 1 (and Figs. 3 and 4) are estimated standard errors, including formal statistical errors plus estimates for possible systematic errors.

DISCUSSION

"Saturation Disk" Model. We shall discuss the relationships between $t_r$, $A$, and $\sigma$ in Figs. 3 and 4 in the framework of a model to be called the "saturation disk" model, which was proposed in earlier studies from this laboratory (3, 4). This model states that the high AcChoR binding site density allows a quantal packet of AcCho to be essentially fully bound within a small postsynaptic area and thus act at saturating concentration. [Adams (18) also favors such a model.] More specifically, let $a_q$ be the postsynaptic area (to be called the minimum quantal area), which contains the number of AcChoR binding sites equal to the number, $N$, of AcCho molecules in a quantal packet (thus $a_q = N/\sigma$). Then, for the normal AcChoR site density, $\sigma \approx$20,000 sites/\mu$m$^2$, and a quantal packet of $N = 10^4$ AcCho molecules (e.g., see ref. 19), $a_q$ is $\approx 0.5 \mu$m$^2$. With a cleft thickness of 50 nm, a quantal packet of AcCho will act over $a_q$ at an average concentration above 500 $\mu$M, which is indeed higher than the highest published values of the dissociation constant for binding of AcCho to AcChoR (20). Similar

$^\dagger$ Previous values for AcChoR density published from this laboratory (3, 4) were $\approx 30\%$ too high due to a net effect of two systematic errors. The major one was our use of bovine serum albumin as the standard for the Lowry protein determination, which gives an overestimate of BTX protein concentration (unpublished data). In addition, we now make a slight correction for self-absorption of $^{125}$I in 100-nm stained autoradiographic sections (12).
Rise Time—σ Correlation. We observed in Fig. 3 that \( t_r \) is almost proportional to \( 1/\sigma \). The saturating disk model predicts such a relationship under certain conditions. Consider that \( t_r \) can be controlled by the time \( (t_b + d) \) for a quantum of AcCho to diffuse to and bind to the postsynaptic AcChoRs, as well as by some time delay \( (t_d) \) contributed by various factors such as AcCho release but primarily by channel opening. Let \( t_d \) (to be defined more quantitatively in Eq. 5) be a characteristic time for AcCho to diffuse over the minimum area \( a_q = N/\sigma \). Because diffusion time is proportional to area covered, \( t_d \) is proportional to \( 1/\sigma \). Let \( t_b \) (to be defined more quantitatively in Eq. 4) be a characteristic time for an AcCho molecule to bind to AcChoR in the absence of competition for binding sites. Because the forward binding rate is proportional to the rate constant \( (k_+ \) and to the concentration of the reactant AcChoR, the time \( t_b \) is also proportional to \( 1/\sigma \). The combined diffusion and binding component \( (t_b + d) \) of \( t_r \) is then also of the form

\[
t_b + d = C/\sigma,
\]

in which \( C \) is a proportionality constant.

We have emphasized that the AcChoR sites within the minimum area \( a_q \) need not necessarily be fully bound. If binding is slower than diffusion \( (t_b \gg t_d) \), a steady state is not reached during the mepc rise time and the actual effective area (to be called \( a_e \)) over which the quantum is bound is larger than \( a_q \). Eq. 1 will, however, still hold as long as the AcCho remains at saturating concentrations and is not lost from the cleft during \( t_r \). The saturating disk model thus predicts that \( t_r \) will be proportional to \( 1/\sigma \) provided \( t_r \) is controlled by \( t_b + d \) and is not dominated by any \( \sigma \)-independent time delays. In Fig. 3 any effect of \( \sigma \)-independent time delays should be most noticeable at low values of \( 1/\sigma \) in the form of an upward deviation from linearity, giving a positive y intercept. Any effect of AcCho loss from the cleft or decreasing efficiency of binding due to decreased AcChoR concentration should be most noticeable at large values of \( 1/\sigma \) as a decreasing slope.

The solid straight line drawn in Fig. 3 is a best fit line, forced to go through the origin to accentuate any experimental deviation from proportionality. No deviation is apparent at large values of \( 1/\sigma \) (which is consistent with saturating AcCho concentration and no loss of AcCho). There is a small, upward deviation from proportionality at small values of \( 1/\sigma \), suggesting the presence of a positive \( t_c \). If we assume that \( t_c \) reflects the conformational change in the ion channel, it can be modelled as an exponential relaxation, \( \exp(-t_c/d) \), in which \( t_c \) equals the relaxation rate constant.

In the limit when \( 1/\sigma \rightarrow 0 \), the rising phase of a mepc would be controlled solely by this relaxation phenomenon. The \( t_r \) would then equal \( t_r = 1.39/t_c \), in which 1.39 is \( \ln(80/20) \), the conversion factor from the exponentiation time constant to the 20–80% rise time. With large values of \( 1/\sigma \) the rising phase of a mepc will reflect a time development due to the combined effect of \( t_c \) and \( t_b + d \). With a simple analytic fit to the waveform of a mepc and an assumed exponential relaxation, computer calculations showed that \( t_c \) is given to a good approximation by the quadratic form:

\[
t_r = \left[ t_c^2 + \left( \frac{C}{\sigma} \right)^2 \right]^{1/2},
\]

in which \( C \) is defined in Eq. 1. (Note that the effect of \( t_c \) on \( t_r \) is small for large values of \( 1/\sigma \).) A maximum likelihood procedure, applied to the three points (plus error bars) in Fig. 3 gave the following fit to the two parameters:

\[
t_c = (58 \pm 16)/\mu sec,
C = (1.24 \pm 0.25) \times 10^6/\mu sec/\mu m^2.
\]

For the normal nmj, Eq. 3 gives \( C/\sigma \approx 68 \mu sec \). The “standard
errors" in Eq. 3 include the random errors obtained from the maximum likelihood procedure and the estimated systematic calibration errors. The broken curve in Fig. 3 shows the fit from Eqs. 2 and 3. If we assume that \( t_d \) is primarily due to the conformational change of the AcChoR-channel complex, then \( t_d < 1/\lambda = 42 \pm 12 \mu s \). If any other \( \sigma \)-dependent time delays contribute to \( t_d \), then the \( 42 \mu s \) is an upper limit to the relaxation time constant for the AcChoR-gating. Although the relaxation rate \( t_r \) is the sum of the rates for gate opening (\( t_a \)) and closing (\( t_c \)), we shall demonstrate (in a later paper) that the high efficiency of an AcCho quantum rate require that \( t_c \) be small compared to \( t_a \) and that the \( 42 \mu s \) reflects primarily the gate opening time constant.

**Limits to Kinetic Parameters in the Cleft.** At present our data do not allow us to separate the influence on \( t_d \) of diffusion and binding. However, the value of \( C/\sigma \) (Eq. 1) can give an upper limit to both \( t_b \) and \( t_d \), and hence we can derive the lower limits to the forward rate constants \( k_+ \) for AcCho binding to AcChoR and the diffusion constant \( D \) for AcCho diffusion in the cleft.

The upper limit for \( t_b \) would be attained if diffusion were rapid relative to binding \( (t_d < t_b) \). In that case there would be little competition for AcChoR sites \( (\text{AcCho would spread over an area } a_q \gg a_k) \) and, in the limit of \( t_b \gg t_d, t_b = t_b + d = C/\sigma \). But \( t_b \) is, by definition, proportional to \( h/(\sigma k_+) \), in which \( h \) is the height of the cleft and \( \sigma/h \) is the effective concentration of AcChoR. Then, when binding is rate limiting, the 20-80% rise time is:

\[
t_r = t_b = C/\sigma = 1.39h/\sigma k_+ \tag{4}
\]

The 1.39 is the conversion factor from the exponential time constant for the decay of free AcCho to the 20-80% rise time.

From our measured value of \( C \) given in Eq. 3 (plus 2.5 times the standard deviation) we then get that \( k_+ \gg 3 \times 10^7 M^{-1} \text{ sec}^{-1} \). This inequality is altered only slightly if the number \( n \) of AcCho binding sites per AcChoR-channel complex is larger than 1. For instance, if \( n = 2 \) (with or without cooperativity in binding), the forward rate constant for the first binding satisfies \( k_+ \geq (3/2) \times 10^7 M^{-1} \text{ sec}^{-1} \). For comparison, Wathey et al. (14) use a rate constant of \( 3 \times 10^8 M^{-1} \text{ sec}^{-1} \) for the first of two binding steps, and Rosenberry (22) uses a forward rate constant of \( 2 \times 10^9 M^{-1} \text{ sec}^{-1} \).

The value for \( t_4 \) would reach its upper limit if binding were fast relative to diffusion: In the limit of \( t_b < t_d, a_q = a_k \), and, from Eq. 1, \( t_4 = t_d + t_b = C/\sigma \). Diffusion time is proportional to area covered divided by the diffusion constant \( D \). But, by definition, \( a_q = N/\sigma \). Thus, in the limit of infinitely fast binding when diffusion rate is limiting, the 20-80% rise time is

\[
t_r = t_d = C/\sigma = K(N/\sigma D) \tag{5}
\]

in which \( K \) is a proportionality constant. We used a computer model (to be described in a later paper) to solve the diffusion equation extrapolated to rapid binding, and obtained a value for \( K \) of about 0.06. With \( N = 10^4 \) AcCho molecules in a quantum packet (19) and using the upper limit of \( C \) (Eq. 3), we calculated \( D \geq 4 \times 10^{-9} \text{ cm}^2 \text{ sec}^{-1} \). This lower limit to the diffusion constant is about half that given for free diffusion by Krsenjevic and Mitchell (23) and is similar to the value quoted by Wathey et al. (14).

There is now general agreement (3, 4, 15, 18, 21) that a quantum of AcCho can act over a small postsynaptic area at high (saturating) concentrations, but, as we already stated, it does not necessarily follow that all the AcChoR within this area need actually be bound. If \( t_d < t_b \approx t_b + d \), then \( a_q \) is larger than the minimum area \( a_k \) by approximately \( t_b/t_d \). The extent of AcChoR saturation is not known, but two indirect arguments on this point can be made: (4) Hartzell et al. (15) find that the postsynaptic response due to an iontophoretically applied AcCho pulse adds linearly to that of a quantum of AcCho without a significant increase in time to peak. This result is compatible with an unsaturated disk—i.e., \( a_q \gg a_k \) (linear part of the dose–response curve) but does not require it. Because their iontophoretic AcCho covered a large area at low concentration, it would bind a small fraction of AcChoR within any postsynaptic area. In order to produce its full response, and add linearly to the iontophoretic response, the quantum of AcCho needs only to spread over a disk area larger than its normal \( a_q \) by this small fraction. (4) The fact that \( t_d \) does not deviate significantly from proportionality with \( 1/\sigma \) at the smallest values of \( \sigma \) used in our experiment means that AcCho remains saturating and that little AcCho is wasted by leakage from the cleft during \( t_b \). The smallest values of \( \sigma \) in our BTX-inactivated nmjs gives an \( a_q \) with a radius of \( \approx 0.7 \mu m \). We estimate that this must be approaching the morphological dimensions of the cleft, and therefore that \( a_q \) cannot be much larger than \( a_q \).

**Amplitude–\( \sigma \) Correlation.** We observed in Fig. 4 that the amplitude was roughly proportional to \( \sigma \). This type of relationship is predicted from the saturating disk model, provided the number \( n \) of AcCho binding sites needed to open the ion channel is larger than 1 (and, for the simplest assumption that binding to one site does not affect binding to other sites, \( n \) is equal to 2). A conclusion that \( n > 1 \), and that most likely \( n \approx 2 \), is consistent with physiological (18, 20, 24), biochemical (25, 26), and morphological studies (27).

The justification for our conclusions from the amplitude–\( \sigma \) correlations is as follows: As \( \sigma \) is decreased, the AcCho packet has to spread further and takes longer to bind, but as long as the AcCho concentration remains high and AcCho is not yet lost from the cleft (which we have claimed above is the case in our experiments), the AcCho molecules will eventually be bound somewhere within the area \( a_q \). If \( n = 1 \), then each AcCho molecule, once bound, would have an equal chance of opening a channel and \( A \) should not decrease with decreasing \( \sigma \). If, however, \( n > 1 \), then any AcCho molecule that binds to an AcChoR–channel complex that already has BTX bound to one of its other binding sites cannot open a channel and is wasted. Therefore, when \( \sigma \) is decreased by BTX binding, \( A \) will also decrease. Because this is what we observe, most channel openings must go with \( n > 1 \). Our results are, however, not accurate enough to exclude a small probability of opening some AcChoR channels with only one AcCho bound to an AcChoR-channel complex (28).

More quantitatively, let \( a_q \) = the original density of AcChoR binding sites and \( q = \) the fraction of sites available for AcCho binding after BTX. Then \( q = \sigma/a_q \), and (without cooperativity in binding) \( q^a = \) the fraction of AcChoR-channel complexes with all \( n \) sites available for binding. Because \( A \) (the number of open channels) \( \propto (q^a a_q) \), \( a_q \approx 1/\sigma \). (28). \( A \approx q^a/\sigma \) or \( \sigma^a \). Thus for \( n = 1, A \) should be independent of \( \sigma \); for \( n = 2 \) (without cooperativity in binding) \( A \) should be proportional to \( \sigma \), as we obtained in Fig. 4; and for \( n > 2, A \) should decrease faster than \( \sigma \).

On the other hand, the saturating disk model makes a different prediction for the relationship between \( A \) and \( \sigma \) if \( \sigma \) is decreased due to the elimination or inactivation of entire AcChoR–channel complexes rather than due to the random elimination of individual AcChoR binding sites as it is by BTX. In that case, even if \( \sigma \) is decreased by a factor of 2 or 3, \( A \) (in the absence of acetylcholinesterase) would not decrease whatever the value for \( n \), because none of the AcCho is wasted by binding.
to partially inactivated AcChoR–channel complexes. This argument has to be modified if AcCho is lost during \( t_f \) by leakage from the cleft. Then a further decrease of AcChoR–channel complexes would result in a decrease in \( A \) and a loss in linearity between \( t_f \) and \( 1/\sigma \). Such seems to be the case in the numerical calculations of Wathey et al. (14), in which a small (\( \approx 0.5 \mu m \)) cleft radius is assumed. However, a more general prediction that the time derivative \( dA/dt \) at early times is proportional to \( \sigma \) should hold in this case whether there is leakage from the cleft or not.

**SUMMARY AND CONCLUSION**

In summary, when AcChoR binding site density (\( \sigma \)) is decreased by BTX in esterase-inactivated lizard intercostal endplates, the mephos rise times lengthen almost proportionately with \( 1/\sigma \). Such a relationship is predicted by the saturating disk model, which requires that a packet of AcCho act during \( t_f \) at a concentration well above its dissociation constant for binding AcChoR and be initially bound without a significant loss from the cleft. We conclude that at a normal nmj the \( \sigma \)-dependent factors (such as the time for AcCho to diffuse to or to bind to the AcChoR) and \( \sigma \)-independent time delays (such as the time to open the receptor-linked ion channel) both influence the rising phase. However, when \( \sigma \) is reduced by as little as a factor of 2 or 3, the \( \sigma \)-dependent factors dominate. Furthermore, the dependence of amplitude on \( \sigma \), seen in Fig. 4, is predicted by our model provided the number of AcCho binding sites necessary to open the AcChoR linked ion channel is greater than 1, and is probably 2.

An AcChoR–channel complex with \( n > 1 \) would tend to suppress the endplate response to a low concentration AcCho leakage (29). However, for AcCho released in concentrated quantal packets, the molecular organization of high \( \sigma \) ensures that the AcCho can be bound over a small postsynaptic area at saturating concentration. Thus, the small postsynaptic area ensures fast time response and the saturating AcCho concentration ensures a high binding efficiency, which is particularly important with \( n > 1 \).

The saturating disk model, which was proposed on the basis of molecular anatomy (3, 4) appears to be compatible with our data. In the present study it has provided a basis for calculating approximate values for the conformational ion gate opening and lower limits to the forward binding rate constant and for the diffusion constant for AcCho in the cleft. We can now expect it to provide a guide for further experiments and for computer modeling, which should give actual values for the kinetic parameters operating in an intact nmj.

We thank Charles Stevens for giving us the voltage clamp design, Ralph Loring for preparing the BTX, Henry Lester for helpful discussions, and Maria Szabo, Mary Johnson, and Joyce Davis for technical assistance. This research was carried out during the tenure of a Postdoctoral Fellowship from the Muscular Dystrophy Association to B.R.L.) and was supported in part by Grant NS 08315 from the National Institutes of Health. This work was presented in preliminary form at the Society for Neuroscience meeting, 1978.