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DRUG-INDUCED MODIFICATION OF IONIC CONDUCTANCE AT THE NEUROMUSCULAR JUNCTION[1]

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INTRODUCTION

At the endplate of the neuromuscular junction the binding of acetylcholine (ACh) to its receptor triggers the opening, for a few milliseconds, of an ionic channel with a conductance of approximately 23 pS (1). The reaction is described by the following sequential scheme:

$$nA + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{closed}}{A_nR} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \underset{\text{open}}{A_nR^*}, \qquad 1.$$

where A is the agonist (ACh) molecule with n, the number of molecules binding to the receptor (R), with an association rate of k_1 and a dissociation

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rate k_{-1}. β is the channel opening rate and α is the channel closing rate. A_nR indicates that the acetylcholine receptor complex is in a nonconductive state; A_nR^* indicates a conducting state.

The ionic channel when activated by agonist is mainly permeable to Na^+ and K^+ ions, which flow inward and outward respectively at a membrane potential of approximately –90mV (2). Some drugs prevent this agonist-induced conductance by competing with ACh for its recognition site on the receptor in a reversible manner, e.g. d-tubocurarine (3). Such receptor antagonists occupy the ACh binding site but do not induce the opening of the ACh ionic channel [however, see (4, 5)].

The interaction of reversible competitive antagonists with R is described according to:

$$B + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BR, \qquad 2.$$

where B is the antagonist molecule and BR is the antagonist-bound but closed receptor channel complex; other symbols are as for equation 1. Similarly many of the α toxins, such as α-bungarotoxin (α-BUTX), appear to bind to the ACh recognition site, but the binding is irreversible or only slowly reversible (6).

Some drugs with diverse structures decrease the agonist-induced ionic endplate conductance in a reversible but noncompetitive manner. A clue to their mechanism of action was provided by the observation that such drugs (e.g. the local anesthetics) modified the decay time of endplate currents (e.p.c.s) and miniature endplate currents (m.e.p.c.s) (7–13). Since Anderson & Stevens (14) had demonstrated that the exponential time constant of m.e.p.c. decay is probably a measure of the randomly distributed lifetime of the ACh-activated ionic channels, this observation indicated that these drugs in some way modified the lifetime of the ACh-activated ionic channel. Adams (15) suggested that local anesthetics did not interact with the ACh binding site on the receptor but rather with the ionic channel associated with the receptor, a mechanism first proposed by Blackman (16) to account for the action of hexamethonium on ganglion cells. Following Adams's model, a cationic local anesthetic is envisaged as entering the ionic channel when the receptor-channel complex is in the activated or open state, thereby blocking the flow of ions through the channel. Drug binding to the activated agonist-receptor complex is seen as not only blocking the channel, but simultaneously "freezing" the agonist-receptor channel complex in the open but blocked state, so that it can repeat its normal life cycle only when

the antagonist dissociates from the ionic channel binding site. This reaction is described by:

$$\begin{array}{ccccccccc} & & k_1 & & \beta & & G\cdot c & & \\ nA + R & \rightleftharpoons & A_NR & \rightleftharpoons & A_nR^* + D & \rightleftharpoons & A_nR^*D, & & 3. \\ & & k_{-1} & \text{closed} & \alpha & \text{open} & F & \text{open blocked} & \end{array}$$

where D is the local anesthetic molecule (e.g. QX-222) at a molar concentration of c with a channel blocking rate constant of G (M^{-1} sec^{-1}) and a channel unblocking rate constant of F (sec^{-1}); other symbols are as for equation 1. A diagrammatic representation of this model is shown in Figure 1.

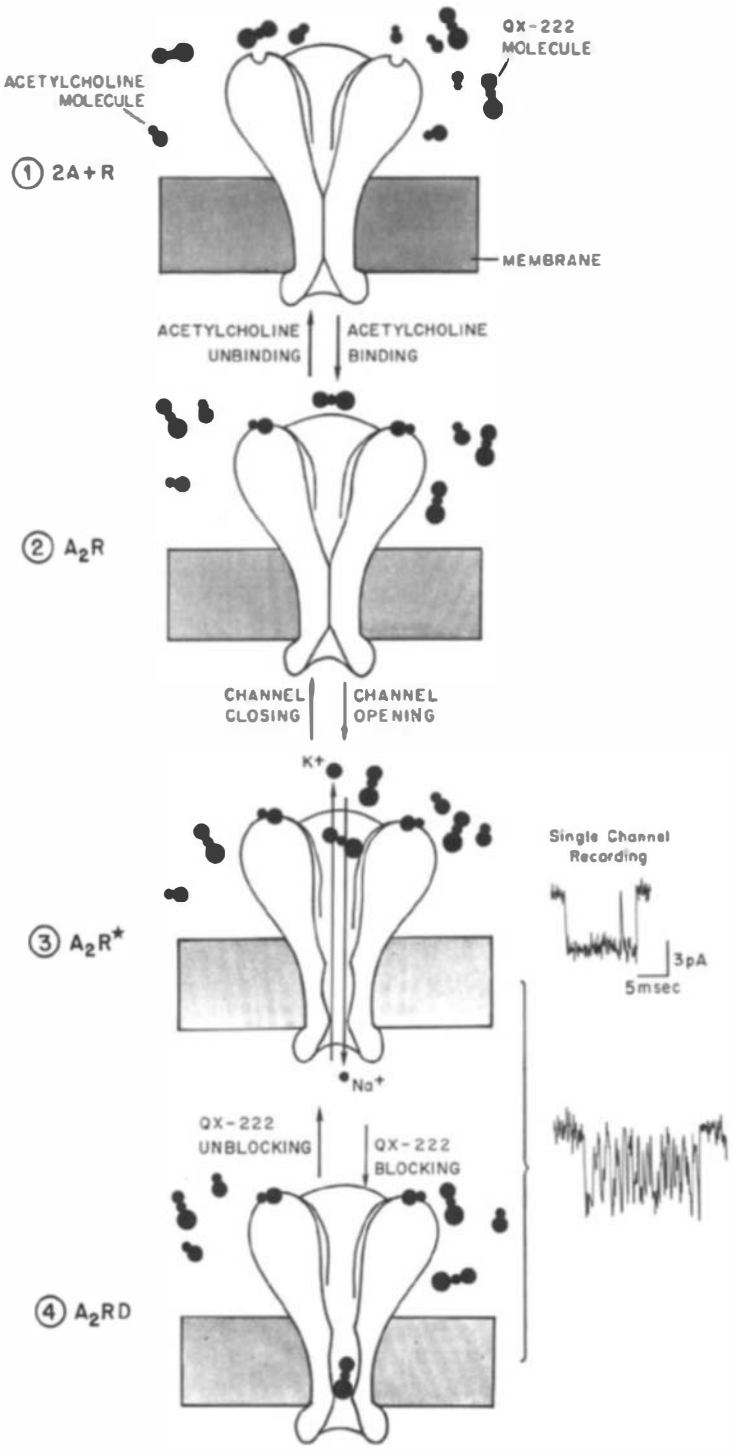
However, the effects of some drugs (17–21) are best explained by a block not only of "open" channels but also of "closed" channels, and a few drugs appear to block selectively the ACh channel in the "closed" conformation (22). Therefore, endplate conductance can be blocked in three ways: 1. by preventing ACh binding to its receptor; 2. by blocking the ACh-activated channel in the open configuration; and 3. by blocking the ACh-activated channel in the closed configuration.

This review focuses mainly on the action of drugs that block the ACh-activated ionic channels at the neuromuscular junction, because this junction lends itself to study by electrophysiological techniques such as voltage clamp, patch clamp, and noise analysis, which in turn has led to a better understanding of the interaction of ACh with its receptor-channel complex. However, as other synapses are investigated, it is apparent that block of transmitter receptor-channel complexes by drugs is not restricted to the ACh receptor of the neuromuscular junction (23–25). The contribution of ion channel block by drugs to their pharmacological and therapeutic effects is not known; however, block of ionic channels should always be taken into account as a way in which drugs can influence synaptic transmission, and the study of such compounds should allow a better understanding of the interaction of ACh and other transmitters with their receptors.

METHODS OF DETERMINING DRUG-CHANNEL INTERACTIONS

Concentration Jump Relaxation

A sudden rise of the endplate concentration of a nicotinic agonist will cause the rapid opening and subsequent closing of the ACh receptor-ionic channel complexes. The use of concentration jump techniques to determine channel kinetic properties is limited by the diffusion rate of agonist in the endplate



region. However, release of ACh from nerve terminals produces a large, rapid rise in transmitter concentration at the receptors because the diffusion distance is relatively small. Furthermore, the rapid breakdown of ACh by acetylcholinesterase produces a rapid decrease of the ACh concentration in the junctional space that greatly exceeds the rate at which channels close. Hence analysis of the time course of the conductance change produced by either the spontaneous (m.e.p.c.s) or evoked release (e.p.c.s) of ACh can be used to measure the kinetics of channel opening and closing.

In order to measure current flow through the endplate it is necessary to keep the membrane potential constant. A common method is to use an electronic system to record the membrane potential of the endplate through one intracellular microelectrode, while a second intracellular microelectrode connected to a feedback circuit passes current into or out of the endplate, effectively "clamping" the membrane potential of the endplate region. Under these conditions ACh-induced currents can be measured (2, 7, 8, 14).

The sudden rise of the ACh concentration at the endplate following presynaptic release of ACh causes a number of channels to open. Each channel will stay open for a random, exponentially distributed period of time. The rising phase of the current reflects the number of channels opening as a function of time and the falling phase reflects the distribution of channel closing. The exponential time constant of the current decay (τ) is a measure of mean channel open time, a finding that has been confirmed by independent techniques such as noise analysis and patch clamp (14, 26).

An alternative method of rapidly jumping the agonist concentration is the use of a photochemically sensitive agonist. The nicotinic agonist 3,3'-*bis*-[α - (trimethylammonium)methyl]azobenzene (Bis Q) exists in both *cis* and *trans* forms, the *trans* isomer being a much more potent agonist of *Torpedo* electroplaque ACh receptors than the *cis* isomer (27). Interconversion of the two isomers occurs very rapidly upon exposure to a brief pulse of light; hence conversion of the *cis* isomer to the *trans* isomer in electroplaque membranes produces a rapid conductance increase, the decay of which reflects the mean channel open time (27).

Voltage Jump Relaxation

In the presence of an agonist, the amount of current that will flow depends upon the agonist concentration and the membrane potential; that is, current

Figure 1 A schematic representation of the sequential model of acetylcholine receptor-ionic channel activation and subsequent blockade by the local anesthetic QX-222. The single channel recording is taken from the unpublished record (M. L. J. Ashford and J. J. Lambert) of an experiment using chick myotubes. The channel is based upon the model of Horn & Brodwick (125).

flow is voltage-dependent (28–30) (see section on electrophysiological properties of the acetylcholine receptor-channel complex). Provided that the agonist concentration is constant, an abrupt jump of the holding potential of the voltage-clamped endplate region, for example from –60 mV to –110 mV, will increase the inward current flow. The initial instantaneous increase of current is followed by a further exponential increase towards a new larger value. The instantaneous increase is due to the greater driving force through the existing open channels at –110 mV than –60 mV. However, net current flow also depends upon how long the channels are open. At more negative membrane potentials channel lifetimes are longer and there will be a greater overlap of channel open time; consequently a larger current will flow. The change in equilibrium caused by a voltage jump is frequently referred to as a "perturbation," and the exponential time constant of the increase of current following a perturbation is a measure of mean channel open time at the new holding potential. Two precautions must be observed when using this technique: low concentrations of agonist should be used so that the rate of channel closing does not become complicated by the rate of opening (31), and the agonist should be applied for relatively short periods of time in order to avoid desentitization.

This technique allows the study of channel kinetics at a fixed, but low, concentration of agonist and has been used to study the kinetics of several agonists including suberyldicholine (29, 30), ACh (29), carbachol (29), and decamethonium (32).

Noise Analysis

Katz & Miledi (33, 34) discovered that when ACh was applied to the neuromuscular junction, the membrane potential observed at a high amplification increased in "noisiness" during the ACh-induced depolarization. They suggested that the "noisiness" arose from the random opening and closing of ionic channels around a mean. Although the initial experiments carried out by Katz & Miledi (33, 34) utilized voltage recordings, there are considerable advantages in recording the current fluctuations (14, 35). Measurements of current fluctuations eliminate the complications produced by the nonlinear relationship between conductance and voltage, and the influence of the membrane time constant (35).

In order to estimate channel life time the noise fluctuations are subjected to Fourier analysis and the resulting spectral density is usually plotted as function of frequency; this plot is flat at low frequencies and curves downward at higher frequencies. The frequency at which the spectral density is half that of the lower frequencies is termed the corner frequency or cut-off frequency (fc) and is related to τ by the relationship (14, 35–37),

$$\tau = 1/(2\pi \text{ fc}). \quad 4.$$

Noise analysis also provides a means of estimating single-channel conductance, (γ) (14, 31, 35, 38, 39), using:

$$\gamma = \frac{\text{var (I)}}{\mu_1 \text{ (V - Veq) pT}}, \quad 5.$$

where var (I) is the variance of the current fluctuations around the mean; μ_1 the mean drug-induced current which flows at holding potential V; V_{eq} the equilibrium potential for the agonist; and pT the fraction of closed channels. At a low agonist concentration, pT will approach unity and will decrease with increasing agonist concentration, thus, it is preferable to use low agonist concentrations (31).

Patch Clamp Analysis of Single ACh-activated Ionic Channels

The patch clamp technique (26, 40, 41) allows the measurement of the current that flows through a single ACh-activated ionic channel. This method consists of pressing a polished glass micropipette against the membrane surface to form a high resistance electrical seal (1–100 GΩ) between the micropipette and membrane, electrically isolating a small (approximately $5\mu m^2$) area of membrane. The micropipette is connected via a Ag/AgCl wire to a high gain, low noise, current-to-voltage converter. If the micropipette contains a nicotinic agonist then square current pulses of random duration and constant amplitude can be recorded. The duration of these events is the open time of individual agonist-activated channels, that is the lifetime of A_nR^* in equations 1 and 3 and Figure 1 (26, 40, 41). The patch-clamp technique has been successfully used to record agonist-activated single channels from a range of muscle cell preparations including the perisynaptic region of frog muscle fibers (41), denervated frog muscle (40), rat myotubes (5, 42), and rat myoballs (43).

A modification of this technique involves the excision from the cell of a patch of membrane containing the ACh receptor-channel complex so that drugs may be applied to either side of the membrane (42, 43). Similarly, the effects of changing the ionic milieu on channel blocking kinetics on either side of the membrane can be studied (44).

Single Channel Recording in Reconstituted Planar Lipid Bilayers

A complete comprehension of drug interaction with ACh-activated ionic channels requires a molecular understanding of the protein subunits that comprise the ACh receptor-channel complex, the influence of membrane

components on the complex, and the direct or indirect modification of the components of the system by drugs.

The ACh-receptor channel complex has been isolated and purified to reasonable homogeneity (45–50). Purified ACh receptors have been fused with lipid bilayers (51) to produce a defined yet functional system (52–57). Addition of an agonist to the ACh receptor-rich side of asymmetric lipid bilayers induces randomly occurring square pulses of current, similar to those recorded with the patch-clamp from denervated frog and rat muscle membrane (54). The dependence of channel open time on membrane potential and the agonist used is similar to that obtained from intact muscle membranes (54). The conductance through purified ACh receptors is blocked by the ACh receptor antagonists *d*-tubocurarine and α-BUTX (52–54), and channel open time is modified by ACh channel blocking agents (54, 57).

Biochemical Analysis of ACh Receptor-Channel Complexes

Postsynaptic inhibitors of neuromuscular transmission block either the ACh receptor recognition site(s) or the open and/or closed ACh ionic channel (58); some inhibitors interact with both receptor and channel (36, 59–65). The most specific drugs are α-BUTX for the ACh receptor sites and histrionicotoxin (HTX), HTX derivatives, and phencyclidine (PCP) for the ACh-activated ionic channel (58). If these agents are radiolabelled, they can be used as probes for either the ACh receptor or the associated channel.

These probes bind with a high affinity to membranes containing the richest known source of ACh receptors, the electric organ of the *Torpedo*. The binding of α-BUTX or ACh to the ACh receptor is not inhibited by HTX or PCP (58, 66), which suggests that the binding of these agents is not to the ACh recognition site of the receptor but to other site(s). Nicotinic agonists increase the binding of HTX or PCP as a function of agonist-concentration. This increased binding is inhibited by *d*-tubocurarine or α-BUTX which suggests that the increased binding in the presence of agonist is due to binding to open ACh-activated ionic channels (A_nR^* in equation 3) (58, 66). The specific binding of HTX and PCP that occurs in the absence of agonist may represent binding to closed ACh ionic channels (58, 66).

Many of the drugs that inhibit PCP or HTX binding to electric organ membranes, with the possible exception of the tricyclic antidepressants (22, 67), have also been shown to accelerate the decay of the e.p.c. or m.e.p.c. recorded from skeletal muscle, indicating that PCP or HTX binding may provide an additional way to study blockade of the ACh-activated ionic channel. For a more detailed description of the use of these radiolabelled ACh-activated ionic channel probes see (58, 66).

PROPERTIES OF THE ACETYLCHOLINE RECEPTOR-CHANNEL COMPLEX

The Acetylcholine Receptor

Knowledge of the molecular structure of the ACh receptor-channel complex is mainly derived from studies of the nicotinic ACh receptors from *Torpedo* membranes, which appear to be biochemically similar to those of mammalian muscle (68).

X-ray diffraction (69), neutron scattering (70), and electron microscopic (71–75) studies of ACh receptor-rich membranes reveal a transmembrane protein structure distributed asymmetrically and projecting 55 Å from the membrane surface on the synaptic side and 15 Å on the cytoplasmic side (69, 73, 75). The complex appears to be roughly cylindrical with an extracellular diameter of 85–90 Å and an overall length through the membrane of 110 Å (50, 69, 75). Electron micrographs of negatively stained ACh receptor-rich membranes reveal closely packed (10,000/μm^2) particles that presumably correspond to the ACh receptors. Each particle has the appearance of a rosette (made up of 5–6 subunits) with a central pit or hole (74).

The molecular weight of the monomer is approximately 250,000 daltons (47, 70, 73, 76) and SDS reducing polyacrylamide gel electrophoresis demonstrated that the complex consists of several subunits 40,000 (α), 50,000 (β), 60,000 (γ), and 65,000 (δ) in the ratio 2:1:1:1 respectively (47, 76). Without reduction this complex exists in the form of a dimer (77, 78). Each dimer appears to carry four α-toxin binding sites and one or two local anesthetic (channel blocking) sites (79, 80). Although it is not known on which subunit(s) these binding sites reside, at least some of the α-toxin sites appear to be on the α chain (81–83), and an agonist-induced increase of binding of some ACh ionic channel blockers to the δ subunit has been demonstrated (84).

Electrophysiological Properties of the Acetylcholine Receptor-Channel Complex

It is generally accepted that each ACh receptor-channel contains two ACh binding sites (85) (i.e. n in equation 3 is equal to 2) and that there is a higher probability of channel opening when both sites are occupied by agonist (86, 87).

The ACh-activated receptor channel can be described in terms of ion selectively, conductance, and open time. Ion selectivity is a function of the highest energy barrier that ions must cross to pass through the channel (the selectivity filter) (88). A measure of ion selectivity is the reversal potential (the membrane potential at which there is no net current flow through the ACh receptor-channel) for nicotinic agonist-induced responses (2, 89). The

reversal potential for the ACh receptor-channel at the frog neuromuscular junction is between 0 and −5mV (90–92). The effects of cation substitution on the ACh receptor-channel reversal potential suggest that Na^+, K^+, and to a lesser extent Ca^{2+} and Mg^{2+}, are the major permeant ions in normal salines (93–95). Ion substitution experiments demonstrate that ACh-activated receptor channels are relatively nonspecific in their cation selectivity, being permeable to all monovalent and divalent cations below 8 Å in diameter (96). Channel selectivity, as measured by reversal potential, appears independent both of the agonist used to activate the ACh-receptor channel and of the temperature (97).

Single channel conductance (γ) is dependent both on the selectivity filter and on how tightly ions bind to sites within the channel (89). If a permeant ion binds tightly inside the channel and other ions cannot pass it, then γ will decrease. Single channel conductance values of between 18 and 32 pS have been reported for normal muscle [see Table 2 in (31)] and are independent of membrane potential (14, 41). The single channel conductance of ACh receptor channel complexes at frog neuromuscular junction shows some dependence on temperature (14, 98); however, conflicting reports about the dependence of γ on temperature in cultured chick myotubes (99–101) and mammalian muscle (102, 103) exist.

Noise analysis studies suggest that γ may vary up to two-fold for frog junctional ACh receptor channels activated by different agonists (104). Other studies reported smaller (29, 38) or no difference (105) in γ values for different agonists. If γ is dependent on the agonist used to activate the ACh receptor channel, then a two-state model depicting a closed channel state of zero conductance and single open state of fixed conductance is an oversimplification. A patch-clamp study of a number of glutamate receptor agonists on single glutamate-receptor channels at the locust neuromuscular junction showed that γ was independent of the agonist used (106). A similar patch-clamp study of nicotinic agonists on the ACh receptor channel complex should resolve this question.

Patch-clamp analysis of single ACh-activated receptor channels of adult frog muscle confirmed that the elementary current is a pulse-like event of unitary amplitude [i.e. ACh opens ionic channels of only two conductance states (107)]; other studies utilizing embryonic rat muscle indicate that the ACh-activated receptor channel complex can adopt several conductance states (5, 108). Clearly the simple two-state model does not apply to all ACh-activated receptor channel complexes and is worthy of further investigation.

Channel open time can be defined as the time a channel conducts before closing and is dependent upon the agonist used to activate the receptor-channel complex, the temperature, and the muscle membrane potential.

Noise analysis, voltage jump, and patch-clamp studies demonstrate that channel open time varies considerably (up to 10-fold) with the agonist [see Table 2 in (31)]. Patch-clamp analysis of glutamate agonists on single glutamate-activated channels of locust muscle (in which receptor desensitization was inhibited by concanavalin A) showed that those agonists that activated channels for a relatively long period of time also activated the receptor more frequently, for a given concentration of agonist, than those agonists that activated the channel for a relatively short period of time (106). These observations, if confirmed for nicotinic agonists on ACh receptor channels, suggest a link between the ability of an agonist to activate the receptor and its ability to keep the channel in the open state once activated.

As stated above, the probability of ACh receptor channel activation is greater when two ACh binding sites are occupied. Trautmann & Feltz (109) reported that the two binding sites could be occupied by different agonists, but channel open time was determined by the agonist that produced the shorter channel open time. Such observations should be taken into account in any proposed model of agonist receptor activation.

Channel open time becomes longer at lower temperatures; the temperature dependence of channel open time is usually expressed in terms of a value of Q_{10} that is between 2 and 3 in amphibian muscle (91, 110).

E.p.c. and m.e.p.c. decay is also dependent on membrane potential becoming prolonged at more hyperpolarized potentials (91, 92, 110) according to the relationship:

$$\tau(V) = \tau(0) \cdot \exp^{(V/H)} \qquad 6.$$

where $\tau(V)$ is the exponential time constant of decay at holding potential V, $\tau(0)$ is the exponential time constant of decay at zero potential, and H is the change of holding potential required to produce an e-fold change of τ. A similar relationship has been observed for average channel open time using noise analysis (1, 14) and the exponential relaxation time constant from voltage jump experiments (28–30). The value of H can be determined from the slope of $\ln\tau(V)$ versus V. Values of H between 70 to 185 mV have been determined at the neuromuscular junction of mammals and amphibians (63, 97); these values are independent of the nicotinic agonist used (88).

A possible explanation for the dependence of ACh receptor channel open time on muscle membrane potential is that the ACh receptor channel protein embedded in the membrane undergoes a voltage-sensitive conformational change upon channel closing (31, 92), although alternative theories involving ion binding to a site inside (111) or outside (88) the channel have been considered.

Until relatively recently channel open time was determined using macroscopic techniques (concentration jump, voltage jump, and noise analysis); however, these techniques only allow the determination of mean channel open time. With the advent of the patch-clamp technique the direct measurement of single ACh receptor channel open times can be determined (26, 40, 41, 43, 101). Histograms of the distribution of many ACh receptor channel open times are well fitted to a single exponential and the time constant of this curve agrees well with that determined by the above macroscopic techniques. However, recent high resolution patch recordings of single ACh receptor channels from perisynaptic region of frog neuromuscular junction have shown that the ACh receptor channel appears to open and close several times during occupancy of the ACh receptor, i.e. it appears to oscillate between $A_nR \rightleftharpoons A_nR^*$ (equation 3) (107). Clearly, high resolution patch techniques should allow a better understanding of the interactions between ACh and its receptor channel complex.

ACTIONS OF DRUGS ON THE ACETYCHOLINE-ACTIVATED RECEPTOR-CHANNEL COMPLEX

Open Channel Block

The ACh-activated receptor channel complexes can be blocked or converted to a low conductance state by a number of drugs of diverse chemical structure. Most investigators explain such effects by an open channel blocking mechanism. This mechanism postulates that the cationic drug molecule, like any other ion, attempts to pass through the open ACh-activated channel. The drug interacts with the channel more strongly than other ions, dwelling inside the channel far longer, and blocking the channel to the ions that normally carry current. The most thoroughly studied drugs are the cationic local anesthetics. Steinbach (112) first reported that the local anesthetic lidocaine (xylocaine) caused the decay of endplate potentials (e.p.p.s) to become biphasic. Similarly the decay of m.e.p.c.s and e.p.c.s (7–13) becomes biphasic in the presence of some local anesthetics. The two phases are exponential with the initial fast component being faster than control and the subsequent slow component slower than control. The effects of local anesthetics on the time course of m.e.p.c. and e.p.c. decay can be described by the following kinetic scheme:

$$I(t) = I_1 \cdot \exp^{-r_s \cdot t} + I_2 \cdot \exp^{-r_f \cdot t} \qquad 7$$

where r_f is the rate constant of the fast component and r_s is the rate constant of the slow component (10–13). In the presence of a local anesthetic r_f is

greater than α, the normal rate constant of e.p.c. decay which in turn is greater than r_s. I_1 and I_2 are the contributions of the slow and fast components respectively to the total current I(t).

r_f and r_s, which can be determined experimentally from analysis of e.p.c. decay, are related to the rate constants of the sequential model (equation 3) by:

$$r_s = [\alpha + F + G \cdot c - \{(\alpha + F + G \cdot c)^2 - 4\ \alpha\ F\}^{1/2}]/2 \quad 8.$$

$$r_f = [\alpha + F + G \cdot c + \{(\alpha + F + G \cdot c)^2 - 4\alpha\ F\}^{1/2}]/2 \quad 9.$$

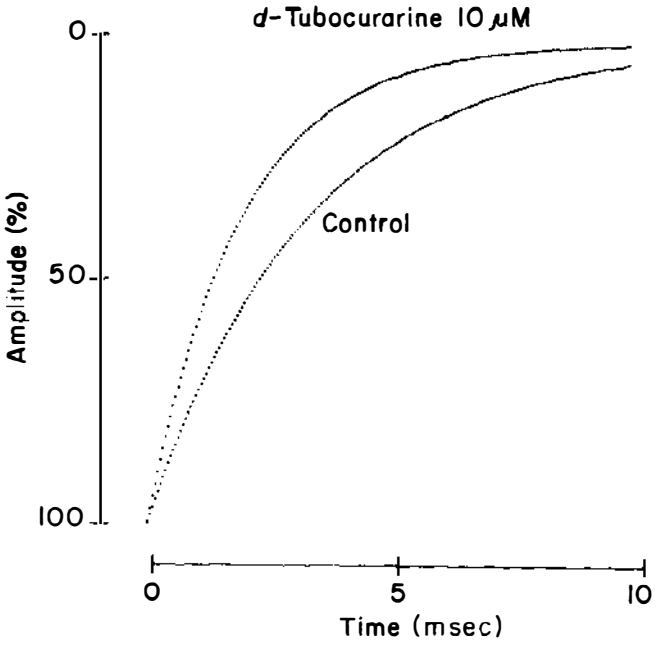
where c is the concentration of local anesthetic. Hence the channel blocking rate constant G and the channel unblocking rate constant F in equation 3 can be determined. Computer generated e.p.c. decay curves for *d*-tubocurarine and gallamine using equations 3, 8, and 9 are shown in Figure 2. The sequential blocking scheme (equation 3) is supported by noise analysis (12, 26), voltage jump (17), and analysis of ACh-activated single channel currents both in lipid bilayers (54, 57) and muscle membrane (26, 114).

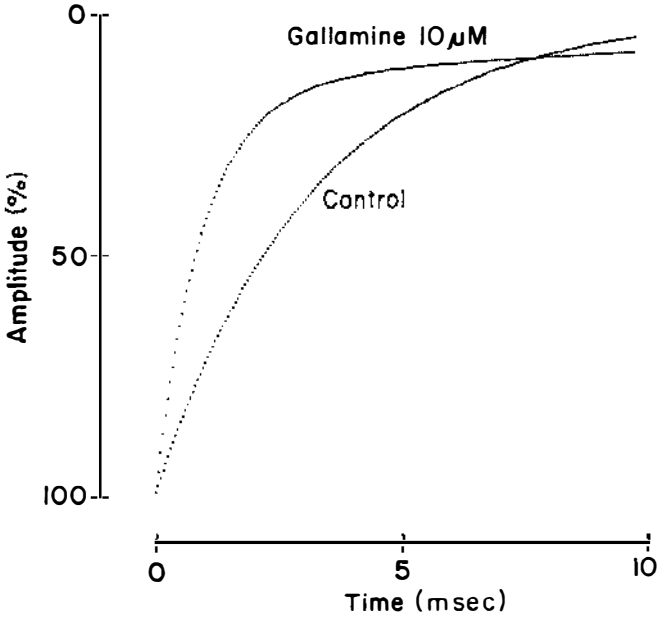
Patch-clamp analysis of single channel currents clearly shows the effect of local anesthetics on ACh-activated channels. Activation of the channels by ACh normally produces occasional square current pulses of random duration and constant amplitude. In the presence of the quaternary local anesthetic QX-222 the square pulse is broken up into a burst of rapid pulses which in terms of equation 3 can be interpreted as representing the blocking and unblocking of the ACh-activated channel by the QX-222 molecule (26) (see Figure 1). Patch-clamp analysis of single channels allows an independent estimate of F and G. From analysis of single channels the mean channel lifetime (t_o) in the presence of QX-222 can be determined. t_o can be described in terms of α, the normal channel closing rate, and G, the channel blocking rate, the rate constants leading away from the open state. At a given membrane potential:

$$t_o = (\alpha + G \cdot c)^{-1}, \quad 10.$$

and a plot of $1/t_o$ versus c yields a straight line the slope of which is equal to G and the intercept of which is equal to α. The value of G for QX-222 is $2 \times 10^7\ M^{-1}\ sec^{-1}$ at a membrane potential of –120 mV (26). This value is similar to that obtained for the channel-blocking effects of the local anesthetics QX-314 (26) and procaine (17) on ACh-activated channels, saxitoxin (STX) and tetrodotoxin (TTX) on Na^+ channels (115), and tetraethylammonium (TEA) on K^+ channels (116), which suggests that the binding reaction for all these drugs is diffusion-limited.

As the unblocking reaction (equation 3) is the only step leading away from the blocked state, an estimate of the unblocking rate (F) can be made





from the distribution of block time intervals (t_b) for a given membrane potential according to:

$$t_b = F^{-1}. \qquad 11.$$

Values of F determined in this way for QX-222 agree well with those determined by m.e.p.c. decay analysis (12, 13) and noise analysis (26).

Assuming that an m.e.p.c. is a superposition of single channel currents from many synchronously opening channels, alignment and addition of many single channels in the presence of QX-222 produced a simulated m.e.p.c. with a biphasic decay (26), similar to that observed experimentally. A number of drugs such as scopolamine (117), benzocaine (118), gallamine (61), lincomycin (119), and decamethonium (32) (see Table 1 at the end of this review) produce a biphasic decay of e.p.c.s or m.e.p.c.s similar to that produced by QX-222, which suggests similar channel blocking and unblocking rates. However, many drugs such as QX-314 (10, 26) produce a monotonic increase of the rate of e.p.c. decay (see Table 1) with no indication of a slow phase within the time course of the m.e.p.c. or e.p.c. Using the sequential blocking model (equation 3), such an effect can be explained if QX-314 has a relatively slow unblocking rate, i.e. $G >> F$. Consistent with this interpretation is the observation that single ACh-activated channels recorded in the presence of QX-314 did not show bursts of pulses as observed with QX-222 but an irregular sequence of very brief widely spaced pulses. Such a pattern of single channel events would be expected if the block interval was long compared to the open time (26). Analysis of single channel events in the presence of QX-314 using equations 10 and 11 show that G is similar for both drugs but that F is much slower for QX-314 (26).

Steinbach in his original study with lidocaine demonstrated, using pH changes, that the positively charged form of lidocaine was more effective in altering e.p.p. decay (112, 113). Similarly, for the positively charged local anesthetics the effects on m.e.p.c. and e.p.c. decay were more evident at hyperpolarized membrane potentials (10–13). Figure 3 shows the normal dependence of the exponential time constant of e.p.c. decay (τ) on membrane potential with τ becoming more prolonged at hyperpolarizing mem-

Figure 2. Computer-generated e.p.c. decay curves at a holding potential of −140 MV using the equations in schemes 3, 8, and 9 for 10 μM *d*-tubocurarine (left) and 10 μm gallamine (right). The decays are normalized for an amplitude of 100% using the following constants: *d*-tubocurarine, $G=2.4 \times 10^6 \exp^{(V/-62)}$ M^{-1} sec^{-1}, $F=3.7\exp^{(V/60)}$ sec^{-1}; gallamine, $G=1.21 \times 10^7 \cdot \exp^{(V/-89)}$ M^{-1} sec^{-1}, $F=1.04 \times 10^4 \exp^{(V/35)}$ sec^{-1}. These values are taken from Colquhoun et al (36) and Colquhoun & Sheridan (61); voltages are expressed in mV the value for k_{-1} was taken as $1 \times 10^3 \cdot \exp^{(V/123)}$ and was derived from unpublished data (N.N. Durant) collected from experiments using e.p.c.s. recorded from untreated transected frog muscle fibers.

brane potentials. In the presence of a positively charged quaternary drug that produces a monophasic shortening of the e.p.c. decay phase, such as the new nondepolarizing neuromuscular blocking agent Org. NC 45 (a derivative of pancuronium, also known as vecuronium) the normal dependence of τ on holding potential is disrupted. The depression of τ is most evident at –140 mV (Figure 3). For a drug that produces a biphasic decay of e.p.c.s or m.e.p.c.s, such as QX-222, τ_{fast} ($1/r_f$) becomes faster at more hyperpolarized membrane potentials; in contrast, τ_{slow} ($1/r_s$) becomes prolonged. In view of the positive charge on such molecules, it is reasonable to envisage that the electrostatic attraction of the drug for the ACh-activated channel will be greater at hyperpolarizing potentials, and in this way the blocking rate (G) increases with hyperpolarization and τ_{fast} becomes shortened. Similarly, the drug will bind to the channel far longer at hyperpolarizing potentials; so that the unblocking rate (F) decreases and τ_{slow} becomes prolonged. The voltage-dependent effects of charged local anesthetics have been confirmed by noise analysis (12), voltage jump (17), and patch-clamp studies (26). However, some uncharged molecules, e.g. barbiturates (120), prednisolone (121), and benzocaine (118) can produce block of the ACh-activated ionic channels and as might be expected, the blocking rate G, and unblocking rate F, of these compounds is independent of membrane potential.

Determination of the voltage-dependence of G and F for positively charged drugs can be used to characterize the location of the binding site within the channel. The equilibrium constant (K_D) for the drug binding reaction from equation 3 is given by:

$$K_D = F/G, \qquad 12.$$

and the dependence of K_D on membrane potential is given by:

$$K_D = K_D(V=0)\cdot \exp^{(z.f.e.V/kT)}, \qquad 13.$$

where V is the membrane potential, z the valency of the drug, f the fraction of the membrane field sensed by the drug, e the electronic charge, k the Boltzman constant, and T the absolute temperature (26, 122). Using this equation it has been calculated that QX-222 senses 78% of the membrane field (26), procaine 50% (17), *d*-tubocurarine either 40 or 80% (36) depending on whether one or two charges are involved, PCP methiodide and piperocaine 6% (123), and quinuclidinyl benzilate 12% (124). Hence, drugs differ in the membrane field sensed, which suggests different binding sites within the ACh-activated channel. Further evidence that channel-blocking agents do not all bind to the same site in the channel comes from studies

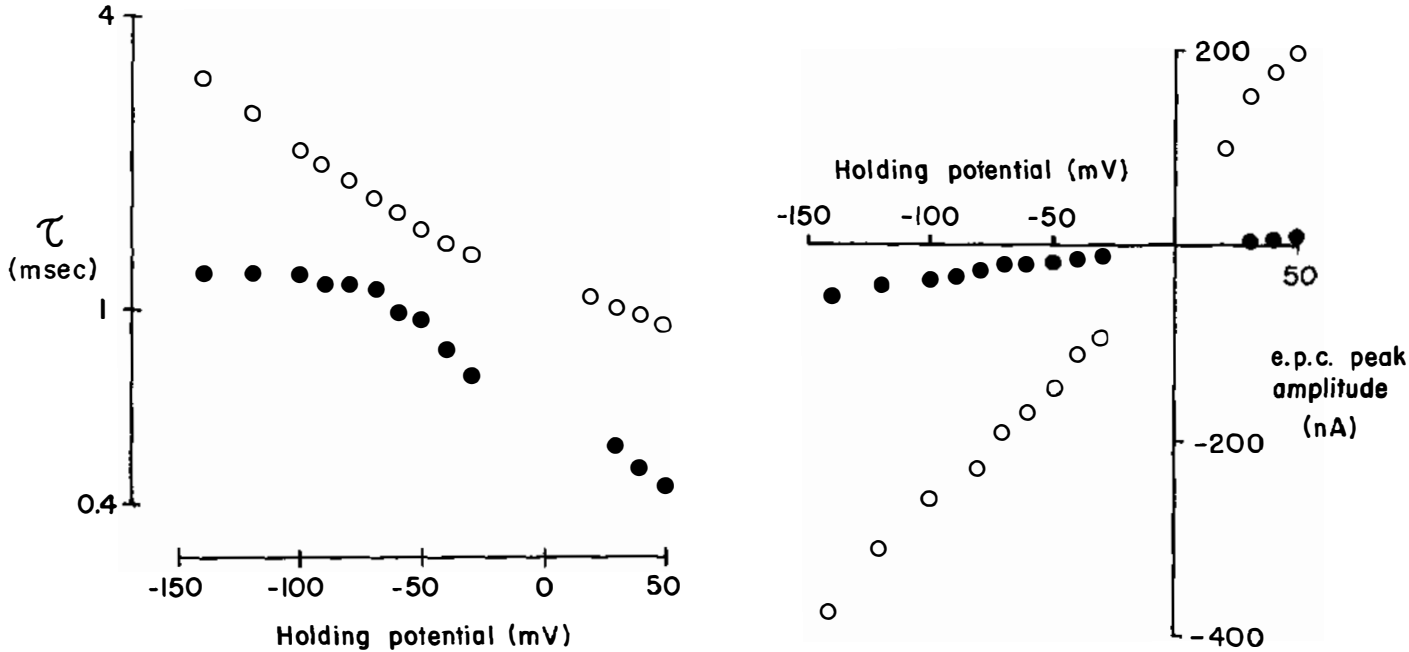


Figure 3 The effects of 15 μM Org. NC 45 (an analogue of pancuronium) on the exponential time constant of e.p.c. decay versus holding potential relationship (*left*), and the peak e.p.c. current versus holding potential relationship (right). Control e.p.c.s. (O) were recorded from the endplates of transected frog muscle (63). Org. NC 45 (●) increases the rate of e.p.c. decay and depresses the peak e.p.c. amplitude.

that apply the channel-blocking agent on the intracellular side of the membrane (123, 125).

Horn & Brodwick proposed a model of the structure of the channel based on the results of experiments in which QX-222 was applied to the intra- and extracellular surfaces of the membrane containing receptor channel complexes obtained from a rat myoball (125). Their model [see Figure 9 of (125)] consists of a funnel-shaped channel that is inserted into the cell membrane with a narrow aperture near the intracellular surface. Another feature of the model is the existence of two energy barriers located within the channel near the intra- and extracellular openings. The inactivity of intracellular applied QX-222 suggests that the drug is too large to fit completely through the channel and hence must enter from the extracellular side. The blockade of open channels by quaternized drug molecules and the voltage dependence of this effect is compatible with this model of the channel (125). Some drugs, such as PCP methiodide, are effective when applied to either side of the membrane, although their channel-blocking effects exhibit an opposite dependence on voltage (123). These results have led to the suggestion that the effects of such drugs on channels may be the mean result of actions on both the inside and the outside of the cell membrane.

If, as has been indicated above, positively charged drugs that block the ACh-activated channel interact at sites within the ACh-activated channel, it is possible that physiological cations might compete for the drug binding site. On the basis of evidence that Mg^{2+} attenuated the shortening of τ induced by lobeline, it was suggested that these two agents compete at a common channel binding site (62). Further studies have revealed that the decrease of τ produced by some drugs, such as lobeline and lidocaine, is attenuated by Mg^{2+} whereas the decrease of τ produced by other drugs, such as *d*-tubocurarine, atropine, and QX-314, is unaffected (126).

Other cations (e.g. Na^+ and Li^+) also appear to compete with QX-222 for its channel binding site (44). Similarly, agonist-induced binding by the ionic channel probe PCP to the ACh-activated receptor channels of *Torpedo* membranes is inhibited by Ca^{2+} ($IC_{50} = 0.7$ to 1 mM) and by Mg^{2+} ($IC_{50} \simeq 6$ mM) (127, 128). These results suggest that permeant ions and some channel-blocking drugs compete for an intra-channel binding site, although the effects of ionic strength on surface potential may be a complicating factor. Hence, for in vitro experiments consideration of the ionic composition of the bathing fluid is essential.

The Channel Blocking Actions of Other Drugs

A variety of drugs are now known to block the ACh-activated ionic channel (see Table 1); among them are the nondepolarizing neuromuscular blocking

drugs, whose main action is to bind and block the ACh receptor recognition site, and some agonists, which bind to the site and cause activation.

The channel-blocking activity of the neuromuscular blocking agent *d*-tubocurarine has been studied in some detail. This drug is normally regarded as a classical competitive antagonist of the nicotinic receptors at the neuromuscular junction (3). Initial work indicated that *d*-tubocurarine competitively blocked ACh receptors with no effect on the channel lifetime (37, 129), despite some earlier evidence that the drug shortened e.p.c. decay (130) and blocked ACh-activated channels in *Aplysia* neurons (131). Manalis (59) demonstrated that the e.p.c. produced by iontophoretic application of ACh at frog endplates was shortened by *d*-tubocurarine, and furthermore that *d*-tubocurarine produced a voltage-dependent decrease of peak e.p.c. amplitude, with this effect becoming more pronounced at hyperpolarizing potentials. These observations led to a "reexamination" of the action of *d*-tubocurarine by Katz & Miledi (60), who confirmed the voltage-dependent channel-blocking action of the drug; in contrast, this effect was not seen with α-BUTX.

Antagonism is usually described in terms of a shift of the log-dose response curve produced by an agonist (dose ratio). A pure competitive antagonist will have a dose ratio (r) described by the Schild equation:

$$r = 1 + C_B, \qquad 14.$$

where the normalized drug concentration C_B is defined as

$$C_B = x_B/K_B, \qquad 15.$$

where x_B is the antagonist concentration and K_B is the equilibrium constant. A pure competitive antagonist should produce a linear relationship between log (r–1) versus log x_B. While this relationship is linear with *d*-tubocurarine at a holding potential of –70 mV, it is not linear at –120 mV. This deviation is more evident with relatively high concentrations of *d*-tubocurarine (36). These results suggest that a voltage-dependent block of ion channels by *d*-tubocurarine contributes under these circumstances to its neuromuscular blocking action.

In contrast to the monotonic shortening of the neurally evoked e.p.c. decay produced by *d*-tubocurarine (60, 62), e.p.c. decay in the presence of another nondepolarizing agent, gallamine, is biphasic, which suggests that the unblocking rate of *d*-tubocurarine from the ACh-activated channel is far slower than that of gallamine (61). Our own studies have demonstrated that a variety of nondepolarizing neuromuscular blocking agents, including pancuronium, Org. NC 45, and Org. 6368, block the ACh-activated ionic channel (65, 132). Below we speculate on the contribution of blockade

of the ACh-receptor channel complex to the actions of these "receptor blockers" at the neuromuscular junction.

In the presence of decamethonium, m.e.p.c.s and e.p.c.s exhibit a biphasic decay, suggesting that decamethonium not only activates the receptor channel complexes but also blocks them (32, 133), an action confirmed by noise analysis and voltage jump techniques (32). Consistent with these observations is the finding that decamethonium displaces the binding of the ACh-activated ionic channel probe HTX (134). Blockade of the ACh-activated ionic channel is consistent with an explanation of the previous observation that radiolabelled decamethonium can penetrate the endplate membrane and that the conditions required for this penetration are those needed to open the endplate channels (135). Taken together these studies indicate that decamethonium can enter channels and pass through at concentrations similar to those needed for the channel-blocking action.

The question that then arises is whether channel block is a common feature of all nicotinic agonists at the neuromuscular junction. Nicotine, but not succinylcholine, has been shown to displace the ionic channel probe HTX from *Torpedo* membranes (134). The muscarinic agonist 4-(m-cholorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343) produced a monotonic shortening of the e.p.c. decay consistent with an action to block ACh-activated ionic channels, whereas the nicotinic agonist 1,1 dimethyl-4-phenylpiperazinium (DMPP) was without effect on e.p.c. decay (136). Taken together, these results suggest that blockade of ACh-activated channels is not a feature of all nicotinic agonists, but neither is it restricted to decamethonium alone. Patch-clamp analysis of single receptor channel complexes activated by different agonists should determine which agonists also block channels. Indeed, single channel records using suberyldicholine as the agonist do show burst-like kinetics similar to those observed in the presence of the channel-blocking agent QX-222 (26, 101) (see Figure 1). A more detailed analysis of this phenomenon has demonstrated that the burst-like behavior seen in the presence of this positively charged agonist is independent of membrane potential, which makes channel block an unlikely explanation (107). These closings within open events probably represent the channel opening and closing several times before agonist dissociation occurs.

It is tempting to invoke a block of ACh-activated ionic channels by agonist to explain such phenomena as partial agonism and ACh receptor desensitization. Certainly agonist channel block by agents such as decamethonium will complicate determination of agonist potency (defined as the "ability to activate the receptor"), but it remains to be shown if such a mechanism is common to all agonists classed as partial agonists. If ACh receptor desensitization can be explained by blockade of open channels,

then one would expect the mean open time of ACh-activated single channels to decrease as a function of increased ACh concentration. However, mean open time appears to be independent of ACh concentration; thus the open channel block hypothesis is unlikely (137).

In summary, these studies illustrate that some agonists, in addition to opening channels, can enter and block the channel. However, to invoke such a mechanism to explain such phenomena as partial agonism and desensitization seems highly speculative and in the latter case unlikely.

Closed Channel Block

The scheme of equation 3 shows the drug binding only to the open or ACh-activated receptor channel complex. For many drugs the predictions of this model agree well with experimental observations; however, for some drugs binding not only to the open conformation (A_nR^*) but also to the closed conformation (A_nR) of the ACh-activated receptor channel-complex has been proposed (17–22).

For such drugs the effects of closed channel block are seen in the peak e.p.c. amplitude versus voltage (I/V) relationship. This plot is usually linear in the absence of drug (see control in Figure 3). In the presence of some drugs, such as HTX (18), piperocaine (19), TEA (20), PCP (21), and some of the tricyclic antidepressant drugs (22), a marked nonlinearity of peak e.p.c. amplitude versus holding potential at hyperpolarized potentials is observed, with e.p.c. amplitude decreasing with increasing hyperpolarization. This effect could not be explained by the block of open channels (determined as a shortening of τ e.p.c.) which in some cases was negligible (22); instead, a voltage-dependent block of closed channels was proposed.

Essentially a similar conclusion was arrived at by Adams (17) to explain the effects of procaine on ACh-activated receptor channels using the voltage jump technique. He suggested that in addition to entering and blocking the open ACh-activated channel, procaine might also interact with the channel via a lipid pathway. Consistent with this proposal is the finding that procaine, in contrast to *d*-tubocurarine, gallamine, and QX-222, was an effective channel blocker when applied on the intracellular side of the endplate region (138). These investigators suggested that procaine penetrated the lipid membrane to access a channel binding site. It is not clear, however, whether this binding site is the same as that which is reached by quaternarized drugs via the lumen of the channel, or why modification of the ACh-activated channel via a lipid pathway should be voltage-dependent. In summary, it appears that the classification of channel blockers into those which block open channels, closed channels, or both may be an oversimplification of a complex system.

Agents That Affect Channels via a Nonspecific Action

The inhalation anesthetics and alcohols affect channel lifetime but in very different ways from the drugs described above. Although the actions of the inhalation anesthetics are not well understood, there is a close correlation between the blood levels expected to produce anesthesia and the concentration that increases the rate of m.e.p.p. (minature endplate potential) decay (139–142). For ether, chloroform, enflurane, and halothane the anesthetic concentrations that cause a 30% increase in the rate of m.e.p.c. decay at the neuromuscular junction correlate well with their lipid solubility (35, 139). Ether at high concentrations prolongs the decay of m.e.p.c.s, an effect attributed to its anticholinesterase activity (143). In contrast, high concentrations of halothane and enflurane cause biphasic e.p.c. decays (141), whereas octanol causes a shortening of e.p.c. decay (144). This rather diverse range of channel effects has led to the development of a model for the site of action of these agents. It was suggested that the membrane lipid is the important site of anesthetic action and that a change in the membrane fluidity may explain the observed effects on channel conductance, although a direct action on the channel protein cannot be ruled out (35). Electrophysiological evidence does not provide a completely clear understanding of the mechanism of action of the inhalation anesthetics.

Ethanol, in contrast to the inhalation anesthetics and octanol, prolonged m.e.p.c. decay, increased channel lifetime, and increased the postsynaptic response to acetylcholine (145–149). Similarly all the alcohols in the series ethanol to pentanol prolonged m.e.p.c. decay and increased channel lifetime, an action not due to anticholinesterase activity (145). It appears that these alcohols slow the rate of channel closing by modifying the lipid environment of the channels, possibly by increasing the membrane dielectric constant, which would alter the rate-limiting reaction involving membrane dipoles (35, 145). Specifically this effect might be achieved by the alcohols dissolving in the lipid layer and changing the dielectric constant of the receptor-channel complex (35) and, as for the inhalation anesthetics, the effect of these alcohols on channels correlates well with their membrane-buffer partition coefficients and solubilities (35).

IMPLICATIONS OF CHANNEL BLOCKADE

The Effect of Channel Blockers on Neuromuscular Transmission

Considering equation 3, whether or not channel blockade will effect neuromuscular transmission will depend on 1. the channel blocking rate (G) and unblocking rate (F); 2. the number of ACh receptors in the open or ac-

tivated state; and 3. the number of ACh receptors at the neuromuscular endplate (the safety factor for neuromuscular transmission).

1. The effects of the channel blocking and unblocking rate on neuromuscular blockade are discussed above.

2. At the neuromuscular junction many channel blockers can only bind and block the ionic channel when the ACh receptor is in the "open" or "active" (A_nR^*) conformation; consequently the number of ionic channels in the blocked state will be increased by any action which "opens" more channels. In view of the number of "spare" receptors at the neuromuscular junction (150, 151) it is unlikely that a channel-blocking action of a drug alone would produce a depression of single nerve evoked e.p.p.s. However, under conditions of relatively high frequency nerve stimulation receptors will be repetitively activated by ACh and a cumulative ionic channel block may occur, which results in a block of neuromuscular transmission.

It has been known for some time that nondepolarizing neuromuscular blocking drugs such as *d*-tubocurarine produce a fade of tetanic muscle tension (152, 153) and a run down of its electrophysiological counterparts, the e.p.p. or e.p.c. (154–156); these effects have been attributed to a prejunctional action of *d*-tubocurarine (155, 156). The observation that in addition to blocking the ACh recognition site of the receptor *d*-tubocurarine also blocks the ACh-activated ionic channel (36, 59, 60, 62) raises the possibility that endplate channel blockade may contribute to the effects of *d*-tubocurarine during high frequency nerve stimulation (36). It may be significant that α-BUTX, which does not produce tetanic fade (157), appears to bind to the ACh recognition site but not to the ACh-activated ionic channel (60). Although the idea that *d*-tubocurarine-induced tetanic fade is due to increased channel block is an attractive one, it does not account for the fact that neostigmine abolishes tetanic fade (152). Indeed it might be expected that the increased channel opening by excess ACh that would occur when acetylcholinesterase is inhibited would result in an increased channel block and an enhancement of tetanic fade.

In an attempt to assess the contribution of *d*-tubocurarine-induced channel block to e.p.c. amplitude run down, Magleby et al (156) investigated the effect of *d*-tubocurarine on trains of e.p.c.s recorded from voltage-clamped neuromuscular junctions of mouse, rat, and frog muscle. Channel block by *d*-tubocurarine is voltage-dependent, being more evident at more hyperpolarized membrane potentials (35, 60, 156), and if channel block by *d*-tubocurarine contributes to the run down of e.p.c.s then this effect should be more evident at hyperpolarized membrane potentials. The run down of e.p.c.s by *d*-tubocurarine was unaffected by holding potential (156), which suggests that cumulative ionic channel block does not contribute to the frequency dependent run down of e.p.c.s. by *d*-tubocurarine.

In contrast to these findings, the voltage-dependent ACh channel blockers HTX (158), trimetazidine (159), and trimethaphan (160) produced a voltage-dependent run down of e.p.c. amplitude at relatively high frequencies of nerve stimulation. Why was this effect not seen with *d*-tubocurarine? In a persuasive argument Magleby et al (156) point out that assuming a channel blocking rate constant of 0.012 $msec^{-1}$ in 1 μM *d*-tubocurarine (36), and assuming 200,000 postsynaptic channels to be open for 1 msec during an e.p.c., approximately 2,400 postsynaptic channels would be blocked by *d*-tubocurarine during the first e.p.c.; thus, assuming no channel unblocking during the train, less than 1% of the 10 million postsynaptic channels would be blocked by *d*-tubocurarine during 15–30 nerve impulses (frequency=100 sec^{-1}). Although the physiological role of prejunctional ACh receptors is controversial (161), the possibility exists that nondepolarizing muscle relaxants may produce tetanic fade and e.p.c. run down by blocking presynaptic ACh-activated ionic channels or receptors (162).

Exogenously applied agonists will cause the repetitive activation of ACh receptors. Under these conditions the effects of an "open" channel blocker on neuromuscular transmission should be evident. Simultaneous addition of carbachol and the ACh-activated ionic channel blocker ketamine (163) depressed the indirectly elicited twitch of the rat phrenic nerve-hemidiaphragm preparation (J. J. Lambert, unpublished observations). The block of neurotransmission was dependent upon the presence of both carbachol and ketamine. As ketamine has no effect on the ACh recognition site, but its binding to the ACh-activated ionic channel is greatly enhanced by the addition of carbachol (64, 163, 164), the simplest interpretation of this result is that carbachol activates a substantial number of the endplate ACh channels that are subsequently blocked by ketamine.

This effect has been clearly demonstrated for a variety of channel blockers by the iontophoretic double pulse technique (60, 120, 121). Iontophoretically applied agonist opens ionic channels (measured as an e.p.c.), allowing a drug to block a fraction of them. If applied shortly after the first pulse of agonist, a subsequent pulse will produce a reduced response (e.p.c.) as a number of channels are in a blocked state. As the time between pulses is increased the amplitude of the second pulse approaches that of the first; a plot of the depression of the second pulse amplitude versus time gives an exponential time course of recovery with a time constant corresponding to the unblocking rate F in equation 3.

Block of ACh-activated ionic channels provides a simple mechanism to explain the so-called enhancement of receptor desensitization that has been reported for a number of agents (165). However, recent work suggests (166) that the drug SKF-525A, an agent that facilitates ACh receptor desensitization, depresses the endplate response to iontophoretically applied agonist in

a frequency-dependent manner at concentrations that do not block ACh-activated ionic channels.

3. Experiments on the dose of a nondepolarizing neuromuscular blocking agent (such as *d*-tubocurarine) required to produce a depression of the indirectly elicited twitch of the cat tibialis-anterior muscle concluded that a large proportion of the receptors (in the order of 75%) had to be occluded before there was any depression of twitch (150). The margin of safety of neuromuscular transmission (150) suggests that under normal conditions it is unlikely that channel block alone, unless extremely marked, will produce a depression of muscle twitch. However, if the number of functional receptors at the endplate is substantially reduced by a neuromuscular blocking agent then the margin of safety will be narrowed and block of even a relatively small number of channels may produce a large depression of twitch height. The enhancement of neuromuscular block produced by nondepolarizing muscle relaxants in the presence of antibiotics (167) or inhalation anesthetics (168) may be explained by such considerations.

The prolonged use of succinylcholine in patients is accompanied by a change in the characteristics of the neuromuscular blockade. With time, the succinylcholine-induced muscle relaxation, instead of being depolarizing in nature ("phase I"), becomes more akin to that produced by a nondepolarizing neuromuscular blocking agent ("phase II") (for review see 169). Some of this change is more than likely due to desensitization, although this would not explain all the effects of "phase II" block. One possibility is that succinylcholine in common with decamethonium (32) enters the ACh-activated channels that it has opened and then causes blockade. Similarly, drugs that enter open channels will tend to do so more easily if the channels have been opened by a drug like succinylcholine; hence channel blockade by another agent might be expected to be more pronounced following succinylcholine. However, some evidence against an effect of succinylcholine on channels is the lack of effect of the drug on $\tau_{e.p.c.}$ (170).

It therefore seems likely that blockade of the ACh-activated ionic channels plays a role in the effects of some drugs at the neuromuscular junction, although the full extent of this role remains to be determined.

Channel Blockade at Other Synapses

There is now evidence that block of agonist-activated ionic channels by drugs is not restricted to the ACh receptor-channel complex of the neuromuscular junction. Investigation of the effects of drugs at other synapses has been limited by the requirement of a preparation suitable for electrophysiological techniques, such as voltage clamp. One such preparation is the neurons of the right pleural ganglion of *Aplysia californica* (111), which gives an excitatory response to ACh. The pharmacology of this response

differs from the ACh receptors of the motor endplate in a way that suggests that the ACh receptor mediating excitation in *Aplysia* neurons more closely resembles that of the vertebrate autonomic ganglionic nicotinic receptor than the nicotinic receptor of the neuromuscular junction. In a study of a variety of ACh antagonists (hexamethonium, *d*-tubocurarine, atropine, and procaine) it was found that the block of the ACh-induced current by these agents was voltage-dependent in a manner consistent with block of activated ACh receptor-channel complexes (23). These studies were extended to include the action of ACh antagonists on the parasympathetic neurons of the rat submandibular ganglion (24, 171). These studies concluded that *d*-tubocurarine, hexamethonium, and decamethonium appeared to act on the ACh-activated ionic channel rather than on the receptor, whereas the action of two other ganglion-blocking agents, trimethaphan and surugatoxin, was compatible with a receptor-blocking effect.

Open channel block does not appear to be restricted to receptor channel complexes activated by ACh. The neuromuscular blocking action of both the antibiotic streptomycin and the venom component, philanthus toxin (δ-PTX), of the solitary digger wasp (*Philanthus triangulum*) at locust neuromuscular junctions, where glutamate is thought to be the excitatory neurotransmitter, can best be explained by effects of these agents on open glutamate-activated ionic channels (25, 172).

CONCLUSION

As an experimental probe, the alteration of ACh receptor channel kinetics by ACh channel blockers has provided a better understanding of the ACh receptor channel complex.

The diverse chemical structures of the drugs that interact with the ACh ionic channel suggests that in comparison to the stereospecificity of the ACh recognition site of the receptor the ionic channel is relatively nonspecific. Agonists and antagonists may bind to a common recognition site on the ACh receptor; however, it appears that several channel blocker binding sites (distinct from the recognition site) exist.

The safety factor for neuromuscular transmission makes it unlikely that ACh channel blockade alone will block neuromuscular transmission, although in some cases it may provide an explanation for drug interactions and drug side effects.

Block of neurotransmitter-activated channels by several agents at other synapses has been observed, although the contribution of channel blockade by these agents to their therapeutic and pharmacological effects remains to be determined.

Table 1 Drugs that affect e.p.c. or m.e.p.c. decay via an action on the ACh-activated ionic channel

Shortened exponential decay	Biphasic exponential decay
Local anesthetics	
QX–314 (10, 11)	QX–222 (10–13, 173)
	Benzocaine (118)
	Procaine (7, 15, 173–75)
	Lidocaine (173)
Nondepolarizing neuromuscular blocking agents	
d-Tubocurarine (36, 59, 60, 62, 156, 176, 177)	Gallamine (60, 61, 65)
	Pancuronium (60, 65)
Antimuscarinic agents	
Atropine (98, 117, 178)	Atropine (173)
	Scopolamine (117, 173)
Quinuclidinyl benzilate (124)	Methscopolamine (173)
Ditran (64, 163)	
Intravenous anesthetics	
Pentobarbital (140, 142)	Pentobarbital (173)
Thiopental (120, 140, 142)	Methyprylone (120)
Methohexitone (120)	
Phencyclidine (21, 179)	
Ketamine (142, 163, 180)	
Althesin (142)	
Phenytoin (181)	Phenytoin (173)
Propanidid (142)	
Diazepam (142)	
Antibiotics	
Clindamycin (119)	Clindamycin (173)
	Lincomycin (119)
Polymyxin B (182)	Streptomycin (173)
Antiviral agent	
Amantadine (183)	
Analgesics	
	Morphine (173)
	Naloxone (173)
Anticholinesterases	
Neostigmine[a] (184)	Physostigmine[a] (184)
	Diisopropylfluorophosphate (185)
Ganglion blocking agents	
Trimethaphan (160)	Hexamethonium (133)
Tetraethylammonium (20)	
Agonists	
McN-A-343 (136)	Decamethonium (32, 133)
Lobeline[b] (62)	
Ligands for channels	
Histrionicotoxin (18, 158)	
Perhydrohistrionicotoxin (186)	
Quinacrine (187, 188)	

Table 1 *(Continued)*

Shortened exponential decay	Biphasic exponential decay
Inhalation anesthetics	
Anesthetic concentrations of: halothane (139, 141) chloroform & enflurane (141)	High concentrations of: Halothane & enflurane (141)
Ether (139, 141, 143, 189)	
Methoxyflurane (189)	
Fluothyl (189)	
Central stimulants	
3M2B (190)	
DBE (190)	
Alcohols	
Octanol (144)	
Prolonged exponential decay	
Alcohols	
Ethanol (173)	
Ethanol-to-hexanol (145)	
Central stimulant	
Bemegride (190)	

[a] This effect was only observed at high concentrations.
[b] A ganglionic agonist.

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Literature Cited

1. Wray, D. 1980. Noise analysis and channels at the postsynaptic membrane of skeletal muscle. *Prog. Drug Res.* 24:9–56
2. Takeuchi, A., Takeuchi, N. 1960. On the permeability of end-plate membrane during the action of transmitter. *J. Physiol.* 154:52–67
3. Jenkinson, D. H. 1960. The antagonism between tubocurarine and substances which depolarize the motor endplate, *J. Physiol.* 152:309–24
4. Jackson, M. B., Lecar, H., Askanas, V., Engel, W. K. 1982. Single cholinergic receptor channel currents in cultured human muscle. *J. Neurosci.* 2:1465–73
5. Trautmann, A. 1982. Curare can open and block ionic channels associated with cholinergic receptors. *Nature* 298: 272–75
6. Lee, C. Y., Tseng, L. F., Chiu, T. H. 1967. Influence of denervation on localization of neurotoxins from elapid venoms in rat diaphragm. *Nature* 215: 1177–78
7. Kordaš, M. 1970. The effect of procaine on neuromuscular transmission. *J. Physiol.* 209:689–99
8. Deguchi, T., Narahashi, T. 1971. Effects of procaine on ionic conductances of end-plate membranes. *J. Pharmacol. Exp. Ther.* 176:423–33
9. Maeno, T., Edwards, C., Hashimura, S. 1971. Difference in effects on endplate potentials between procaine and lidocaine as revealed by voltage clamp experiments. *J. Neurophysiol.* 34:32–46
10. Beam, K. G. 1976. A voltage-clamp study of the effect of two lidocaine de-

rivatives on the time course of end-plate currents. *J. Physiol.* 258:279–300
11. Beam, K. G. 1976. A quantitative description of end-plate currents in the presence of two lidocaine derivatives. *J. Physiol.* 258:301–22
12. Ruff, R. L. 1977. A quantitative analysis of local anaesthetic alteration of minature end-plate currents and end-plate current fluctuations. *J. Physiol.* 264:89–124
13. Ruff, R. L. 1982. The kinetics of local anesthetic blockade of end-plate channels. *Biophys. J.* 37:625–31
14. Anderson, C. R., Stevens, C. F. 1973. Voltage clamp analysis of acetylcholine produced end-plate current fluctuations [redacted] neuromuscular junction. *J.* 235:665–91
15. Adams, P. R. 1975. A model for the procaine end-plate current. *J. Physiol.* 246:61–63P
16. Blackman, J. G. 1970. Dependence on membrane potential of the blocking action of hexamethonium at a sympathetic ganglionic synapse. *Proc. Univ. Otago Med. Sch.* 48:4–5
17. Adams, P. R. 1977. [redacted]ump analysis of procaine acti[redacted] end-plate *J. Physiol.* 268:291–318
18. Masukawa, L. M., Albuquerque, E. X. 1978. Voltage- and time-dependent action of histrionicotoxin on the endplate current of the frog muscle. *J. Gen. Physiol.* 72:351–67
19. Tiedt, T. N., Albuquerque, E. X., Bakry, N. M., Eldefrawi, M. E., Eldefrawi, A. T. 1979. Voltage- and time-dependent actions of piperocaine on the ion channel of the acetylcholine r ceptor. *Mol. Pharmacol.* 16:909–21
20. Adler, M., Oliveira, A. C., Albuquerque, E. X., Mansour, N. A., Eldefrawi, A. T. 1979. Reaction of tetraethylammonium with the open and closed conformations of the acetycholine receptor ionic channel complex. *J. Gen. Physiol.* 74:129–52
21. Albuquerque, E. X., Tsai, M.-C., Aronstam, R. S., [redacted] B., Eldefrawi, A. T., Eldefraw[redacted] 1980. Phencyclidine interactions with the ionic channel of the acetylcholine receptor and electric membrane. *Proc. Natl. Acad. Sci. USA* 77:1224–28
22. Schofield, G. G., Witkop, B., Warnick, J. E., Albuquerque, E. X. 1981. Differentiation of the open and closed states of the ionic channels of nicotinic acetylcholine receptors by tricyclic antidepressants. *Proc. Natl. Acad. Sci. USA* 78:5240–44
23. Ascher, P., Marty, A., Neild, T. O. 1978. The mode of action of antagonists of the excitatory response to [redacted] line in *Aplysia* neurones. *J.* [redacted] 278:207–35
24. Rang, H. P. 1982. The action of ganglionic blocking drugs on the synaptic responses of rat submandibular ganglion cells. *Br. J. Pharmacol.* 75:151–68
25. Gration, K. A. F., Lambert, J. J., Usherwood, P. N. R. 1980. Glutamate-activated channels in locust muscle. *Adv. Physiol. Sci.* 20:377–83
26. Neher, E., Steinbach, J. H. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.* 277:153–76
27. Lester, H. A., Nerbonne, J. M. 1982. Physiological and pharmacological manipulations with light flashes. *Ann. Rev. Biophys. Bioeng.* 11:151–75
28. Adams, P. R. 1975. Kinetics of conductance changes during hyperpolarization at frog endplates. *Br. J. Pharmacol.* 53:308–10
29. Neher, E., Sakmann, B. 1975. Voltage-dependence of drug-induced conductance in frog neuromuscular junction. *Proc. Natl. Acad. Sci. USA* 72:2140–44
30. Adams, P. R. 1977. Relaxation experiments using bath-applied suberyldicholine. *J. Physiol.* 268:271–89
31. Colquhoun, D. 1979. The link between drug binding and response: theories and observations. In *The Receptors. A Comprehensive Treatise,* ed. R. D. O'Brien, pp. 93–142. New York: Plenum
32. Adams, P. R., Sakmann, B. 1978. Decamethonium both opens and blocks endplate channels. *Proc. Natl. Acad. Sci. USA* 75:2994–98
33. Katz, B., Miledi, R. 1970. Membrane noise produced by acetylcholine. *Nature* 226:962–63
34. Katz, B., Miledi, R. 1971. Further observations on acetylcholine noise. *Nature New Biol.* 232:124–26
35. Gage, P. W., Hamill, O. P. 1981. Effects of anesthetics on ion channels in synapses. *Int. Rev. Physiol.* 25:1–45
36. Colquhoun, D., Dreyer, F., Sheridan, R. E. 1979. The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol.* 293:247–84
37. Katz, B., Miledi, R. 1973. The binding of acetylcholine to receptors and its r - moval from the synaptic cleft. *J. Physiol.* 231:549–74
38. Neher, E., Sakmann, B. 1976. Noise analysis of drug induced voltage clamp currents in denervated frog muscle fibres. *J. Physiol.* 258:705–29

39. Colquhoun, D., Large, W. A., Rang, H. P. 1977. Analysis of the action of a false transmitter at the neuromuscular junction. *J. Physiol.* 266:361–95
40. Neher, E., Sakmann, B. 1976. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* 260:799–802
41. Neher, E., Sakmann, B., Steinbach, J. H. 1978. The extracellular patch clamp: a method for resolving currents through individual open channels in biological membranes. *Pflügers Arch.* 375:219–28
42. Horn, R., Patlak, J. 1980. Single channel currents from excised patches of muscle membrane. *Proc. Natl. Acad. Sci. USA* 77:6930–34
43. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., Sigworth, F. J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391:85–100
44. Redmann, G. A., Clark, R. B., Adams, P. R. 1982. Single acetylcholine channel block in elevated sodium and lithium. *Biophys. J.* 37:324a
45. Lindstrom, J., Anholt, R., Einarson, B., Engel, A., Osame, M., Montal, M. 1980. Purification of acetylcholine receptors, reconstitution into lipid vesicles, and study of agonist-induced cation channel regulation. *J. Biol. Chem.* 255:8340–50
46. Sobel, A., Weber, M., Changeux, J.-P. 1977. Large-scale purification of the acetylcholine-receptor protein in its membrane-bound and detergent extracted forms from *Torpedo marmorata* electric organ. *Eur. J. Biochem.* 80: 215–24
47. Neubig, R. R., Krodel, E. K., Boyd, N. P., Cohen, J. B. 1979. Acetylcholine and local anesthetic binding to torpedo nicotinic postsynaptic membrane after removal of nonreceptor peptides. *Proc. Natl. Acad. Sci. USA* 76:690–94
48. Raftery, M., Blanchard, S., Elliott, J., Hartig, P., Moore, H.-P., et al. 1979. Properties of *Torpedo californica* acetylcholine receptor. In *Neurotoxins: Tools in Neurobiology*, ed. B. Ceccarelli, F. Clementi, pp. 159–82. New York: Raven
49. Barrantes, F. J. 1979. Endogenous chemical receptors: some physical aspects. *Ann. Rev. Biophys. Bioeng.* 8:287–321
50. Raftery, M. A., Changeux, J.-P. 1982. The nicotinic acetylcholine receptor (AChR). *Neurosci. Res. Prog. Bull.* 20:277–301
51. Montal, M., Mueller, P. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA* 69:3561–66
52. Nelson, N., Anholt, R., Linstrom, J., Montal, M. 1980. Reconstitution of purified acetylcholine receptors with functional ion channels in planar bilayers. *Proc. Natl. Acad. Sci. USA* 77:3057–61
53. Schindler, H., Quast, U. 1980. Functional acetylcholine receptor from *Torpedo marmorata* in planar membranes. *Proc. Natl. Acad. Sci. USA* 77:3052–56
54. Boheim, G., Hanke, W., Barrantes, F. J., Eibl, H., Sakmann, B., et al. 1981. Agonist-activated ionic channels in acetylcholine receptor reconstituted into planar lipid bilayers. *Proc. Natl. Acad. Sci. USA* 78:3586–90
55. Hanke, W., Eibl, H., Boheim, G. 1981. A new method for reconstitution: fusion of protein-containing vesicles with planar bilayer membranes below lipid phase transition temperature. *Biophys. Struct. Mech.* 7:131–37
56. Anholt, R. 1981. Reconstitution of acetylcholine receptors in model membranes. *Trends Biochem. Sci.* 6:288–91
57. Labarca, P., Linstrom, J., Montal, M. 1982. Studies on the properties of the purified acetylcholine receptor reconstituted in planar lipid bilayers. *Biophys. J.* 37:170a
58. Eldefrawi, M. E., Eldefrawi, A. T. 1980. Coupling between the nicotinic receptor site and the ionic channel site. *Ann. NY Acad. Sci.* 358:239–52
59. Manalis, R. S. 1977. Voltage-dependent effect of curare at the frog neuromuscular junction. *Nature* 267:366–68
60. Katz, B., Miledi, R. 1978. A re-examination of curare action at the motor endplate. *Proc. R. Soc. London B* 203: 119–33
61. Colquhoun, D., Sheridan, R. E. 1981. The modes of action of gallamine. *Proc. R. Soc. London B* 211:181–203
62. Lambert, J. J., Volle, R. L., Henderson, E. G. 1980. An attempt to distinguish between the actions of neuromuscular blocking drugs on the acetylcholine receptor and on its associated ionic channel. *Proc. Natl. Acad. Sci. USA* 77:5003–7
63. Lambert, J. J., Durant, N. N., Reynolds, L. S., Volle, R. L., Henderson, E. G. 1981. Characterization of end-plate conductance in transected frog muscle: modification by drugs. *J. Pharmacol. Exp. Ther.* 216:62–69

64. Smith, C. J., Epstein, P. M., Lambert, J. J., Volle, R. L., Henderson, E. G. 1982. Comparative aspects of nicotinic receptor blockade by ketamine, ditran and lobeline. *Fed. Proc.* 41:1112
65. Durant, N. N., Lambert, J. J., Katz, R. L. 1981. The effects of nondepolarizing neuromuscular blocking agents on endplate currents. *Fed. Proc.* 40:263
66. Eldefrawi, M. E., Eldefrawi, A. T., Aronstam, R. S., Maleque, M. A., Warnick, J. E. Albuquerque, E. X. 1980. (^{3}H)Phencyclidine: a probe for the ionic channel of the nicotinic receptor. *Proc. Natl. Acad. Sci. USA* 77:7458–62
67. Shaker, N., Eldefrawi, A. T., Miller, E. R., Eldefrawi, M. E. 1981. Interaction of tricyclic antidepressants with the ionic channel of the acetylcholine receptor of *Torpedo* electric organ. *Mol. Pharmacol.* 20:511–18
68. Lindstrom, J., Walter, B., Einarson, B. 1979. Immunochemical similarities between subunits of acetylcholine receptors from *Torpedo, Electrophorus* and mammalian muscle. *Biochemistry* 18: 4470–80
69. Ross, M. J., Klymkowsky, M. W., Agard, D. A., Stroud, R. M. 1977. Structural studies of a membrane-bound acetylcholine receptor from *Torpedo californica. J. Mol. Biol.* 116: 635–59
70. Wise, D. S., Karlin, A., Schoenborn, B. P. 1979. An analysis by low-angle neutron scattering of the structure of the acetylcholine receptor from *Torpedo californica* in detergent solution. *Biophys. J.* 28:473–96
71. Rosenbleuth, J. 1975. Synaptic membrane structure in *Torpedo* electric organ. *J. Neurocytol.* 4:697–712
72. Cartaud, J., Benedetti, L. E., Sobel, A., Changeux, J. -P. 1978. A morphological study of the cholinergic receptor protein from *Torpedo marmorata* in its membrane environment and its detergent-extracted purified form. *J. Cell Sci.* 29:313–37
73. Klymkowsky, M. W., Stroud, R. M. 1979. Immunospecific identification and three-dimensional structure of a membrane-bound acetylcholine receptor from *Torpedo californica. J. Mol. Biol.* 128:319–34
74. Heuser, J. E., Salpeter, S. R. 1979. Organization of acetylcholine receptors in quick-frozen, deep-etched, and rotatory-replicated *Torpedo* postsynaptic membrane. *J. Cell Biol.* 82:150–73
75. Kistler, J., Stroud, R. M., Klymkowsky, M. W., Lalancette, R. A., Fairclough, R. H. 1982. Structure and function of an acetylcholine receptor. *Biophys. J.* 371–83
76. Moore, H. -P. H., Hartig, P. R., Raftery, M. A. 1979. Correlation of polypeptide composition with functional events in acetylcholine receptor-enriched membranes from *Torpedo californica. Proc. Natl. Acad. Sci. USA* 76: 6265–69
77. Chang, H. W., Bock, E. 1977. Molecular forms of acetylcholine receptor. Effects of calcium ions and a sulfhydryl reagent on the occurence of oligomers. *Biochemistry* 16:4513–20
78. Hamilton, S. L., McLaughlin, M., Karlin, A. 1979. Formation of disulfide-linked oligomers of acetylcholine receptor in membrane from Torpedo electric tissue. *Biochemistry* 18:155–63
79. Krodel, E. K., Beckman, R. A., Cohen, J. B. 1979. Identification of a local anesthetic binding site in nicotinic postsynaptic membranes isolated from *Torpedo marmorata* electric tissue. *Mol. Pharmacol.* 15:294–312
80. Elliott, J., Raftery, M. A. 1979. Binding of perhydrohistrionicotoxin to intact and detergent-solubilized membranes enriched in nicotinic acetylcholine receptor. *Biochemistry* 18:1868–74
81. Witzemann, V., Raftery, M. A. 1978. Affinity directed crosslinking of acetylcholine receptor polypeptide components in post-synaptic membranes. *Biochem. Biophys. Res. Commun.* 85: 623–31
82. Nathanson, N. M., Hall, Z. W. 1979. Subunit structure and peptide mapping of junctional and extrajunctional acetylcholine receptors from rat muscle. *Biochemistry* 18:3392–3401
83. Hamilton, S., McLaughlin, M., Karlin, A. 1978. Cross-linking of the acetylcholine receptor from *Torpedo* electric tissue. *Fed. Proc.* 37:529
84. Oswald, R., Changeux, J. -P. 1981. Ultraviolet light-induced labeling by noncompetitive blockers of the acetylcholine receptor from *Torpedo marmorata. Proc. Natl. Acad. Sci. USA* 78:3925–29
85. Karlin, A. 1980. Molecular properties of nicotinic acetylcholine receptors. In *Cell Surface and Neuronal Function,* ed. C. W. Cotman, G. Poste, G. L. Nicolson. pp. 191–260. New York: Elsevier/North Holland. 546 pp.
86. Dreyer, F., Peper, K., Sterz, R. 1978. Determination of dose-response curves by quantitative ionophoresis at the frog neuromuscular junction. *J. Physiol.* 281:395–419

87. Dionne, V. E., Steinbach, J. H., Stevens, C. F. 1978. An analysis of the dose-response relationship at voltage-clamped neuromuscular junctions. *J. Physiol.* 281:421–44
88. Steinbach, J. H. 1980. Activation of nicotinic acetylcholine receptors. See Ref. 85, pp. 119–56
89. Lewis, C. A. 1979. Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Physiol.* 286:417–45
90. Kordaš, M. 1969. The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. *J. Physiol.* 204:493–502
91. Magleby, K. L., Stevens, C. F. 1972. The effect of voltage on the time course of end-plate currents. *J. Physiol.* 223: 151–71
92. Magleby, K. L., Stevens, C. F. 1972. A quantitative description of end-plate currents. *J. Physiol.* 223:173–97
93. Takeuchi, N. 1963. Some properties of conductance changes at the endplate membrane during the action of acetylcholine. *J. Physiol.* 167:128–40
94. Takeuchi, N. 1963. Effects of ca cium on the conductance change of the end-plate membrane during the action of transmitter. *J. Physiol.* 167:141–55
95. Ritchie, A., Fambrough, D. M. 1975. Ionic properties of the acetylcholine receptor in cultured rat myotubes. *J. Gen. Physiol.* 65:751–67
96. Adams, D. J., Dwyer, T. M., Hille, B. 1980. The permeability of endplate channels to monovalent and divalent metal cations. *J. Gen. Physiol.* 75:493–510
97. Steinbach, J. H., Stevens, C. F. 1976. Neurmuscular transmission. In *Frog Neurobiology,* ed. R. Llinás, W. Precht. pp. 33–92. Berlin:Springer-Verlag
98. Feltz, A., Large, W. A., Trautmann, A. 1977. Analysis of atropine action at the frog neuromuscular junction. *J. Physiol.* 269:109–30
99. Sachs, F., Lecar, H. 1977. Acetylcholine-induced fluctuations in tissue-cultured muscle cells under voltage clamp. *J. Gen Physiol.* 17:129–43
100. Fischbach, G. D., Lass, Y. 1978. A transition temperature for acetylcholine channel conductance in chick myoballs. *J. Physiol.* 280:527–36
101. Nelson, D. J., Sachs, F. 1979. Single ionic channels observed in tissue cultured muscle. *Nature* 282:861–63
102. Dreyer, F., Mü ler, K. -D., Peper, K., Sterz, R. 1976. The M. omohyoideus of the mouse as a convenient mammalian muscle preparation. *Pflügers Arch.* 367: 115–22
103. Bevan, S., Kullberg, R. W., Rice, J. 1979. An analysis of cell membrane noise. *Ann. Stat.* 7:237–57
104. Colquhoun, D., Dionne, V. E., Steinbach, J. H., Stevens, C. F. 1975. Conductance of channels opened by acetylcholine-like drugs in muscle end-plate. *Nature* 253:204–6
105. Dreyer, F., Walther, C., Peper, K. 1976. Junctional and extrajunctional acetylcholine receptors in normal and denervated frog muscle fibres: noise analysis experiments with different agonists. *Pflügers Arch.* 366:1–9
106. Gration, K. A. F., Lambert, J. J., Ramsey, R. L., Rand, R. P., Usherwood, P. N. R. 1981. Agonist potency by patch clamp analysis of single glutamate receptors. *Brain Res.* 20:400–5
107. Colquhoun, D., Sakmann, B. 1981. Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. *Nature* 294:464–66
108. Hamill, O. P., Sakmann, B. 1981. Multiple conductance states of sing e acetylcholine receptor channels in embryionic muscle cells. *Nature* 294:462–64
109. Trautmann, A., Feltz, A. 1980. Open time of channels activated by binding of two distinct agonists. *Nature* 286: 291–93
110. Gage, P. W., McBurney, R. N. 1975. Effects of membrane potential, temperature and neostigmine on the conductance change caused by a quantum of acetylcholine at the toad neuromuscular junction. *J. Physiol.* 244:385–407
111. Ascher, P., Marty, A., Neild, T. O. 1978. Life time and elementary conductance of the channels mediating the excitatory effects of acetylcholine in *Aplysia* neurones. *J. Physiol.* 278:177–206
112. Steinbach, A. B. 1968. Alteration by xylocaine (lidocaine) and its derivatives of the time course of the end plate potential. *J. Gen Physiol.* 52:144–61
113. Steinbach, A. B. 1968. A kinetic model for the action of xylocaine on receptors for acetylcholine. *J. Gen. Physiol.* 52: 162–80
114. Ashford, M. L. J., Henderson, E. G., Lambert, J. J., Reynolds, L. S., Volle, R. L. 1982. Hemicholinium-3 blocks currents through single acetylcholine-receptor channels. *Br. J. Pharmacol.* 76:185P
115. Wagner, H. -H., Ulbricht, W. 1975. The rates of saxitoxin action and of saxitox-

in-tetrodotoxin interaction at the node of Ranvier. *Pflügers Arch.* 364:297–315
116. Armstrong, C. M. 1966. Time course of TEA^+-induced anomalous rectification in squid giant axons. *J. Gen. Physiol.* 50:491–503
117. Adler, M., Albuquerque, E. X. 1976. An analysis of the action of atropine and scopolamine on the end-plate current of frog sartorius muscle. *J. Pharmacol. Exp. Ther.* 196:360–72
118. Ogden, D. C., Siegelbaum, S. A., Colquhoun, D. 1981. Block of acetylcholine-activated ion channels by an uncharged local anesthetic. *Nature* 289:596–98
119. Fiekers, J. F., Marshall, I. G., Parsons, R. L. 1979. Clindamycin and lincomycin alter miniature endplate current decay. *Nature* 281:680–82
120. Adams, P. R. 1976. Drug blockade of open end-plate channels. *J. Physiol.* 260:531–52
121. Dreyer, F., Peper, K., Sterz, R., Bradley, R. J., Müller, K. -D. 1979. Drug-receptor interaction at the frog neuromuscular junction. *Prog. Brain Res.* 49:213–23
122. Woodhull, A. M. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* 61:687–708
123. Aguayo, L. G., Pazhenchevsky, B., Daly, J. W., Albuquerque, E. X. 1981. The ionic channels of the acetylcholine receptor: regulation by sites outside and inside the cell membrane which are sensitive to quaternary ligands. *Mol. Pharmacol.* 20:345–55
124. Schofield, G. G., Warnick, J. E. Albuquerque, E. X. 1981. Elucidation of the mechanism and site of action of quinuclidinyl benzilate (QNB) on the electrical excitability and chemosensitivity of the frog sartorius muscle. *Cell. Mol. Neurobiol.* 1:209–30
125. Horn, R., Brodwick, M. S. 1980. Acetylcholine-induced current in perfused rat myoballs. *J. Gen. Physiol.* 75:297–321
126. Henderson, E. G., Lambert, J. J., Reynolds, L. S. 1980. Protection by Mg^{++} of acetylcholine activated ionic channel blockade by lidocaine and lobeline in transected frog muscle. *Soc. Neurosci. Abstr.* 6:780
127. Epstein, P. M., Smith, C. J., Henderson, E. G., Lambert, J. J. 1982. Antagonism of phencyclidine binding to *Torpedo* electroplaque membrane by divalent cations. In preparation.
128. McKay, D. B., Carrier, G. O., Aronstam, R. S. 1980. Influence of calcium on ligand binding to the ion channel associated with the nicotinic acetylcholine receptor. *Soc. Neurosci. Abstr.* 6:298
129. Katz, B., Miledi, R. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol.* 224:665–99
130. Beránek, R., Vyskočil, F. 1968. The effect of atropine on the frog sartorius neuromuscular junction. *J. Physiol.* 195:493–503
131. Marty, A., Neild, T., Ascher, P. 1976. Voltage sensitivity of acetylcholine currents in *Aplysia* neurones in the presence of curare. *Nature* 261:501–3
132. Durant, N. N., Horn, R. S., Lambert, J. J. 1982. The effects of the neuromuscular blocking agent Org.6368 on the endplate channels of amphibian muscle. *Soc. Neurosci. Abstr.* 8:498
133. Milne, R. J., Byrne, J. H. 1981. Effects of hexamethonium and decamethonium on end-plate current parameters. *Mol. Pharmacol.* 19:276–81
134. Eldefrawi, A. T., Miller, E. R., Eldefrawi, M. E. 1982. Binding of depolarizing drugs to ionic channel sites of the nicotinic acetylcholine receptor. *Biochem. Pharmacol.* 31:1819–22
135. Creese, R., England, J. M. 1970. Decamethonium in depolarized muscle and the effects of tubocurarine. *J. Physiol.* 210:345–61
136. Alkadhi, K., Branisteanu, D. D., Henderson, E. G., Lambert, J. J., Volle, R. L. 1980. Effects of McN-A-343, a cholinomimetic drug, on endplate currents. *Naunyn-Schmied. Arch. Pharmakol.* 312:117–21
137. Sakmann, B., Patlak, J., Neher, E. 1980. Single acetylcholine-activated channels show burst-kinetics in presence of desensitizing concentrations of agonist. *Nature* 286:71–73
138. Katz, B., Miledi, R. 1980. Blockade of endplate responses by intracellular application of procaine. In *Ontogenesis and Functional Mechanisms of Peripheral Synapses,* ed. J. Taxi, pp. 171–78. Amsterdam: Elsevier-North Holland
139. Gage, P. W., Hamill, O. P. 1975. General anaesthetics: synaptic depression consistent with increased membrane fluidity. *Neurosci. Lett.* 1:61–65
140. Torda, T. A., Gage, P. W. 1976. Effect of barbiturates on synaptic currents. *Anaesth. Intens. Care* 4:199–202
141. Gage, P. W., Hamill, O. P. 1976. Effects of several inhalation anaesthetics on the kinetics of postsynaptic conductance

changes in mouse diaphragm. *Br. J. Pharmacol.* 57:263–72
142. Torda, T. A., Gage, P. W. 1977. Post-synaptic effect of I. V. anaesthetic agents at the neuromuscular junction. *Br. J. Anaesth.* 49:771–76
143. Gage, P. W., Hamill, O. P., van Helden, D. 1979. Dual effects of ether on end-plate currents. *J. Physiol.* 287:353–69
144. Gage, P. W., McBurney, R. N., van Helden, D. 1978. Octanol reduces end-plate channel lifetime. *J. Physiol.* 274:279–98
145. Gage, P. W., McBurney, R. N., Schneider, G. T. 1975. Effects of some aliphatic alcohols on the conductance change caused by a quantum of acetylcholine at the toad end-plate. *J. Physiol.* 244:409–29
146. Quastel, D. M. J., Linder, T. M. 1975. Pre- and postsynaptic actions of central depressants at the mammalian neuromuscular junction. In *Progress in Anesthesiology.* Vol. 1. *Molecular Mechanisms of Anesthesia,* ed. B. R. Fink, pp. 157–68. New York: Raven
147. Gage, P. W. 1965. The effect of methyl, ethyl and n-propyl alcohol on neuromuscular transmission in the rat. *J. Pharmacol. Exp. Ther.* 150:236–43
148. Inoue, F., Frank, G. B. 1967. Effects of ethyl alcohol on excitability and neuromuscular transmission in frog skeletal muscle. *Br. J. Pharmacol. Chemother.* 30:186–93
149. Okada, K. 1970. Effects of divalent cations on the spontaneous transmitter release at the amphibian neuromuscular junction in the presence of ethanol. *Jpn. J. Physiol.* 20:97–111
150. Paton, W. D. M., Waud, D. R. 1967. The margin of safety of neuromuscular transmission. *J. Physiol.* 191:59–90
151. Barnard, E. A., Wieckowski, J., Chiu, T. H. 1971. Cholinergic receptor molecules and cholinesterase molecules at mouse skeletal muscle junctions. *Nature* 234:207–9
152. Bowman, W. C., Webb, S. N. 1976. Tetanic fade during partial transmission failure produced by non-depolarizing neuromuscular blocking drugs in the cat. *Clin. Exp. Pharm. Physiol.* 3:545–55
153. Bowman, W. C. 1980. Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. *Anesth. Analg.* 59:935–43
154. Hubbard, J. I., Wilson, D. F. 1973. Neuromuscular transmission in a mammalian preparation in the absence of blocking drugs and the effect of *D*-tubocurarine. *J. Physiol.* 228:307–25
155. Glavinovič, M. I. 1979. Presynaptic action of curare. *J. Physiol.* 290:499–506
156. Magleby, K. L., Pallotta, B. S., Terrar, D. A. 1981. The effect of (+)-tubocurarine on neuromuscular transmission during repetitive stimulation in the rat, mouse, and frog. *J. Physiol.* 312:97–113
157. Lee, C., Chen, D., Katz, R. L. 1977. Characteristics of nondepolarizing neuromuscular block. 1. Post-junctional block by alpha-bungarotoxin. *Can. Anaesth. Soc. J.* 24:212–9
158. Albuquerque, E. X., Kuba, K., Daly, J. 1974. Effect of histrionicotoxin on the ionic conductance modulator of the cholinergic receptor: a quantitative analysis of the end-plate current. *J. Pharmacol. Exp. Ther.* 189:513–24
159. Kuba, K., Chikazawa, K., Koketsu, K. 1976. Novel action of a piperazine derivative on the end-plate of the frog. *Jpn. J. Physiol.* 26:159–75
160. Gibb, A. J., Marshall, I. G. 1982. The effects of trimetaphan on tetanic fade and on endplate ion channels at the rat neuromuscular junction. *Br. J. Pharmacol.* 76:187P
161. Miyamoto, M. D. 1978. The action of cholinergic drugs on motor nerve terminals. *Pharmacol. Rev. 29:221–47*
162. Standaert, F. G. 1982. Release of transmitter at the neuromuscular junction. *Br. J. Anaesth.* 54:131–45
163. Volle, R. L., Alkadhi, K. A., Branistaneau, D. D., Reynolds, L. S., Epstein, P. M., et al. 1982. Ketamine and ditran block end-plate ion conductance and (^{3}H)-phencyclidine binding to electric organ membrane. *J. Pharmacol. Exp. Ther.* 221:570–76
164. Aronstam, R. S., Narayanan, L., Wenger, D. A. 1982. Ketamine inhibition of ligand binding to cholinergic receptors and ion channels. *Eur. J. Pharmacol.* 78:367–70
165. Terrar, D. A. 1974. Influence of SKF-525A congeners, strophanthidin and tissue culture-media on desensitization in frog skeletal muscle. *Br. J. Pharmacol.* 51:259–68
166. Magazanik, L. G., Nikolsky, E., Vyskočil, F. 1982. Effect of the desensitization-potentiating agent SKF-525A on frog end-plate currents. *Eur. J. Pharmacol.* 80:115–19
167. Singh, Y. N., Marshall, I. G., Harvey, A. L. 1980. The mechanisms of the muscle paralysing actions of antibiotics, and their interaction with neuromuscu-

lar blocking agents. *Rev. Drug Metab. Drug Interact.* 3:129–53

168. Ngai, S. H. 1975. Action of general anesthetics in producing muscle relaxation: Interaction of anesthetics with relaxants. In *Muscle Relaxants,* ed. R. L. Katz. pp. 163–91. New York: American Elsevier
169. Durant, N. N., Katz, R. L. 1982. Suxamethonium. *Br. J. Anaesth.* 54:195–208
170. Durant, N. N., Lee, C., Katz, R. L. 1981. The effects of 4-aminopyridine on neuromuscular block produced by succinyldicholine. In *Advances in the Biosciences,* Vol. 35: *Aminopyridines and Similarly Acting Drugs,* ed. P. Lechat, S. Thesleff, W. C. Bowman, p. 241. New York: Pergamon
171. Ascher, P., Large, W. A., Rang, H. P. 1979. Studies on the mechanism of action of acetylcholine anatagonists on rat parasympathetic ganglion cells. *J. Physiol.* 295:139–70
172. Clark, R. B., Gration, K. A. F., Lambert, J. J., Piek, T., Ramsey, R., et al. 1982. Philanthus toxin blocks neuromuscular transmission in locusts. *Brain Res.* In press
173. Pennefather, P., Quastel, D. M. J. 1980. Actions of anesthetics on the function of nicotinic acetylcholine receptors. In *Progress in Anesthesiology.* Vol 2. *Molecular Mechanisms of Anesthesia,* ed. B. R. Fink, pp. 45–58. New York: Raven
174. Maeno, T. 1966. Analysis of sodium and potassium conductances in the procaine end-plate potential. *J. Physiol.* 183:592–606
175. Katz, B., Miledi, R. 1975. The effect of procaine on the action of acetylcholine at the neuromuscular junction. *J. Physiol.* 249:269-84
176. Mallart, A., Molgó, J. 1978. The effects of pH on the time course of end-plate currents at the neuromuscular junction of the frog. *J. Physiol.* 276:343–52
177. Guinan, J. J. 1980. The decay of end-plate currents in neostigmine-treated frog muscle blocked by acetylcholine or tubocurarine. *J. Physiol.* 305:345–55
178. Katz, B., Miledi, R. 1973. The effect of atropine on acetylcholine action at the neuromuscular junction. *Proc. R. Soc. London B* 184:221–26
179. Albuquerque, E. X., Tsai, M. -C., Aronstam, R. S., Eldefrawi, A. T., Eldefrawi, M. E. 1980. Sites of action of phencyclidine. II. Interaction with the ionic channel of the nicotinic receptor. *Mol. Pharmacol.* 18:167–78
180. Maleque, M. A., Warnick, J. E., Albuquerque, E. X. 1981. The mechanism and site of action of ketamine on skeletal muscle. *J. Pharmacol. Exp. Ther.* 219:638–45
181. Gage, P. W., Lonergan, M., Torda, T. A. 1980. Presynaptic and postsynaptic depressant effects of phenytoin sodium at the neuromuscular junction. *Br. J. Pharmacol.* 69:119–21
182. Durant, N. N., Lambert, J. J. 1981. The action of polymyxin B at the frog neuromuscular junction. *Br. J. Pharmacol.* 72:41–47
183. Albuquerque, E. X., Eldefrawi, A. T., Eldefrawi, M. E., Mansour, N. A., Tsai, M. -C. 1978. Amantadine: neuromuscular blockade by suppression of ionic conductance of the acetylcholine receptor. *Science* 199:788–90
184. Kordaš, M., Brzin, M., Majcen, Ž. 1975. A comparison of the effect of cholinesterase inhibitors on end-plate current and on cholinesterase activity in frog muscle. *Neuropharmacology* 14: 791–800
185. Kuba, K., Albuquerque, E. X., Daly, J., Barnard, E. A. 1974. A study of the irreversable cholinesterase inhibitor, diisopropylfluorophosphate, on time course of end-plate currents in frog sartorius muscle. *J. Pharmacol. Exp. Ther.* 189:499–512
186. Eldefrawi, A. T., Eldefrawi, M. E., Albuquerque, E. X., Oliveira, A. C., Mansour, N., et al. 1977. Perhydrohistrionicotoxin: a potential ligand for the ion conductance modulator of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 74:2172–76
187. Adams, P. R., Feltz, A. 1980. Quinacrine (mepacrine) action at frog end-plate. *J. Physiol.* 306:261–81
188. Tsai, M. -C., Oliveira, A. C., Albuquerque, E. X., Eldefrawi, M. E., Eldefrawi, A. T. 1979. Mode of action of quinacrine on the acetylcholine receptor ionic channel complex. *Mol. Pharmacol.* 16:382–92
189. Landau, E. M., Richter, J., Cohen, S. 1979. The mean conductance and open-time of the acetylcholine receptor channels can be independently modified by some anesthetic and convulsant ethers. *Mol. Pharmacol.* 16:1075–83
190. Gage, P. W., Sah, P. 1982. Postsynaptic effects of some central stimulants at the neuromuscular junction. *Br. J. Pharmacol.* 75:493–502