

# TRAPPING OF AN OPEN-CHANNEL BLOCKER AT THE FROG NEUROMUSCULAR ACETYLCHOLINE CHANNEL

ALAN NEELY AND CHRISTOPHER J. LINGLE

*Department of Biological Sciences, The Florida State University, Tallahassee, Florida 32306*

**ABSTRACT** At the ganglionic nicotinic acetylcholine channel (Gurney, A. M., and H. P. Rang, 1984, *Br. J. Pharmacol.*, 82:623–642) and on some cholinergic neuromuscular synapses of Crustacea (Lingle, C., 1983a, *J. Physiol. (Lond.)*, 339:395–417; Lingle, C., 1983b, *J. Physiol. (Lond.)*, 339:419–437), some agents that block cholinergic currents by an open-channel block mechanism appear to become trapped within the channel when it subsequently closes. It is unknown whether trapping of some open-channel blockers might also occur at the neuromuscular nicotinic acetylcholine channel. Here we show that the long-lived cholinergic blocking action of chlorisondamine, a ganglionic nicotinic blocker, can in part be most simply explained by an open-channel block mechanism followed by a subsequent trapping of the blocking molecule within the closed ion channel. Unique structural characteristics of the chlorisondamine molecule place several provocative constraints on the mechanism by which trapping may be occurring.

## INTRODUCTION

The study of membrane currents and their modification by particular drugs continues to provide valuable information about the functional architecture of ion channels, which might otherwise be rather inaccessible to direct structural methods (Miller, 1982). For example, pharmacological agents have proven useful in providing estimates of the minimum diameter of channel permeation pathways (Dwyer et al., 1980) and in the elucidation of molecular changes that may be associated with channel gating and inactivation (Armstrong, 1971). One type of channel block phenomenon that any model of ion channel structure must account for is the apparent occurrence of trapping (Armstrong, 1971) of particular channel blockers within the closed channel. Until recently, trapping of molecules within channels had been observed exclusively in the sodium (Hille, 1977) and potassium (Armstrong, 1971) channels of squid axon and appeared to be restricted primarily to quaternary ammonium compounds of a rather lipophilic nature. More recently, trapping of much simpler diquaternary ammonium compounds of various chain lengths (Gurney and Rang, 1984) and of secondary, tertiary, and quaternary amines (Lingle, 1983a, b) in different nicotinic cholinergic channels has been reported.

As yet it is unclear to what extent trapping of molecules may be a general feature of ion channels. Clearly, in cases where it has been examined, trapping is likely to occur with only a small fraction of all channel closures. However, the existence of any trapping at all certainly has powerful implications for models of channel architecture. Nevertheless, trapping has received substantially less consideration than the ultimately less informative type of blockade in which blocker is transiently associated solely with the open conformation of the ion channel protein.

Here we present evidence that at the frog neuromuscular junction, chlorisondamine, in addition to acting as an open-channel blocker, leads to a stable blocked state that can only be unblocked by subsequent addition of agonist. The existence of a very long-lasting, perhaps irreversible component of blockade is also discussed. A preliminary account of this report has been presented (Neely and Lingle, 1985).

## METHODS

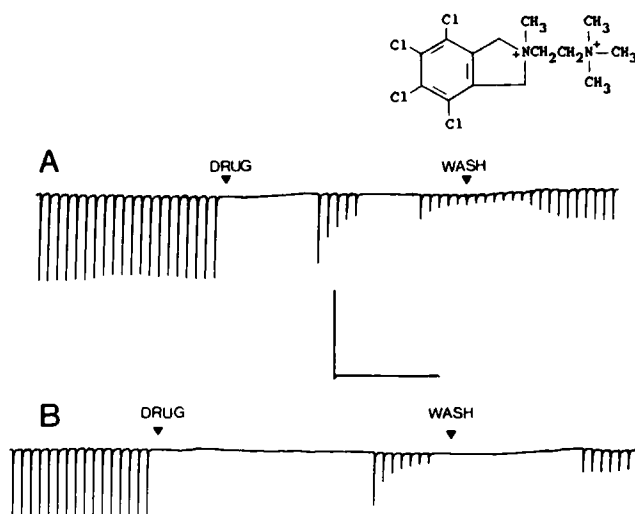
All experiments were performed on the *cutaneous pectoris* of *Rana pipiens*, which were obtained from Charles O. Sullivan Co., Nashville, TN. Conventional two-microelectrode voltage-clamp with a virtual ground current monitor was used to record spontaneous miniature end plate currents (MEPCs) and currents induced by iontophoretic application of acetylcholine (ACh). Recording microelectrodes were filled with 4 M potassium acetate/0.5 KCl (<10 M $\Omega$ ) and iontophoretic electrodes with 1 M ACh (>60 M $\Omega$ ). A braking current of 10–15 nA was routinely applied to the iontophoretic electrode to minimize leakage of ACh from the pipette. Duration, intensity, and the frequency of pulses of ACh were adjusted carefully to obtain cholinergic currents of constant amplitude. Additionally, the iontophoretic electrode was positioned such that a fast rising phase in the agonist-induced current was obtained, while at the same time minimal desensitization during a series of agonist pulses was observed. For selection of impalements, the rise time of the MEPCs and the quality of clamping of the MEPC current were used as the primary criteria after fine nerve branches had been visualized under the dissecting microscope (model M5A,  $\times 100$ , Wild Heerbrugg Ltd, Heerbrugg, Switzerland). For recording of MEPCs, the saline contained the following: 110 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 1.15 mM maleic acid, 2.50 mM Tris, pH 7.3 with 10 nM tetrodotoxin (TTX) to prevent contraction. In iontophoretic experiments, 4 mM MnCl<sub>2</sub> was added to the saline to increase input resistance and improve space clamp conditions. MEPCs were digitized and captured on line by a microcomputer using a single-level threshold criteria. Averaging of MEPCs was performed off line to optimize alignment of MEPC rising phases. Alignment was accomplished using a matchfilter algorithm. MEPC decay times were obtained by a nonlinear least-squares fitting routine based on the RMSSQ subroutine from the International Math and

Statistics Library, Bellaire, TX, in which the amplitude of the exponential component, the exponential decay time constant, and baseline were independently adjusted. Preparations were continuously perfused with saline at  $\sim 10$  ml/min with a bath volume of 2–3 ml. Application of drugs and exchanges of solutions were accomplished by solenoid-controlled switches in the perfusion lines. All experiments were done at room temperature (22–25°C). Acetylcholine and TTX were obtained from Sigma Chemical Co., St. Louis, MO and chlorisondamine chloride was a gift from Ciba Pharmaceutical Co., Summit, NJ.

## RESULTS

### Effects on Iontophoretic Currents

The basic qualitative features of blockade and recovery from chlorisondamine action on cholinergic currents at the frog end plate are summarized in Fig. 1. Three primary aspects of chlorisondamine action can be discerned within the context of simple iontophoretic applications of ACh. First, virtually no blockade of cholinergic currents occurs until agonist application is initiated, which suggests that blockade by chlorisondamine reflects an action on a state of the channel occurring subsequent to the opening reaction. Second, once blockade is initiated, the approach to a steady-state level of block occurs quite slowly. Third, after washout of chlorisondamine, recovery is very slow and, to some extent, irreversible.



**FIGURE 1** Qualitative features of blockade by chlorisondamine of iontophoretic currents activated by acetylcholine at the frog end-plate. Inward currents were activated by repetitive pulses of acetylcholine applied at 10-s intervals to minimize desensitization between pulses. *A* and *B* correspond to the same muscle fiber voltage-clamped at  $-100$  mV. Record *B* starts after a recovery period of 500 s following record *A*.  $5 \times 10^{-7}$  M chlorisondamine was introduced into the perfusion line and washed out as indicated by the arrows. During introduction of drug into the preparation, agonist application was suspended. In *A*, agonist application was reinitiated  $\sim 80$  s after drug application, while in *B*  $\sim 200$  s elapsed prior to agonist application. In *A* the first response was reduced 15% relative to the control response, while in *B* the first response was reduced 18% relative to the control. The slow development of blockade once agonist application is initiated and the slow recovery from chlorisondamine action are also illustrated. Vertical calibration, 100 nA; horizontal calibration, 100 s.

In the experiment illustrated in Fig. 1, after a stable control amplitude was maintained for a period of at least 2 min in normal saline, a solution containing  $0.5 \mu\text{M}$  chlorisondamine was perfused over the preparation. To test for the existence of an agonist-independent component of blockade, the drug-containing saline was allowed to fully exchange and bathe the preparation for 2 min before any agonist was applied. When agonist application was subsequently initiated, the response to the first iontophoretic pulse in the presence of drug showed a 15% blockade. When the same sequence was repeated in the same cell with a somewhat longer interval before the first agonist pulse, the first agonist pulse was blocked 18% (Fig. 1 *B*). These results indicate that little or no blockade of the ACh response occurs until agonist application is initiated as expected for an open-channel blocker. Given additional evidence presented below that chlorisondamine blocks the open-state of the ACh channel, it is likely that the small component of blockade observed during the first agonist pulse reflects blockade of agonist-opened ion channels during the rising phase of the cholinergic current. Although we can not rigorously exclude the possibility that a small component of block may occur either at the ACh receptor site or by a direct action on the closed channel, such effects, if they occur, are clearly secondary to the main effect of chlorisondamine under consideration here.

Fig. 1 also shows the slow onset of blockade in the presence of chlorisondamine. That this slowly developing block reflects an action on the opened ion channel is indicated by a comparison of Fig. 1, *A* and *B*. In Fig. 1 *A* the slowly developing block results in a  $>75\%$  reduction in the amplitude of the cholinergic currents. In Fig. 1 *B* after a similar period of time during which agonist was not applied, blockade remains undeveloped. This indicates that exposure to agonist is required for the slow blockade to develop and is consistent with the action of an open-channel blocker with a slow dissociation rate (Adams and Feltz, 1980; Colquhoun, Dreyer, and Sheridan, 1979).

Finally, Fig. 1 illustrates some qualitative features of unblocking from chlorisondamine action. After the short series of ACh pulses produced a significant level of blockade, agonist application was suspended for  $\sim 1$  min. The amount of recovery revealed by the subsequent response to an ACh pulse is minimal, even after washout of the drug and a longer recovery time (Fig. 1 *B*). When the agonist pulses are maintained during washout, a multiplicity of components in the recovery kinetics becomes apparent. However, even after an extensive period of wash (500 s) separating the records in Fig. 1, *A* and *B*, the response (Fig. 1 *B*) only reaches 80% of the amplitude attained before the cell has been exposed to chlorisondamine (Fig. 1 *A*). We have been unable to identify conditions that lead to full recovery of these blocked channels whether by voltage steps or by agonist application, although this point will be considered further below.

## Blockade of MEPCS

Further evidence that the blocking action of chlorisondamine involves a direct action on the open form of the ACh channel is provided by the effect of chlorisondamine on the decay time of MEPCs (Fig. 2). In controls, a typical single exponential decay with a time constant that increased with hyperpolarization was observed. In the presence of chlorisondamine, MEPCs can also be fit by single exponential functions but with faster time constants. The shortening of the decay increases steeply with hyperpolarization (Fig. 2 *B*), revealing a strong voltage dependence of the action of chlorisondamine.

During application of chlorisondamine, a gradual reduction in mean MEPC amplitude was also observed. This is consistent with the idea suggested above that, although only a small fraction of opened channels may become blocked during any individual MEPC, recovery from that blockade is quite slow. This is also supported by the absence of any significant slow component in the MEPC decay. In accordance with the standard sequential blocking model, the absence of a slow component suggests that the processes leading to unblocking are much slower than the time course of the synaptic current. As such, the rate of decay of the MEPCs would be expected to comply with the following relationship:

$$\tau^{-1} = \alpha + f^* [\text{chlorisondamine}], \quad (1)$$

where  $\alpha$  represents the apparent rate of channel closure and  $f^*$  is the forward rate of blockade. Consistent with this idea that blockade by chlorisondamine results from a pseudo-first-order blocking reaction, the MEPC decay rate exhibited an approximately linear dependence on chlorisondamine concentration with a forward rate of  $1.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  at  $-140 \text{ mV}$  (Fig. 3 *A*). In Fig. 3 *B*, the forward rate of block is plotted as a function of membrane potential and exhibits an approximately *e*-fold increase per 30 mV hyperpolarization. These features of the action of chlorisondamine are consistent with a simple channel block mechanism in which rates of unblocking are slow. The large voltage dependence of the forward blocking rate perhaps reflects the relatively close juxtaposition of two quaternary ammonium groups in the chlorisondamine molecule, both of which may sense the electric field of the membrane.

## Agonist-dependent Recovery from Chlorisondamine Blockade

The experiments illustrated in Fig. 4 were designed to examine aspects of unblocking from chlorisondamine. The muscle cell membrane potential was stepped between two voltages ( $-45 \text{ mV}$  and  $-115 \text{ mV}$  in Fig. 4 *A*;  $-60 \text{ mV}$  and  $-115 \text{ mV}$  in Fig. 4 *B*) while cholinergic currents were elicited by iontophoretic application of acetylcholine in the

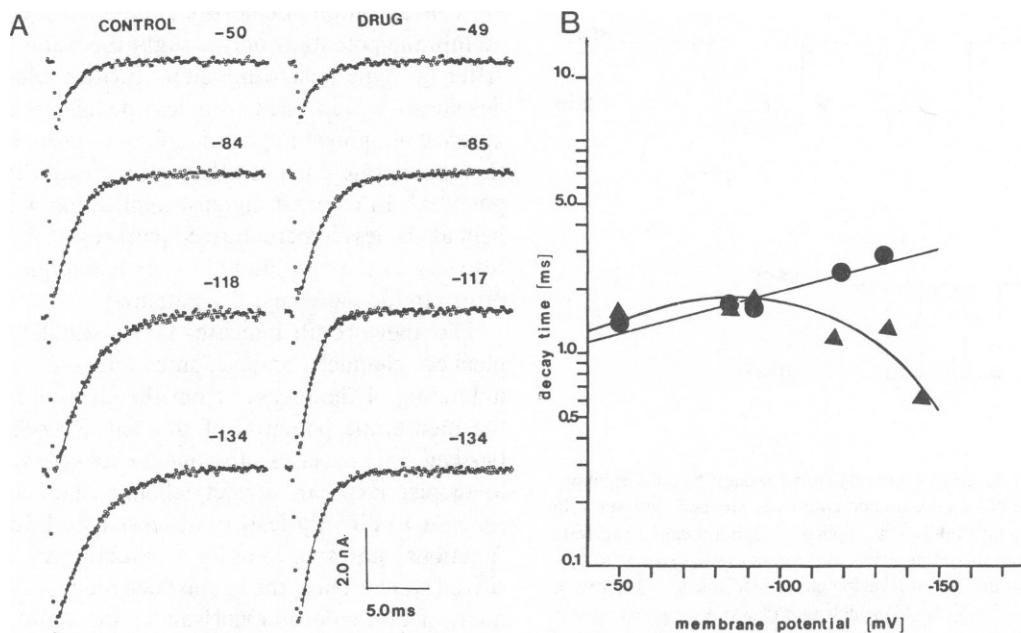


FIGURE 2 Effect of chlorisondamine on spontaneous miniature synaptic currents. (*A*) Left, averages of MEPCs in normal saline obtained at the indicated membrane potentials are illustrated. Right, averages of MEPCs after application of  $8 \mu\text{M}$  chlorisondamine are shown. Each trace corresponds to the average of at least 30 individual MEPCs. Points correspond to the digitized raw data and lines are single exponential fits to the experimental data. Holding potentials are indicated on each trace and time constants are as follows: controls from top to bottom, 1.40, 1.60, 2.43, and 2.91 ms;  $8 \mu\text{M}$  chlorisondamine from top to bottom, 1.66, 1.51, 1.13 and 1.30 ms. Vertical calibration, 1.0 nA; horizontal calibration, 4.8 ms. (*B*) The MEPC decay time constant is plotted as a function of membrane voltage both in the presence (filled triangles) and absence (filled circles) of  $8 \times 10^{-6} \text{ M}$  chlorisondamine. Data were obtained from a single cell. The line over control points was a least-squares fit with a slope of  $103 \text{ mV}/e$ -fold change in MEPC decay time while the line over points obtained in chlorisondamine was drawn by eye.

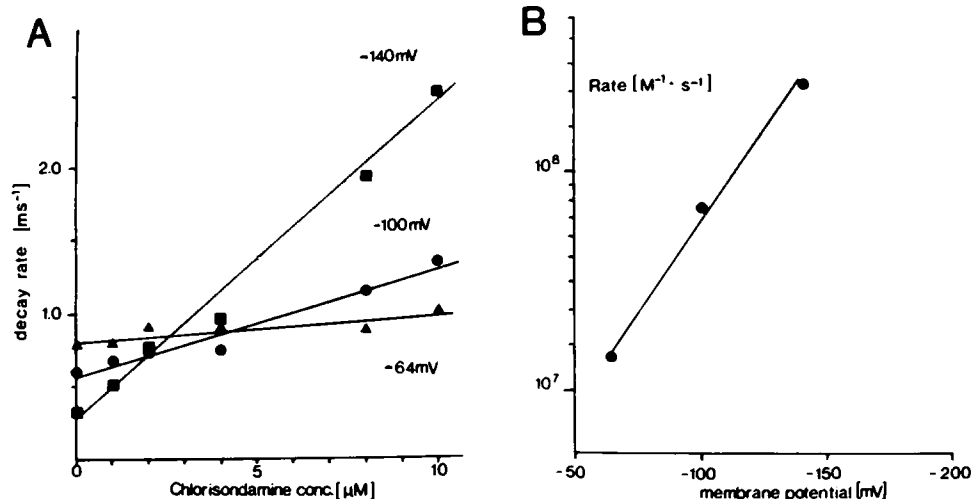


FIGURE 3 Concentration-dependence of shortening of MEPC decay times. (A) the MEPC decay rate is plotted as a function of chlorisondamine concentration for three different membrane voltages with all data obtained from one cell. The points at each voltage were fit by linear regression and the slope of the lines were  $1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  at  $-64 \text{ mV}$ ,  $7.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  at  $-100 \text{ mV}$ , and  $1.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  at  $-140 \text{ mV}$ . Over this concentration range no indication of saturation in decay rate with concentration was observed. (B) The forward blocking rate constants obtained from the slopes of the lines in A are plotted as a function of membrane potential. The forward blocking rate varies approximately  $e$ -fold for a 30-mV change in membrane potential.

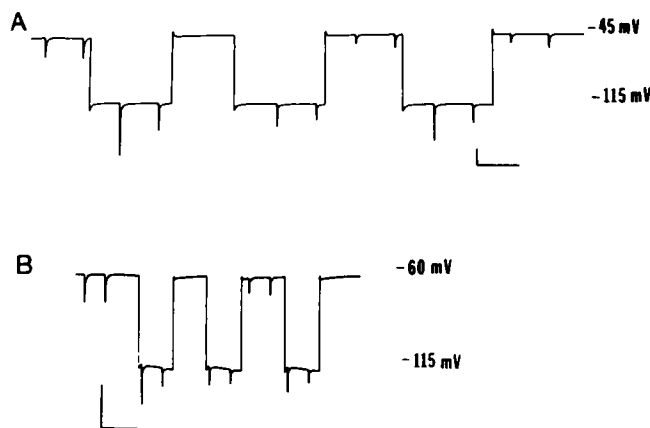
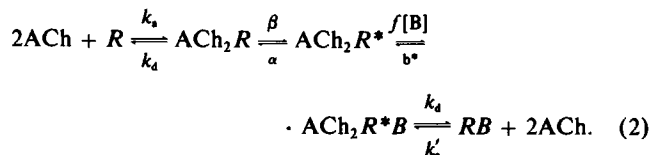


FIGURE 4 Agonist-dependent recovery from blockade by chlorisondamine. (A) The muscle cell membrane potential was stepped between  $-45$  and  $-115 \text{ mV}$  and acetylcholine was applied iontophoretically to activate inward currents while the preparation was continuously exposed to  $5 \times 10^{-7} \text{ M}$  chlorisondamine. After development of blockade at  $-115 \text{ mV}$ , a recovery period at  $-45 \text{ mV}$ , during which agonist was not applied, results in no recovery in sensitivity to acetylcholine. After a recovery period at  $-45 \text{ mV}$  in which two pulses of acetylcholine were applied, a substantial recovery in the response at  $-115 \text{ mV}$  was observed. (B) An experiment similar to that shown in A is illustrated except that the cell was stepped between  $-60$  and  $-115 \text{ mV}$ . In addition, the time of initial application of acetylcholine following return steps to  $-115 \text{ mV}$  was kept strictly fixed. Again, a component of blocked channels requires reactivation by agonist to produce recovery. In both A and B the capacitive transients that occur at the time of a voltage-step have been truncated for clarity. Vertical calibration, 50 nA for both A and B; horizontal calibration, 5 s in A and 10 s in B.

presence of  $0.5 \text{ μM}$  chlorisondamine. At least in part because of the strong voltage dependence of the forward blocking rate, a marked difference in the blocking equilibrium between the two potentials is observed. At this rather low concentration of chlorisondamine, at the more positive membrane potentials only a slight blockade was observed. After a hyperpolarizing step, further blockade slowly develops. A step back to a less depolarized level in the absence of agonist application fails to produce recovery in the cholinergic current after return to the more negative potential. In contrast, agonist application when the cell is held at the less hyperpolarized level results in a substantial recovery in the amplitude of the cholinergic current after return to the more negative potential.

The above result indicates that a significant portion of blocked channels enters some state that prevents the unbinding of the blocker from the channel irrespective of the membrane potential of the cell. Recovery from that blocked state requires subsequent exposure of the channels to agonist. Exposure of acetylcholine channels to agonist is thought to directly lead to at least two different types of functional states: open or ion-conducting states and desensitized states. Thus, the agonist dependence of the development of blockade by chlorisondamine could conceivably result from a preferential affinity for either of these types of states. However, in addition to the agonist dependence of blockade, chlorisondamine exhibits an agonist-dependent recovery. If the effect of chlorisondamine were to stabilize the desensitized state, further application of agonist would only be expected to increase the number of channels in that nonconducting state. Alternatively, if chlorisondamine binds specifically to the open state, which subsequently undergoes closure, such a closed-blocked state would be

expected to be reversed by the application of agonist as is observed. The simplest scheme (Lingle, 1983a) that qualitatively accounts for these phenomena is therefore the following:



This model, although related to previously described cyclic schemes (eg., Adams, 1977; and Gurney and Rang, 1984) suggests that the channel can close while blocked but that neither blockade of the closed channel nor unblocking of the blocked-closed channel can occur. Thus, the presence of the blocking molecule in its blocking site does not completely hinder the gating of the channel and the drug binding site is only accessible when the gate is open.

The above model does not explain the irreversible component of the blocking action of chlorisondamine at the frog end-plate, which we consistently observe. This irreversibility has compromised our attempts to quantitatively characterize the recovery from blockade by chlorisondamine and we have little information about the irreversible processes. We have noted, using iontophoretic experiments, that the extent of irreversibility was correlated with the length of time that channels were allowed to remain in the blocked-closed state without reactivation. Repeated activation of channels in the presence of chlorisondamine appeared to minimize the slow irreversible degradation in cholinergic sensitivity. This would argue against the possibility that the irreversible component of chlorisondamine action is unrelated to the other phenomena described above. Rather, these observations would suggest that a blocked-closed channel can undergo some subsequent transition to a state exceedingly refractory to subsequent activation and unblocking. In this scheme, a channel would be protected from this state by reactivation and unblocking.

## DISCUSSION

The vertebrate end-plate ACh channel has been the subject of extensive pharmacological characterization, and a wide variety of molecules appear to occlude the ion permeation pathway after channel opening. It is perhaps surprising that, to our knowledge, this is the first explicit demonstration that any blocker may become trapped in the closed neuromuscular ACh channel, although trapping has been proposed and discarded as a possible mechanism of desensitization (Adams, 1975) and as an explanation for some aspects of blockade by procaine (Gage and Wachtel, 1984). However, without the use of experimental protocols similar to those of Fig. 4 (Gurney and Rang, 1984; Lingle, 1983a, b), an agonist-dependent component of recovery

from blockade may easily go undetected. Even with such protocols, if both the dissociation rate of the blocker from its binding site is rapid and the reopening for the blocked-closed channel is not too dissimilar from the normal opening rate, the existence of the blocked-closed state would be unobserved.

Is there evidence that other agents may be trapped in neuromuscular ACh channels? For a phenomenon that may occur in only a small fraction of all channel blocking events, the usual kinetic tests of channel blocking mechanisms would, in general, be inadequate (eg., Adams and Sakmann, 1978; Adams and Feltz, 1980). One type of observation is at least suggestive that with some agents trapping may occur. For blockers with rapid unblocking rates, the simple channel block model predicts that, as blocker concentration is increased, the duration of the bursts of openings should increase linearly with blocker concentration. Recently, bursts of single channel openings produced in the presence of either QX-222 (Neher, 1983) or mecamylamine (Varanda et al., 1985) have been shown to be shorter than would be predicted by a simple channel block scheme. This effect on burst length, although consistent with any mechanism in which the channel can enter long-lived closed states, could be explained by trapping. This action of mecamylamine is of particular interest, since mecamylamine clearly results in the trapping phenomenon at the crustacean acetylcholine channel (Lingle, 1983b). The possibility of closure of blocked-open channels has also been suggested as an explanation for some aspects of the blockade by barbiturates of the ACh-activated sodium channels in *Aplysia* (Wachtel and Wilson, 1983).

Irrespective of the extent to which trapping may be occurring, the fact that it occurs at all may have particular significance for models of channel structure. Two particular physical views of trapping are worth considering. In one, in accordance with the earlier pictures of Armstrong (1971) and Hille (1977) to explain blockade of potassium and sodium channels by quaternary ammonium molecules, the blocking molecule is completely trapped within the channel between the permeation barrier and the gate of the channel. The molecules examined in these studies in general contained rather bulky hydrophobic domains, which were imagined to interact with particular regions of the channel walls. For chlorisondamine, the rather polar nature of the molecule both in its diquaternary region and in the tetrachloroindole ring (Fig. 1, *inset*) suggests that solubility into the channel protein will be minimal. Thus, entrapment of chlorisondamine between a channel gate and permeation barrier would require the presence of a rather large aqueous space within the closed channel conformation. Given recent models of internal pore dimensions of at least one energy-minimized conformation of the acetylcholine channel (Bash, Langridge, and Stroud, 1985), this view is not particularly attractive.

On the other hand, an alternative picture of the underlying physical mechanism is that only a portion of the

trapped molecule is pinched within the closed channel. The results of Gurney and Rang (1984) are perhaps most easily reconciled with this view in that, for bis-trimethylammonium compounds of C4 through C10, trapping was most observable for C5 through C8, less so for C9 and C10, and not observed for C4. The less pronounced trapping observed for C9 and C10 may reflect the folding of molecules of chain length greater than 8 (Coronado and Miller, 1980), which may place additional constraints on the ability of the channel to close. Based on evidence that some molecules as large as C10 can permeate at least some nicotinic channels (Creese and England, 1970; Lingle, 1983b), it might be expected that inward unblocking could occur for molecules simply trapped in an aqueous space. On the other hand, molecules pinched within the closed ion channel would not be expected to exhibit any recovery in the absence of reopening of the channel. Given current views of the pentasymmetric nature of ACh channels (Brisson and Unwin, 1985) and the possibility of a concerted rotation of all subunits in the gating process, pinching within the closed channel may provide a more accurate description of drug entrapment. Additionally, the energy-minimized conformation cited above (Bash, Landridge, and Stroud, 1985) leaves open the possibility that, although permeation by cations would be impossible, some space within the core of the channel may be present. Specific alignment of quaternary residues on a blocking molecule with negative sites on amphipathic chains of the ACh channel subunits might in some way stabilize what might otherwise be an energetically unfavored conformation.

The main purpose of this paper has been to show the trapping of drug molecules within an apparently closed-unliganded conformation of the neuromuscular acetylcholine channel. The structural issues raised above are clearly beyond the scope of the present study and would require a systematic investigation with a series of structurally related agents. As yet, for the vertebrate neuromuscular junction, an appropriate series of blockers capable of producing trapping has not been identified. The simple diquaternary agents that have been investigated on the ganglionic preparation (Gurney and Rang, 1984) have complicating agonist and receptor blocking actions at the endplate (Adams and Sakmann, 1978; Milne and Byrne, 1981). Attempts to fully characterize the trapping phenomenon in other ACh channels have been limited by particular experimental complications (Gurney and Rang, 1984; Lingle, 1983a). Despite experimental difficulties in the characterization of trapping, further elucidation of this phenomenon promises to provide important information concerning structural features of the closed channel.

This work was supported in part by grant NS-19139 to Dr. Lingle and by a grant from the Muscular Dystrophy Association. Dr. Lingle is the recipient of a Research Career Development Award (NS-00836).

Received for publication 21 February 1986 and in final form 21 May 1986.

## REFERENCES

- Adams, P. R. 1975. A study of desensitisation using voltage clamp. *Pfluegers Arch.* 360:135-144.
- Adams, P. R. 1977. Voltage jump analysis of procaine action at the frog end-plate. *J. Physiol. (Lond.)*. 268:291-318.
- Adams, P. R., and A. Feltz. 1980. Quinacrine (mepacrine) action at frog end-plate. *J. Physiol. (Lond.)*. 306:261-281.
- Adams, P. R., and B. Sakmann. 1978. Decamethonium both opens and blocks end-plate channels. *Proc. Natl. Acad. Sci. USA*. 75:2994-2998.
- Armstrong, C. M. 1971. Interaction of tetramethylammonium derivatives with the potassium channels of squid giant axons. *J. Gen. Physiol.* 58:413-437.
- Bash, P. A., R. Langridge, and R. M. Stroud. 1985. Model for the ion channel in the acetylcholine receptor. *Biophys. J.* 47:43a.
- Brisson, A., and P. N. T. Unwin. 1985. Quaternary structure of the acetylcholine receptor. *Nature (Lond.)*. 315:474-477.
- Colquhoun, D., F. Dreyer, and R. S. Sheridan. 1979. The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 293:247-284.
- Coronado, R., and C. Miller. 1980. Decamethonium and hexamethonium block  $K^+$  channels of sarcoplasmic reticulum. *Nature (Lond.)*. 288:495-497.
- Creese, R., and J. M. England. 1970. Decamethonium in depolarized muscle and the effects of tubocurarine. *J. Physiol. (Lond.)*. 210:345-361.
- Dwyer, T. M., D. J. Adams, and B. Hille. 1980. The permeability of the endplate channel to organic cations in frog muscle. *J. Gen. Physiol.* 75:469-492.
- Gage, P. W., and R. E. Wachtel. 1984. Some effects of procaine at the toad end-plate are not consistent with a simple channel-blocking model. *J. Physiol.* 346:331-339.
- Gurney, A. M., and H. P. Rang. 1984. The channel-blocking action of methonium compounds on rat submandibular ganglion cells. *Br. J. Pharmacol.* 82:623-642.
- Hille, B. 1977. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497-515.
- Lingle, C. 1983a. Blockade of cholinergic channels by chlorisondamine on a crustacean muscle. *J. Physiol. (Lond.)*. 339:395-417.
- Lingle, C. 1983b. Different types of blockade of crustacean acetylcholine-induced currents. *J. Physiol. (Lond.)*. 339:419-437.
- Miller, C. 1982. Bis-quaternary ammonium blockers as structural probes of the sarcoplasmic reticulum  $K^+$  channel. *J. Gen. Physiol.* 79:869-891.
- Milne, R. J., and J. H. Byrne. 1981. Effects of hexamethonium and decamethonium on end-plate current parameters. *Mol. Pharmacol.* 19:276-281.
- Neely, A., and C. J. Lingle. 1985. Open-channel blockade by chlorisondamine (CHL) leads to a stable non-conducting state of the acetylcholine (ACh) channel at the frog end-plate. *Soc. Neurosci. Abstr.* 11:850.
- Neher, E. 1983. The charge carried by single-channel currents of rat cultured muscle cells in the presence of local anesthetics. *J. Physiol. (Lond.)*. 339:663-678.
- Varanda, W. A., Y. Aracava, S. M. Sherby, W. G. VanMeter, M. E. Eldefrawi, and E. X. Albuquerque. 1985. The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol. Pharmacol.* 28:128-137.
- Wachtel, R. E., and W. A. Wilson. 1983. Barbiturate effects on acetylcholine-activated channels in *Aplysia* neurons. *Mol. Pharmacol.* 24:449-457.