Current-dependent Block of Endplate Channels by Guanidine Derivatives

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ABSTRACT Methyl- and ethylguanidine block the endplate current in frog muscle. Both derivatives blocked inward-going endplate currents without affecting outward endplate currents. Repetitive stimulation that evoked several inward endplate currents enhanced the block, which suggests that these agents interact with open endplate channels. The relative conductance vs. potential curve exhibited a transition from a low to a high value near the reversal potential for the endplate current, both in normal and in 50% Na solution. In the latter solution, the reversal potential for endplate current was shifted by a mean value of 16 mV in the direction of hyperpolarization. The results suggest that methyl- and ethylguanidine block open endplate channels in a manner dependent on the direction of current flow rather than on the membrane potential.

INTRODUCTION

Whereas guanidine has been found permeant in the endplate channel, certain guanidine derivatives block the channels (Watanabe and Narahashi, 1979). The iontophoretically evoked acetylcholine potential was blocked with apparent dissociation constants of 15, 4, 0.5, 0.4, 0.065, and 0.006 mM for amino-, ethyl-, methyl-, propyl-, amyl-, and octylguanidine, respectively (Farley et al., 1979, 1981; Watanabe and Narahashi, 1979).

Normally, the current-voltage (I-V) relation of endplate currents (EPCs) is almost linear at membrane potentials ranging from -100 to +50 mV (Kordas, 1977; Deguchi and Narahashi, 1971; Gage, 1976). The I-V relation becomes nonlinear in the presence of certain drugs and toxins. This is generally interpreted as being due to the voltage dependence of the block (Fiekers and Henderson, 1982; Masukawa and Albuquerque, 1978; Tsai et al., 1978; Albuquerque et al., 1974, 1978).
In some instances, the nonlinearity of the I-V relationship caused by blockers can be influenced by the direction and/or magnitude of the membrane current. Current-dependent block of ionic channels has indeed been demonstrated for tetraethylammonium ions acting on K channels (Armstrong, 1969, 1971) and for paragracine acting on Na channels (Seyama et al., 1980). In these cases the blocking ions present inside the axon can be swept into the ionic channels when membrane currents flow through the channels in the outward direction. This will result in an intensification of block. However, if the currents flow in the inward direction, the blocking ions can be swept away from the channels, which results in a decrease in block. Thus, the block depends solely on the direction of current flow, irrespective of the membrane potential.

We suspected that the short-chain guanidine derivatives acting on the endplate could cause current-dependent block because methylguanidine and ethylguanidine induced rectification in the I-V relationship, with the inflection point always occurring near the reversal potential for the endplate current (Farley et al., 1981). In the present study we have found that the endplate channel block caused by short-chain guanidine derivatives is dependent on the direction of current flow rather than on the magnitude of current or the membrane potential.

METHODS

The endplate current was measured with the sciatic nerve–sartorius muscle or cutaneous pectoris muscle preparations from the frog Rana pipiens using two-microelectrode voltage clamp techniques (Takeuchi and Takeuchi, 1959). A microelectrode with 5–10 MΩ impedance was inserted near the endplate to measure membrane potential. A current injection microelectrode of 2–5 MΩ impedance was inserted into the muscle fiber within 50 μm of the voltage-measuring microelectrode. Acetylcholine (ACh) was applied iontophoretically to the endplate from a microelectrode filled with 1 M ACh chloride. This microelectrode had an impedance ranging from 50 to 100 MΩ. A small breaking current was applied to prevent diffusion of ACh from the iontophoretic microelectrode.

The muscle preparations were pre-soaked in normal Ringer's solution containing 2 M formamide to abolish muscle contraction (del Castillo and de Motta, 1977). The formamide was washed out for a period of 1 h prior to experiments. The Ringer's solution used contained 115 mM Na, 2.5 mM K, 1.8 mM Ca, and 2 mM HEPES. The pH was adjusted to 7.3 with NaOH. In order to improve the space clamp in the sartorius muscle preparation, 1–2 μM d-tubocurarine was added to Ringer's solution to reduce the size of the EPCs.

All experiments were carried out at a room temperature of 20–22°C.

RESULTS

The endplate block caused by methyl- and ethylguanidine was studied in detail by observation of the nerve-evoked endplate currents. The most striking effects of these guanidine derivatives on the EPC are as follows. (a) The current-voltage relation became nonlinear, being rectified for the inward-going EPCs; (b) the time constant of decay of the EPC was altered; (c) block of the inward EPC was intensified by repetitive stimuli; (d) the outward EPC did not exhibit block unless preceded by one or more inward EPCs, which induced block. These effects are described in detail in the following sections.
Amplitude and Decay Time Course of the EPC

Fig. 1A shows families of EPCs recorded from an endplate bathed in normal Ringer's solution containing d-tubocurarine with or without 3 mM methylguanidine. Methylguanidine had marked effects on the peak amplitude of the EPC. The inward-going EPCs were reduced in amplitude, whereas the outward-going EPCs were increased by methylguanidine. The increase in the outward EPC is due largely to the well-documented presynaptic action of methylguanidine in stimulating evoked transmitter release (Farley et al., 1979). The reduction of
inward-going EPCs reflects the postsynaptic blocking action of methylguanidine, which is sufficient to overcome the presynaptic stimulatory effect of the compound.

Fig. 1B illustrates the effect of 2 mM ethylguanidine on the nerve-evoked EPC recorded in normal Ringer's solution in the absence of d-tubocurarine. Ethylguanidine caused the EPC to increase at all membrane potentials tested (−60 to +60 mV), because of the presynaptic stimulatory action (Farley et al., 1979). However, the inward EPCs were less augmented than the outward EPCs, which suggests that the inward EPCs were partially blocked by ethylguanidine.

The decay rate of the control EPCs is voltage dependent, becoming accelerated at more positive potentials. This voltage dependence was reversed in the presence of methylguanidine, such that the decay time course was accelerated at negative potentials and slowed at positive potentials. Ethylguanidine reduced the apparent potential dependence of the EPC decay rate. The slope of the relationship between the time constant of decay and the membrane potential became slightly positive. The decay time constant of the EPC under control conditions represents the gating kinetics of the single channel (Anderson and Stevens, 1973). However, in the presence of guanidine derivatives that markedly stimulate transmitter release, the decay phase of the EPC becomes more complicated and can no longer be assumed to represent solely the gating kinetics of single channels. The kinetic changes in the EPC induced by methyl- and ethylguanidine will be reported in a future communication.

**Outward EPCs Are Not Blocked by Methyl- or Ethylguanidine**

**ACH-INDUCED CURRENT** In order to demonstrate the direct blocking action of methylguanidine on the endplate, transient currents induced by iontophoretic applications of ACh at the endplate were observed. Whereas the inward ACh-induced currents were suppressed by 0.5 mM methylguanidine, the outward currents were totally unaffected (Fig. 2). This experiment clearly demonstrates that the methylguanidine-induced block of endplate is current and/or voltage dependent.

**WASH-IN EXPERIMENT** Another way of demonstrating the direct blocking action of guanidine derivatives on the endplate is to monitor EPCs during wash-in of drug. This is possible because the postsynaptic effect of guanidines develops quickly, while the presynaptic effect, if any, develops much more slowly (Farley et al., 1979, 1981). Fig. 3 illustrates the time course of the action of 2 mM ethylguanidine on inward and outward EPCs. Two preparations were voltage-clamped during wash-in of ethylguanidine, one to +40 mV and the other to −60 mV. The EPC was elicited by nerve stimulation at an interval of 10 s in both cases. During the first 40 s of wash-in, the EPC at −60 mV was depressed, but the EPC at +40 mV was not (Fig. 3A). It appears, therefore, that ethylguanidine did not block the outward-going EPC. The delayed increase in the EPC amplitude at both potentials reflects the presynaptic stimulatory action of ethylguanidine, which develops more slowly than the postsynaptic blocking action. Similar results were obtained with methylguanidine.
As illustrated in Fig. 4A, methylguanidine (3 mM) caused a pronounced change in the I-V relation for EPCs. The reversal potential was -7 mV and was not affected by the guanidine derivative. However, the I-V relation became nonlinear...
in the presence of methylguanidine, and inward EPCs were markedly suppressed, with an inflection point occurring near the reversal potential. These results suggest that rectification in the I-V relation caused by methylguanidine is related to the current flow during the EPC. This idea was tested by experiments in which the reversal potential was shifted in the hyperpolarizing direction. To do this, the external Na ion concentration was lowered to 50% of the normal value by substitution with sucrose on an isosmotic basis.

Fig. 4B shows the I-V relationship measured in 50% Na Ringer's solution. The reversal potential was shifted to -28 mV, and the I-V relationship remained linear. In the presence of 3 mM methylguanidine, the I-V curve again became nonlinear. When Fig. 4A is compared with Fig. 4B, it becomes clear that rectification occurs whenever the potential is more negative than the reversal potential. This result supports the idea that the methylguanidine block of the EPC is dependent on the direction of current flow. Similar results were obtained with ethylguanidine, which indicates that this derivative also exhibits block that is current-direction dependent.
Conductance-Voltage Relation

Quantitative analysis of the blocking action of methylguanidine and ethylguanidine is complicated because they stimulate transmitter release from the presynaptic nerve terminal. For qualitative comparison, the chord conductance, $g$, was first calculated from the equation $g = \text{EPC}/(E_m - E_r)$, where $E_m$ and $E_r$ represent the membrane potential and the EPC reversal potential, respectively, and then the calculated conductance was normalized to its maximum value. The normalized conductance is plotted as a function of the membrane potential in Fig. 5A. The outward EPC is unaffected by methylguanidine; hence, the curve gives an indication of the degree of block caused by methylguanidine. Additionally, the large transition in conductance induced by methylguanidine is easily recognized in the conductance-voltage plot.

This large change in conductance always occurred near the reversal potential for the EPC, which had a mean value of $-10 \pm 1$ mV ($n = 5$ fibers) in normal
Na and −26 ± 6 mV (n = 5 fibers) in 50% Na (Fig. 5A). For example, in normal Ringer’s solution, the relative conductance was very low at $E_m = -20$ mV, where the EPC is inward. In contrast, in 50% Na Ringer’s solution, the relative conductance was near unity at −20 mV, where the current direction is outward. This indicates that block is a function of the current direction and not the membrane potential per se.

In support of this view, we plotted the normalized conductance as a function of the driving force, $E_m - E_r$. As shown in Fig. 6, A and B, the abrupt change in conductance in the presence of methylguanidine always occurred when $E_m - E_r = 0$. Hence, when $E_m - E_r$ was >0 (current direction outward), the relative conductance was near unity, whereas when $E_m - E_r$ was <0 (current direction inward), the relative conductance was low, regardless of the absolute value of $E_m$ or $E_r$. The results obtained with ethylguanidine were qualitatively similar to those obtained with methylguanidine.

In order to better document the sharpness of the transition in conductance at potentials near the reversal potential, the membrane potential was varied in 1-mV steps. As can be seen in Fig. 5B, the relative conductance takes on intermediate values in the vicinity of the reversal potential. Outside of this transition region, the relative conductance is nearly constant.

**Conditioned Block of the EPC**

In the presence of methylguanidine, the degree of block of an inward-going EPC was intensified by repetitively evoking the EPC. It took several inward-going EPCs to establish a steady state level of block. Once the block attained a steady state, it could be relieved by generating several outward-going EPCs. To demonstrate the relief of block, the membrane was first held at −50 mV and then stepped rapidly to +50 mV. Endplate currents were evoked at a rate of 1/10 s. In normal Ringer’s solution, the amplitude of the outward-going EPCs at +50 mV was almost constant during repetitive stimuli (Fig. 7A). In the presence of 3 mM methylguanidine, however, the outward EPCs were gradually increased during repetitive stimuli (Fig. 7B). Next, to demonstrate conditioned block, the membrane was held at +50 mV and stepped to −50 mV, and repetitive stimuli were applied at 1/10 s. There was no marked change in the EPC amplitude in the control (Fig. 7C). In methylguanidine, however, the inward-going EPCs were gradually decreased in amplitude during repetitive stimuli (Fig. 7D).

Thus, with methylguanidine, both establishment and removal of block require several evoked EPCs. The time course of block development and recovery in 3 mM methylguanidine is plotted in Fig. 7E. Changing the holding potential without evoking an EPC did not affect the blocking action (not illustrated). These observations provide evidence that the opening of endplate channels is a prerequisite for the blocking or unblocking action to occur.

The question arises as to whether the removal of block of the outward-going EPCs depends on the frequency of EPCs or the number of EPCs. In order to differentiate between these two possibilities, ACh currents were induced by iontophoresis at various intervals. Steady state block of the inward ACh current was first established at a negative holding potential (−80 to −50 mV), and then the holding potential was changed to a positive potential (+30 to +50 mV), and
removal of block of the outward EPC was evaluated as a function of the interval between stimuli. An example of such an experiment is illustrated in Fig. 8, in which the amplitude of the outward EPC is normalized as \((I_{\text{max}} - I)/I_{\text{max}}\), where \(I_{\text{max}}\) and \(I\) are the amplitudes of maximum EPC and of EPC, respectively, and

\[
\begin{align*}
0.4 & \quad 0.2 \\
0.6 & \quad 0.8 \\
1.0 & \quad 1.2 \\
-60 & \quad -40 \\
-20 & \quad 0 \\
20 & \quad 40 \\
60 & \quad 80
\end{align*}
\]

**Figure 6.** Conductance during the peak endplate current normalized to the maximum conductance plotted as a function of the driving force \((E_m - E_r)\) (mV) in the presence of 3 mM methylguanidine. (A) 115 mM Na; (B) 57.5 mM Na. Each symbol represents data obtained from different endplates of the frog sartorius muscle. This plot gives the steady state block caused by methylguanidine (see legend to Fig. 4).
are plotted against the number of ACh currents delivered at an interval of 10 or 20 s. As can be seen, the recovery from block is a function of the number of pulses rather than the frequency of pulses. This indicates that the total time spent at the positive potential is not an important determinant of recovery, whereas outward current flow is a principal determinant.

Ethylguanidine block of the inward EPC, like that of methylguanidine, was intensified by repetitive stimuli. As shown in Fig. 9B, application of four stimuli, with a 2-s interval between each stimulus, depressed the nerve-evoked inward-
FIGURE 8. Recovery of repetitive outward ACh currents in the presence of 0.5 mM methylguanidine. The experimental protocol was similar to that in Fig. 7B except that iontophoretic ACh currents were measured instead of endplate currents. The membrane was stepped from -80 to +40 mV, and the change in the amplitude (I) of outward ACh currents at +40 mV was normalized to its maximum amplitude (I_max). The logarithm of the normalized change is plotted as a function of the number of ACh current pulses delivered at an interval of 10 (open circles) or 20 s (filled circles).

going EPC at -60 mV by ~25%, but had no effect on the outward EPC at +40 mV. Block could be conditioned at any potential at which the current direction is inward (Fig. 10). Block of outward-going EPCs could not be induced by repetitive stimuli (Fig. 10).

FIGURE 7. (Opposite) Stimulus dependence of endplate currents before and during application of 3 mM methylguanidine. Endplate currents were evoked at a rate of 1/10 s. (A) The membrane potential was held at -50 mV and stepped rapidly to +50 mV in control solution. Several endplate currents are superimposed at +50 mV. (C) Similar to A except that the membrane potential was stepped from +50 to -50 mV. Several endplate currents are superimposed at -50 mV. (B and D) The same protocols as in A and C were used except that 3 mM methylguanidine was present. Note that upon stepping the membrane potential from -50 to +50 mV (B) the endplate currents increase in amplitude with each successive stimulus. Stepping the membrane from +50 to -50 mV yields a successive decrease in the endplate current amplitude. Records A and B and records C and D were obtained at the same endplates of the frog sartorius muscle. (E) Time course of block development and recovery in 3 mM methylguanidine (C_M). The relative amplitude of the test EPCs is plotted as a function of time following the last conditioning stimulus (see inset). Time course of block development at -50 mV is indicated by open symbols; time course of relief of block at +50 mV is indicated by the filled symbols. In the absence of drug, repetitive pulsing did not influence EPC amplitudes either at +50 or at -50 mV. The stimulation rate was 0.1 Hz.
FIGURE 9. Influence of repetitive stimuli on EPCs in the presence and absence of 3 mM ethylguanidine (C2). (A) In control experiments, four repetitive stimuli at 0.5 Hz had no effect on EPCs either at -60 or +40 mV. (B) Application of four stimuli at 0.5 Hz depressed inward EPCs (-60 mV) to 75% of control, but had no effect on outward EPCs (+40 mV). (C) Rest period of 2 min removed the block (traces 1–4) established by repetitive pulsing at 0.5 Hz (compare traces 4 and 5). Block was produced after resumption of frequent stimulation (traces 5–8). Currents in B were elicited after a rest period of ~2 min. Records in B and C were taken from the same endplate. Frog cutaneous pectoris muscle.

FIGURE 10. Influence of repetitive stimuli on EPCs in the presence of 3 mM ethylguanidine (C2). Four EPCs, elicited at 0.5 Hz (dots in inset), were recorded during a clamp step to a test potential. Holding potential was -60 mV. Interval between steps was 2 min. Measurements represented by circles and triangles give peak amplitude of first and fourth EPCs, respectively. Note that pulsing diminished inward EPCs only. Frog cutaneous muscle.
FIGURE 11. Time course of recovery from conditioned block in 3 mM ethylguanidine (C₂). Conditioned block of EPC was established at -60 mV with a train of four stimuli delivered at a frequency of 1 Hz. The recovery of the test current is plotted as a function of time (Δt) after the last conditioning stimulus. The solid line was fitted to data points by eye; the recovery time constant at -60 mV was 26 s. Frog cutaneous pectoris muscle.

In ethylguanidine, a rest period of 2 min at -60 mV completely removed the additional block that had been established by repetitive pulsing (Fig. 9C). The inward-going EPC was gradually decreased in amplitude during four stimuli applied to the nerve with a 2-s interval between each stimulus (traces 1–4). After 2 min without stimulation, the EPC amplitude was restored (trace 5), and block could be re-established by resumption of stimuli (trace 8). The time constant for recovery from conditioned block caused by ethylguanidine was 26 s at -60 mV (Fig. 11). This time constant is independent of the membrane potential at which the endplate is held during the recovery period (not illustrated). With methyl-

FIGURE 12. Relationship between relative conductance and membrane potential in the presence of 10 μM octylguanidine in normal and 50% Na. Reversal potential shifted from 0 to -20 mV on changing the solution to low Na, but the conductance-voltage curve shifted slightly in the depolarizing direction. Data were obtained from a single endplate of the frog cutaneous pectoris muscle.
guanidine, however, waiting periods of 2 min did not noticeably relieve the block induced by repetitive stimuli.

Conductance-Voltage Curve for Octylguanidine

If a guanidine derivative blocks the channel in a manner dependent solely upon the membrane potential, then lowering the Na concentration is not expected to shift the conductance-voltage curve in the face of a change in the reversal potential for EPC. Farley et al. (1981) postulated that long-chain guanidine derivatives such as amylguanidine (five-carbon alkyl chain) and octylguanidine block the closed endplate channel in a voltage-dependent manner, and this accounts for the potential dependence of the block of the EPC. This was indeed the case, as illustrated in Fig. 12, which shows the conductance-voltage curve in the presence of 10 μM octylguanidine in normal and in 50% Na Ringer’s. Although lowering the Na caused the reversal potential to shift by some 20 mV in the direction of hyperpolarization, the conductance-voltage curve in 50% Na remained similar, with a slight shift in the depolarizing direction. Unlike methylguanidine (Fig. 5A), there was no sharp break in the curve relating conductance to membrane potential. In addition, the slight shift of curve in octylguanidine occurred in the direction opposite to what would be expected from the current-dependent block. These results are consistent with the idea that the block caused by octylguanidine is voltage dependent rather than current-direction dependent.

DISCUSSION

The present results demonstrate that the direction of current flow is crucial to the action of methyl- and ethylguanidine in blocking the endplate channels. The most clear-cut evidence for this view is that inward EPCs were blocked by methyl- and ethylguanidine, whereas outward EPCs were unaffected (Figs. 2 and 3). This results in an abrupt transition near the reversal potential for EPC in the curve relating conductance to membrane potential in both normal and 50% Na concentrations (Fig. 5A). If plotted as a function of the driving force, the curves of relative conductance for normal and 50% Na solutions are superimposable (Fig. 6). These results can be interpreted to mean that the block is determined primarily by the direction of current flow rather than the membrane potential per se.

In order to shift the EPC reversal potential, the external Na concentration was reduced using sucrose to replace NaCl. Although this maintained the osmolality of the solution, the change in ionic strength could have altered the membrane surface potential. We have calculated the theoretical change in surface potential for a 50% reduction in the Na concentration, using the Grahame equation (Grahame, 1947). Within the range of surface charge density reported for the endplate membrane (0.01–0.002 electron charge/A²) (Lewis, 1979), the change in surface potential is calculated to be −5.6 to −10 mV. Also, for a 50% reduction in the Na concentration of Ringer’s solution, the EPC reversal potential is predicted to shift by 17 mV at 22°C according to the Goldman constant field equation with a P₉/P₆ ratio of 1.0 (Watanabe and Narahashi, 1979). If the block caused by methyl- and ethylguanidine is potential dependent, the conductance-
voltage curve should shift by no more than the change in the surface potential. If the block is dependent on the direction of current flow, the conductance-voltage curve should shift to the same extent as the reversal potential. The latter was indeed the case with an average shift of 16 mV in the direction of hyperpolarization. Therefore, the results are consistent with the concept of current-dependent block.

Farley et al. (1981) showed that octylguanidine blocks the peak endplate current in a highly voltage-dependent manner. From the data presented in their Fig. 6A, we have calculated the fractional distance in the membrane electric field of the octylguanidine binding site to be 0.46 from the external surface. Since the surface potential is calculated to change by $-5.6$ to $-10$ mV by a reduction of ionic strength when sucrose is substituted for 50% Na concentration, in the presence of octylguanidine, the conductance-voltage curve is not expected to shift more than $2.6-4.6$ mV ($5.6-10 \times 0.46$) in the direction of hyperpolarization when the Na concentration is reduced to 50% of control. The observed shift was indeed negligible (Fig. 12). This result indicates that the block caused by octylguanidine is not dependent on the current direction. The absence of an abrupt transition in the curve relating conductance to membrane potential at the EPC reversal potential (Fig. 12) provides further evidence for this conclusion.

In general, the rectification of the $I-V$ relation in the presence of blocking ions can be accounted for by at least two distinct types of mechanisms, one based on current-dependent block and the other on voltage-dependent block. Two examples for current-dependent block are tetraethylammonium (TEA) block of K channels (Armstrong, 1969, 1971) and paragracine block of Na channels (Seyama et al., 1980). TEA block of K channels has been demonstrated to depend on the direction of current flow. TEA blocks outward-going K currents but does not affect inward-going currents when it is applied internally to squid axons. In contrast, paragracine, when applied internally, blocks the outward-going Na currents but has no effect on the inward Na currents. Methylguanidine resembles TEA and paragracine in that it blocks the ACh-activated channel only when the current flows in one direction, inward-going in this case.

For current-dependent blockers such as methylguanidine, the binding strength between an alkylguanidine molecule and a site in the channel is strongly dependent on the direction of current flow. The outward current weakens the binding strength and the guanidine derivative is swept away from the blocking site. For inward-going current, this type of interaction is negligible, and the blocking molecule would be able to bind to a site in the channel, resulting in a block. In contrast to the short-chain derivatives, octylguanidine, which also causes a strong rectification in the $I-V$ curve, exhibits only potential-dependent block.

Ca ion causes a change of the current-voltage characteristic from an almost linear to a strongly rectifying behavior in gramicidin channels and in Na channels of squid axons (Bamberg and Lauger, 1977; Taylor et al., 1976). The current is larger when positive permeant ions are driven from the Ca-free solution to the Ca-containing solution than in the opposite direction. Taylor et al. (1976) interpreted the change of the $I-V$ relation as all-or-none voltage-dependent block.
of Na channels by Ca ions. In their model, the energy barrier within the Na channel is high for Ca ions such that Ca ions cannot readily pass through Na channels. This prevents other ions from passing through the channels. The applied voltage can affect the binding strength in such a way that the positive potential weakens the binding strength and therefore removes the blocking effect caused by external Ca.

In the model of Bamberg and Lauger (1977), the blocking effect is not all-or-none and is interpreted in terms of the potential energy profile of the permeant ion in the channel being raised by the presence of the blocking ion. The blocking ion is thought to bind to a site that is near the mouth of the channel but is different from the channel path. This means that the permeant ion may pass by the blocking ion, which, however, modifies the flow rate. This model predicts that the block is only weakly dependent on the potential.

Our recent study of single Na channels of neuroblastoma cells has demonstrated that Ca ions block the channels in a highly voltage-dependent manner (Yamamoto et al., 1984). The Ca block can be accounted for in terms of an energy barrier model similar to that used by Taylor et al. (1976). In the energy model, an ion can block the channel in a manner dependent on either voltage or current. The energy profile and ion occupancy in the channel will determine whether the block is mainly dependent on voltage or current direction.

The stimulus-dependent block of endplate channels by short-chain guanidine derivatives appears to be similar to the use-dependent block of Na channels in excitable membranes by local anesthetics (Courtney, 1975; Yeh, 1978, 1979; Hille, 1977; Cahalan, 1978; Strichartz, 1973). In both cases many stimuli are required to enhance or remove the block while the channels are open. The use-dependent block of the Na channels has been explained simply by assuming that the h-gate of the drug-bound channel can close with the drug molecule trapped inside the channel. By analogy, we propose that the methylguanidine-bound endplate channel can close while trapping the drug molecule within the channel. The drug can be released upon re-opening of the channel, provided that the current direction is outward. If the current direction is inward, more channels become blocked. Consistent with this view, recovery from the block in methylguanidine depended on the number of stimuli used to activate the channels rather than the time interval between the stimuli. This model is in contrast to the conventional model for endplate channel block (Ruff, 1977; Neher and Steinbach, 1978), in which the drug-bound channel is assumed not to undergo a conformational transition to the closed state.

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