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An attempt to distinguish between the actions of neuromuscular blocking drugs on the acetylcholine receptor and on its associated ionic channel

(tubocurarine/lobeline/endplate kinetics)

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ABSTRACT **The effects of lobeline and tubocurarine on the voltage-clamped endplates of frog sartorius and cutaneous pectoris muscles were examined at room temperature (20–23°C). Like tubocurarine, lobeline causes nondepolarizing neuromuscular blockade. The half-time of decay ($t_{1/2}$) of endplate currents (e.p.c.s) recorded at a holding potential (V_m) of −90 mV was significantly shorter in endplates treated with lobeline (50 μM; mean $t_{1/2}$ ± SEM = 0.41 ± 0.02 ms) or tubocurarine (11.4 μM; $t_{1/2}$ = 0.64 ± 0.04 ms) than in those treated with Mg^{2+} (13 mM; $t_{1/2}$ = 1.39 ± 0.11 ms) or a low concentration of tubocurarine (3 μM; $t_{1/2}$ = 0.87 ± 0.05 ms). Similarly, lobeline (10 μM) shortened the $t_{1/2}$ of untreated miniature e.p.c.s by 35%; tubocurarine, however, abolished miniature e.p.c.s at the concentration required to observe its actions on e.p.c. decay kinetics. The $t_{1/2}$ of e.p.c.s recorded from preparations treated with Mg^{2+} (13 mM), tubocurarine at low concentrations (3 μM), or untreated miniature e.p.c.s was logarithmically related to V_m, being slower at more hyperpolarized values. By contrast, the $t_{1/2}$s of e.p.c.s recorded in either lobeline (50 μM) or tubocurarine (11.4 μM) were independent of voltage in the range −150 to −80 mV. The ability of lobeline to shorten $t_{1/2}$ and to remove the voltage dependence of $t_{1/2}$ was partially antagonized by Mg^{2+} (13 mM). As expected, when lobeline or tubocurarine was removed from the bath or when acetylcholine release from the motor nerve terminals was increased by 4-aminopyridine (20 μM) and Ca^{2+} (10 mM) (in the presence of lobeline or tubocurarine), the amplitude of e.p.c.s increased as a function of time. However, the $t_{1/2}$ of the decay phase of the e.p.c.s remained shortened (i.e., unaltered from the earlier treatment). These results suggest that both tubocurarine and lobeline have at least two distinct postjunctional actions including: (*i*) a block of the acetylcholine receptor and (*ii*) a block of the ionic channel associated with the acetylcholine receptor.**

Tubocurarine acts both on the acetylcholine (AcCho) receptor and on the AcCho receptor-activated ionic channel at the neuromuscular junction (1–3) to block transmission. The neuromuscular blocking properties of lobeline (4, 5) suggest that lobeline, like tubocurarine, may have blocking actions both at the receptor and at the receptor-activated ionic channels. We compared the actions on endplate currents (e.p.c.s) of lobeline with those of tubocurarine and found that both lobeline and tubocurarine act on the ionic channel.

In addition, we were able to discriminate between the actions of the drugs on the AcCho receptor and on the ionic channels. By increasing AcCho release, we could selectively antagonize the blocking action of lobeline and tubocurarine on the receptor without affecting the action on the ionic channel. Conversely, in the case of lobeline, we found that Mg^{2+} selectively antagonizes the blockade of the ionic channel. Furthermore, by studying the washout of lobeline and tubocurarine from the endplates, we demonstrated that the reversal of blockade of the receptor occurs long before the reversal of the blockade of the channel. Some results have been reported (6, 7).

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METHODS

Drugs used were lobeline·HCl (Sigma), tubocurarine (Sigma), and 4-aminopyridine (4-AP) (J. T. Baker).

All the experiments reported here were done on voltage-clamped endplates of frog (*Rana pipiens*) sartorius or cutaneous pectoris muscles at 20–23°C in Ringer's solution of the following composition (mM): NaCl, 98.5; KCl, 2.5; $CaCl_2$, 2; NaH_2PO_4, 1.9; Na_2HPO_4, 4.8. Ringer's solution containing 10 mM Ca^{2+} had the following composition (mM): NaCl, 98.5; KCl, 2.5; $CaCl_2$, 10; Tris, 2.0.

The two-microelectrode voltage clamp described by Deguchi and Narahashi (8) was used. Two microelectrodes filled with 3 M KCl were placed in endplates with an interelectrode distance of 50 μm. One electrode was used to measure membrane potential and the other to pass current for the membrane holding potential (V_m). The clamping circuit had a rise time less than 20 μsec and cells with a clamping error greater than 5% were rejected. e.p.c.s produced by stimulation of the nerve at 0.2 Hz and miniature e.p.c.s (m.e.p.c.s) were displayed on a Tektronix 502A or 5113 oscilloscope and recorded either on moving 35-mm film by a Grass (Quincy, MA) oscilloscope camera or processed by a Zonic Technical Laboratories (Cincinnati, OH) 200-kHz analog-to-digital converter and analyzed by an on-line LSI-11 computer.

Where mean values of several experiments were presented, the standard error of the mean is included.

RESULTS

Lobeline (50 μM) and tubocurarine (11.4 μM) caused a cessation of muscle twitch in response to motor nerve stimulation within 12.4 ± 0.74 and 2.0 ± 0.26 min, respectively. The reversal potential (V_r) of e.p.c.s evoked in the presence of lobeline (50 μM) alone was not different from V_rs obtained for e.p.c.s in Mg^{2+} alone (−5 to 0 mV) (Fig. 1). However, the amplitudes of the e.p.c.s for V_ms in the range 0 to +50 mV were more depressed than those recorded in the range 0 to −150 mV.

e.p.c.s recorded from either lobeline- or tubocurarine-depressed junctions at a V_m of −90 mV decayed with a single exponential, as did e.p.c.s recorded from Mg^{2+}-depressed junctions (Fig. 2). However, e.p.c.s recorded in either lobeline (half-time of decay = $t_{1/2}$ = 0.41 ± 0.02 ms; mean ± SEM) or tubocurarine ($t_{1/2}$ = 0.64 ± 0.04 ms) decayed at a faster rate than did e.p.c.s recorded from preparations treated with Mg^{2+}

Abbreviations: AcCho, acetylcholine; e.p.c., endplate current; m.e.p.c., miniature e.p.c.; 4-AP, 4-aminopyridine.

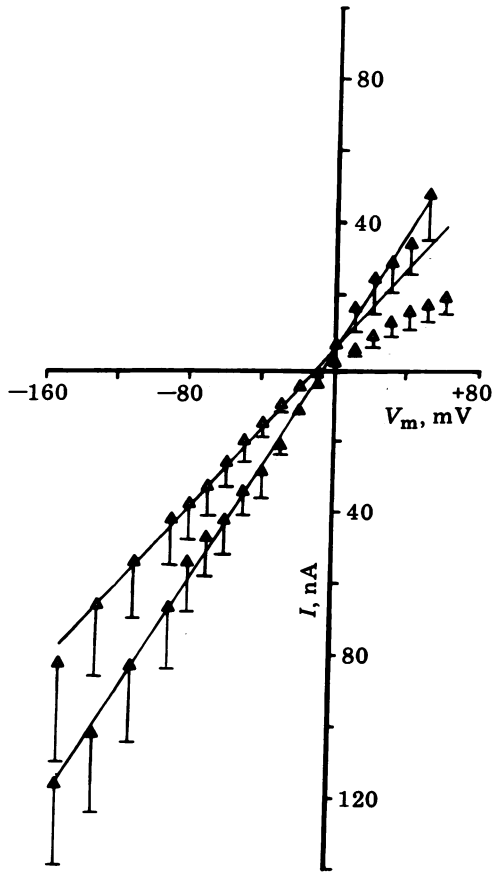

FIG. 1. Relationship between peak e.p.c. amplitude and V_m for e.p.c.s recorded from endplates treated with 13 mM Mg^{2+} (▲) or 50 μM lobeline (△). Each point represents the mean of 10 e.p.c.s per endplate recorded from four endplates. The error bars show the SEM. The lines through the points are linear regression lines (range −150 to +50 mV for Mg^{2+}-treated endplates and −150 to −10 mV for lobeline-treated endplates). Note in the positive quadrant that the peak e.p.c.s recorded from the lobeline-treated endplates deviate from the regression line.

($t_{1/2}$ = 1.39 ± 0.11 ms) or treated with a low concentration of tubocurarine (3 μM; $t_{1/2}$ = 0.87 ± 0.05 ms). Similarly, lobeline (10 μM) produced a shortening of the decay of untreated m.e.p.c.s by 35% (n = 4; Fig. 3). High concentrations of lobeline or tubocurarine could not be studied because they depressed the m.e.p.c. amplitude to the noise level of the recording system.

The $t_{1/2}$ of e.p.c.s recorded from endplates treated with glycerol (9, 10), low concentrations of tubocurarine (11), or α-bungarotoxin (2), and the $t_{1/2}$ of untreated m.e.p.c.s (12) vary with V_m, becoming prolonged at more hyperpolarized potentials. In the presence of lobeline alone (50 μM) or a relatively high concentration of tubocurarine (11.4 μM), the $t_{1/2}$ was independent of V_m in the range −150 to −80 mV. However, at more depolarized potentials the $t_{1/2}$ of tubocurarine-treated e.p.c.s showed a normal voltage dependence, whereas the $t_{1/2}$ of lobeline-treated e.p.c.s remained independent of voltage. On the other hand, the $t_{1/2}$ of e.p.c.s recorded from Mg^{2+}-treated (13 mM) or tubocurarine-treated (3 μM) endplates showed the expected voltage dependence (Fig. 4) throughout the range studied.

The $t_{1/2}$ of m.e.p.c.s observed over the V_m range of −70 to −150 mV had a voltage-dependent slope of 0.0098 ± 0.0007 mV^{-1} (n = 6), a value identical to that found by others (12). The $t_{1/2}$s of m.e.p.c.s treated with 5 or 10 μM lobeline and studied

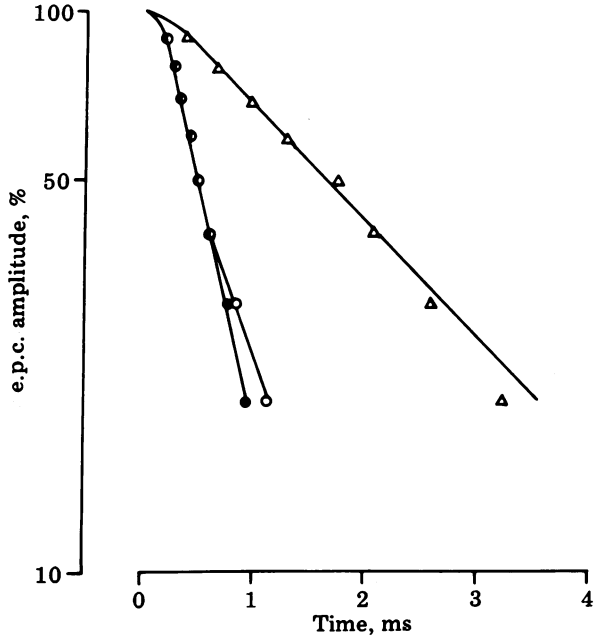

FIG. 2. Semilogarithmic plot of the decay of e.p.c.s recorded at −90 mV in 13 mM Mg^{2+} (△), 11.4 μM tubocurarine (○), or 50 μM lobeline (●). Each point represents the mean of five e.p.c.s recorded from three endplates. See text for average $t_{1/2}$s.

over the same range of V_ms were less dependent on V_m, having slopes of 0.0068 ± 0.0009 mV^{-1} and 0.0049 ± 0.006 mV^{-1}, respectively.

The ability of lobeline (50 μM) to shorten $t_{1/2}$ and to eliminate the voltage dependence of $t_{1/2}$ of e.p.c.s was reduced at endplates treated with 13 mM Mg^{2+} (Fig. 5*A*). When 50 μM lobeline was added to endplates treated with 3 μM tubocurarine, the $t_{1/2}$ was shortened to values similar to those recorded from endplates treated with 50 μM lobeline alone. Under these conditions, the $t_{1/2}$ of e.p.c.s showed no dependence on voltage (Fig. 5*B*). These results suggest that Mg^{2+} and lobeline may compete for common binding sites in the ionic channels.

As expected, washing muscles previously treated with lobeline with lobeline-free solutions resulted in a large increase in amplitude of the e.p.c.s. Unexpectedly, however, little change occurred in the drug-induced shortening of the $t_{1/2}$ of the e.p.c. (Fig. 6). This result is consistent with two sites of action for lobeline, one (the depression of e.p.c. amplitude) readily reversible on washout, the other (the shortening of e.p.c. decay time) less readily reversible.

Somewhat similar results were obtained when the effects of changing the AcCho concentration at the junction were studied at endplates blocked by either lobeline or tubocurarine. 4-AP increases AcCho release at the neuromuscular junction (13, 14)

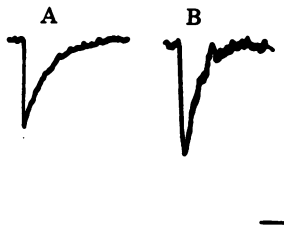

FIG. 3. Representative photographic records showing m.e.p.c.s recorded at −90 mV from the same endplate in control (*A*) and 10 μM lobeline-containing Ringer's solution (*B*). The vertical calibration bar is 4 nA for *A* and 2 nA for *B*; the horizontal calibration bar is 4 ms in *A* and *B*.

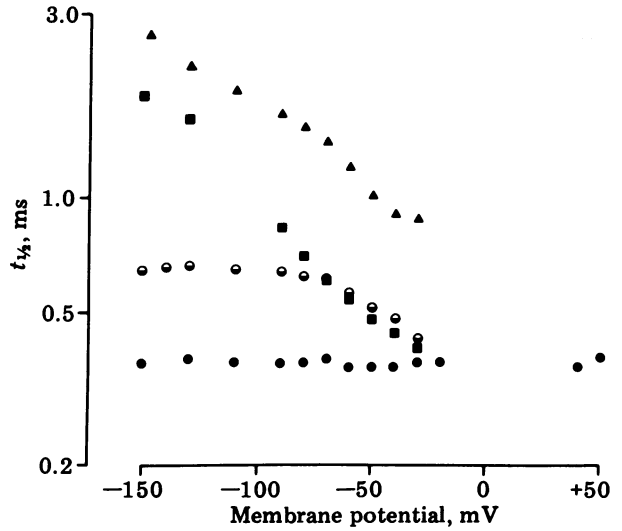

FIG. 4. The relationship between the decay half-time of the e.p.c. and the membrane potential in Ringer's solution containing 13 mM Mg^{2+} (▲), 3 μM tubocurarine (■), 11.4 μM tubocurarine (◒), or 50 μM lobeline (●). Each point represents the mean of at least 10 cells from at least three preparations.

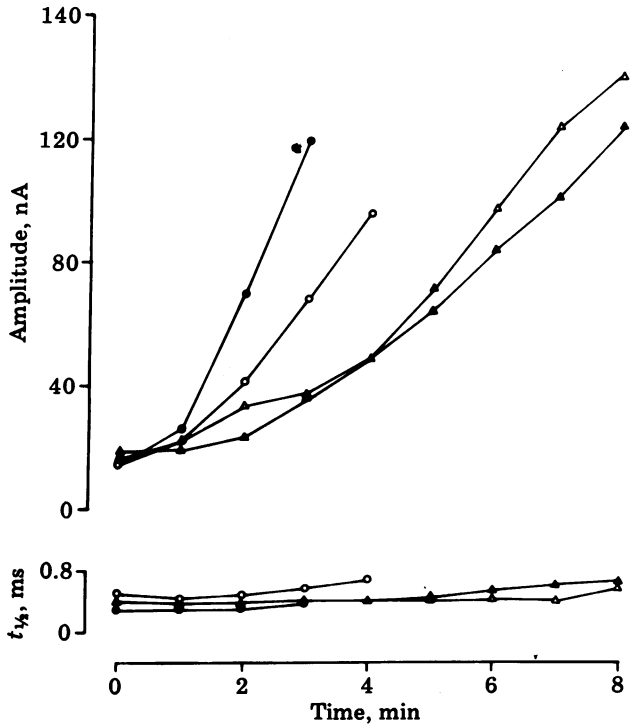

FIG. 6. Change in e.p.c. amplitude (*Upper*) and $t_{1/2}$ (*Lower*) with time after superfusing a lobeline-treated preparation with normal Ringer's solution. The results of four experiments are shown (●, ○, ▲, △). Between 3 and 9 min after the start of perfusion of normal Ringer's solution the muscle began twitching in response to nerve stimulation, making subsequent intracellular recording impossible. The holding potential in each experiment was −90 mV.

but does not appear to have actions on the ionic channel (13). 4-AP (20 μM) added to a muscle pretreated with either lobeline or tubocurarine caused a large increase in e.p.c. amplitude with little or no effect on $t_{1/2}$ (Fig. 7). Like the addition of 4-AP, raising external Ca^{2+} concentration to 10 mM resulted in an increase in e.p.c. amplitude with no change in $t_{1/2}$ (Fig. 7). In addition, the voltage independence of the $t_{1/2}$ of e.p.c.s recorded from tubocurarine-treated endplates was not altered by 4-AP or 10 mM Ca^{2+}.

DISCUSSION

Lobeline, like tubocurarine, acts both on the nicotinic AcCho receptor and on the receptor-activated ionic channel. The depression of m.e.p.c. and e.p.c. amplitudes by lobeline and tubocurarine is due mainly to their known ability to block the AcCho receptor. The shortening of the m.e.p.c. $t_{1/2}$ by lobeline and the e.p.c. $t_{1/2}$ by both lobeline and tubocurarine can be explained by an action on the receptor-activated ionic channel, as has been proposed for tubocurarine (2, 3) and other agents (15, 16). It is probable that lobeline and tubocurarine cause the shortening of the e.p.c. decay by reacting with open endplate channels (17).

The decay phase of e.p.c.s recorded from preparations

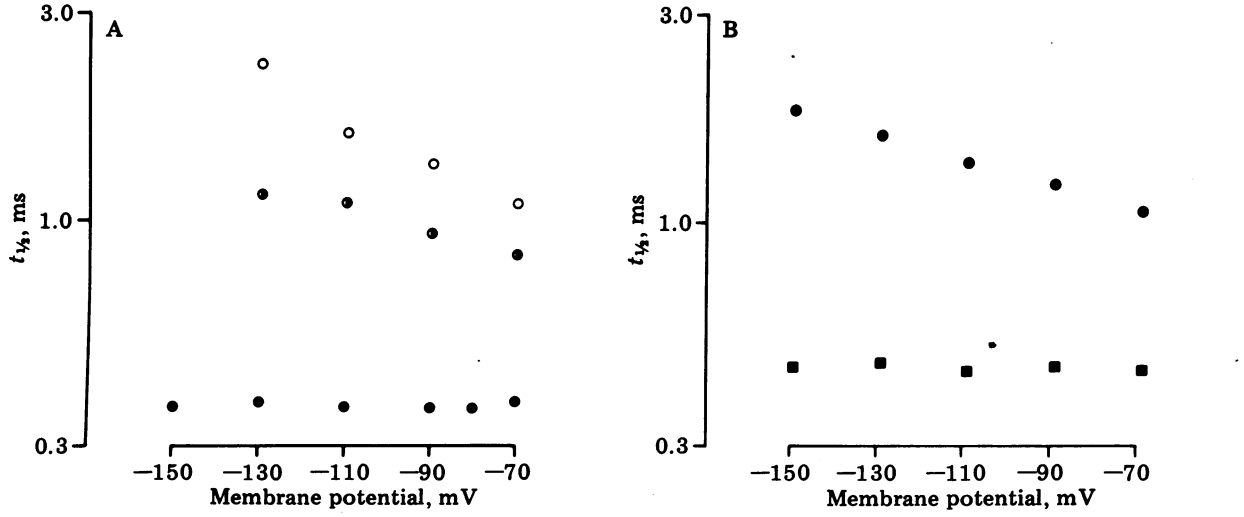

FIG. 5. (*A*) Relationship between the decay half-time of the e.p.c. and the membrane potential for e.p.c.s recorded in 13 mM Mg^{2+} (○) and from the same endplate in the continued presence of Mg^{2+} 10 min after exposure to 50 μM lobeline (◑). For comparative purposes the data for e.p.c.s recorded in lobeline (50 μM) alone are shown (●). (*B*) Relationship between the decay half-time of the e.p.c. and the membrane potential for e.p.c.s recorded in 3 μM tubocurarine (●) and from the same endplate in the continued presence of tubocurarine 10 min after exposure to 50 μM lobeline (■). Each point in *A* and *B* represents the mean of four experiments.

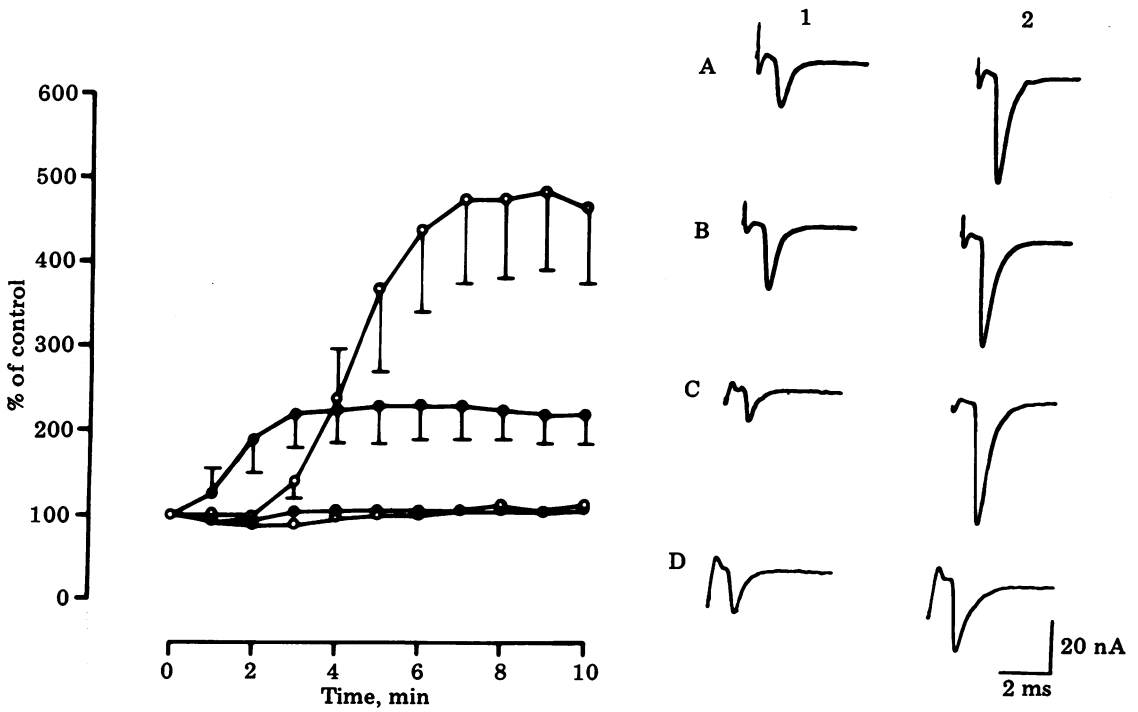

FIG. 7. Effects of adding 20 μM 4-AP or 10 mM Ca^{2+} to muscles treated with lobeline and tubocurarine. The holding potential in each experiment was −90 mV. (*Left*) The time-dependent changes of e.p.c. amplitude (two upper curves) and $t_{1/2}$ (two lower curves) as a percentage of values in tubocurarine (11.4 μM) alone. ○, Mean values of four experiments in which 4-AP was added to the tubocurarine-treated muscles; ●, mean values of four experiments in which Ca^{2+} was added. The SEM of the $t_{1/2}$ values was too small to plot. (*Right*) Representative records of e.p.c.s in lobeline (50 μM) (A1, B1) and tubocurarine (11.4 μM) (C1, D1) alone and after the addition of 4-AP (20 μM) (A2, C2) to the lobeline- and tubocurarine-treated endplates, respectively, or after the addition of Ca^{2+} (B2, D2) to the lobeline- and tubocurarine-treated endplates.

treated with either lobeline (50 μM) or tubocurarine (11.4 μM) was described by a single exponential function. Both lobeline and tubocurarine abolished the voltage dependence of the decay phase of the e.p.c.s at more hyperpolarized potentials. This suggests either that channel closing rates are accelerated by these compounds to the extent that the decay phase of the e.p.c. is no longer rate limiting or that the binding of the drugs to channel sites is voltage dependent (increasing with hyperpolarization) (2). e.p.c.s, recorded in the presence of local anesthetics, show multiphasic decay suggesting rapid channel blocking and unblocking. The rapid monophasic decay of e.p.c.s observed with lobeline and tubocurarine predicts a low channel-unblocking rate—i.e., one too slow to be detected by these methods (2). The dependence of $t_{1/2}$ of e.p.c.s on holding potential in tubocurarine at more depolarized potentials suggests that when the membrane is held at a potential below −80 mV the channel environment is not favorable for the binding of tubocurarine to the channel. A similar observation has been made for tetraethylammonium at the neuromuscular junction (18).

The lobeline effect of shortening the e.p.c. $t_{1/2}$ and removing the voltage dependence of $t_{1/2}$ was reduced by 13 mM Mg^{2+}. It seems unlikely that this effect is secondary to the well-established action of Mg^{2+} in decreasing evoked AcCho release; rather, Mg^{2+} and lobeline compete for some common channel binding site. There is some evidence that Mg^{2+} prolongs m.e.p.c. decay (19), indicating an action of Mg^{2+} on endplate ionic channels.

Clear evidence for two distinct postjunctional actions of the drugs was obtained when the drugs were washed out from the muscle and when AcCho release from the motor nerve terminals was increased. In both cases the amplitude of e.p.c.s was increased at a time when there was no change of the $t_{1/2}$ of the decay phase. Thus, it was possible to distinguish between the blocking actions of these drugs on the AcCho receptor and their action on the ionic channel.

These results can be explained in a number of ways. First, lobeline and tubocurarine may have a greater affinity for some channel site(s) than for the AcCho receptor. If this is so, then the reversal of the blockade of the AcCho receptor during washout of the drug or the increased concentration of AcCho in the junction would be expected to occur before the reversal of the blockade of the channel. Second, the results might be explained by a differential distribution of the drugs. It is possible that lobeline and tubocurarine accumulate in the region of the ionic channel to an extent greater than is found in the region of the AcCho receptor, with the result that it is easier to remove the drugs from the receptor area upon washout. Third, the inability of increased transmitter release to antagonize the blockade of ionic channels may be due to the inaccessibility of the channels to endogenous AcCho.

An action of lobeline, blocking outward-going current when the endplate membrane was held at positive potentials (0 to +50 mV), was suggested by the depression of e.p.c. amplitude in this range. It appears unlikely that this effect is related to the effect on e.p.c. decay, which was evident at all holding potentials (−150 to +50 mV).

In conclusion, both tubocurarine and lobeline have at least two distinct postjunctional actions. They appear to interact with both the AcCho receptor and its ionic channel. It would be of interest to compare the binding characteristics of these two sites and to determine if lobeline, tubocurarine, and local anesthetics share a common channel binding site or sites.

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