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Meproadifen Reaction with the Ionic Channel of the Acetylcholine Receptor: Potentiation of Agonist-Induced Desensitization at the Frog Neuromuscular Junction

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SUMMARY

The actions of the nicotinic noncompetitive antagonist meproadifen on both the acetylcholine (ACh) receptor-ion channel complex and electrically excitable membrane were examined in frog sciatic-nerve sartorius muscle preparations. Meproadifen (10–25 μM) blocked the nerve-evoked twitch without affecting the directly evoked twitch, the threshold, overshoot, amplitude, rate of rise, or falling phase of the directly elicited action potential in muscle. This suggests that this agent, at the concentrations that affect the nicotinic receptor, had negligible effect on the excitable membrane. In addition, the drug did not affect either the quantal content or quantal size of the end-plate potential. Meproadifen caused a voltage- and time-dependent decrease in the peak amplitude of the end-plate current (EPC) without significantly shortening the time constant of EPC decay. The voltage- and time-dependent effects of meproadifen were more pronounced at more negative potentials, as evidenced by hysteresis loops and nonlinearity in the current-voltage relationship of the EPC. Both hysteresis and nonlinearity in the current-voltage relationship of the EPC were eliminated when brief conditioning pulses were used for stepwise changes of membrane potentials. The decay time constant of the EPC in the presence of meproadifen remained an exponential function of time. Meproadifen blocked iontophoretically elicited EPCs but did not affect single-channel lifetime, conductance, or the decay time constant of the miniature EPC. Thus, the blockade was more marked on iontophoretically elicited EPCs than on miniature EPCs. Meproadifen also caused desensitization of both the junctional and extrajunctional ACh receptors, but, more important, meproadifen accelerated steady-state desensitization by several-fold (compared with the agonist). The marked depression of peak EPC amplitude and miniature EPC, its high affinity for the binding sites in the presence of the agonist, and acceleration of agonist-induced desensitization suggest that meproadifen interacts with the ACh-bound but nonconducting state of the ACh receptor-ion channel complex. Therefore, it appears that meproadifen interacts with the closed ionic channel of the ACh receptor in its resting and activated but nonconducting states, and only slightly affects the open conformation of the ionic channel.

INTRODUCTION

At the neuromuscular junction, the reaction of ACh[4] with its recognition site at the nicotinic receptor results in an intramolecular transformation culminating in the opening of the ion channel. Recently, a number of novel, noncompetitive antagonists of the AChR have allowed us to study channel properties more closely. Compounds such as atropine, scopolamine, quinuclidinyl benzilate, ketamine, and certain local anesthetics (1–5) appear to react preferentially with the ionic channel of the ACh receptor in its open conformation. HTX and its ana-

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[4] The abbreviations used are: ACh, acetylcholine; AChR, ACh receptor; HTX, histrionicotoxin; PCP, phencyclidine; EPC, end-plate current; MEPC, miniature end-plate current; τ_{EPC}, decay time constant of EPC; τ_{MEPC}, decay time constant of miniature end-plate current; γ, single-channel conductance; τ_I, channel lifetime.

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logues, tricyclic antidepressants and PCP, also react with sites which are distinct from the ACh recognition site (6–10). As demonstrated by Masukawa and Albuquerque (7) and by Schofield *et al.* (9), the analysis of the current-voltage relationship of the peak amplitude of the EPC disclosed a voltage- and time-dependent depression. This time-dependent depression of the EPC occurred independently of the presence of an agonist. These compounds appear to interact primarily with the AChR-ion channel molecule in its closed conformation (both the resting and activated but nonconducting states) and, to a lesser extent, with the open channel state (7, 10). The marked voltage and time dependence of the decrease of EPC amplitude induced by these agents is practically abolished when the duration of the conditioning pulse is shortened from 3 sec to 20 msec (7, 9, 11). Since some of their actions appear to be agonist-independent, the results obtained with these agents support the notion that the nonconducting species of the AChR-ion channel complex is distinct from the desensitized form that results in an increase in affinity of ACh for its recognition site (12, 13). Another agent, meproadifen [2-(diethylmethylaminoethyl)-2,2-diphenylvalerate iodide)] (Fig. 1), a quaternary local anesthetic and noncompetitive antagonist of the nicotinic AChR, has been shown to increase the affinity of ACh for its binding site in isolated *Torpedo* membranes (14, 15). The similarity of this effect to that which accompanies prolonged treatment of the AChR with agonists has suggested that this high-affinity state may correspond to desensitization. In fact, meproadifen, HTX, PCP, and certain tricyclic antidepressants affect the ion channel of the nicotinic receptor (8–10, 16) and increase the affinity of ACh for its recognition site (17–21). Several features of meproadifen's action on the junctional membrane of the nicotinic synapse remain to be elucidated. First, meproadifen is a quaternary compound, thus most of its effects are probably limited to the outer portion of the membrane. In contrast, HTX, a tertiary amine, also interacts with intracellular sites.[5] Second, meproadifen's actions may allow us to clarify the voltage and time dependence of the EPC as demonstrated by our preliminary experiments (16). Third, the drug may provide some additional electrophysiological evidence for the concept that the AChR-ion channel may have the ability to assume various conformations (upon activation), inducing a conducting species which may interchange with a short-term desensitized state; the latter appears to be different from slow receptor desensitization induced by an excess of agonist (12). Since these aspects remain to be clarified, we decided to investigate the effects of meproadifen on various properties of the excitable membrane as well as the ionic channel of the AChR.

MATERIALS AND METHODS

Electrophysiological techniques. All experiments were carried out at room temperature (20–22°) on sartorius and cutaneous pectoris muscles from the frog, *Rana pipiens*. The physiological solution had the following composition (millimolar): NaCl, 116; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7. The solution was bubbled with 100% O_2 and the pH was 6.9–7.1. For twitch studies, the sciatic nerve was stimulated (via a bipolar platinum electrode) with supramaximal rectangular pulses having a duration varying from 0.05 to 0.1 msec. Direct stimulation of the muscle was accomplished by applying supramaximal rectangular pulses having durations varying from 2.0 to 3.0 msec, again via a bipolar platinum electrode. Direct and indirect stimulation was applied alternatively, each at a rate of 0.05 Hz.

For recording intracellular potentials, the muscles were pinned under slight tension to a Sylgard plate and placed in a 10-ml bath. Resting membrane potentials were recorded only from surface fibers using conventional intracellular recording techniques (22). Intracellular electrodes had resistances of 1–3 Mohm and were filled with 3 M KCl. Action potentials were evoked by passing a 30-msec depolarizing pulse through a microelectrode inserted into a surface fiber and recorded by a second microelectrode inserted about 50 μm away in the same fiber. The threshold depolarization for the generation of action potentials was measured as the amplitude of the potential step preceding the self-regenerative response; the amplitude was taken between threshold and summit. The repolarization phase was represented by the time to half-decay of the action potential amplitude. The maximal rate of rise of the action potential was measured by an R.C. circuit (1 Mohm–100 pF) and was recorded on an oscilloscope. Delayed rectification was determined in surface fibers of frog sartorius muscles continuously exposed to tetrodotoxin (1.0 μM). The muscle fibers were polarized to −90 mV and stimulated with rectangular depolarizing and hyperpolarizing current pulses. Quantal content was determined in high-magnesium (12 mM) Ringer's solution as described previously (23). The microiontophoresis technique used to apply ACh to the extrajunctional region of the chronically (10–15 days) denervated soleus muscle of the rat is similar to that previously described (24).

Voltage-clamp and EPC analysis. The sciatic nerve-sartorius muscle preparations were treated with 600 mM glycerol to disrupt excitation-contraction coupling (25). The voltage-clamp technique was similar to that of Takeuchi and Takeuchi (26) as modified by Kuba *et al.* (27). Voltage- and time-dependent effects were determined by using various membrane potential conditioning sequences (7, 9). The voltage sequence used for a 3-sec pulse duration was similar to that used to examine the effect of HTX on EPCs in frog sartorius muscles (7). The sequence consisted of 10-mV steps made in the depolarizing and then hyperpolarizing directions between the extremes of +50 mV and −150 mV. EPCs were elicited at the end of each conditioning step. Three second conditioning steps were used to avoid any frequency-dependent effects of the drug. A voltage sequence using brief pulse duration was used to test the influence of conditioning step duration on the relationship between EPCs and membrane potential. The voltage sequence consisted of voltage excursions of 50-msec duration every 3 sec from a holding potential of −50 mV. The conditioning steps were again made first in the depolarizing and then in the hyperpolarizing direction between the voltage extremes of +50 mV and −150 mV. EPCs were evoked 20 msec after the initiation of the conditioning step (9). The

[5] E. X. Albuquerque, unpublished observations.

d.c. output of a Tektronix oscilloscope, which displayed the nerve-evoked EPC and the membrane potential, was digitized at a 10 KHz interval and analyzed by a laboratory computer (PDP 11/40; Digital Equipment Corporation, Maynard, Mass.). The τ_{EPC} was determined by linear regression on the logarithms of the digitized decay points (20%–80%) against time.

MEPC and EPC fluctuations. Conventional procedures were used for recording and analyzing MEPC and EPC fluctuation data (9). For noise analysis, an EPC induced by iontophoretically applied ACh was recorded on a low-gain d.c. trace for mean EPC measurement and on a high-gain a.c. trace for measurement of current fluctuations. The EPC was displayed on a Mingograf 81 having a frequency response from d.c. to 700 Hz and recorded on an FM tape recorder (Racal Store 4DS, Rockville, Md.). MEPCs were filtered (1–2500 Hz) using a Krohn-Hite 3700 bandpass filter and captured by a digital oscilloscope (Gould OS4000) before being transmitted to the computer for averaging and analysis. The sampling rates for the PDP 11/40 computer were 2 KHz for noise and 8 kHz for MEPCs, and the EPC signal was filtered between 1 and 800 Hz. Thirty segments of 0.256 sec (512 points) of baseline and of ACh fluctuations were obtained from each response. Power-density spectra of ACh noise and of MEPCs were fitted to a single Lorentzian curve using a nonlinear regression program for computation of γ and τ_I. τ_I was estimated from the formula $\tau_I = 1/(2\pi f_c)$, where f_c is the half-power frequency of the Lorentzian curve. Channel conductance was estimated from the formula $\gamma = S(0)/[4\mu(V_m - V_{eq})\tau_I]$, where $S(0)$ is the zero-frequency asymptote, μ is the mean d.c. current, V_m is the holding potential, and V_{eq} is the equilibrium potential, taken to be −15 mV.

Statistics. All values are expressed as means ± standard error of the mean. Student's *t*-test was used to examine the difference between control and drug. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effects on twitch and electrical properties of the frog sartorius muscle. The quaternary agent meproadifen blocked the indirectly evoked muscle twitch without affecting the directly evoked twitch. Figure 1 shows the effects of one concentration of meproadifen (20 μM) which blocks neuromuscular transmission of the frog sciatic-nerve sartorius muscle preparation in about 10 min. At concentrations of 1, 5, and 10 μM, the indirectly evoked muscle twitch was blocked by 29% and 57% and 100%, respectively, in 60 min (Fig. 1). There was neither potentiation nor blockade of the directly evoked muscle twitch at any of the above concentrations. Washing the muscle for 60 min resulted in a 70% recovery of the indirectly evoked twitch (Fig. 1). At concentrations varying from 1 to 20 μM, meproadifen had a negligible effect on the muscle action potential and did not significantly change the membrane potential of the junctional and extrajunc-

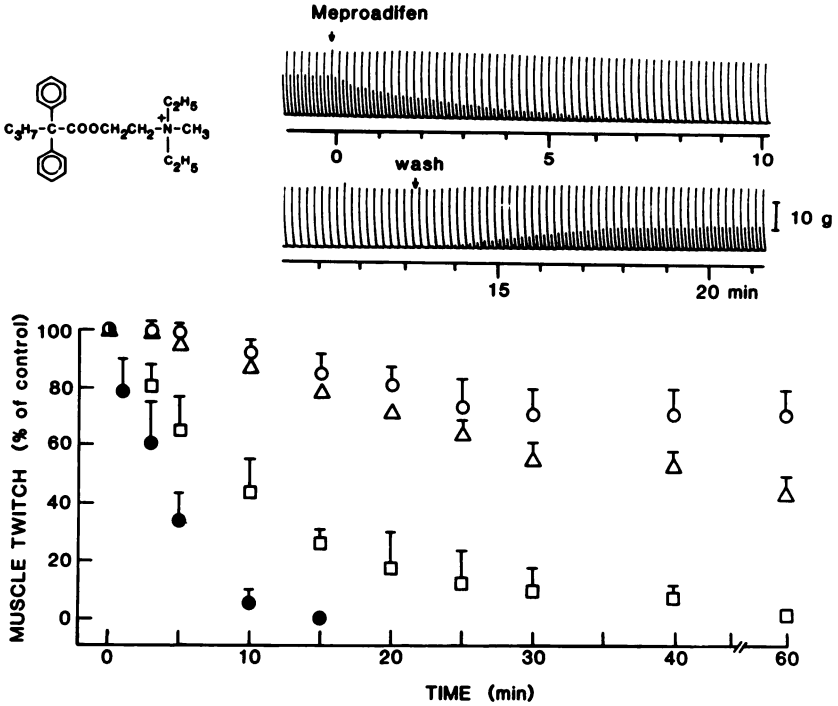

FIG. 1. *Effect of meproadifen on directly and indirectly evoked isometric twitch of frog sartorius muscle*

Polygraph record of an isometric twitch of frog sartorius muscle in response to alternating supramaximal direct and indirect stimulation (0.1 Hz) are shown. The addition of meproadifen (20 μM, *arrow*) to the preparation blocked the indirectly evoked twitch. Note the partial (70%) recovery of the indirectly evoked twitch after washing the preparations with normal physiological solution. The effects of meproadifen at 1 μM (○), 5 μM, (△), 10 μM (□), and 20 μM (●) on the indirectly evoked twitch of the frog sartorius muscle are expressed as percentage of control tension plotted against time in minutes (*lower*). Each point represents the mean ± standard error of the mean from three to five muscles.

TABLE 1

Effects of meproadifen on the amplitude, rise time, and decay time constant of the EPC at −90 mV

The values are mean ± standard error of the mean from three to nine muscles. Numbers in parentheses indicate number of determinations.

Condition	Amplitude	Rise time	τ_{EPC}
	$\times 10^{-7}$ amp	msec	msec
Control	3.82 ± 0.32 (68)	0.80 ± 0.02	1.82 ± 0.09
Meproadifen			
2 μM	2.36 ± 0.48 (5)[a]	0.88 ± 0.04	1.77 ± 0.08
5 μM	1.45 ± 0.19 (26)[b]	0.83 ± 0.09	1.68 ± 0.06
10 μM	0.60 ± 0.18 (10)[b]	0.80 ± 0.03	1.52 ± 0.11
25 μM	0.44 ± 0.13 (4)[b]	0.72 ± 0.02	1.45 ± 0.20
Washing (60 min)	2.15 ± 0.17 (5)	0.73 ± 0.04	1.64 ± 0.06

[a] $p < 0.01$.
[b] $p < 0.001$.

tional region of surface fibers. In addition, no alteration was observed in the threshold, overshoot, amplitude, or rate of rise of the directly evoked action potential. There was no prolongation of the falling phase of the action potential when evoked at a stimulation rate of 1 or 10 Hz. Furthermore, delayed rectification, which reflects a qualitative evaluation of potassium conductance (28), remained unaffected in the presence of meproadifen (20 μM).

Effects on amplitude and time course of the EPC. The effects of various concentrations of meproadifen (2 to 25 μM) on amplitude, rise time, and τ_{EPC} are shown in Table 1. Families of EPCs recorded under control conditions and after incubation with 5 μM meproadifen for 30–60 min are shown in Fig. 2. Under control conditions, the EPC recorded at −90 mV had an average rise time of 0.8 msec, a peak amplitude of 382 namp, and a τ_{EPC} of 1.82 msec. These values agree with those reported earlier (3). Exposure of the end-plate to meproadifen (5–25 μM) for 30–60 min produced a concentration-dependent decrease in peak amplitude with minor effects on τ_{EPC} (Table 1). For example, at −90 mV, 5 and 10 μM meproadifen decreased the peak EPC amplitude to 25% and 16% but shortened τ_{EPC} only to 92% and 84% of control, respectively. The onset of the action of meproadifen began within 0.5 min of incubation, and the effect of the agent was reversed by about 50% when the preparation was washed with normal physiological solution for 60 min (Fig. 2; Table 1). Figure 3 shows the effect of meproadifen on EPCs recorded at various membrane potentials. The peak amplitude under control conditions was voltage-sensitive and linearly related to membrane potential. At 2 μM, meproadifen depressed the peak amplitude while inducing only negligible changes in the linearity of the current-voltage relationship. However, as the concentrations were raised (from 5 to 25 μM), meproadifen caused a significant decrease in the voltage sensitivity of the EPC amplitude and induced a marked nonlinearity in the current-voltage relationship. The depression of peak amplitude was evident at all membrane potentials, but the depression increased progressively with membrane hyperpolarization, despite an increase in driving force, which led to a nonlinear current-voltage relationship.

Voltage- and time-dependent actions of meproadifen on EPC amplitude. To characterize the voltage-dependent action of meproadifen, we used conditioning pulses similar to that used by Masukawa and Albuquerque (7). The membrane was clamped initially at −50 mV, was shifted sequentially in 10-mV steps to +50 mV then to −150 mV, and returned to −50 mV. At each potential the membrane was held for approximately 3.0 sec. The EPC amplitude in most cells was observed to be larger during the initial hyperpolarizing steps (from −50 to −150 mV) than it was at the corresponding potentials on the return excursion (from −150 to −50 mV) (Fig. 4). This hysteresis loop resembled those seen with HTX (7) and to a lesser extent PCP (10). On the other hand, when the current-voltage relationship was studied with a brief-duration conditioning pulse of only 20 msec, a low-voltage sensitivity or decreased slope-conductance was recorded but a linear relationship was obtained regardless of the direction of the voltage steps (Fig. 4). In the absence of meproadifen, the current-voltage relationship was identical with either 3-sec or 20-msec conditioning pulses. Since both the nonlinearity and hysteresis disappeared when conditioning durations of 20 msec were used, this indicates that both the voltage and time dependence were caused by the same process, evident after 3-sec but not after 20-msec conditioning times.

To determine whether the change in potential itself is sufficient to depress the peak EPC amplitude or whether activation of the AChR at hyperpolarizing potentials is required, two series of experiments were performed (7).

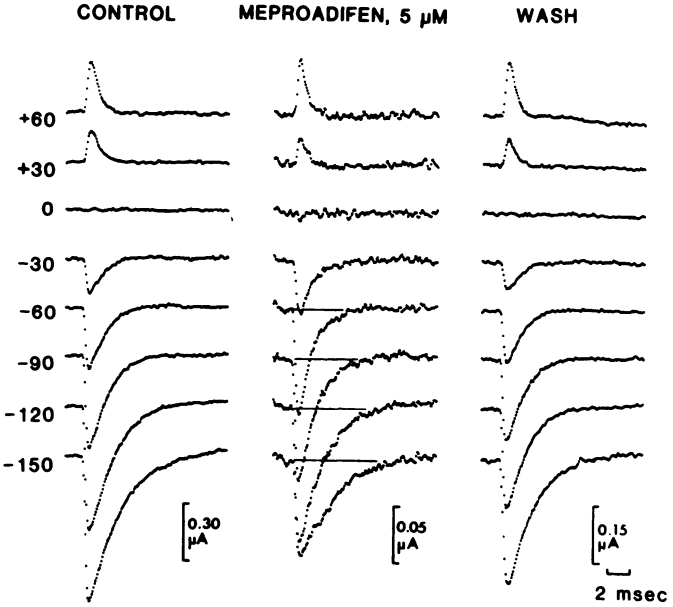

FIG. 2. *Digitized EPC at various membrane potentials under control conditions and in the presence of meproadifen*

Series of EPCs recorded at different membrane potentials prior to and after a 30- to 60-min exposure to meproadifen (5 μM), and after a 60-min washing. Each series was obtained from a single surface fiber on frog sartorius muscle. Note the different calibration for current in each column.

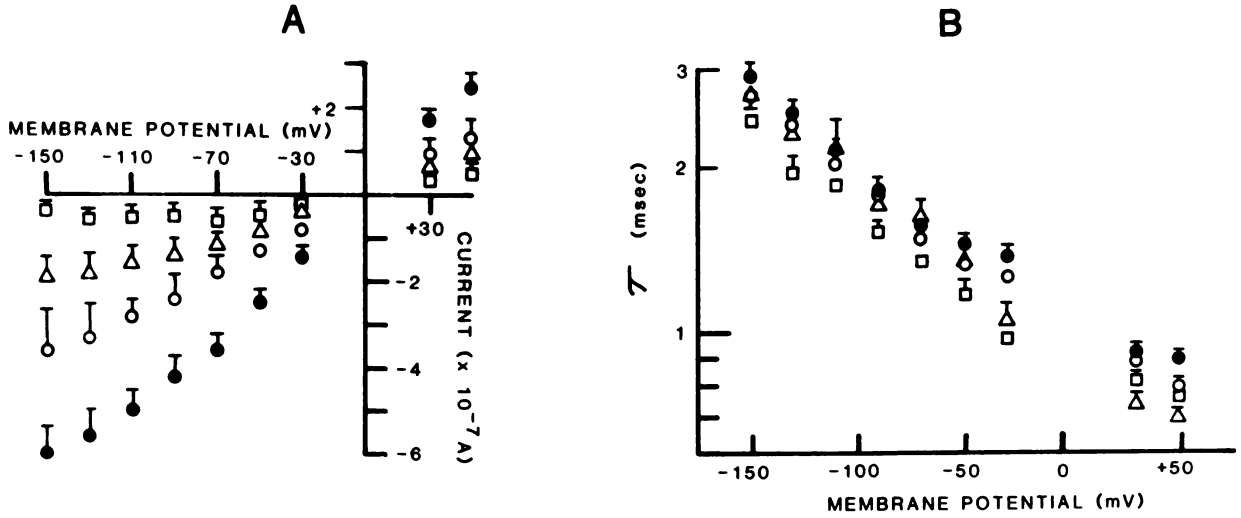

FIG. 3. *Effect of meproadifen on peak EPC amplitude and* τ_{EPC}

A. The relationship between peak amplitude of the EPC and the membrane potential is shown under control conditions (●) and after a 30- to 60-min exposure to meproadifen, 2.0 μM (○), 5.0 μM (△), and 10.0 μM (□). Each point represents the mean ± standard error of the mean from 12 to 46 surface fibers from at least 5 muscles.

B. The relationship between the logarithm of the τ_{EPC} and membrane potential under control conditions and in the presence of meproadifen, 2.0 μM (○), 5.0 μM (△), and 10.0 μM (□).

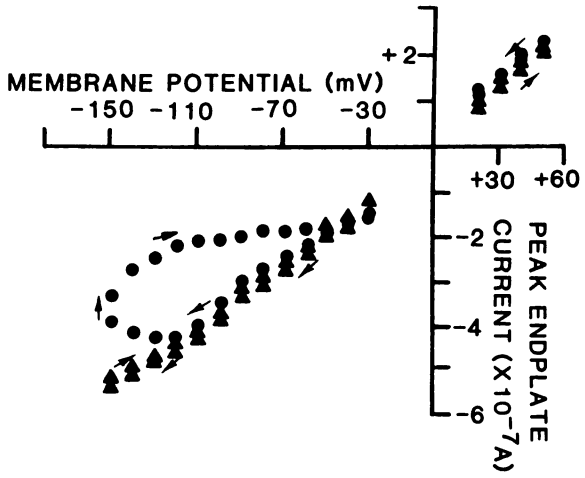

FIG. 4. *Voltage- and time-dependent blockade of EPCs by meproadifen (5 μM)*

Membrane potential conditioning steps of 20-msec duration (▲) initiated from a holding potential of −50 mV linearized the current-voltage relationship of the cell that showed a distinct hysteresis when recorded using membrane potential conditioning steps of 3-sec duration (●).

The membrane potential was changed in one step from −50 mV to −150 mV, where it was held for 30 sec before being returned to −50 mV. In one series, EPCs ceased during the step, and in the other they continued throughout. The effect on EPC peak amplitudes after the step was the same, showing that the change in potential alone augments meproadifen's blockade (data not shown).

Effect on τ_{EPC}. Although meproadifen significantly decreased the peak EPC amplitude in a voltage-, time-, and concentration-dependent manner, its effects on τ_{EPC} were very much less evident (Fig. 3B). Meproadifen (1–25 μM) did not change the single exponential decay of the EPC and MEPC. A sample of the semilogarithmic plots of the falling phase of the EPC recorded at +60, −90, and −150 mV under control conditions and in the presence of meproadifen (5 μM) is shown in Fig. 5. In the absence of meproadifen, the EPC decayed faster as the membrane potential was depolarized from −150 mV to +50 mV, and a plot $\log_{10} \tau$ versus voltage was characterized by a slope of −3.16 ± 0.16 V^{-1} (Fig. 3B). For meproadifen, at concentrations of 2, 5, and 10 μM, the relationship between log τ versus membrane potential remained linear and had slopes of −3.12 ± 0.38, −3.14 ± 0.25, and −3.04 ± 0.32 V^{-1}, respectively (Fig. 3B). At 40 μM, where the EPC amplitudes were so depressed that measurements were unreliable, τ_{EPC} was only slightly affected. Although the effect of meproadifen on EPC peak amplitude was time-dependent, its effect on τ_{EPC} remained unaltered with either long or short conditioning pulses. The depression of EPC amplitudes by meproadifen, without altering the slope of the log τ_{EPC}, versus the membrane potential plot supports the notion that peak amplitude and τ_{EPC} are two independent phenomena which may involve more than one binding site for the drug.

Effect on the EPC evoked by repetitive stimulation. Binding studies indicate that meproadifen's effects on the ionic channel are enhanced by the presence of the agonist (14). Such a biochemical reaction could be tested electrophysiologically by applying repetitive stimulation in a manner similar to that used with HTX(28). Figure 6 shows the peak amplitude under control conditions and in the presence of meproadifen (5 μM) during tetanic stimulation at several membrane potentials. In a manner similar to H_{12}-HTX (29), but to a lesser extent, meproadifen produced a use-dependent depression, or rundown, of EPC amplitude when the nerve was stimulated at 25 Hz. After 30- to 60-min exposure to meproadifen (5 μM), the control peak EPC amplitude at −90 mV was reduced to 40% of the control value. Following a facilitation, also seen in control condition, the EPC amplitude decreased

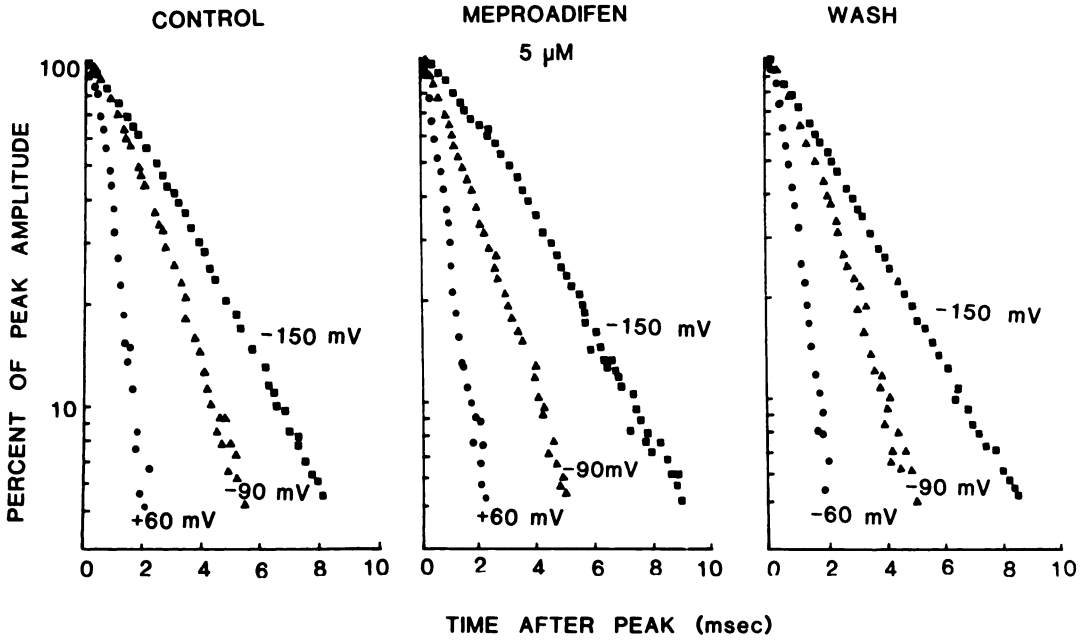

FIG. 5. *Effects of meproadifen on the decay phase of EPC recorded at three membrane holding potentials*

A representative sample of the decay phase of EPC at three membrane potentials under control conditions in the presence of meproadifen (5 μM) and after washout. Note that the decay phase of EPC remains a single-exponential function of time under all conditions.

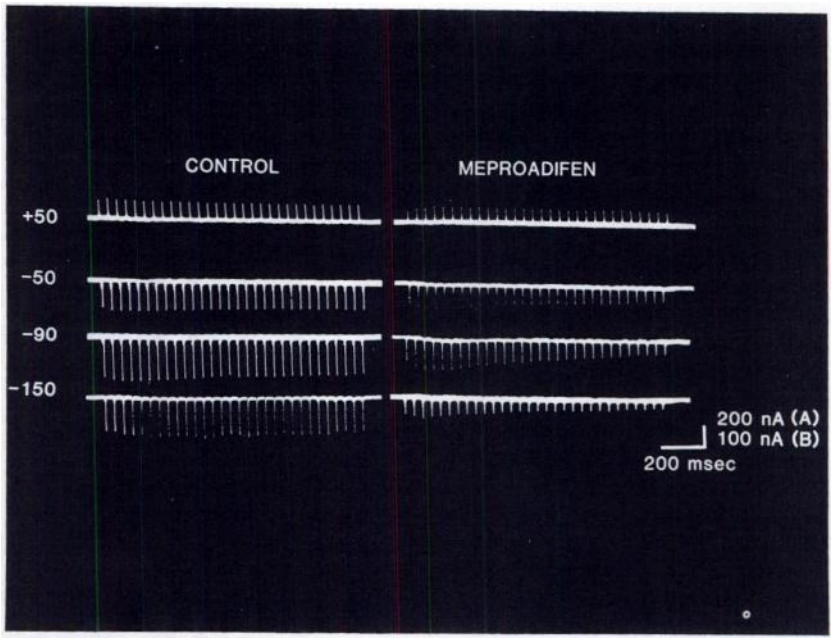

FIG. 6. *Effect of membrane potentials on the amplitude of the EPCs evoked by repetitive stimulations (25 Hz) in physiological solution and after treatment with meproadifen*

Repetitive stimulations were given every 1.5 min under control conditions and every 2 min in the presence of meproadifen (5 μM). The vertical scale shows current (*A*, control; *B*, meproadifen); the horizontal scale shows time in milliseconds.

as a single exponential function of time up to the 15th pulse and then reached a plateau (Fig. 7A and B). This pattern of depression was observed in each of the three consecutive trains, applied at 2-min intervals at −90 mV. Values for the relative depression of EPC amplitude (compared with the EPC of the highest amplitude, fifth pulse) were 32%, 30%, and 29% in the first, second, and third train, respectively. However, the initial value of the second and third train did not quite reach the initial value of the first train following a 2-min interval between trains. The frequency-dependent depression induced by meproadifen was also voltage-sensitive. Note that, despite the decrement of EPC amplitude, the decay time constant of EPC remained unaffected (Fig. 7C, *inset*). When the frequency of trains was increased to 50 and 100 Hz, a further significant depression of the peak EPC amplitude recorded at −90 mV was observed (Fig. 7C). Indeed, the frequency-dependent decrease of peak am-

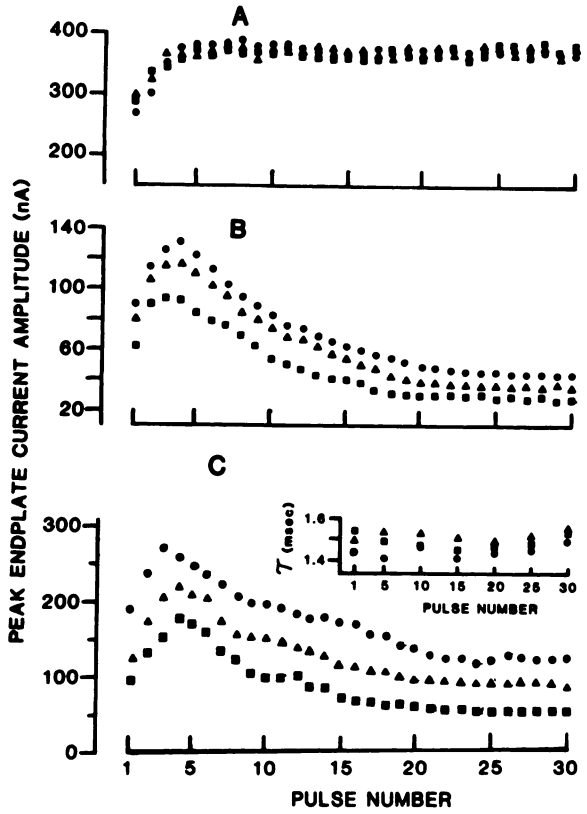

FIG. 7. *Effects of tetanic stimulations on the amplitude of the EPC under control conditions (A) and in the presence of meproadifen (B and C)*

Three trains of repetitive stimulations (25 Hz for 1.2 sec) were given at intervals of 10 sec. The symbols for A, B, and C indicate the sequence of trains, i.e., ●, first; ▲, second; ■, third. In C is shown the frequency-dependent blockade of EPC when meproadifen is present in the muscle bath. The membrane potential was maintained at −90 mV. The ordinate shows EPC in nanoamperes; the abscissa shows pulse number. The *inset* shows the magnitude of τ_{EPC} for 1–30 potentials of train at a frequency of 25, 50, and 100 Hz. Note that the τ_{EPC} remains unaffected by the frequency-dependent stimulation.

TABLE 2

Decay time constants of EPC under control conditions and in the presence of meproadifen (5 μM) during tetanic stimulation (25 Hz)

The results are mean ± standard error of the mean of at least three determinations from two to three muscles. First, fifth, and last refer to the number of an EPC in a train of 2-sec durations.

Condition	Holding potential	τ_{EPC}		
		First	Fifth	Last
	mV	*msec*	*msec*	*msec*
Control	−90	1.65 ± 0.07	1.63 ± 0.05	1.67 ± 0.06
	−150	1.95 ± 0.03	2.02 ± 0.09	2.04 ± 0.08
Meproadifen	−90	1.40 ± 0.08	1.49 ± 0.07	1.57 ± 0.05
	−150	2.27 ± 0.10	2.28 ± 0.14	2.28 ± 0.90

plitude occurred without any significant shortening of τ_{EPC} (Table 2). Since the depression of each peak amplitude in the train occurred in frequency-dependent manner, the possibility existed that some of the observed depression resulted from a decrease in the quantal content. However, when determination of the quantal content was made, no significant alteration of either quantal content or quantal size was observed in the presence of 5 μM meproadifen (Table 3). Thus, the frequency-dependent depression of peak EPC amplitude suggests that channel activation increases inhibition by meproadifen.

Effect of meproadifen on junctional and extrajunctional ACh sensitivity of chronically denervated muscles. Meproadifen produced a concentration-dependent decrease in the amplitude of the depolarization induced

TABLE 3

Effects of meproadifen on the quantal content and quantal size of the end-plate potential of frog sartorious muscles

End-plate potentials were recorded in the presence of a physiological solution containing 12 mM $MgCl_2$. Determinations were made from the same end-plate region before, during, and after washout of meproadifen. All values are mean ± standard error of the mean of results from three end-plates.

Condition	Amplitude	Quantal content	Quantal size
	mV		*mV*
Control	2.76 ± 0.02	15.50 ± 0.09	0.19 ± 0.006
Meproadifen (5 μM)	3.60 ± 0.97	12.38 ± 0.46	0.32 ± 0.09
Wash	2.50 ± 0.63	12.62 ± 2.63	0.21 ± 0.01

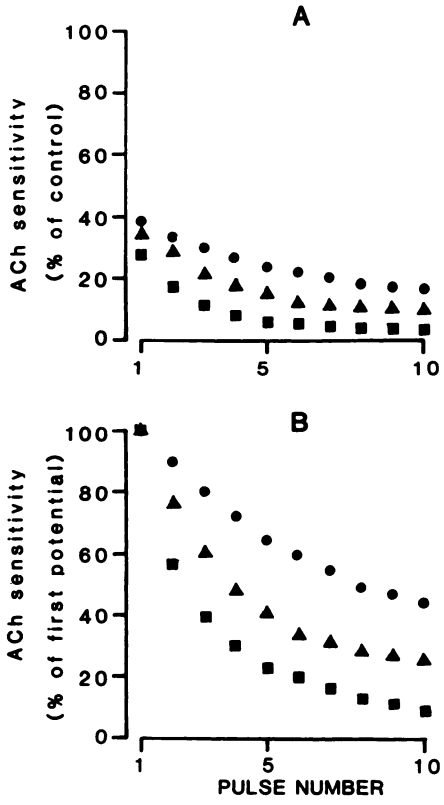

FIG. 8. *Influence of stimulus frequency on ACh sensitivity of the extrajunctional region of chronically denervated rat soleus (8–15 days)*

Under control conditions, ACh sensitivity following a train of 10 stimuli at 1 Hz remained unaltered, whereas in the presence of meproadifen, ACh sensitivity was decreased following the similar train of stimuli. A shows ACh sensitivity expressed as percentage of the control potentials; B shows ACh sensitivity expressed as percentage of the first potential in the presence of meproadifen: ●, 1 μM ▲, 2.5 μM; ■, 5 μM. The duration of the ACh pulse was 1.0 msec; stimulus frequency was 1 Hz. ACh sensitivity was determined from the same region before and during the presence of the drug.

by microiontophoretic application of ACh to the extrajunctional region of chronically denervated rat muscle. ACh sensitivity was reduced from a control mean value of 1350 mV/nC to <10 mV/nC in the presence of 10 μM meproadifen. Repetitive stimulation (1 Hz) caused a further depression of ACh potential amplitude, a phenomenon resembling neurally evoked EPCs (Fig. 8). In the presence of 2.5 μM meproadifen, the first extrajunctional ACh potential was decreased by 62% relative to control, and the 10th potential was further reduced to only 25% of control when the stimulation was continued. By the 25th pulse the ACh potential was too small to be recorded accurately. Upon completion of a set of 10 pulses at a rate of 1 Hz, complete recovery required 2–3 min (Fig. 9). Regardless of the interval between trains, the rate constant of decay of the ACh amplitude was similar.

The effect of meproadifen on the junctional region of the frog cutaneous pectoris muscle was also studied. In this case, ACh was applied with a double-barreled micropipette. One barrel applied a continuous (30-sec) pulse while the second repetitively applied a brief (50-μsec) test pulse. The test pulse produced a depolarization characterized by an average rise time of 850 μsec and a half-decay time of 3.7 ± 0.7 msec (data not shown). In the absence of meproadifen, test pulses applied at 1 Hz produced no desensitization, and a 30-sec conditioning pulse resulting in a depolarization of 5–10 mV produced a partial, reversible desensitization (Fig. 10). After exposure to 5 μM meproadifen, the test pulse at 1 Hz was characterized by a rapid decrement and, in the presence of conditioning ACh pulse, test pulses were completely blocked (Fig. 11). Upon cessation of the conditioning pulse, ACh sensitivity recovered to that value observed before the conditioning pulse. Further recovery occurred within 18 sec after the 1-Hz test pulse was terminated (Fig. 11). When the same technique was applied to the extrajunctional region of chronically denervated rat soleus muscle, meproadifen caused desensitization identical with that of junctional receptor desensitization.

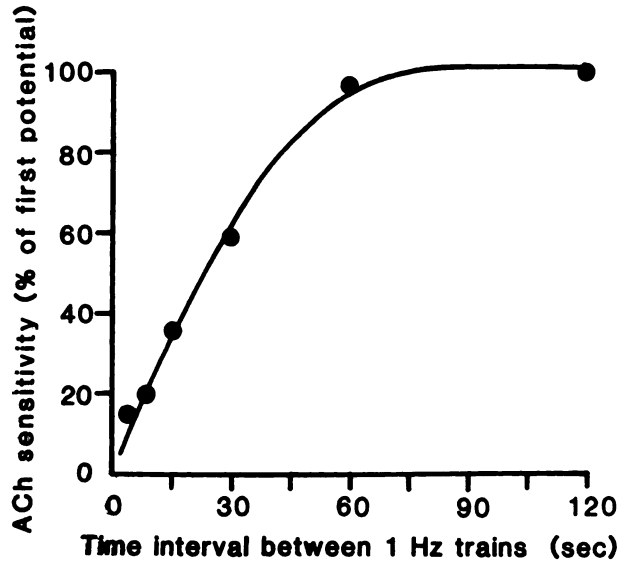

FIG. 9. *Recovery of extrajunctional ACh sensitivity after repetitive stimulation at 1 Hz*

The recovery of extrajunctional ACh sensitivity of the first ACh potential recorded at various time intervals between trains of stimuli at 1 Hz in the presence of 2.5 μM meproadifen is shown. ACh sensitivity is expressed as percentage of the first potential. The duration of current, applied through an ACh-containing pipette, for each single potential was 1.0 msec.

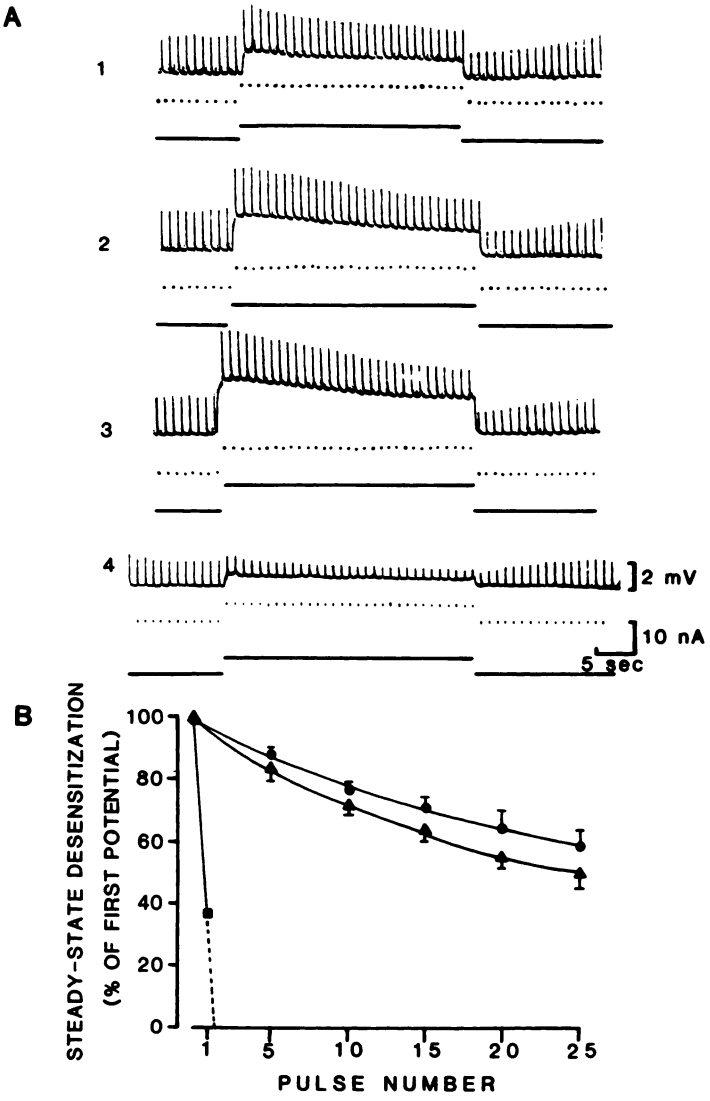

FIG. 10. *Desensitization and recovery of junctional ACh receptor of a frog cutaneous pectoris muscle*

A. Typical responses of repetitive (1-Hz) brief microiontophoretic test pulses (50 μsec, *small dots*; these dots were drawn because of the brief nature of the pulse) and a long conditioning pulse (30 sec, *long square pulse*) delivered from each barrel of a double-barreled ACh-containing pipette are shown for each series of traces. A1 to A3 represent controls at different conditioning pulses which caused in A1 5-mV, in A2 8-mV, and in A3 12-mV membrane depolarization from the same functional region. A4 shows responses in the presence of atropine (100 μM). Note that no additional desensitization of the ACh receptor channel molecule occurred as a result of the presence of atropine.

B. The time course of the maximal desensitization is expressed as percentage of the first potential. ●, Control; ▲, atropine (100 μM); ■, meproadifen (5 μM). Results are means ± standard deviation of three and four determinations. Meproadifen blocked the test pulse immediately following the conditioning pulse. However, only the first pulse could be seen if it came just at the onset of the conditioning pulse (see Fig. 11D). The *dotted line* represents extrapolation of the results.

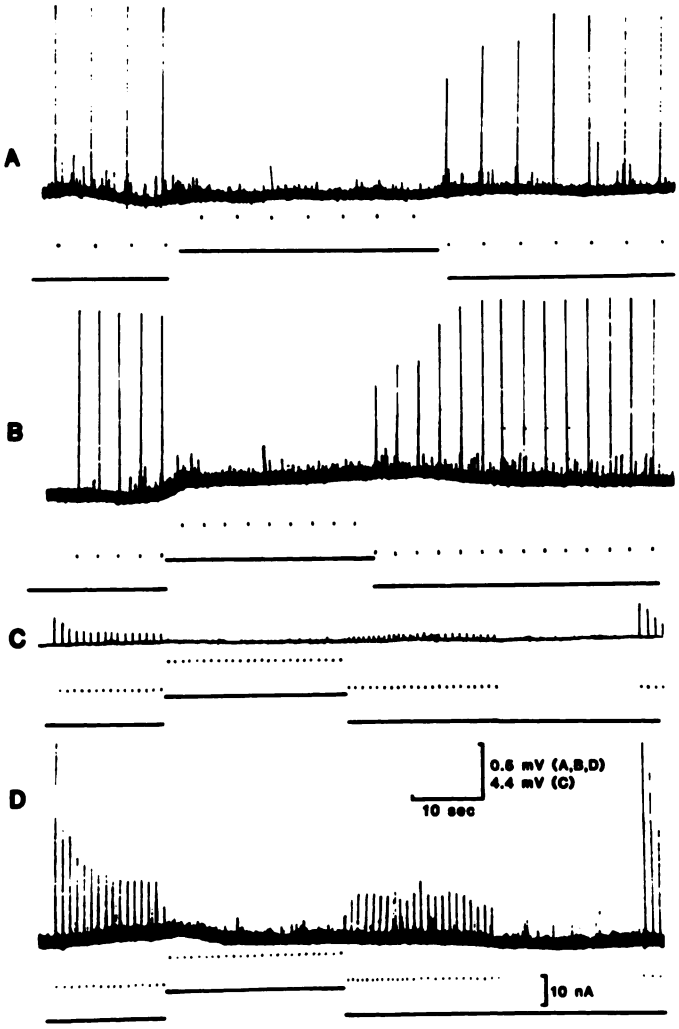

FIG. 11. *Desensitization and recovery of junctional ACh receptors in the presence of meproadifen*

Similar to Fig. 10, a series of ACh responses induced by repetitive brief test pulses (50 μsec) and a long conditioning pulse (30 sec) delivered from each barrel of a double-barreled ACh-containing pipette are shown. The instantaneous blockade of ACh potentials by meproadifen (5 μM) is shown at different frequencies (A, 0.2 Hz; B, 0.3 Hz; C and D, 1 Hz). Control responses from the same fiber are shown in Fig. 10. ACh potentials remained depressed after cessation of conditioning pulse if stimulated at 1 Hz, but recovered if discontinued (see C and D). Recovery occurred at about 18 sec after cessation of conditioning pulse. The times of exposure to meproadifen were 5 min (C and D), 20 min (A), and 35 min (B). Note the absence of membrane depolarization during the conditioning pulse (same current as in control) and the presence of spontaneous MEPCs while test potentials were blocked. However, only the first potential could be seen if it appeared just at the onset of the conditioning pulse (see D).

Assuming that agents such as meproadifen (14, 15) and HTX (6, 14, 20) increase the affinity of ACh recognition site to the agonist, and presumably induce an intramolecular transformation of the AChR-ionic channel macromolecule which yield desensitized forms, one would like to verify this hypothesis by testing the effect of certain channel blockers such as atropine (Fig. 10A4 and B), which does not increase the affinity of ACh to its recognition sites. Indeed, such an agent did not induce potentiation of the desensitization induced by junctional ACh sensitivity (Fig. 10A4 and B).

Effect on MEPC and EPC fluctuations. The voltage-dependent decrease and nonlinear current-voltage relationship of peak EPC amplitude produced by meproadifen suggest that the agent affects the ionic channel of the AChR. However, the lack of marked effect on τ_{EPC} suggests a negligible effect on τ_I activated by applied ACh. This suggestion was pursued further by studying the possible interactions of meproadifen with end-plate channels by ACh noise analysis. The γ and τ_I, under control conditions in the frog sartorius muscle at −90 mV, were

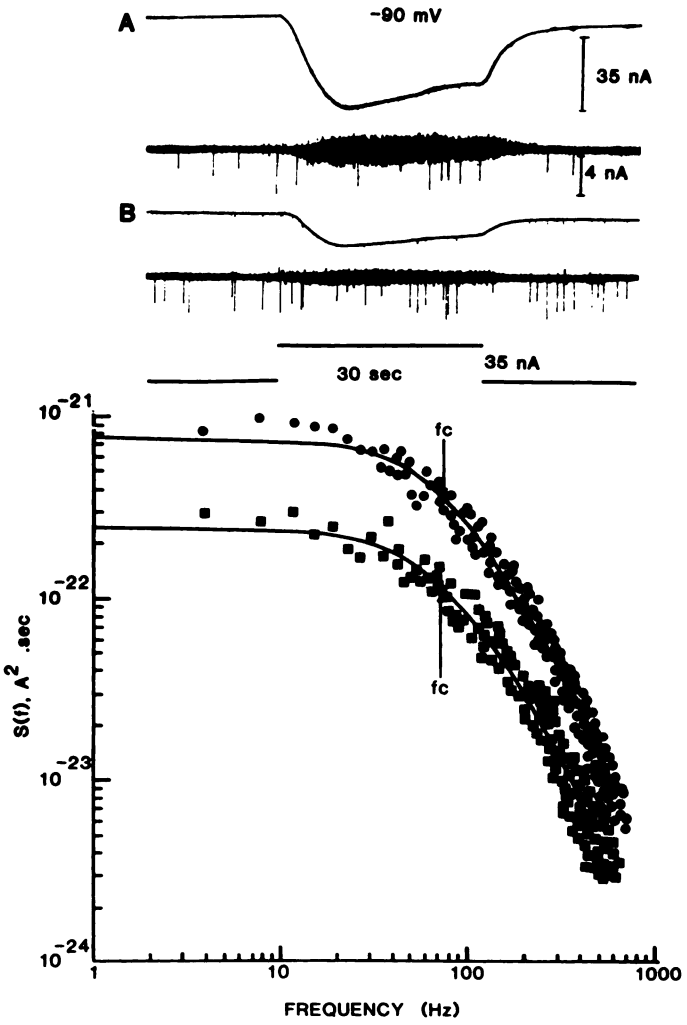

FIG. 12. *Effect of meproadifen on single-channel conductance and channel lifetime*

Microiontophoretically induced EPCs recorded from the synaptic junction of the frog sartorius muscle in control (*A*, ●) and after 30 min of exposure to meproadifen (1 μM) (*B*, ■) at a holding potential of −90 mV. Records *A* and *B* show EPCs in the absence and presence of ACh recorded on a low-gain, d.c.-coupled (*upper trace*), and a high-gain, a.c.-coupled (*lower trace*). The small downward sharp deflections are the spontaneous MEPCs. The lower records illustrate the microiontophoretic current, which was identical under control conditions and in the presence of meproadifen. The spectra of ACh-induced fluctuations shown *below* is plotted in a double-logarithmic scale under control conditions and after exposure to meproadifen. The curves represent the best fit by nonlinear regression to a single Lorentzian function. The half-power frequencies (*fc*) (indicated by *arrows*) were 76.5 Hz and 76.8 Hz for control and meproadifen, respectively. γ and τ_I were, respectively, 36 pS and 2.08 msec for control, and 34 pS and 2.07 msec in the presence of meproadifen. The experiment was performed at 22°.

TABLE 4

Effects of various concentrations of meproadifen on the amplitude and decay time constant of the MEPCs

Each value represents mean ± standard error of the mean obtained after a 30- to 60-min exposure to meproadifen; *N* represents the number of MEPCs measured in three to five preparations.

Condition	Holding potential	*N*	Amplitude	τ_{MEPC}
	mV		*namp*	*msec*
Control	−90	91	4.93 ± 0.40	1.48 ± 0.09
Meproadifen				
0.025 μM	−90	22	4.83 ± 0.13	1.56 ± 0.12
0.050 μM	−90	12	4.70 ± 0.25	1.32 ± 0.04
0.100 μM	−90	11	3.78 ± 0.35	1.36 ± 0.09
1.0 μM	−90	35	3.34 ± 0.01[a]	1.30 ± 0.09
2.0 μM	−90	28	2.81 ± 0.30[a]	1.39 ± 0.08
4.0 μM	−90	9	1.84 ± 0.09[a]	1.36 ± 0.14
Control	−110	34	5.51 ± 0.92	2.17 ± 0.25
Meproadifen				
0.025 μM	−110	21	4.53 ± 0.50	2.23 ± 0.38
0.050 μM	−110	16	5.80 ± 0.12	1.97 ± 0.04
0.100 μM	−110	7	3.97 ± 0.22	1.75 ± 0.10
1.0 μM	−110	24	3.63 ± 0.27[a]	1.97 ± 0.07
2.0 μM	−110	36	3.35 ± 0.38[a]	1.81 ± 0.08
4.0 μM	−110	7	1.44 ± 0.18[a]	1.94 ± 0.22

[a] $p < 0.01$.

37.75 ± 1.0 pS and 1.92 ± 0.10 msec, respectively. In the presence of 1 μM meproadifen, γ and τ_I were unchanged (Fig. 12). Because of a large blockade of the ACh-induced EPC with 1 μM meproadifen, higher concentrations could not be used for ACh noise analysis. Although 1 μM and higher concentrations of meproadifen depressed ACh-induced EPC and MEPC amplitude, they had no effect on τ_{MEPC} (Table 4). For example, in the presence of 2 and 4 μM of meproadifen, the peak amplitude was decreased to 57% and 34% of control, whereas τ_{MEPC} was reduced to only 94% and 92% of control, respectively. There was no enhanced effect on amplitude or τ_{MEPC} by the agent when MEPCs were recorded during the iontophoresis of ACh. A similar differential effect on intrinsic and extrinsic response of ACh have been observed with HTX, PCP, and tricyclic antidepressants (9, 10, 30).

DISCUSSION

The present study demonstrates that meproadifen blocks neuromuscular transmission in frog sartorius and cutaneous pectoris muscles. At the concentrations used, meproadifen does not alter the electrical excitability of the muscle membrane; however, it does block the AChR in its resting (closed conformation) and activated but nonconducting states. It also induces "desensitization" of the AChR which appears to be related to its ability to increase the affinity of the agonist to its binding site. These conclusions are based upon the following actions of the agent: (a) it blocks muscle twitch without potentiating either directly or indirectly elicited twitches (Fig. 1); (b) it does not affect the directly evoked action potential or block delayed rectification; (c) it reduces the peak amplitude and causes a voltage-, time-, and concentration-dependent nonlinearity in the current-voltage relationship of the EPC (Figs. 2 and 3); (d) it does not significantly affect τ_{EPC}, τ_{MEPC}, or τ_I, and it also does not alter the single-exponential nature of the decay of either EPC or MEPC (Figs. 3 and 5); and (e) it depresses the peak EPC amplitude and junctional and extrajunctional ACh potentials in a frequency-dependent manner and enhances desensitization in the presence of agonist (Figs. 6 and 7).

The voltage-dependent decrease and nonlinear current-voltage relationship of the peak amplitude produced by meproadifen suggest that the agent interacts with the ionic channel component of the AChR. Indeed, recent biochemical studies show that there is a site for meproadifen binding to *Torpedo* postsynaptic membrane that is distinct from ACh binding sites (14, 15). In addition, the concentration of H_{12}-HTX, a channel probe, necessary to displace competitively one-half of the meproadifen (C_{50} = 0.7 μM) is similar to the dissociation constant (K_d = 0.3 μM) reported for the interaction of [^{3}H]H_{12}-HTX with receptor-rich membranes isolated from *T. californica* (14, 15, 18). The voltage-dependent inhibition of EPC amplitude and the marked nonlinearity in the current-voltage relationship are qualitatively similar to those seen with other agents that interact with the ion channel, such as HTX and its analogues (7, 8), PCP (10), and tricyclic antidepressants (9). Similar to HTX and PCP, meproadifen also produces a time-dependent decrease in the peak EPC amplitude, seen as a hysteresis loop in the current-voltage relationship at more negative potentials, but with a small concomitant change in the τ_{EPC} (Figs. 3 and 4). Since a reduction of the conditioning pulse from 3 sec to 20 msec abolished the voltage- and time-dependent effects of the agent on the EPC, one may conclude that the reaction of the drug with this site is relatively slow (> ~100 msec). In addition to its effects in the absence of agonist, meproadifen produces a use-dependent inhibition of neurally or iontophoretically released ACh. That effect occurred without any detectable alteration of the conductance or lifetime of the individual ion channels.

The progressive decrement of EPC amplitude did not occur in the presence of meproadifen (5–20 μM) when EPCs were generated at a low frequency (0.3 Hz), but decrement was observed at higher rates of stimulation (25, 50, and 100 Hz). For ACh applied microiontophoretically to the junctional and extrajunctional receptor, a significant decrement was observed during a pulse frequency as low as 1 Hz with meproadifen, a rate much faster than that seen with agonist alone. The decrease in amplitude occurred without changes in the time of decay of the potentials. Under these experimental conditions, the time course of this depression is fast (<1 sec) and its recovery is of the order of many seconds.

On the basis of the electrophysiological results presented here, meproadifen enhances the rate and extent of desensitization of the responses to ACh, both neurally and iontophoretically released. It is interesting to contrast the actions of meproadifen and other noncompetitive antagonists as analyzed by electrophysiological and biochemical techniques. Ligands such as meproadifen, PCP, HTX, nortryptiline, and SKF 525-A (proadifen) (14, 17–20, 31) produce the agonist-dependent loss of sensitivity (desensitization). On the other hand, atropine and (apparently) piperocaine (20, 31) do not produce a

comparable desensitization at those concentrations having a significant effect on τ_I (1, 11). In biochemical studies, all of these ligands have been shown to bind to a site in the *Torpedo* AChR that is distinct from the site of binding of ACh, (+)-tubocurarine and α-bungarotoxin (14, 18, 20). However, all of the compounds which, like meproadifen, produce desensitization at the frog neuromuscular junction are bound to the *Torpedo* AChR with higher affinity in the presence of agonist than in the absence of agonist (e.g., carbamylcholine). On the other hand, piperocaine and atropine are bound with the same affinity in the presence or absence of agonist (20). For the *Torpedo* AChR, the noncompetitive antagonists that are bound with higher affinity in the presence of agonist do so because they bind preferentially to a receptor conformation that also binds agonist with enhanced affinity (14, 18, 32). This conformation is not associated with open ion channels and may be a desensitized conformation. Piperocaine and atropine do not bind preferentially to that conformation and do not desensitize *Torpedo* AChR in the absence of agonist or enhance desensitization in the presence of agonist. Although additional studies are necessary to identify the effect of these ligands—particularly piperocaine—on AChR conformational equilibria, it is striking that the effects of nicotinic noncompetitive antagonists appeared to be highly conserved from *Torpedo* to frog.

Although meproadifen greatly reduces the peak EPC amplitude, its effect on τ_{EPC} was small even at concentrations of 15 μM (see Fig. 3) and, like controls, it is exponentially related to membrane potential (Fig. 5). At high concentration (25 μM), τ_{EPC} is reduced by only 20% from control whereas the peak amplitude is reduced by 86% at −150 mV (Fig. 3; Table 1). The small effect on τ_{EPC} produced by meproadifen is in contrast to that seen with PCP or HTX but in close conformity with the tricyclic antidepressants (9). Both PCP and HTX are thought to interact with both closed and open conformations of the ionic channel. The open-channel effects of HTX and PCP are manifested as a voltage-dependent decrease in τ_{EPC} whereas the closed-channel effects appear as a voltage- and time-dependent decrease in EPC amplitude, a consequent hysteresis loop, and a nonlinear current-voltage relationship of the EPC. On the other hand, the retention of linearity in the log τ_{EPC} versus membrane potential relationship, despite hysteresis and nonlinearity in the current-voltage relationship of EPC amplitude produced by meproadifen, demonstrates that the decay phase and peak amplitude of the EPC are independent. The absence of any alteration of γ (up to 1 μM) suggests that the decrease in EPC and MEPC amplitudes (Figs. 2 and 3; Table 4) induced by meproadifen cannot be explained by a decrease in the conductance of the channels opened by ACh. However, because very high concentrations of meproadifen did affect τ_{EPC} slightly at very negative membrane potentials, some degree of open-channel block would be expected, yet undetectable by noise analysis. Thus it is clear that meproadifen cannot produce the decrease in peak amplitude by shortening τ_I. Rather, meproadifen can cause a decrease in the peak amplitude by the following mechanisms: initially the agent blocks the receptor channel molecule in its resting conformation ($R + D \rightleftharpoons RD$); this would account for the initial marked decrease in peak EPC amplitude prior to activation. During activation, the agent at concentrations <10 μM increases the affinity of the agonist for its recognition site (ACh + $RD \rightleftharpoons$ AChR′D) and appears to increase the population of desensitized species (14, 15) with a fast rate of recovery upon hydrolysis and diffusion of ACh from the cleft (see Fig. 9). Simultaneously, the agent also reacts with a site on the ionic channel, thus causing the formation of an intermediate nonconducting species (ACh + $RD \rightleftharpoons$ AChRD) which could be responsible (at least in part) for the nonlinearity of the voltage pathway (see Figs. 3 and 4). Higher concentration of the agent increases the number of the above-mentioned states, but also slightly decreases τ_{EPC}, a phenomenon which could be explained as a reaction with the open state of the receptor-channel molecule. It is obvious that the three species, i.e., the closed (RD), apparent desensitized (AChR′D), and intermediate nonconducting state (AChRD), are the most predominant targets for meproadifen.

In summary, meproadifen appears to be selective at the nicotinic AChR-ion channel complex. It does not interact with the ACh recognition site (14) but to some site(s) located at the ionic channel that can undergo conformational change without opening. It depresses the peak EPC amplitude in a voltage- and time-dependent manner while altering τ_{EPC} only slightly. Therefore, the most likely explanation for the actions of the agent appears to be a reaction of the agent with the resting channel and activated but nonconducting species of the ionic channel, thus causing a voltage-dependent reduction of effective channel number. Meproadifen appears to be a unique probe for differentiating the ionic channel in its resting or activated but nonconducting states (both of which are closed conformations) and also from the open conformation.

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REFERENCES

1. Adler, M., E. X. Albuquerque, and F. J. Lebeda. Kinetic analysis of endplate currents altered by atropine and scopolamine. *Mol. Pharmacol.* **14**:514–529 (1978).
2. Schofield, G. G., J. E. Warnick, and E. X. Albuquerque. 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**:209–230 (1981).
3. Maleque, M. A., J. E. Warnick, and E. X. Albuquerque. The mechanism and site of action of ketamine on skeletal muscle. *J. Pharmacol. Exp. Ther.* **219**:638–645 (1981).
4. Beam, K. G. A quantitative description of endplate currents in the presence of two lidocaine derivatives. *J. Physiol. (Lond.)* **258**:301–322 (1976).
5. Ruff, R. L. A quantitative analysis of local anesthetic alteration of miniature endplate currents and endplate current fluctuations. *J. Physiol. (Lond.)* **264**:89–124 (1977).
6. Albuquerque, E. X., M. Adler, C. E. Spivak, and L. Aguayo. Mechanism of nicotinic channel activation and blockade. *Ann. N. Y. Acad. Sci.* **358**:204–238 (1980).
7. Masukawa, L. M., and E. X. Albuquerque. Voltage- and time-dependent action of histrionicotoxin on the endplate current of the frog muscle. *J. Gen. Physiol.* **72**:351–367 (1978).
8. Spivak, C. E., M. A. Maleque, A. C. Oliveira, L. Masukawa, T. Tokuyama, J. W. Daly, and E. X. Albuquerque. Actions of the histrionicotoxins at the ion channel of the nicotinic acetylcholine receptor and at the voltage-sensitive ion channels of muscle membranes. *Mol. Pharmacol.* **21**:351–361 (1981).
9. Schofield, G. G., B. Witkop, J. E. Warnick, and E. X. Albuquerque. Differ-

entiation of the open and closed states of the ionic channels of nicotinic acetylcholine receptors by tricyclic antidepressants. *Proc. Natl. Acad. Sci. U. S. A.* **78**:5240–5244 (1981).
10. Albuquerque, E. X., M.-C. Tsai, R. S. Aronstam, A. T. Eldefrawi, and M. E. Eldefrawi. Sites of action of phencyclidine. II. Interaction with the ionic channel of the nicotinic receptor. *Mol. Pharmacol.* **18**:167–178 (1980).
11. Tiedt, T. N., E. X. Albuquerque, N. M. Bakry, M. E. Eldefrawi, and A. T. Eldefrawi. Voltage- and time-dependent actions of piperocaine on the ion channel of the acetylcholine receptor. *Mol. Pharmacol.* **16**:909–921 (1979).
12. Katz, B., and S. Thesleff. A study of the desensitization produced by acetylcholine at the motor endplate. *J. Physiol.* (*Lond.*) **138**:63–80 (1957).
13. Magleby, K. L., and B. S. Pallotta. A study of desensitization of acetylcholine receptors using nerve released transmitter. *J. Physiol.* (*Lond.*) **316**:225–250 (1981).
14. Krodel, E. K., R. A. Beckman, and J. B. Cohen. Identification of local anesthetic binding site in nicotinic postsynaptic membranes isolated from *Torpedo marmorata* electric tissue. *Mol. Pharmacol.* **15**:294–312 (1979).
15. Cohen, J. B., N. D. Boyd, and N. S. Shera. Interaction of anesthetics with nicotinic postsynaptic membranes isolated from *Torpedo* electric tissue, *Molecular Mechanisms of Anesthesia* (Program in Anesthesiology, Vol. 2) (B. R. Fisk, ed.). Raven Press, New York, 165–174 (1980).
16. Souccar, C., M. A. Maleque, E. Driyer, J. B. Cohen, and E. X. Albuquerque. Meproadifen (MEP) interaction with ionic channels of nicotinic acetylcholine receptors. Abstracts of the VII International Biophysics Congress, Mexico City, August 23–28, 331 (1981).
17. Burgermeister, W., W. A. Catterall, and B. Witkop. Histrionicotoxin enhances agonist-induced desensitization of acetylcholine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **12**:5754–5758 (1977).
18. Eldefrawi, M. E., R. S. Aronstam, N. M. Bakry, A. T. Eldefrawi, and E. X. Albuquerque. Activation, inactivation and desensitization of acetylcholine receptor channel complex detected by binding of perhydrohistrionicotoxin. *Proc. Natl. Acad. Sci. U. S. A.* **77**:2309–2313 (1980).
19. Aronstam, R. S., M. E. Eldefrawi, A. T. Eldefrawi, E. X. Albuquerque, K. F. Jim, and D. J. Triggle. Sites of action of phencyclidine. III. Interactions with muscarinic receptors. *Mol. Pharmacol.* **18**:179–184 (1980).
20. Aronstam, R. S., A. T. Eldefrawi, and M. E. Eldefrawi. Similarities in the binding sites on the muscarinic receptor and the ionic channel of the nicotinic receptor. *Biochem. Pharmacol.* **29**:1311–1314 (1980).
21. Aronstam, R. S. Interactions of tricyclic antidepressants with a synaptic ion channel. *Life Sci.* **28**:59–64 (1981).
22. Albuquerque, E. X., and R. J. McIsaac. Fast and slow mammalian muscles after denervation. *Exp. Neurol.* **26**:183–202 (1970).
23. Del Castilo, J., and B. Katz. Quantal components of the endplate potential. *J. Physiol.* (*Lond.*) **297**:423–442 (1954).
24. Lapa, A. J., E. X. Albuquerque, J. M. Sarvey, J. Daly, and B. Witkop. Effects of histrionicotoxin on the chemosensitive and electrical properties of skeletal muscle. *Exp. Neurol.* **47**:558–580 (1975).
25. Eisenberg, E. S., and P. W. Gage. Frog skeletal muscle fibers: changes in electrical properties after disruption of the transverse tubules. *Science* (*Wash. D. C.*) **158**:1700–1701 (1967).
26. Takeuchi, A., and N. Takeuchi. Active phase of frog's endplate potential. *J. Neurophysiol.* **22**:395–411 (1959).
27. Kuba, K., E. X. Albuquerque, J. Daly, and E. A. Barnard. A study of the irreversible cholinesterase inhibitor, diisopropylfluorophosphate, on time course of endplate currents in frog sartorius muscle. *J. Pharmacol. Exp. Ther.* **189**:499–512 (1974).
28. Hodgkin, A. L. and A. F. Huxley. Currents carried by sodium and potassium ions through the membrane of the giant axon of liligo. *J. Physiol.* (*Lond.*) **116**:449–472 (1952).
29. Albuquerque, E. X., K. Kuba, and J. Daly. Effect of histrionicotoxin on the ionic conductance modulator of the cholinergic receptor: a quantitative analysis of the endplate current. *J. Pharmacol. Exp. Ther.* **189**:513–524 (1974).
30. Albuquerque, E. X., P. N. Gage, and A. C. Oliveira. Differential effect of perhydrohistrionicotoxin on "intrinsic" and "extrinsic" endplate response. *J. Physiol.* (*Lond.*) **297**:423–442 (1979).
31. Magazanik, L. G., and Vyskocil, F. Desensitization at the neuromuscular junction, in *Motor Innervation of Muscle* (S. Thesleff, ed.). Academic Press, London, 151–176 (1976).
32. Heidmann, T., and J. P. Changeux. Interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from *Torpedo marmorata* in the millisecond range: resolution of an intermediate conformational transition and evidence for positive cooperative effects. *Biochem. Biophys. Res. Commun.* **97**:889–896 (1980)

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