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Mode of Action of Quinacrine on the Acetylcholine Receptor Ionic Channel Complex

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SUMMARY

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The effect of quinacrine was studied on neuromuscular transmission in frog sartorius and rat soleus muscles and on the binding of ligands to the electric organ of *Torpedo ocellata*. Quinacrine (30-200 μM) blocked neuromuscular transmission in both muscles, and inhibited the carbamylcholine-induced membrane depolarization at the endplate. The most pronounced effect of quinacrine was on the acetylcholine (ACh)-receptor mediated postsynaptic conductance. It reduced the endplate current (EPC) peak amplitude, without marked departure from linearity of the current voltage relationship, and it shortened the EPC rise time. The rate of decay of the EPC was also altered by quinacrine, becoming less voltage-dependent at concentrations of about 5 μM and completely voltage-independent at drug concentrations of 30-100 μM. Biochemical studies on membranes from electric organ of the electric ray *Torpedo ocellata* revealed that quinacrine inhibited the binding of [^{3}H]ACh and [^{3}H]H_{12}-HTX to the membrane-bound ACh-receptor and its ionic channel, respectively. The inhibition constant (K_i) values were 7.4 μM and 14 μM, respectively. It is suggested that the mode of action of quinacrine on neuromuscular transmission is complex and reflects interaction with both the ACh-receptor and its ionic channel.

INTRODUCTION

At nicotinic neuromuscular junctions, the binding of ACh[2] to its recognition site on the ACh-receptor, initiates a chain of reactions leading to the opening of the ionic channel, and subsequent increase in ionic flux across the postsynaptic membrane (1, 2). Drugs and toxins that influence such postsynaptic events belong to three groups, depending on their primary site of action. One group of drugs interacts mainly with the ACh-receptor and either activates (e.g., carbamylcholine, nicotine) or blocks (e.g., α-bungarotoxin, *d*-tubocurarine (2-4)) it at concentrations that do not affect the ionic channel. The second group modulates ionic conductances at concentrations that do not inhibit ACh binding to its receptor. Exam-

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[2] Abbreviations used are: ACh, acetylcholine; H_{12}-HTX, perhydrohistrionicotoxin; DFP, diisopropylfluorophosphate; MEPC, miniature endplate current; EPC, endplate current; EPP, endplate potential; MEPP, miniature endplate potential; TTX, tetrodotoxin.

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ples of such drugs are histrionicotoxin (the toxin isolated from the skin of the Colombian frog, *Dendrobates histrionicus*) (3, 5), local anesthetics (e.g., procaine and lidocaine) (6–8), atropine (9), and amantadine (10). The third group of drugs (e.g., organophosphates, carbamates) inhibits ACh-esterase, thereby maintaining high concentrations of ACh in the synaptic gap (11). However, many of these drugs interact with more than one of these proteins, e.g., DFP, which irreversibly phosphorylates ACh-esterase, yet at higher concentrations binds to the ACh-receptor (12) and its ionic channel (11).

The effects of drugs and toxins on EPCs may cause a reduction in peak amplitude of the EPC by either blocking the ACh-receptor or its ionic channel. The block of the latter usually induces marked nonlinearity of the current voltage relationship of the EPC while the former reduces the peak amplitude without changes in the linearity (2, 5, 8).

It has been suggested that the ACh-receptor and its ionic channel may be two separate proteins that are closely associated in the membrane (13–15). This is based on the partial separation of the protein which binds ACh and α-bungarotoxin (i.e., the ACh receptor) from the one which binds H_{12}-HTX and amantadine (i.e., the ionic channel) by means of detergent solubilization followed by affinity adsorption of all of the ACh-receptor molecules or their precipitation with anti-ACh-receptor antibodies, while retaining much of the ionic channel protein in solution (13, 14).

Quinacrine, the antimalarial drug, was found by Grunhagen and Changeux (16, 17) to act like local anesthetics in noncompetitively blocking carbamylcholine-induced depolarization and in enhancing *in vitro* the binding of [^{3}H]ACh to membranes from the electric organ of the electric ray, *Torpedo marmorata*. Their discovery that quinacrine fluorescence was enhanced upon its binding to electric organ membranes, and the further increase caused by receptor agonists, led to the utilization of quinacrine as a probe for the study of the ACh-receptor-channel complex. They suggested that the fluorescence increase occurred during conformational transition of the ACh-receptor from the resting to its active state. Quinacrine was also utilized by Sobel *et al.* (15) as a label of a protein present in the receptor-rich membranes and different from the ACh-receptor protein. Subsequent electrophysiological analysis made by Adams and Feltz (18) of the kinetic behavior of the postsynaptic membrane of intact vertebrate muscle fibers treated with quinacrine, led to the proposal that the increase in fluorescence induced by the drug did not monitor the activation of the receptor, but rather the blockade of an already activated receptor-ionic-channel complex.

The present investigation was initiated to evaluate the mode of action of quinacrine on neuromuscular transmission and to determine whether quinacrine had more than one site of action at the nicotinic synapse, using both biochemical and electrophysiological techniques. Transmission was studied in amphibian and mammalian muscles, whereas biochemical studies were performed on *Torpedo* electric organ because of its high content of ACh-receptors and ionic channels whose pharmacological properties appear to be similar to those in skeletal muscle endplates (19).

MATERIALS AND METHODS

A. *Electrophysiological Experiments*

Animals and preparation. Experiments were carried out at room temperature (22–24°) on sartorius muscle preparation of the frog, *Rana pipiens*, and the soleus muscles of rat (Wistar females, 180–200 g). During dissection and throughout the experiment the muscles were bathed in a physiological solution having the following composition (mM concentration): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4, 1.3, NaH_2PO_4, 0.7 (frog muscles) and NaCl, 135.0; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 15.0; NaH_2PO_4, 1.0 and glucose, 11.0 (mammalian). Frog physiological solution had a pH of 6.9–7.1 and was bubbled with 100% O_2, while the mammalian one was continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2 giving a final pH of 7.1–7.3.

The technique used to study directly and indirectly elicited muscle twitch has previ-

ously been described (20). For intracellular recording, the muscles were stretched slightly beyond their resting length in a Plexiglas plate, which had a planoconvex lens at its center, and immersed in a 15 ml bath. Nerve stimulation was delivered by two platinum electrodes. All recordings were made from surface fibers using glass microelectrodes filled with 3 M KCl (5–15 MΩ).

The potentials were displayed on a Tektronix oscilloscope and recorded on a Mingograph 81. Data from the oscilloscope were recorded on film and measured under magnification or recorded and analyzed by an on-line PDP 11/40 computer.

In voltage clamp experiments, the circuit used was similar to that previously described (11). The muscles were pretreated with 400–600 mM glycerol to disconnect functionally the sarcotubular system from the sarcolemmal membrane and allow the superimposition of several action potentials without causing contraction of the muscle fibers (21). Voltage clamp errors were less than 5%. The rise time of the clamping circuit (10–90%) with 2–8 MΩ microelectrodes was about 50 μsec. Changes in membrane potentials from the holding potentials were evoked either from a DC source under manual control or an isolated stimulus unit controlled by a programmable digital timer (5).

Preparation of microsacs. The electric organ of *Torpedo ocellata* was homogenized (20 g) in 200 ml of 90 mM KCl, 10 mM NaCl and 1 mM Na_2HPO_4, pH 7.4, centrifuged at 5,000 × *g* for 10 min, then the supernatant centrifuged at 30,000 × *g* for 60 min at 4° in a Sorvall centrifuge. The final pellet was suspended in a solution of 10 mM NaCl, 90 mM KCl and 1 mM Na_2HPO_4, pH 7.4, and 0.02% NaN_3 at an average protein concentration of 1.5 mg/ml. Maximum number of binding sites for [^{3}H]ACh and [^{3}H]H_{12}-HTX was 0.7 and 0.5 nmoles/mg protein.

Equilibrium dialysis. Binding of [^{3}H]-ACh to the electric organ membranes was studied at 23° for 4 hr as described previously (13). In order to inhibit all cholinesterases without affecting the binding of ACh to its receptor, DFP was added to the membranes, at a final concentration of 1 mM, 1 hr before the start of dialysis, and 100 μM DFP was present in the dialysis bath. When the effect of quinacrine on [^{3}H]ACh binding was determined, quinacrine was added to the bath.

Centrifugal assay. Binding of [^{3}H]H_{12}-HTX to the microsacs was assayed by a centrifugal assay to save on the radiolabeled toxin. Binding data obtained from centrifugal assay were similar to those obtained from equilibrium dialysis (14). Siliconized 1.5 ml polypropylene centrifuge tubes (BioRad 1.5 ml disposable test tubes) were used as described previously (14). Samples (0.9 ml) of microsac preparation (1 mg protein/ml) were pipetted into each centrifuge tube, then the quinacrine was added in 0.1 ml, and after 30 min at 22° the radioactive toxin (10 μl) was added. The tubes were centrifuged in SS-34 rotor with adaptors in a Sorvall RC-2 at 17,000 rpm for 30 min at 4°. Three samples of 50 μl were taken from the mixture before, as well as from the supernatant after, centrifugation and the radioactivity of each counted in 4 ml of liquid scintillation solution in a disposable 5 ml glass minivial (Rochester Scientific) placed in a glass liquid scintillation vial. The difference in radioactivity was taken to be the bound toxin. Each experiment was duplicated with a microsac preparation immersed in boiling water for 30 min, whose binding was taken to represent nonspecific binding, which amounted to 20 ± 5% (in 12 experiments) of control at 10 nM [^{3}H]H_{12}-HTX. Every experiment was run in triplicate.

Chemicals. Refrigerated stock solutions (10^{-2} M) of quinacrine-2 HCl (Atabrine from Sterling Winthrop) and *d*-tubocurarine (K & K Laboratories) in distilled H_2O were diluted with physiological solution immediately before use. Carbamylcholine was dissolved in appropriate physiological solution at the time of its use.

Isodihydrohistrionicotoxin was reduced with $^3H_2$ to [^{3}H]H_{12}-HTX and purified as previously described (9). Its radiochemical purity was 90% as determined by scanning of a thin layer chromatogram, its specific radioactivity was 21 Ci/mmol, and its effectiveness in blocking neuromuscular trans-

mission in the frog sartorius was routinely determined using the half-decay times and peak amplitudes of the endplate and miniature endplate currents as parameters (13). [*Acetyl*-^{3}H]acetylcholine ([^{3}H]ACh) (49.5 mCi/mmol; New England Nuclear) was utilized and its purity checked periodically by thin layer chromatography. The α-neurotoxin of the cobra species *Naja naja siamensis* was purified from the venom as described previously (22).

RESULTS

Effect on muscle twitch tension and resting membrane potential. Quinacrine (6 and 10 μM) reduced the amplitude of the indirect twitch tension by 20% and 40% of control, respectively (Fig. 1). Using higher concentration (200 μM), the drug induced a block of the indirect twitch tension within 12 min. Repetitive washing of the muscle (once every 10 min) with Ringer's solution resulted in partial dissociation of the drug from its reactive sites with about 50–70% of the indirect twitch tension recovered in 180 min.

The normal resting membrane potential of frog sartorius muscles was -97.8 ± 1.1 mV ($n = 11$), and after two hour incubation in quinacrine (100 μM), it became -93.4 ± 0.5 mV ($n = 30$). The normal resting membrane potential of rat soleus muscles was -72.1 ± 0.7 mV ($n = 34$), after one hour incubation in quinacrine (100 μM), it became -71.4 ± 1.6 mV ($n = 23$).

Effect of carbamylcholine-induced depolarization. When carbamylcholine (0.7 mM) was applied to rat soleus muscles previously exposed to TTX (0.6 μM) for 30 min, the membrane potential at the endplate was significantly reduced. In a typical experiment (Fig. 2), the control membrane potential before application of carbamylcholine was -72.0 ± 0.6 mV (mean ± SE, n = 16 fiber/muscle) and declined to -24.0 ± 4.8 mV (n = 8 fiber/muscle) in a few minutes after carbamylcholine application. After 30 min, the muscle membrane repolarized to about 95% of control values, similar to effects reported by Garrison *et al.* (23) on frog sartorius muscle. When the muscle was first exposed to TTX (0.6 μM) for 30 min, then to quinacrine (50 μM) plus TTX for 30 min, the initial depolarizing response to carbamylcholine (0.7 mM), applied in the presence of quinacrine plus TTX, was reduced by 80%. Therefore, quinacrine significantly inhibited the depolarizing action of carbamylcholine (Fig. 2).

Effects of quinacrine on the amplitude of the EPC. Records of EPCs before and during quinacrine application are presented in Fig. 3. Under control condition, the relationship between peak EPC amplitude and membrane potential was approximately linear from +50 to −100 mV, but exhibited a slight upward concavity at potentials greater than −100 mV. This small deviation in the current-voltage relationship has been previously observed (9, 11, 24, 25). The amplitude of the normal EPC increased with hyperpolarization and de-

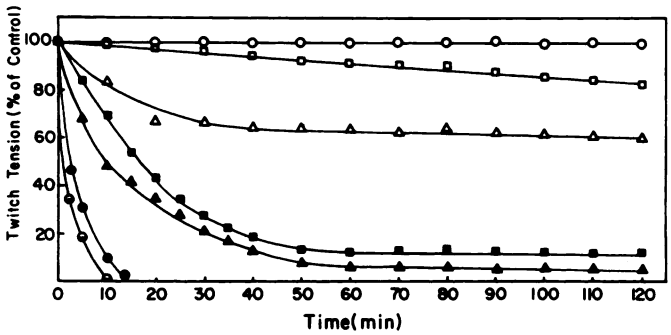

FIG. 1. *Time course of the block of the indirect twitch tension of the frog sartorius muscle produced by various concentrations of quinacrine at 23°*

○ 1 μM, □ 6 μM, △ 10 μM, ■ 30 μM, ▲ 60 μM, ● 100 μM, ⊖ 200 μM. Each symbol represents the mean ± SEM of 4 muscles. The SEM values were too small to be shown.

creased with depolarization, reversing at about 0 mV (−3.2 ± 1.7 mV, n = 7) and becoming outward thereafter (Fig. 4). Quinacrine (1–100 μM) decreased the amplitude of the EPC in a concentration-dependent manner. For example, at holding membrane potential of −90 mV the drug decreased the EPC peak amplitude to 22% at 30 μM and 11% at 100 μM (Table 1). Although after 60 min incubation in quinacrine (2.5 to 30 μM), the EPC amplitude was significantly decreased, the linearity of the peak amplitude-membrane potential relationship remained similar to that seen under control conditions (Fig. 4). The drug affected the current-voltage relationship both at positive and negative potentials, without altering the reversal potential of the EPC (30 μM, −3.0 ± 1.7 mV, n = 9) (Fig. 4). Upon washing the muscle with normal Ringer's solution for 3 hr a partial recovery (44% of control) of the peak amplitude of the EPC was observed (Table 1).

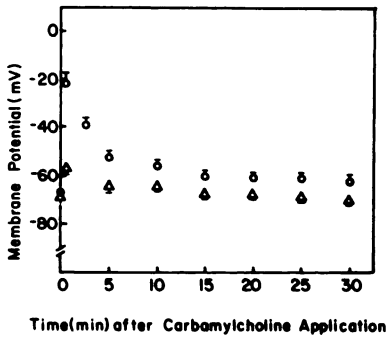

FIG. 2. *Effect of quinacrine (50 μM) on endplate depolarization induced by bath applied carbamylcholine in rat soleus muscle*

The control muscle (○) was exposed to tetrodotoxin (TTX, 0.6 μM) for 30 min prior to the addition of carbamylcholine (0.7 mM). The other muscle of the pair (△) was first exposed to TTX (0.6 μM) for 30 min, followed by quinacrine (50 μM) and TTX (0.6 μM) for another 30 min and then exposed to carbamylcholine (0.7 mM) (plus TTX and quinacrine). Each point is the mean ± standard error for 5–20 surface muscle fibers.

Effect of quinacrine on the decay time of the EPC. A sample of the logarithmic plots of the falling phase of the EPC recorded at −150, −100 and +50 mV under control conditions and in the presence of quinacrine is shown in Fig. 5. The decay phase of the EPC was a single exponential function of time in both situations. However, the slope of the falling phase was increased in the presence of quinacrine as expected from a

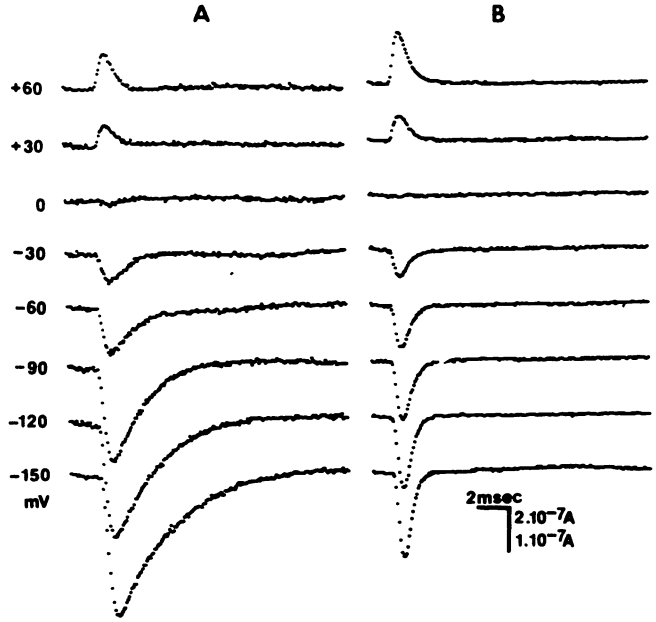

FIG. 3. *Digitized computer output of endplate currents at various membrane potentials (from −150 to +60 mV) in (A) control and (B) preparation treated with 30 μM quinacrine*

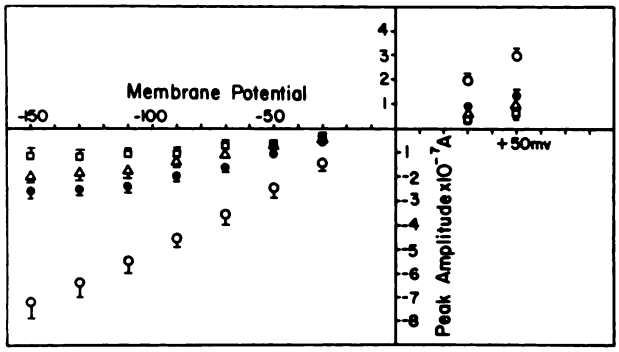

FIG. 4. *The relation between the peak amplitude of the EPC and membrane potential in control* (○) *and in the presence of quinacrine: 2.5 μM* (●), *5 μM* (△) *and 30 μM* (□)

Each symbol represents the mean ± SEM of 6–9 fibers of frog sartorius muscles. Where no SEM appears, the value was too small to be shown.

TABLE 1

Effect of various concentrations of quinacrine on the amplitude, rise time and the decay time constant (τ) of endplate currents recorded at −90 mV

Condition	Amplitude	Rise time[a]	Decay time (τ)[b]
	(×10⁻⁷A)	*(msec)*	*(msec)*
Control	4.51 ± 0.42 (9)[c]	1.0	1.7
Quinacrine			
1 μM (60–180 min)[d]	4.28 ± 0.048 (10)	1.0	1.6
5 μM (60–120 min)	1.43 ± 0.29[e] (6)	1.0	1.1[e]
30 μM (60–90 min)	1.01 ± 0.21[e] (9)	0.9	0.7[e]
60 μM (30–60 min)	0.44 ± 0.09[e] (3)	0.7[g]	0.6[e]
100 μM (30–60 min)	0.49 ± 0.20[e] (4)	0.7[e]	0.4[e]
Washing[f] (60–180 min)	3.00 ± 0.52[g] (7)	1.0	1.6

[a] Standard error values for all conditions are <0.1 msec.

[b] Standard error values are 0.1 msec or less except for the washing condition where it is ±0.4 msec.

[c] The values in parentheses refer to the numbers of surface fibers sampled.

[d] The time intervals in parenthesis refer to the recorded time interval after quinacrine application.

[e] Differs significantly from control ($p < 0.001$).

[f] Values refer only to muscle previously exposed to 30 μM quinacrine.

[g] Differs significantly from control ($p < 0.01$).

reduction of the time constant of the decay phase of the EPC (Fig. 6). Quinacrine (1–100 μM) accelerated the rise and decay times of the EPC in a concentration dependent manner (Table 1). The decay phase of the normal EPC was found to vary with changes in the membrane electric field, such that the EPC decayed progressively faster as the membrane was depolarized from −150 to +50 mV thus giving a slope of −3.5 msec/V (Fig. 6). In the presence of quinacrine (5 μM), the slope of the time constant-membrane potential relationship decreased to −2.2 msec/V. When higher concentrations were applied (30 and 100 μM), the time constant-membrane potential relationship underwent a change in slope to +0.28 msec/V and +0.42 msec/V, respectively, although the relationship remained log linear (Fig. 6). This occurred without a further change in the slope of the time constant-membrane potential relationship.

Effects of quinacrine on the binding of ligands to Torpedo membranes. Quinacrine bound to the ACh-receptor of *Torpedo* membranes, as judged by its inhibition of [^{3}H]ACh binding (Fig. 7). The K_i was 7.2 μM. α-Neurotoxin from the cobra, species *Naja siamensis*, which is a classic

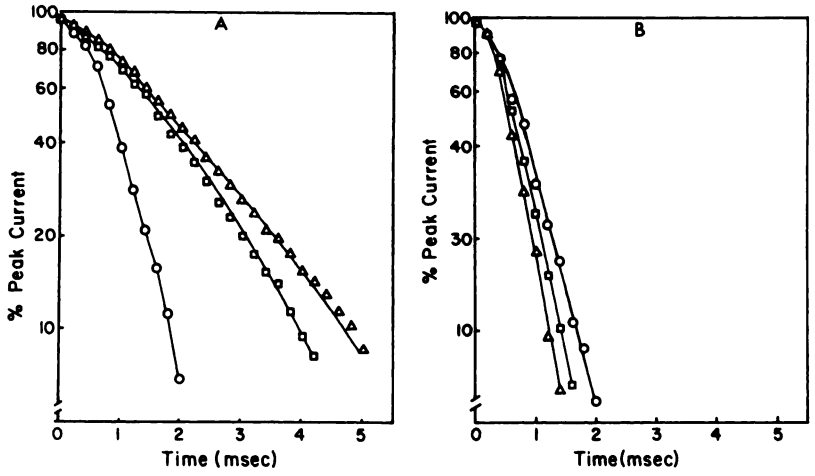

FIG. 5. *Semilogarithmic plots of the EPC falling phase as a function of time in control endplate of frog sartorius muscles (Fig. 5A) and 60 min after quinacrine (30 μM) treatment (Fig. 5B), at various membrane potentials*

○ = +50 mV; □ = −100 mV; △ = −150 mV.

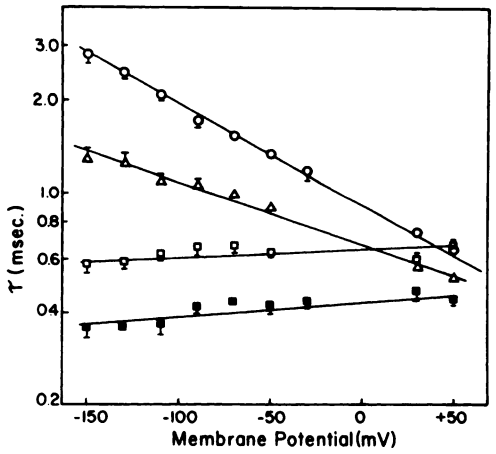

FIG. 6. *The relation between the time constant of the EPC and membrane potential in control* (○) *and in the presence of quinacrine, 5 μM* (△), *30 μM* (□) *and 100 μM* (■)

Each symbol represents the mean ± SEM of 6-9 fibers of frog sartorius muscles. Where no SEM appears, the value is too small to be shown.

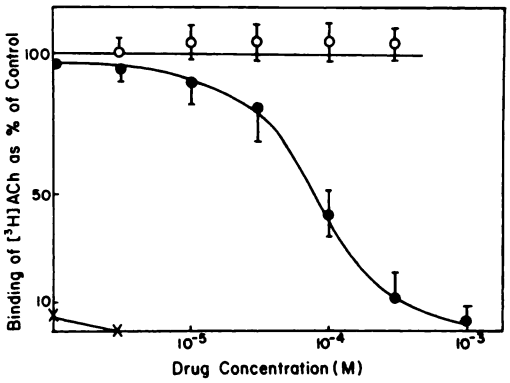

FIG. 7. *Inhibition of binding of [³H]ACh (1 μM) to Torpedo membrane by various concentrations of quinacrine* (●), *H_{12}-HTX* (○) *and Naja α-neurotoxin* (×)

Each bar represents ± SD of three experiments.

ACh-receptor inhibitor, displaced [³H]ACh much more effectively than did quinacrine. By comparison, H_{12}-HTX, which inhibits neuromuscular transmission by binding to the ionic channel of the ACh-receptor (13, 14), did not inhibit [³H]ACh binding even at concentrations of up to 300 μM.

A double reciprocal plot of the binding of [³H]ACh to *Torpedo* membranes in the absence and presence of two concentrations of quinacrine (Fig. 8) suggests that the inhibition is not competitive.

[³H]H_{12}-HTX binds not only to proteins but also to lipids and the detergent Triton X-100 (14). Thus, its specific binding was obtained after subtraction of the nonspecific, nonsaturable binding to heat-resistant membrane components. Quinacrine effectively inhibited the specific binding of [³H]H_{12}-HTX to *Torpedo* membranes (Fig.

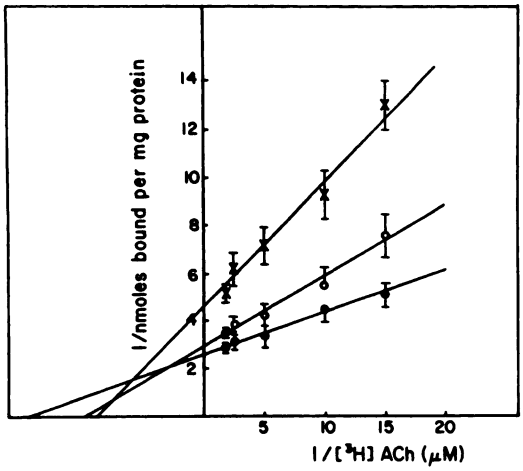

FIG. 8. *Double reciprocal plot of the binding of [³H]ACh to the membrane-bound Torpedo ACh-receptors and its inhibition by quinacrine*

[³H]ACh alone (●) and in presence of 20 μM quinacrine (○) and 100 μM quinacrine (×). Each bar represents ± SD of three experiments.

9). It was less potent than H_{12}-HTX, whereas α-neurotoxin from *Naja* toxin did not inhibit [³H]H_{12}-HTX binding. The inhibition of [³H]H_{12}-HTX by quinacrine was competitive, as shown by the common intercept of the ordinate in the double reciprocal plot of [³H]H_{12}-HTX binding to *Torpedo* membranes in absence and presence of quinacrine (Fig. 10). The K_i was 14 μM.

The effect of quinacrine on the binding of [³H]ACh and [³H]H_{12}-HTX to *Torpedo* membranes was mostly reversible. Incubation of *Torpedo* membranes with 30 μM quinacrine for 30 min reduced the binding of [³H]ACh and [³H]H_{12}-HTX to 65% and 35% of control levels, respectively. To remove the dissociated quinacrine, the membrane preparation was centrifuged and to reverse the bound quinacrine, the pellet was resuspended in 10-fold excess volume of buffered saline then recentrifuged and suspended. This resulted in 100% recovery of [³H]ACh binding but only 80% of [³H]H_{12}-HTX binding. Further washing resulted in no more change.

DISCUSSION

The present study demonstrates that quinacrine blocks neuromuscular transmission at concentrations higher than 10 μM. This blockade is only partially reversible, even after 180 min of washing. It is evident that the major action of quinacrine on neuromuscular transmission is due to inhibition of postsynaptic events as previously reported (16–18). The drug inhibits the depolarizing action of carbamylcholine in rat soleus muscle preexposed to TTX (Fig. 2), as was reported earlier for *Electrophorus* electroplax (16). On endplates the drug reduces EPC peak amplitudes at negative and positive membrane potentials while the relationship between EPC peak amplitude and membrane potential remains slightly nonlinear (Fig. 4, Table 1). It also shortens the rise and decay time constants of the EPC (Figs. 5, 6).

The reduction in peak amplitude of the EPC could be due to inhibition of either or both the ACh receptor and its ionic channel. Biochemical data suggest that it is due to inhibition of both proteins as shown by the inhibition of binding of [³H]ACh to the ACh-receptor and [³H]H_{12}-HTX to its ionic channel in *Torpedo* membranes (Figs. 7–10). It had previously been observed that binding of [³H]ACh to *Torpedo* ACh-receptors was slightly enhanced with concentrations of quinacrine below 2 μM, an effect interpreted in terms of allosteric interactions between the ACh-receptor and ionophore sites (26). However, higher concentrations were inhibitory, and this was proposed to be due to a direct competition with

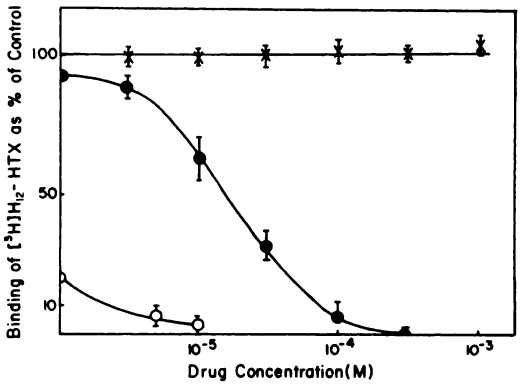

FIG. 9. *Inhibition of binding of [³H]H_{12}-HTX (0.01 μM) to Torpedo membranes by quinacrine* (●), *H_{12}-HTX* (○) *and Naja α-neurotoxin* (×)

Bars as in Fig. 7.

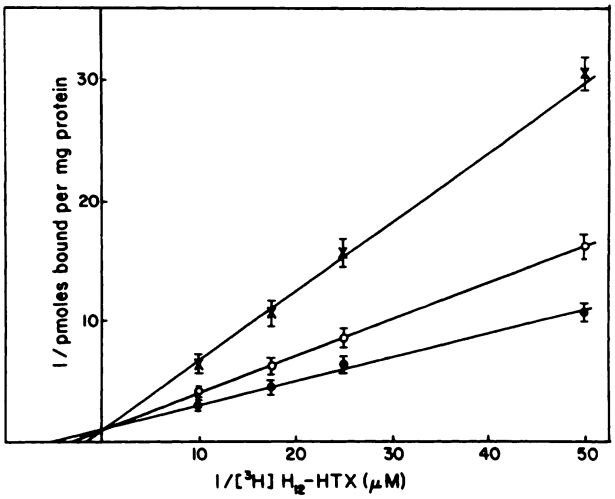

FIG. 10. *Double reciprocal plot of the binding of [^{3}H]H$_{12}$-HTX (0.01 μM) to Torpedo membranes and its inhibition by quinacrine*

[^{3}H]H$_{12}$-HTX alone (●) and in the presence of 20 μM quinacrine (○) and 100 μM quinacrine (×). Each bar represents ± SD of these experiments.

ACh at the level of the ACh-receptor site (27). The double reciprocal plot (Fig. 8) suggests that blockade of [^{3}H]ACh binding to its receptor sites by quinacrine is not competitive. This inhibition may be the result of quinacrine binding to sites on the receptor molecule itself or to closely associated molecules such as the ionic channel. However, we found that quinacrine bound to the purified ACh-receptor, which did not bind [^{3}H]H$_{12}$-HTX.[3] This suggests that quinacrine may bind to the H$_{12}$-HTX binding site on the ionic channel as evidenced by its competitive inhibition (Fig. 10) as well as to a site on the ACh-receptor, which may be different from the ACh binding site.

Although quinacrine is less effective on the ACh-receptor (Fig. 9) than a typical ACh-receptor inhibitor such as *α-Naja* toxin, the latter has no affect on [^{3}H]H$_{12}$-HTX binding to the ionic channel. This makes the toxin a specific ACh-receptor inhibitor, while quinacrine is quite effective on both the receptor and its ionic channel, although it is a 2-fold stronger inhibitor of the receptor (K_i of 14 μM for the channel vs. 7.4 μM for the receptor).

The specific binding of [^{3}H]H$_{12}$-HTX is saturable and is to proteins, as judged by its sensitivity to protein group reagents, exposure to proteases and heat sensitivity (13, 14). Binding of [^{3}H]H$_{12}$-HTX to the ionic channel of the ACh receptor is blocked by drugs and toxins that modulate the EPC. The competitive inhibition of [^{3}H]H$_{12}$-HTX binding to *Torpedo* ionic channel by quinacrine (Fig. 10), and the reduction it causes in rise and decay times of the EPC (Table 1, Fig. 6), suggest that quinacrine also binds to the ionic channel of the ACh-receptor, as was proposed earlier (16–18, 27). Since the reversal potential of the EPC remains the same in the presence and absence of quinacrine, it should be concluded that the drug does not affect the selectivity of the ionic channels opened by ACh.

In view of the marked acceleration of decay time of the EPC observed with quinacrine at concentrations from 30 to 100 μM (Fig. 6), it is suggested that quinacrine reacts with the ionic channel in the open conformation (27, 28) in a way similar to atropine (2, 9), i.e., causing a shortening of the channel open time. Quinacrine (Fig. 6) also shares with atropine (9) a marked ability to decrease or abolish the voltage dependence of the EPC's falling phase. Among many possible explanations is that quinacrine could perhaps decrease the dipole moment of the macromolecules mod-

[3] Unpublished results.

ulating the increase in the conductance of the postjunctional membrane of the endplate (24, 25). In addition, quinacrine reacts with the nicotinic ACh receptor, thus accounting for the decrease in peak amplitude of the EPC without significant departure from linearity of the current-voltage relationship (Fig. 4). Indeed, we find that the correlation between inhibition of these same electrophysiologic parameters with inhibition of [^{3}H]ACh binding at 1 μM of *Torpedo* membranes is lower, 0.72 and 0.86, respectively. However, we find that the correlation coefficients between inhibition of [^{3}H]H_{12}-HTX binding (10 nM) to *Torpedo* membranes and peak EPC amplitude and decay are 0.83 and 0.97, respectively.

The above data suggest that the decrease in amplitude and acceleration of the decay phase of the EPC by quinacrine is due to its interactions with the ACh-receptor and its ionic channel. These biophysical and biochemical studies disclose a high correlation between inhibition of [^{3}H]H_{12}-HTX binding and decay time and a lower correlation between inhibition of [^{3}H]ACh binding and EPC amplitude in the presence of quinacrine. These findings could be taken to indicate that the preferential effect of quinacrine is on the ionic channel of the ACh-receptor.

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