Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 310–314, January 1983
Neurobiology

Electrophysiological and biochemical studies on enhancement of desensitization by phenothiazine neuroleptics

(neuromuscular synapse/chlorpromazine/trifluoperazine/prochlorperazine/endplate currents)

J. S. CARP†, R. S. ARONSTAM‡, B. WITKOP§, AND E. X. ALBUQUERQUE†

†Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201; ‡Department of Pharmacology, Medical College of Georgia, Augusta, Georgia 30912; and §Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

Contributed by Bernhard Witkop, September 7, 1982

ABSTRACT **The actions of the phenothiazines chlorpromazine, prochlorperazine, and trifluoperazine were studied on the acetylcholine receptor–ionic channel complex of frog and rat skeletal muscle and of *Torpedo californica* to determine their role in pharmacological desensitization and their interactions with different states of the receptor–ionic channel complex. The phenothiazines depressed the peak amplitude of spontaneous and evoked endplate currents while having negligible effect on the decay time constants. Mean channel lifetime and single channel conductance were not altered by these drugs. They also produced a frequency-dependent depression of the peak amplitude of endplate potentials evoked by repetitive microiontophoresis at the extrajunctional region. In addition, these drugs enhanced the ability of carbamoylcholine to displace ^{125}I-labeled α-bungarotoxin from receptor-rich membrane preparations of *T. californica* when used in concentrations that had no effect on ^{125}I-labeled α-bungarotoxin binding alone (10 μM). Similarly, the phenothiazines inhibited the binding of tritiated ionic channel ligands, such as phencyclidine and perhydrohistrionicotoxin, a process also enhanced by the presence of carbamoylcholine. These data suggest that the phenothiazines augment agonist-induced desensitization primarily by interacting with the receptor–ionic channel complex prior to channel opening.**

Desensitization of the nicotinic receptor at the neuromuscular junction has been described as a gradual decrease in depolarization upon continued application of agonist (1). The progressive decrease in endplate responsiveness has been suggested to be associated with a concomitant increase in receptor affinity for the agonist (2, 3). In light of these observations, desensitization has been proposed to result from an agonist-induced conformational change, generating a nonconducting species of the acetylcholine (AcCho) receptor–ionic channel complex (4–6).

Drugs such as meproadifen (6, 7) and chlorpromazine (ClPZ) (8–11) have been shown to produce a phenomenon resembling desensitization. These drugs produce a progressive decrease in endplate responsiveness to AcCho under conditions in which the agonist alone did not produce desensitization. The similarity of the pharmacologically enhanced desensitization to the phenomenon initially described by Thesleff (1) and Katz and Thesleff (4) is further borne out by the observation that these agents increase the affinity of the agonist for its binding site. Despite these similarities, it remains unclear whether these two phenomena proceed by identical processes. One point of controversy is whether the interaction between these agents and the receptor–ionic channel complex that produces these effects occurs before (8) or after (9) opening of the ionic channel.

The objective of the present investigation is to provide an electrophysiological and biochemical analysis of the action of the phenothiazines ClPZ, trifluoperazine (TFP), and prochlorperazine (PClP) on the nicotinic receptor–ionic channel complex of the frog and rat neuromuscular synapse as well as of the electric organ of *Torpedo californica* and to establish the mechanism(s) by which the phenothiazines and other agents that enhance desensitization act.

METHODS AND MATERIALS

Electrophysiology. Endplate currents (EPCs), miniature endplate currents (MEPCs), and AcCho-induced noise were recorded from glycerol-treated (12, 13), voltage-clamped superficial fibers of cutaneous pectoris muscles of the frog *Rana pipiens* as described (14–16). MEPCs and AcCho-induced noise were recorded in the presence of 0.3 μM tetrodotoxin (Sigma). Endplate potentials (EPPs) evoked by iontophoretic application of AcCho were recorded from superficial fibers of denervated rat soleus and extensor digitorum longus muscles as described (17). Ten consecutive EPPs were evoked by iontophoretic application of brief pulses (each of 0.1- to 0.5-msec duration) of AcCho at frequencies of 0.5, 1.0, 2.0, and 5.0 Hz. In all electrophysiological experiments, the following drugs were dissolved in the appropriate physiological solutions: ClPZ hydrochloride, PClP edisylate, and TFP dihydrochloride (Smith Kline & French).

Biochemical Techniques. Membranes were prepared from the electric organs of *T. californica* (Pacific Bio-Marine, Venice, CA) and were stored in liquid nitrogen. Tissue samples were homogenized in 5 vol of 50 mM Tris·HCl (pH 7.4) containing 0.1 mM phenylmethylsulfonic acid to prevent proteolysis by using a glass Waring blender. The homogenate was filtered through four layers of cheesecloth and was centrifuged at 20,000 × g for 20 min. The resulting pellet was suspended in 50 mM Tris·HCl (pH 7.4) at a concentration of 1–2 mg of protein per ml. Sodium azide (0.02%) was added and the tissue was kept on ice for up to 3 days before use.

^{125}I-Labeled α-bungarotoxin (^{125}I-BGT; 10–20 Ci/μg, New England Nuclear; 1 Ci = 3.7 × 10^{10} becquerels) binding to the nicotinic AcCho receptor was measured at room temperature by using a filtration procedure as described (18, 19).

RESULTS

Effects of Phenothiazines on Nerve-Evoked EPCs. At a membrane potential of −90 mV, ClPZ in concentrations of 3, 5, and 7.5 μM depressed significantly the peak amplitude of the EPC to 63%, 51%, and 30% of control, whereas the decay

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Abbreviations: AcCho, acetylcholine; BGT, α-bungarotoxin; ClPZ, chlorpromazine; EPC, endplate current; EPP, endplate potential; H_{12}-HTX, perhydrohistrionicotoxin; MEPC, miniature endplate current; PClP, prochlorperazine; PCP, phencyclidine; TFP, trifluoperazine.

time constant of the EPC (τ_{EPC}) was only 95%, 87%, and 82% of control, respectively (Fig. 1). PClP in concentrations of 5 and 10 μM depressed the peak amplitude of the EPC to 88% and 32% of control, whereas the τ_{EPC} was 103% and 101% of control, respectively. TFP in concentrations of 5, 7, 10, and 20 μM depressed the peak amplitude to 62%, 31%, 18%, and 3% of control, whereas the τ_{EPC} was 100%, 82%, 74%, and 81% of control, respectively. For each of these drugs, the current–voltage relationships remained linear at all concentrations used. No differences in the peak amplitude of the EPC were found at any membrane potential betweeen −150 and −50 mV when the membrane potential was changed in either the hyperpolarizing or the depolarizing direction (i.e., no hysteresis was observed) (20). Thus, ClPZ, PClP, and TFP do not affect the voltage- and time-dependent nature of the EPC.

Effects of Phenothiazines on MEPCs. Fig. 2 shows the effects of the phenothiazines on the peak amplitude (Fig. 2 *A–C*) and the decay time constant of the MEPC (τ_{MEPC}) (Fig. 2 *D–F*). ClPZ in concentrations of 2 and 5 μM depressed ($P < 0.05$) the peak amplitude of the MEPC at membrane potentials of −60, −90, and −120 mV, whereas the τ_{MEPC} was depressed ($P < 0.05$) only by 5 μM at membrane potentials of −90 and −120 mV, respectively. After 5 μM ClPZ, the peak amplitude was 36% and 31% of control, whereas the τ_{MEPC} was 70% and 65% of control at membrane potentials of −90 and −120 mV, respectively. At 2 μM, PClP depressed ($P < 0.05$) the peak amplitude at a membrane potential of −90 mV and, at 5 μM, it depressed the peak amplitude at membrane potentials of −60, −90, and −120 mV. τ_{MEPC} was not affected by PClP at any of the concentrations or membrane potentials studied. TFP at a concentration of 5 μM depressed ($P < 0.05$) the peak amplitude at membrane potentials of −90 and −120 mV, whereas τ_{MEPC} was depressed ($P < 0.05$) by concentrations of 2 and 5 μM at a membrane potential of −60 mV. In the presence of 5 μM TFP, the peak amplitude was 55% and 52% of control at membrane potentials of −90 and −120 mV, respectively. At a membrane potential of −60 mV, the τ_{MEPC} was 80% and 86% of control in the presence of 2 and 5 μM TFP, respectively.

Effect of Phenothiazines on EPCs Evoked by Microiontophoretic AcCho Application. ClPZ at a concentration of 1 μM did not affect single channel conductance (γ) and channel lifetime (τ_I). The value (mean ± SEM for six fibers) recorded at −80 mV for γ under control conditions was 25 ± 2 pS and, after 30–60 min of exposure to ClPZ (1 μM), the value was 20 ± 1 pS ($n = 4$). Similarly, τ_I was 1.34 ± 0.19 msec ($n = 6$) and 1.04 ± 0.12 msec ($n = 4$) for control and after the ClPZ exposure, respectively. Under similar conditions neither PClP nor TFP had any statistically significant effect ($P > 0.05$) on either the γ or τ_I.

Effect of Phenothiazines on EPPs Elicited by Repetitive Application of AcCho. As shown in Fig. 3, there was no time dependence under control conditions in the peak amplitude of consecutive EPPs evoked by iontophoretic application of AcCho. The mean peak amplitudes of the second to the tenth EPPs in a train of 10 EPPs did not vary by >10% from the amplitude of the first EPP when elicited at frequencies from 0.5 to 5.0 Hz. However, all three phenothiazines induced a time-dependent depression of the peak amplitude of consecutively evoked EPPs. In the presence of 1 and 5 μM ClPZ, the mean (± SEM) peak amplitude of the tenth EPP of a train of 10 EPPs evoked at 0.5 Hz was 96% ± 5% and 69% ± 1% of control, respectively (Fig. 3*A*). The time-dependent depression of the peak amplitude of the EPP was more pronounced at higher frequencies. At 1 and 5 μM ClPZ, the mean (± SEM) amplitude of the tenth EPP of a train of 10 EPPs evoked at 5.0 Hz was 68% ± 3% and 22% ± 2% of control, respectively. Similar effects were observed with PClP (Fig. 3*B*) and TFP (Fig. 3*C*). At concentrations of 1 and 5 μM PClP, the mean (± SEM) peak amplitude of the tenth EPP of a train of 10 EPPs evoked at 0.5 Hz was 98% ± 2% and 71% ± 2%, and at 5.0 Hz it was 96% ± 2% and 59% ± 1% of control, respectively. At concentrations of 1 and 5 μM TFP, the mean (± SEM) peak amplitude of the tenth

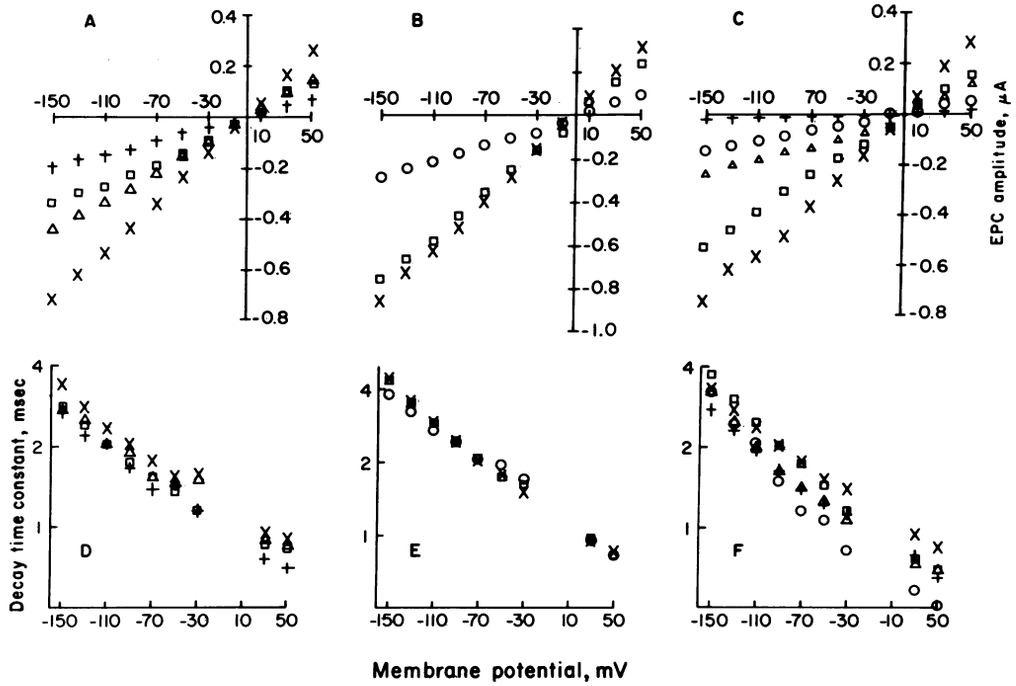


FIG. 1. Effects of phenothiazines on EPCs. (*A–C*) Voltage dependence of the peak amplitude of the EPC; (*D–F*) voltage dependence of the τ_{EPC}. The effects of ClPZ, PClP, and TFP are shown in *A* and *D*, *B* and *E*, and *C* and *F*, respectively. Each point represents the mean ± SEM for controls (×, $n \geq 5$); ClPZ at 3 μM (△, $n = 5$), 5 μM (□, $n = 10$), and 7.5 μM (+, $n = 5$); PClP at 5 μM (□, $n = 5$) and 10 μM (○, $n = 5$); TFP at 5 μM (□, $n = 9$), 7 μM (△, $n = 6$), 10 μM (○, $n = 4$), and 20 μM (+, $n = 4$). The SEM for each point is <15% of the mean.

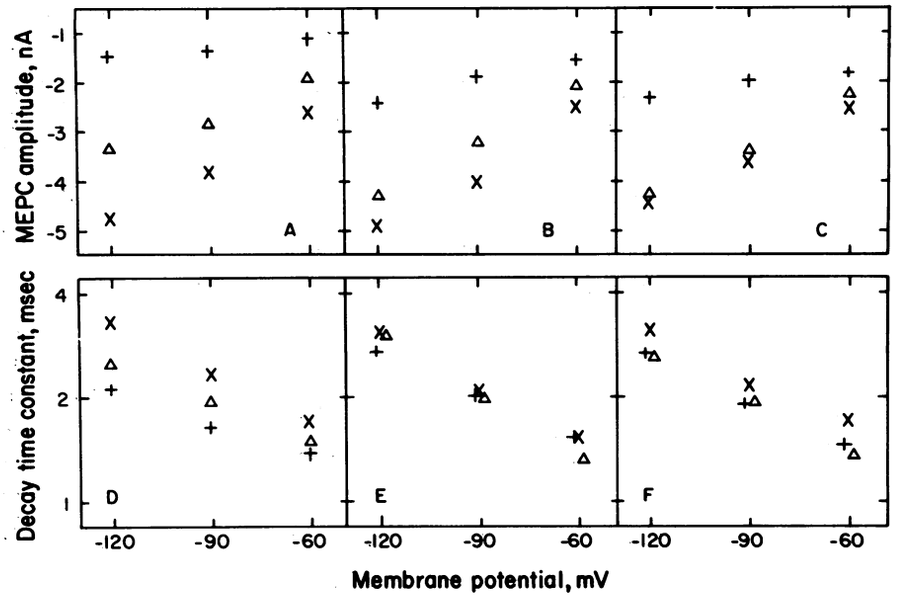


FIG. 2. Effects of phenothiazines on MEPCs. (*A–C*) Voltage dependence of the peak amplitude of the MEPC; (*D–F*) voltage dependence of the τ_{MEPC}. The effects of ClPZ, PClP, and TFP are shown in *A* and *D*, *B* and *E*, and *C* and *F*, respectively. Each point represents the mean ± SEM for controls (×, $n = 4–7$) and for all drugs at concentrations of 2 μM (△, $n = 4–6$) and 5 μM (+, $n = 4–8$). The SEM for each point is <15% of the mean.

EPP of a train of 10 EPPs evoked at 0.5 Hz was 81% ± 8% and 33% ± 3%, and at 5.0 Hz it was 81% ± 3% and 13% ± 1% of control, respectively.

Effects of Phenothiazines on Ionic Channel and AcCho Receptor Binding. As summarized in Table 1, the phenothiazines blocked the specific (i.e., amantadine-sensitive) binding of ^{3}H-labeled perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) and ^{3}H-labeled phencyclidine ([^{3}H]PCP) to sites associated with the ionic channel. In the absence of receptor ligands, all drugs inhibited [^{3}H]H_{12}-HTX and [^{3}H]PCP binding with EC_{50} values between 1 and 4 μM. In the presence of 1 μM carbamoylcholine, phenothiazine affinity was increased 2- to 8-fold so that the EC_{50} values were in the range of 0.3 to 1.4 μM. In contrast, the phenothiazines were weak inhibitors of ^{3}H-labeled AcCho and ^{125}I-BGT binding to the AcCho receptor. None of the three compounds inhibited receptor binding at 0.1 mM. However, the phenothiazines increased carbamoylcholine's affinity for the receptor, as identified by the increased ability of the agonist to inhibit the binding of ^{125}I-BGT (Fig. 4). A carbamoylcholine concentration of 4.3 μM inhibited the binding of ^{125}I-BGT (1 nM) by 50%. In the presence of 10 μM of ClPZ, PClP, and TFP, the concentration of carbamoylcholine needed to inhibit ^{125}I-BGT binding by 50% was decreased to 1.6, 1.8, and 2.3 μM, respectively. ^{125}I-BGT binding (1 and 5 nM) in the absence of carbamoylcholine was not affected by any of the phenothiazines at this concentration.

DISCUSSION

Both the electrophysiological and biochemical evidence suggest that the phenothiazines produce a phenomenon that may resemble desensitization at the neuromuscular junction. First, ClPZ, TFP, and PClP all cause a frequency-dependent depression of consecutive EPPs evoked by microiontophoretic application of AcCho. The similarities between frequency-dependent depression of EPPs caused by ClPZ and agonist-induced desensitization observed in denervated rat muscle have been demonstrated previously (8, 9). Second, the phenothiazines, in concentrations that have no effect on BGT binding alone, enhance the ability of carbamoylcholine to displace BGT from the AcCho receptor. This suggests that the phenothiazines may produce a relative increase in the affinity of the AcCho receptor for the agonist compared with that for the antagonist. Enhanced affinity of the AcCho receptor for agonist coupled with a depression of ion flux have been shown to occur upon exposure of receptor-rich *Torpedo* membrane preparations to agonist, suggesting that formation of a high affinity state of the AcCho receptor is a characteristic phenomenon of desensitization (3, 4). Because phenothiazines produced both a frequency-dependent decrease in endplate responsiveness and an apparent relative increase in receptor affinity for agonist, it seems likely that these drugs enhance agonist-induced desensitization at the neuromuscular junction.

The effects of the phenothiazines on EPCs and spontaneous MEPCs may explain how these drugs interact with the receptor–ionic channel complex and cause the pharmacological desensitization. All three phenothiazines depressed the peak amplitude of the EPC and MEPC at concentrations that had little or no effect on the τ_{EPC} and τ_{MEPC}. At higher concentrations τ_{EPC} and τ_{MEPC} were decreased, but always to a lesser degree than were the peak amplitudes of EPCs and MEPCs. Inasmuch as the τ_{EPC} and τ_{MEPC} reflect τ_I (21–23), the phenothiazines would appear to be only weak blockers of the ionic channel in its "open" conformation. The minimal effect of the phenothiazines on the τ_{EPC} and τ_{MEPC} is consistent with the observation that these drugs had no significant effect on τ_I or γ, as determined by noise analysis. However, other drugs that produce pharmacological desensitization do not share this profile of effects. Although meproadifen also produces a depression of the peak amplitude of the EPC with little or no effect on the τ_{EPC} (6), histrionicotoxin causes a marked reduction in both peak amplitude and τ_{EPC} (24). Thus, contrary to the hypothesis of Anwyl and Narahashi (9), these data suggest that a drug interaction with the receptor–ionic channel complex in its open conformation may not be essential for the expression of pharmacological desensitization (see step 3 in the reaction scheme below, in which A represents the agonist, D represents the drug, and RI, RI*, and RI′ represent the receptor–ionic channel

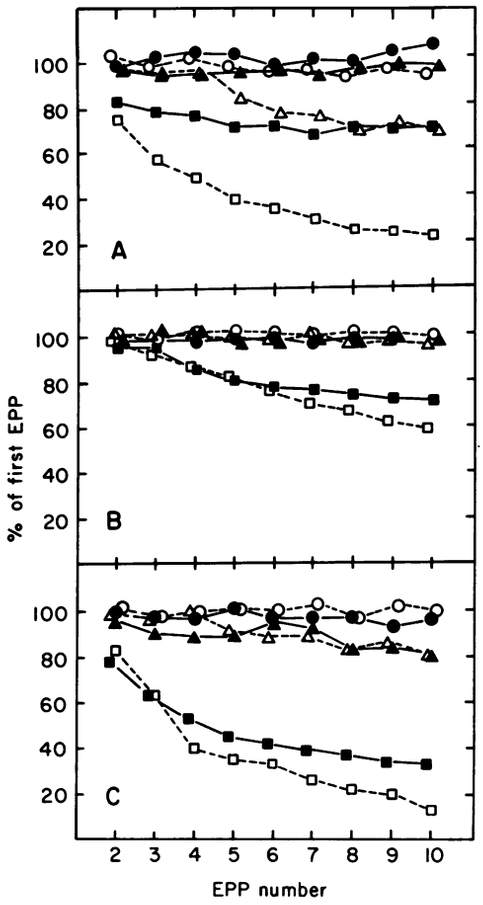
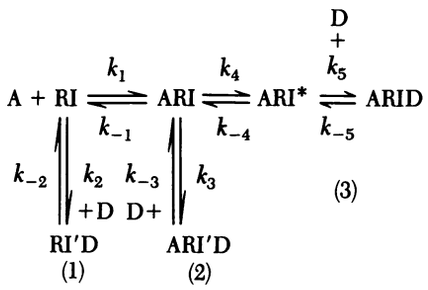


FIG. 3. Effect of ClPZ (*A*), PClP (*B*), and TFP (*C*) on EPPs evoked by repetitive microiontophoretic application of AcCho. Trains of EPPs were evoked at frequencies of 0.5 Hz (solid line with closed symbols) and 5.0 Hz (broken lines with open symbols). Each point represents the mean peak amplitude ($n = 3$) of the second to the tenth EPP expressed on the ordinate as a percentage of the peak amplitude of the first EPP for controls (○, ●) and for all drugs at concentrations of 1 μM (△, ▲) and 5 μM (□, ■). The SEM for each point is <10% of the mean.

complex in the "closed," open, and "desensitized" conformations, respectively; the k_n values refer to micro-rate constants).

These data suggest that the phenothiazines exert their effects



primarily through an interaction with the receptor–ionic channel complex prior to channel opening. It has been proposed that, even in the absence of agonist, ClPZ and meproadifen stabilize a species of the receptor–ionic channel complex that has a high affinity for agonist (7, 10, 25). If this high affinity species were similar to the high affinity species that is incapable of activation and is produced by exposure to agonist alone, then the phenothiazines could be producing a shift in the equilibrium concentrations of receptor–ionic channel complexes in favor of

Table 1. Phenothiazine inhibition of the binding of ion channel blockers to *Torpedo* electrocyte membranes

	[^{3}H]H_{12}-HTX			[^{3}H]PCP		
	EC_{50}*			EC_{50}*		
Drug	Control	Carbamoylcholine	R†	Control	Carbamoylcholine	R†
ClPZ	3.5	1.4	2.5	1.3	0.66	2.0
PClP	2.3	0.44	5.2	1.3	0.28	4.6
TFP	2.3	0.48	4.8	3.3	0.42	7.9

* Phenothiazine concentration (μM) that inhibited the specific binding of 2 nM [^{3}H]H_{12}-HTX or 3 nM [^{3}H]PCP by 50%. Binding was measured in the absence (control) and presence of 1 μM carbamoylcholine. Each value is the mean of three determinations that varied by <20%.

† R, the ratio of EC_{50} values measured in the absence and presence of carbamoylcholine.

a species that is incapable of activation (see steps 1 and 2 in the reaction scheme above). The depression in the peak amplitude of EPCs elicited at low frequencies could be explained by the phenothiazine-induced increase in the fraction of receptor–ionic channel complexes in the state that is incapable of activation (step 1 in the reaction scheme). To explain the frequency-dependent depression of the peak amplitude of EPPs, one has to assume that $k_3 > k_{-3}$. Under this condition, the species ARI'D that is incapable of activation could accumulate over the course of consecutive stimulations when the time interval between successive stimuli was sufficiently brief that equilibrium concentrations of the species that are capable and incapable of activation could not be reestablished.

Meproadifen, which also induces pharmacological desensitization, has been demonstrated to produce hysteresis and nonlinearity in the current–voltage plots for EPCs (7). These irregularities in the current–voltage relationship have been attributed to this drug's interaction with the receptor–ionic channel complex in its closed conformation at a site that is sensitive to membrane potential (20). However, the phenothiazines did not alter the voltage or time dependence of the peak am-

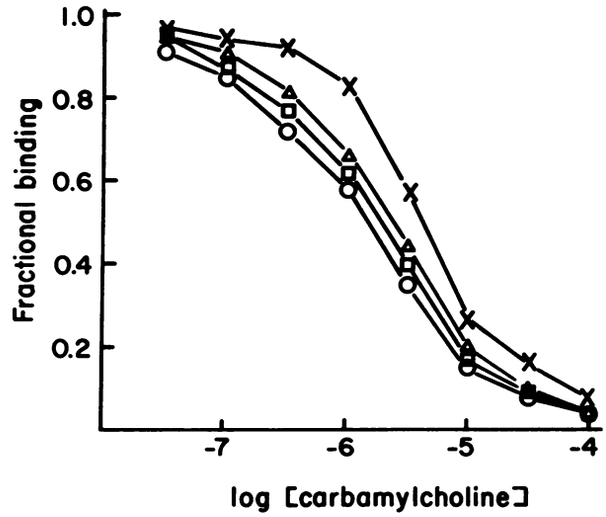


FIG. 4. Influence of phenothiazines on carbamoylcholine inhibition of ^{125}I-BGT binding to the AcCho receptor. The specific binding of 1 nM ^{125}I-BGT, expressed on the ordinate as the fraction of total specific binding, was measured in the presence of the concentration of carbamoylcholine indicated on the abscissa. Binding was measured in the absence of phenothiazines (×) or in the presence of 10 μM PClP (□), ClPZ (○), or TFP (△). Each point represents the average of three determinations that varied by <15%.

plitude of the EPC, which suggests an interaction with a membrane site that is not voltage-sensitive. Two classes of noncompetitive antagonist binding sites have been proposed for drugs that stabilize the high affinity form of the receptor–ionic channel complex (10, 25). ClPZ has been proposed to bind to one of these sites, whereas meproadifen binds preferentially to the other. Thus, it is possible that the differential binding of these agents could reflect their interactions with voltage-sensitive and voltage-insensitive sites at the receptor–ionic channel complex.

Despite the apparent lack of effect of the phenothiazines on τ_I, it is possible that open channel blockade might contribute to decremental postjunctional membrane responsiveness to agonist by a mechanism unrelated to desensitization under certain kinetic conditions. Upon channel activation, if $k_{-4} > k_5$, the phenothiazines would not be expected to be potent in decreasing the τ_{EPC} and τ_{MEPC}. Furthermore, if $k_5 > k_{-5}$, then the fraction of receptor–ionic channel complexes that is capable of activation during a train of evoked responses would be dependent on the time interval between successive stimulations. Under such kinetic conditions, it is possible that both the frequency-dependent depression of EPPs and the weak depression of τ_{EPC} and τ_{MEPC} result in part from a slow interaction of the phenothiazines with the ionic channel in its open conformation relative to the channel closure event. In this case, the lack of effect of the phenothiazines on τ_I might result from the rates of association and dissociation of the drug–ionic channel complex being sufficiently slow that a drug-induced effect may be unmeasurable by noise analysis in the frequency range studied (approximately 4–800 Hz). A more rigorous kinetic and single channel analysis of the effects of the phenothiazines must be performed to determine the extent to which the mechanisms discussed in this paper may be involved in the precise actions of these phenothiazines.

The authors are greatly indebted to Dr. J. W. Daly (Laboratory of Bioorganic Chemistry, National Institutes of Health) for the generous supply of [^{3}H]H_{12}-HTX. We thank Dr. J. E. Warnick (Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine) for his help during the initial part of this research, Ms. Mabel A. Zelle and Mrs. Lauren Aguayo for their excellent technical assistance and computer programing and analysis, and Mrs. Margaret Shimkaveg for her careful typing of this manuscript. This work was supported in part by National Institutes of Health Grant DA-03303 and U.S. Army Medical Research and Development Contract DAMD 17-81-C-1279.

1. Thesleff, S. (1955) *Acta Physiol. Scand.* **34**, 218–231.
2. Weber, M., David-Pfeuty, T. & Changeux, J.-P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3443–3447.
3. Weiland, G., Georgia, B., Wee, V. T., Chignell, C. F. & Taylor, P. (1976) *Mol. Pharmacol.* **12**, 1091–1105.
4. Katz, B. & Thesleff, S. (1957) *J. Physiol. (London)* **138**, 63–80.
5. Steinbach, J. H. & Stevens, C. F. (1976) in *Frog Neurobiology*, eds. Llinas, R. & Precht, W. (Springer, New York), pp. 33–92.
6. Maleque, M. A., Souccar, C., Cohen, J. B. & Albuquerque, E. X. (1982) *Mol. Pharmacol.* **22**, 636–647.
7. Krodel, E. K., Beckman, R. A. & Cohen, J. B. (1979) *Mol. Pharmacol.* **15**, 294–312.
8. Magazanik, L. G. & Vyskocil, F. (1976) in *Motor Innervation of Muscle*, ed. Thesleff, S. (Academic, London), pp. 151–176.
9. Anwyl, R. & Narahashi, T. (1980) *Br. J. Pharmacol.* **69**, 99–106.
10. Heidemann, T. & Changeux, J.-P. (1981) *FEBS Lett.* **131**, 239–244.
11. Carp, J. S., Warnick, J. E., Heinonen, E. & Albuquerque, E. X. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1721 (abstr.).
12. Fujino, M., Yamaguchi, T. & Suzuki, K. (1961) *Nature (London)* **192**, 1159–1161.
13. Gage, P. W. & Eisenberg, R. S. (1976) *Science* **158**, 1702–1703.
14. Takeuchi, A. & Takeuchi, N. (1959) *J. Neurophysiol.* **22**, 395–411.
15. Kuba, K., Albuquerque, E. X., Daly, J. & Barnard, E. A. (1974) *J. Pharmacol. Exp. Ther.* **189**, 499–512.
16. Schofield, G. G., Witkop, B., Warnick, J. E. & Albuquerque, E. X. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5240–5244.
17. Albuquerque, E. X. & McIsaac, R. J. (1970) *Exp. Neurol.* **26**, 183–202.
18. Aronstam, R. S., Eldefrawi, A. T., Pessah, I. N., Daly, J. W., Albuquerque, E. X. & Eldefrawi, M. E. (1981) *J. Biol. Chem.* **256**, 2843–2850.
19. Aronstam, R. S., Narayanan, L. & Wenger, D. A. (1982) *Eur. J. Pharmacol.* **78**, 367–370.
20. Spivak, C. E. & Albuquerque, E. X. (1982) in *Progress in Cholinergic Biology: Model Cholinergic Synapses*, eds. Hanin, I. & Goldberg, A. M. (Raven, New York), pp. 323–357.
21. Magleby, K. L. & Stevens, C. F. (1972) *J. Physiol. (London)* **223**, 151–171.
22. Magleby, K. L. & Stevens, C. F. (1972) *J. Physiol. (London)* **223**, 173–197.
23. Anderson, C. R. & Stevens, C. F. (1973) *J. Physiol. (London)* **235**, 655–691.
24. Masukawa, L. M. & Albuquerque, E. X. (1978) *J. Gen. Physiol.* **72**, 351–367.
25. Heidemann, T. & Chageux, J.-P. (1979) *Eur. J. Biochem.* **94**, 281–296.