Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4563–4567, September, 1978
Neurobiology

Volatile anesthetic facilitation of *in vitro* desensitization of membrane-bound acetylcholine receptor from *Torpedo californica*

(mechanism of general anesthesia/ligand-induced conformational change/protein–lipid interactions)

A. P. YOUNG*, F. F. BROWN†, M. J. HALSEY‡, AND D. S. SIGMAN*

* Department of Biological Chemistry, UCLA School of Medicine and Molecular Biology Institute, University of California, Los Angeles, California 90024;
† Department of Biochemistry, Oxford University, Oxford, England; ‡ Division of Anaesthesia, Clinical Research Centre, Harrow, Middlesex, England

Communicated by Paul D. Boyer, May 16, 1978

ABSTRACT **Incubation of membrane fragments bearing acetylcholine receptors from *Torpedo californica* under an atmosphere of 3% halothane, 1% chloroform, or 6% diethyl ether greatly facilitates the carbamoylcholine-induced structural transition of the acetylcholine receptor reflected by alterations in the rate of binding of ^{125}I-labeled α-bungarotoxin. The half-time of this ligand-induced conformational change is decreased to 10% of the original value after incubation of the membranes with these volatile anesthetics at or near their clinical concentrations. The synergistic effects observed with the general anesthetics and carbamoylcholine are abolished if the membranes are incubated under a stream of air after exposure to the inhalational agents. The antagonist *d*-tubocurarine exerts a smaller yet measurable time-dependent effect on the toxin-binding properties of the membrane fragments. Treatment of membranes with general anesthetics facilitates this antagonist-induced conversion of the receptor protein as well. The synergism between ligands and general anesthetics may be due to the disruption by these inhalational agents of interactions at the protein–lipid interface, which may play a significant role in determination of receptor conformation. In addition, if the conformational change induced by carbamoylcholine observed in the snake toxin binding assay corresponds to desensitization of the receptor *in vivo*, facilitation of this conformational change by volatile anesthetics provides an attractive model for the pharmacological action of these compounds.**

The inhibition by carbamoylcholine of the rate of snake neurotoxin binding to membrane-bound acetylcholine receptor protein from *Torpedo* electroplaques increases upon pretreatment with this cholinergic agonist (1–3). This observation has been interpreted in terms of a reversible agonist-induced structural transition in the receptor to yield a conformational state of the protein that binds the agonist more tightly. The half-time of the observed structural transition of the receptor protein is approximately 1 min and is diminished by calcium ions and by increased temperature.

Calcium-dependent pharmacological desensitization has been well documented by electrophysiological methods at the neuromuscular junction (4, 5) and in electroplaques from eel (6, 7) and possibly from *Torpedo* (8). A desensitization-like phenomenon is also demonstrable *in vitro*, using ^{22}Na-enriched microsacs prepared from *Torpedo* electroplaques (9). The desensitized state of the postsynaptic membrane is induced by prolonged ionophoretic treatment with a cholinergic agonist such as carbamoylcholine. In this condition the membrane is refractory and no postsynaptic depolarization can occur upon further application of cholinergic agonist. The temporal relationship between the onset of desensitization observed electrophysiologically (4) and the structural transition of the receptor protein characterized *in vitro* by its increased affinity for carbamoylcholine suggests that the two processes might be intimately related (1–3).

Solubilization of *Torpedo* receptor protein with various detergents alters its agonist-binding affinity (10–15). Moreover, agonist-induced conformational changes are not observed with detergent-solubilized receptor (3). The requirement for the protein to be associated with membrane in order to exhibit these ligand-induced structural changes strongly suggests that protein–lipid interactions exert an important conformational constraint on the receptor protein and could play a crucial role in its function.

Since precedence exists for disruption of a protein–lipid interface by the small hydrophobic molecule benzyl alcohol (16), it seems likely that general anesthetics such as halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), chloroform, and diethyl ether are capable of perturbing protein–lipid interactions. We have investigated the effect of these volatile anesthetics on ligand-induced conformational changes of membrane-bound acetylcholine receptor protein prepared from *Torpedo californica*. Although these inhalational agents have only a marginal effect on the rate of snake toxin binding to the receptor, the carbamoylcholine-induced structural transition of the receptor protein observed in a snake toxin binding assay is accelerated at least 10-fold if receptor-containing membrane fragments are incubated under an atmosphere of 3% halothane, 1% chloroform, or 6% diethyl ether. These are concentrations used to induce clinical anesthesia. The effect of these volatile anesthetic agents is reversible and is observed with *d*-tubocurarine, which induces changes on the toxin-binding properties of the receptor that are similar, though smaller, than those observed with carbamoylcholine. There is no synergistic effect involving anesthetic and carbamoylcholine on the kinetics of snake toxin binding to receptor protein that has been solubilized with Triton X-100 detergent. If the conformational change induced by carbamoylcholine observed in the snake toxin binding assay corresponds to desensitization of the receptor *in vivo* (4), an interesting model system for the mode of action of general anesthetics emerges from these studies. A preliminary report of these data has appeared (17).

MATERIALS AND METHODS

The following materials were obtained commercially: carbamoylcholine chloride, phenylmethylsulfonylfluoride, Triton X-100, and 1× recrystallized bovine serum albumin (Sigma); *d*-tubocurarine chloride (K & K Laboratories, Brunswick, NJ); lactoperoxidase (EC 1.11.1.7) (Calbiochem); Na^{125}I (New England Nuclear); VC 02500 nitrocellulose filter papers (Millipore); DEAE 81 filter disks (Whatman); halothane, containing

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: α-Bgt, α-bungarotoxin; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

0.01% thymol (Halocarbon Laboratories, Hackensack, NJ); and analytical grade chloroform and diethyl ether (Mallinckrodt). Compressed air of medicinal grade was purchased from Liquid Carbonics (Los Angeles, CA). *T. californica* were obtained live from Pacific Biomarine (Venice, CA). α-Bungarotoxin (α-Bgt) was purchased from the Miami Serpentarium (Miami, FL) and migrated as one band on sodium dodecyl sulfate gel electrophoresis (18). All buffer components were of the highest purity available.

Preparation of Acetylcholine Receptor. Receptor-bearing membrane fragments were prepared essentially by the procedure of Reed *et al.* (19) from freshly excised electric organs of *T. californica*, which were either used immediately or quick frozen in liquid nitrogen and stored at −70°. For certain preparations, purification by sucrose gradient centrifugation was omitted. Specific activities ranged from 0.3 to 1.2 nmol of α-Bgt binding sites per mg of protein assayed by the method of Lowry *et al.* (20). Receptor protein was solubilized by 1:10 dilution of membrane fragments, originally in the low affinity state, into 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.4/1 mM $CaCl_2$/0.1 M NaCl/0.02% NaN_3/1% Triton X-100 detergent (vol/vol) and left at 4° for 12 hr. After centrifugation to remove the membrane lipid, 95% of the toxin binding sites originally present had been solubilized. For kinetic runs, this material was diluted into the above buffer without detergent to yield final concentration of 0.25% Triton X-100.

Assay of α-Bgt Binding to Acetylcholine Receptor. α-Bgt was labeled with ^{125}I using lactoperoxidase (21) and purified by gel filtration with Sephadex G-50 (fine) followed by ion-exchange chromatography with DEAE-cellulose. Specific activities of 4.0–6.6 × 10^6 cpm/nmol of toxin were generally obtained from iodination of 1 mg of α-Bgt with 1 mCi of carrier-free $Na^{125}I$.

The binding of ^{125}I-labeled α-Bgt to membrane-bound acetylcholine receptor was assayed using nitrocellulose filter disks in our initial experiments (Figs. 1 and 3). VC papers (Millipore) were wetted by passage of 0.3 ml of 15% Triton X-100 (vol/vol) through the filter paper by suction. Then 40 μl of the solution containing receptor and ^{125}I-labeled α-Bgt were pulled through the filter paper, which was then washed twice with 1-ml aliquots of 0.5 M NaCl and 1 mg of bovine serum albumin per ml. In all experiments with the detergent-solubilized acetylcholine receptor and in other experiments with the membrane-bound protein (Figs. 2 and 4), ^{125}I-labeled α-Bgt binding was assayed by the method of Schmidt and Raftery (22). In these experiments 100 μl of solution was assayed. In all experiments retention of the ^{125}I-labeled α-Bgt–receptor complex to the filter paper was quantitative. Background binding of the radioactive toxin to the filter paper, determined by pretreatment of the membranes with unlabeled α-Bgt prior to the addition of the ^{125}I-labeled α-Bgt, is shown at the ordinate of the figures. This binding was always less than 10% of that observed when comparable concentrations of receptor and ^{125}I-labeled α-Bgt were allowed to react for 10 hr prior to addition of the unlabeled toxin. Radioactivity was determined with a Beckman Biogamma counter.

Measurement of Rate of ^{125}I-Labeled α-Bgt Binding to Acetylcholine Receptor. Membrane fragments were equilibrated at 21° for 30 min in a closed vessel, under a gentle stream of air, or under an atmosphere of the indicated concentration of the general anesthetic. When the time course for the carbamoylcholine-induced structural transition was monitored (Figs. 3 and 4), this treatment was followed by incubation with carbamoylcholine for the time indicated in the figure legend. In certain indicated cases, cholinergic ligand was present during the initial equilibration. Reactions were initiated by addition of ^{125}I-labeled α-Bgt and, where appropriate, cholinergic ligand. The time course of toxin binding was monitored by addition of an aliquot of the reaction mixture to a 65-fold excess of unlabeled α-Bgt at various times followed by assay using one of the procedures described above. Concentrations of α-Bgt binding sites and ^{125}I-labeled α-Bgt, given in the figure legends, were determined by titration, with unlabeled α-Bgt as the primary standard. Reaction mixtures contained 0.1 M NaCl, 1 mM $CaCl_2$, 0.02% NaN_3, and 1 mM sodium phosphate (pH 7.4) (Figs. 1 and 3) or 10 mM Hepes (pH 7.4) (Figs. 2 and 4). When a preparation of membrane fragments was diluted into either buffer, the observed carbamoylcholine-induced structural transition of the receptor protein was unaltered. Triton-solubilized receptor protein was diluted into Hepes buffer as described above and pretreated in the presence or absence of 1% chloroform at 21° for 30 min. Kinetic methods were the same as described for membrane-bound receptor.

Delivery of Inhalational Anesthetics. Gaseous mixtures of 3% halothane, 1% chloroform, and 6% diethyl ether were generated by a simple gas flow system (23). To generate 3% halothane, we passed air at 50 cm^3/min flow rate through the anesthetic at 21° in a closed system. A second stream of air of flow rate 725 cm^3/min was mixed with the effluent emerging from the halothane. Most of the resulting air flow was bled to the atmosphere, but a small but constant flow was passed through a small vial of distilled water to humidify it prior to delivery over the receptor solution. Mixtures of 1% chloroform and 6% diethyl ether were generated at 21° in an analogous fashion. The appropriate flow rates were 25 and 625 cm^3/min for chloroform and its dilutant air, respectively, and 40 and 825 cm^3/min for diethyl ether and its dilutant air, respectively.

RESULTS

The effect of exposure of receptor-enriched membrane fragments to 0.5 μM carbamoylcholine for 30 min prior to initiation of the toxin binding reaction is illustrated in Fig. 1. Membranes

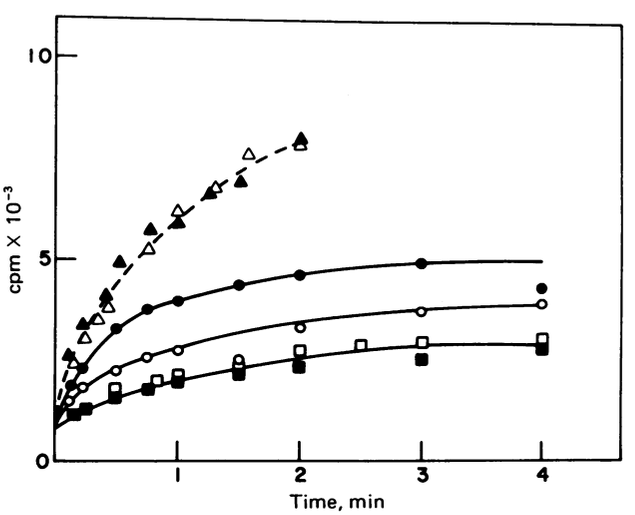

FIG. 1. Time-dependent inhibition by 0.5 μM carbamoylcholine of α-Bgt binding to receptor-enriched membrane fragments in the presence or absence of 3% halothane. Membrane solutions (0.05 μM α-Bgt binding sites) were added to ^{125}I-labeled α-Bgt (0.2 μM). Membranes pretreated under air (▲) or 3% halothane (△); membranes pretreated with 0.5 μM carbamoylcholine under air (■) or 3% halothane (□); membranes pretreated under air (●) or 3% halothane (○), 0.5 μM carbamoylcholine present only in the reaction mixture. Infinity values were 10,800 ± 500 cpm for all time courses.

treated in this fashion showed a diminished rate of toxin binding relative to the rate observed for membranes to which carbamoylcholine was added only upon initiation of the toxin binding reaction. After 10 hr, equivalent amounts of α-Bgt were bound under either experimental condition. The time-dependent inhibition in the presence of carbamoylcholine and the rate of toxin binding in the absence of ligand are not perturbed by passage of humidified air above the receptor solution. Other authors have observed similar effects of pretreatment by carbamoylcholine and have presented evidence that strongly suggests that the time-dependent agonist inhibition of toxin binding monitors a reversible change in affinity of the receptor for ligand (1–3, 24).

Fig. 1 also shows the effect of membrane treatment under 3% halothane on these toxin binding kinetics. Several features of this figure are of special interest. First, the rate of toxin binding in the absence of carbamoylcholine was unperturbed by halothane treatment. Second, the rate of toxin binding was similar for membrane fragments that had been pretreated with carbamoylcholine in the presence and absence of halothane. Third, and of great significance, is that membranes that had been incubated under 3% halothane and then added to a toxin solution containing carbamoylcholine exhibited significantly slower kinetics of toxin binding than membrane fragments that had been incubated under air and then added to an identical solution of α-Bgt and carbamoylcholine. This synergistic effect of carbamoylcholine and halothane, which could be reversed within 15 min by passage of air over halothane-treated samples, is consistent with the general anesthetic accelerating the time-dependent change that carbamoylcholine itself exerts. Fully parallel data have been obtained with 0.1 μM carbamoylcholine.

Fig. 2 demonstrates that the anesthetic agents chloroform and diethyl ether also facilitate the carbamoylcholine-induced conformational change of the membrane-bound acetylcholine receptor. While membranes treated with chloroform or diethyl ether show only a slight diminution in their rate of toxin binding in the absence of ligand relative to the rate for untreated membranes (Fig. 2*A*), membranes treated with one of these anesthetics and added to a solution of carbamoylcholine and ^{125}I-labeled α-Bgt show a substantial inhibition of the rate of toxin binding relative to the control (Fig. 2*B*). As was seen with halothane, the effect of chloroform and diethyl ether could be reversed by passing air over anesthetic-treated samples.

Direct demonstrations that general anesthetics accelerate the time-dependent effect on toxin binding observed in the presence of carbamoylcholine are summarized in Figs. 3 and 4. For one preparation of membrane fragments, a halftime of 30 sec for the conformational change induced by 0.5 μM carbamoylcholine can be estimated from the initial rate of toxin binding (Fig. 3*A*). Fig. 3*B* shows that, in the presence of halothane, this time-dependent effect of carbamoylcholine is complete within 12.5 sec. If the agonist-induced change is 95% complete at this time, a half-time of approximately 3 sec can be inferred. The net effect of halothane, therefore, appears to be at least a 10-fold acceleration of the rate for the carbamoylcholine-induced change of the acetylcholine receptor observed in the snake toxin binding assay. From the data in Fig. 4, obtained with a different preparation of membrane fragments, halftimes for the carbamoylcholine-induced conformational change of the membrane-bound acetylcholine receptor of 50 sec and 6 sec can be estimated for untreated and chloroform-treated membranes, respectively. A similar acceleration for this conformational change was observed after membrane pretreatment under an atmosphere of 6% diethyl ether.

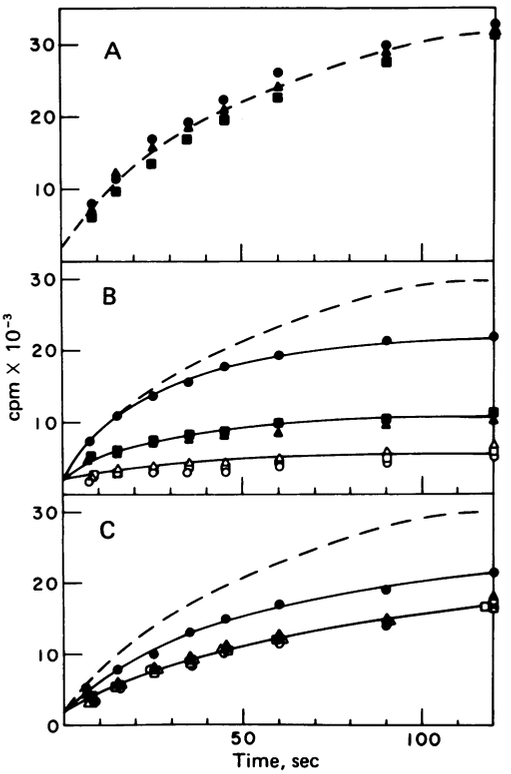

FIG. 2. Time-dependent inhibition by ligands of α-Bgt binding to membrane-bound acetylcholine receptor in the absence or presence of 1% chloroform or 6% diethyl ether. Membrane solutions (0.11 μM α-Bgt binding sites) were added to ^{125}I-labeled α-Bgt (0.2 μM). Infinity values were 46,700 ± 1100 cpm for all time courses. (*A*) Membranes pretreated in the absence (●) or presence of 1% chloroform (■) or 6% diethyl ether (▲) in the absence of ligand. (*B*) Time-dependent inhibition by 0.5 μM carbamoylcholine. Membranes pretreated with 0.5 μM carbamoylcholine in the absence (○) or presence of 1% chloroform (□) or 6% diethyl ether (△); membranes pretreated in the absence (●) or presence of 1% chloroform (■) or 6% diethyl ether (▲), 0.5 μM carbamoylcholine present only in the reaction mixture. (*C*) Time-dependent inhibition by 0.2 μM *d*-tubocurarine. Membranes pretreated with 0.2 μM *d*-tubocurarine in the absence (○) or presence of 1% chloroform (□) or 6% diethyl ether (△); membranes pretreated in the absence (●) or presence of 1% chloroform (■) or 6% diethyl ether (▲), 0.2 μM *d*-tubocurarine present only in the reaction mixture. Dashed line in *B* and *C* shows the control rate of toxin binding in the absence of ligand.

Although we have observed variability in the rate of the carbamoylcholine-induced structural transition of the acetylcholine receptor, treatment of the membrane preparation with one of the general anesthetics always resulted in a substantial facilitation of the ligand-induced conformational change. Accordingly, our qualitative observation with respect to the general anesthetics tested is independent of the observed control rate.

We have examined the effect of pretreatment of membrane fragments with the pharmacological antagonist *d*-tubocurarine on the kinetics of toxin binding. As shown in Fig. 2*C*, we have observed a slight increase in the inhibition of the rate of snake toxin binding upon pretreatment with this ligand with some preparations of membrane fragments. Others have demonstrated a synergistic effect between agonists and some antagonists on the conformation of the membrane-bound acetylcholine receptor (1, 2, 24). These authors have suggested a relationship of this agonist-antagonist synergism to the metaphilic behavior of agonists with respect to some antagonists observed *in vivo* at the neuromuscular junction (25). Consistent with the reported synergism is our demonstration (Fig. 2*C*) that the

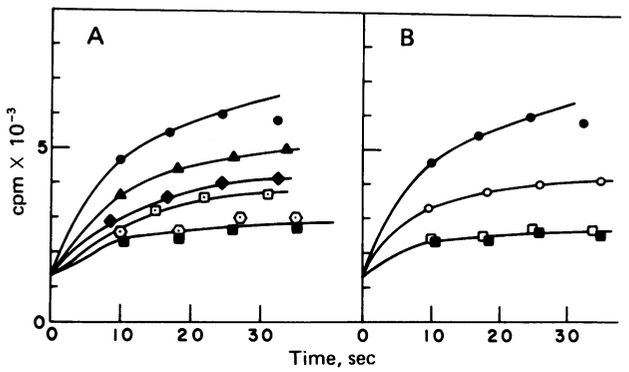

FIG. 3. Kinetics of the carbamoylcholine-induced change in toxin binding of receptor-enriched membrane fragments in the absence (*A*) or presence (*B*) of 3% halothane. Membrane solutions incubated with 0.5 μM carbamoylcholine. The reaction was initiated by addition of the carbamoylcholine-treated membranes (0.049 μM α-Bgt binding sites) to ^{125}I-labeled α-Bgt (0.44 μM). (*A*) Air alone. Carbamoylcholine pretreatment for 0 sec (●), 20 sec (▲), 40 sec (♦), 90 sec (⊡), 10 min (⊙), or 30 min (■). (*B*) 3% halothane. Carbamoylcholine pretreatment for 0 sec (○) and 12.5 sec (□); data from *A* shown for comparison (● and ■).

anesthetic agents chloroform and diethyl ether facilitate the *d*-tubocurarine-induced structural transition of the receptor protein as well as the conversion induced by the agonist carbamoylcholine.

In agreement with published reports (3), we found no increase for the inhibition of toxin binding in the presence of 0.5 μM carbamoylcholine after pretreatment with this ligand using receptor protein that had been solubilized with Triton X-100 detergent. Moreover, no alteration in these kinetics could be observed after pretreatment of the solubilized receptor under an atmosphere of 1% chloroform. These observations suggest that the lipid environment provides an important constraint on the receptor protein and that volatile anesthetics as well as local anesthetics (3) alter protein–lipid interactions.

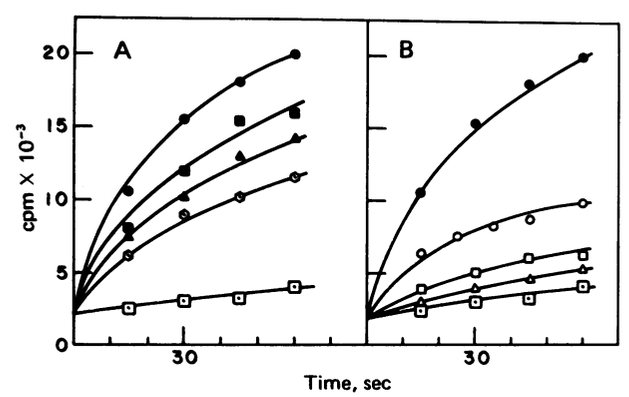

FIG. 4. Kinetics of the carbamoylcholine-induced change in toxin binding of membrane-bound acetylcholine receptor in the absence (*A*) or presence (*B*) of 1% chloroform. Membrane solutions incubated with 0.5 μM carbamoylcholine. The reaction was initiated by addition of the carbamoylcholine-treated membranes (0.11 μM α-Bgt toxin binding sites) to ^{125}I-labeled α-Bgt (0.2 μM). (*A*) No anesthetic treatment. Carbamoylcholine pretreatment for 0 sec (●), 20 sec (■), 40 sec (▲), 60 sec (⊙), or 30 min (⊡). (*B*) 1% chloroform. Carbamoylcholine pretreatment for 0 sec (○), 10 sec (□), and 30 sec (△); data from *A* shown for comparison (● and ⊡).

DISCUSSION

The central observation of the present communication is that the general anesthetics halothane, chloroform, and diethyl ether, at their approximate clinical concentrations, facilitate a structural transition of membrane-bound acetylcholine receptor protein induced by the agonist carbamoylcholine. The data in Figs. 3 and 4 show that the effect is sufficiently dramatic that it is difficult to measure with conventional mixing techniques. A reasonable estimate is that the carbamoylcholine-induced structural transition of the receptor protein is accelerated 10-fold by the anesthetics tested.

Although general anesthetics could act at hydrophobic domains within the protein, we feel that these agents exert their primary effect by disruption of protein–lipid interactions. The absence of a carbamoylcholine-induced conformational change of detergent-solubilized receptor protein suggests that the membrane lipid has an important function with regard to the conformation of the acetylcholine receptor. Interestingly, agonists that contain an alkyl sidechain or an aromatic ring show an increased ability relative to carbamoylcholine to induce desensitization in frog or chick muscle (26). Such compounds might interact at both the acetylcholine binding site and the protein–lipid interface to facilitate desensitization. Furthermore, a protein–lipid boundary site for the action of halothane, chloroform, and diethyl ether is supported by the recent observation that limited phospholipase A treatment of postsynaptic membrane vesicles from *T. californica* inhibits carbamoylcholine-induced ^{22}Na efflux from these vesicles (27). Both phospholipase A and the anesthetics could be acting to facilitate desensitization of the acetylcholine receptor by disruption of protein–lipid interactions which provide a conformational restraint on the receptor.

These results provide an interesting insight into a potential mechanism of general anesthesia. Interruption of synaptic transmission rather than axonal transmission is a more likely mechanism for general anesthesia (28). The multineural polysynaptic pathways of the brain stem reticular formation are regarded as crucial toward the maintenance of consciousness. General anesthetics selectively depress transmission through these pathways (29). Moreover, concentrations of chloroform or ether causing surgical anesthesia inhibit synaptic transmission, but not axonal transmission, in cat sympathetic ganglia (30).

Electrophysiological studies at the neuromuscular junction for a variety of volatile anesthetics, including diethyl ether and halothane, support the view that general anesthetics interrupt synaptic transmission by reduction of the sensitivity of the postsynaptic membrane to neurotransmitter (31, 32). The demonstration that general anesthetics do not affect the binding of *d*-tubocurarine to the functional and nondesensitized receptors at the isolated guinea pig lumbrical muscle (33) is consistent with the anesthetics inhibiting net depolarization by facilitation of desensitization of the acetylcholine receptor and not by inhibition of ligand binding. Assuming the time-dependent inhibition of toxin binding can be related to desensitization in the synapse, our observations provide a molecular basis for these effects. The relative potency of various inhalational anesthetics in their ability to depress synaptic transmission at the neuromuscular junction suggests that the neuromuscular junction is an appropriate system for the study of the mechanisms of action of general anesthetics (32). Inasmuch as receptor desensitization could be a general feature of synaptic transmission and not merely confined to the nicotinic acetylcholine receptor from muscle or electroplaques, anesthetic-assisted desensitization of the acetylcholine receptor is a reasonable

model for the pharmacological activity of volatile anesthetics as well as local anesthetics (3).

The excellent technical assistance of Ms. Janis Oshiki is gratefully acknowledged. We appreciate the suggestion of Dr. Michael A. Raftery and Dr. Michael Schimerlik to further purify ^{125}I-labeled α-Bgt by passage through a DEAE-cellulose column and their hospitality in inviting us to their laboratory to observe the DEAE-disk assay. Financial support of this research was provided by U.S. Public Health Service Grant 21199. A.P.Y. was a recipient of a graduate student award from the University of California, Los Angles, providing funds for travel to England.

1. Weber, M., David-Pfeuty, T. & Changeux, J. P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3443–3447.
2. Lee, T., Witzemann, V., Schimerlik, M. & Raftery, M. A. (1977) *Arch. Biochem. Biophys.* **183,** 57–63.
3. Weiland, G., Georgia, B., Lappi, S., Chignell, C. F. & Taylor, P. (1977) *J. Biol. Chem.* **252,** 7648–7656.
4. Katz, B. & Thesleff, S. (1957) *J. Physiol. (London)* **138,** 63–80.
5. Nastuk, W. L. & Giessen, A. J. (1966) in *Nerve as a Tissue,* eds. Rodahl, K. & Issekutz, B. (Harper and Row, New York), pp. 305–320.
6. Larmie, E. T. & Webb, G. D. (1973) *J. Gen. Physiol.* **61,** 263.
7. Lester, H. A., Changeux, J. P. & Sheridan, R. E. (1975) *J. Gen. Physiol.* **65,** 797–816.
8. Moreau, M. & Changeux, J. P. (1976) *J. Mol. Biol.* **106,** 457–467.
9. Sugiyama, H., Popot, J. L. & Changeux, J. P. (1976) *J. Mol. Biol.* **106,** 485–496.
10. O'Brian, R. D. & Gibson, R. E. (1975) *Arch. Biochem. Biophys.* **169,** 458–463.
11. Weber, M. & Changeux, J. P. (1974) *Mol. Pharmacol.* **10,** 35–40.
12. Sugiyama, H. & Changeux, J. P. (1975) *Eur. J. Biochem.* **55,** 505–515.
13. Martinez-Carrion, M. & Raftery, M. A. (1973) *Biochem. Biophys. Res. Commun.* **55,** 1156–1164.
14. Eldefrawi, M. E., Eldefrawi, A. T. & Wilson, P. B. (1975) *Biochemistry* **14,** 4304–4310.
15. Eldefrawi, M. E. & Eldefrawi, A. T. (1973) *Arch. Biochem. Biophys.* **159,** 362–373.
16. Hesketh, R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsall, N. J. M., Metcalfe, C. & Warren, G. B. (1976) *Biochemistry* **15,** 4145–4151.
17. Young, A. P. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1627.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Reed, K., Vandlen, R., Bode, J., Duguid, J. & Raftery, M. A. (1975) *Arch. Biochem. Biophys.* **167,** 138–144.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
21. Thorell, J. I. & Johnsson, B. G. (1971) *Biochim. Biophys. Acta* **251,** 363–369.
22. Schmidt, J. & Raftery, M. A. (1973) *Anal. Biochem.* **52,** 349–354.
23. Sadove, M. S. & Wallace, V. E. (1962) *Halothane* (F. A. Davis, Philadelphia, PA), pp. 58–59.
24. Quast, U., Schimerlik, M., Lee, T., Witzemann, V., Blanchard, S. & Raftery, M. A. (1978) *Biochemistry,* **17,** 2405–2414.
25. Rang, H. P. & Ritter, J. M. (1970) *Mol. Pharmacol.* **6,** 383–390.
26. Rang, H. P. & Ritter, J. M. (1970) *Mol. Pharmacol.* **6,** 357–382.
27. Andreasen, T. J. & McNamee, M. G. (1977) *Biochem. Biophys. Res. Commun.* **79,** 958–965.
28. Barker, J. L. (1975) in *Molecular Mechanisms of Anesthesia,* ed. Fink, B. R. (Raven, New York), pp. 135–153.
29. Larrabee, M. G. & Posternak, J. M. (1952) *J. Neurophysiol.* **15,** 91–114.
30. Davis, H., Collins, S., Randt, W. F. & Dillon, W. H. (1957) *Anesthesiology* **18,** 634–642.
31. Giessen, A. J., Karis, J. M. & Nastuk, W. L. (1966) *J. Am. Med. Assoc.* **197,** 116–120.
32. Waud, B. E. & Waud, D. R. (1975) *Anesthesiology* **43,** 540–547.
33. Waud, B. E. & Waud, D. R. (1975) *Anesthesiology* **42,** 275–280.