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Ultraviolet light-induced labeling by noncompetitive blockers of the acetylcholine receptor from *Torpedo marmorata*

(affinity labeling/ionic channel/receptor polypeptide)

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ABSTRACT **Reversible ligands were attached covalently to membrane-bound acetylcholine receptor from *Torpedo marmorata* by a method which is generally applicable and does not require the synthesis of specially designed molecules. UV irradiation of the receptor in the presence of [^{3}H]trimethisoquin, [^{3}H]phencyclidine, or [^{3}H]perhydrohistrionicotoxin resulted in the labeling of the binding site(s) for these noncompetitive blockers of the permeability response. The labeling of the δ chain was enhanced by carbamoylcholine, and this increase was blocked by snake α-toxins. The effect of carbamoylcholine on [^{3}H]trimethisoquin binding was more pronounced than with the other two noncompetitive blockers; in all instances, the labeling was abolished by unlabeled histrionicotoxin. These three compounds therefore interact with the high-affinity site for noncompetitive blockers. Incorporation of radioactivity also occurred into the α chain but either was insensitive to cholinergic effectors or decreased in the presence of carbamoylcholine (or snake α-toxin), probably as a result of an interaction with the acetylcholine-binding site. In contrast to the other noncompetitive blockers tested, [^{3}H]chlorpromazine heavily labeled the four receptor polypeptides (α, β, γ, δ), and this labeling also was enhanced by carbamoylcholine and decreased by histrionicotoxin. These data indicate a contribution of the δ chain to the binding site(s) of several well-characterized noncompetitive blockers and suggest that other receptor polypeptides may also contribute to this binding.**

Photoaffinity labeling of a specific site on a macromolecule usually requires synthesis of a ligand with a photolabile group (1). With some ligands, such as cyclic nucleotides (2–4), nucleic acids (5, 6), or benzodiazepines (7, 8), simple UV irradiation in the presence of the protein substrate appears sufficient to cross-link covalently the ligand and the protein. Although not necessarily as quantitative (9), this technique provides sufficient selectivity to identify the polypeptide chain that interacts with a radioactive ligand.

The present communication explores the use of UV irradiation to identify the binding site for noncompetitive blockers of the physiological response to acetylcholine (AcCho) on the membrane-bound acetylcholine receptor (AcChoR) from *Torpedo marmorata*. These agents include aminated local anesthetics (10–12), quinacrine (13), histrionicotoxin (HTX) and perhydrohistrionicotoxin (H_{12}-HTX) (ref. 14 and references in ref. 15), phencyclidine (16–18), and various other ligands (reviewed in ref. 19). Several of these compounds may have diverse effects in other systems, but all of them block the electrophysiological response of the motor end plate or the electroplaque to AcCho without changing the apparent affinity for AcCho. *In vitro* studies (reviewed in refs. 19 and 20) have shown that these compounds bind to a site distinct from, but in reciprocal allosteric interaction with, the agonist binding site. Equilibrium binding of radioactive local anesthetics (12), H_{12}-HTX (15), and phencyclidine (17) provided a set of criteria for the characterization of their common binding site. Two of these are: (*i*) their mutually exclusive binding, and (*ii*) their enhanced affinity caused by the binding of agonists to the AcCho site.

The AcChoR of *T. marmorata* electroplaque is composed of four different types of subunits of apparent molecular weights 40,000 (α), 50,000 (β), 60,000 (γ), and 66,000 (δ) (reviewed in refs. 19, 21, 22). The α chain is labeled by affinity reagents specific for the AcCho-binding site (19, 21) and, therefore, carries this site. The δ chain can be labeled with a photoaffinity derivative of a local anesthetic, 5-azido[^{3}H]trimethisoquin (23) and thus is implicated in the binding of noncompetitive agents (24, 25). The role of the β and γ [referred to as the 60,000-dalton subunit (19, 21) but with an apparent molecular weight of 57,000 in most $NaDodSO_4$ gel systems (25)] chains is not known. The four chains are closely packed together, and their accepted stoichiometry is $\alpha_2\,\beta\,\gamma\,\delta$ (19, 21, 22).

We report here the UV-induced labeling of the membrane-bound AcChoR from *T. marmorata* with various reversible noncompetitive blockers of the physiological response to AcCho. These include, in addition to the local anesthetic [^{3}H]-trimethisoquin, the frog toxin derivative [^{3}H]H_{12}-HTX, the hallucinogenic drug [^{3}H]phencyclidine, and the sedative [^{3}H]chlorpromazine. The results support the idea that the δ chain is involved in the binding of all these noncompetitive blockers and suggest the participation of other receptor subunits in the binding of chlorpromazine.

MATERIALS AND METHODS

AcChoR-rich membranes were prepared from *T. marmorata* electric organ, as described (25), in 3 mM EDTA/1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid/0.1 mM phenylmethanesulfonyl fluoride/50 mM Tris·HCl, pH 7.5, containing 5 units of aprotinin and 5 μg of pepstatin per ml (buffer A). The membranes were either used directly, or stored at −70°C until used, at a concentration of 20–25 μM in ^{125}I-labeled α-bungarotoxin (^{125}I-BuTx) sites. The concentration of these sites was determined by DEAE-filter disk assay (26).

$NaDodSO_4$/polyacrylamide gel electrophoresis (Tris glycine) was performed with two different 10% acrylamide systems in 1.1–1.3 mm slabs (25). Gels were stained and fixed in 0.05% Coomassie blue R-250 in 10% ethanol/10% acetic acid and destained in the same solvent. Radioactivity was detected by fluo-

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Abbreviations: AcCho, acetylcholine; AcChoR, acetylcholine receptor; BuTx, α-bungarotoxin; HTX, histrionicotoxin; H_{12}-HTX, perhydrohistrionicotoxin; CbmCho, carbamoylcholine; ID_{50}, dose that decreases the response to 25 μM CbmCho by 50%.

rography (27); dried gels were exposed to Kodak X-Omat R film preflashed to an absorbance of 0.2. Alternatively, selected sections of the dried gel were cut out and assayed (28).

Irradiation with UV light was performed essentially as described (25). Membranes were washed twice in *Torpedo* physiological solution (250 mM NaCl/5 mM KCl/4 mM $CaCl_2$/2 mM $MgCl_2$/5 mM Na phosphate, pH 7.0) by centrifugation (Beckman Airfuge, full speed) and resuspension to a final concentration of 2–5 μM BuTx sites. To 10 μl of the suspension, 10 μl of *Torpedo* physiological solution or nonradioactive pharmacological agent was added followed by 10 μl of the radioactive ligand. The solutions were placed in a chamber, flushed with nitrogen for 15 min, and irradiated for 5 min with a Mineralight short-wave UV lamp (Ultra-Violet Products, San Gabriel, CA) placed outside the chamber at a distance of 15 cm (254 nm; 1200 $\mu W/cm^2$) from the samples. The solutions were separated from the light source by a thin sheet of cellophane which absorbed less than 5% of the light. Immediately after illumination, 30 μl of $NaDodSO_4$ buffer [2% $NaDodSO_4$/5% (vol/vol) 2-mercaptoethanol/0.001% bromphenol blue/10% (vol/vol) glycerol/0.06 M Tris·HCl, pH 6.8] was added and 25-μl samples were loaded on the gel. The samples were not heated prior to loading on the gel. Heating had little effect on the labeling pattern except for a slight degradation of the α chain (28).

The reversible binding of [^{3}H]phencyclidine was measured by incubating receptor-rich membranes (1 μM in BuTx sites) with 0.1 mM carbamoylcholine (CbmCho), the unlabeled noncompetitive blockers, and [^{3}H]phencyclidine in a total volume of 100 μl for 45 min. After the incubation, the solution was pelleted at top speed in an Eppendorf Minifuge for 15 min, the supernatant was aspirated, and the pellet was solubilized in 12% Triton X-100 and assayed in 4 ml of Bray's solution. Background (i.e., the radioactivity in the pellet in the presence of 0.1 mM phencyclidine) constituted approximately 10% of the total radioactivity in the pellet in the absence of unlabeled noncompetitive blockers.

Electroplaques isolated from the Sachs organ of *Electrophorus electricus* was used for the electrophysiological measurements. CbmCho (25 μM) and noncompetitive blockers were applied to the innervated face of the cell, and the transmembrane potential was measured by using an intracellular electrode (29).

Dimethisoquin was a gift of B. Roques, and histrionicotoxin was kindly provided by J. Daly. [^{3}H]Trimethisoquin (6.5 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was synthesized by the methylation of dimethisoquin with [^{3}H]methyliodide; its purity (>99%) was verified by thin-layer chromatography with ethyl acetate/isopropanol/H_2O/formic acid, 2:1:1:0.04 (vol/vol). [^{3}H]H_{12}-HTX (46 Ci/mmol) was synthesized by the reduction of histrionicotoxin with $^3H_2$ (Commissariat à l'Energie Atomique, Saclay) and its purity (>99%) was verified by thin-layer chromatography with chloroform/methanol/ammonium hydroxide, 9:1:0.1 (vol/vol). Unlabeled phencyclidine was a gift of M. Sokolovsky; [^{3}H]phencyclidine (48 Ci/mmol) was provided by E. F. Domino and New England Nuclear; [^{3}H]chlorpromazine (21 Ci/mmol) was from New England Nuclear.

RESULTS

Preliminary Characterization of Relevant Compounds. The pharmacological action of the four ligands used in these studies was first tested on *E. electricus* electroplaque. All of them caused a decrease of the maximal amplitude of the steady-state depolarization caused by CbmCho without significantly affecting the apparent dissociation constant for CbmCho. In other words, they behaved as noncompetitive blockers. The concentrations at which H_{12}-HTX, phencyclidine, trimethisoquin, and chlorpromazine decreased the response to 25 μM CbmCho by 50% (ID_{50}) were 0.26, 1.9, 0.3, and 15 μM, respectively.

The effect of these ligands on the reversible equilibrium binding of one of them, [^{3}H]phencyclidine, to *T. marmorata* receptor-rich membranes was also investigated. In the presence of 0.1 mM CbmCho, [^{3}H]phencyclidine bound to a single population of sites equal to 0.5 the total number of BuTx sites, with a dissociation constant K_d = 0.7 μM (see ref. 19 for a review of the ratio of the number of noncompetitive blocker sites to the number of BuTx sites). In the absence of added ligand, the affinity for [^{3}H]phencyclidine decreased ≈6-fold [K_d = 3.5 μM (unpublished data)]. Under these conditions, H_{12}-HTX, trimethisoquin, and chlorpromazine displaced [^{3}H]phencyclidine bound to its site with K_i of 0.69, 4.4, and 6.0 μM, respectively, in the range (except for trimethisoquin) of the ID_{50} found by following the electrophysiological response of *E. electricus* electroplaque. It is probable that all of them block the electrogenic action of CbmCho by binding to a common site referred to as the site for noncompetitive blockers (10–12, 19, 20).

The inhibition of the electrophysiological response to AcCho by trimethisoquin had an ID_{50} value (0.3 μM), much lower than the K_i of the binding of [^{3}H]phencyclidine to the *Torpedo* receptor (4.4 μM). Results similar to those found with [^{3}H]phencyclidine were observed for trimethisoquin in *Torpedo* receptor-rich membranes by stopped-flow kinetic techniques [2.2 μM (30)] and by the direct binding of [^{3}H]trimethisoquin [1 μM (28)]. One possibility for the discrepancy is a species difference between *Torpedo* and *Electrophorus*.

Principle of the Method. In the course of labeling studies with 5-azido[^{3}H]trimethisoquin, an experiment was performed in which the reversible analog of the photoaffinity label, [^{3}H]trimethisoquin, was UV irradiated in the presence of AcChoR-rich membranes. A labeling of the receptor polypeptides was found that persisted after denaturation by 2% $NaDodSO_4$/5% 2-mercaptoethanol at 100°C and denaturing gel electrophoresis in $NaDodSO_4$. In the absence of UV illumination, the radioactivity migrated with or faster than the tracking dye and presumably corresponded to unbound reagent. Irradiation of either the membranes or of [^{3}H]trimethisoquin alone followed by the addition, within 5 sec after the termination of illumination, of the omitted component did not give rise to any labeling. Also, UV irradiation of either the membranes or [^{3}H]trimethisoquin alone had no effect on any subsequent UV-catalyzed labeling with the same sample. The labeling thus required the presence of both [^{3}H]trimethisoquin and membranes during UV irradiation. Further investigation revealed that the method could be extended to the noncompetitive blockers [^{3}H]phencyclidine, [^{3}H]H_{12}-HTX, and [^{3}H]chlorpromazine. [^{14}C]Lidocaine and [^{3}H]tetraethylammonium ion did not give any significant labeling under the same conditions.

The selectivity of the labeling of the AcChoR peptides was tested with *T. marmorata* receptor-rich membranes both in the presence and in the absence of 0.1 mM CbmCho under four conditions: without further addition, or in the presence of 1 μM HTX, 50 μM HTX, or 10 μM erabutoxin b. The labeled membranes were then subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis in one dimension and the radioactivity was detected either by fluorography or by direct assay of slices of the dried gel corresponding to individual receptor subunits.

Trimethisoquin. The labeling by [^{3}H]trimethisoquin (Fig. 1) was quite similar to that observed with 5-azido[^{3}H]trimethisoquin (24, 25), although with a lower yield; at 100 μM or higher, [^{3}H]trimethisoquin labeled less than half of the number of sites labeled by a saturating concentration (>100 μM) of 5-azidotrimethisoquin. The most remarkable labeling pattern was that of the δ chain. In the presence of 14 μM [^{3}H]trimethisoquin the

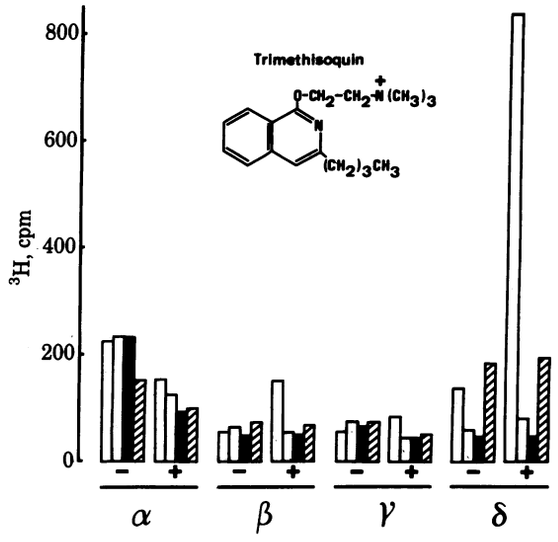

FIG. 1. Incorporation of [^{3}H]trimethisoquin (14 μM) into AcChoR subunits by irradiation for 5 min with 254-nm UV light. Sections of the gel corresponding to the α, β, γ, and δ subunits were cut out, solubilized, and assayed. The four bars in each set represent the following conditions used both in the presence (+) and in the absence (−) of 0.1 mM CbmCho: no further additions; 1 μM HTX; 50 μM HTX; 10 μM erabutoxin b. HTX was incubated with the receptor-rich membranes in the presence or absence of CbmCho for 5 min before the addition of [^{3}H]trimethisoquin. Erabutoxin b was incubated with the membranes for 10 min before the addition of CbmCho followed by an additional 5-min incubation before the addition of [^{3}H]trimethisoquin.

radioactivity incorporated into the δ chain increased 6-fold in the presence of 0.1 mM CbmCho but decreased to background levels in the presence of 1 μM and 50 μM HTX. Erabutoxin b, a selective label of the AcChoR site, did not affect the labeling of the δ chain in the absence of CbmCho at the [^{3}H]trimethisoquin concentration in Fig. 1 but abolished the increased labeling caused by CbmCho most likely by blocking its effect at the level of the AcCho binding site. When the incorporation of label into the δ chain was studied as a function of [^{3}H]trimethisoquin concentration, the same number of sites were labeled in the presence as in the absence of CbmCho, but the apparent affinity for [^{3}H]trimethisoquin was higher in the presence of CbmCho. Thus, the increased labeling in the presence of CbmCho reflects an increased affinity for [^{3}H]trimethisoquin rather than an increase in the number of available sites. A dose–response curve in the presence of erabutoxin b revealed an apparent affinity lower than that observed in the absence of CbmCho. A similar effect was observed with 5-azido[^{3}H]trimethisoquin (data not shown).

Dimethisoquin, dibucaine, chlorpromazine, tetracaine, and procaine blocked the incorporation of radioactivity into the δ chain (data not shown) in the same range of concentration as they blocked the electrophysiological response of *E. electricus* electroplaque. The covalent labeling of the δ chain by [^{3}H]trimethisoquin, therefore, has the same characteristics as the reversible equilibrium binding of local anesthetics to their high-affinity site (12, 28).

The radioactivity recovered at the level of the β chain (in the presence of CbmCho) did not belong to this chain but to a proteolytic fragment of the δ chain, the 50 *bis* fragment (25), which could be separated from the β chain in a different gel system. The labeling of the α chain was not affected by HTX but was decreased in the presence of erabutoxin b or CbmCho, probably resulting from the interaction of [^{3}H]trimethisoquin with the AcChoR site.

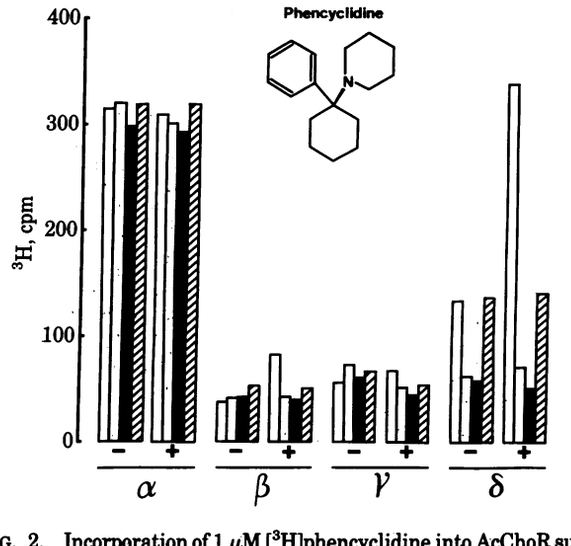

FIG. 2. Incorporation of 1 μM [^{3}H]phencyclidine into AcChoR subunits. Conditions as for Fig. 1 except that 1 μM [^{3}H]phencyclidine replaced 14 μM [^{3}H]trimethisoquin.

Phencyclidine and H_{12}-HTX. [^{3}H]Phencyclidine and [^{3}H]H_{12}-HTX have similar structures (16) and gave rather similar labeling patterns (Figs. 2 and 3). Both labeled the δ chain and, as in the case of [^{3}H]trimethisoquin, this labeling was enhanced by CbmCho and abolished by 1 and 50 μM HTX.

In the case of [^{3}H]phencyclidine (Fig. 2), a nearly 3-fold increase in labeling of the δ chain was observed in the presence of CbmCho. The increase in labeling with 1 μM [^{3}H]phencyclidine was exactly the same as the increase in reversible binding of 1 μM [^{3}H]phencyclidine observed in the presence, compared to the absence, of CbmCho. A significant incorporation of radioactivity into the α chain also occurred. In the case of [^{3}H]phencyclidine this labeling was unaffected by the presence of CbmCho, erabutoxin b, or HTX and therefore did not result from an interaction with the AcCho site or with the high-affinity site for noncompetitive blockers. With [^{3}H]H_{12}-HTX (Fig. 3), a slight decrease of the α-chain labeling was observed in the presence of 50 μM HTX or 0.1 mM CbmCho but was not studied quantitatively.

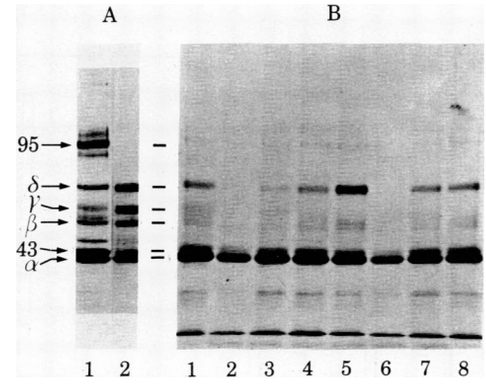

FIG. 3. (*A*) Coomassie blue stain of a 10% acrylamide/NaDodSO$_4$ gel. Lanes: 1, AcChoR-rich membranes; 2, sucrose gradient-purified AcChoR (24). (*B*) Labeling of AcChoR-rich membranes with 5.7 μM [^{3}H]H_{12}-HTX. Shown here is a fluorogram of a 10% acrylamide/NaDodSO$_4$ gel exposed for 11 days to Kodak X-Omat film preflashed to an optical density of 0.2. Same conditions as for Fig. 1. Lanes: 1, no CbmCho, no inhibitor; 2, no CbmCho, 50 μM HTX; 3, no CbmCho, 1 μM HTX; 4, no CbmCho, 10 μM erabutoxin b; 5, 0.1 mM CbmCho, no inhibitor; 6, 0.1 mM CbmCho, 50 μM HTX; 7, 0.1 mM CbmCho, 1 μM HTX; 8, 0.1 mM CbmCho, 10 μM erabutoxin b.

Chlorpromazine. The labeling pattern observed in the presence of 0.5 μM [^{3}H]chlorpromazine (Fig. 4) strikingly differed from that found with the other noncompetitive blockers. Significant radioactivity was recovered in all four receptor subunits (α, β, γ, and δ), and 0.1 mM CbmCho enhanced the labeling of the four chains. For each chain, incorporation of [^{3}H]chlorpromazine was blocked by 1 and 50 μM HTX and the potentiating effect of CbmCho was prevented by preincubation with erabutoxin b. Under the experimental conditions used, CbmCho enhanced the labeling of the four chains to different extents (α, 1.6-fold; β, 3.5-fold; γ, 1.9-fold; δ, 5-fold); a contribution of the 50 *bis* fragment (25) to the radioactivity recovered at the level of the β-chain band is plausible but cannot exceed 20%. HTX (1 or 50 μM) decreased the incorporation of [^{3}H]chlorpromazine into the four chains but to a larger extent (95%) at the level of the δ chain. In the presence of CbmCho, a minor but significant HTX-sensitive labeling of three bands of apparent molecular weight greater than 100,000 was noticed (Fig. 5). Because no precursors of the receptor peptides of such size are yet known, these bands might result from the crosslinking of receptor peptides by chlorpromazine.

A low concentration of [^{3}H]chlorpromazine was used in Figs. 4 and 5 (0.5 μM) because the high reactivity of the compound resulted in a high level of nonspecific labeling at higher concentrations. Nevertheless, at a concentration of 15 μM (ID_{50} of chlorpromazine for inhibition of the response to AcCho on the eel electroplaque), CbmCho enhanced and HTX inhibited the labeling of all four receptor subunits. This pattern was superimposed on a background of nonspecific (i.e., unaffected by CbmCho or HTX) labeling.

Labeling of Nonreceptor Peptides. With all ligands tested, faint but systematic labeling of two nonreceptor peptides was noticed. One was the 43,000-dalton peripheral polypeptide (31) bound to the cytoplasmic face (32) of the receptor-rich membranes; the other, ≈95,000 daltons, is known to belong to different membrane fragments (19, 21) and might be one chain of Na^+,K^+-ATPase. CbmCho, erabutoxin b, or HTX did not affect the labeling of these chains, which were thus taken as controls. In the case of [^{3}H]H_{12}-HTX, a significant decrease of the labeling of the 43,000-dalton polypeptide occurred in the presence of 50 μM HTX (see ref. 33), but the incorporation of radioactivity into this chain was not enhanced by CbmCho.

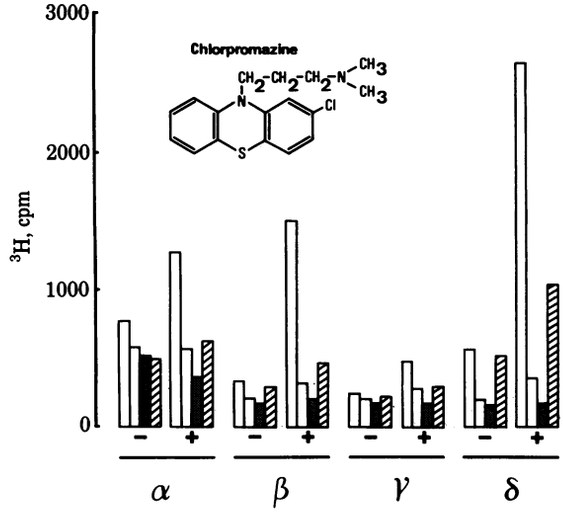

FIG. 4. Incorporation of 0.5 μM [^{3}H]chlorpromazine into AcChoR subunits. Conditions as for Fig. 1 except that 0.5 μM [^{3}H]chlorpromazine replaced 14 μM [^{3}H]trimethisoquin.

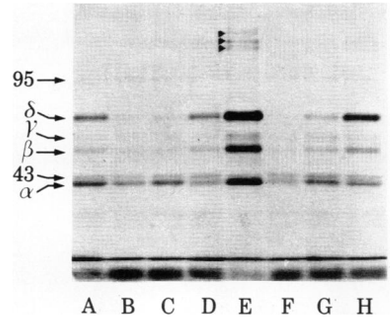

FIG. 5. Fluorogram of a 10% acrylamide/$NaDodSO_4$ gel run with the same samples as in Fig. 4. Arrowheads, receptor subunits crosslinked by chlorpromazine. Lanes: A, no CbmCho, no inhibitors; B, no CbmCho, 50 μM HTX; C, no CbmCho, 1 μM HTX; D, no CbmCho, 10 μM erabutoxin b; E, 0.1 mM CbmCho, no inhibitors; F, 0.1 mM CbmCho, 50 μM HTX; G, 0.1 mM CbmCho, 1 μM HTX; H, 0.1 mM CbmCho, 10 μM erabutoxin b.

DISCUSSION

Compared with the traditional method of designing ligands with photolabile groups, the UV-induced labeling of the AcChoR polypeptides by noncompetitive blockers offers the advantages of simplicity and potentially increased selectivity. The structural features of the ligand molecule required for its bonding to an amino acid side chain of the labeled polypeptide have not yet been identified, although all the radioactive ligands that gave successful labeling had a cyclic carbon ring. The substitutions on the ring may be important, judging from the fact that lidocaine does not become covalently attached even though it has a cyclic carbon ring. A disadvantage of the method is that the efficiency of labeling appeared lower than that found with standard photoaffinity ligands. At this stage of the work, such low yields preclude direct estimation of site stoichiometries.

The most striking result obtained with the noncompetitive blockers used in this study is that all of them labeled the δ chain of the AcChoR and this labeling was blocked by HTX and potentiated by CbmCho. Similar results have been reported with 5-azido[^{3}H]trimethisoquin (24, 25). They are consistent with the interpretation that the high-affinity site for noncompetitive blockers is at least partially carried by, or in the vicinity of, the δ chain. Significant labeling of the α chain also occurred with several of the compounds tested. In the case of trimethisoquin, the labeling was decreased by CbmCho and erabutoxin b. Therefore, it occurred at the AcChoR site, a result consistent with the presence of a quaternary nitrogen in its molecule (see ref. 10 for discussion). In the case of phencyclidine, the labeling of the α chain was unaffected by HTX, CbmCho, and erabutoxin b, thus reflecting unidentified sites distinct from the agonist and noncompetitive blocker binding sites.

Labeling by [^{3}H]chlorpromazine was quite different from labeling with the other ligands tested. The four chains of the AcChoR, including the α chain, incorporated radioactivity with the characteristics of the labeling of the δ chain by the other ligands. In particular, this incorporation was potentiated by CbmCho and blocked by HTX. Although the concentration used in the present study (0.5 μM) was somewhat lower than the ID_{50} value (15 μM) for the inhibition of the electrophysiological response to AcCho, the same general characteristics of the labeling pattern (enhancement by CbmCho; inhibition by HTX) were observed at higher concentrations. At least two alternative models may account for the paradoxical labeling of all four chains. Each of them carries a distinct site for [^{3}H]chlorpromazine and the binding of this ligand is regulated

by a common molecular transition elicited by the agonist. The four chains contribute to a single common binding site, such as a crevice through the central part of the 250,000-dalton oligomer, [which could be analogous to the phosphoglycerate site in hemoglobin (34)], and the agonists regulate the interaction of [^{3}H]chlorpromazine with this site in an allosteric manner. Determination of the precise stoichiometry of [^{3}H]chlorpromazine and of trimethisoquin or phencyclidine binding sites should lead to an unambiguous distinction between the two models. The second model is consistent with the known pictures of the AcChoR molecule disclosed by electron microscopy after negative staining (35); as in the case of the gap junction connections (36), the ion channel could correspond to the hydrophilic pit observed in the center of the multisubunit receptor rosettes (35).

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