

ACCELERATED COMMUNICATION

Selective Enhancement of the Interaction of Curare with the Nicotinic Acetylcholine Receptor

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Received April 5, 1993; Accepted May 18, 1993

SUMMARY

Alteration of the ligand-binding domain of the nicotinic acetylcholine receptor through site-directed mutagenesis offers a powerful approach to the elucidation of structure-function relations in the receptor. Several conserved tyrosine residues in the large extracellular amino terminus of the α subunit of the receptor have been implicated by both chemical labeling and mutagenesis studies as playing an important role in the interaction of acetylcholine with the receptor. We and others have previously shown that substitution of phenylalanine for tyrosine at position 198 of the α subunit (α Y198F) leads to a rightward shift in the dose-response curve for acetylcholine-elicited currents. We have further investigated this particular mutation by examining the inter-

action of the competitive antagonist *d*-tubocurarine (curare) with the receptor. In contrast to the effect on the interaction of agonists with the receptor, this mutation leads to a marked increase in the affinity of the receptor for curare. Furthermore, this enhancement in affinity is selective for curare and is not seen with other competitive antagonists (pancuronium, β -erythroidine, and gallamine). Examination of the structures of these competitive antagonists leads to the proposal that this enhancement is due to the formation of an aromatic-aromatic interaction between the phenylalanine ring at position α 198 in the mutant and one of the aromatic rings of curare and that this can provide information about the spatial arrangement of this residue in the binding site.

The nicotinic AChR is a transmembrane protein composed of four different subunits in the stoichiometry $\alpha_2\beta\gamma\delta$ (for recent reviews, see Refs. 1 and 2). The binding of two agonist molecules such as ACh to the receptor induces a conformational change that results in the formation of a cation-selective ion channel. Competitive antagonists such as *d*-tubocurarine (curare) can also bind to the agonist binding site; however, they are unable to elicit this conformational change. [Note, however, that under some conditions curare can open channels in embryonic muscle (3, 4).] In the context of the Monod-Wyman-Changeux model for cooperativity (5), one can consider that the agonist binds more tightly to the "open" configuration of the binding site and thus can drive the equilibrium towards that state, whereas an antagonist binds more tightly to the "closed" conformation and thus biases the equilibrium toward the closed state.

A number of elegant protein chemistry studies have localized at least a portion of the ligand binding site to a region between residues 190 and 200 of the α subunit (6-14). We and others have applied the technique of site-directed mutagenesis to examine this region, to better define the interactions between

ACh and the receptor and to understand the process of ligand-activated channel opening (15-18). To extend these studies, we have begun to examine the effects of various mutations on the interaction of antagonists with the ligand-binding domain, with the goal of defining structural components that are important for antagonist-binding site interaction. In this communication, we report that substitution of a phenylalanine for tyrosine at position 198 in the α subunit (α Y198F) of the mouse AChR, which decreases the apparent affinity for ACh of both *Torpedo* (17) and mouse (18) receptors, markedly increases the affinity for curare and this increase in affinity is seen only for curare and not for three other competitive antagonists.

Materials and Methods

Plasmids and site-directed mutagenesis. Mouse muscle AChR subunit cDNAs were obtained from Drs. J. P. Merlie (Washington University) (α subunit), N. Davidson (California Institute of Technology) (β and δ subunits), and S. Heinemann (Salk Institute) (γ subunit). The α subunit was subcloned into the pALTER-1 vector (Promega, Madison, WI).

Mutagenesis of the α subunit was carried out using the commercially available Altered Sites system (Promega). The mutagenic primer was 21 nucleotides long and was synthesized using an Applied Biosystems model 391 oligonucleotide synthesizer. The mutation was confirmed by sequence analysis through the entire coding region using the Sanger

This work was supported by National Institutes of Health Grant NS23885 awarded to M.M.W. M.M.W. is an Established Investigator of the American Heart Association.

deoxy termination method (Sequenase; United States Biochemicals, Cleveland, OH), to verify that only the desired nucleotide changes were present.

In vitro transcription and expression in *Xenopus* oocytes. Plasmid DNAs were linearized with the appropriate restriction enzymes and transcribed *in vitro* using SP6 RNA polymerase, as described previously (19). Oocytes were harvested from mature female *Xenopus laevis* (*Xenopus* One, Ann Arbor, MI) and the adhering follicle cell layer was removed using collagenase (type IA; Sigma). Isolated, follicle-free oocytes were maintained in SOS (100 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.6) supplemented with 2.5 mM sodium pyruvate and 50 µg/ml gentamicin. AChR subunit RNAs were mixed in a molar stoichiometry of 2:1:1:1 α : β : γ : δ , and 8–15 ng were injected into the oocyte cytoplasm. Oocytes were maintained in standard oocyte saline supplemented with pyruvate and gentamicin at 19° for 18–72 hr before recording.

Electrophysiology. Currents elicited by bath application of various concentrations of ACh (with or without antagonist) were measured using a standard two-microelectrode voltage clamp [either a Gene-Clamp 500 (Axon Instruments, Foster City, CA) or an OC-725 (Warner Instruments, Hamden, CT)] at a holding potential of –60 mV. Electrodes were filled with 3 M KCl and had resistances of 0.5–3 M Ω . The recording chamber was continuously perfused with a low-Ca²⁺ saline (100 mM NaCl, 2 mM KCl, 0.1 mM CaCl₂, 5 mM MgCl₂, 10 mM HEPES, pH 7.6) containing 0.3 µM atropine to block any residual muscarinic AChRs that remained after removal of the follicle cells. The low-Ca²⁺ saline was used to reduce receptor desensitization, which requires external Ca²⁺ (20). ACh blocks AChR channels in a voltage-dependent fashion, with a zero-voltage dissociation constant on the order of 5 mM (21). Such a block would lead to a reduction in the amplitude of macroscopic (and single-channel) currents at high ACh concentrations and negative membrane potentials. At –60 mV this block does not become significant until the ACh concentration exceeds 2 mM; therefore, in this study, ACh concentrations were limited to \leq 1 mM.

Data analysis. Inhibition curves were determined by measurement of the currents elicited by ACh in the presence of varying concentrations of the selected antagonist. Oocytes were continuously perfused with low-Ca²⁺ saline and then the perfusate was switched to one containing a fixed concentration of ACh with a selected concentration of antagonist for 1 min, before being switched back to the ACh- and antagonist-free saline. Applications of antagonist-containing saline were bracketed by ones containing ACh only, to reliably measure the percentage of inhibition. The data were fit to a single-site inhibition curve (eq. 1) using a Levenberg-Marquart algorithm in a commercially available Apple Macintosh-based analysis program (IGOR; Wave-Metrics, Lake Oswego, OR):

$$\theta = (I(X)/I(0)) = [1 + (X/IC_{50})]^n \quad (1)$$

where θ is the fractional current remaining in the presence of the antagonist at concentration [X] and IC₅₀ is the inhibitor concentration required to give 50% inhibition. The inhibition constant, K_i, was obtained by measuring the IC₅₀ at several concentrations of ACh and extrapolating the data to [ACh] = 0.

For receptor activation, dose-response data were fit to eq. 2, as described previously (17):

$$\Phi = I/I_{\max} = [1 + (K_{1/2}/[A])^n]^{-1} \quad (2)$$

where I and I_{\max} are the currents at a given concentration of ACh and the maximal value, respectively, K_{1/2} is the concentration of agonist required to obtain half-maximal current, and n is the apparent Hill coefficient.

Chemicals. ACh chloride, *d*-tubocurarine chloride, pancuronium bromide, and gallamine triethiodide were obtained from Sigma. β -Erythroidine was the generous gift of Dr. G. B. Koelle (University of Pennsylvania).

Results

Fig. 1A shows that substitution of phenylalanine for tyrosine at position 198 of the α subunit (α Y198F) of the mouse muscle AChR results in a shift in the dose-response curve for currents evoked by bath application of ACh towards higher concentrations. As has been previously reported for both the mouse muscle (18) and *Torpedo* (17) AChRs expressed in *Xenopus* oocytes, this mutation results in an approximately 6-fold increase in the concentration of ACh needed to elicit half-maximal currents (K_{1/2}), with no effect on the apparent Hill coefficient. In the data presented here, K_{1/2} was changed from 19.3 \pm 0.6 µM to 88.1 \pm 2.9 µM for the wild-type and mutant receptors, respectively. This decrease in apparent affinity has been ascribed to either a decrease in agonist affinity (18) or a decrease in the coupling of agonist binding to channel opening (17). In either case, it is clear that this mutation leads to an alteration in the interaction of ACh with the receptor.

As an alternate probe of the structure of the ligand-binding domain, we have investigated the effect of this mutation on the inhibition of ACh-elicited currents by the competitive antagonist *d*-tubocurarine (curare). Fig. 1B shows that, in contrast to the effect of the mutation on agonist action at the receptor, the α Y198F receptor shows a marked increase in affinity for curare, with the IC₅₀ value changing from 20 nM for the wild-type to 0.5 nM for the mutant receptor. Measurement of the IC₅₀ values at various concentrations of ACh and extrapolation to zero agonist concentration to estimate K_i, the true affinity (see Fig.

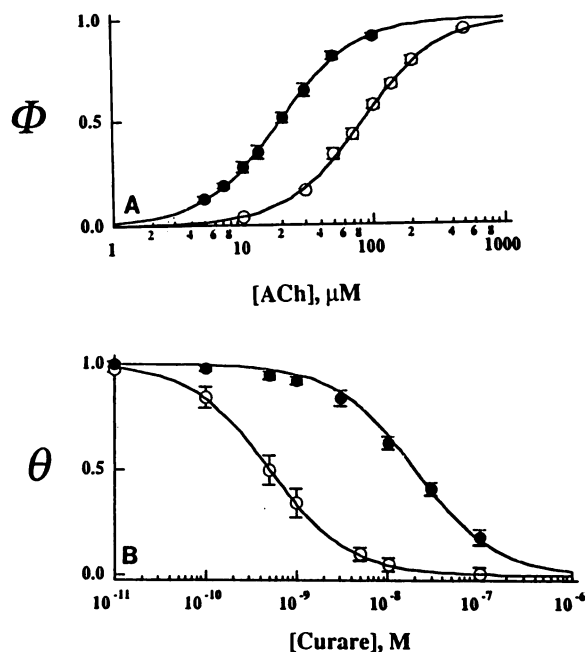


Fig. 1. Phenylalanine substitution has opposite effects on agonist and antagonist dose-response curves. A, ACh dose-response curves were determined for wild-type (●) and α Y198F (○) receptors as described in the text. Each point represents the mean \pm standard error of five to seven determinations. The solid curves are fits to eq. 2, with K_{1/2} values of 19.3 and 88 µM and apparent Hill coefficients of 1.49 and 1.47 for wild-type and α Y198F receptors, respectively. B, Curare inhibition curves were determined for wild-type (●) and α Y198F (○) receptors at ACh concentrations of 2 and 20 µM for wild-type and mutant receptors, respectively. Each point represents the mean \pm standard error of three to seven determinations. The solid curves represent fits to eq. 1, with IC₅₀ values of 20 nM and 0.5 nM for wild-type and α Y198F receptors, respectively.

2 for an example), provides values of 14.2 ± 1.1 nM for the wild-type and 0.1 ± 0.03 nM for the mutant receptor, a >100-fold increase in affinity.

As a first step towards an understanding of the structural features involved in this enhancement of affinity, we have examined several other antagonists of varying structure, i.e., pancuronium, gallamine, and β -erythroidine (Fig. 3). Each of these compounds is a competitive antagonist, albeit of different affinities. Fig. 4 and Table 1 compare the potency of all four antagonists as inhibitors of wild-type and α Y198F receptors.

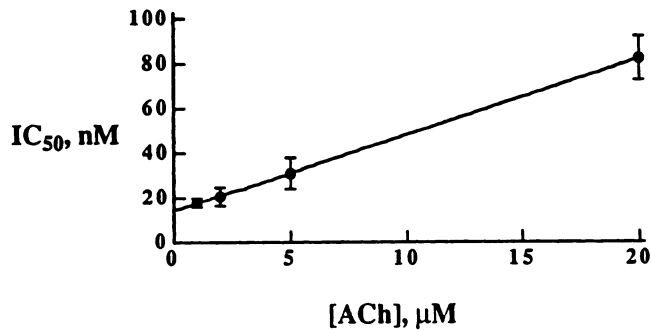


Fig. 2. Determination of the K_i for curare with wild-type receptors. IC_{50} values were determined at various ACh concentrations as described in the text, and the K_i value was estimated by extrapolation to zero ACh concentration by linear regression. Each point represents the mean \pm standard error of three to seven determinations. The value of K_i obtained from this plot is 14 nM.

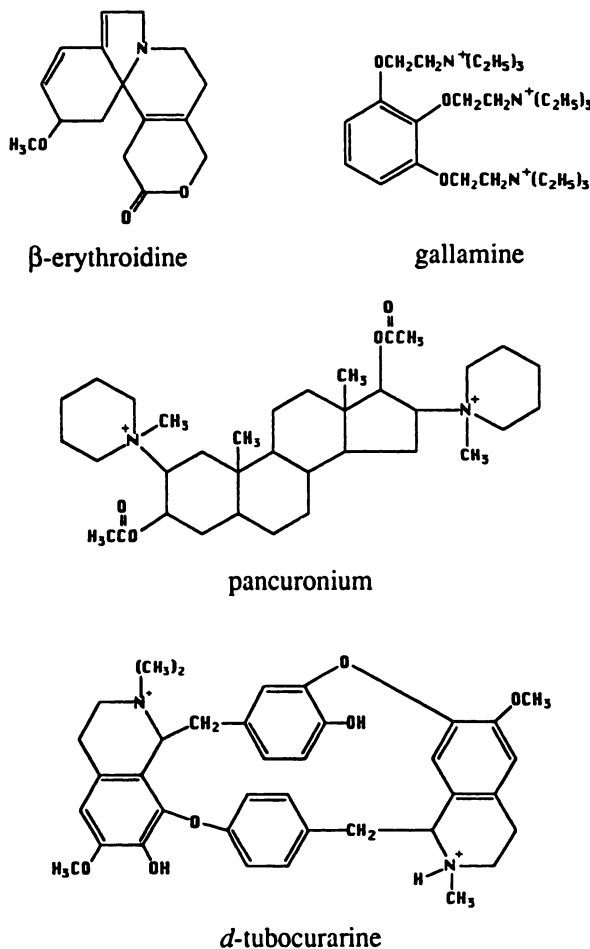


Fig. 3. Structures of antagonists used in this study.

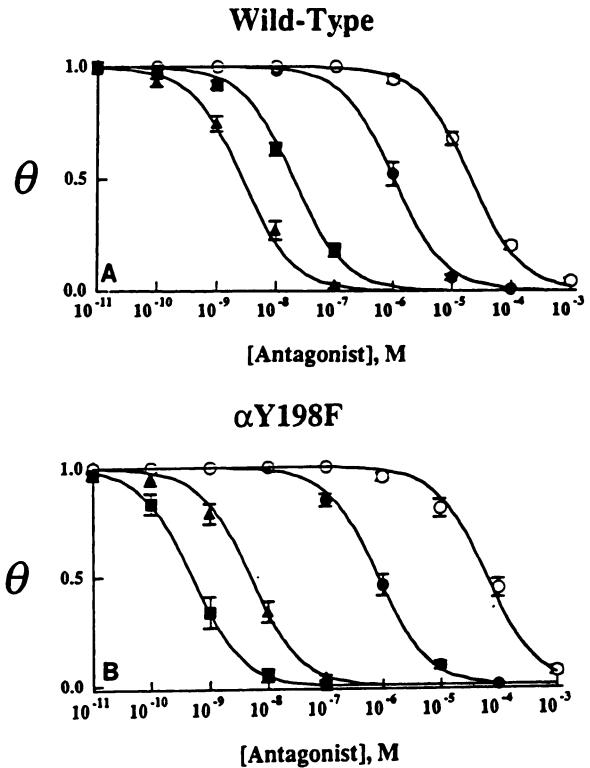


Fig. 4. Inhibition curves were determined for wild-type (A) and α Y198F (B) receptors, at ACh concentrations of 2 and 20 μ M for wild-type and mutant receptors, respectively, with pancuronium (Δ), curare (\blacksquare), gallamine (\circ), and β -erythroidine (\circ). Each point represents the mean \pm standard error of three to seven determinations. The solid curves represent fits to eq. 1 with the following IC_{50} values: wild-type: pancuronium, 3 nM; curare, 20 nM; gallamine, 900 nM; β -erythroidine, 20 μ M; α Y198F: pancuronium, 5 nM; curare, 0.5 nM; gallamine, 700 nM; β -erythroidine, 60 μ M.

TABLE 1

Comparison of K_i values for wild-type and mutant receptors

IC_{50} values were obtained at several ACh concentrations for each antagonist, and the K_i was estimated by extrapolation to $[ACh] = 0$. Each determination represents the mean \pm standard error of three to seven determinations.

| Antagonist | K_i | | Ratio (α Y198F/wild-type) |
|-----------------------|--------------------|--------------------|--------------------------------------|
| | Wild-type | α Y198F | |
| | nM | | |
| d-Tubocurarine | 14.2 ± 5.0 | 0.1 ± 0.03 | 0.01 |
| Pancuronium | 0.9 ± 0.3 | 1.5 ± 0.4 | 1.6 |
| Gallamine | 390 ± 70 | 340 ± 40 | 0.9 |
| β -Erythroidine | $10,700 \pm 1,600$ | $51,300 \pm 6,300$ | 4.8 |

The rank order of antagonist potency for wild-type receptors is pancuronium > curare > gallamine > β -erythroidine. For the mutant receptor, the positions of pancuronium and curare are reversed, with curare being the most potent. With the exception of curare, all antagonists examined show either no change (gallamine) or a decrease (pancuronium and β -erythroidine) in affinity, relative to that seen for wild-type receptors. Therefore, the effect of this mutation is to promote an interaction that selectively increases the affinity of curare for the receptor.

Discussion

Alterations in receptor structure by site-directed mutagenesis have provided information concerning the structural determinants of ligand-receptor interactions. In the case of ligand-

gated channels, several groups have used site-directed mutagenesis to identify residues important for agonist and antagonist binding and/or channel activation for γ -aminobutyric acid type A receptors (22–24), glycine receptors (25–27), and nicotinic AChRs (15, 17, 18). In this study we have used mutagenesis coupled with variation of the structure of the ligand to show that alteration of the structures of both the ligand-binding domain of a receptor and the ligand itself can prove useful in the elucidation of structural details of the geometry of the binding site that cannot be easily obtained by alteration of only one of the components, much as been done for adrenergic receptors (28, 29). In addition, the examination of antagonist interactions allows one to examine directly the affinity of a ligand for the binding site, as opposed to studies using agonists, with the uncertainty regarding the mechanism that underlies alterations in apparent affinities determined from agonist dose-response curves (i.e., binding versus coupling) (17).

The marked increase in curare affinity produced by substitution of phenylalanine for tyrosine at position α 198 in the mouse muscle AChR may provide some insight into the spatial arrangement of the residues in the binding domain. Of the four antagonists examined here, pancuronium and curare have much higher affinities for the receptor than do gallamine and β -erythroidine. Unlike the two lower affinity antagonists, curare and pancuronium have two charged nitrogens separated by a network of either aromatic (curare) or saturated (pancuronium) rings. It is likely that these features are responsible for the higher affinities of these two antagonists for the binding site, relative to those of gallamine and β -erythroidine. Whereas curare and pancuronium are both high affinity antagonists, only curare shows enhancement of affinity when tyrosine is replaced by phenylalanine at position α 198. We propose that this enhancement is due to the creation of an additional interaction between one of the aromatic rings of curare and the phenylalanine at position 198. Edge-to-face aromatic-aromatic interactions between amino acid residues in proteins and between aromatic amino acids and aromatic regions of ligands can provide on the order of 1–2.5 kcal/mol of stabilizing energy (30, 31), which is sufficient to account for the enhanced affinity for curare seen with the mutant receptor. In a statistical survey of protein structures, Burley and Petsko (30) noted that phenylalanine was much more likely to make this type of interaction than tyrosine, which would be consistent with our hypothesis that phenylalanine, but not tyrosine, at position α 198 provides this extra binding energy in the AChR.

Karlin and co-workers (11, 12, 32) proposed that the charged nitrogen of agonists and antagonists is located within 9 Å (agonists) or 12 Å (antagonists) of an easily reducible disulfide, now known to be formed between residues α C192 and α C193. Our data provide additional information on the spatial arrangement of residues in the ligand binding site. Our data suggest that one of the aromatic rings of curare is positioned at α Y198 (phenylalanine in the mutant) in the antagonist-bound receptor. Examination of the crystal structure of *O,O',N*-trimethyl-*d*-tubocurarine (33) shows that the centers of the rings are either 3.8–5.2 Å or 6.8–9.9 Å from a given nitrogen. The lesser value refers to the two rings nearest the nitrogen, whereas the greater value refers to the two rings away from the nitrogen. Due to the relative symmetry of the curare molecule, the ranges hold for either of the nitrogens in the molecule, because two rings are near a given nitrogen and the other two are more

distant. The nitrogen under consideration defines which rings are considered “near” or “distant.” The single aromatic ring of gallamine, a lower affinity antagonist that is unaffected by the mutation, is located about 6–7 Å from the nitrogens, which is approximately between the positions of the two pairs of rings in curare and pancuronium, relative to the nitrogens, and therefore unfortunately sheds no light on which pair may be the important one for the increase in affinity.

Based on conformational studies of a variety of agonists and antagonists, Beers and Reich (34) hypothesized that nicotinic ligands contain a hydrogen bond acceptor located about 5.9 Å from the nitrogen. Curare contains several ether oxygens about this distance from the nitrogens that have been postulated to function as this hydrogen bond acceptor (33); likewise, pancuronium contains ester groups that could play a similar role. Our data suggest that, if this type of interaction takes place in the binding of antagonists such as curare or pancuronium, it is unlikely that α Y198 is the hydrogen bond donor. Because curare and pancuronium show no decrease in affinity with the mutant receptor, this postulated hydrogen bond is still present in the α Y198F receptor, in which phenylalanine at position α 198 cannot serve as the donor.

Our data do provide some spatial constraints on the geometry of the binding site, which, in conjunction with other data obtained from previous and future experiments involving site-directed mutagenesis or affinity labeling, should provide a better picture of ligand-receptor interactions and potentially some insight into the mechanism of agonist and antagonist actions at the AChR.

Acknowledgments

We thank R. Anand, H. Juul, S. Garber, and J. Russell for helpful comments and J. Tanaka for assistance with the analysis of the crystal structures of the antagonists.

References

- Changeux, J.-P., J.-L. Galzi, A. Devillers-Thiery, and D. Bertrand. The functional architecture of the acetylcholine receptor explored by affinity labelling and site-directed mutagenesis. *Q. Rev. Biophys.* **25**:395–432 (1993).
- Karlin, A. Explorations of the nicotinic acetylcholine receptor. *Harvey Lect.* **85**:71–107 (1991).
- Trautmann, A. Curare can open and block ionic channels associated with cholinergic receptors. *Nature (Lond.)* **298**:282–285 (1982).
- Ziskind, L., and M. J. Dennis. Depolarising effect of curare on embryonic rat muscles. *Nature (Lond.)* **276**:622–623 (1978).
- Monod, J., J. Wyman, and J.-P. Changeux. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12**:88–118 (1965).
- Abramson, S. N., Y. Pi, P. Culver, and P. Taylor. An analog of lophotoxin reacts covalently with Tyr¹⁹⁰ in the α subunit of the nicotinic acetylcholine receptor. *J. Biol. Chem.* **264**:12666–12672 (1989).
- Cohen, J. B., S. D. Sharp, and W. S. Liu. Structure of the agonist-binding site of the nicotinic acetylcholine receptor: [³H]acetylcholine mustard identifies residues in the cation-binding site. *J. Biol. Chem.* **266**:23354–23364 (1991).
- Czajkowski, C., and A. Karlin. Agonist binding site of *Torpedo* electric tissue nicotinic acetylcholine receptor: a negatively charged region of the δ subunit within 0.9 nm of the α subunit binding site disulfide. *J. Biol. Chem.* **266**:22603–22612 (1991).
- Dennis, M., J. Giraudat, F. Kotzyba-Hibert, M. Goeldner, C. Hirth, J.-Y. Chang, C. Lazure, M. Chretien, and J.-P. Changeux. Amino acids of the *Torpedo marmorata* acetylcholine receptor α subunit labeled by a photoaffinity ligand for the acetylcholine binding site. *Biochemistry* **27**:2346–2357 (1988).
- Galzi, J.-L., D. Revah, D. Black, M. Goeldner, C. Hirth, and J.-P. Changeux. Identification of a novel amino acid tyrosine 93 within the cholinergic ligand-binding sites of the acetylcholine receptor by photoaffinity labeling. *J. Biol. Chem.* **265**:10430–10437 (1990).
- Kao, P. N., A. J. Dwork, R.-R. J. Kaldany, M. L. Silver, J. Wideman, S. Stein, and A. Karlin. Identification of α subunit half-cystine specifically labelled by an affinity reagent for the acetylcholine receptor binding site. *J. Biol. Chem.* **259**:11662–11665 (1984).
- Kao, P. N., and A. Karlin. Acetylcholine receptor binding site contains a disulfide cross-link between adjacent half-cystinyl residues. *J. Biol. Chem.* **261**:8085–8088 (1986).

13. Pedersen, S. E., E. B. Dreyer, and J. B. Cohen. Location of ligand-binding sites on the nicotinic acetylcholine receptor α -subunit. *J. Biol. Chem.* **261**:13735-13743 (1986).
14. Pedersen, S. E., and J. B. Cohen. d -Tubocurarine binding sites are located at α - γ and α - δ subunit interfaces of the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* **87**:2785-2789 (1990).
15. Galzi, J.-L., D. Bertrand, A. Devillers-Thiery, F. Revah, S. Bertrand, and J.-P. Changeux. Functional significance of aromatic amino acids from three peptide loops of the $\alpha 7$ neuronal nicotinic receptor site investigated by site-directed mutagenesis. *FEBS Lett.* **294**:198-202 (1991).
16. Bertrand, D., A. Devillers-Thiery, F. Revah, J.-L. Galzi, N. Husay, C. Mulle, S. Bertrand, M. Ballivet, and J.-P. Changeux. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc. Natl. Acad. Sci. USA* **89**:1261-1265 (1992).
17. O'Leary, M. E., and M. M. White. Mutational analysis of ligand-induced activation of the *Torpedo* acetylcholine receptor. *J. Biol. Chem.* **267**:8360-8365 (1992).
18. Tomaselli, G. F., J. T. McLaughlin, M. E. Jurman, E. Hawrot, and G. Yellen. Mutations affecting agonist sensitivity of the nicotinic acetylcholine receptor. *Biophys. J.* **60**:721-727 (1991).
19. Buller, A. L., and M. M. White. Control of *Torpedo* acetylcholine receptor biosynthesis in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **85**:8717-8721 (1988).
20. Manthey, A. A. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. *J. Gen. Physiol.* **49**:963-976 (1966).
21. Sine, S. M., and J. H. Steinbach. Agonists block currents through acetylcholine receptor channels. *Biophys. J.* **46**:277-284 (1984).
22. Pritchett, D. B., and P. H. Seeburg. γ -Aminobutyric acid type A receptor point mutation increases the affinity of compounds for the benzodiazepine site. *Proc. Natl. Acad. Sci. USA* **88**:1421-1425 (1991).
23. Wieland, H. A., H. Luddens, and P. H. Seeburg. A single histidine in GABA_A receptors is essential for benzodiazepine agonist binding. *J. Biol. Chem.* **267**:1426-1428 (1991).
24. Sigel, E., R. Baur, S. Kellenberger, and P. Malherbe. Point mutations affecting antagonist affinity and agonist dependent gating of GABA_A receptor channels. *EMBO J.* **11**:2017-2023 (1992).
25. Schmieden, V., J. Kuhse, and H. Betz. Agonist pharmacology of neonatal and adult glycine receptor α subunits: identification of amino acid residues involved in taurine action. *EMBO J.* **11**:2025-2032 (1992).
26. Vandenberg, R. J., C. A. Handford, and P. R. Schofield. Distinct agonist- and antagonist-binding sites on the glycine receptor. *Neuron* **9**:491-496 (1992).
27. Vandenberg, R. J., C. R. French, P. H. Barry, J. Shine, and P. R. Schofield. Antagonism of ligand-gated ion channel receptors: two domains of the glycine receptor α subunit form the strychnine-binding site. *Proc. Natl. Acad. Sci. USA* **89**:1765-1769 (1992).
28. Dohlman, H. G., J. Thorner, M. G. Caron, and R. J. Lefkowitz. Model systems for the study of seven-transmembrane-segment receptors. *Annu. Rev. Biochem.* **60**:653-688 (1991).
29. Strader, C. D., T. Gaffney, E. E. Sugg, M. R. Candelore, R. Keys, A. A. Patchett, and R. A. F. Dixon. Allele-specific activation of genetically engineered receptors. *J. Biol. Chem.* **266**:5-8 (1991).
30. Burley, S. K., and G. A. Petako. Aromatic-aromatic interaction: a mechanism of protein structure stabilization. *Science (Washington D. C.)* **229**:23-28 (1985).
31. Burley, S. K., and G. A. Petako. Weakly polar interactions in proteins. *Adv. Protein Chem.* **39**:125-189 (1988).
32. Karlin, A. Chemical modification of the active site of the acetylcholine receptor. *J. Gen. Physiol.* **54**:245a-264a (1969).
33. Sobell, H. M., T. D. Sakore, S. S. Tavale, F. G. Canepa, P. Pauling, and T. J. Petcher. Stereochemistry of a curare alkaloid: *O,O'*-*N*-trimethyl-*d*-tubocurarine. *Proc. Natl. Acad. Sci. USA* **69**:2212-2215 (1972).
34. Beers, W. H., and E. Reich. Structure and activity of acetylcholine. *Nature (Lond.)* **228**:917-922 (1970).

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