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Changeux J-P. The acetylcholine receptor: an "allosteric" membrane protein. *Harvey Lect.* 75:85-254, 1981; and **Changeux J-P, Thiéry J, Tung Y & Kittel C.** On the cooperativity of biological membranes. *Proc. Nat. Acad. Sci. USA* 57:335-41, 1967.

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A critical step in chemical synaptic transmission is the opening of an ion channel by the neurotransmitter. Evidence is presented in favor of the notion that, in the case of the nicotinic synapse, this process is accounted for by a membrane-bound allosteric protein, the acetylcholine receptor, whose functional architecture has been largely deciphered in the past decades. [The *SCI*® indicates that these papers have been cited in over 240 and 380 publications, respectively.]

Functional Architecture of the Acetylcholine Receptor

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The Harvey Lecture was written during the summer of 1980 primarily to review the work done on the acetylcholine receptor, following its initial *in vitro* identification using jointly fish electric organ and snake venom α toxins.[1] It was also for me the opportunity to test the validity of an idea I first briefly evoked in the conclusions of my PhD thesis in 1964 and further developed in the 1967 *Proceedings of the National Academy of Sciences of the USA* paper. Are there allosteric mechanisms involved in the pharmacological response of a synaptic membrane to acetylcholine? In particular, does the interaction acetylcholine-ion channel take place between topographically distinct sites? Is the cooperativity of the response to acetylcholine associated with a symmetrical organization of the receptor protein and with discrete conformational transitions of the receptor molecule?

This hypothesis was, at a glance, shaken by the observations of Karlin and Raftery's groups that the receptor protein was composed of five subunits of four different kinds. The receptor molecule thus looked nonsymmetrical. Yet their finding was basically structural. Four main arguments convinced me that my initial idea was nevertheless still valid: (1) Pharmacological agents that block the permeability response to acetylcholine in a noncompetitive manner were shown to interact with an *allosteric site* distinct from the acetylcholine receptor site but positively modulated by agonists binding to their site; moreover, affinity ligands of the site for noncompetitive blockers resulted in the labelling of subunits different from that primarily labelled by the agonists (alpha),[2] thus disclosing a functional role for the non-alpha subunit(s); (2) Electron microscopy of the purified and membrane-bound protein disclosed *regular* rosettes on *en face* views;[3] (3) Raftery and coworkers revealed important *sequence homologies* between the different subunits (subsequently documented with the complete sequence by Numa's group), thus making plausible a *quasi*-symmetrical organization of the receptor protein with an axis of rotational symmetry perpendicular to the plane of the membrane and two interacting acetylcholine binding subunits; (4) Rapid kinetic methods showed that the receptor protein undergoes transitions between *discrete* conformational states, some of which present *before* the binding of acetylcholine. In other words, the acetylcholine receptor exhibits several properties typical of allosteric proteins but with distinctive features associated with its transmembrane organization.

In the Harvey Lecture, I also emphasized that two main issues remained to be solved: (1) the identification of the ion channel that reconstitution experiments had shown to be part of the receptor protein and (2) the mechanisms of gene expression involved in the biogenesis of the postsynaptic membrane where the receptor protein accumulates in the adult endplate.

Since then, amino acids from the ion channel have been identified. Jérôme Giraudat and Michael Dennis in my laboratory have shown that serine 262 is labelled by the noncompetitive "channel" blocker chlorpromazine on the delta subunit.[4] This amino acid is located within the transmembrane segment MII, whose contribution to the ion channel has subsequently been supported and extended by site-directed mutagenesis experiments done in other laboratories.

Another outcome was the analysis of the regulation of acetylcholine receptor gene expression during endplate formation by recombinant DNA technology with the identification and sequencing of the α-subunit promoter and DNA regulatory sequences in the chick,[5] the demonstration of a compartmentation of gene expression in junctional *vs.* extrajunctional nuclei by *in situ* hybridization,[6] and the presentation of evidence in favor of a contribution of protein kinase C in the electrical activity dependent repression of α-subunit gene expression in nonjunctional areas.[7]

A reason for the success of the Harvey Lecture is, I believe, that it dealt with the functional architecture of the first known receptor for neurotransmitter that rapidly became the prototype of a super family of ligand gated-ion channels.

The awards received because of this work include, in particular, the Gairdner Foundation Award (1978), the Lounsbery Prize (1982), the Wolf Prize (1983), the F.O. Schmitt Prize for Neuroscience (1986), and the Rita Levi-Montalcini Award lecture from Fidia (1988).

1. **Changeux J-P, Kasai M & Lee C Y.** The use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. Nat. Acad. Sci. USA* 67:1241-7, 1970. (Cited 355 times.)
2. **Oswald R, Sobel A, Waksman G, Roques B & Changeux J-P.** Selective labeling by [^{3}H-]trimethisoquin azide of polypeptide chains present in acetylcholine receptor rich membranes from *Torpedo marmorata*. *FEBS Lett.* 111:29-34, 1980. (Cited 35 times.)
3. **Cartaud J, Benedetti L, Cohen J B, Meunier J C & Changeux J-P.** Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. *FEBS Lett.* 33:109-13, 1973. (Cited 165 times.)
4. **Giraudat J, Dennis M, Heidmann T, Chang J Y & Changeux J-P.** Structure of the high affinity binding site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the δ subunit is labeled by ^{3}H chlorpromazine. *Proc. Nat. Acad. Sci. USA* 83:2719-23, 1986. (Cited 65 times.)
5. **Klarsfeld A, Daubas P, Bourachot B & Changeux J-P.** A 5' flanking region of the chicken acetylcholine receptor alpha-subunit gene confers tissue-specificity and developmental control of expression in transfected cells. *Mol. Cell. Biol.* 7:951-5, 1987. (Cited 30 times.)
6. **Fontaine B, Sassoon D, Buckingham M & Changeux J-P.** Detection of the nicotinic acetylcholine receptor α-subunit mRNA by *in situ* hybridization at neuromuscular junctions of 15-day old chick striated muscles. *EMBO J.* 7:603-9, 1988. (Cited 40 times.)
7. **Klarsfeld A, Laufer R, Fontaine B, Devillers-Thiéry A, Dubreuil C & Changeux J-P.** Regulation of muscle AChR α-subunit gene expression by electrical activity: involvement of protein kinase C and Ca^{++}. *Neuron* 2:1229-36, 1989.

