ACETYLCHELONE RECEPTORS FROM ELECTROPLAX MEMBRANES: IN VITRO AND
IN SITU PROPERTIES

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INTRODUCTION

Cell surface receptors are capable of recognizing chemical mediators of cellular communication and generate a specific signal for a biological response. At certain neuronal synapses, the vertebrate neuromuscular end plate and the electric tissue of certain elasmobranch, the chemical mediator is acetylcholine and the receptor is a postsynaptic transmembrane protein. Acetylcholine binding induces opening of short lived (≈ 3 ms) membrane channels permeable to monovalent or divalent cations of less than 8 Å in diameter. This event leads to a conductance increase and a depolarization of the membrane. While it is agreed that the acetylcholine receptor (AcChR) and the ionic channel are coupled in the membrane, the relationship of the receptor binding sites and the ionic channel sites is still unclear. When the neurotransmitter concentration or that of a variety of compounds eliciting similar responses (agonists) is maintained, the number of active receptor channels slowly declines and the phenomenon is known as desensitization. In general, activation of the receptors results from recognition of agonists with low affinity for the resting state of the AcChR. As binding slowly develops desensitization ensues which parallels the appearance of a state of high AcChR affinity for those agonists.1,2 Voltage clamp

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*Abbreviations: AcChR, acetylcholine receptor; DPH, 1,6-diphenyl-1,3,5-hexatriene; MBTA, 4-(N-maleimido)benzoyltrimethylammonium; PC, phosphatidylcholine; EP, ethanolamine phosphoglycerides; PySA, pyrenesulfonyl azide; TMA-DPH, 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene.

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and radioactive tracers ion flux studies of the rapid activation
process are consistent with a mechanism in which binding of two or
more agonist molecules to the receptor is required to open the ion
channel.\textsuperscript{1,2} Elapid \(\alpha\)-neurotoxins appear to share binding domains
with agonists and they have become valuable probes in determining
the degree of occupation of the receptor by agonists and in the
molecular characterization of the receptor.

Because of high concentration of AcChR in the electroplax of
electric fish, this tissue is preferred for molecular studies of
this receptor which is the most extensively characterized neuro-
receptor to date. The electric ray Torpedo californica electroplax
membranes share many analogies with those of muscle receptors and
this tissue has been a favorite for the isolation of solubilized
receptor and the preparation of membrane vesicles in which over 50%
of the protein is represented by the AcChR and in which ligand bind-
ing, desensitization and ion flux can be reproduced with responses
similar to those detected through electrophysiologic measurements
in whole cells. In this work, we describe the molecular and func­
tional properties of the AcChR when, after isolation, it is incorporated
into a lipid bilayer in a process of membrane reconstitution in
which the properties of reconstituted membranes are compared to
those of freshly isolated whole electroplax membranes.

MOLECULAR PROPERTIES OF AcChR

AcChR can be solubilized from Torpedo californica and other
electric fish species through the use of detergents. Nonionic
detergents are best and Triton X-100 has been preferred. However,
this detergent binds very tightly to AcChR and can amount to as
much as 50\% of the total weight of the receptor preparation and
cannot be easily separated from the protein. More recently, \(8\)D-
Octylglucopyranoside has been introduced as an effective solubilizer
of membrane electroplax proteins and removal of this detergent by
dialysis is complete.\textsuperscript{3} Solubilized AcChR can be purified through
the use of affinity chromatography methods using agonists or anta­
gonists (compounds which prevent ionic conductance), including
\(\alpha\)-neurotoxins, as ligands attached to the solid chromatographic
matrix. SDS polyacrylamide gels reveal four types of subunits
(Fig. 1) of 40, 50, 60, 65,000 daltons for the purified AcChR with
stoichiometry of 2;1;1;1. The molecular weight of the receptor is
260,000 and it may exist as a dimer of about 500,000 daltons in
which the monomers are linked by a disulfide bond between the 65,000
dalton subunits. The Stokes radius of detergent free receptor is
70 Å and has an acidic isoelectric point.\textsuperscript{3} The first 60 amino acid
residues in the \(N\)-terminal sequence of the anion subunits are highly
homologous, yet, only the 40,000 subunit has been associated, through
covalent affinity binding studies, as that containing the site(s)
for agonist and antagonist. Separation of the subunits is achieved
through the use of SDS detergent, yet subunit interchanging between