Mapping noncompetitive antagonists binding sites in the nicotinic acetylcholine receptor

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Abstract

The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel that is opened upon the binding of agonist to the extracellular surface. Noncompetitive antagonists of the nAChR block the response of the nAChR to agonist without preventing the binding of agonist. While most aromatic amine noncompetitive antagonists appear to bind within the lumen of the ion channel, the binding site of the fluorescent noncompetitive antagonist ethidium has been predicted by fluorescence resonance energy transfer studies to lie at the most extracellular aspect of the receptor. [^{3}H]Ethidium diazide, a photoactivatible analog of ethidium, was used to map the binding site of ethidium in the desensitized state. Sequence analysis showed that [^{3}H]ethidium diazide photoincorporated into the α and δ M2 segments, which are known to contribute to the lumen of the channel, and particularly into residues which have been shown to line the channel. Additionally, photoincorporation was also evident in the M1 segments of these two subunits, indicating that the M1 segment contributes to the formation of the lumen of the channel.

[^{3}H]3-Azioctanol is a photoaffinity probe that is a general anesthetic that inhibits the nAChR. Sequence analysis of nAChR photolabeled with this probe showed that the primary site of [^{3}H]3-azioctanol incorporation in the desensitized state of the nAChR was αGlu-262, at the extracellular end of αM2, indicating binding within the lumen of the channel. In addition, [^{3}H]3-azioctanol incorporated at lower efficiency into residues at the protein-lipid interface, at equal levels in the presence or absence of agonist. In the absence of agonist, [^{3}H]3-azioctanol also reacted with low efficiency with αTyr-190 and αTyr-198, residues contributing to the binding site of agonist.

[^{3}H]Progestin aryl azide is a photoaffinity analog of the steroid anesthetic progesterone. Although most noncompetitive antagonists appear to bind within the lumen of the channel, the high hydrophobicity of steroids suggests that they may interact at the protein-lipid interface. The primary site of [^{3}H]progestin aryl azide incorporation in the α-subunit was mapped within a large fragment containing αM4, known to form the protein-lipid interface. However, the instability of the photoadducts to HPLC and sequencing conditions precluded identification of labeled residues.

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Abbreviation List

ACh	acetylcholine
1-AP	1-azidopyrene
CPZ	chlorpromazine
FRET	fluorescence resonance energy transfer
GluR	glutamate receptor
HTX	histrionicotoxin
HPLC	high-performance liquid chromatography
nAChR	nicotinic acetylcholine receptor
NCA	noncompetitive antagonist
PAGE	polyacrylamide gel electrophoresis
PCP	phencyclidine
PTH	phenylthiohydantoin
SDS	sodium dodecyl sulfate
TFA	trifluoroacetic acid
TID	3-(trifluoromethyl)-3-m-(iodophenyl)diazirine
TPP	triphenylphosphonium
TPS	*Torpedo* physiological saline
V8	*staphylococcus aureus* V8 protease