NICOTINIC-ACETYLCHOLINE RECEPTOR (nAChR): STRUCTURE AND FUNCTION

INTRODUCTION

The nicotinic-acetylcholine receptor (nAChR) is a ligand-gated ion channel, made up of five transmembrane protein subunits. It belongs to a large superfamily of receptors called "cys-loop" ligand-gated ion channels, which are characterised by a loop of 13 highly conserved amino acid residues between two Cysteine residues covalently linked together. Two types of nAChR are found in mammals: one found in skeletal muscle cells and the other in the central nervous system. The channel opens when a ligand (acetylcholine and nicotine) binds to the extracellular binding site of the receptor; when the channel is open, ions flow through the vestibule and after a series of selectivity filter, only cations (K^+, Na^+, Ca^{2+}) manage to reach the cytoplasm. At the neuromuscular junction, the synapse between a motor neuron and a skeletal muscle cell, nAChRs are responsible to receive the message of the neurotransmitter Acetylcholine to activate muscle contraction; whereas in the central nervous system, nicotine binds to nAChR, opens the channel and initiate a cascade of reactions that ultimately culminates in the excitatory effect associated with smoking tobacco. Recent studies show also a correlation between Alzheimer Disease, which is associated with neuronal loss and loss of acetylcholine levels in the brain, and nAChR, highlighting thus these receptors as potential therapeutic targets.

STRUCTURE

The high resolution structure of the Nicotinic-Acetylcholine receptors has been determined by the studies of ray *Torpedo* electric organ, which is densely packed with muscle-type nicotinic receptors (Sine, 2012); the structure of the extracellular domain, as it is water soluble, has been easily determined by X-Ray crystallography, whereas that of the transmembrane domain has been determined using a neurotoxin found in krait snakes (α-bungarotoxin), which binds to the receptor, contracts the membrane bilayer and thus extract the domain, that can be then studied with cryo-electron microscopy (Young et al., 2003). The use of cryo-EM gives a very accurate high resolution structure because the protein is not fixed nor stained but observed in its native

environment and so avoids any unwanted folding, which sometimes occurs after X-ray crystallography.

NAChRs are composed of 5 protein subunits, each one containing 4 transmembrane spanning α-helices, an extracellular domain which is largely β sheets and a cytoplasmic domain that varies depending on the type of receptor. The pentameric complex forms a ring structure, arranged around a central hydrophilic pore, which traverses the plasma membrane (Unwin, 2005). The subunits that form the pentamer depend on the specificity of the receptor; in muscle tissues 2 types of nAChRs have been found: one is 2 α_1, 1β, 1γ, 1δ and the other one is 2α_1, 1β, 1δ, 1ε, whereas neuronal nAChRs can be formed by 7 different types of α subunits (α_2, α_3, α_4, α_5, α_6, α_7, α_9, α_{10}) and 3 types of non α subunits (β_2, β_3, β_4); muscle nAChRs are always heteropentamer, while neuronal nAChRs can also be homopentamer (Albuquerque et al., 2009), (see figure 1 below).

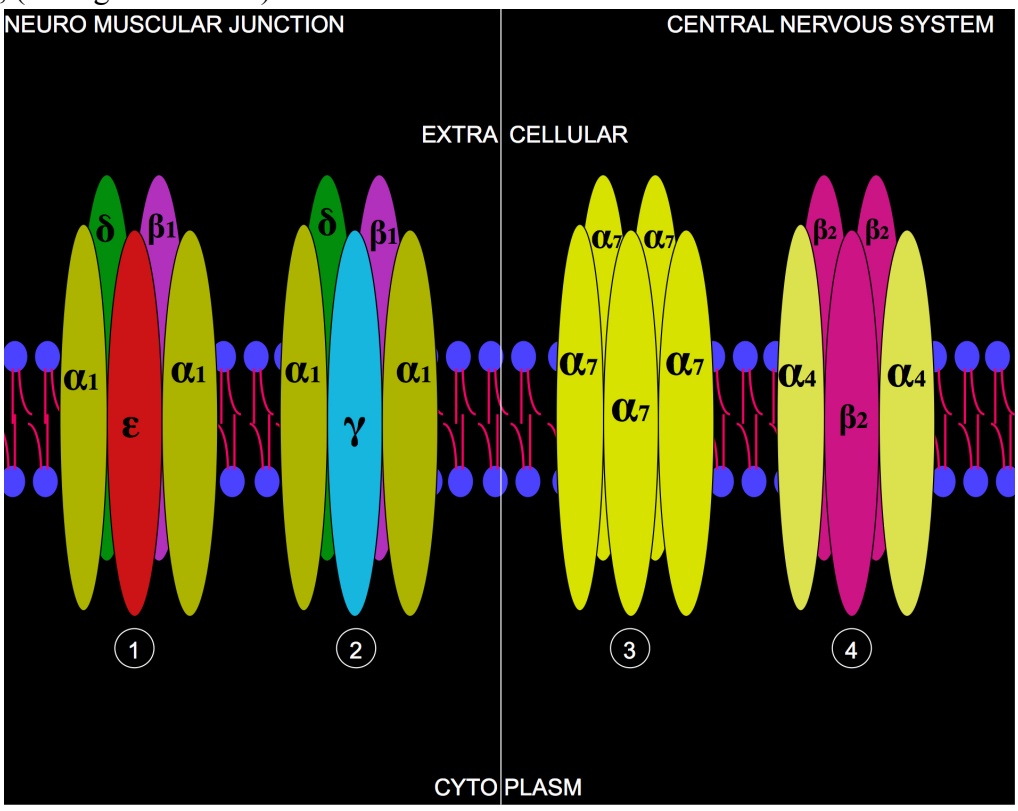
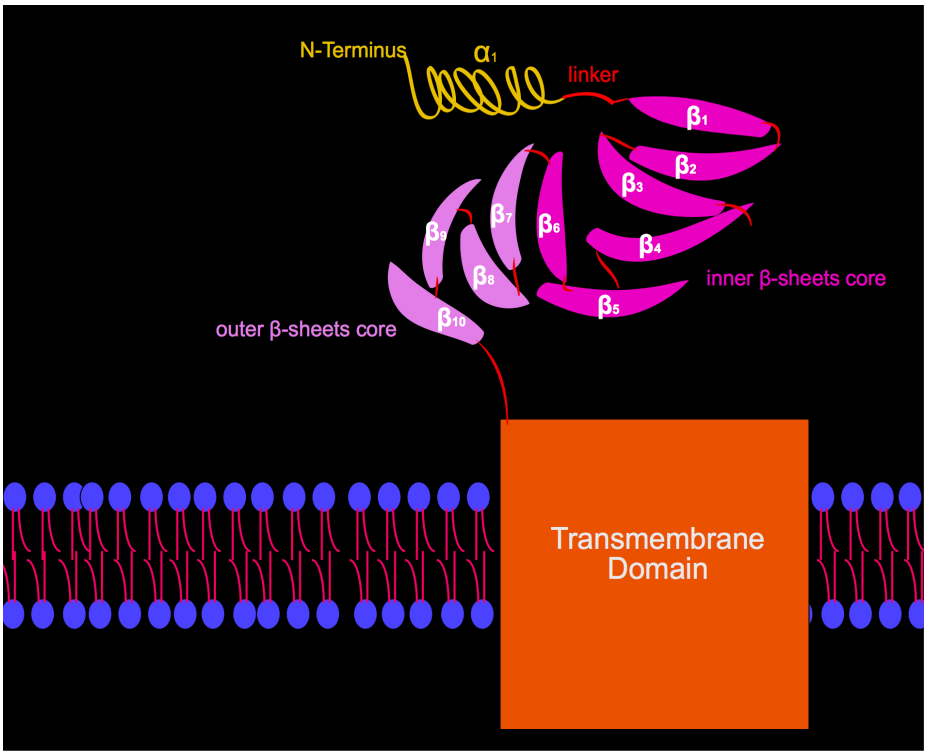


Fig.1) Pentamer frontal view with hydrophilic pore between the five subunits. Different types of subunits compose different types of receptors:
1: $(\alpha_1)_2\beta_1\delta\varepsilon$ nAChR in adult skeletal muscle tissues; **2**: $(\alpha_1)2\beta_1\gamma\delta$ nAChR in fetal skeletal muscle tissues; **3**: $(\alpha_7)_5$ homopentamer nAChR in central nervous system, responsible for nicotine addiction; **4**: $(\alpha_4)_2(\beta_2)_3$ nAChR in CNS

The extracellular domain of each subunit begins with an N-Terminus followed by a sequence of amino acid residues (linker) that link the terminus to 10 β-strands; 6 β-strands form an inner β-sheet core and the remaining 4 form an outer β-sheets core (fig. 2). In α subunits is present

also a Cys-loop, which is a sequence of 13 highly conserved amino acid residues within 2 Cysteine residues linked together by a disulfide bond; this loop is only present in α subunits and plays a fundamental role in the binding of the ligand. In addition to forming the binding site for the ligand, the extracellular domain constitutes also a first vestibule through which ions have to pass and thus act as first selectivity filter. There is a first ring of 5 negatively charged Aspartic Acid residues that form a ring (αAsp97, αAsp97, βAsp97, γAsp97, δAsp97) and prevent anions to pass (Stokes, 2015).

Fig.2) Topology of one subunit with focus on the β-strands of the extracellular domain, structure modified from Unwin, 2005



The transmembrane domain of each subunit is formed by 4 α- helices (TM1, TM2, TM3, TM4), the surface of the pore is made up of one α helix of each subunit, in particular TM2, which contains a larger number of hydrophilic residues and thus form the hydrophilic surface of the pore; the other 3 α-helices of each subunit are buried within the lipid bilayer. Five Leucines residues for each TM2 (Stokes, 2015) form the gate of the receptor, in its closed conformation the Leu residues, which are in the middle of the pore facing towards the outside of the helix, occlude the pore, whereas in its open conformation the Leu residues rotate towards the inside of the helix, opening the channel for the cations; in its open conformation the pore has a diameter of less than 0.65 nm (Alberts, 2015). Leucine residues are not only important in the gating mechanism of the receptor but also in the dehydration of the ions: as mentioned before the vestibule and the pore is mostly hydrophilic and thus hydrated ions are able to pass, but

when they reach the hydrophobic gate formed by the hydrophobic Leucine residues, the ions lose their water shell and get dehydrated. Before and after the Leucine gate, other negatively charged amino acids (aspartic and glutamic acid) attract cations and repell anions and form the subsequent selectivity filters (fig 3).

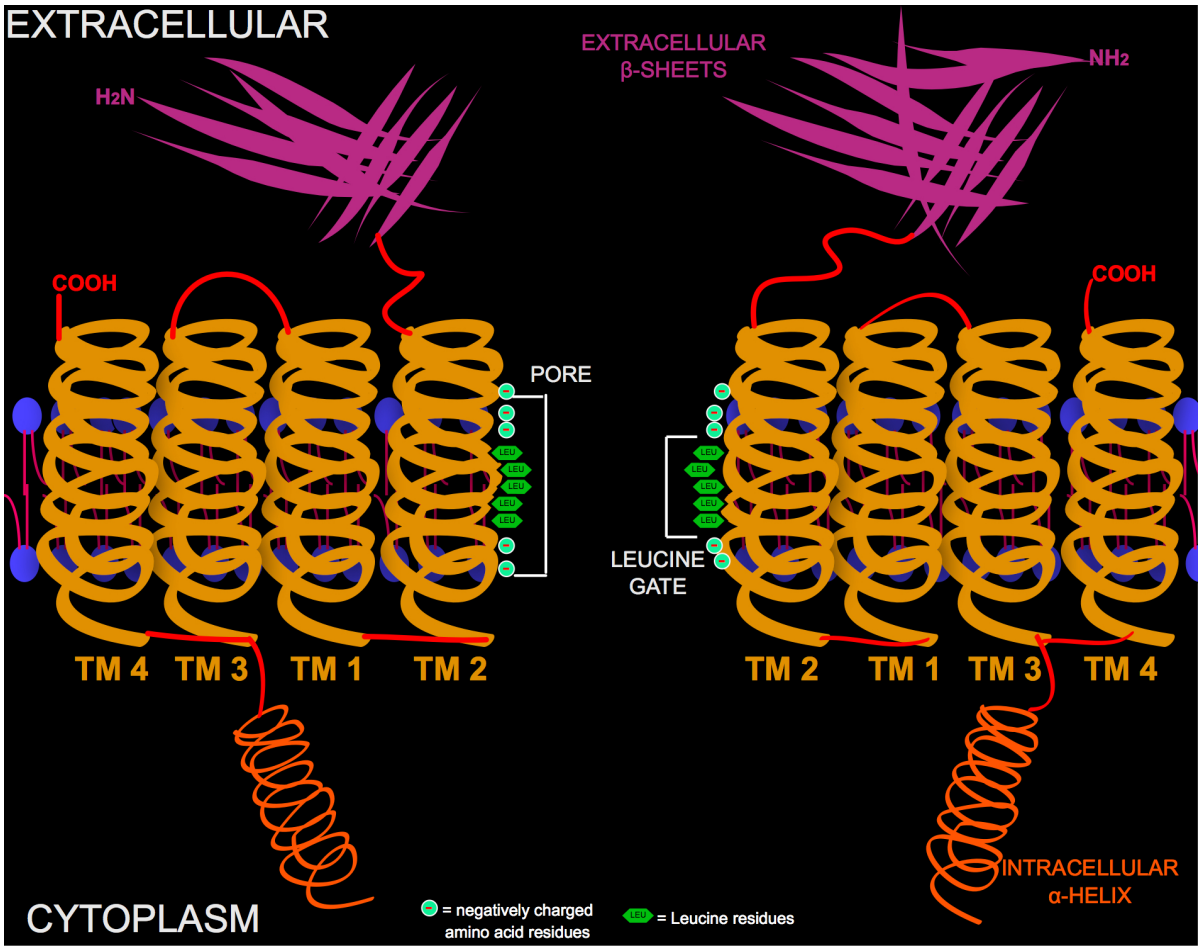


Fig.3) Transmembrane topology of the receptor, frontal view of just 2 subunits for clarity. TM2 of each subunit lines up with the hydrophilic pore; the gate is formed by five hydrophobic Leucine residues, negatively charged amino acid residues attract cations.
Structure modified from Unwin, 2005

The intracellular domain in *Torpedo* is formed by a single and large α-helix, located between TM3 and TM4 (Lodish, 2008), whereas in human nAChRs a composition of α-helices and β-strands is found depending on the location and specific cellular function of the receptor.

FUNCTION

As all ligand-gated ion channels, nAChRs activate when a ligand (acetylcholine in normal conditions, nicotine when assumed) bind to the receptor's outer surface, the channel then opens, allowing ions to pass through the pore. In nAChR there are two ligand binding sites,

pocket is called “front” or positive side and is adjacent to the “back” or negative side of the located in the two α-subunits that compose the receptor; two molecules of acetylcholine or nicotine are so required to activate the receptor. Each ligand binding site of the two α-subunits consists in a hydrophobic pocket that traps the ligand, the side of the α-subunit that forms the adjacent subunit (β, γ, δ or ε). In the front side is required a loop termed “C-loop”, which has a cys-cys pair at the end of it, and several hydrophobic aromatic amino residues, such as Tyrosine and Tryptophan; in the back side the residues involved in the interactions between the ligand and the ligand binding site are Leucine, Methionine and Tryptophan, which also contribute in the hydrophobicity of the pocket. The C-loop sticks out from the α-subunit and extend to the face of the adjacent subunit, it is responsible for the shape of the pocket, burying the ligand and forming tight interactions between ligand and receptor (fig 4).

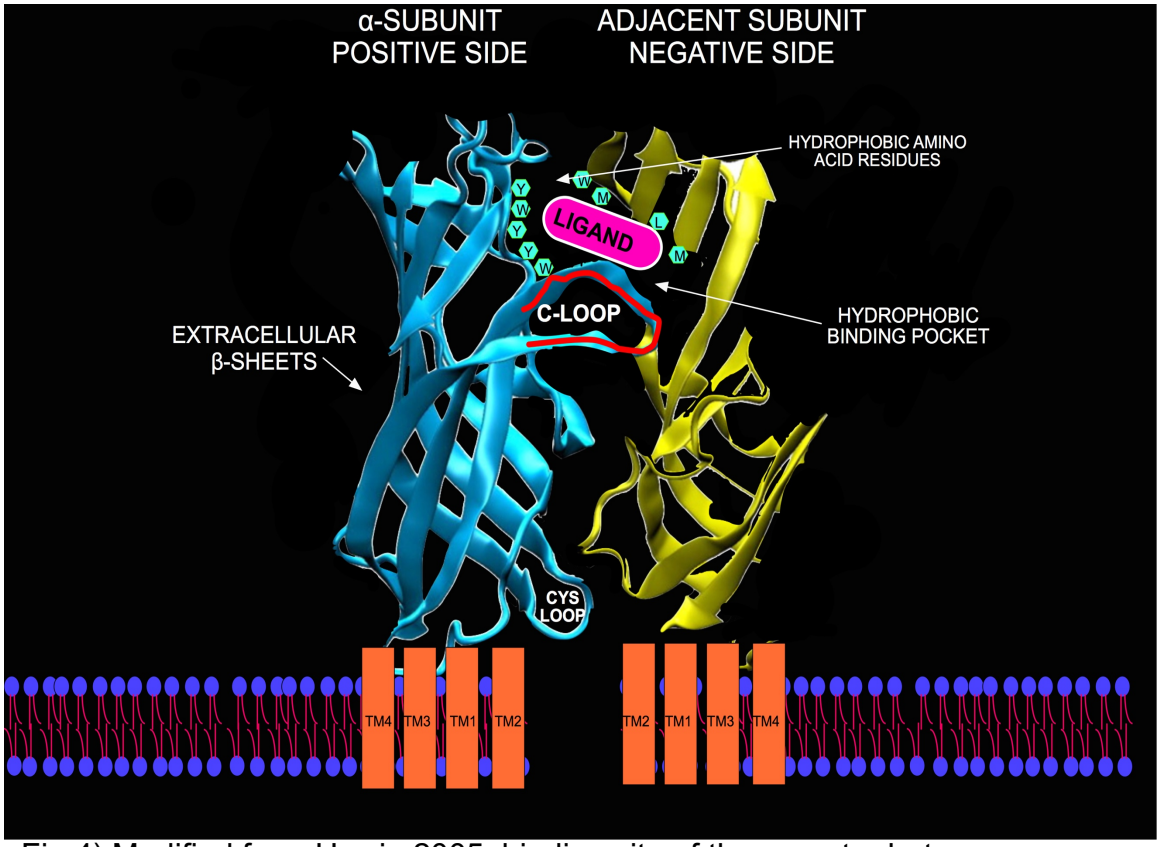


Fig.4) Modified from Unwin 2005, binding site of the receptor between an α-subunit and an adjacent one (β,γ,δ or ε). The C-loop connects the front side with the back and creates a hydrophobic pocket that binds the ligand.

When the ligand binds to the hydrophobic pocket, there is a rearrangement of Hydrogen bonds, which results in a movement of the C-loop toward the core of the receptor (Sine, 2012). The movement of the loop allows the Cys-Cys pair to interact even more tightly with the ligand and trap it in the inside of the pocket. When two ligands bind the receptor at the same time, enough

movement is generated and is transferred to the transmembrane domain; the binding causes a rotation of the extracellular β-barrel that consequently rotates the second transmembrane α-helix, TM2 (Albuquerque et al., 2009). This results in a widening of the pore and in a rotation of the hydrophobic Leucine residues that form the gate and occlude the pore. The hydrophobic residues hide in the lipid core of the lipid bilayer and new hydrophilic residues face the surface of the pore, attracting ions and thus opening the channel.

Negatively charged amino acid residues then attract cations and repulse anions, allowing the passage of just K^+, Na^+ and Ca^{2+}. Unwin (2005) suggested that the rotation of the extracellular domain is transferred to the helix via interactions between the Cys-loop and TM2, which explains why exclusively α-subunits are ligand binding sites.

The flow of cations from the outside to the inside of the cell results in a depolarization of the membrane, which, depending on the nature of the ligand and the specificity of the receptor, may result in muscle contraction or activation of enzymatic reactions.

TRANSMEMBRANE PREDICTION AND CONCLUSIONS

Nicotinic Acetylcholine receptors are protein complexes of fundamental importance in animals; 17 different genes encode for the 17 different protein subunits that can form the pentamer (Sine, 2012) and each subunit is an integral membrane protein, which owes its existence across the lipid bilayer to the four membrane spanning α-helices. The 4 α-helices in fact contain a high number of hydrophobic residues and are thus able to interact with the hydrophobic tails of the phospholipids that make up the plasma membrane; the helical structure then maximise Hydrogen bonds between carbon and nitrogen atoms of the backbone, increasing the stability of the helix and minimising its polarity. Using the online tool TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) and the sequence of a human neuronal nAChR found on the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein), it is possible to predict the transmembrane domains of each subunit (in this case an α_4) and the results shown in figure 5 confirm that there are 4 transmembrane domains, one (TM1) from amino acid residue number 220 to 242, TM2 from 249 to 271, TM2 from 281 to 303 and TM4 from 576 to 598.

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# CAA60959.1 Length: 602
# CAA60959.1 Number of predicted TMHs:  4
# CAA60959.1 Exp number of AAs in TMHs: 89.6055900000000001
# CAA60959.1 Exp number, first 60 AAs:  0.08721
# CAA60959.1 Total prob of N-in:        0.00598
CAA60959.1      TMHMM2.0        outside      1   219
CAA60959.1      TMHMM2.0        TMhelix    220   242
CAA60959.1      TMHMM2.0        inside     243   248
CAA60959.1      TMHMM2.0        TMhelix    249   271
CAA60959.1      TMHMM2.0        outside    272   280
CAA60959.1      TMHMM2.0        TMhelix    281   303
CAA60959.1      TMHMM2.0        inside     304   575
CAA60959.1      TMHMM2.0        TMhelix    576   598
CAA60959.1      TMHMM2.0        outside    599   602
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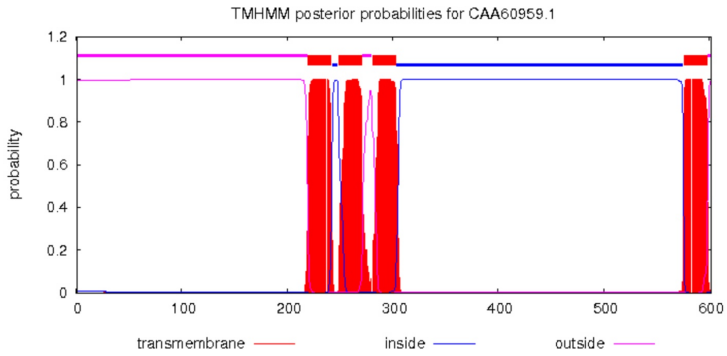

Fig.5) Transmembrane prediction of human neuronal α_4 subunit of nAChR. The sequence is from NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/CAA60959.1), the tool used is TMHMM from the Center for Biological Sequence Analysis (CBS) of the Technical University of Denmark (http://www.cbs.dtu.dk/services/TMHMM/).

NAChRs of the central nervous system are now studied intensively as it has been shown a correlation between Alzheimer disease and malfunction of these receptors; potential therapeutic drugs would mimic the behaviour of Acetylcholine and bind to the α-subunit, compensating the loss of Acetylcholine in people affected by the disease (Lombardo and Maskos, 2015).

1392 Words
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