University of Ottawa/ Department of Biochemistry, Microbiology, Immunology

Role of the M4 α-helix in the function of human muscle-type nicotinic acetylcholine receptors

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INTRODUCTION

Cell signalling at the neuromuscular junction is crucial for communication between neurons and muscle cells resulting in muscular contractions. The muscle-type nicotinic acetylcholine receptor (nAChR) is a pentameric ligand-gated ion channel that translates a chemical message released by a presynaptic neuron into an electrical impulse on a postsynaptic membrane.

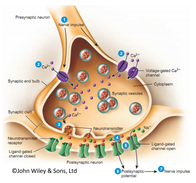


Figure 1. The neuromuscular junction. Acetylcholine is released by the presynaptic neuron at the neuromuscular junction to bind to nAChRs on the post synaptic cellular membrane, causing a conformation change which opens the ion channel. The flux of cations signals muscle contraction.

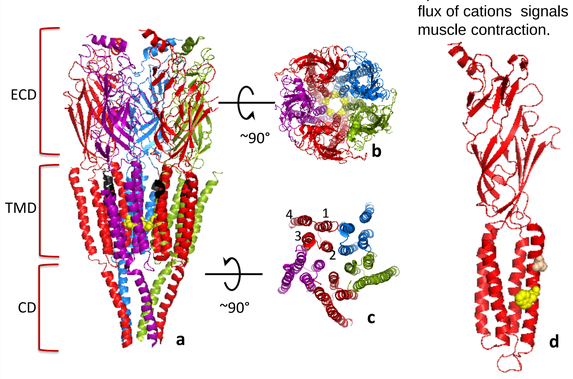


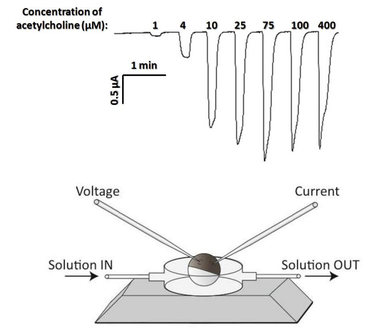
Figure 2. The structure of nicotinic acetylcholine receptor. (**a**) The sideview of nAChR shows the extracellular domain (ECD), transmembrane domain (TMD) and cytoplasmic domain (CD). The residues involved in ion channel gating can be seen in yellow. (**b**) The *Torpedo* nAChR is composed of five subunits; β (blue),γ (purple), δ (green) and two α subunits (red). In the adult human muscle-type nAChR, γ is replaced by the ε subunit. (**c**) The 4 transmembrane α-helices of each subunit are shown, the furthest from the ion channel is M4. (**d**) The C418W mutation and the F426A mutation, in yellow and tan respectively are shown located on the M4 α-helix of the α subunit.

Subtle changes in the structure of nAChR can have drastic effects on its activity and on human biology. Previous research has shown that an amino acid change from Cys418 to Trp gives a 20-25 fold gain of function and is implicated in Congenital Myasthenic Syndrome (CMS), group of conditions characterized by muscle weakness that worsens with physical exertion. The C418W mutation is located on the most lipid-exposed transmembrane helix, M4, of the alpha subunit suggesting that αM4 can allosterically modulate channel activity. The sensitivity of nAChR with its lipid environment, i.e. the interactions of M4, is of additional interest due to age-related diseases; e.g., Alzheimer's and Parkinson's, thought to be associated with changes in the lipid environment of neuronal nAChRs. Using site-directed mutagenesis on αM4, we explore here the interaction of αM4 with its environment and its effect on nAChR function in diseased versus wild-type states.

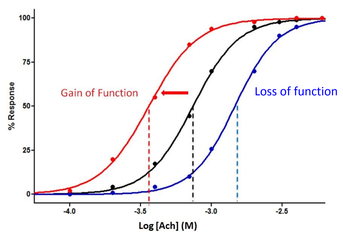
METHODS

1. Oocytes are injected with cRNA.

2. Oocytes are incubated at 16° C for 2 to 3 days.



3. Channel response to Ach binding is measured and dose response curve is plotted



RESULTS

Table 1. Averaged EC_{50} values and hill slope coefficients, ± standard deviation, of mutants from induced mutations on the M4 α-helix of the α-subunit of the human muscle-type nicotinic acetylcholine receptor. EC_{50} value corresponds to the acetylcholine concentration needed to achieve half-maximal activation of the receptor.

Mutation	EC_{50} (μM)	Hill slope n_H	Number of repeats n
wild-type	6.29 ± 1.18	1.63± 0.26	8
C418W	0.88 ± 0.13	1.81 ± 0.81	9
F426A	1.23 ± 0.19	1.24 ± 0.75	4
C418W+F426A	0.67 ± 0.21	1.46 ± 0.13	8

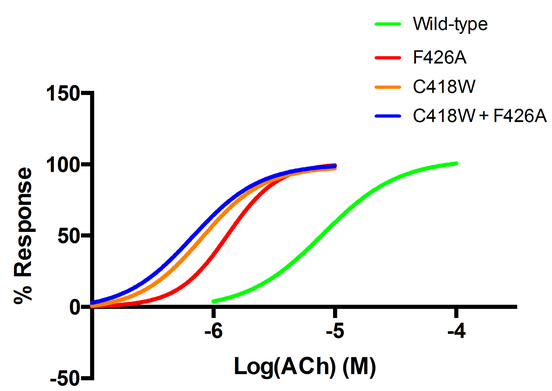


Figure 3. Acetylcholine dose response curve of human muscle-type nicotinic acetylcholine receptor mutants.

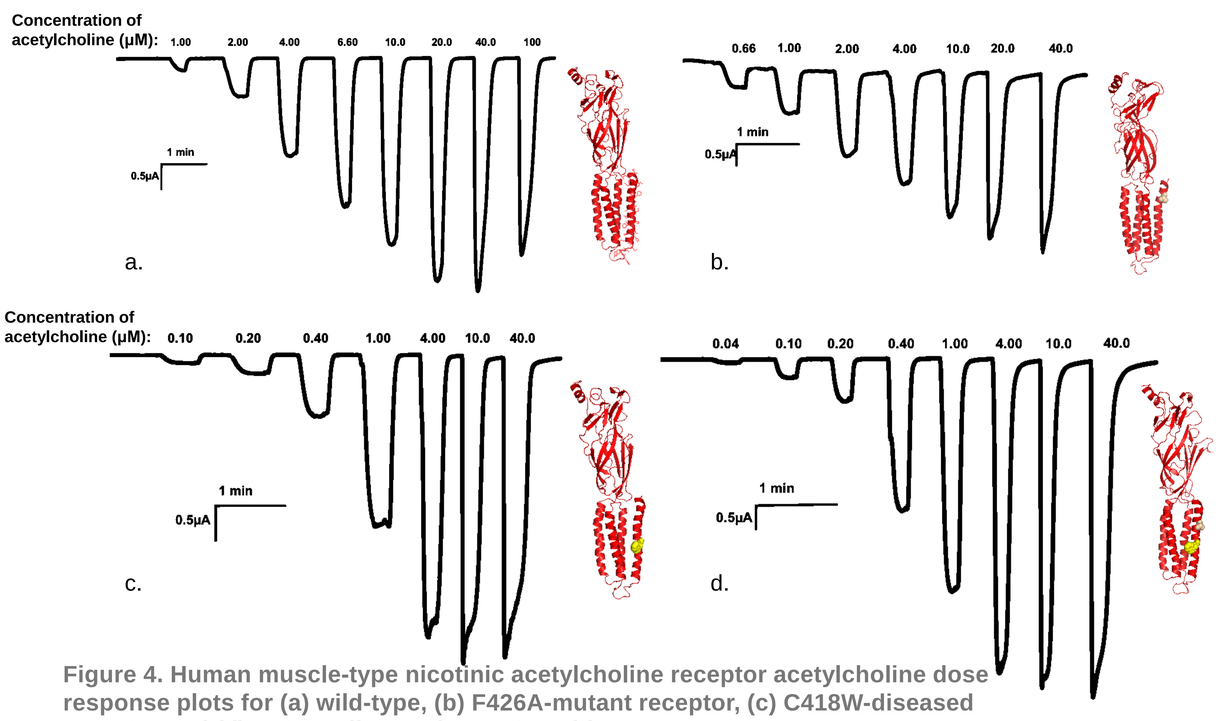


Figure 4. Human muscle-type nicotinic acetylcholine receptor acetylcholine dose response plots for (a) wild-type, (b) F426A-mutant receptor, (c) C418W-diseased receptor and (d) C418W-diseased receptor with F426A.

CONCLUSION

The lipid-facing F426A mutation affects the wild-type and C418W-diseased receptor differently by a respective 5 and 1.3-fold gain of function. This significant difference in effect suggests a difference in interaction of the M4 α-helix with its lipid environment in the wild-type and diseased state. The lesser effect of the F426A mutation on the, CMS-causing, mutant receptor channel gating suggests an unfavourable interaction with the lipid environment at the C418W location. The replacement of Cys with Trp may cause a tighter association of the transmembrane domain α-helices, potentiating the channel function. In contrast, the M4 alpha helix in the wild-type receptor is less "hidden" within the protein and responds more to the lipid environment, allowing changes in the environment caused by lipid facing mutations, such as F426A, to have a greater effect. The M4 α-helix and its lipid interactions are, again, shown to play a role in the allosteric modulation of the channel gating in the muscle-type nicotinic acetylcholine receptor.

FURTHER STUDIES

Further mutagenesis to pinpoint the specific interactions of the M4 alpha helix with adjacent transmembrane α-helices and lipid environment, causing gain of function in the diseased receptor, are needed to better understand nicotinic acetylcholine receptor function and to better treat CMS alongside diseases associated with receptor- lipid interactions.

References

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