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Computational Analysis of nAChR a4 and b2 Subunit Stability and NMR Study of Protein Anesthetic Interaction

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Abstract

Because the α4 and β2 subunits of the transmembrane domain of nAChRs are naturally unstable in solution suitable for NMR experimentation and structural determination, mutation of the subunit sequences has been performed to lower subunit pI. However, as α4 stability is much greater than β2 stability, further mutation of the β2 sequence at key residues has been attempted to increase β2 stability. Computer modeling and simulation of the α4 and β2 subunits provide a basis for assessing the mutant subunit stability. NMR experiments run both with and without anesthetic were also performed to provide insight as to which specific residues within the α4 subunit interact with anesthetic based on observed differences in chemical shifts.

Introduction

General anesthetics are characterized by their ability to induce unconsciousness and prevent painful stimuli from being recognized. These drugs have been used since the late 1800s without a clear understanding of the mechanism by which they bring about their effects. Study of the anesthetic mechanism of action is challenging due to the difficulties associated with isolation and manipulation of the membrane-bound proteins that play a role in general anesthesia.

NMR spectroscopy is rarely used to determine the structure of membrane-bound proteins due to the inherent instability of these proteins in aqueous solution. NMR is, however, a useful technique for providing insight into the interaction between general anesthetics and protein receptors within the cell membrane. NMR experiments are useful in that they can be used to identify specific residues to which anesthetics bind as indicated by chemical shifts of the residue's peak. NMR can also indicate effects on protein motion caused by exposure to general anesthetics through the use of rmsd calculations to determine protein relaxation times. The focus of this experiment has been to observe the interaction of anesthetics with certain residues of the α4 nicotinic acetylcholine receptor (nAChR) subunit.

Methods

NMR Sample:
- 250 µl α4
- 80 mM LDAO detergent
- pH 4.7
- ^{15}N labeled

NMR Spectrometer:
- 700 MHz
- 45°C
- p3919gp and TROSY spectra collected

Experimental parameters:

1) p3919gp spectra: NS=16
D_1=1s Sw=16 ppm TD=16k

2) TROSY-HSQC NS=64 D_1=1s
Sw=13 ppm TD=1K (^{1}H), 128 (^{15}N)

Bruker 600 MHz Spectrometer

Computational Analysis:
- Native sequences placed in water boxes
- Energy minimization of the system
- Charge of solution neutralized
- Dynamics simulations to observe stability
- Subunit dimerization
- Repeat sim. with membrane-like solution
- Repeat simulations with α4/β2 dimer
- Repeat sim. with mutant seqeunces
- Repeat sim. with heteropentameric transmembrane nAChR

α4 nAChR subunit in a water box

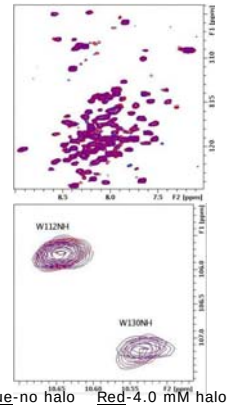
Results

Computational Study

Water Boxes → Energy Minimization → Charge Neutralization → Dynamics Simulations

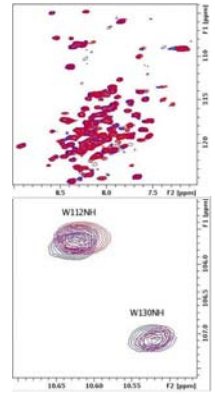
NMR Study

Effects of Halothane on α4



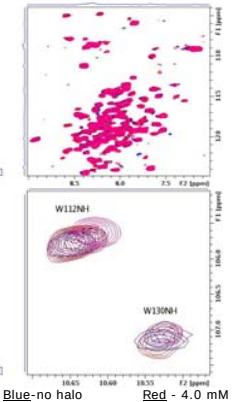
Blue-no halo Red-4.0 mM halo
Purple-1.7 mM halo

Effects of Isoflurane on α4



Blue-no IsoF Red-4.0 mM IsoF
Purple-1.7 mM IsoF

Halo Effects vs. IsoF Effects



Blue-no halo Red - 4.0 mM halo
Purple-halo removed Pink - 5.0 mM isoF

Conclusions

This experiment indicates that isoflurane is a more potent general anesthetic than halothane. Tryptophan residues displayed notable chemical shifts upon addition of anesthetics with W130NH being the more reactive of the two trp residues. This difference in reactivity is probably due to a closer proximity to the edge of the protein.

Future Research

Continuation of the incomplete molecular dynamics modeling of the nAChR subunits could yield valuable insight into novel mutations increasing β2 stability. Further NMR studies using β2 could then provide a basis for comparing the strengths of isoflurane and haothane.

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