Introduction

Nicotinic Acetylcholine Receptors (nAChRs) are ligand gated ion channels found in both the peripheral and central nervous systems. These receptors can be activated by nicotine as well as their native ligand acetylcholine and have been associated with several health-related phenomena. Nicotine is the major addictive component of tobacco, and chronic tobacco use (smoking) has been implicated in many types of cancer as well as heart disease. Other related phenomena include an inverse correlation between smoking and Parkinson's disease and the observation that patients with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) who smoke have fewer seizures [1].

Nicotinic acetylcholine receptors belong to the Cys-loop family of ionotropic receptors. Individual subunits consist of an extracellular domain (ECD), four α-helical transmembrane domains, and an intracellular loop between the M3 and M4 transmembrane domains. Agonists such as acetylcholine and nicotine bind to the ECD; as a result, the pore opens and cations flow according to their electrochemical gradient.

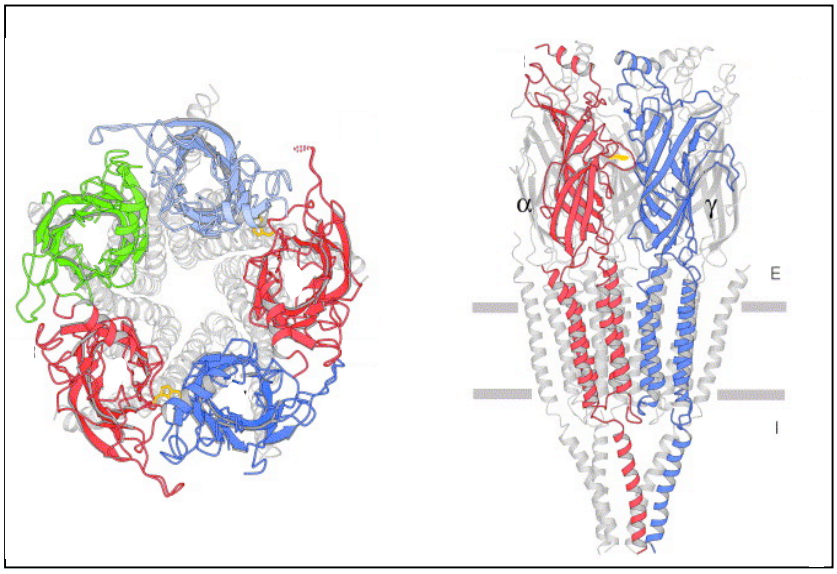

Figure 1 [2]
A. View of an assembled nAChR from the extracellular surface. Individual subunits are indicated by shading: **α4** and **β2**. The **auxiliary position** can be occupied by α5.

B. Side view of the receptor. E and I designate extracellular and intracellular regions respectively. The I regions vary markedly in size and sequence among subunits, and in this view are only partially resolved.

nAChRs found in muscle are composed of two α and one β, γ (or ε) and δ subunits [2-3]. Neuronal nAChRs are composed of α2-α11 and β2-β4 subunits and assemble in α and β or α only pentamers. The neuronal α4β2 receptor subtype is one of the two most abundant nAChRs in the central nervous system (CNS). Two α4β2 pentameric stoichiometries are reported: $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$ [4-5]. The latter stoichiometry displays a higher sensitivity to nicotine than most other neuronal nAChRs. The subunit stoichiometry of nAChRs is important in determining its pharmacology, stability, and subcellular location. Perturbations to these properties contribute to the development of disease or dependence states. ADNFLE is a very rare monogenic disease of α4β2 nAChRs. The study of ADNFLE has yielded important information about nAChR stoichiometry. Point mutations associated with ADNFLE shift α4β2 stoichiometry to $(\alpha4)_3(\beta2)_2$ [6]. This apparently affects the trafficking and pharmacology of the receptor by shifting localization to the plasma membrane (PM) and decreasing sensitivity to ACh. Nicotine use leads to reduced seizures in ADNFLE patients [1]. We know that in mammalian cells nicotine acts as a pharmacological chaperone to overcome the point mutation bias and shift stoichiometry towards $(\alpha4)_2(\beta2)_3$. Thus, ADNFLE provides a relatively simple model of how changes in α4β2 stoichiometry and trafficking contribute to disease [6].

Nicotine dependence is more complex than ADNFLE, although selective upregulation of $(\alpha4)_2(\beta2)_3$ receptors is certainly involved. Several brain regions express α4, α5 and β2 subunits and assemble α4β2α5 receptors including the substantia nigra pars compacta, subthalamic nucleus, medial habenula, prefrontal cortex, and hippocampus [7]. Receptors containing α5 also play a part in nicotine self-administration and nicotine withdrawal [8-9]. These receptors are also important for dopamine release and attention tasks [7, 10-11].

The α5α4β2 receptors are more permeable to Ca2+ than α4β2 receptors and have a higher sensitivity to nicotine [12]. Relative to $(\alpha4)_3(\beta2)_2$, the α5α4β2 receptor exhibits a higher sensitivity to acetylcholine (ACh), has increased Ca^{2+} permeability and may be resistant to upregulation by nicotine [13-14]. The α5 subunit does not participate in functional agonist binding sites, and it may serve as an auxiliary subunit that modulates nAChR function when coassembled with other α and β subunit isoforms (see figure 1).

nAChRs containing the α5 subunit are especially interesting because genome wide association studies and candidate gene studies have identified polymorphisms in the α5 gene that are linked to an increased risk for nicotine dependence, lung cancer, and/or alcohol addiction [15-17]. We have chosen to examine the only known coding-region polymorphism. The single nucleotide polymorphism (SNP), rs16969968, encodes an aspartic acid to asparagine mutation at position 398 in the flexible, intracellular loop that connects two transmembrane domains (the M3-M4 loop) of the human α5 protein. This mutation, α5D398N, is of interest because of its association with increased risk for nicotine dependence [18]. It was hypothesized that the M3-M4 loop localization of the D398N mutation may contribute to changes in intracellular trafficking or localization of the mutant protein. Live-cell high resolution fluorescence microscopy techniques have been used to study changes to α4β2 receptor stoichiometry, trafficking and pharmacology. We proposed to use similar techniques to examine behavior of this receptor after inclusion of an α5 or α5D398N subunit.