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Quantitative Molecular Imaging of Neuronal Nicotinic Acetylcholine Receptors in the Human Brain with A-85380 Radiotracers

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Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) have been implicated in a spectrum of cognitive functions as well as psychiatric and neurodegenerative disorders, including tobacco addiction and Alzheimer's Disease. The examination of neuronal nAChRs in living humans is a relatively new field. Researchers have developed brain-imaging radiotracers for nAChRs, with radiolabeled A-85380 compounds having the most widespread use. We provide a brief background on nAChRs, followed by a discussion of the development and application of A-85380 radiotracers in human imaging studies. We describe potential future studies using nicotinic receptor radioligands for the study of tobacco addiction, including the mechanism of action of the smoking-cessation therapy varenicline. Throughout this review, we focus on the significant potential that resides in the identification and quantification of nAChRs in the living human brain.

Keywords

Nicotinic acetylcholine receptors; positron emission tomography; single-photon emission computed tomography; human brain imaging; tobacco dependence; varenicline; 2FA; 5IA; A-85380

I. INTRODUCTION

A. Acetylcholine, Nicotine, and Neuronal Nicotinic Receptors

Acetylcholine is the endogenous neurotransmitter known to bind to muscarinic and nicotinic acetylcholine receptors (nAChRs). The endogenous functions of acetylcholine are believed to be important for cognitive behaviors such as attention, motivation, memory, reward, and awareness (i.e. consciousness). Nicotine, on the other hand, is an exogenous agent, believed to be the main psychoactive component in cigarettes leading to reward and addiction [1]. Imitating the basic functions of acetylcholine, nicotine mediates functional outcomes through its interactions with neuronal nAChRs. Its primary role is to modulate release of various neurotransmitters including dopamine [2]. Illnesses associated with alterations in the distribution of nAChRs in the human brain include addiction, anxiety, Parkinson's Disease,

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attention deficit hyperactivity disorder, schizophrenia, major depressive disorder, epilepsy, autism, and Alzheimer's Disease (for review: [3]).

B. nAChR Structure and Function

Nicotinic receptors in the central nervous system are pentameric ligand-gated ion channels composed of α (2–10) and β (2–4) protein subunits (for review: [2, 4]). The most common nicotinic receptor in the human brain is made from the α4β2* subtype, found in the majority of neurons throughout the brain [5, 6]. In particular, α4β2* receptors are expressed on dopaminergic neurons projecting to the ventral striatum and are developmentally regulated [7]. The α4β2* subunits play critical roles in the modulation of dopamine release in the nucleus accumbens, an effect known to be a hallmark of drug reward [8]. The modulation of neurotransmitter release is mediated through a ligand-receptor interaction most notably due to nicotine binding to α4β2* nAChRs. As a consequence, the ion channel opens allowing sodium and calcium to permeate the cell membrane, thereby triggering the release of neurotransmitters [2]. Nicotinic receptor-mediated release of neurotransmitters can occur through both pre- and post-synaptic mechanisms. In general, while post-synaptic mechanisms require an action potential to be propagated, pre-synaptic release is mediated through the modulatory role of nAChRs (without the requirement of an action potential). Modulation of neurotransmitter release through a presynaptic mechanism is proposed to be the major function of nicotinic receptors in the brain (for review: [2, 4]).

C. Anatomy and Age Dependence of Neuronal nAChR Expression

Subunit expression has been shown to be anatomically specific and age dependent. High-affinity α4β2* nicotinic receptors are located throughout the human brain, with relative densities: thalamus > basal ganglia > cerebral cortex > cerebellum [9]. Reduction in binding of radiolabeled nicotine and epibatidine (a naturally-occurring frog poison with high affinity for the α4β2* nAChR ligand) has been observed in the frontal, temporal, and cerebellar regions of older individuals, signifying reductions in α4β2* nAChR density with age [9, 10].

D. The Behavioral Functional Significance of α4β2* nAChRs in Animal Studies

With the innovation of genetic engineering, researchers have started to evaluate the functional roles of α4β2* nicotinic receptors in the brain. Recent evidence suggests that α4β2* nAChRs are associated with learning and executive functions, particularly associated with nicotine reinforcement behavior. By "knocking-out" either the α4 or β2 nAChR subunit in the mouse genome, researchers have demonstrated a loss of (i) nicotine-mediated high-affinity binding, (ii) nicotine self-administration behavior, and (iii) nicotine-induced dopamine release in reward centers of the brain [11–13]. The reduction in nicotine self-administration behavior could then be 'rescued' through selective α4 or β2 subunit re-expression in localized reward-mediating centers of the mouse brain [11]. Growing evidence also implicates these receptors in a host of other behaviors, including passive avoidance, locomotor sensitization, tolerance, and conditioned placed preference (for review: [14]). α4β2* nicotinic receptors may also interact with accessory subunits, like α5, to influence attentional performance [15]. Furthermore, re-expression of β2-containing nicotinic receptors in the ventral tegmental area can rescue complex cognitive behaviors, including exploration, navigation, and transition probability [16]. The findings suggest that endogenous acetylcholine may influence cognitive function through its interactions with β2-containing nicotinic receptors, which presents a rich area of future research.

In the human population, less is known about how the presence or absence of α4β2* nAChRs influences cognitive function (reviewed further below). It has been shown, however, that patients with Alzheimer's Disease, Parkinson's Disease, and Lewy-Body

Dementia have reductions in nAChR density in the caudate and putamen [17]. Whether these effects are mediated through the selective elimination of α4β2* nicotinic receptors or through the loss of neurons containing these receptors needs further investigation. Given that cholinesterase inhibitors are reported to be effective for the treatment of AD [18], cholinergic systems may be important for mediating cognitive functions related to such disorders.

II. MOLECULAR IMAGING OF HUMAN NEURONAL NACHRS

A. Early nAChR Imaging

Imaging of nAChRs in the living human brain is a relatively new field. The first human brain-imaging studies to evaluate nAChR availability were performed with positron emission tomography (PET) and [^{11}C]nicotine, which binds with high affinity to the α4β2* nAChR [19, 20]. However, this method was shown to be limited in its ability to quantify specific and non-specific binding, and demonstrated cerebral-blood-flow dependence of binding [19, 21]. Other compounds structurally similar to nicotine like [^{11}C]ABT-418 and [^{11}C]N-methyl cytisine (a plant alkaloid) were found inadequate for reasons including low brain uptake and rapid washout [22]. Epibatidine, a ligand with very high affinity for the α4β2* nAChR, reportedly has excessive toxicity and binding to non-α4β2* nAChRs at high doses [23, 24].

B. Radiotracers Based on A-85380

In 1996, A-85380 was first synthesized at Abbot Laboratories in a program to develop drugs that bind specifically to nAChRs [25]. In 1998, Koren *et al.* synthesized halogenated analogs of A-85380, including [^{18}F]2-F-A-85830 (2FA), suitable for PET, and [^{123}I]5-I-A-85830 (5IA) and [^{125}I]5-IA-85830, suitable for single-photon emission computed tomography (SPECT) and autoradiography, respectively [26]. Early brain-imaging studies were performed in the baboon [27] and subsequently in the human [28, 29].

C. Pharmacology and Toxicology of A-85380

A-85380 binds with high affinity to α4β2* nicotinic receptors [30] and, at a sufficient dose, can maintain self-administration behavior [31]. Mukhin *et al.* (2000) demonstrated that the Ki values for A-85380 binding at 22°C range from 0.017 nM – 320 nM, depending on the nAChR subtype [32]. A-85380 has the highest affinity for the α4β2* nAChR, as compared with all other receptors. A-85380 has greater ED50 values than those of nicotine for inducing convulsions [27] and requires significantly higher doses to elevate blood pressure. While A-85380 and epibatidine have similar affinities for nicotinic receptors, differences have been observed between the two compounds, including a lower binding in the thalamus for epibatidine as compared with A-85380 [33]. The differences are hypothesized to relate to differences in lipophilicity and/or nonspecific binding [33]. After the addition of the ^{18}F or ^{123}I atom to the A-85380 compound, only small differences (less than a factor 2) in binding affinity were observed for α4β2* nAChRs [26–32]. Drug-interaction studies demonstrate that the A-85380 radioligands are displaced by cytisine and epibatidine [32, 33]. A-85380 compounds showed lower toxicity than epibatidine and the radiolabel is without effect in the Ames test for mutagenicity [27]. For these reasons, 2FA and 5IA are the radiotracers of choice in human brain-imaging studies.

D. Kinetics and Anatomical Distribution of Radiolabeled A-85380

Shortly after radiolabeled A-85380 was first synthesized, time-activity curves and regional distributions following intravenous bolus injection of the radiotracer were reported for the mouse, rat, primate, and human brains [29, 34–40]. Radiotracer uptake was found to be highest in the thalamus followed by superior colliculus, hippocampus, and cerebellum, in

that order, with peak uptake around 30–60 minutes [29, 35, 40]. Whole-body distribution demonstrated high radioactive concentrations immediately after bolus injection of 2FA and 5IA in the kidneys, bladder, liver, and intestines, with mean residence times ranging from minutes to hours, depending on the organ [28, 29, 41–43]. 5IA is reported to have faster kinetics than 2FA [44, 45]. In a two-tissue compartment model, the distribution volumes for 5IA and 2FA in the thalamus are 51.4 and 15.43, respectively [44, 45]. As discussed by the investigators [44], the faster kinetics of 5IA versus 2FA may relate to 5IA's higher lipophilicity or affinity for α4β2* nAChRs. Regional distributions between 2FA and 5IA are similar and reported to be highly correlated [44]. The advantage of 5IA is faster kinetics, while the advantage of 2FA is the better resolution of PET imaging compared with SPECT. Additional studies have demonstrated that over 70% of both 2FA and 5IA is cleared from the system within 24 hrs [29, 43]. Fujita *et al.*, 2003 calculated the average clearance rate for 5IA to be equal to 1.49±0.52/min [45].

E. Experimental Use of Radiolabeled A-85380

(i) Sex, Age, and Cognitive Functions and α4β2* nAChR Density—Researchers have studied the relationships between sex, age, and cognitive functions and α4β2* nAChR density using 2FA-PET and 5IA-SPECT. Cosgrove *et al.* evaluated sex differences in nAChR density in the brains of healthy nonsmoking women and men using 5IA-SPECT [46]. They demonstrated that neither sex nor phase of menstrual cycle is a significant predictor of α4β2* nAChR density. However, sex-dependent differences were observed in the rate of radiotracer metabolism and plasma-protein binding [46].

Mitsis *et al.* examined 47 subjects using 5IA-SPECT, and reported an age-related decline in α4β2* nAChR density in 7 of the 8 brain regions studied (ages 18–85 (average age = 46 ± 22)) [47]. In contrast, Ellis *et al.* examined 26 healthy participants (aged 21–83; average age = 54 ± 20) using 2FA-PET, and demonstrated no association between age and α4β2* nAChR density [48]. Future studies are needed to resolve this disparity, as the difference between the average ages of the groups does not appear to be the mediating factor. In the Ellis *et al.* study, α4β2* nAChR density and cognitive measures were assessed. No associations were found between 2FA distribution volumes and working memory, attention, language, executive function, visual-spatial ability, verbal learning, or verbal memory. In subjects with mental illness or cognitive impairment, however, abnormalities in α4β2* nAChR density have been observed. In a study of patients with post-traumatic stress disorder, Czermak *et al.* reported increased α4β2* nAChR density in the mesiotemporal cortex and a significant positive correlation between re-experiencing symptoms and 5IA distribution volume [49]. In a 5IA-SPECT study of patients with amnestic mild cognitive impairment (MCI), Terriere *et al.* reported reduced α4β2* nAChR density in the medial temporal cortex [50]. Studies of Alzheimer's Disease patients with 5IA by O'Brien *et al.* and Terriere *et al.* found reduced α4β2* nAChR densities in the brain [51, 52]; however Ellis *et al.*, in a 2FA-PET study, found no abnormality in early Alzheimer's Disease, no correlation between α4β2* nAChR density and cognitive measures [53], and no treatment effect or correlation between α4β2* nAChR density and galantamine-induced improvement in cognitive function [54]. Since galantamine could indirectly increase acetylcholine through acetylcholinesterase inhibition [55], the latter finding would suggest that either (i) higher doses of galantamine are required to influence these relationships, (ii) modulation of the levels of acetylcholine through galantamine exposure may not be enough to modify the density of α4β2* nAChR, or (iii) the method is not sensitive enough for detection of these differences. Future longitudinal studies with 5IA-SPECT and 2FA-PET are required to resolve the role of nAChR density in Alzheimer's Disease.

(ii) Cigarette Smoking Studies Using Radiolabeled A-85380—The mechanisms of tobacco addiction remain elusive. However, researchers have hypothesized that smoking-induced modifications of α4β2* nAChR density may be a mediating factor leading to continued use. A study by our group demonstrated the effect of cigarette smoking on α4β2* nAChR occupancy, showing that smoking causes displacement of 2FA for prolonged periods of time (i.e., at least several hours) [56]. Dose-dependent reductions in 2FA displacement were observed by both controlling (i) the number of puffs smoked during the experiment and (ii) the concentration of nicotine in the smoked cigarette [57]. These findings suggest that nicotine mediates 2FA displacement by occupying α4β2* nAChRs [57]. Several authors, using 5IA-SPECT and 2FA-PET, have shown that habitual cigarette smoking is associated with up-regulation of α4β2* nAChRs [58–62]. The nAChR density returns to normal after a prolonged abstinence of weeks to months [60, 61]. Taken together, these results suggest that exposure to cigarette smoke, most likely through the effects of nicotine, influences α4β2* nAChR density in the human brain. The functional role of nAChR density and its association with continued tobacco consumption needs further investigation.

(iii) Genetic Variability Influencing nAChR Density—Using imaging techniques, researchers have started evaluating genetic influences on α4β2* nAChR density in the human brain. A recent study by Picard *et al.*, using 2FA-PET, studied subjects with autosomal-dominant nocturnal frontal-lobe epilepsy (ADNFLE), a hereditary epilepsy associated with mutations in the genes for the α4 and β2 nicotinic subunits [63]. The data revealed brain-region-specific abnormalities in α4β2* nAChR density in ADNFLE patients, including increases in the mesencephalon, pons, and cerebellum, and a decrease in the right prefrontal cortex [63]. The latter abnormality was associated with hypometabolism in the right orbitofrontal cortex in the same patients examined with [^{18}F]-fluorodeoxyglucose (FDG) PET, compatible with focal epilepsy involving the frontal lobe.

III. POTENTIAL OF NICOTINIC RECEPTOR IMAGING FOR SMOKING RESEARCH

Imaging with 5IA-SPECT and 2FA-PET has only recently started to have an impact in the fields of psychiatry and neurodegenerative disease. We now discuss possible studies that could be performed with these radiotracers in the area of smoking research.

A. The Therapeutic Mechanisms of Nicotine Replacement Therapies

The nicotinic-receptor partial agonist, varenicline (tradename: Chantix), has shown effectiveness in smoking cessation studies, with drug-assisted quit rates as high as 40–50% (versus 10–20 % for placebo). Varenicline is commonly prescribed for smoking-cessation therapy in humans, with nearly 11 million people having been given this medication. However, little is known about its mechanism of action in the human brain. While animal studies have provided significant evidence that varenicline acts as a partial agonist on high affinity α4β2* nAChRs [64], this property has not yet been demonstrated in humans. Varenicline may block the binding of nicotine to nAChRs, thus partially inhibiting the release of neurotransmitters, such as dopamine, in reward centers of the brain. This mechanism, observed in animal studies, is believed to mediate diminished withdrawal effects from smoking cessation and inhibit further smoking-related reward, thus facilitating smoking cessation. It is important to determine whether these effects are also observed in humans. Studies using radiolabeled A-85380 and radioligands for dopaminergic and other neurotransmitter systems may illuminate (i) the pharmacological action of varenicline in the human brain, (ii) the neurochemical mechanism mediating varenicline-induced decrease in tobacco withdrawal symptoms and reward, and (iii) the long-term effects of varenicline on

the density of α4β2 nAChR in the human brain. Furthermore, as the radiolabeled A-85380 method can quantify nAChR densities throughout the brain, which appear to correlate with the motivation to self-administer nicotine [65], brain-imaging studies with 2FA-PET or 5IA-SPECT may be predictive of success for smoking cessation therapies.

B. Consequences of Developmental Exposure to Cigarettes

Cigarette smoking is clearly known to negatively affect the health and wellbeing of humans [66, 67]. One particular population of subjects that is at risk consists of individuals who are exposed to maternal cigarette smoking during pregnancy [68]. Currently, over 700,000 newborns are exposed to maternal cigarette smoking each year in the United States [69], yet little is known about its consequences on the offspring. A recent study by Lotfipour *et al.* has demonstrated that prenatal exposure to maternal cigarette smoking is associated with increased substance use and also modifications of the orbital frontal cortex, as evaluated through structural magnetic resonance imaging [70]. A subsequent study by the same group suggests that polymorphisms in the α6 nAChR subunit may increase substance use as well as influence the volume of striatum [71]. The findings suggest a gene (α6 nAChR) by environment (prenatal cigarette exposure) interaction associated with an increase in the likelihood for drug experimentation. Abnormalities in nAChR densities could potentially mediate the structural and functional changes observed in both humans and animals [72–76]. No studies have demonstrated whether *in utero* cigarette exposure influences nicotinic-receptor-densities in the adult human brain. However, one human study in the aborted fetus [72] and several animal studies in the rodent and primate have shown effects of development exposure to tobacco or nicotine on nAChR density, with the animal findings demonstrating long-lasting consequences [75–79]. Based on animal and human studies, one might expect relatively higher nAChR density in humans developmentally exposed to cigarette smoking, with a positive relationship between nAChR density and the severity of neuroanatomical and behavioral abnormalities [17, 72, 75–77, 80, 81]. Future studies using radiolabeled A-85380 could evaluate the consequences in humans of *in utero* exposure to maternal cigarette smoking. It is possible that such studies will demonstrate the consequences of maternal cigarette smoking on the developing brain and potentially identify mechanisms leading to disorders associated with prenatal exposure to maternal cigarette smoking. Such studies could only be performed in adult humans (18 years or older) or in non-human-primates, and will require careful design to minimize confounding factors [82].

IV. SIGNIFICANCE OF MOLECULAR IMAGING OF HUMAN NACHRS

The value of imaging techniques that can quantify the nAChR density and that of other receptors in the brain is great. The techniques we have described may improve our understanding of the pathophysiology and longitudinal progression of diseases associated with nAChRs; these include tobacco addiction and degenerative disorders including Alzheimer's Disease. For neurodegenerative diseases, the resulting understanding may lead to improved methods for early and specific diagnosis prior to the onset of severe symptoms, allowing early treatment and potentially facilitating specific treatment tailored to the neuroreceptor and genetic profile of the individual patient. Treatment monitoring using neuroreceptor imaging could potentially improve the efficacy of treatment, much as metabolic imaging of malignancies allows clinicians to adjust therapies to the response of the disease to treatment [83]. Similarly, for tobacco addiction and potentially other substance-abuse disorders, the brain imaging techniques described above may also facilitate tailoring therapies and treatment monitoring specifically to the neuroreceptor and genetic profile of the individual patient.

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