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INVITED REVIEW

Activities of nicotinic acetylcholine receptors modulate neurotransmission and synaptic architecture

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Abstract

The cholinergic system is involved in a broad spectrum of brain function, and its failure has been implicated in Alzheimer's disease. Acetylcholine transduces signals through muscarinic and nicotinic acetylcholine receptors, both of which influence synaptic plasticity and cognition. However, the mechanisms that relate the rapid gating of nicotinic acetylcholine receptors to persistent changes in brain function have remained elusive. Recent evidence indicates that nicotinic acetylcholine receptors activities affect synaptic morphology and density, which result in persistent rearrangements of neural connectivity. Further investigations of the relationships between nicotinic acetylcholine receptors and rearrangements of neural circuitry in the central nervous system may help understand the pathogenesis of Alzheimer's disease.

Key Words: *cholinergic system; nicotinic acetylcholine receptors (nAChRs); Alzheimer's disease (AD); synaptic transmission; synaptic plasticity; synaptic morphology; dendritic spine remodeling; cognition*

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Nicotinic receptors in the central nervous system (CNS) are candidate targets for the treatment of dementia

Dementia is one of the biggest global public health challenges. Accordingly, over 40 million people suffer from dementia globally, and this number is expected to double by 2030 and more than triple by 2050 (Alzheimer's disease International, World Alzheimer Report 2014). Alzheimer's disease (AD) is the most common type of dementia, and accounts for an estimated 60 to 80% of cases.

Loss of cholinergic neurons is a pathological hallmark of AD, and is also assessed in human imaging studies (Nordberg et al., 2010). Cholinergic neurons of the nucleus basalis of Meynert and the medial septum innervate the cerebral cortex and the hippocampus, respectively, and are involved in cognition. Hence enhancement of cholinergic transmission with acetylcholinesterase inhibitors ameliorates cognitive deficits in AD patients (Rogers et al., 1998). Consistently, deprivation of cholinergic transmission by either genetic disruption of acetylcholine receptors or denervation of cholinergic nerve fibers severely impairs learning and memory in animal experiments (Champtiaux and Changeux, 2004; Wess, 2004).

The cholinergic system also plays pivotal roles in other psychiatric disorders (Scarr et al., 2013). For example, an imbalance between cholinergic and dopaminergic systems contributes to the pathophysiology of schizophrenia, and adjunctive use of acetylcholinesterase inhibitors alleviates visual hallucinations (Patel et al., 2010). In contrast, acetylcholine receptor antagonism ameliorates depressive symptoms in major depression and bipolar disorders (Drevets et al., 2013).

The neurotransmitter acetylcholine transduces signals through muscarinic and nicotinic acetylcholine receptors. Nicotinic acetylcholine receptor (nAChR) comprises five subunit polypeptides. Brain-type nAChRs consist of heteromeric combinations of α2–10 and β2–4 subunits, as well as α7 homopentamers; $\alpha4\beta2^*$ and α7 nAChRs are predominantly expressed in the brain (an asterisk indicates that the receptor may include other subunits than the described ones). In the pathophysiology of AD, amyloid β (Aβ), the peptide in senile plaques characteristic of AD, binds with high affinity to α7 nAChRs, and impairs their channel functions. The selective vulnerability of nAChR-expressing neurons to Aβ toxicity may lead to the failure of cholinergic neurotransmission and cognitive dysfunctions in AD (Dineley, 2007).

Several nAChR-targeting compounds have been developed to relieve neuropsychiatric symptoms in AD patients. However, clinical trials of compounds that target $\alpha4\beta2^*$ and α7 nAChRs (**Table 1**) have produced discouraging results (see review, Hurst et al., 2013). For example, varenicline, a partial agonist of $\alpha4\beta2^*$ nAChR, is the only clinically available nAChR-targeting drug, and was approved for smoking cessation, but failed to improve cognition in AD patients (Kim et al., 2014).

The failure of nAChR-targeting therapy for AD may be at-

Table 1 Development of nicotinic acetylcholine receptor (nAChR) ligands for the treatment of Alzheimer's disease and cognitive disorders

Code name	Generic name	Company	Molecular mechanism	Indication	Development status	Notes
EVP-6124, MT-4666	Encenicline hydrochloride	FORUM Pharmaceuticals	α7 nAChR agonist	Alzheimer's disease	Phase 3	A phase 3 program called cognitive Alzheimer's disease is set to run through 2016.
ABT-126		AbbVie	α7 nAChR agonist	Alzheimer's disease	Phase 2	ABT-126 is also in phase 3 development for cognitive impairment associated with schizophrenia.
AZD-3480, TC-1734	Ispronicline	Targacept	α7 nAChR agonist	Alzheimer's disease	Phase 2	Phase 2b clinical trial in Alzheimer's disease does not show superiority of TC-1734 over donepezil in July, 2014.
DMXBA, GTS-21	DMXB-anabaseine	CoMentis	α7 nAChR partial agonist	Alzheimer's disease	Phase 2	
AQW051		Novartis	α7 nAChR partial agonist	Cognitive impairment associated with schizophrenia	Phase 2	Phase 2 trial in mild Alzheimer's disease was discontinued.
ABT-560, ABT-PR2		Abbott, NeuroSearch	α4β2 nAChR agonist	Cognitive disorders	Phase 1	
CP-526555-18	Varenicline tartrate	Pfizer	α4β2 partial nAChR agonist	Alzheimer's disease	Discontinued	Development of varenicline for Alzheimer's therapy was discontinued in 2011, based on negative results from phase 2 trial conducted in South Korea.
TC-5619	Bradanicline	Targacept	α7 nAChR agonist	Alzheimer's disease	Discontinued	In 2013, Targacept announced negative results for phase 2b clinical trials of schizophrenia, and decided not to pursue further development for the treatment of either schizophrenia or Alzheimer's disease.
MEM 3454, R3487, RG3487, RO5313534	Facinicline hydrochloride	Roche	α7 nAChR partial agonist 5-HT_3 antagonist	Alzheimer's disease	Discontinued	
SSR 180711		Sanofi	α7 nAChR partial agonist	Alzheimer's disease	Discontinued	
AZD-1446, TC-6683		AstraZeneca	α4β2 nAChR agonist	Alzheimer's disease	Discontinued	
A-87089, ABT-089	Pozanicline hydrochloride	Abbott	nAChR partial agonist	Alzheimer's disease	Discontinued	
A-81418, ABT-418		Abbott	nAChR agonist	Alzheimer's disease	Discontinued	
ABT-107, ABT-PR1		Abbott, NeuroSearch	α7 nAChR agonist	Cognitive disorders	Discontinued	In 2009, development of ABT-107 was discontinued in phase 1 trials.
AR-R23465XX, AZD-0328		AstraZeneca	α7 nAChR agonist	Alzheimer's disease	Discontinued	In 2009, AstraZeneca discontinued the development of AZD-0328 as a potential treatment option for Alzheimer's disease in phase 2 trials in Europe.

Drug information was sourced from Alzforum, the Schizophrenia Research Forum, and a recent review article (Hurst et al., 2013). This source was amended upon availability of more accurate information on company websites. 5-HT_3: 5-Hydroxytryptamine 3.

tributed to several reasons. First, the expression of nAChRs is down-regulated in the brains of AD patients (Nordberg et al., 2010). Moreover, the expression of α4β2* nAChRs is decreased in these patients, especially in affected brain areas, and appears to be correlated with the severity of cognitive impairment and the amount of amyloid deposition (Sabri et al., 2008; Okada et al., 2013). Conditions relating to α7 nAChRs have been more controversial, with reports of decreased (Burghaus et al., 2000), stable (Ikonomovic et al., 2009), and increased (Nordberg, 2001) α7 nAChR expression in AD patients. Second, tested compounds have insufficient potencies, and under conditions of decreased nAChR subunit expression, these reportedly activate only a small fraction (about 10%) of nAChRs (Kim et al., 2014). Third, adverse effects of nAChR-targeting compounds, such as gastrointestinal and CNS-related side effects (Hurst et al., 2013), may limit therapeutic doses of compounds. To circumvent these side effects, partial agonists for nAChRs have been developed with the expectation of broader therapeutic windows than full agonists (**Table 1**). Nonetheless, a better understanding of the relationships between nAChRs and plasticity or rearrangements of neural circuitry may be required for the successful treatment of AD.

Nicotinic activity modulates plasticity and various synaptic transmissions

In addition to acetylcholine, major neurotransmitters in

Table 2 Nicotinic acetylcholine receptors modulate synaptic architecture

Receptor type	Outcome	Brief description	Setting	Reference
α7 nAChR	Presynaptic enlargement	Inactivation of axonal α7 nAChRs enlarges glutamatergic presynaptic boutons.	Cultured cortical neurons	Lin et al. (2010)
α7 nAChR	Glutamatergic synapse formation	Activation of α7 nAChRs increases the number of glutamatergic synapses.	Hippocampal slice and dispersed cell culture	Lozada et al. (2012a)
α4β2* nAChR	Postsynaptic enlargement	Activation of presynaptic α4β2* nAChRs induces dendritic spine enlargement.	Cultured hippocampal neurons	Oda et al. (2014)
β2* nAChR	Induction of dendritic spines	Activation of β2* nAChRs elevates the number of spines.	Hippocampal slice and dispersed cell culture	Lozada et al. (2012b)
β2 nAChR	Dendritic arbor complexity and density of spines	Basal dendritic size and complexity are low and homogeneous in mice lacking β2 nAChR.	Mutant mice lacking β2 nAChR	Ballesteros-Yanez et al. (2010)
α7 nAChR	Accumulation of AMPARs on dendritic spines	Activation of postsynaptic α7 nAChR induces the stabilization and accumulation of AMPARs on dendritic spines.	Hippocampal slice and dispersed cell culture	Halff et al. (2014)

the CNS include glutamate, GABA, dopamine, serotonin, and noradrenaline. These molecules are involved in various physiological processes, such as learning, memory, mood, appetite, anxiety, and fear. Corresponding synaptic transmissions mediated by these neurotransmitters are known to be modulated by nAChRs, which are localized to pre- and postsynaptic membranes, and have been demonstrated in multiple brain regions including the cerebral cortex, striatum, hippocampus, thalamus, and cerebellum (Gotti et al., 2006).

Presynaptically, changes in the probabilities of transmitter release from synaptic vesicles have been reported (Placzek et al., 2009). Among these, spontaneous release of glutamate, as reflected in the frequency of miniature excitatory postsynaptic currents, is enhanced by the activation of presynaptic nAChRs. In addition, stimulus-dependent glutamate release is facilitated by the activation of nAChRs (Radcliffe et al., 1999; Rousseau et al., 2005). The mechanism underlying these processes reportedly involves increased presynaptic calcium levels following the actions of nAChRs (Placzek et al., 2009).

Long-term potentiation (LTP), a well-known form of synaptic plasticity in glutamatergic synapses, is modulated by postsynaptic nAChRs. Postsynaptic nAChR activities in pyramidal neurons boost the induction of LTP in hippocampal slices (Ji et al., 2001). Induction of LTP depends on the precise timing of presynaptic action potentials relative to postsynaptic depolarization (Dan and Poo, 2004). Coincidence or precedence of mild presynaptic stimulation with nAChR-mediated action potentials also leads to the induction of LTP (Ge and Dani, 2005; Gu and Yakel, 2011).

The excitability of GABAergic interneurons is reportedly modulated by postsynaptic nAChRs, and release of GABA from interneurons in turn modulates the excitability of pyramidal neurons, which further influence the tone of neural circuits (Placzek et al., 2009). Local treatments of interneurons with acetylcholine activate nAChRs, resulting in the burst firing of action potentials of interneurons (Frazier et al., 1998; Alkondon et al., 1999).

Dual modulatory mechanisms of synaptic transmission through pre- and postsynaptic nAChRs may influence brain activity and behavior. Thus, the impairment of nAChR functions in AD results in the failure of broad spectrum of synaptic transmissions.

Activation of nAChRs leads to rearrangements of synaptic architecture

Although nicotine rapidly modulates synaptic transmissions through nAChRs, exposure to nicotine produces persistent cravings for nicotine after long periods of abstinence (Dani and De Biasi, 2001). However, the mechanisms that relate rapid gating of nAChR to persistent changes in brain function have remained elusive. Recent evidence indicates that nAChR activities affect synaptic morphology and density, and neurotransmitter receptor distributions (**Table 2**).

Excitatory synapses in the CNS are usually formed on dendritic spines, which are small protrusions on dendrites. Dendritic spines receive glutamatergic inputs from apposing presynaptic termini, and are morphologically modulated by released glutamate (Okamura et al., 2004; McKinney, 2010). Enlargement and shrinkage of these spines are associated with LTP and long-term depression, respectively (Tada and Sheng, 2006). Although neuronal α4β2* and α7 nAChRs have been shown to modulate glutamatergic neurotransmission, little is known of the accompanying alterations of synaptic architecture. In a recent study, nicotine was shown to induce dendritic spine enlargement through the activation of α4β2* nAChRs, which localize to presynaptic termini as indicated by super-resolution structured illumination microscopy (Oda et al., 2014). Moreover, nicotine induces acute *de novo* glutamatergic synaptogenesis via β2* nAChRs *in vitro* and *in vivo* (Lozada et al., 2012a). In the absence of β2 nAChRs, the density of spines is reduced (Ballesteros-Yanez et al., 2010) and excitatory synaptic loci shift from spines to dendritic shafts (Lozada et al., 2012a). These observations are consistent with the hypothesis that activation of α4β2* nAChRs influences the modulation and maintenance of synaptic architecture.

Chronic exposure to nicotine promotes glutamatergic synaptogenesis, which is mediated by α7 nAChRs (Lozada et al., 2012b). Moreover, significant reductions in numbers of glutamatergic synapses have been observed in α7 nAChR knockout mice, resulting in imbalances of excitatory/inhibitory inputs to neurons (Lozada et al., 2012b). In contrast, chronic inactivation of α7 nAChRs leads to increased numbers of presynaptic boutons by enhancing presynaptic N-methyl-D-aspartate receptor (NMDAR) function (Lin et

al., 2010), indicating that the desensitization of α7 nAChRs also contributes to changes in synaptic architecture.

Recently, the molecular mechanisms of nAChR-mediated modulation of synaptic function were demonstrated using super ecliptic pHluorin-tagged α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Ashby et al., 2004); surface insertion and internalization of AMPARs follows nicotinic stimulation (Halff et al., 2014). Postsynaptic morphological plasticity is often accompanied by changes in numbers of cell surface glutamate receptors, and stimulation of postsynaptic α7 nAChRs results in stabilization and accumulation of GluA1-containing AMPARs on dendritic spines (Halff et al., 2014). Hence, insertion and removal of synaptic AMPARs may be related to nicotine-induced modifications of synaptic structure and function, as was the case with glutamate-induced plasticity (Kessels and Malinow, 2009).

Current treatments for AD are limited to symptomatic relief and fail to prevent disease progression. Thus, AD treatments that produce persistent rearrangements of neural connectivity are required and may substantially ameliorate the pathogenesis of AD. Further investigations of the relationships between nAChR and persistent rearrangements of neural circuitry in the CNS may elucidate therapeutic targets within the CNS cholinergic system.

Conflicts of interest: *Oda A is an employee of Takeda Pharmaceutical Company Limited. The authors declare no competing financial interests.*

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