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Nicotinic ACh Receptors as Therapeutic Targets in CNS Disorders

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Abstract

The neurotransmitter acetylcholine (ACh) can regulate neuronal excitability by acting on the cys-loop cation-conducting ligand-gated nicotinic ACh receptor channels (nAChRs). These receptors are widely distributed throughout the central nervous system, being expressed on neurons and non-neuronal cells, where they participate in a variety of physiological responses such as anxiety, the central processing of pain, food intake, nicotine seeking behavior, and cognitive functions. In the mammalian brain, nine different subunits have been found thus far, which assemble into pentameric complexes with much subunit diversity; however the α7 and α4β2 subtypes predominate in the CNS. Neuronal nAChR dysfunction is involved in the pathophysiology of many neurological disorders. Here we will briefly discuss the functional makeup and expression of the nAChRs in the mammalian brain, and their role as targets in neurodegenerative diseases (in particular Alzheimer's disease), neurodevelopmental disorders (in particular autism and schizophrenia), and neuropathic pain.

Keywords

α7 receptor; α4β2 receptor; nAChR; cys-loop receptor

FUNCTIONAL ROLE of NICOTINIC ACh RECEPTORS IN THE BRAIN

The nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of cys-loop receptors (Fig. 1), which also includes the serotonin 5-HT_3, $GABA_A$ and $GABA_C$, and glycine receptors, and participate in a variety of physiological functions, including the

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regulation of neuronal excitability and neurotransmitter release [1–4]. The nAChRs are widely distributed throughout the peripheral and central nervous systems, as well as the immune system and various peripheral tissues [5–9]. In the mammalian brain, nine different nAChR subunits are known to exist (α2–7 and β2–4), which combine as either homo- or heteromeric complexes into multiple functionally diverse pentameric receptors [3, 10, 11]. The predominant subtypes functionally expressed in the brain are categorized as $\alpha7^*$ subunit-containing receptors (either homo- or heteromeric), or those composed of both α and β subunits, including the $\alpha4\beta2^*$ and $\alpha3\beta4^*$ subtypes [12–17]; the * denotes that these nAChRs can contain other α and β subunits as well.

The $\alpha4\beta2^*$ receptor subtype was initially found to be the major nAChR subtype in the brain (where it comprises 90% of the high affinity nicotine binding sites [16, 18]), while the $\alpha3\beta4^*$ nAChR is known primarily as a ganglionic receptor in the peripheral nervous system. The $\alpha3\beta4^*$ nAChR is also expressed in a variety of brain areas, including (but not limited to) the interpeduncular nucleus and medial habenula [16, 17]. In addition, the α2, α5, α6 and β3 subunits participate in nAChRs expressed in various brain regions, although they represent a minority population of the total.

The $\alpha7^*$ nAChR subunit is a particularly intriguing subunit as these receptors are expressed on a variety of cell types in the periphery, including immune cells [19–23] and neurons [24], as well as in brain regions that underlie learning and memory [25, 26]. Furthermore, these receptors are highly permeable to calcium, implicating them as significant modulators of intracellular signaling and neurotransmitter release from neurons. In the brain, the $\alpha7^*$ receptors are expressed on both neurons and non-neuronal cells [20], including (but not limited to) astrocytes, microglia, oligodendrocyte precursor cells, endothelial cells, and chondroitin sulfate proteoglycan NG2-expressing (NG2) cells [21–23, 27–30]. Expression of $\alpha7^*$ receptors in these non-neuronal cells suggests a possible role in brain innate immunity, inflammation, and neuroprotection [31, 32]. Immune cell expression of $\alpha7^*$ receptors has been shown to modulate inflammatory responses by regulating the production of inflammatory cytokines and chemokines [33, 34].

While the $\alpha7^*$ nAChR was initially thought to be functionally expressed as homomeric receptors, it has recently been shown to be capable of co-assembling with other subunits, which provides an explanation for the incongruent properties of *in situ* α7-containing receptors and *in vitro* expressed homomeric α7 receptors [35–39]. Initially, we found that α7 and β2 subunits co-assembled *in vitro* [14, 15, 40]; subsequently it was found that basal forebrain cholinergic neurons express functional α7β2 receptors with an enhanced sensitivity to the amyloid-β (Aβ) peptide associated with Alzheimer's disease [41] (see below).

In the brain, the nAChRs are expressed and function at the synapse (both pre- and postsynaptically) as well as extrasynaptically [13, 16, 42–44], and participate in nAChR-mediated postsynaptic responses [45]. While $\alpha7^*$ nAChR-mediated synaptic responses have previously been observed in hippocampal interneurons utilizing electrophysiological techniques [46, 47], optogenetic stimulation of direct cholinergic inputs to the hippocampus revealed new evidence for $\alpha4\beta2^*$ nAChR-mediated postsynaptic responses from

interneurons [48] and pyramidal cells [45]. Various subtypes of nAChRs have been shown to modulate synaptic transmission in other areas of the brain, including (but not limited to) the visual cortex, cortical interneurons, supraoptic nuclei, and thalamic nuclei [49–52]. In addition to presynaptic and postsynaptic locations in which they function to promote neurotransmitter release and excitability, nAChRs are also functionally expressed extrasynaptically where they participate in non-synaptic communication [4, 53]. Although most cholinergic presynaptic neurotransmitter terminals do not make direct postsynaptic contacts, they are able to release ACh. However the precise role that extrasynaptic nAChRs play in brain circuit excitability and plasticity remains to be determined.

Several layers of complexity contribute to the challenge of deciphering the role of nAChRs in brain circuit excitability and plasticity, as well as in disorders and diseases of the brain. Subunit composition directly contributes to nAChR channel permeability and kinetics, whereas subcellular localization within the neural network determines the precise contribution of a given nAChR population in a spatial- and time-dependent manner. The unique anatomical distribution of each nAChR subtype within the brain implicates particular ones in brain disease (Table 1). As such, the $\alpha7^*$ and non-$\alpha7$ nAChR subtypes are active targets for therapeutic development in neurodegenerative disease, neurodevelopmental disorders, and chronic pain. Below we will discuss these receptors as they are understood in the etiology and treatment of Alzheimer's disease, autism, schizophrenia, and neuropathic pain.

ALZHEIMER'S DISEASE

Neuronal nAChR dysfunction is involved in the pathophysiology of various neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) diseases. Here we will focus solely on AD since there are excellent recent reviews discussing the complexity between nAChR distribution within the nigro-striatal pathway, how nAChR functional contributions to this network, and PD pathophysiology [54].

Prevalence and causes

Alzheimer's disease (AD) is a neurodegenerative disease characterized by memory and cognitive loss, and represents the leading cause of dementia in people aged >60 years [55]. Sporadic, or non-inherited, forms of AD comprise the majority of diagnosed cases of the disease. However, identification of the genetic mutations that underlie the familial, or inherited, forms of AD have provided enormous insight into fundamental mechanisms, namely that the trigger for synaptic and neural network dysfunction is the aberrant accumulation of misfolded amyloid-β (Aβ).

Aβ is formed through proteolytic cleavage of its precursor protein, a type 1 membrane protein called amyloid precursor protein (APP). Aβ peptide length can vary from 38–43 amino acids in length depending on the γ-secretase C-terminal cleavage site and N-terminal posttranslational truncation events (Fig. 2A).

The nAChRs are integral to early AD cholinergic hypofunction. In addition to the complex biochemical processes involved in neuronal degeneration due to misfolded Aβ accumulation

and development of neurofibrillary tangles comprised of misfolded tau, the cholinergic deficit due to the loss of basal forebrain cholinergic neurons and production of ACh significantly contributes to early AD dementia [56]. The cholinergic deficit is evident by reduced choline acetyltransferase (ChAT) protein and activity, and vesicular ACh transporter (vAChT) protein. In presynaptic nerve terminals, ChAT synthesizes ACh, and the vAChT is responsible for the transport of ACh into synaptic vesicles for storage until exocytotic release into the synapse [57]. Acetylcholinesterases (AChE), resident within the synaptic cleft, hydrolyse ACh to rapidly terminate the availability of neurotransmitter and nAChR activation. Acetylcholinesterase inhibitors (Aricept, Exelon, Razadyne, Cognex) were the first FDA-approved therapies to treat the cognitive symptoms (memory loss, confusion, and problems with thinking and reasoning) of early AD that are thought to be due in part to the cholinergic deficit and are a first line of therapy today.

Brain regions associated with attention, spatial, and episodic memory lose cholinergic innervation in early AD; thus, cholinergic hypofunction is most evident in the neocortex and temporal lobes inclusive of the hippocampus [58]. The current model for the cholinergic deficit in AD posits that the septo-hippocampal pathway preferentially accumulates misfolded Aβ and tau, which leads to loss of the cholinergic phenotype, e.g., loss of cholinergic markers (ChAT, VAChT) and eventually cholinergic neurons from the basal forebrain nuclei through altered nAChR function and disruption of the nerve growth factor (NGF) trophic support system [59]. A compelling overlap exists between NGF-mediated signaling and the $\alpha7^*$ nAChR subtype as α7 nAChR activation also promotes the cholinergic phenotype [31]; thus, loss of $\alpha7^*$ nAChR function in particular likely contributes to early AD cholinergic hypofunction and cognitive deficits.

As briefly discussed above, nAChRs (primarily $\alpha4\beta2^*$ and $\alpha7^*$ subtypes) are expressed within the cholinergic forebrain nuclei, as well as at pre- and postsynaptic locations within their projection areas. For example, in the hippocampus, α4β2- and α7-containing nAChRs have been localized presynaptically and somato-dendritically on glutamatergic principal cells that drive synaptic plasticity; however, these nAChRs are also enriched on GABAergic interneurons that play a significant role in modulating principal cell activity [60]. Furthermore, nAChR subtypes within the basal forebrain are important players in modulating synaptic transmission and plasticity, learning and memory [45, 61].

Distinct nAChR subtypes are differentially affected in AD. The observation that Aβ preferentially accumulates in brain regions that are also enriched for $\alpha4\beta2^*$ and $\alpha7^*$ nAChRs may provide an important clue for the selective vulnerability of the hippocampus and neocortex to Aβ toxicity given the high affinity interaction between Aβ and these nAChRs [62, 63]. Importantly, $\alpha7^*$ nAChRs exhibit an exceptionally high Aβ affinity (picomolar range) [64], suggesting a physiological interaction that may influence synaptic transmission and plasticity [65–67], as well as contribute to Aβ-mediated synaptic neural network dysfunction (Fig. 2B) [66, 68]. While the extant literature appears contradictory, if one considers the stunning complexity of nAChR subunit stoichiometry, brain region, and subcellular localization, one must acknowledge that the most parsimonious interpretation of nAChR-Aβ experimental results is that Aβ can either activate or antagonize $\alpha4\beta2^*$ and $\alpha7^*$ nAChRs, depending on the anatomical microenvironment as well as the concentration,

length, and conformation of Aβ peptides[41, 69–76]. Given that *in vivo* Aβ constituents and conformations will be highly heterogeneous, while concentration will increase with disease state, it is likely that nAChR responses to the Aβ microenvironment will vary and be in constant flux. The next challenge is to decipher these dynamics to refine therapeutic strategies.

In advanced AD, it is unequivocal that muscarinic AChR levels remain relatively intact [77–79], and upwards of 50% of α4β2* nAChR binding sites are lost from neocortex and hippocampus compared to unaffected humans at similar age, and α7* nAChRs remain relatively stable in neocortex, although some reports vary [80, 81]. The status of nAChRs in mild cognitive impairment (MCI) due to probable AD [82] reflects a different dynamic in that ChAT activity and α7* nAChR expression levels have been reported to be elevated in the hippocampus and frontal neocortex [83–85]. In fact, individuals' levels inversely correlated with neuropsychological tests that are used to help diagnose AD (e.g., Global Cognitive Score and Mini-Mental State Examination) [85], suggesting that α7* nAChR expression in early AD is a reflection of cognitive reserve; (a term that refers to superior memory function for the stage of AD pathology seen with currently accepted biomarkers for AD staging [86, 87]).

With few exceptions [76], α4β2* nAChRs are antagonized by Aβ [41] and lost in early AD, whereas α7* nAChR-Aβ interaction may result in transient receptor activation [88, 89] and downstream signal transduction cascades that promote neuronal survival and function [67, 90] (however see [91]); a potential explanation for recent studies reporting that α7* nAChRs are preserved in early AD. However as Aβ concentration increases, receptor desensitization and *functional* down-regulation likely ensues with concomitant receptor *upregulation* to transiently repopulate the functional receptor pool [31, 62, 92]. This model is supported by reports that α7* nAChRs are upregulated in several AD patient cell types, including astrocytes and neurons [93–96], as well as in AD animal models [97], and neuronal cell cultures chronically exposed to Aβ [98]. Furthermore, the majority of α7* nAChR protein in AD brain is associated with Aβ [99, 100], which upon dissociation, can resurrect α7* nAChR function. While this suggests a potential therapeutic intervention, a recent *in vitro* study indicates that neuronal hyperexcitability may be an unintended consequence of therapeutic interventions that reverse neuronal Aβ-α7 nAChR interaction [98].

An additional level of complexity for the role of α7* nAChRs in the pathophysiology of AD stems from work focused on these receptors expressed by astrocytes. It is now recognized that astrocytes express α7* nAChRs [21, 22, 27] and participate in synaptic communication through intimate interactions with neurons [101–103]. A principal mechanism is through the release of gliotransmitters, such as glutamate, in response to astrocytic calcium elevations that can activate neuronal glutamate receptors. Recently, two independent studies [66, 68] demonstrated that biologically-relevant concentrations of Aβ elicited α7* nAChR-dependent calcium elevations in astrocytes and induced glutamate gliotransmission to activate neuronal glutamate receptors. Furthermore in AD mouse models for Aβ over-production and accumulation, tonic glutamate receptor [68] and spontaneous astrocytic calcium elevations [66] were of higher frequency compared to controls; observations that laid the foundation

for the newly explored hypothesis that a significant aspect of AD pathophysiology is epileptiform dysfunction [104, 105].

The cholinergic deficit in early AD has led to the development of drugs able to prevent ACh hydrolysis (e.g. AChE inhibitors). While these drugs do not change the course of the disease, AChE inhibitors improve memory and other cognitive functions throughout most of the duration of the disease. The pharmacological activity of these drugs suggests an effect beyond the mere increase of ACh levels; e.g. galantamine (Razadyne) has been shown to act also as a positive allosteric modulator (PAM) on $\alpha7^*$ and $\alpha4\beta2^*$ nAChRs [106, 107], and both donepezil (Aricept) and galantamine increase nAChR density [108]. Nonetheless, long-term clinical assessments indicate that the main effect of AChE drugs is symptomatic treatment with limited disease modifying actions [109].

The $\alpha4\beta2^*$ and $\alpha7^*$ nAChRs within the basal forebrain cholinergic system are important for the types of cognitive performance that are impaired in early AD. Thus, several subtype-selective agonists and partial agonists that target $\alpha4\beta2^*$ as well as $\alpha7^*$ nAChRs have been tested [110]. While study design and placebo effects may account for some of the variability, it is now evident that many AD clinical trials likely ‘fail’ due to enrollment of patients at advanced disease stages. There is an emerging consensus that the best strategy for AD clinical efficacy is to treat people during the earliest stages of disease [111]; e.g., prodromal Alzheimer’s or MCI due to AD [112]. The_current challenge is to reliably diagnose at such early stages using a combination of biomarkers, brain imaging, and cognitive testing [86, 113]. Currently in trial development (clinicaltrials.gov) targeting nAChRs for mild to moderate AD is EVP-6124 (FORUM Pharmaceuticals), also known as MT-4666 (Mitsubishi Tanabe Pharma Corporation), an $\alpha7$ nAChR partial agonist, varenicline (Pfizer), an $\alpha4\beta2^*$ nAChR partial agonist, and AZD-3480 (AstraZeneca), a partial agonist for $\alpha4\beta2$ and $\alpha2\beta2$ nAChRs [114]. Nicotine and acetylcholinesterase inhibitors (e.g. galantamine and donepezil) continue to be evaluated for efficacy in AD as well. Furthermore, since $\alpha4\beta2^*$ nAChRs are lost early in AD, $\alpha4\beta2^*$ nAChR single photon emission computed tomography (SPECT) and positron emission tomography (PET) radioligands are being developed and tested in clinical trials as potential diagnostic tools and biomarkers for AD staging. For example [^{123}I]5-I-A-85380 (sponsored by the National Institutes of Health) is being tested for use in SPECT and [^{18}F]XTRA (Johns Hopkins University) is currently being tested for use in PET.

The continued design and synthesis of nAChR ligands with the desired pharmacokinetics and pharmacodynamics to target the desired receptor population, in combination with continued elucidation of the disease-stage properties of nAChRs, may ultimately achieve the goal of developing an interventional tool to combat the loss of cholinergic basal forebrain connectivity in this most prevalent of the devastating neurodegenerative disorders.

NEURODEVELOPMENTAL DISORDERS

The nAChRs are widely expressed on both differentiated and undifferentiated CNS cells, and are known to influence development and modulate multiple pathways of neurogenesis

during brain development [115, 116]. Here we discuss the role of nAChRs in two of the most common neurodevelopmental disorders, autism and schizophrenia.

Autism

Autism is a neurodevelopmental disorder, also known as autism spectrum disorders (ASD), which is characterized by deficits in socialization and a lack of verbal as well as non-verbal communication with pronounced cognitive impairment [117, 118]. Neuropathological data suggests that abnormalities in the cholinergic system may be related to autism [119]. In a study carried out on adult post-mortem autistic brains using quantitative RT-PCR for measuring mRNA expression together with protein level expression and specific radioligand receptor binding, it was found that there was a decrease of 40–50% in the expression of the α3, α4, and β2 nAChR subunits in the cerebral cortex, while there was no change in the expression of the α7 subunit [120]. The reduced gene expression of the α4 and β2 receptor subunits in the cerebral cortex is a major feature of the neurochemical pathology of ASD [120]. If there is a loss of cholinergic tone in the autistic brain, then nAChR ligands that restore or improve cholinergic transmission may be used to compensate for such loss [121, 122]. Therefore agonists and PAMs for $\alpha4\beta2^*$ nAChRs may be employed to compensate for the loss of nAChR function in the autistic brain.

In contrast to this, it has also been suggested that nicotinic cholinergic antagonism may provide symptomatic relief in ASD [123]. This was based primarily on the low prevalence of smoking in the ASD population [124, 125], although this may due to the loss of expression of the $\alpha4\beta2^*$ nAChRs early in development and presumably loss of sensitivity to the rewarding properties of nicotine since the $\alpha4\beta2^*$ receptors are primarily responsible for the addictive properties of nicotine [126, 127]. In support of this notion, in a placebo-controlled pilot study with a nonselective and noncompetitive antagonist of the nAChRs, mecamylamine, it was found that while this drug was well tolerated, it lacked efficacy in treating autistic symptoms [128].

Recently it has been reported that AChE inhibitors (e.g. donepezil and galantamine), which, in general, increase cholinergic tone at the synapse [129], cause a pronounced improvement in typical symptoms associated with autism (e.g. verbal communication, eye to eye contact, and emotional responsiveness) in a single case report [130]. Similarly in a randomized, double-blind, placebo-controlled clinical trial, galantamine was shown to be safe and relatively effective in alleviating some autism-related symptoms (e.g. irritability and lethargy/social withdrawal) [131]. The lack of efficacy of the nAChR antagonist mecamylamine, combined with the effectiveness of galantamine and donepezil, suggest that nAChR agonists and PAMs (rather than antagonists) are more effective in providing symptomatic relief in autism and ASD. While further studies are needed to investigate and develop nAChR drugs as a treatment option for symptoms related to autism and ASD, the FDA-approved AChE inhibitors may provide symptomatic relief in the interim.

Schizophrenia

Schizophrenia is a neurodevelopmental disorder that is characterized by cognitive deficits and disordered thoughts that manifest as hallucinations, delusions, and paranoia.

Schizophrenia has both genetic and environmental etiologies, and has been previously genetically linked to dysfunction in the hippocampal nAChR system [132]. In contrast to ASD, the schizophrenia patient population exhibits a much higher prevalence for smoking than the general population which some consider to be a form of self-medication as it may normalize some of the cognitive and sensory deficits [133, 134]. Nicotine exposure (either through smoking, nicotine gum, nasal spray, or the patch) appears to improve or normalize sensory deficits in schizophrenia [134]; however due to the adverse health effects and toxicity of nicotine, other ligands acting on nAChRs may prove useful as a treatment option for symptoms related to schizophrenia.

Indeed the nAChR partial agonist, varenicline, improves cognition in schizophrenic patients [135, 136]. For example in a randomized, double-blind, placebo-controlled clinical trial for patients diagnosed with schizophrenia, varenicline improved some aspects of cognitive function (e.g. Digital Symbol Substitution and the Wisconsin Card Sorting tests) compared to controls [137]. However it is unclear which nAChR subtypes may be involved since it is a partial agonist for the a4β2 nAChRs and a full agonist for the α7 nAChRs [138].

A robust feature of the schizophrenic brain is a decrease in the number of $\alpha7^*$ nAChRs (particularly in the hippocampus) [132]. In fact, a direct genetic linkage to the α7 subunit was first reported in 1997 where it was found that a polymorphism existed at chromosome site 15q13–14, the site of the α7 nAChR subunit gene [139, 140]. In support of a mechanistic role for $\alpha7^*$ nAChRs in schizophrenia, studies on auditory gating (investigated using the P50 auditory-evoked response), in which deficits are known to be associated with schizophrenia, have been done. The systemic administration of the type II α7 nAChR PAMs (PNU-120596 or JNJ-1930942) improved auditory gating deficits caused by amphetamine or MK-801 in rodent models that reflect circuit level disturbances associated with schizophrenia [141–143]. Furthermore several clinical trials support the use of α7 nAChR agonists to treat cognitive deficits in schizophrenia. For example, cognitive and/or sensory deficits were improved with the α7 partial agonists GTS-21[144], DMXB-A [145], or EVP-6124 [146] in schizophrenia cohorts. Additionally in a randomized, double-blind, placebo-controlled study of schizophrenic patients, the novel and selective α7 nAChR agonist, EVP-6124, provided significant improvement in a number of areas of cognitive function that are compromised in schizophrenia [146].

At the moment, $\alpha7^*$ nAChR agonists and PAMs appear to be the most effective hope for the future in the treatment of schizophrenia. What is needed next is a determination of the mechanism of synaptic failure in schizophrenia and the potential cellular mechanisms of the positive cognitive actions of these selective $\alpha7^*$ nAChR ligands in these patients. This information will provide further insight into the development of therapeutic treatments for the cognitive impairments associated with schizophrenia. Along these lines, we recently found that presynaptic $\alpha7^*$ nAChRs, via a mechanism involving PKA, enhanced hippocampal CA3 mossy fiber glutamatergic transmission [147]. These types of studies may help to provide a mechanistic explanation for the hypothesis that nicotine enhances the mossy fiber glutamatergic transmission in the hippocampal CA3 region to compensate for a loss of synaptic connection in schizophrenic patients [148].

PAIN

Pain is an unpleasant sensory feeling that includes neuropathic, nociceptive and psychogenic pain [149]. If the pain persists for longer than a few months, the condition is said to be chronic (as opposed to acute) pain [150]. The most common reason for neuropathic pain is a disruption in the normal functioning of the somatosensory system [151]. The nAChRs have been known for some time to be involved in the process of mediating pain, and as such the analgesic effects of nAChR agonists as well as positive allosteric modulators (PAMs) have long been investigated [152, 153]. The microinjection of nicotine into different regions of the brainstem produced an antinociceptive effect [154]. In rats, the antinociception induced by nicotine was further shown to be inhibited by the administration of the general nAChR antagonist mecamylamine [155]. Apart from nicotine, the frog skin-derived alkaloid epibatidine, which acts as a non-selective agonist for nAChRs, was shown to have analgesic properties [156, 157]. When epibatidine and morphine were tested in the mouse straub tail response, both compounds were able to reduce pain [158, 159]. As expected, the analgesic effect of morphine was blocked by naloxone, but not that of epibatidine [158, 160]. Upon further investigation it was found that while epibatidine caused anti-nociception in animal models of pain, it also produced several major adverse effects like dose-dependent decreases in locomotor activity, and a similar decrease in body temperature [156]. Epibatidine is a non-selective agonist since it activates multiple subtypes of the neuronal nAChRs, as well as the neuromuscular receptor [161], which accounts for its various adverse effects [162], limiting its use as a therapeutic.

Based upon these observations, the research focus has since shifted to develop compounds that would selectively target specific neuronal nAChR subtypes with the purpose of maintaining the analgesic effect while reducing or eliminating the adverse effects that are seen with non-selective agonists such as epibatidine. The $\alpha4\beta2$ subtype-selective ligands were the first to be developed. ABT-594, which is a structural analogue of epibatidine, was shown to be a potent full agonist for the $\alpha4\beta2$ nAChRs with a strong analgesic effect [158, 163, 164]. In various animal models of pain, the analgesic activity of ABT-594 was inhibited by the administration of the general antagonist mecamylamine [162, 165]. At lower doses, ABT-594 does not induce any significant analgesic effects, while at higher doses (150 to 300 mg), the analgesic effects of ABT-594 were accompanied by significant adverse effects like nausea, dizziness, and vomiting [166], indicating that ABT-594 has a narrow therapeutic window and may act upon peripheral nAChRs at higher doses. Subsequently other selective agonists for the $\alpha4\beta2$ nAChRs (e.g. TC-2696 and TC-6499) were developed [167]. While both TC-2696 and TC-6499 showed significant analgesic effects in animal models for neuropathic pain, they were not developed further because both had a narrow therapeutic window (i.e. the difference in doses at which they produce analgesic effects and adverse effects was small) [167, 168], similar to that seen with ABT-594. Recently, another drug that targets the $\alpha4\beta2$ nAChRs, A-366833, was been reported to cause significant analgesia in a variety of models for neuropathic pain [169, 170]. In this scenario, it is thought that the activation of presynaptic $\alpha4\beta2^*$ receptors within the spinal cord that modulate GABA and glycinergic neurons may lead to an inhibitory effect on nociception [153, 168, 171–175]. However, while the activation of $\alpha4\beta2^*$ receptors

may be required, it may not be sufficient to induce an analgesic effect; the activation of other subtypes of nAChRs, specifically the α3-containing nAChRs, may also be required [168, 176].

In addition to α4β2* and α3-containing nAChRs, compounds that act on the α7 nAChRs have also been studied for their effects on pain. Choline, a by-product of ACh enzymatic degradation by AChE and a selective agonist for the α7 receptors, has analgesic effects in rodent models of pain [177, 178]. However other agonists that target α7 nAChRs, such as SSR-180711 and tropisetron, fail to induce analgesia when tested in the formalin model for pain [168], suggesting that targeting α7* nAChRs for pain relief is modality selective. Based on these contradictory results, further work needs to be done to clearly define the role of α7 nAChR agonists in analgesia.

A variety of α4β2 and α7 nAChR PAMs have been evaluated in preclinical models for pain [122, 179–181]. For instance the α7 nAChR PAM PNU-120596 caused a significant reduction in mechanical hyperalgesia in rats [182]. Additionally, PNU-120596 is able to decrease formalin-induced pain by itself, and in combination with the α7 nAChR agonists choline, nicotine and PHA-543613 [183]. PNU-120596 is also able to reduce edema induced by a hind paw infusion of carrageenan in mice [184]. Another α7 nAChR PAM, NS-1738, has also been tested for its effects on pain. Both NS-1738 and PNU-120596 were able to reduce heat-induced hyperalgesia [184]. In a chronic constriction injury model for neuropathic pain, an anti-hyperalgesic and anti-allodynic effect of PNU-120596 was observed, however NS-1738 did not have any effect in the same study [184]. One possible mechanism for this may be the ability of PNU-120596 to enhance α7 nAChR activation over a certain threshold, which would indirectly stimulate inhibitory neurons located in the neural circuits for neuropathic pain.

The α4β2 nAChR PAM, NS-9283, administered alone did not affect mechanical allodynia in the spinal nerve ligation test (a model of neuropathic pain), nor on nociception and inflammation in various rodent models of pain [185, 186]. However, when NS-9283 was coadministered with the α4β2 receptor agonist ABT-594, the presence of the α4β2 nAChR PAM increased the anti-allodynic effects of the agonist alone [185]. In addition NS-9283 was able to strengthen the antinociceptive actions of the α4β2 nAChR partial agonist NS-3965 [187]. Therefore combining an α4β2 receptor PAM with a nAChR full agonist may be a viable strategy in therapeutic drug development for treatment of acute as well as chronic pain to avoid the major adverse effects commonly seen with a nAChR agonist alone.

Besides the α4β2* and α7* receptors, other nAChRs are also known to be involved in the processing of pain [153]. For example, it is emerging that the α5 nAChR subunit plays a significant role in nociception. The α5 subunit can participate in α4β2*, α3β2* or α3β4* nAChRs [188]. The anti-nociception effect of nicotine in α5 subunit knockout mice was reduced compared to wildtype mice, indicating that the α5 subunit may play an important role in the processing of pain [188]. In addition there may be a role for the α9/α10 nAChRs in pain. Dorsal root ganglion neurons express the α9/α10 nAChR subtypes [189].

There is accumulating evidence, based upon pharmacological testing and behavioral studies, which strongly implicates nAChRs in the process of nociception and the treatment of pain. However given the complexity of the nAChR subtypes expressed within the CNS and PNS, as well as the complexity and plasticity of the neuronal circuits involved in the processing of pain, developing therapeutic strategies that target nAChRs to treat acute and chronic pain poses significant challenges.

CONCLUDING REMARKS

The distribution of nAChRs in the nervous system is extensive, yet discrete, and this has made them an active target of drug development; historically for treating neurodegenerative diseases such as AD. However, the nAChRs are increasingly appreciated for their roles in neurodevelopment and sensory processing, and thus have been identified as therapeutic targets in autism, schizophrenia, and neuropathic pain. Unfortunately, no nAChR compounds have demonstrated disease-modifying properties for any of the disorders in which these receptors are implicated to date. Thus we contend that a new generation of nAChR ligands is needed for studies on their ability to treat neurodegenerative or neurodevelopmental disorders, as well as neuropathic pain.

What are the prospects for the future? Currently there are at least two main obstacles. First, more mechanistic details about the stoichiometry, expression, and developmental regulation of the various subtypes of nAChRs in the brain and nervous system are needed, both in neurons and non-neuronal cells, and how these receptors are regulating brain circuit function, excitability, plasticity, and development. It is only through this knowledge that we can know which nAChRs we need to target for the design of therapeutics in order to treat disorders and diseases linked with nAChRs. This will also help us to understand how we want to alter receptor function; e.g. in some instances we may need to enhance function (either competitively or via allosteric modulation), while in other instances we may need to block or reduce function (either short- or long-term). Great strides are now being made and will continue, in part through the use of optogenetics, *in vivo* physiological studies in live animals, and genetic methods to remove or modify specific nAChR subtypes from specific brain regions, cell types, and at specific developmental stages.

Second, a more targeted approach to develop therapeutics acting selectively and effectively on the various nAChR subtypes is needed. For example, more structural and biophysical information on the properties of the nAChRs and their binding site will help in the targeting of potential therapeutic drugs to various regions of the extracellular domain of the receptor (e.g. binding site, pore, or putative allosteric site). The various ACh-binding proteins (AChBPs), which are a family of pentameric and soluble protein analogous to the extracellular ligand-binding domain of nAChRs and have been crystalized with several nAChR ligands including varenicline [190], will certainly open new perspectives for the design of new drugs targeting nAChRs. Furthermore the recent determination of the crystal structures of the neurotransmitter-gated members of the cys-loop receptor family (e.g. the 5-HT_3 and $GABA_A$ receptors; [191, 192]), and the ligand-binding domain of the $\alpha 7$ nAChR [193]), will not only enhance our understanding of the structure and possible function of mammalian cys-loop receptors, but will also further aid in the targeted development of new

compounds that one day may be used to treat various disorders and diseases linked with nAChRs.

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- Neuronal nicotinic ACh receptor (nAChR) dysfunction is involved in the pathophysiology of many neurological disorders
- nAChRs are increasingly appreciated for their roles in neurodevelopment and sensory processing
- $\alpha4\beta2$ and $\alpha7$ nAChRs in the basal forebrain cholinergic system are important for cognitive performance

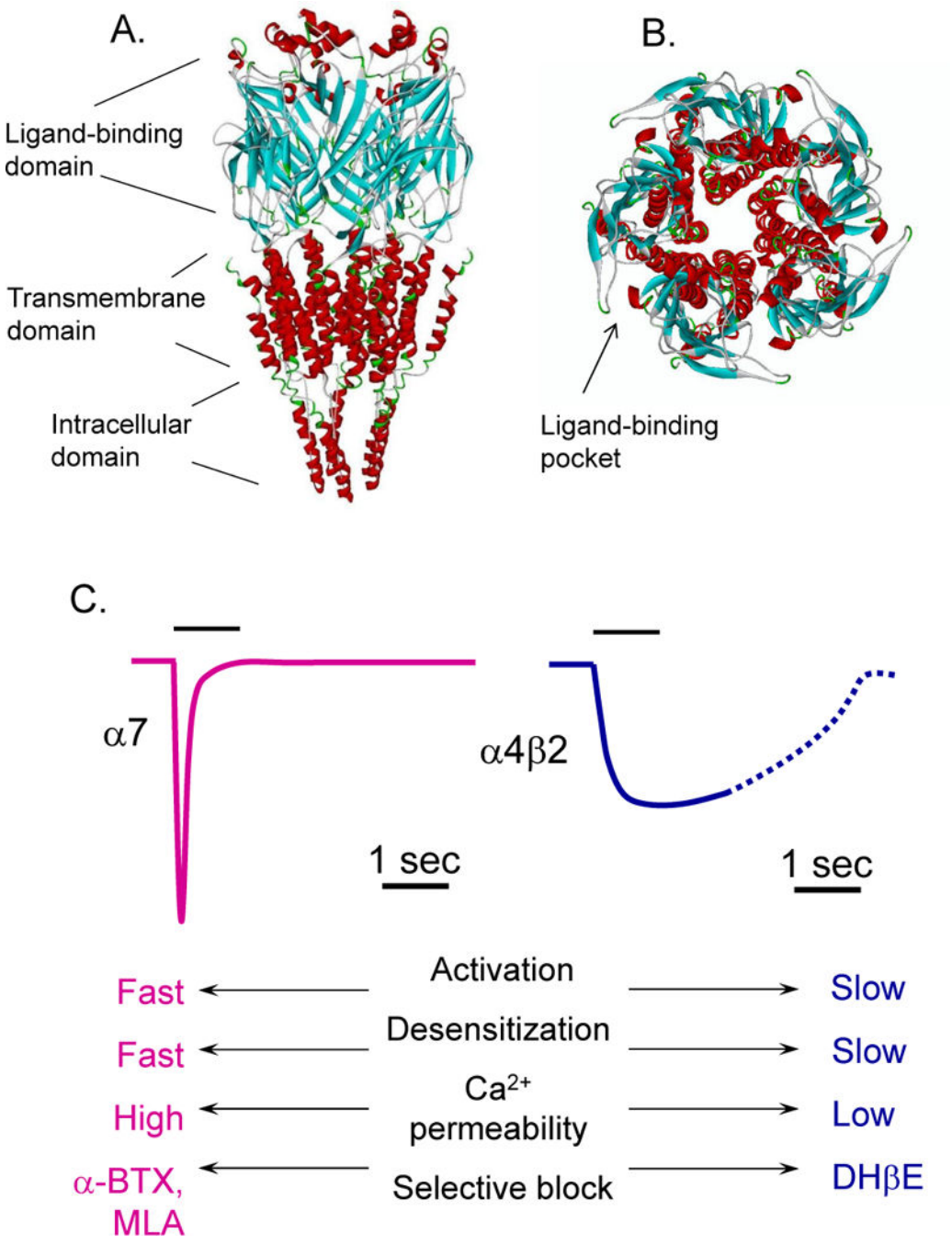

Figure 1. Nicotinic ACh receptor channel; basic structure and functional properties

Molecular model of the rat α7 nAChR with ligand-binding from a side view showing the ligand-binding, transmembrane, and intracellular domain **(A)**, and a top down view **(B)** showing the pentameric nature of the receptor with the ligand-binding pocket at the interface between two subunits. **C**, The basic functional and pharmacological properties of the α7 and α4β2 nAChR subtypes.

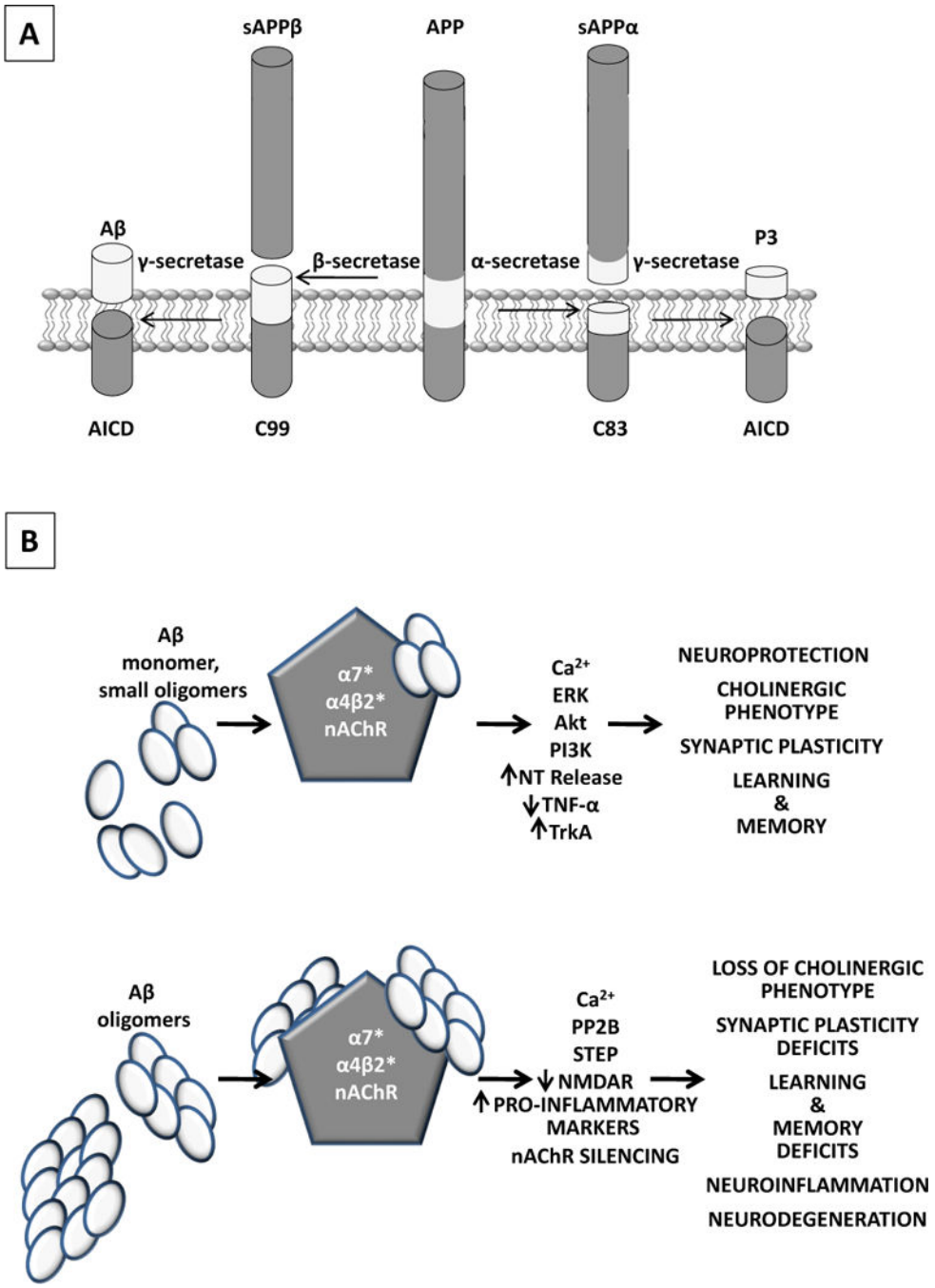

Figure 2.

A, Aβ is formed through proteolytic cleavage of its precursor protein, a type 1 membrane protein called amyloid precursor protein (APP). APP cleavage by β- and γ-secretases lead to the formation of the N- and C-terminal residues of Aβ, respectively, in addition to soluble APP (sAPPβ) and a C-terminal fragment (CTF) of 99 amino acids (C99). If APP is cleaved by the α-secretase, then Aβ formation is precluded as this enzyme cleaves APP within the Aβ sequence to generate sAPPα and CTF83. When cleaved by γ-secretase, CTFs generate the AICD (APP intracellular domain) and either P3 or Aβ resulting from α- and β-secretase

cleavage, respectively. **B,** As Aβ accumulates one can postulate that at lower concentration and smaller oligomer aggregates (upper panel), transient nAChR activation may occur depending on the nAChR subunit composition (Table 2). If activation ensues, it can lead to membrane depolarization, increased intracellular/presynaptic calcium and synaptic transmission, activation of kinases important for synaptic plasticity, learning and memory as well as the induction of genes and proteins necessary for the cholinergic phenotype and neuroprotection. As disease progresses and Aβ concentration and oligomer status increase (lower panel), nAChR function is lost either through loss of receptor protein ($\alpha4\beta2^{*}$) or function ($\alpha7^{*}$) leading to synaptic dysfunction and loss of the cholinergic phenotype, learning and memory deficits, increased inflammatory status and progressive neurodegeneration.

Table 1

Summary table for nAChRs as targets in CNS diseases

Categorized by the CNS diseases discussed in the present review, brain regions implicated in each disease, and the nAChR subunits implicated in each disease as well as potential pharmacological interventions currently identified using preclinical and clinical trial design.

CNS Disease	Brain Regions Implicated	nAChR Subunits Implicated	Potential Pharmacological Strategies
Alzheimer's disease	Basal forebrain cholinergic nuclei, hippocampus, cerebral cortex	α7, α4, β2	Agonists, partial agonists and PAMs for α7* nAChRs, α4β2* agonists
Autism, Autism Spectrum Disorders	Cerebral cortex, hippocampus, amygdala, basal ganglia, cerebellum	α3, α4, β2	agonists or PAMs for α4β2* nAChRs
Schizophrenia	Hippocampus, cortex	α7, α4β2	agonists, partial agonists, or PAMs for α7* nAChRs or partial agonists for α4β2* nAChRs
Pain	Dorsal root ganglia, Brainstem rostral ventromedial medulla, midbrain periaqueductal gray, spinal cord dorsal horn	α3, α4, α5, α7, α9, α10, β2	agonists or PAMs for α4β2* nAChRs or type II or PAMs for α7* nAChRs

Table 2

In situ and heterologous expression studies have revealed varied nAChR responses to Aβ

nAChR responses to Aβ appear to depend upon the species of receptor, subunit composition, have revealed receptor activation and receptor inhibition for the α7 nAChR subtype

nAChR Subtype	Experimental Preparation	Type of Interaction	Downstream Consequences	Reference
human α7*	human brain: control & AD	co-localization & co-IP	n/a	Wang et al., 2000a
human α7*	human & rat cell lines	high-affinity binding	competitive binding: BTX vs. Aβ	Wang et al., 2000a, 2000b
rat α7	*Xenopus* oocytes	receptor activation	Ca^{2+} influx, ERK activation	Dineley et al., 2001, 2002
rat α7*, non-α7*	isolated presynaptic terminals: hippocampus, neocortex	receptor activation	increased presynaptic Ca^{2+}	Dougherty et al., 2003
rat α4*	diagonal band nucleus	receptor activation	membrane depolarization, increased mEPSC frequency	Fu & Jhamandas, 2003; Chin et al., 2007
mouse α7*, β2*	NG108-15 cells, isolated presynaptic terminals: hippocampus, neocortex	receptor activation	increased presynaptic Ca^{2+}	Khan & Nichols, 2007
rat α7*, non-α7*	acute hippocampal slice, GABAergic interneurons	functional antagonism, reversible	decreased open p_o	Pettit et al., 2001
rat α7β2*	medial septum/diagonal band cholinergic neurons	functional antagonism, reversible	noncompetitive (choline)	Liu et al., 2009
rat α7*	cultured hippocampal neurons	functional antagonism, reversible	noncompetitive (ACh)	Liu et al., 2001
human α7	*Xenopus* oocytes	functional antagonism, reversible	noncompetitive (ACh)	Grassi et al., 2003; Pym et al., 2005
rat α7	*Xenopus* oocytes	no effect	n/a	Lamb et al., 2005
human α4β2	SH-EP1 cells	functional antagonism, reversible	noncompetitive (nicotine)	Wu et al., 2004
human α4β2	*Xenopus* oocytes	agonist potentiation (ACh)	membrane depolarization	Pym et al., 2005
rat α4β2, α2β2, α4α5β2	*Xenopus* oocytes	functional antagonism, reversible	n/a	Lamb et al., 2005
human α3β4	*Xenopus* oocytes	no effect	n/a	Pym et al., 2005
mouse muscle γ- or ε-nAChR	BOSC 23 cells	functional antagonism, reversible	noncompetitive	Grassi et al., 2003