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Alpha7 nicotinic acetylcholine receptor-specific agonist DMXBA (GTS-21) attenuates Aβ accumulation through suppression of neuronal γ-secretase activity and promotion of microglial amyloid-β phagocytosis and ameliorates cognitive impairment in a mouse model of Alzheimer's disease

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ABSTRACT

We previously demonstrated that stimulation of nicotinic acetylcholine receptors (nAChRs) increases amyloid-β (Aβ) phagocytosis in rat microglia and is closely associated with the decrease of brain Aβ and amelioration of memory dysfunction in a transgenic mouse model of Alzheimer's disease (AD). Here, we examined the subtypes of nAChRs involved in these beneficial effects. In primary cultures of rat microglia, the α7 nAChR selective agonist 3-[(2,4-dimethoxy)benzylidene]-anabaseine dihydrochloride (DMXBA) promoted Aβ and fluorescent latex bead phagocytosis, whereas selective α7 nAChR antagonists suppressed the enhanced Aβ phagocytosis. In a transgenic mouse model of AD, administration of DMXBA attenuated brain Aβ burden and memory dysfunction. Moreover, DMXBA suppressed γ-secretase activity in solubilized fractions of human neuroblastoma cells and transgenic mouse brain. These results suggested that selective activation of α7 nAChRs promoted microglial Aβ phagocytosis and suppressed neuronal γ-secretase activity to contribute to the attenuation of the brain Aβ burden and cognitive impairment. Thus, we propose neuronal and microglial α7 nAChRs as new therapeutic targets in the treatment of AD.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels that play a central role in intercellular communication in the central nervous system (CNS) by converting the binding of the neurotransmitter acetylcholine into an ion flux through the postsynaptic membrane of neurons and microglia in the brain (Cecchini and Changeux, 2015). Although various subtypes of nAChRs are expressed in the mammalian brain, the majority are the heteromeric α4β2 (α4β2 nAChR) and homomeric α7 (α7 nAChR) subtypes (Buisson and Bertrand, 2002). Stimulation of neuronal nAChRs by agonists directly elicits neuroprotection through the α4β2 nAChR or α7 nAChR, at least in part, by inhibiting cellular apoptotic pathways (Akaike et al., 2010; Kihara et al., 2001; Quik et al., 2015).

Alzheimer's disease (AD) is the most common cause of dementia. The neuropathological hallmarks of AD are the presence of

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senile plaques and neurofibrillary tangles along with extensive neuronal loss (Goedert, 2015; Selkoe and Hardy, 2016). Senile plaques and neurofibrillary tangles consist mainly of amyloid-β (Aβ) peptides and intracellularly accumulated hyperphosphorylated tau proteins, respectively. Senile plaques are present extracellularly and are accompanied by microglial activation (Cameron and Landreth, 2010). The Aβ is derived from sequential proteolysis of amyloid precursor protein (APP) by β- and γ-secretases (Tomita, 2017) and is composed of 36–43 amino acid residues because γ-secretase, which is a complex consisting of 4 individual proteins, including presenilin, generates the C-terminal of Aβ with different lengths (Masters and Selkoe, 2012). Among the variations in Aβ, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42) are the major species found in the brains of patients with AD. The most predominant species deposited in Aβ plaques is Aβ42, which is prone to aggregation and indicates increased neurotoxicity (Iwatsubo et al., 1994). By contrast, Aβ40 is the major soluble species; its secretion is 10-fold more than that of Aβ42 in normal brains. A previous study demonstrated that the deposition of Aβ40 in AD brains is particularly correlated with synaptic and neuronal loss (Lue et al., 1999). Longitudinal studies of neuroimaging and biomarkers in patients with autosomal dominant familial AD suggest that Aβ deposition occurring through increased production or decreased degradation is the primary event before the emergence of brain atrophy, tau phosphorylation, and chronic symptoms (Bateman et al., 2012; Reiman et al., 2012). Moreover, a recent double-blind clinical phase Ib randomized trial examining antibody-based immunotherapy against Aβ demonstrated a reduction of brain Aβ with a slowing of clinical decline in patients with AD (Sevigny et al., 2016). Such results have led to the acceptance of the amyloid hypothesis, which asserts that Aβ accumulation in the brain is responsible for the development of AD, by most researchers worldwide, and Aβ is currently considered the most common disease-modifying target in AD therapy (Selkoe and Hardy, 2016).

The 3 cholinesterase inhibitors and an *N*-methyl-D-aspartate receptor antagonist currently available for drug treatment of AD are purely for symptomatic relief as they may not affect Aβ accumulation or other substantial pathology in AD. However, the cholinesterase inhibitors donepezil and galantamine used in the treatment of AD show direct neuroprotective effects mediated through neuronal nAChRs (Akaike et al., 2010; Kihara et al., 2004; Kim et al., 2017; Shen et al., 2010). Thus, additional therapeutic mechanisms provided by cholinesterase inhibitors have gained attention, and neuronal nAChRs are expected to become new targets for drug development in the treatment of AD.

Microglia, the primary immune effector cells, are distributed ubiquitously throughout the CNS. Under the pathological conditions of AD, microglia accumulate on the Aβ deposits (Cameron and Landreth, 2010). We (Kakimura et al., 2002; Kitamura et al., 2003; Matsumura et al., 2015; Takata et al., 2003) and others (Bamberger et al., 2003; El Khoury et al., 1996; Koenigsknecht and Landreth, 2004; Paresce et al., 1996; Rogers et al., 2002; Wilkinson and El Khoury, 2012) previously demonstrated that microglia have effective phagocytic ability of Aβ. Aging (Njie et al., 2012) and genetic risk factors, such as CD33 and triggering receptor expressed on myeloid cells 2, identified by genome-wide association studies (Lambert et al., 2013) may induce dysfunction in microglial phagocytosis (Griciuc et al., 2013) and the sensing of Aβ (Wang et al., 2016; Yuan et al., 2016), respectively, and may be critically involved in the pathogenesis of AD. Therefore, approaches to promote microglial Aβ phagocytosis are currently being explored as new therapeutic strategies in AD.

Similar to neurons, microglia express functional nAChRs, and microglial nAChRs modulate immunological functions, such as the production of cytokines and reactive oxygen species (Egea et al., 2015; Sadigh-Eteghad et al., 2016; Shytle et al., 2004; Suzuki et al., 2006). We previously demonstrated that stimulation of microglial nAChRs with nicotine (a nonselective nAChR agonist) or galantamine (an allosterically potentiating ligand at nAChRs) increases phagocytosis of Aβ by microglia, and oral administration of galantamine ameliorates brain Aβ accumulation and memory impairment in a transgenic mouse model of AD (Takata et al., 2010). Thus, targeting the stimulation of microglial nAChRs may provide additional opportunities to suppress pathogenesis progression in AD. However, it is not yet known which nAChR subtypes on microglia promote Aβ phagocytosis. Thus, the present study was conducted using various selective agonists and antagonists for the α4β2 and α7 nAChR subtypes. We found that the microglial α7 nAChR is critically involved in the promotion of Aβ phagocytosis in primary cultures of rat microglia and that 3-[(2,4-dimethoxy)benzylidene]-anabaseine dihydrochloride (DMXBA; also known as GTS-21), a selective agonist for α7 nAChRs, effectively reduces the Aβ burden in the brain and improves spatial memory and learning in a transgenic mouse model of AD. Furthermore, we found that DMXBA suppresses γ-secretase activity in solubilized fractions of human neuroblastoma cells and of the brain from a transgenic mouse used as a model of AD.

2. Material and methods

2.1. Primary cultures of rat microglia and drug treatments

Primary cultures of rat microglia were prepared as previously described (Kakimura et al., 2002; Kitamura et al., 2003; Takata et al., 2003, 2007, 2010). The forebrains from newborn Wistar rats were minced and filtered through a 70-μm-diameter nylon mesh. The cells were resuspended in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Carlsbad, CA, USA) with 10% fetal bovine serum, 100-units/mL penicillin, and 100-μg/mL streptomycin and plated onto 100-mm-diameter dishes at 37 °C in humidified 5% CO_2/95% air. At 21 days, the floating microglia were isolated from the mixed glial cell cultures. The purified microglia (>97% pure as determined using CD11b immunostaining) were transferred to 24-well plates (3.0×10^5 cells/well) and allowed to adhere at 37 °C overnight. The cells were treated with synthetic human Aβ42 hydrochloride (1 μM; AnaSpec; San Jose, CA, USA) alone or simultaneously with (−)-nicotine (a nonselective nAChR agonist; Sigma; St. Louis, MO, USA), 3-methyl-5-[(2S)-1-methylpyrrolidin-2-yl]-1,2-oxazole hydrate (ABT-418, an α4β2 nAChR selective agonist; Sigma), epibatidine (an α4β2 nAChR selective agonist; Sigma), N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide monohydrochloride hydrate (PNU-282987, an α7 nAChR selective agonist; Sigma), or DMXBA (an α7 nAChR selective agonist) for 12 hours. DMXBA was synthesized by the reaction of anabaseine dihydrochloride and 2,4-dimethoxybenzaldehyde in acidic alcohol as described previously (Kem et al., 2004). Antagonists for nAChRs, including methyllycaconitine (MLA, an α7 nAChR selective antagonist; Sigma) or dihydro-β-erythroidine (DHβE, an α4β2 nAChR selective antagonist; Sigma), were added individually to microglia 10 minutes before the Aβ42 treatment. After the Aβ42 treatment, adherent microglia were rinsed with phosphate-buffered saline (PBS) and then collected with PBS containing 0.1% Triton X-100. The amount of Aβ in the cell lysate was measured using a human Aβ-specific enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories Co, Ltd, Gunma, Japan) according to the manufacturer's instructions.

Primary cultures of rat microglia were also seeded on glass-bottom dishes and treated with drugs as previously described. In addition, fluorescent latex beads (Fluoresbrite carboxylate microspheres; yellow green, 1.0 μm; 4.0×10^5 beads/mL; Polyscience Inc,