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Journal of Alzheimer's Disease 33 (2013) 841–851
DOI 10.3233/JAD-2012-121447
IOS Press

Modulation of α7 Nicotinic Acetylcholine Receptor and Fibrillar Amyloid-β Interactions in Alzheimer's Disease Brain

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Handling Associate Editor: Shun Shimohama

Accepted 10 September 2012

Abstract. The nicotinic receptors (nAChRs), which play a critical role in cognitive function, are impaired early in the course of Alzheimer's disease (AD). We have previously demonstrated that amyloid-β (Aβ) assemblies bind to α7 nAChRs and form complexes in AD brain, suggesting that this cooperative process may contribute to disruption of synaptic function in AD. In the current study, we further characterized the interaction between different nAChR subtypes and fibrillar Aβ by binding assays in postmortem brain from AD and control cases using a wide range of drugs with different molecular targets, including selective nAChR subtype agonists, and the amyloid ligand Pittsburgh compound B (PIB) that binds with high (nanomolar) affinity to fibrillar Aβ. The α7 nAChR agonists varenicline and JN403, but not the α4β2 nAChR agonist cytisine, increased the ^{3}H-PIB binding in autopsy tissue homogenates from AD and control frontal cortex. This effect was blocked in the presence of the α7 nAChR antagonists methyllycaconitine, α-bungarotoxin, and mecamylamine, but not by the α4β2 nAChR antagonist dihydro-β-erythroidine. Increases in ^{3}H-PIB binding evoked by varenicline and JN403 were also prevented by pre-incubation with another amyloid ligand, BF-227. The acetylcholinesterase inhibitor and allosteric nAChR modulator galantamine as well as the N-methyl-d-aspartate receptor blocker memantine did not significantly alter ^{3}H-PIB binding levels in AD brain. The present findings further support a specific interaction between fibrillar Aβ and α7 nAChRs in the brain, suggesting that treatment with α7 nAChR stimulatory drugs can modulate Aβ/α7 nAChR pathogenic signaling mechanisms in AD brain.

Keywords: α7 nicotinic acetylcholine receptors, Alzheimer's disease, fibrillar amyloid-β, ^{3}H-PIB binding, postmortem brain

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by excessive deposition of amyloid-β (Aβ) plaques and neurofibrillary tangle formation in the brain, as well as impairment of cholinergic neurotransmission leading to cognitive deficits. The accumulation of Aβ in the brain is considered to play a central role in the cascade of pathological events during the progression of disease [1]. Nicotinic acetylcholine receptors (nAChRs) have a widespread distribution in the brain, and the most abundant are the α4β2 and α7 subtypes. These receptors play an important role in learning and memory, regulating neurotransmission,

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and synaptic plasticity mechanisms [2–4]. A pronounced loss of α4β2 nAChRs in the cerebral cortex has been reported in autopsy studies of AD brain as well as *in vivo* by positron emission tomography (PET) imaging with ^{11}C-nicotine in AD patients [5]. Moreover, the decline in cortical ^{11}C-nicotine binding *in vivo* seems to correlate with impairment of executive and attentional cognitive functions [6]. We recently also reported a negative correlation between total amount of Aβ oligomers in the insoluble fraction and ^{3}H-nicotine binding (α4 nAChRs) in AD frontal cortex [7], suggesting a link between Aβ and nicotinic receptors.

An interaction between Aβ and α7 nAChRs has been described in several studies (for review [8]). Initially, Wang and colleagues suggested that Aβ bound to α7 nAChRs in postmortem AD brain tissue [9]. This observation was confirmed by other studies in AD brain tissue [10] and in rat hippocampal slices [11], as well as in xenopus oocytes [12–14]. However, later functional studies showed somewhat inconsistent observations of either activation [12, 15] or inhibition effect [11, 16], which might be due to different Aβ composition and concentrations [17–19]. When knocking out α7 nAChRs in AD transgenic mice, both protective effects against Aβ toxicity [20] and improved cognitive function [21] as well as an exacerbating effect on the pathology have been reported [22]. These conflicting findings could be attributed to the different AD transgenic mouse models used as well as the age of these mice, reflecting different stages of Aβ pathology.

The recent rapid progresses in molecular PET imaging have resulted in development of new amyloid PET tracers, such as Pittsburgh compound B (PIB), which is a Thioflavin T analogue that binds to fibrillar amyloid in AD brain with affinity in the nanomolar range [23, 24]. Regional *in vivo* ^{11}C-PIB retention positively correlates with ^{3}H-PIB binding and Aβ plaque distribution when analyzed in autopsy brain tissue [25].

To further understand the physiological relevance and binding properties of fibrillar Aβ aggregates with different nAChR subtypes in AD brain, we investigated in the present study the effects of α7 and α4β2 nAChR selective agonists (varenicline, JN403, cytisine), the cholinesterase inhibitor (ChEI) and nAChR modulator galantamine, the N-methyl-D-aspartate receptor (NMDA) blocker memantine, and anti-histamine drug dimebon on fibrillar Aβ levels in cortical homogenates measured by ^{3}H-PIB binding. This study supports a specific interaction between fibrillar Aβ and α7 nAChRs in AD brain.

MATERIALS AND METHODS

AD and control autopsy cases

Autopsy frontal cortex brain tissues from five AD cases (mean age 69.6 ± 4.2 years; mean postmortem delay 7.3 ± 1.3 h) and five age-matched normal controls (mean age 67.4 ± 3.9 years; mean postmortem delay 8.5 ± 1.1 h) were obtained from the Brain Bank at Karolinska Institutet and the Netherlands Brain Bank, Netherlands Institute for Brain Research (see demographic description of patients in Table 1). The clinical diagnosis of AD was confirmed at autopsy by pathological examination according to NINCDS-ADRDA and CERAD criteria. The control cases had no clinical history of psychiatric or neurological disorders and showed no neuropathological changes indicative for dementia. There was no significant

Table 1
Demographic data of Alzheimer's disease (AD) and control cases

Case no.	Age (y)	Postmortem delay (h)	Gender	Braak stage	APOE alleles
Control 1	54	9.0	M	NA	NA
Control 2	65	11.0	M	NA	33
Control 3	68	10.5	F	2	33
Control 4	74	5.9	M	2	33
Control 5	76	6.0	M	NA	34
Mean ± S.E.M	67.4 ± 3.9	8.5 ± 1.1	F/M 1/4		APOE ε4 (+/–) 1/3
AD 1	58	5.3	M	6	33
AD 2	64	5.5	F	6	34
AD 3	68	5.3	M	6	34
AD 4	78	8.3	F	5	33
AD 5	80	12	F	4[a]	44
Mean ± S.E.M	69.6 ± 4.2	7.3 ± 1.3	F/M 3/2		APOE ε4 (+/–) 3/2

[a]Histopathological dementia score graded according to [62]; NA, not available.

difference in age, gender, or postmortem delay between AD and control cases (χ^2 tests). Permission to use autopsy brain tissues in experimental procedures was granted by the Regional Human Ethics committee in Stockholm and the Swedish Ministry of Health.

Drugs

The following drugs were used: varenicline (kind gift from Pfizer, Groton, CT, USA), JN403 (kind gift from Novartis, Basel, Switzerland), BF-227 (kind gift from Dr. Nobuyuki Okamura, Department of Pharmacology, Tohoku University School of Medicine, Sendai, Japan), dimebon (kind gift from Dr. Manfred Windisch, JSW Research, Grambach, Austria), BTA-1, cytisine, galantamine hydrobromide, memantine hydrochloride, methyllycaconitine (MLA), mecamylamine (Mec) (all purchased from Sigma Aldrich, St Louis, MO, USA), Dihydro-β-erythroidine hydrobromide (DHβE), and α-bungarotoxin (α-BgTX) (Tocris Bioscience, Bristol, UK).

Membrane tissue preparations

Brain tissues from the frontal cortex (gray matter) were homogenized in cold phosphate-buffered saline (PBS) (137 mM NaCl, 3 mM KCl, 10 mM sodium phosphate; pH 7.4) containing phosphatase and protease inhibitors (10 μL/mL PBS). The homogenates were centrifuged at $1000 \times g$ for 10 min at 4°C and the resulting pellet was collected and resuspended in PBS buffer. The supernatant fraction (S1) was further centrifuged at $25{,}000 \times g$ for 30 min at 4°C. The resulting pellet (P2, membrane fraction) was resuspended in PBS buffer. The crude homogenates, P1, P2, and S2 fractions (cytosolic soluble fraction) were stored at –80°C until use.

^{3}H-PIB binding assays

For saturation binding assays, crude cortical homogenates (100 μg tissue) obtained from five AD cases were incubated with 0.01–20 nM ^{3}H-PIB (specific activity 68–85 Ci/mmol, customer synthesis, GE Healthcare, Germany) for 2 h at room temperature (RT). Non-specific binding was determined in the presence of 1 μM BTA-1. The specific binding was calculated by subtracting the non-specific from the total binding.

^{3}H-PIB binding in AD and control brain in the presence of drugs were performed as described earlier [19]. Briefly, cortical homogenates from AD brain were pre-incubated with 1 nM ^{3}H-PIB together with 10^{-11} to 10^{-5} M concentrations of varenicline, JN403, dimebon, cytisine, galantamine hydrobromide, memantine hydrochloride, and 10^{-9} to 10^{-5} M MLA, Mec, DHβE, and α-BgTX for 30 min prior to the application of ^{3}H-PIB. For the ^{3}H-PIB binding assays performed with respective drug in combination with nAChR antagonists (MLA, Mec, DHβE, or α-BgTX), samples from both AD and control brains were pre-incubated with antagonist for 30 min at RT.

Competitive binding assays were carried out with 1 nM ^{3}H-PIB and BTA-1 (10^{-12}–10^{-6} M) or BF-227 (10^{-13}–5×10^{-6} M). For binding assays with BF-227 in combination with nAChR drugs, varenicline or JN403 (1 μM) were pre-incubated for 30 min before incubation with BF-227 (20 nM or 100 nM).

All incubations were terminated by filtering samples through Whatman GF/C glass filters pre-soaked with 0.3% polyetylenamine solution. The filters were washed three times with ice-cold PBS buffer, transferred to scintillation vials and the radioactivity counted in a LS-6500 liquid scintillation counter (Beckman Coulter AB, Sweden). The assays were performed in triplicates and the specific binding expressed as fmol/mg tissue.

Statistical analyses

The binding data from saturation and displacement binding assays were analyzed using a nonlinear least-squares curve-fitting program (GraphPad Prism) to determine K_d, B_{max}, and K_i values. Comparisons between ^{3}H-PIB binding levels in AD and control cases in the presence of drugs were analyzed by one way ANOVA followed by Dunnett's *post hoc* test or two tail *student t* test, using GraphPad Prism program 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Significance level was set at $p < 0.05$. All values are shown as means ± S.E.M.

RESULTS

^{3}H-PIB binding in the frontal cortex of AD and control cases

Saturation binding studies with ^{3}H-PIB (0.01–20 nM) in frontal AD autopsy brain homogenates revealed a high affinity site (K_d = 3.6 ± 0.9 nM, B_{max} = 1201 ± 99 fmol/mg tissue ($n = 5$) (Fig. 1A, B). Therefore, 1 nM ^{3}H-PIB was used for following measurements of fibrillar Aβ amount. Significantly higher ^{3}H-PIB (1 nM) binding was

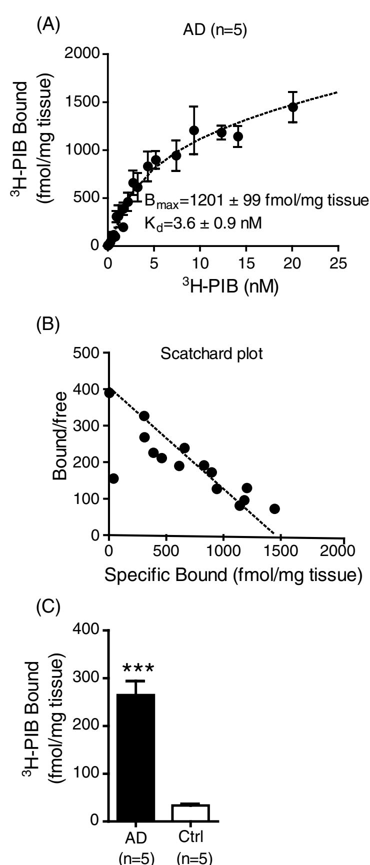

Fig. 1. A) Representative saturation binding of ^{3}H-PIB (0.01 nM–20 nM) on frontal cortical homogenates from autopsy AD brain ($n = 5$). B) Scatchard plot of binding data shown in (A). C) Specific bound ^{3}H-PIB (fmol/mg tissue) on frontal cortical homogenates from AD ($n = 5$) and control cases ($n = 5$). Results are means ± S.E.M; ***$p < 0.001$, AD group compared to control group.

observed in AD brain (264.4 ± 29.5 fmol/mg tissue) compared to controls (33.4 ± 3.9 fmol/mg tissue) ($p < 0.001$; Fig. 1C).

Effects of JN403, varenicline, cytisine, galantamine, memantine, dimebon on ^{3}H-PIB binding levels in AD brain

The effects of several classes of drugs (at concentrations varying from 10^{-11}–10^{-5} M) including varenicline (partial α4 and full α7 nAChR agonist), JN403 (α7 nAChR agonist), cytisine (α4 nAChR agonist), galantamine (ChEI), dimebon, and memantine (NMDAR blocker) on ^{3}H-PIB binding levels in the AD frontal cortex were measured. Brain tissue homogenates were pre-incubated with drugs for 30 min prior to the addition of 1 nM ^{3}H-PIB. When different nicotinic drugs were added to the homogenates, the level of specific ^{3}H-PIB binding increased compared with control when no drug was added. Varenicline and JN403 showed the largest increases in ^{3}H-PIB binding at 10^{-6} M ($p < 0.01$ for both) followed by dimebon at 5×10^{-8} M ($p < 0.05$) and galantamine at 10^{-7} M (ns) (Fig. 2A, B). No significant alterations in ^{3}H-PIB binding levels were observed following pre-incubations with cytisine or memantine, or with any of the nAChR antagonists MLA, Mec, α-BgTX, as well as DHβE (Fig. 2A, B, C).

MLA, Mec, DHβE, and α-BgTX inhibit increases in ^{3}H-PIB binding induced by JN403, varenicline, galantamine, and dimebon in AD brain

To determine whether fibrillar Aβ interacts with different nAChR subtypes in AD ($n = 5$) and control ($n = 5$) brain, the effects of varenicline and JN403 (1 μM), galantamine (100 nM), and dimebon (50 nM) on ^{3}H-PIB binding levels were investigated in combination with subtype selective antagonists. The elevated ^{3}H-PIB binding in AD brain induced by varenicline (+20%, $p < 0.01$) and JN403 (+31%, $p < 0.001$) was blocked by a 30-min pre-incubation with 1 μM Mec, 1 μM MLA, or 100 nM α-BgTX, but not with 1 μM DHβE (Fig. 3A). Pre-incubation with of 1 μM MLA, 1 μM Mec, or 100 nM α-BgTX did not prevent the galantamine (+17%, ns) and dimebon (+21%, $p < 0.01$) induced increase in ^{3}H-PIB binding in AD brain (Fig. 3B). In contrast to the effects observed in AD brain, no significant alterations in ^{3}H-PIB binding levels was observed in control cases brain in the presence of these drugs (Fig. 3C, D).

The amyloid PET ligand BF-227 prevents JN403 and varenicline-induced increases in ^{3}H-PIB binding levels in AD brain

To confirm that the effects of JN403 and varenicline on ^{3}H-PIB binding were mediated via specific binding of ^{3}H-PIB to released fibrillar Aβ, we preincubated samples with the amyloid PET tracer BF-227. Competitive binding with ^{3}H-PIB and BF-227 demonstrated a K_i value of 37 nM for BF-227 binding in AD frontal cortex (Fig. 4A). ^{3}H-PIB binding in the presence of 1 μM varenicline or 1 μM JN403 was significantly reduced by BF-227 (20 nM and 100 nM) by approximately 90% in AD brain ($n = 5$) (Fig. 4B).

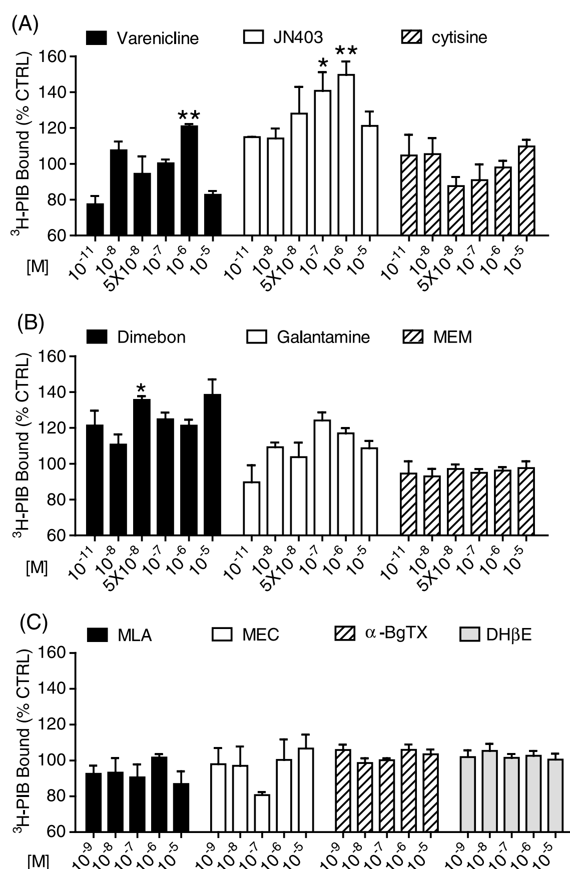

Fig. 2. ^{3}H-PIB bound (% CTRL) on frontal cortical homogenates from AD ($n = 3$) in the presence of (A) 10^{-11}–10^{-5} M JN403, varenicline, and cytisine, (B) 10^{-11}–10^{-5} M dimebon, galantamine, and memantine. C) 10^{-9}–10^{-5} M methyllycaconitine (MLA), mecamylamine (Mec), α-bungarotoxin (α-BgTX), and dihydro-β-erythroidine (DHβE). Results are means ± S.E.M; $^*p < 0.05$; $^{**}p < 0.01$, compared to untreated.

Next, we determined whether there is a change in the binding affinity of amyloid PET ligand PIB for fibrillar Aβ in the presence of an α7 nAChR agonist. Competitive assays with ^{3}H-PIB and BTA-1 in the presence of JN403 (which showed the largest effect from earlier findings of this study) were performed. However, no significant differences in K_i values were found in the presence and absence of JN403 in AD brain tissue (Fig. 4C).

Effect of JN403 on ^{3}H-PIB binding in different fractions of the frontal cortical homogenates of AD brain

To further determine the subcellular localization of this fibrillar Aβ-α7 nAChR interaction, we examined how different fractions of frontal cortical homogenates contributed to the JN403-induced increase of ^{3}H-PIB binding in AD brain. ^{3}H-PIB binding levels in the different cellular compartments of AD frontal cortex showed the following rank order of distribution; P1 fraction (75.1 ± 11.2%) >P2 membrane fraction (17.6 ± 3.5%) >S2 cytosolic fraction (5.2 ± 1.3%) (Fig. 5A). JN403 significantly increased ^{3}H-PIB binding in the crude homogenate fractions (+31%, $p < 0.01$), while the P2 membrane fractions contributed to approximately a 2% of the increase in ^{3}H-PIB binding measured in the crude homogenates fractions (Fig. 5B).

DISCUSSION

A current research focus is to identify the molecular targets and the mechanisms by which various Aβ assemblies are involved in mediating reduced synaptic connections, memory, and other cognitive impairment during the neurodegenerative processes in AD [26]. It has been shown that Aβ binds to phospholipid

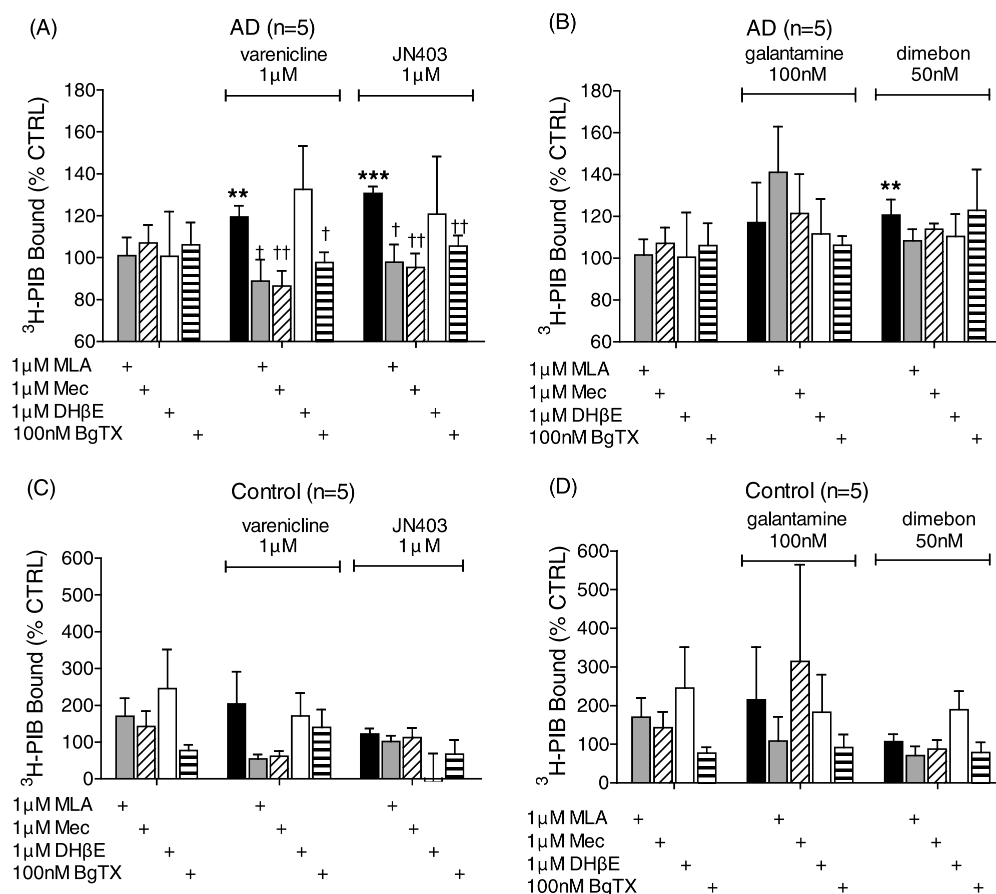

Fig. 3. Effects of 1 μM JN403, 1 μM varenicline (A, B), 100 nM and 1 μM galantamine, 50 nM dimebon (C, D) in combination with 1 μM methyllycaconitine (MLA) or 1 μM methyllycaconitine (Mec), 1 μM dihydro-β-erythroidine hydrobromide (DHβE), or 100 nM α-bungarotoxin (α-BgTX) on ^{3}H-PIB binding in frontal cortical homogenates from AD ($n = 5$) and control ($n = 5$) cases. Results are means ± S.E.M; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to untreated; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ compared to JN403/varenicline/galantamine/dimebon.

membranes with a relatively high affinity, and that modulation of the composition of the membrane can alter both membrane-Aβ interactions and toxicity [27]. Several other possible membrane receptors to which Aβ binds have been reported, including the α7 nAChR, the NMDAR, the neurotrophin receptor p75, the prion receptor, as well as the receptor for advanced glycation endproducts [28]. This multitude of molecular substrates pinpoints the complexity in finding suitable drug targets having a significant effect on disease pathogenesis. Earlier studies have suggested the α7 nAChRs as possible drug candidates for treatment of AD. However, the molecular mechanisms underlying the interaction between Aβ and α7 nAChRs is still not well understood.

In the present study, we demonstrated a specific interaction between α7 nAChRs and fibrillar Aβ by using labeled amyloid ligand ^{3}H-PIB in AD brain. We showed that the α7 nAChR agonists JN403 and varenicline, but not the α4β2 nAChR agonist cytisine, significantly increased fibrillar Aβ levels in cortical tissue of AD brain, measured by the amyloid ligand ^{3}H-PIB. JN403 binds to the α7 nAChRs as a partial agonist with high binding affinity ($K_i = 55$ nM) and selectivity [29, 30]. Varenicline on the other hand, works as a partial agonist for the α4β2 nAChRs ($K_i = 0.06$ nM) and a full agonist for the α7 nAChRs ($K_i = 322$ nM) [31]. Herein, we found that the increase in ^{3}H-PIB binding induced by JN403 and varenicline was abolished by the α7 nAChR antagonists MLA, α-BgTX and the nAChR channel blocker Mec, but not by the α4β2 nAChR antagonist DHβE, suggesting a selective interaction of fibrillar Aβ with the α7 nAChR subtype.

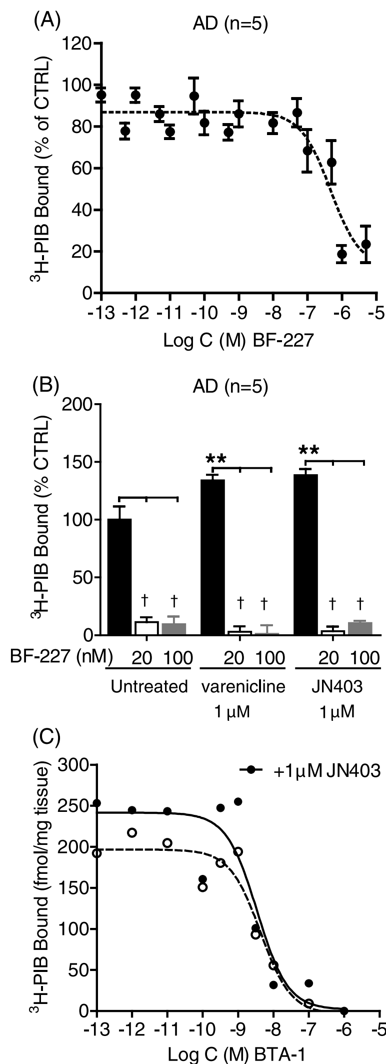

Fig. 4. A) Representative competitive binding of ^{3}H-PIB (1 nM) against BF-227 (10^{-13}–5×10^{-6}M) on the frontal cortical homogenates from one AD brain tissue. B) Effects of 1 μM JN403, 1 μM varenicline, in the presence of BF-227 (20 nM or 100 nM) on ^{3}H-PIB binding on frontal cortical homogenates from AD brain ($n = 5$). Results are means ± S.E.M; $^{**}p < 0.01$, compared to control; $^{\dagger}p < 0.001$ compared to drug. C) Representative competitive binding of ^{3}H-PIB (1 nM) against BTA-1 (10^{-12}–10^{-6} M) on the frontal cortical homogenates from AD brain in the presence and absence of 1 μM JN403.

One possible explanation for the increased ^{3}H-PIB binding could be the dissociation of preformed fibrillar Aβ-α7 nAChR complexes in the brain. Immunohistochemical studies have shown that Aβ is co-localized with α7 nAChRs on Aβ positive cortical pyramidal neurons and astrocytes. The Aβ/α7 nAChR complex could also be detected inside Aβ plaques [10, 32, 33]. However, it is not clear whether Aβ binds directly to α7 nAChRs or involves other membrane components [34–36], and if this interaction could be modulated by nicotinic drugs and what impact this could have on the disease progression.

Variables in studies on amyloid-receptor interaction is that the external Aβ assemblies used could be of different concentrations and aggregation states and interact differently with α7 nAChRs [17, 19] under physiological or pathological conditions [37]. Moreover, it is possible that heterogeneous α7 subunit compositions in different brain regions influence amyloid receptor interactions, and further studies are needed to elucidate the physiological relevance of other α7 nAChR subunit-containing subtypes in the human brain. To investigate Aβ interactions in AD brain in natural aggregation states, we performed measurements in brain homogenates in PBS buffer without further extraction steps. Our findings are consistent with a previous study demonstrating that Aβ binds to α7 nAChRs in a non-competitive manner in cultured hippocampal neurons as well as in human brain tissue as measured by ^{125}I-α-BgTX binding assays [16]. One limitation of our study is that we were not able to differentiate between $A\beta_{40}$ and $A\beta_{42}$, since the amyloid ligand PIB binds to both peptides with similar affinity [23].

Our *in vitro* findings as regards to the binding profile of amyloid ligands PIB and BF-227 are consistent with earlier reports demonstrating high affinity binding of these tracers in AD cortex [23, 38]. Furthermore, we found that although fibrillar Aβ was detectable in the P2 membrane fraction, ^{3}H-PIB binding levels were more abundant in the P1 fraction. In addition, the P1 fraction contributed the most to the JN403-induced increase in ^{3}H-PIB binding, suggesting that the Aβ-α7 nAChR interaction probably occurs on the extracellular side. The involvement of lipid rafts in this Aβ-α7 nAChRs interaction has been suggested [34]. Whether Aβ perturbs the membrane directly or its effect is mediated by membrane receptors is debated. Recent evidence supports that a specific aromatic residue within the agonist binding domain of α7 nAChR is required for this interaction [35, 39].

Furthermore, a distinct pattern of Aβ-α7 nAChR interaction was observed in controls compared to AD brain. One explanation might be that there is a different mechanism involved, due to low amounts of fibrillar Aβ in control cases compared to high (pathological) amounts in AD brain. Recent evidence suggests a physiological role of Aβ in synaptic plasticity and memory, mediated by the activation of α7 nAChRs *in vivo* [37, 40]. It is also possible that Aβ could exert

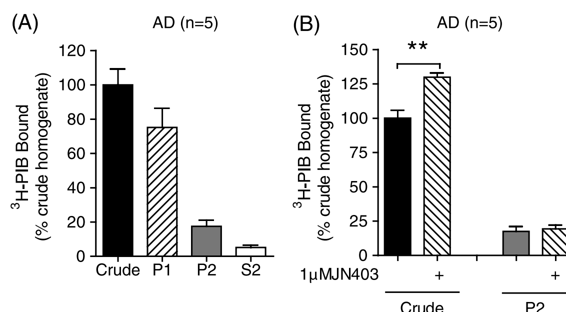

Fig. 5. A) Specific ^{3}H-PIB binding (% crude homogenate) on frontal cortical crude homogenates, P1, P2 (membrane) fraction, S2 fraction from AD cases ($n = 5$). B) Effects of 1 μM JN403 on ^{3}H-PIB binding on the frontal cortical crude homogenates and P2 fraction of AD brain ($n = 5$). Results are means ± S.E.M. $^{**}p < 0.01$.

different effects on α7 nAChRs during specific stages of the disease.

The binding between Aβ and α4β2 nAChRs has earlier been shown to be 1000-fold lower compared to that of α7 nAChRs (pM range) [9]. In the context of pM Aβ concentrations measured in the AD brain, it is possible that α4β2 nAChR agonists and antagonists may not affect the ^{3}H-PIB binding level effectively. Interestingly, a novel α7β2 nAChR that is highly sensitive to the functional antagonism of Aβ was identified in the basal forebrain cholinergic neurons in rodent brain [41].

The cholinergic deficit is an early feature of AD and the density of α7 nAChRs has been reported to increase, decrease, or remain stable at different stages of AD [42–45]. These different observations might reflect the changes in distribution and numbers of α7 nAChRs on neurons and astrocytes, respectively [33, 46]. Moreover, α7 nAChRs have been shown to increase with higher Aβ plaque load [45], especially in AD cases carrying APP_{swe} mutations compared to sporadic AD cases [33]. The pathological Aβ-α7 nAChR interactions could play a critical role in inducing incipient Aβ neurotoxicity mediated in part through the formation of Aβ-α7 nAChR complexes [19], and in turn disrupt receptor signaling and result in intracellular Aβ accumulation and synaptic dysfunction and contribute to the selective vulnerability of the cholinergic system in Aβ-burdened brain regions [8, 16, 32].

Interestingly, we observed no significant change in ^{3}H-PIB binding in AD brain in the presence of galantamine (a weak AChEI and allosteric potentiating ligand, ALP, for both α7 and α4β2 nAChRs [47]). One possible reason might be that Aβ and galantamine bind to different sites on the α7 nAChRs, since the APL-binding site of galantamine is located close to, but is distinct from, the acetylcholine binding site on nAChRs [48].

Memantine did not alter the ^{3}H-PIB measures of fibrillar Aβ in the present study. The NMDAR is suggested to be a membrane target of oligomeric Aβ, and blockage of the NMDARs by memantine, reversed synaptic dysfunction caused by Aβ, and stimulated neuroprotecive effects in AD APP_{swe} transgenic mice [49–51]. However, whether NMDAR and Aβ directly interact [52] or require another receptor such as an Aβ-α7 nAChR interaction is not clear [32, 53].

Similar to our present observations, dimebon has been shown to increase extracellular Aβ levels both *in vitro* and *in vivo* [54]. Interestingly, here we demonstrated that dimebon induced an elevation in ^{3}H-PIB binding in AD brain, which seems to be an effect mediated independent of nAChRs. This could be in line with the finding that dimebon binds to a wide range of membrane receptor targets, including α-adrenergic, histamine, and serotonin receptors [55, 56]. α7 nAChR agonists are reported to protect neuronal cells against Aβ induced toxicity, rescue receptor function, and dissociate Aβ from α7 nAChRs [20, 57, 58], and confer cognitive and neuroprotective benefits, as reported in a recent Phase IIb clinical trial in AD patients (EVP-6124) [59] as well as in AD mouse models [60, 61]. In addition to a neuromodulatory role, the involvement of α7 nAChRs in Aβ pathogenesis renders it as a potential target for treatment of AD.

In conclusion, our study suggests that α7 nAChR agonists such as JN403 and varenicline disrupt the interaction between fibrillar Aβ and α7 nAChRs in the frontal cortex of AD brain. A possible mechanism is that the binding site of these drugs overlaps partly with the fibrillar Aβ binding site to α7 nAChRs as illustrated in Fig. 6. The α7 nAChR agonists may therefore

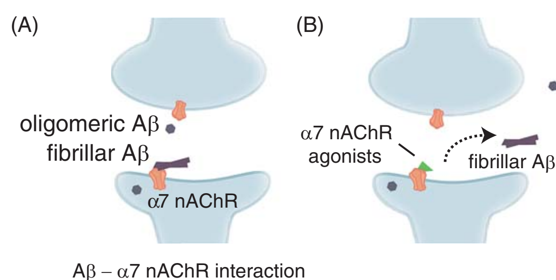

Fig. 6. An illustration of the proposed interactions between different forms of Aβ and α7 nAChRs (A) and of α7 nAChR agonists interrupting this interaction (B).

induce a release of Aβ from preformed complexes. Treatment with α7 nAChR stimulatory drugs modulate Aβ/α7 nAChR pathogenic signaling mechanisms in AD brain and hold great promise for new disease modifying therapies in AD.

ACKNOWLEDGMENTS

The authors thank Professor Nobuyuki Okamura (Department of Pharmacology, Tohoku University School of Medicine, Sendai, Japan) for the kind gift of BF-227. The authors thank Pfizer (Groton, CT, USA) for the kind gift of varenicline and Novartis (Basel, Switzerland) for the kind gift of JN403. The authors thank Professor Manfred Windisch (JSW research, Grambach, Austria) for the kind gift of dimebon.

This work was supported by grants the Swedish Research Council (project 05817), the Strategic Research Program in Neuroscience at Karolinska Institutet, the Swedish Brain Power, the Old Servants foundation, the Gun and Bertil Stohne's foundation, Magnus Bergvalls Foundation, Karolinska Institutet's Foundation for Aging Research, the Lars Hierta Memorial Foundation, the Olle Engkvist Byggmästare Foundation, the Alzheimer Foundation in Sweden, Brain Foundation, the Regional Agreement on Medical Training and Clinical Research (ALF) between Stockholm County Council and the Karolinska Institutet.

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1514).

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