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Cortical α7 Nicotinic Acetylcholine Receptor and β-Amyloid Levels in Early Alzheimer's Disease

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Abstract

Objective—To examine α7 nicotinic acetylcholine receptor (nAChR) binding and β-amyloid (Aβ) peptide load in superior frontal cortex (SFC) across clinical and neuropathological stages of Alzheimer's disease (AD).

Design—Quantitative measures of α7 nAChR by [^{3}H]methyllycaconitine binding and Aβ concentration by enzyme-linked immunosorbent assay in SFC were compared across subjects with antemortem clinical classification of no cognitive impairment (NCI), mild cognitive impairment (MCI), or mild-moderate AD (mAD), and with post-mortem neuropathological diagnoses.

Setting—Academic medical center.

Subjects—Twenty-nine elderly retired clergy.

Results—Higher concentrations of total Aβ peptide in SFC were associated with clinical diagnosis of mAD (p=0.015), lower Mini Mental State Examination scores (p=0.0033), presence of cortical Aβ plaques (p=0.015), and likelihood of AD diagnosis by the NIA-Reagan criteria (p=0.0015). Increased α7 nAChR binding was associated with NIA-Reagan diagnosis (p=0.021) and, albeit weakly, the presence of cortical Aβ plaques (p=0.079). There was no correlation between the two biochemical measures.

Conclusions—These observations suggest that during the clinical progression from normal cognition to neurodegenerative disease state, total Aβ peptide concentration increases, while α7 nAChRs remain relatively stable in SFC. Regardless of subjects' clinical status, however, elevated α7 nAChR binding is associated with increased Aβ plaque pathology, supporting the hypothesis that cellular expression of these receptors may be up-regulated selectively in Aβ plaque-burdened brain areas.

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Keywords

acetylcholine; mild cognitive impairment; cholinergic; nicotinic; receptors; amyloid; dementia; frontal cortex

Introduction

Cholinergic synaptic dysfunction contributes to cognitive impairment in Alzheimer's disease (AD). These changes may be due, in part, to increased concentrations of β-amyloid (Aβ) peptides[1] and their interactions with nicotinic acetylcholine receptors (nAChRs), which are essential for normal cognitive function[2, 3]. Aβ binds to nAChRs, particularly the α7 subclass[4, 5]; this may alter receptor function[6–10] and also result in Aβ internalization, fibrillization, and deposition into plaques and cerebral vasculature[4, 11–13]. The status of α7 nAChRs in AD is controversial as there are reports of increases, decreases, or stability in AD[14–18]. While non-α7 nAChR binding in frontal cortex declines early in AD[19], quantitative biochemical studies specific for α7 nAChRs in subjects with preclinical and early AD remain to be performed. The current study quantified α7 nAChR binding and total Aβ peptide concentration in the superior frontal cortex (SFC) from subjects who participated in the Religious Orders Study[20, 21]. The status of these two biochemical measures was examined across subjects' groups defined by clinical diagnoses of no cognitive impairment (NCI), mild cognitive impairment (MCI), and early AD stage (mild-moderate AD, mAD), or neuropathological diagnosis.

Materials and Methods

Subjects

This study included 29 participants in the Religious Orders Study, a longitudinal clinical-pathological study of aging and AD in retired Catholic nuns, priests, and brothers[20]. Inclusion criteria and a description of the clinical evaluation have been published[20, 21]. At the last clinical evaluation (<12 months prior to death), subjects were classified as NCI, MCI, or mild-moderate AD (see Table 1). Diagnosis of AD dementia was made using standard criteria[22]. MCI was defined as impairment on neuropsychological testing, but without a diagnosis of dementia by the examining neurologist[23], criteria similar to those describing patients who were not cognitively intact, but nonetheless did not meet the criteria for dementia[24–27]. A consensus conference of neurologists and neuropsychologists reviewed all the clinical and neuroimaging data, medical records and interviews with family members, and assigned a final diagnosis.

Neuropathological evaluation

Neuropathological diagnosis of AD (possible, probable, or definite AD) or Not AD (Table 1) was based on modified criteria by the Consortium to Establish a Registry for AD (CERAD) 28 which applied semi-quantitative estimates of neuritic plaque density by a board-certified neuropathologist blinded to the clinical diagnosis[29]. Subjects were also assigned an NIA Reagan neuropathological diagnosis[30] and a Braak score based on the presence of neurofibrillary tangles (NFTs)[31]. Subjects with pathology other than AD were excluded from the study.

[^{3}H]MLA binding assay

Fresh frozen SFC (Brodmann area 9) gray matter was divided into aliquots for nAChR binding and Aβ peptide enzyme linked immunoadsorbent assay (ELISA). For α7 nAChR binding, samples were homogenized in 10 volumes of 50 mM Tris HCl buffer (pH=7.0), centrifuged twice at 40,000 × *g* for 10 minutes, re-suspended in Tris buffer and stored at −80°C. Samples

were thawed and re-suspended in an equal volume of Tris buffer containing 0.1% bovine serum albumin (BSA)[32, 33]. Samples, 0.5 mg protein each, were combined with 9.5 nM [^{3}H]MLA (43 Ci/mmol, Tocris Cookson Ltd., Bristol, UK) in Tris HCl buffer containing 0.1% BSA. Non-specific binding was measured in the presence of 1 mM nicotine. After a 2 hr incubation on ice, bound ligand was separated from free ligand using Whatman GF/B filters, presoaked in 0.3% polyethyleneimine. Filters were rinsed with Tris HCl buffer, placed in scintillation vials, and shaken in scintillation fluid for 1 hour before radioactivity was determined. Specific binding was calculated as the difference between total and non-specific binding. Results were expressed as femtomoles/mg protein.

Aβ ELISA Assay

The Aβ assay was performed using a previously reported protocol[34]. Frozen SFC samples were homogenized (150mg tissue wet weight/ml PBS buffer, pH = 7.4) and 30 mg of homogenized tissue were sonicated in 70% formic acid (FA), and centrifuged at 109,000 × *g* at 4°C for 1 hour, resulting in samples containing both soluble and FA-extracted insoluble Aβ peptides. The supernatant was neutralized with 1M Tris and 0.5M sodium phosphate, and the samples assayed using a fluorescent-based ELISA (BioSource, Carlsbad, CA) following the kit's instructions, with a capture antibody specific for the NH_2-terminus of Aβ(amino acids 1–16), and detection antibodies specific for Aβ40 and Aβ42 peptides. Values were determined from standard curves using synthetic $Aβ_{1–40}$ and $Aβ_{1–42}$ peptides (BioSource) and expressed as pmoles/gram wet brain tissue. "Total" Aβvalues represent a sum of $Aβ_{1–40}$ and $Aβ_{1–42}$ peptide values.

Statistical analysis

Aβ levels were log-transformed due to data skewness. Comparisons of demographic characteristics, MLA binding and Aβ levels between clinically- or neuropathologically-defined groups were performed using the Wilcoxon rank-sum test, Kruskal Wallis test, or the Fisher's exact test, as appropriate. The association between demographic characteristics and MLA binding or Aβ levels was assessed by Spearman rank correlation or Wilcoxon rank-sum test. Partial correlation was used for additional analyses adjusting for age. The correlation between MLA binding and Aβ levels was assessed by Spearman correlation. The level of statistical significance was set at 0.05 (two-sided).

Results

Clinical and Pathological Analyses

The three clinical groups differed in MMSE scores ($p<0.0001$), with the mAD group performing worse than both the NCI and MCI groups (Table 1). Clinical diagnostic groups also differed in CERAD diagnosis ($p=0.040$), Braak staging ($p=0.036$), and the NIA-Reagan diagnosis ($p=0.018$), with the mAD subjects being more advanced neuropathologically compared to both the MCI and NCI groups (Table 1).

Subjects with a CERAD diagnosis of AD (CERAD < 4, possible, probable or definite AD; positive for cortical plaques) had lower MMSE scores ($p=0.055$), and more advanced Braak stages and NIA-Reagan neuropathologic diagnoses ($p=0.004$ and $p < 0.0001$; Table 1) than the Not AD group (CERAD = 4; no cortical plaques).

MLA binding and Aβ concentrations across clinical and neuropathologic categories

MLA binding levels were slightly higher in mAD subjects, however, the difference was not statistically significant (Table 2). Clinical groups differed in total Aβ (combined Aβ42 and

Aβ40; p=0.015; Figure 1) and Aβ42 (p=0.022), but not Aβ40, concentrations, with mAD subjects having the highest levels.

Subjects with a CERAD diagnosis of AD had higher MLA binding (p = 0.079; Figure 1), and higher concentrations of Aβ42 (p = 0.021), Aβ40 (p = 0.063), and total Aβ (p = 0.015; Table 2; Figure 1) in the SFC when compared to those with CERAD diagnosis of Not AD.

MLA binding and Aβ concentration: association with clinical-neuropathological factors

There was no association of MLA binding with any of the demographic or clinical variables examined. Higher MLA binding levels correlated with greater likelihood of AD by the NIA-Reagan diagnosis (r=-0.47, p=0.021; Table 3), and weakly with Braak staging. There was an association of higher Aβ concentrations with more advanced age (r=0.48–0.56, p=0.011–0.032; Table 3), but not with sex, education, the presence of *APOE*ε4 allele, or post-mortem interval. Higher concentrations of total Aβ and Aβ42, but not Aβ40, correlated with lower MMSE scores (r=−0.62 for both, p=0.0033 and 0.0038; Table 3). In addition, higher total Aβ and Aβ42 concentrations correlated with worse neuropathological scores (r=0.62–0.70, p<0.01), as did Aβ40 concentrations, although to a lesser extent (Table 3). Adjusting for age, partial correlation showed similar results, although it yielded smaller correlation coefficients. There was no correlation between MLA binding and Aβ protein concentrations.

Comment

This study examined SFC α7 nAChR binding and Aβ peptide concentrations across the clinical and neuropathologic categories of AD. Both markers were assayed in the same samples of cortical tissue from subjects who were clinically characterized within 12 months before death, and neuropathologically evaluated postmortem. We did not detect significant changes in SFC α7 nAChR binding across clinical diagnostic groups. However, a trend toward elevated α7 nAChR binding levels was evident in subjects with CERAD diagnoses of AD (possible, probable or definite) relative to Not AD subjects (without neuritic Aβ plaques). Total (sum of Aβ42 and Aβ40) and Aβ42 concentrations were elevated in the CERAD-AD group compared to the CERAD-Not AD group, as well as in the clinical mAD compared to the MCI and NCI groups. The increase in α7 nAChRs in Aβ plaque-positive subjects, despite the lack of an association with Aβ concentrations, indicates that cellular expression of this receptor is influenced, either directly or indirectly, by the presence of senile plaques. This is in agreement with previous studies in AD patients and animal models[35, 36], and is supported further by the current observation that the correlation between α7 nAChR levels and neuropathological staging was stronger using NIA-Reagan criteria compared to Braak staging, the latter relying only on NFTs for stage designation31.

The apparent stability of α7 nAChRs across clinical categories of NCI, MCI and mAD, and the lack of an association with MMSE scores, could be explained by the presence of plaques in all clinical groups. Cortical plaques were present in more than half of our NCI cases (Table 1), in agreement with previous reports of a substantial AD pathology in cognitively normal aged individuals[29, 37–41]. Although these studies would benefit from examining larger numbers of cognitively intact subjects free of any Aβ pathology, such individuals are rare, as Aβ plaques are a common feature in brains of elderly individuals[37, 38, 40, 41]. Furthermore, the pathological burden of Aβ includes not only insoluble fibrils in plaques, but also soluble Aβoligomers[42]. The impact of these distinct pools of Aβ upon α7 nAChR binding in preclinical, early/moderate and severe end-stage AD cases will be an important question to answer in future studies.

There are several possible explanations for the observed association between Aβ plaques and increased α7 nAChR binding. Plaques may serve as reservoirs of soluble Aβ species[1], which

can bind with high affinity to neuronal α7 receptors[4,5] into a complex that is subsequently internalized[12]. This may result in a compensatory increase in expression of α7 nAChR on the cell surface. Additionally, excessive intracellular accumulation of Aβ42 and subsequent neuronal lysis may contribute to plaque pathology[12], potentially creating a cycle of neuronal degeneration and Aβ plaque deposition in AD. High concentrations of fibrillar Aβ in plaques, or soluble Aβ in the vicinity of these structures, may also influence the up-regulation of α7 nAChR by reactive astrocytes. Astrocytes proliferate and display increased α7 nAChR density in the presence of Aβ plaques[36, 43, 44], and up-regulate nAChR mRNA expression and protein levels when exposed to Aβ *in vitro*[45]. Receptor binding assays cannot differentiate the relative contribution of different cell types to the overall regional expression of α7 nAChRs detected in tissue homogenates. Adding to this complexity are the post- and pre-synaptic sites of expression of α7 nAChRs, involving both local neuronal circuitry and afferent projections from distant neuronal cell populations. In this regard, a recent single-cell expression profiling study demonstrated up-regulation of α7 nAChR mRNA in cortical-projecting basal forebrain cholinergic neurons in mAD subjects[46], suggesting that changes in cortical α7 protein levels involve presynaptic elements on an important cholinergic afferent system. Collectively, these studies suggest that in the SFC, α7 nAChRs levels reported here reflect changes both in cortical-projecting cholinergic basal forebrain neurons and regional cell-specific expression of these receptors in response to Aβ pathology.

In conclusion, the present findings demonstrate that cognitive decline in mAD is not associated with detectable changes in cortical α7 nAChR binding levels. In contrast, Aβ concentrations increased in mAD and correlated with cognitive impairment, in accord with reported associations of increased Aβ load with cognitive decline in AD[47, 48]. The observed trend for increased SFC α7 in subjects with plaques is in agreement with a previously reported positive correlation between α-bungarotoxin binding and Aβ plaque density[35], and warrants further investigation. These changes are in contrast with reports of reduced cortical α4 nAChR immunoreactivity with increased Aβ plaque densities, and a loss of epibatidine binding with increased Aβ42 concentrations[35]. Thus, α7 and non-α7 nAChRs may be differentially affected by Aβ pathology. *In vivo* PET imaging techniques using radiolabeled probes for early detection of Aβ plaques[49, 50] and changes in select nAChRs[51] may act as early biomarkers for AD and will enable the timely implementation of appropriate therapies.

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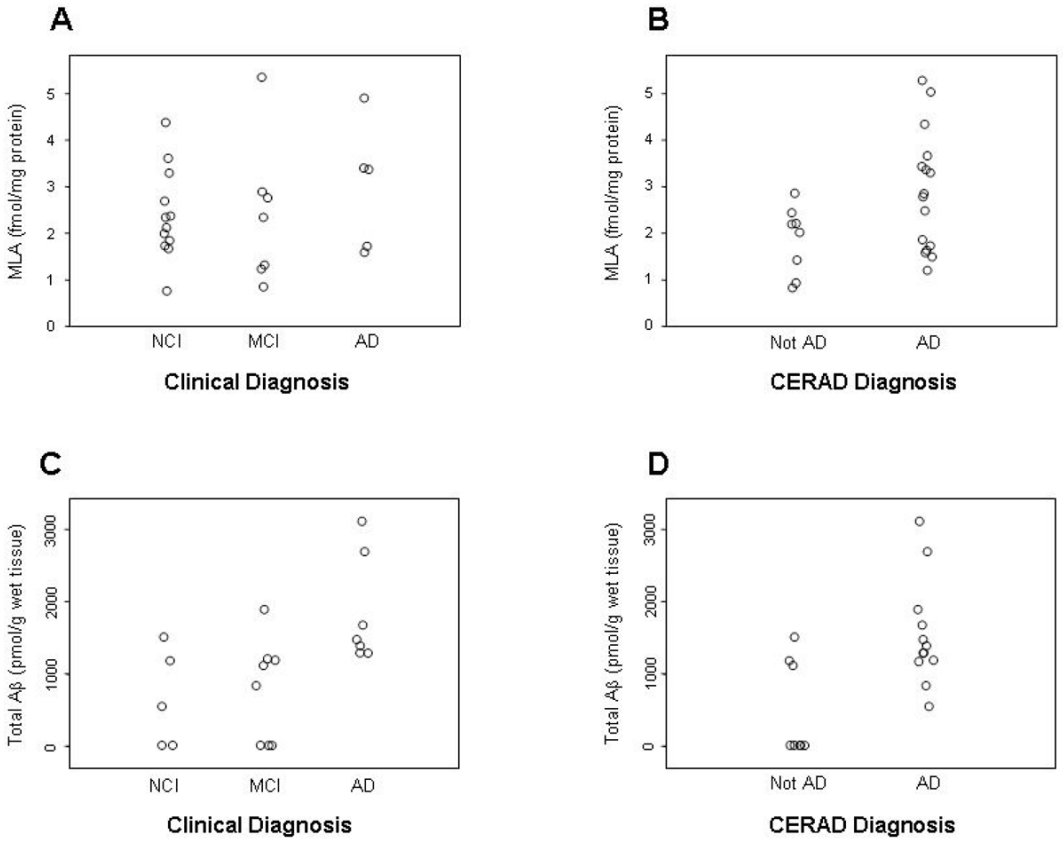

Figure 1.

α7 binding (A,B) and total Aβ peptide concentrations (C,D) in the frontal cortex from subjects categorized into clinical diagnostic groups of NCI, MCI and AD (A,C), or into neuropathological groups of Not AD and AD (possible, probable, definite) by modified CERAD criteria (B,D).

Table 1

Demographic, clinical and neuropathological characteristics by diagnostic groups

		Clinical Diagnosis				CERAD Diagnosis		
Variable		**NCI (N=12)**	**MCI (N=9)**	**mAD (N=8)**	***P* value**	**Not AD (N=10)**	**AD[§] (N=19)**	***P* value**
Age, mean (SD), yr		86.1 (5.5)	85.3 (3.6)	89.4 (5.0)	0.1 [a]	85.0 (4.5)	87.7 (5.0)	0.2 [a]
Male, N (%)		4 (33)	3 (33)	3 (38)	1.0 [b]	4 (40)	6 (32)	0.7 [b]
Education, mean (SD), yr		18.5 (2.7)	18.9 (2.5)	15.9 (3.3)	0.1 [a]	17.8 (3.0)	17.9 (3.1)	0.9 [a]
APOE ε4, N (%)		0	0	2 (25)	0.074 [b]	0 (0)	2 (11)	1.0 [b]
MMSE, mean (SD)		28.7 (1.1)*	27.1 (2.5)	12.8 (5.8)	< 0.0001 [a]	27.7 (2.5)	21.4 (8.8)*	0.055 [a]
PMI, mean (SD), hr		5.3 (3.3)	5.0 (3.6)	4.4 (2.1)	0.8 [a]	5.8 (3.9)	4.5 (2.5)	0.6 [a]
Braak Stage, N	I – II	1	3	1		4	1	
	III – IV	10	6	2	0.036 [a]	6	12	0.0040 [a]
	V	1	0	5		0	6	
NIA Reagan Dx, N	LL	5	5	0		10	0	
	IL	7	4	5	0.018 [a]	0	16	< 0.0001 [a]
	HL	0	0	3		0	3	
CERAD Dx, N	Not AD	5	5	0	0.040 [b]	--	--	
	AD[§]	7	4	8		--	--	

[§] Includes CERAD diagnosis of possible, probable, and definite AD

* MMSE not available for 1 case

[a] Wilcoxon rank-sum test or Kruskal-Wallis test

[b] Fisher's exact test

Table 2

Superior frontal cortex MLA binding and Aβ ELISA levels by diagnostic groups

	Clinical Diagnosis				CERAD Diagnosis		
Variable	**NCI**	**MCI**	**mAD**	***P* value** [a]	**Not AD**	**AD**[§]	***P* value** [a]
MLA, mean (SD)	2.4 ± 1.0 (N=12)	2.4 ± 1.5 (N=7)	3.0 ± 1.4 (N=5)	0.7	1.8 ± 0.7 (N=8)	2.9 ±1.3 (N=16)	0.079
Aβ40*, mean (SD)	2.3 ± 1.6 (N=5)	2.5 ± 2.2 (N=8)	4.3 ± 1.7 (N=7)	0.1	1.7 ± 1.9 (N=8)	4.0 ±1.6 (N=12)	0.063
Aβ42*, mean (SD)	5.0 ± 2.5 (N=5)	5.2 ±2.4 (N=8)	7.3 ± 0.3 (N=7)	0.022	4.1 ± 2.5 (N=8)	7.1 ±0.4 (N=12)	0.021
Total Aβ*, mean (SD)	5.1 ± 2.5 (N=5)	5.3 ±2.4 (N=8)	7.5 ± 0.4 (N=7)	0.015	4.2 ± 2.4 (N=8)	7.3 ±0.5 (N=12)	0.015

[§] Includes CERAD diagnosis of possible, probable, and definite AD

* Aβ levels were log-transformed

[a] Wilcoxon rank-sum test or Kruskal-Wallis test

Table 3

Association between clinical/neuropathological variables and superior frontal cortex MLA binding and Aβ ELISA levels

Variable	MLA (N=24)	Aβ40 (N=20)	Aβ42 (N=20)	Total Aβ (N=20)
Age	--	r = 0.48, p = 0.032	r = 0.56, p = 0.011	r = 0.55, p = 0.012
Sex	--	--	--	--
Education	--	--	--	--
APOE ε4	--	--	--	--
MMSE	(r = −0.25, p = 0.26)	(r = −0.36, p = 0.12)	r = −0.62, p = 0.0038	r = −0.62, p = 0.0033
PMI	--	--	--	--
Braak stage	(r = 0.40, p = 0.053)	(r = 0.36, p = 0.12)	r = 0.70, p = 0.0005	r = 0.62, p = 0.0035
NIA Reagan Dx	r = −0.47, p = 0.021	r = −0.52, p = 0.020	r = −0.63, p = 0.0031	r = −0.66, p = 0.0015

r: Spearman rank correlation coefficient

--: Not statistically significant