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Does apolipoprotein E (Apo-E) genotype influence nicotinic receptor binding in Alzheimer's disease

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Summary. We wished to determine the influence of the apolipoprotein E (Apo-E) genotype on the loss of high affinity nicotinic acetylcholine receptor (nAChR) binding in Alzheimer's disease (AD). The interaction between ε4 allele gene dose and cholinergic loss in AD remains controversial. We have demonstrated that nicotinic binding is significantly lost in AD. Tissue from the midfrontal (MF) cortex of 7 subjects with no ε4 allele copies (ε−/ε−) (mean death age 75.1 ± 10.4 years) was compared to MF cortex of 14 subjects heterozygous for the ε4 allele (ε4/ε−) (mean death age 81.4 ± 7.3 years) and MF cortex of 10 subjects homozygous for the ε4 allele (ε4/ε4) (mean death age 79.6 ± 5.0 years). All subjects were autopsy confirmed AD (using NIA and CERAD criteria) and met NINCDS-ADRDA clinical criteria for probable or possible AD. Nicotine AChR binding was assayed using the high affinity nicotinic agonist ^{3}H-epibatidine ([^{3}H]-Epi). Apo-E genotype was determined in blood samples or in post-mortem tissue. The mean age at death was not significantly different among the groups ($p = 0.19$). There was no difference in mean [^{3}H]-Epi total binding among the three groups (6.7 ± 4.6, 6.1 ± 2.4, and 6.0 ± 1.0 fmol/mg protein for ε−/ε−, ε4/ε−, and ε4/ε4 respectively. We conclude that the presence or absence of the Apo-E4 genotype does not influence the loss of high affinity nAChR in AD.

Keywords: Nicotinic receptors, Alzheimer's disease, apolipoprotein E, choline acetyltransferase, synaptophysin.

Introduction

Alzheimer's disease (AD) is a progressive dementia characterized by memory loss, functional decline, and global impairment in cognition. While the etiol-

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ogy remains largely unknown, several risk factors have been identified. Included in these are genetic risk factors that predispose to the development of AD. One of these, the apolipoprotein E4 (Apo-E4) allelle is associated with an increased risk of AD as the allele dose increases (Corder et al., 1993; Saunders et al., 1993; Roses and Saunders, 1994). How Apo-E4 mechanistically affects AD is unknown. Research indicates that Apo-E may interact with Aβ or tau. Apo-E4 may promote a higher cholinergic deficit due to aberrant trafficking of phosphotidyl choline, a source of precursor for acetylcholine (Poirier et al., 1995; Beffert and Poirier, 1996).

The loss of nicotinic receptor ligand binding in AD has been reported by ourselves and others and is a consistent finding in the disease (Flynn and Mash, 1986; Nordberg and Winblad, 1986; Perry et al., 1990, 1995; Rinne et al., 1991; Aubert et al., 1992; Nordberg et al., 1992; Sabbagh et al., 1998; Reid et al., 2000; Sparks et al., 2000). However, the relationship between high affinity nicotine acetylcholine receptor (nAChR) binding and Apo-E4 genotype is still controversial. In this study we sought to determine if there was an association between Apo-E4 gene dosage and the post mortem level of high affinity nAChR binding in the frontal cortex of AD subjects. If the inverse relationship between cholinergic loss and Apo-E4 gene dosage exists, one might predict that nAChR loss might also be inversely related to increasing Apo-E4 gene dosage.

Material and methods

Subjects

The AD subjects in the present study were followed clinically at the University of California, San Diego's (UCSD) Alzheimer's Disease Research Center (ADRC), by the Senior's Only Care (SOCARE) group, or in the private practices of the senior clinicians. Included were 31 autopsy-confirmed AD patients diagnosed by NIA (Khachaturian, 1985) and CERAD (Mirra et al., 1991) criteria for definite or probable AD who also met NINCDS-ADRDA criteria for a clinical diagnosis of probable or possible AD (McKhann et al., 1984). Patients with a pathological diagnosis of dementia with Lewy bodies according to Consortium on Dementia with Lewy bodies criteria were excluded (McKeith et al., 1996). Pathological assessment was performed at the UCSD neuropathology laboratory. The post-mortem interval varied from two to 12 hours. Autopsy was performed using a protocol described by Terry et al. (1981, 1991).

[^{3}H]-(±)epibatidine binding

Mid-frontal (MF) cortex (Brodmann area 38, 39 and 46) was homogenized and assayed for [^{3}H]-(±)Epi binding as previously described (Sabbagh et al., 1998). Briefly, nAChR levels were determined by incubating 0.75–2.5 mg protein in 1 ml assay buffer containing 5 nM [^{3}H]-(±)Epi (45–65 Ci/mmole, NEN, Boston MA). Non-specific binding was determined in the presence of 1 μM unlabeled (±)Epi (RBI; Natick, MA). Samples were incubated on ice for 2 hours, and the assay terminated by rapid filtration through GF/C filters, presoaked in 0.5% polyethyleneimine for at least one hour, using a Brandell cell harvester (Brandell Instruments; Gaithersburg, MD) and radioactivity quantified by liquid scintillation spectrometry (Tri-Carb 1600TR, Packard Instruments; Meriden, CT). There were three replicates per experiment, with the data representing the average of one to three separate experiments per sample. Protein content in the samples was deter-

mined using the BCA protein assay (Pierce Chemical; Rockford, IL) with BSA as a standard.

Choline acetyltransferase (ChAT) measurements

Samples were taken from MF areas adjacent to samples used for [^{3}H]-Epi binding of frozen unfixed right hemibrain neocortex and homogenized in 1 mM ethylenediamine tetra-acetic acid (EDTA), pH 7.0 containing 0.1% Triton X-100. Analysis of ChAT activity was performed in triplicate by the modified Fonnum technique (Fonnum, 1969; Hansen et al., 1988).

Synapse density measurements

Synaptic density measurements from the right MF cortex were performed by the dot-immunobinding assay for synaptophysin (Syn) immunoreactivity described by Alford et al. (1994).

Apo-E genotyping

The Apo-E genotype was determined from analysis of post-mortem brain tissue by homogenizing 500 mg of frozen brain tissue over ice, adding lysis buffer and proteinase K, and rocking overnight at 37°C, followed by phenol/chloroform extraction. Genomic DNA was amplified by PCR, using primers prescribed by Wenham et al. (1991). After amplification, DNA was digested with the *Hha* restriction enzyme, electrophoresed on 6% denaturing polyacrylamide gels, and visualized by ethidium bromide staining.

Statistical analysis

Differences across groups were examined using analysis of variance (ANOVA) for continuous variables. The criterion for significance was $p < 0.05$. Analyses were performed using PRISM statistical programs.

Results

Included in the study were 7 AD patients that were ε−/ε−, 14 patients that were ε4/ε−, and 10 patients that were ε4/ε4. The data is summarized in Table 1. The mean ages for these three groups did not significantly differ among the groups ($p = 0.19$). Syn levels for the three groups also did not significantly differ among the groups ($p = 0.58$).

There was no significant difference in [^{3}H-Epi] binding among the groups ($p = 0.87$, Table 1 and Fig. 1). Although ChAT activity was not significantly different between Apo ε−/ε− and Apo ε4/ε4, both groups have significantly less ChAT activity than Apo ε4/ε− ($p < 0.002$) (Table 1 and Fig. 2).

Table 1. Clinical and neurochemical data

Apo E dosage (n)	Age (years)	^{3}H-EPI (fmol/mg protein)	ChAT (nmole Ach/hr/100 mg)	Syn (AU/mg protein)
ε–/ε– (7)	75.1 ± 10.4	6.7 ± 4.6	107.8 ± 66.6*	82.4 ± 37.7
ε4/ε– (14)	81.4 ± 7.3	6.1 ± 2.4	189.3 ± 67.1	82.2 ± 34.9
ε4/ε4 (10)	79.6 ± 5.0	6.0 ± 1.0	80.5 ± 57.7*	97.4 ± 30.8

*$p < 0.002$ vs. Apo ε4/ε–

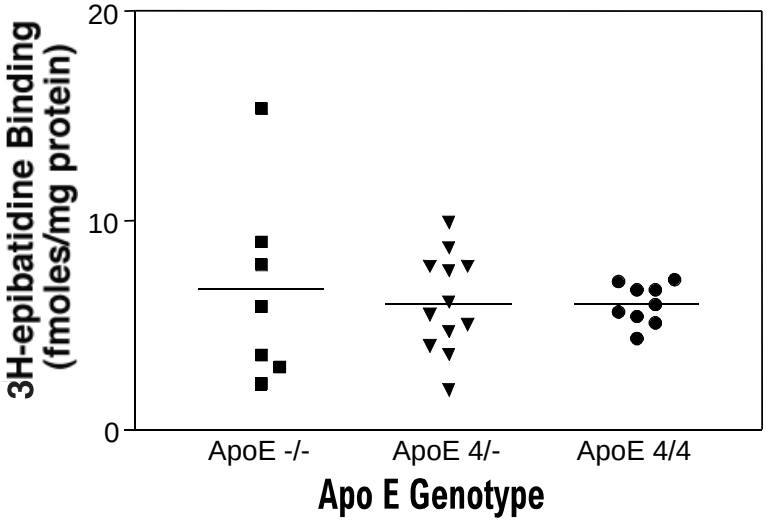

Fig. 1. Scatter plot of ^{3}H-epibatidine binding for cortical membranes from Apo E-/- (■), Apo E 4/- (▼) and Apo E4/4 (●). [^{3}H]-epibatidine binding and Apo-E genotype were determined as described as described in Materials and methods

Discussion

The results of these experiments indicate that Apo-E4 allele burden does not influence level of nAChR loss in AD. These findings confirm earlier published reports of a lack of correlation between Apo-E4 allele burden and loss of nAChR binding in AD (Svensson et al., 1997). Our work differs somewhat from Svensson et al. (1997) in that we examined frontal cortex and have a larger ε4/ε4 homozygous sample. This report also differs from previously

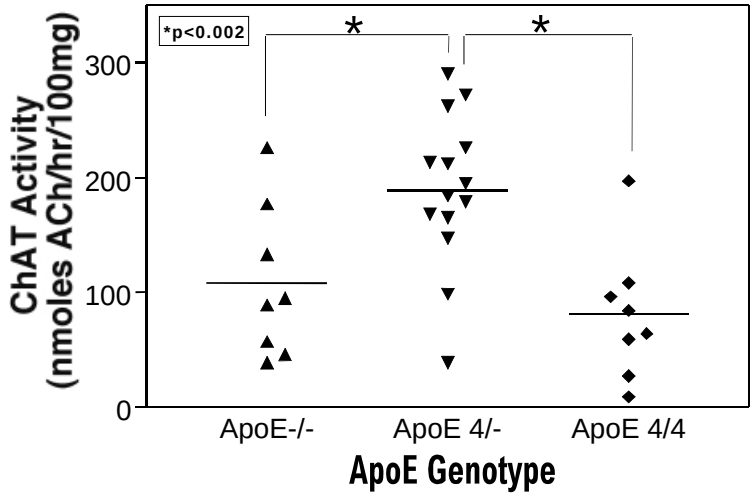

Fig. 2. Scatter plot of ChAT activity for cortical membranes from Apo E-/- (▲), Apo E 4/- (▼) and Apo E 4/4 (◆). [^{3}H]-epibatidine binding and Apo E genotype were determined as described as described in Materials and methods

published reports of a decremental loss of nAChR binding inverse to the Apo-E4 allele burden (Poirier et al., 1995). The reason for the different findings might be explained by the use of a different ligand, examination of a different region of cortex, or a more clearly defined sample. One possible explanation for a lack of interaction between Apo-E status and nAChR binding is that receptor levels were examined in post-mortem tissue. It may be that the receptor levels and Apo-E genotype interacted during the course of AD. An alternate explanation might be that [^{3}H-Epi] might label a different receptor population than the methylcarbamoylcholine used by Poirier et al. (1995) since [^{3}H-Epi] labels the nAChR with very high afftinity (Houghtling et al., 1995; Warpman and Nordberg, 1995). However, since two separate studies do not demonstrate a progressive loss of nAChR as the Apo-E4 gene dose increases, it appears unlikely that the Apo-E genotype influenced nicotinic receptors during the course of AD.

Another finding of this study is a lack of loss of ChAT activity with increasing Apo-E4 allele burden. In fact, the heterozygotes had the highest ChAT activity but there was no significant difference between non-ε4 carriers and ε4 homozygotes. This finding corroborates the findings of Corey-Bloom et al. (2000) and others who failed to show an inverse relationship between Apo-E4 allele burden and declining ChAT activity (Morris et al., 1996; Svensson et al., 1997; Corey-Bloom et al., 2000). However, others have found that ChAT activity, like nicotinic binding is progressively lost with increasing Apo-E4 allele burden (Poirier, 1994; Poirier et al., 1995; Beffert and Poirier, 1996). Like Corey-Bloom et al. (2000), the reason for the lack of decrement may include use of a matched cohort for age and disease duration, and the use of frontal cortex rather than temporal lobe or hippocampus. Nevertheless, Svensson et al. (1997) used temporal cortex and still did not find a loss of ChAT activity with increasing Apo-E4 allele burden. The reason Apo-E4 heterozygotes have the highest ChAT activity remains unclear but it suggests the lack of an inverse association between ChAT and Apo-E is unlikely to be a spurious finding and further supports the absence of effect that the Apo-E genotype has on cholinergic activity.

In this study, we confirm the findings of Corey-Bloom et al. (2000) that synapse loss is not influenced by Apo-E genotype.

The absence of an interaction between Apo-E status and cholinergic activity or synapse loss does not mean that Apo-E is not important mechanistically. In fact, Apo-E appears to interact with Aβ and tau. Apo-E has been localized to plaques and dystrophic neurites immunohistochemically (Gearing et al., 1995; Dickson et al., 1997) and Apo-E4 may increase amyloid and neuritic pathology (Nagy et al., 1995). Apo-E4 homozygotes have been shown to have a reduced ability to suppress amyloid formation in vivo (Evans et al., 1995) and have higher Aβ amyloid deposition (Schmechel et al., 1993; Berr et al., 1994; Heinonen et al., 1995; Ohm et al., 1995, 1999). Alternatively Apo-E may affect AD pathogenesis by affecting tangle pathology. Studies show that the presence of Apo-E4 is associated with neurofibrillary tangle formation (Ohm et al., 1995, 1999). Ohm et al. (1999) demonstrated that the Apo-E4 genotype is associated with a higher Braak stage and higher tangle counts.

Tau and microtubule-associated proteins (MAPs) bind to Apo-E3 but not Apo-E4, suggesting isoform specific interactions that may regulate intraneuronal tau metabolism in AD (Huang et al., 1994; Strittmatter et al., 1994). Others have shown that Apo-E binds to tau and Aβ and that it is not isoform specific (Richey et al., 1995).

Taken together, the lack of association between Apo-E4 allele burden and the loss of nAChR binding or loss of ChAT activity suggests that cholinergic activity is not affected by Apo-E status. It is likely that other factors such as amyloid burden or synaptic losses affect these neurochemical markers in AD. While it is clear that Apo-E affects the neuropathology of AD, it does not appear to influence cholinergic loss.

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