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Acetylcholine receptor and behavioral deficits in mice lacking apolipoprotein E

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Abstract

Apolipoprotein E (apoE) is involved in the risk to develop sporadic Alzheimer's disease (AD). Since impaired central acetylcholine (ACh) function is a hallmark of AD, apoE may influence ACh function by modulating muscarinic ACh receptors (mAChRs). To test this hypothesis, mAChR binding was measured in mice lacking apoE and wild type C57BL/6J mice. Mice were also tested on the pre-pulse inhibition, delay eyeblink classical conditioning, and 5-choice serial reaction time tasks (5-SRTT), which are all modulated by ACh transmission. Mice were also given scopolamine to challenge central mAChR function. Compared to wild type mice, mice lacking apoE had reduced number of cortical and hippocampal mAChRs. Scopolamine had a small effect on delay eyeblink classical conditioning in wild type mice but a large effect in mice lacking apoE. Mice lacking apoE were also unable to acquire performance on the 5-SRTT. These results support a role for apoE in ACh function and suggest that modulation of cortical and hippocampal mAChRs might contribute to genotype differences in scopolamine sensitivity and task acquisition. Impaired apoE functioning may result in cholinergic deficits that contribute to the cognitive impairments seen in AD.

Keywords: Acetylcholine; Apolipoprotein E; Alzheimer's disease; Muscarinic; Stress; Attention

1. Introduction

Apolipoprotein E (apoE) plays a critical role in lipid transport and metabolism in the brain (Mahley, 1988) and is involved in CNS repair after injury (Arendt et al., 1997). The gene encoding for apoE is polymorphic in humans, yielding apoE2, apoE3, and apoE4 isoforms (Mahley, 1988). ApoE interacts with the brain acetylcholine (ACh) system in an isoform-specific manner in humans, such that apoE4 is associated with decreased nucleus basalis neuronal activity (Salehi et al., 1998) and choline acetyltransferase activity in the cortex and hippocampus compared to apoE2 and apoE3 (Allen et al., 1997; Lai et al., 2006; Poirier et al., 1995). Compared to apoE3, apoE4 is also associated with an increased risk of developing sporadic Alzheimer's disease (AD) (Farrer et al., 1997; Saunders et al., 1993). Deterioration of the brain ACh system in the basal forebrain, cortex, and hippocampus is a hallmark of AD (Geula and Mesulam, 1996; Svensson et al., 1997; Whitehouse et al., 1982). As cognition is highly modulated by the ACh system (Berger-Sweeney, 2003), apoE-isoform-specific effects on ACh function may be directly related to the development of cognitive impairments associated with AD. These differential effects of apoE could be due to lack of function or gain of misfunction effects. Therefore, mice deficient in apoE are valuable to define the potential role of mouse apoE in cholinergic function.

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Experiments using animal models also support a role for apoE in the maintenance of ACh function, but the data are incomplete. Mice lacking apoE (*Apoe*$^{-/-}$) show decreased cholinergic functioning compared to wild type (*Apoe*$^{+/+}$) mice (Buttini et al., 2002; Chapman and Michaelson, 1998; Gordon et al., 1995; Kleifeld et al., 1998). However, not all studies have found such effects (Anderson and Higgins, 1997; Bronfman et al., 2000; Krzywkowski et al., 1999). Thus, the relationship between apoE and the ACh system remains elusive. As most previous studies have focused on enzymatic and structural markers of ACh function, it is unclear whether apoE affects the number or function of ACh receptors in the brain.

There are two classes of ACh receptors; nicotinic and muscarinic (Cummings, 2000). The M_1- and M_2-type muscarinic ACh receptors (mAChRs) are two of the most highly expressed ACh receptors in brain (von Bohlen and Dermietzel, 2002) and are expressed in the cortex and hippocampus (Palacios, 1982; Porter et al., 2002; Probst et al., 1988; Schwab et al., 1992), two areas important for cognitive function and conditioned learning (Christian and Thompson, 2003). M_2 receptors function as presynaptic autoreceptors, regulating ACh release (Cummings, 2000). Furthermore, mAChR binding and function is impaired in AD (Claus et al., 1997; Koch et al., 2005). Pre-pulse inhibition (PPI), the delay eyeblink classical conditioning (DEBCC) task, and the 5-choice serial reaction time task (5-SRTT), are behavioral tasks sensitive to alterations in ACh function. PPI is a measure of sensorimotor gating that is impaired in AD (Ueki et al., 2006) and mAChR antagonism causes PPI impairments in rats (Jones and Shannon, 2000) that are reversed by acetylcholinesterase inhibitors (Ballmaier et al., 2002). DEBCC is a type of associative learning that is also impaired in AD and is affected by mAChR blockade in rabbits (Woodruff-Pak et al., 2002; Woodruff-Pak and Hinchliffe, 1997) and mice (Takatsuki et al., 2002). Finally, attention, as measured by the 5-SRTT, increases ACh levels (Arnold et al., 2002) and the ability to perform well on attention tasks is diminished with mAChR antagonism in rats (Gill et al., 2000; Mirza and Stolerman, 2000). Attention is also one of the earliest cognitive domains to deteriorate in AD (Perry and Hodges, 1999). Thus, PPI, DEBCC, and the 5-SRTT can be used to determine ACh functioning in rodents and model cognitive deficits associated with early AD.

As apoE is important for ACh function, *Apoe*$^{-/-}$ mice might have lower M_1 and M_2 mAChR densities than *Apoe*$^{+/+}$ mice. Such changes might be associated with altered performance on tasks sensitive to ACh function and increase the sensitivity of *Apoe*$^{-/-}$ mice to ACh challenges. In this study we examined the role of apoE in ACh function by determining mAChR binding in the cortex, hippocampus, and cerebellum of *Apoe*$^{+/+}$ and *Apoe*$^{-/-}$ mice and assessing behavioral performance on tasks sensitive to alterations in ACh function under baseline conditions and following an ACh challenge with scopolamine, a mAChR antagonist. To rule out non-specific effects of scopolamine on sensitivity to the stimuli used in the behavioral tests, we measured the effect of scopolamine on stimulus sensitivity thresholds. Since the stress hormone corticosterone can modulate DEBCC performance (Shors et al., 1992), we also measured corticosterone levels induced by one day of DEBCC training after scopolamine administration. We hypothesized that, compared to *Apoe*$^{+/+}$ mice, *Apoe*$^{-/-}$ mice would show reduced mAChR binding in the cortex and hippocampus and that as a result of this decreased binding would also show impaired performance in PPI, DEBCC, and the 5-SRTT. We further hypothesized that disruption of ACh transmission by scopolamine would impair performance to a greater degree in *Apoe*$^{-/-}$ mice than in *Apoe*$^{+/+}$ mice, thus supporting a role for apoE in central ACh function.

2. Methods

2.1. Animals

Three- to five-month-old naïve male C57BL/6J *Apoe*$^{+/+}$ and *Apoe*$^{-/-}$ mice, bred in our colony, were used for all experiments with the exception of the 5-SRTT in which both female and male mice were used. The mice were maintained on a 12 h light/dark schedule (lights on at 06:00). Behavioral testing took place during the light cycle. Lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO, USA) and water were given *ad libitum*. Separate groups of animals were used for each procedure. All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

2.2. Muscarinic acetylcholine receptor saturation binding

Receptor saturation binding experiments were performed using cortical, hippocampal, and cerebellar membrane preparations from *Apoe*$^{+/+}$ ($N=16$) and *Apoe*$^{-/-}$ ($N=17$) mice using radioligands specific for M_1 ([^{3}H]pirenzepine) or M_2 ([^{3}H]AF-DX-384) mAChRs (see Supplementary data, Vaucher et al., 2002; Watson et al., 1986). The number of binding sites (B_{max}) and the equilibrium dissociation constants (K_d) were determined according to the Hill equation (Whiteaker et al., 2000), using non-linear regression analysis performed in Graphpad Prism 4.0 (Graphpad, San Diego, CA, USA).

2.3. Foot-shock and acoustic startle

Mice were tested for foot-shock ($N=8$) or acoustic startle thresholds ($N=8$) following saline or scopolamine injections to rule out potential genotype differences in sensitivity to stimuli or scopolamine (see Supplementary data).