Blockade of Nicotinic Acetylcholine Receptors Suppresses Hippocampal Long-Term Potentiation in Wild-Type but Not ApoE4 Targeted Replacement Mice

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Both impaired nicotinic neurotransmission and the inheritance of apoE4 are associated with increased risk for Alzheimer disease (AD) as well as other deficiencies in memory-related behavior. Long-term potentiation (LTP), a cellular model of memory, is known to be altered by nicotinic agents. Recent studies also support an emergent role for apoE in LTP. We compared the effects of mecamylamine, a nonspecific antagonist of nicotinic acetylcholine receptors (nAChRs), on basal synaptic transmission and LTP in hippocampal slices from wild-type (wt) mice and targeted replacement mice expressing human apoE4 (apoE4-TR). Field excitatory postsynaptic potentials (EPSPs) were recorded in the dentate gyrus (DG) in response to medial perforant path activation, and theta burst stimulation was used to induce LTP. Bath application of mecamylamine (3 μM) did not alter input–output relationships or paired pulse depression in either mouse strain. Under control conditions, apoE4-TR mice showed significantly less LTP than wt mice (17.5% ± 3.2%, n = 9, vs. 30.1% ± 3.9%, n = 11, P < 0.02). Mecamylamine reduced LTP in wt mice to a level that was similar to control levels for apoE4-TR mice (15.7% ± 3.4%, n = 9), whereas apoE4-TR showed no further reduction of LTP (16.6% ± 3.7%, n = 8) by mecamylamine. Thus mice expressing human apoE4 differ from wt mice both in their capacity for LTP and in the effect on LTP of nicotinic cholinergic blockade. It is possible that nicotinic neurotransmission is already compromised in apoE4-TR mice and, hence, that interference with the integrity of this cholinergic system represents a mechanism by which inheritance of the apoE4 allele contributes to cognitive risk.

Key words: mecamylamine; hippocampal slices; dentate gyrus; Alzheimer's disease

The human APOE gene has three alleles, ε2, ε3, and ε4, which encode the apolipoproteins apoE2, apoE3, and apoE4, respectively. ApoE is a major contributor to the transport of cholesterol and phospholipids to and from the blood stream and is required for dendritic and synaptic remodeling (Poirier, 1994). Inheritance of apoE4 is the primary genetic risk factor for Alzheimer’s disease (AD; Corder et al., 1993) and is associated with poor cognitive outcome in other clinical settings, such as head injury and stroke (Teasdale et al., 1997; Slooter et al., 1997; Deary et al., 2002). Mice expressing human apoE4 also show behavioral and cognitive deficits (Raber et al., 2000; Levi et al., 2003; Grootendorst et al., 2005).

Acetylcholine and nicotinic acetylcholine receptors (nAChRs) also play an important role in learning and memory (Bartus et al., 1982). Nicotinic agonists improve memory in both humans (Warburton et al., 1992) and animals (Buccafusco and Jackson, 1991; Abdulla et al., 1993; Levin et al., 1993; Decker, 1995), whereas memory function is impaired by the nAChR antagonist mecamylamine (Levin and Simon, 1998). Post-mortem studies of AD brains have shown significant reductions in the number of cholinergic neurons in basal forebrain (Perry et al., 1977; Whitehouse et al., 1982) as well as reductions in nAChR expression in frontal and temporal cortex (Wevers et al., 2000).

Long-term potentiation (LTP) is a widely accepted model for the cellular basis of learning and memory.
(Bliss and Collingridge, 1993). Nicotinic agonists facilitate LTP in hippocampal CA1 (Hunter et al., 1994; Fujii et al., 1999; Mann and Greenfield, 2003) and dentate gyrus (DG) in vitro (Sawada et al., 1994; Curran and O’Connor, 2003) and reverse the age-related decline of LTP in CA1 in vitro (Fujii and Sumikawa, 2001). Conversely, blockade of nAChRs inhibits LTP in CA1 (Freir and Herron, 2003) and DG in vivo (Matsuyama et al., 2000). In addition, nicotine increases glutamatergic synaptic transmission in hippocampal CA3 in vitro (Gray et al., 1996) and DG in vivo (Matsuyama et al., 2000).

ApoE can play an important role in the modulation of LTP in hippocampus. For example, several investigators have shown that apoE-deficient (apoE-KO) mice show a significant reduction of LTP compared with wild-type (wt) mice (Kruegers et al., 1997; Veinbergs et al., 1998; Krzywkowski et al., 1999; Valastro et al., 2001). In addition, our recent studies have found that targeted replacement mice expressing human apoE4 (apoE4-TR) have both reduced LTP compared with wt mice and enhanced susceptibility to LTP impairment by oligomeric amyloid β1-42 (Trommer et al., 2004, 2005). In the present study, we compared the effects of mecamylamine, a nonspecific antagonist of nAChRs, on basal synaptic transmission and LTP in hippocampal slices from wt and apoE4-TR mice. Recordings were made in DG, because this region is vital to normal learning and memory (Naie and Manahan-Vaughan, 2004) and is specifically implicated in AD (Palop et al., 2003). DG receives extensive innervation from septohippocampal cholinergic afferents, and nAChRs in the DG molecular layer play a significant role in the regulation of information processing (Frazier et al., 2003). We report that, under control conditions, LTP magnitude is smaller in apoE4-TR than in wt mice. LTP in wt mice is reduced by mecamylamine and becomes similar to control levels for apoE4-TR mice. In contrast, apoE4-TR mice show no further reduction of LTP by mecamylamine.

MATERIALS AND METHODS

In Vitro Slice Preparation

Male mice 2–3 months of age of two genotypes were used for these experiments: wt (C57BL/6) and targeted replacement mice expressing human apoE4 (apoE4-TR). The apoE4-TR mice were back-crossed to C57BL/6 more than eight times to establish a strain background consistent with the wt mice, and they are maintained in a homozygous background (Sullivan et al., 1997). Both strains were obtained from colonies maintained at Taconic Laboratories. Hippocampal slices (350 μm) were prepared as previously described (Trommer et al., 2004). In brief, animals were decapitated after anesthesia with isoflurane, and their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 124, KCl 3, CaCl2 2.4, MgSO4 1.3, NaH2PO4 1.25, NaHCO3 26, and glucose 10, gassed with 95% O2/5% CO2, pH 7.4. Picrotoxin (100 μM) was included during recording. Slices were allowed to recover for ≥1 hr at room temperature and were transferred as needed to a submersion chamber maintained at 32°C.

Field EPSP Recording

Stimulating and recording electrodes were placed in the middle one-third of the suprapyramidal limb of DG in the middle one-third of the molecular layer to activate the medial perforant path and to record dendritic field excitatory post synaptic potentials (EPSPs). Electrophysiological measures of basal synaptic transmission included input/output (I/O) relationships and short-term plasticity (paired pulse depression; PPD). I/O relationships were measured by comparing the ratio of EPSP amplitudes to stimulus intensity by using a pulse width of 0.1 msec and a current range of 20–100 μA. The current that produced about 40% of maximal responses (usually 20–40 μA) was used throughout the experiment for monitoring test pulses. The same current was used to evoke PPD, which was calculated as the ratio of EPSP slopes (pulse 2/pulse 1) in response to pairs of stimuli delivered at an interpulse interval 50 msec; the presence of PPD also served to confirm placement of electrodes in the medial perforant path. Data were sampled at 10 kHz and low-pass filtered at 3 kHz. Baseline EPSP slope was recorded in response to 0.05 Hz for 10 min. LTP was induced by applying theta burst stimulation (TBS) that consisted of 10 high-frequency bursts (four pulses, 100 Hz) repeated at 5 Hz. During TBS, stimulus intensity was increased to evoke 70%–of-maximal responses. Magnitude of LTP was assessed by measuring the percentage increase compared with baseline of averaged responses at 30–40 min following TBS; all responses were normalized to the 10-min baseline period to allow comparison among slices. pClamp v. 8.0 (Axon Instruments, Burlingame, CA) was used for data collection and analysis. Mecamylamine (Sigma, St. Louis, MO) was prepared as 100 mM stock solutions in sterile water and was diluted to 3 μM in ACSF for bath application. This concentration of mecamylamine was chosen because it is in the range (1–5 μM) that block nAChRs and inhibits nicotinic currents in vitro (Pidoplichko et al., 1997; Nomura and Nishizaki, 2000; Roberto et al., 2001) and because this concentration (3 μM) has previously been shown to block the facilitation of LTP by nicotine in hippocampal area CA1 (Fujii et al., 1999, 2000).

Statistical Analysis

All values are given as mean ± SEM. I/O functions were analyzed by repeated-measures ANOVA, with treatment (ACSF vs. mecamylamine) and strain (wt vs. ApoE4-TR) as the grouping variables. PPD was analyzed by two-way ANOVA, with strain and treatment (ACSF vs. mecamylamine) as the grouping variables. Student’s t-test was used to compare LTP magnitude between groups (wt vs. apoE4-TR, ACSF vs. mecamylamine).

RESULTS

I/O Functions and Short-Term Plasticity

Basal synaptic transmission was comparable in both strains in both ACSF and mecamylamine (Fig. 1). PPD
ratios (Fig. 1A) were 68.5% ± 3.2% and 66.1% ± 3.2% in control conditions for wt and apoE4-TR, respectively. In the presence of mecamylamine, PPD ratios were 73.5% ± 4.7% and 72.6% ± 2.7% for wt and apoE4-TR, respectively. There were no differences between strains (P > 0.1) or treatment groups (P > 0.6), and there was no interaction effect (P > 0.8). Stimulus–response curves for all groups overlapped (Fig. 1B), and there were no statistical differences in the ratios of EPSP amplitude to stimulus intensity in any group (P > 0.1).

**LTP**

In control ACSF, apoE4-TR mice showed LTP of significantly smaller magnitude than wt mice (17.5% ± 3.2%, n = 9, vs. 30.1% ± 3.9%, n = 11, P < 0.02; Figs. 2A, 3). In the presence of 3 μM mecamylamine, wt mice showed LTP of significantly smaller magnitude than in ACSF (30.1% ± 3.9%, n = 11, vs. 15.7% ± 3.4%, n = 9, P < 0.01; Figs. 2, 3). In contrast, apoE4-TR mice showed comparable LTP in the absence and presence of mecamylamine (17.5% ± 3.2%, n = 9, vs. 16.6% ± 3.7%, n = 8, P > 0.8). Interestingly, the average magnitude of LTP in the wt mice treated with mecamylamine was reduced to the control level for apoE4-TR mice (Fig. 3).

It is possible that transient exposure to mecamylamine followed by washout could confound interpretation of the LTP results by inducing a gradual change in synaptic transmission, as has previously been shown for neurotrophins (Kang and Schuman, 1995) and carbachol (Yun et al., 2000). To control for this possibility, we monitored the EPSP slope in slices from wt mice (n = 5) over a time course and mecamylamine exposure identical to those used in the LTP experiments but without TBS. As shown in Figure 4, mecamylamine did not alter EPSP slope compared with baseline either during the 15-min exposure (mean slope 99.1% ± 1.1% of baseline, Student's t-test, P > 0.4) or for 35 min following washout (mean slope during last 10 min of recording 103.2% ± 4.5% of baseline, Student's t-test, P > 0.5).

**DISCUSSION**

The main results of this study are that LTP magnitude is smaller in apoE4-TR mice than in wt mice and that 3 μM mecamylamine inhibits LTP in wt but not apoE4-TR mice. There were no differences in basal synaptic transmission between mouse strains, and mecamylamine did not alter basal transmission in either strain. These results are consistent with our previous finding of a relative impairment of tetanus-induced LTP in apoE4-TR compared with wt (Trommer et al., 2004) as well as with prior work showing that blockade of nAChRs inhibits hippocampal LTP in vivo in rat (Freir and Herron, 2003) and mouse (Matsuyama et al., 2000).

The mechanism underlying apoE isoform-specific effects on cognition and LTP is unknown. It is known, however, that apoE can bind members of the low-density lipoprotein (LDL) receptor family, which are abundantly expressed on neurons (Herz and Beffert, 2000; Strittmatter, 2001). General inhibition of ligand binding to these receptors by the receptor-associated protein...
(RAP) effectively abolishes LTP in hippocampal CA1 area (Zhuo et al., 2000; Weeber et al., 2002), and knockout of a specific LDL receptor (apoER2) leads to a profound deficit in LTP (Weeber et al., 2002). ApoE4 has the highest binding affinity for LDL receptors among apoE subtypes (Kowal et al., 1990; Weisgraber, 1994) and is thought to associate preferentially with larger lipoprotein particles (i.e., VLDL vs. HDL; Dong and Weisgraber, 1996). Although the brain contains only HDL-like species, apoE4 may preferentially associate with larger HDLs, which could impact its binding affinity in the brain. For example, it has been proposed that apoE4, in association with such relatively large particles, may inhibit LTP by competing for LDL receptors with physiologic ligands (e.g., Reelin) that are known to enhance LTP (Weeber et al., 2002).

The role of nAChRs in LTP is complex. Short-term application of nicotine itself can enhance synaptic transmission in hippocampus in vivo and in vitro by increasing neurotransmitter release through presynaptically located α7 nAChRs (Gray et al., 1996; Radcliffe and Dani, 1998), inducing what has been called nicotinic LTP (nLTP). Nicotinic agonists enhance LTP in hippo-
The lack of a mecamylamine response in apoE4 mice is of interest and, together with the relative diminution of LTP in these mice compared with wt, may suggest that nAChR activity is already compromised in apoE4. This might occur, for example, if the expression of apoE4 were to alter the lipid composition of synaptic membranes in a manner that affects nAChR number or function. Human studies have shown that carriers of the apoE4 allele have diminished nAChR number as well as decreased choline acetyltransferase activity and nicotinic receptor binding (Poibier et al., 1995; Soininen et al., 1995; Arendt et al., 1997). Alternatively, the presence of apoE4 may directly interfere with nicotinic cholinergic neurotransmission. For example, a recent patch clamp study showed that coapplication of apoE-mimetic peptides with acetylcholine inhibits the nicotinic current evoked by acetylcholine alone (Klein and Yakel, 2004). Thus apoE4 may interfere with LTP by impairing the integrity of nicotinic cholinergic neurotransmission.

In summary, our data demonstrate that mice expressing human apoE4 differ from wt mice both in their capacity for LTP and in the effect on LTP of nicotinic cholinergic blockade. Interference with LDL receptor function and compromise of nicotinic cholinergic receptor function represent plausible means by which inheritance of the apoE4 allele might contribute to cognitive risk and AD.

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