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Featured Articles

Apolipoprotein E4 domain interaction: Synaptic and cognitive deficits in mice

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Abstract

Background: Apolipoprotein E4 (apoE4), the major genetic risk factor for Alzheimer's disease (AD) and other neurodegenerative diseases, has three structural and biophysical properties that distinguish it from the other isoforms—domain interaction, reduced stability, and lack of cysteine. Assessing their relative contributions to effects of apoE4-associated pathogenesis in AD is important from a mechanistic and therapeutic perspective, that is not possible using human apoE transgene or knock-in models.

Methods: We analyzed Arg-61 apoE mice, a gene-targeted model that selectively displays domain interaction.

Results: The mice displayed age-dependent loss of the synaptic protein synaptophysin in neocortex and hippocampus and had lower levels of the postsynaptic neuroligin-1. Activation of dentate gyrus granule neurons increased Arc expression 3.5-fold in wildtype mice but only 2.3-fold in Arg-61 mice. The losses of synaptic proteins caused a mild memory deficit in Arg-61 mice in the water-maze test. Since synaptic integrity requires efficient glutamate uptake, we measured astrocyte glutamate transporter 1 in the hippocampus. The level was reduced in Arg-61 mice, suggesting that inefficient glutamate uptake by astrocytes causes chronic excitotoxicity. Consistent with the reduced secretion of Arg-61 apoE by astrocytes in this model, cholesterol secretion was also reduced 34%. This reduction could also contribute to the synaptic deficits by limiting the availability of cholesterol for neuronal repair.

Conclusions: Domain interaction in the absence of other structural characteristics of apoE4 is sufficient to cause synaptic pathology and functional synaptic deficits, potentially associated with astrocyte dysfunction and impaired maintenance of neurons. Therapeutic targeting of domain interaction might blunt effects of apoE4 in neurodegenerative disease.

Keywords: apolipoprotein E; Alzheimer's disease; apoE4 domain interaction; Arg-61 apoE mouse model; synaptophysin; activity-regulated cytoskeleton-associated protein; neuroligin-1; glutamate transporter

1. Introduction

Human apolipoprotein E (apoE) is critical for neuronal maintenance and repair [1,2]. The three common isoforms of apoE, apoE2, apoE3, and apoE4, differ in their ability to perform these functions. apoE4 is the major known genetic risk factor for late-onset familial and sporadic Alzheimer's disease (AD), which accounts for more than 95% of AD cases [3]. In AD subjects and transgenic mice, apoE4 displays a gene dose effect in reducing presynaptic synaptophysin immunoreactivity and dendritic spine density [4,5]. apoE4 also impairs synaptic functions [6]. Human apoE4 knock-in mice have reduced long-term potentiation in the dentate gyrus compared with apoE3 mice, without evidence of advanced neurodegeneration [7]. apoE4 mice also show reduced excitatory synaptic transmission and dendritic arborization in the amygdala [8].

Although several mechanisms have been proposed (Mahley et al [1], Huang et al [9]), the molecular basis of the apoE

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isoform-specific effects remains unclear. However, the detrimental effects of apoE4 must reflect its unique structural and biophysical properties [9–11]. apoE4 has three major structural characteristics that distinguish it from apoE2 and apoE3: domain interaction (an interaction between the amino- and carboxyl-terminal domains mediated by a salt bridge between Arg-61 and Glu-255) [12], a lower resistance to protein unfolding (resulting in a propensity to form a reactive folding intermediate or a molten globule state) [10,13], and the lack of cysteine [14]. Molten globule formation and aggregation in apoE4 are neurotoxic to cultured neuronal cells, and the single cysteine in apoE3 results in hetero- and homo-disulfide–linked dimers that modulate lipid binding and low-density receptor-binding activity [10,14]. Therefore, to understand mechanisms and to design potential therapeutic approaches, it is necessary to distinguish the relative contribution of these structural and biophysical differences to apoE4-associated neurodegeneration. This cannot be accomplished with the human apoE4 knock-in mouse model, which displays all apoE4-specific structural properties. Previously, domain interaction was proposed to underlie the association of apoE4 with AD and other neurodegenerative diseases [12]. However, this hypothesis has not been formally tested in vivo because of the lack of a suitable animal model specific for domain interaction.

To test this hypothesis directly, we took advantage of the fact that wild-type (WT) mouse apoE does not exhibit either domain interaction or molten globule formation. Regarding domain interaction, mouse apoE contains the equivalents of Arg-112 and Glu-255 but lacks the equivalent of the critical Arg-61 (instead, it contains threonine), making it functionally similar to apoE3 with a preference of high-density lipoproteins [11]. To introduce domain interaction into mouse apoE, the threonine codon was replaced with an arginine codon in mouse apoE by gene targeting, resulting in an "apoE4-like" functional molecule [15] with similar stability to WT mouse apoE and human apoE3 [16]. Thus, the Arg-61 apoE mouse provides a specific model of domain interaction, and any phenotype observed in this model, compared with WT, implicates domain interaction. Other features of the model include that expression of Arg-61 apoE is under the control of the natural mouse control elements and that the single mutation in WT mouse apoE minimizes any species effects. Secretion of Arg-61 apoE by astrocytes is decreased in these mice, leading to lower brain levels of apoE than in WT mice, suggesting that Arg-61 apoE might be selectively degraded by astroctyes [17].

In this study, the Arg-61 apoE model was examined to determine whether domain interaction contributes to the synaptic deficits associated with apoE4 independent of an added Aβ stress. Our results demonstrated that domain interaction is associated with both morphologic and functional synaptic deficits.

2. Materials and methods

2.1. Mice

Arg-61 apoE mice were generated as described [15] and backcrossed with WT C57BL/6J mice for eight generations [17]. In most experiments, 12-month-old male Arg-61 mice and WT controls were used. Male and female Arg-61 apoE mice express similarly lower levels of apoE than WT mice as reported [17]. Mice were housed and handled in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

2.2. Novel environment

Mice were singly housed for 3 days before the experiment. Mice (seven mice for each group) assigned to the novel environment exploration were then transferred to a larger uncovered cage (45 × 25 × 20 cm) that contained different bedding and five novel objects and was located in an adjacent room that differed markedly in size, shape, lighting, and furnishings. The mice were allowed to explore the new environment for 2 hours; control mice (six WT and seven Arg-61 apoE mice) remained undisturbed in their home cages. At the end of the experiment, mice were removed from either the novel environment or home cage and immediately killed.

2.2. Morris water maze

Mice were trained to find a square platform (14 × 14 cm) submerged 1.5 cm below the surface in a 122-cm pool containing water (18°C ± 2°C) made opaque by the addition of nontoxic tempera powder. In a series of four trials, mice were pretrained to remain on the platform in which they were placed in a restricted (14 × 122 cm) alley within the pool and allowed to swim until they encountered the platform. The trial ended when the mouse remained on the platform for 5 seconds without jumping off. If the mouse did not accomplish this task within 90 seconds of testing, it was led to the platform by hand, placed on it, and allowed to sit for 5 seconds.

After 1 day of pretraining, mice were trained to find the visibly cued platform with a 10-cm-high, black-and-white-striped marker at its center. Mice were trained in two daily sessions of two trials each during a period of 3 consecutive days. The platform and the visual cue were moved for each trial. The mice were then trained to find a hidden platform in two daily sessions of three trials each during a period of 5 consecutive days. The platform location was kept constant, but the starting point where each mouse was placed into the water was changed between trials. For both hidden and cued training, the intertrial interval was 10 minutes, and the time between sessions was 3 hours. Maximum trial length was 60 seconds. Time to reach the platform (latency), path length, and swim speed were recorded with an EthoVision video tracking system (Noldus, Wageningen, The Netherlands).