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Research report

Hippocampal infusions of apolipoprotein E peptides induce long-lasting cognitive impairment

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ABSTRACT

The inheritance of the ε4 allele of apolipoprotein E (ApoE4) and cholinergic system dysfunction have long been associated with the pathology of Alzheimer's disease (AD). Recently, *in vitro* studies have established a direct link between ApoE and cholinergic function in that synthetic peptides containing segments of the ApoE protein ($ApoE_{133-149}$ and $ApoE_{141-148}$) interact with α7 nicotinic acetylcholine receptors (nAChRs) in the hippocampus. This raises the possibility that ApoE peptides may contribute to cognitive impairment in AD in that the hippocampus plays a key role in cognitive functioning. To test this, we acutely infused ApoE peptides into the ventral hippocampus of female Sprague–Dawley rats and assessed the resultant effects on radial-arm maze choice accuracy over a period of weeks after the infusion. Local ventral hippocampal infusion of ApoE peptides caused significant cognitive impairment in radial-arm maze learning that persisted several weeks after the acute infusion. This persisting deficit may be an important model for understanding the relationship between ApoE protein-induced neurotoxicity and cognitive impairment as well as serve as a platform for the development of new therapies to avoid neurotoxicity and cognitive decline.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive loss of cognitive functioning. Apolipoprotein E4 (ApoE4) is of particular interest to understanding AD because it has been identified as a major risk factor for developing AD. People who are homozygous for ApoE4 have a 4.5 times greater risk of acquiring AD and average an earlier age of onset by 16 years [1,20]. While genetic links between AD and ApoE protein have been shown [7], it is not known whether the protein itself directly contributes to the cognitive impairment associated with AD or if the relationship between ApoE proteins and cognitive impairment is less direct.

The hippocampus is of critical importance with regard to the cognitive impairment of AD. A great many studies show the importance of this brain region for integrating learning and memory processes [17]. Additionally, AD is characterized by hippocampal dysfunction, in particular a decrease in the number of hippocampal cholinergic neurons and decreased hippocampal expression of nicotinic acetylcholine receptors (nAChRs) [2]. Thus the progressive loss of cholinergic signaling within the hippocampus may be an important mechanism underlying the cognitive defects associated with AD. Cholinergic systems are also linked to several protein factors associated with AD including β-amyloid (Aβ) [9], the β-amyloid precursor protein (APP) [22], and apolipoprotein E (ApoE) [11,19]. It is our hypothesis that ApoE4 in the hippocampus may provide a critical link between cholinergic systems and the functional impairment associated with AD.

Our hypothesis is based on two separate, but important findings regarding the effects of endogenous ApoE cleavage products and synthetically derived ApoE peptides on hippocampal neurons/receptors. First, an endogenous ApoE cleavage product (tApoE) and ApoE peptides ($ApoE_{277-299}$) have been found to produce AD-like neurodegeneration in the cortex and hippocampus [16,21]. Additionally, tApoE has been found in the brains and cerebral spinal fluid of AD patients and interact with phosphorylated tau proteins and neurofibrils [8]. The fact that tApoE and small ApoE peptide fragments are cytotoxic and interact with AD-related proteins raises the possibility that ApoE4 contributes to AD and AD-related cognitive impairment by inducing hippocampal dysfunction.

Secondly, ApoE peptides derived from the LDL-receptor binding domain, $ApoE_{133-149}$ and $ApoE_{141-148}$, block ACh-evoked maximal current responses of homomeric α7 nAChRs expressed in *Xenopus* oocytes [4] and native α7 nAChRs in hippocampal slices [10]. The blockade of α7 nAChRs by these ApoE-derived peptides is dose-dependent, voltage-independent, and shows receptor specificity where blockade of α4β2 and α2β2 nAChRs is significantly smaller than that of α7 nAChRs. This is important in that hippocampal α7

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nAChRs are critically important to proper spatial cognitive function (for review see [12]). Thus, it is plausible that ApoE peptides through either the neurodegenerative processes or by the blockade of α7 nAChRs may lead to cognitive defects.

The current study was conducted to determine whether $ApoE_{133-149}$ and $ApoE_{141-148}$ peptides infused into the rat ventral hippocampus would impair learning and memory as measured in the 8-arm radial maze task. This study focused on the effects of a single bolus dosing of ApoE peptides in an attempt to simulate a pathological state in which AD-related events have existed for a number of years.

2. Methods

2.1. Subjects

Adult female Sprague–Dawley rats (Taconic Labs, Germantown, NY, $N = 45$) were singly housed in plastic cages with corn-cob shavings. The rats lived in a vivarium (AAALAC-approved facility) immediately adjacent to the behavioral test facility, and maintained on a reverse 12:12 light–dark cycle with testing during the behaviorally active, dark phase. All rats had *ad libitum* access to water and with one daily meal being administered after behavioral testing. These studies were conducted under approved procedures of the Animal Care and Use Committee of Duke University.

2.2. ApoE peptide infusion

ApoE-derived peptides were synthesized by Sigma–Genosys (The Woodlands, TX, USA) at a purity of 95% and reconstituted in sterile, deionized water yielding stock concentrations of 15–20 mM. The peptides used in this study were acetylated at the amino terminus and amide-capped at the carboxyl terminus. Stock solutions were stored at −20 °C and diluted to desired concentrations in sterile filtered artificial cerebral spinal fluid (150 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1 mM NaH_2PO_4, pH 7.4) on the day of the experiment.

A guide cannula (Plastics One, Roanoke, VA) was stereotaxically implanted into the ventral hippocampus according to Pellegrino et al. [18] while rats were anesthetized with ketamine (0.6 mg/kg) and domitor (0.15 mg/kg). One week after cannula implantation ApoE peptides (1 or 5 μg per side) was infused into the ventral hippocampus at a flow rate of 0.126 μL/min (a total volume of 0.378 μL was infused over 3 min). Control animals were infused with equal volumes of artificial cerebral spinal fluid. Fig. 1 shows representative bilateral cannula placements in the ventral hippocampus.

2.3. 8-Arm radial maze

Behavioral testing began 3 days after ApoE peptide infusion. The behavioral task consisted of 18 sessions on the 8-arm radial maze. The rats were tested an average of two times per week. The 8-arm radial maze was constructed of wood and consisted of a central platform 50 cm in diameter, elevated 30 cm from the floor, with eight arms (10 cm × 60 cm) extending radially. Each arm was baited with a treat, and the rats were placed in a plastic cylinder (30 cm in diameter and 20 cm high) on the central platform. To begin the session, the cylinder was lifted allowing the rat to move freely about the maze. Arm choices were recorded when the rat placed all of its paws into the arm. Since the arms were not rebaited during the session, only the first entry into an arm was rewarded. Subsequent entries into an arm previously entered were counted as an error. The session was continued until either the rat entered all baited arms or 5 min elapsed. The choice accuracy measurement of working memory function is entries to repeat (ETR) defined as the number of correct arm entries in a session before the first error was made. Higher ETR scores indicate better memory of entering more correct arms as the session progressed and the task became more difficult. The response latency was measured by determining the seconds taken per arm entry, calculated by dividing the total session length by the number of arm entries. Higher scores indicated slower responding.

2.4. Cannula placement verification

After completion of the behavioral task the brains were removed and cannula placements were verified. Briefly, the rats were anesthetized with sodium pentobarbital. The rats were then perfused with a 0.9% phosphate-buffered saline solution followed by 4% paraformaldehyde solution. The brains were removed and preserved in 4% formaldehyde. Before being sliced on a cryostat to make histological slides, the brains were frozen on dry ice. Histological slides were then made and studied under a microscope for placement verification.

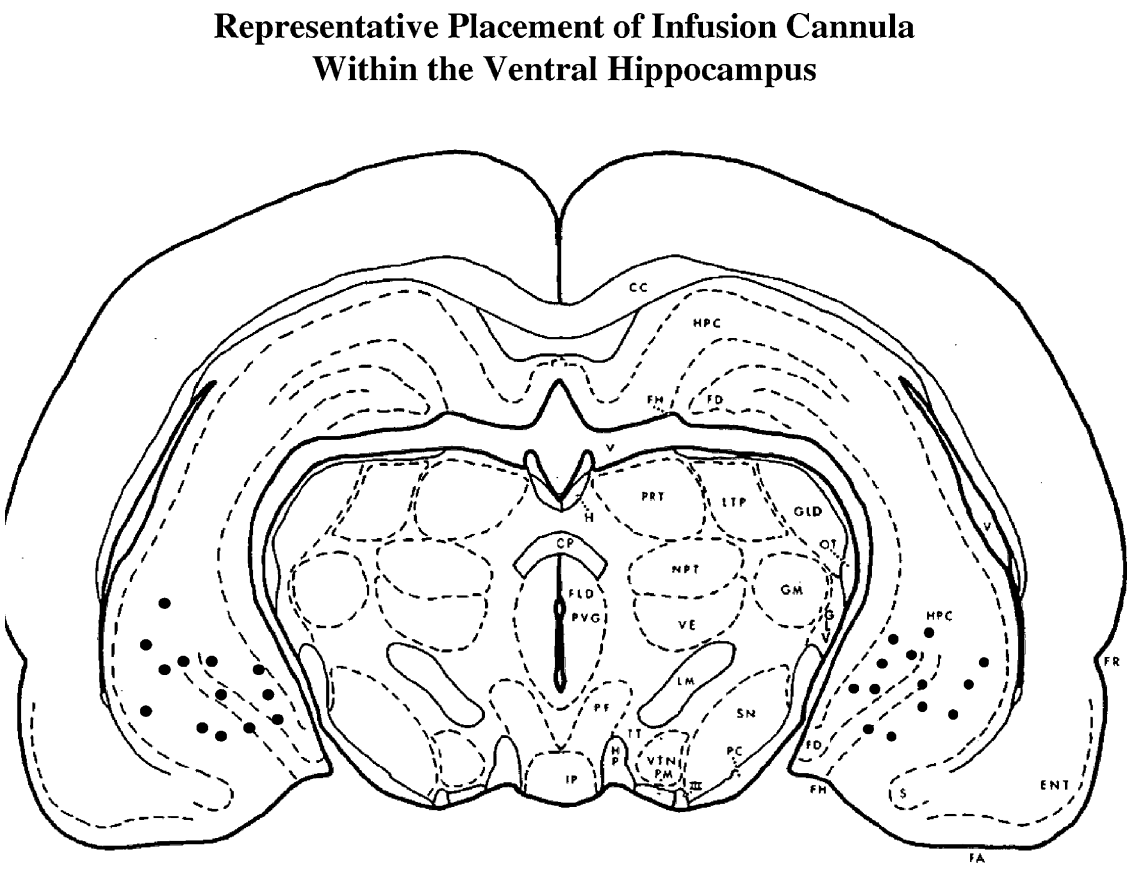

Fig. 1. Representative localization of cannula placements within the ventral hippocampus. Infusion cannula were stereotaxically implanted bilaterally into the ventral hippocampus according to coordinates of Pellegrino et al. [18]. The coordinates were −3.2 anterior/posterior, ±5.0 medial/lateral, and −7.0 dorsal/ventral.