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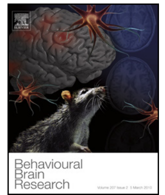

Research report

Cerebral dopamine neurotrophic factor improves long-term memory in APP/PS1 transgenic mice modeling Alzheimer's disease as well as in wild-type mice

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HIGHLIGHTS

- Intrahippocampal CDNF protein or gene improved long-term memory in mice.
- CDNF did not influence short-term memory, spontaneous activity or object neophobia.
- CDNF did not significantly affect brain amyloid load or adult neurogenesis.

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ABSTRACT

Cerebral dopamine neurotrophic factor (CDNF) protects and repairs dopamine neurons in animal models of Parkinson's disease, which motivated us to investigate its therapeutic effect in an animal model of Alzheimer's disease (AD). We employed an established APP/PS1 mouse model of AD and gave intrahippocampal injections of CDNF protein or *CDNF* transgene in an AAV2 viral vector to 1-year-old animals. We performed a behavioral test battery 2 weeks after the injections and collected tissue samples after the 3-week test period. Intrahippocampal CDNF-therapy improved long-term memory in both APP/PS1 mice and wild-type controls, but did not affect spontaneous exploration, object neophobia or early stages of spatial learning. The memory improvement was not associated with decreased brain amyloid load or enhanced hippocampal neurogenesis. Intracranial CDNF treatment has beneficial effects on long-term memory and is well tolerated. The CDNF molecular mechanisms of action on memory await further studies.

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1. Introduction

In recent years, growing body of evidence has accumulated pointing to an important role of altered neurotrophic factor signaling in a wide array of neurodegenerative diseases. Most studied neurotrophic factors include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and more recently, also glial cell line-derived neurotrophic factor (GDNF) and cerebral dopamine neurotrophic factor (CDNF). For instance, BDNF levels are reported to decrease in Alzheimer's disease (AD) inflicted brains [1,2] and proapoptotic NGF levels to increase [3]. Also expression patters of novel human GDNF isoforms are reported to be deregulated in human AD brains [4].

Alterations in trophic signaling in AD have inspired studies for therapeutic use of neurotrophic factors. Indeed, in several trials neurotrophic factors as therapeutic agents have been able to protect neurons from degeneration and save neural function in animal models and even in humans. BDNF injections to entorhinal cortex were able to restore neuronal loss and memory functions in AD mice, aged rats and primates [5]. Further, intracranial NGF-treatment has been reported to rescue cholinenergic neurons and memory functions in aged [6] and IgG-saporin lesioned [7] rats and even in AD patients [8]. In a recent study, also GDNF gene therapy was able to improve spatial learning in APP/PS1/tau triple transgenic mice [9].

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A newcomer in the neurotrophic factor field is CDNF which has displayed strong neuroprotective and neurorestorative effects in animal models of Parkinson's disease (PD). CDNF can protect dopaminergic cells in the 6-OHDA rat model [10,11] and the MPTP mouse model [12] when given prior to lesioning but also when administered weeks after the lesion. CDNF and related protein mesencephalic astrocyte-derived neurotrophic factor (MANF) also regulate endoplasmic reticulum (ER) stress and unfolded protein response (UPR) [13]. Since protein aggregation triggers ER stress and neuronal death in AD, we hypothesized that CDNF may reduce ER stress, block neuronal cell death, partially regenerate hippocampal neurons and thus improve cognitive functions in mouse model of AD. To our knowledge, no study so far has addressed the potential of CDNF in AD models.

In order to investigate the potential therapeutical role of CDNF in AD we used intrahippocampal protein and gene therapy in APPswe/PS1dE9 (APP/PS1) mice modeling AD. In these mice, the hallmark pathology of AD, amyloid plaques, appear in the cortex and hippocampus at ~4 months of age [14], while decline of spatial memory manifests later on around 12 months of age [15]. Thus, these mice perfectly mimic the order of disease progression in the human AD in which brain amyloid accumulation precedes memory impairment and other clinical manifestations by 15–20 years as revealed by PET imaging studies with amyloid-binding ligand [16,17] and assays of CSF biomarkers [18]. Our hypothesis was that CDNF renders protection against downstream effects of amyloid-β accumulation in the brain, therefore we focused on the age around manifestation of memory impairment.

2. Methods

2.1. Animals

Female 12–13-month-old APP/PS1 mice and their wild-type (WT) littermates were used in the study. We chose female mice since the majority of AD patients are female and in even the mouse model, the brain amyloidosis advances faster in females [19]. The APPswe/PS1dE9 (APP/PS1) founder mice were obtained from Johns Hopkins University, Baltimore, MD, USA (D. Borchelt and J. Jankowsky, Department of Pathology). These mice carry mouse APP695 harboring a human Aβ domain and mutations K595 N and M596L linked with Swedish familial AD pedigrees and human PS1-dE9 (deletion of exon 9) vectors controlled by independent mouse prion protein promoter elements [20]. This line was originally maintained in a hybrid C3HeJ × C57BL6/J F1 background, but the mice used in the present study were derived from backcrossing to C57BL6/J in the Laboratory Animal Center, Kuopio, Finland, for 12–14 generations.

The animals were group-housed in controlled environment (temperature 22 ± 1 °C, light 7:00–19:00; humidity 50–60%), and food and water were freely available. All behavioral tests were conducted during the light phase. The experiments were conducted according to the Council of Europe (Directive 86/609) and Finnish guidelines, and approved by the State Provincial Office of Eastern Finland.

2.2. Protein production and viral vectors

Recombinant human CDNF (rhCDNF) was produced using baculoviral expression in Sf9 insect cells as described previously [11] with additional thrombin cleavage to remove Flag and 6×His tags.

In addition to single protein administration, we wanted to study the effects of long-term protein expression. This was achieved by using adeno-associated-viruses (AAV) for human CDNF gene transfer [10]. As a control, we used AAVs carrying GFP. Production of the AAV2-CDNF and AAV2-GFP viruses used in this study has been described previously in detail [10]. Briefly, a cDNA-encoding human CDNF was cloned into pAAV-MCS vector (Stratagene). For the production of virus particles, pAAV2-CDNF or pAAV2-GFP (Stratagene) vector was cotransfected with pHelper and pAAV-RC plasmids (Stratagene) into AAV-293 cells. Virus particles were purified from lysed cells and viral titers were measured by quantitative polymerase chain reaction (qPCR) using primers for the cytomegalovirus (CMV) promoter.

2.3. Intrahippocampal administration of treatments

For surgeries, the mice were randomly assigned to following treatment groups:

$\text{WT} + \text{NaCl}\; n = 12$	$\text{APP/PS1} + \text{NaCl}\; n = 11$
$\text{WT} + \text{CDNF}\; n = 14$	$\text{APP/PS1 CDNF}\; n = 12$
$\text{WT} + \text{AAV2} - \text{GFP}\; n = 16$	$\text{APP/PS1} + \text{AAV2} - \text{GFP}\; n = 17$
$\text{WT} + \text{AAV2} - \text{CDNF}\; n = 16$	$\text{APP/PS1} + \text{AAV2} - \text{CDNF}\; n = 18$

Before surgery, each mouse was anesthetized with halothane (3% for induction, 1–1.5% for maintenance) in 70/30 N_2O/O_2. Carprofen (Rimadyl Vet, Pfizer, Dundee, UK) at 5 mg/kg (s.c.) was given prior operation. The mice were placed in a stereotactic frame (David Kopf) and skull exposed and punctured in order to administer treatments. CDNF (10 μg), NaCl (0.9%), AAV2-CDNF (titer: 1.0 × 10$\hat{1}$2 virus genome/ml) or AAV2-GFP (titer: 4.0 × 10$\hat{1}$0 virus genome/ml) in 1.4 μl volume were injected bilaterally into the dentate gyrus at the following coordinates: ±1.6 mm medial/lateral, -1.7 mm anterior/posterior, -2.7 mm dorsal/ventral from the bregma. Injection speed was 0.2 μl/min, and a Hamilton 10 μl syringe with 26 G blunt needle was used. After injection, the needle was left in place for 6 min to prevent fluid surging upwards. Core temperature was maintained at 36–37 °C throughout the surgery with an automated heating pad and after the surgery, mice were allowed to recover in a heated chamber until fully alert and moving. Animals in CDNF protein experiment were treated in four and AAV2-CDNF in three cohorts. Due to short storage time of the viral vector construct, all three cohorts received a different batch of AAV2-CDNF.

2.4. Behavioral testing

After 2 weeks of post-operative recovery, the mice underwent a neurological test battery comprising tests for novel object recognition and spatial learning and memory, exploratory activity, and object neophobia to assess memory functions and general behavior.

The *Morris swim task* (water maze) was used to test spatial learning and memory. For protein-treated animals, the apparatus consisted of a black plastic pool (Ø = 120 cm) and a black escape platform (14 × 14 cm) hidden below 1.0 cm the water surface. The temperature of the water was kept at 20 ± 0.5 °C throughout the experiment, and a 10 min recovery period was allowed between the trials. First, the mice were pretrained (2 days) to find and climb onto the submerged platform, aided by a guiding alley (1 m × 14 × 25 cm) leading to the platform. In the testing phase (days 1–4), five 60 s daily trials were conducted with a submerged platform. The platform location was kept constant and the starting position varied between four constant locations at the pool edge, with all mice starting from the same position in any single trial. Each mouse was placed in the water with its nose pointing toward the wall. Having found the platform, the mouse was allowed to stay on it for 10 s. If the mouse failed to find the escape platform within 60 s, it was placed there for 10 s by the experimenter. On day 5, the trial length was set to 40 s. The first and last trials on that day were run without the platform to test the search bias. On day 9, a subset

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