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Reelin has a preventive effect on phencyclidine-induced cognitive and sensory-motor gating deficits

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ABSTRACT

Reelin has recently attracted attention because of its connection to several neuropsychiatric diseases. We previously reported the finding that prior transplantation of GABAergic neuron precursor cells into the medial prefrontal cortex (mPFC) of mice significantly prevented the induction of cognitive and sensory-motor gating deficits induced by phencyclidine (PCP). The majority of the precursor cells transplanted into the mPFC of the recipient mice differentiated into members of a somatostatin/Reelin-expressing class of GABAergic interneurons. These findings raised the possibility that Reelin secreted by the transplanted cells plays an important role in preventing the deficits induced by PCP. In this study, we investigated whether Reelin itself has a preventive effect on PCP-induced behavioral phenotypes by injecting conditioned medium containing Reelin into the lateral ventricle of the brains of 6- to 7-week-old male mice before administrating PCP. Behavioral analyses showed that the prior Reelin injection had a preventive effect against induction of the cognitive and sensory-motor gating deficits associated with PCP. Moreover, one of the types of Reelin receptor was found to be expressed by neurons in the mPFC. The results of this study point to the Reelin signaling pathway as a candidate target for the pharmacologic treatment of neuropsychiatric diseases.

1. Introduction

The extracellular protein Reelin has been concluded to be essential to the development of the laminar structure of the cerebral cortex because the layers of the cerebral cortex of Reelin-deficient mice, *reeler*, are disorganized (Bar et al., 1995; D'Arcangelo et al., 1995; Ogawa et al., 1995). Reelin has been reported to be related to several neuropsychiatric diseases. *Reelin* mRNA and protein levels are significantly reduced in several areas of the brains of patients with schizophrenia (Fatemi et al., 2000; Guidotti et al., 2000; Impagnatiello et al., 1998) and bipolar disorder with psychosis (Guidotti et al., 2000), and similar abnormal Reelin levels have been seen in autism (Fatemi et al., 2002). Furthermore, multiple lines of evidence have pointed to the fact that Reelin plays key roles in Alzheimer's disease (AD) (Botella-Lopez et al., 2006; Bothwell and Giniger, 2000). A role for Reelin in schizophrenia (Liu et al., 2010; Shifman et al., 2008), bipolar disorders (Goes et al., 2010), autism (Skaar et al., 2005), and AD (Kramer et al., 2011) has been supported by studies that have examined genetic associations between Reelin and those diseases.

Reelin is required for normal development of dendritic structures (Niu et al., 2004), developmental maturation of *N*-methyl-D-aspartate (NMDA) receptors (Sinagra et al., 2005), and enhancement of glutamate-mediated function in the adult brain (Beffert et al., 2005; Chen et al., 2005; Qiu et al., 2006; Weeber et al., 2002). Reelin treatment not only increased α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/NMDA receptor current ratios (Qiu and Weeber, 2007), but also regulated homeostasis of the subunit composition of NMDA receptors (Campo et al., 2009). Reelin injected into the lateral ventricle of the mouse brain has been found to be transported to the hippocampus, to increase dendritic spine density and synaptic plasticity, and to enhance associative and spatial learning and memory performance (Rogers et al., 2011).

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Moreover, Reelin-overexpressing transgenic mice are resistant to the effects of chronic corticosterone and psychostimulant treatments (Teixeira et al., 2011). Based on the above findings the Reelin signaling pathway has been pointed to as a candidate target for the pharmacologic treatment of a range of neuropsychiatric diseases.

Noncompetitive NMDA receptor antagonists, including phencyclidine (PCP), induce schizophrenia-like positive and negative symptoms as well as cognitive deficits in healthy humans (Javitt and Zukin, 1991) and similar cognitive and sensory-motor gating deficits in rodents (Mouri et al., 2007). We previously reported finding that prior GABAergic neuron precursor cell transplantation into the medial prefrontal cortex (mPFC) of mice significantly prevented the induction of cognitive and sensory-motor gating deficits by PCP (Tanaka et al., 2011). The majority of the precursors transplanted into the mPFC of the recipient mice differentiated into members of a somatostatin/Reelin-expressing class of GABAergic interneurons. These findings raised the possibility that Reelin secreted by the transplanted cells plays an important role in preventing the deficits induced by PCP. In order to investigate whether Reelin itself has a preventive effect against PCP-induced behavioral alterations, in this study we assessed the effect of Reelin injection prior to administering PCP.

2. Materials and methods

2.1. Animals

Pregnant ICR female mice and adult C57BL/6 female mice were purchased from Japan SLC (Shizuoka, Japan). The animals were housed under a standard 12 h light–dark cycle (light phase 9:00–21:00) at a constant temperature of 23 ± 1 °C, with free access to food and water throughout the experiments. GAD67-GFP mice were kindly provided by Dr. Y. Yanagawa (Gunma University). Mice homozygous for the Gad67-GFP allele were crossed with C57BL/6 wild-type mice to obtain heterozygous (Gad67gfp/+) mice for immunohistochemistry.

All animal experiments were performed in accordance with protocols approved by the Keio University Institutional Animal Care and Use Committee and Nagoya University Institutional Animal Care and Use Committee in compliance with the Institutional Guidelines for Animal Experimentation at Keio University and Nagoya University, and the Japanese Government Law Concerning the Protection and Control of Animals and Japanese Government Notification of Feeding and Safekeeping of Animals.

2.2. Preparation of Reelin

The 293T cells were transfected with a full-length mouse *reelin* (kindly provided by Dr. T. Curran [University of Pennsylvania, Philadelphia, PA]) expression construct under cytomegalovirus-immediate early enhancer (CAG) promoter (Kubo et al., 2010; Niwa et al., 1991) (kindly provided by Dr. J. Miyazaki [Osaka University]) or the control vector, pCAGGS1. GeneJuice Transfection Reagent (Novagen, Madison, WI) was used to transfect 293T cells. The cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). Two days after transfection, the conditioned medium from mock and Reelin-transfected cells was collected into Viva Spin tubes (100,000 molecular weight cut-off) and concentrated 70 fold. The conditioned media were solubilized with a sample buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 0.005% bromophenol blue, 10% glycerol, and 100 mM DTT). The solubilized materials were boiled for 3 min at 98 °C and subjected to SDS-PAGE (8% acrylamide). Reelin was detected by staining with an anti-Reelin antibody (G10) as follows or by Coomassie blue staining. The concentration of Reelin was estimated by comparison with bovine serum albumin (BSA). We obtained approximately 60 ng/μl Reelin in the concentrated medium, which corresponded to 150 nM of Reelin having a molecular weight of 400 K (D'Arcangelo et al., 1997; Kubo et al., 2002; Utsunomiya-Tate et al., 2000).

2.3. Western blot analysis

The conditioned media were subjected to SDS-PAGE (8% acrylamide), and then electrotransferred onto a PVDF membrane using an iBlot gel transfer system (Invitrogen). The blots were treated with a blocking buffer, 5% skimmed milk in PBS containing 0.05% Tween 20, for 1 h at room temperature (RT), incubated overnight at 4 °C with mouse G10 anti-Reelin (de Bergeyck et al., 1997, 1998), washed three times, incubated for 1 h at RT with HRP-labeled goat anti-mouse IgG (1:2000; Dako), and then washed again three times. After the final wash, the blots were treated with ECL Plus Western blotting detection reagents (GE Healthcare). The signals were detected and measured using a cooled charge-coupled device camera (LAS-4000mini; Fujifilm).

2.4. Immunohistochemistry

Coronal brain slices were prepared as described previously (Tabata and Nakajima, 2003). The sections were first washed with 0.05% Triton X-100 in PBS and then blocked for 30 min in 10% normal goat serum (NGS) or 10% normal donkey serum (NDS) and PBS. Next, the sections were incubated with the primary antibody in 5% NGS or NDS, 0.05% Triton X-100, and PBS at 4 °C overnight. The following primary antibodies were used: rabbit anti-ApoER2 (1:1500, abcam), goat anti-VLDLR (1:300, R&D), mouse anti-CaM Kinase II alpha subunit antibody (1:200, upstate biotechnology), and rabbit anti-parvalbumin (1:1000, oncogene). To detect VLDLR, CaMKII and parvalbumin, the sections were incubated at 70 °C in HistoVT One (Nacalai tesque) for 20 min prior to the incubation with the primary antibody. The sections were then rinsed several times with 0.05% Triton X-100 in PBS and incubated with fluorescence-conjugated secondary antibodies (donkey Alexa 555 anti-rabbit, anti-mouse, or anti-goat, donkey Alexa 647 anti-rabbit or anti-goat, 1:1000, Invitrogen, Molecular probes, Eugene, OR) for 1 h at room temperature. The nuclei in some sections were labeled with 4′,6-diamidino-2-phenylindole (DAPI 1:5000, Invitrogen, Molecular probes, Eugene, OR). Images were acquired through a confocal microscope (FV1000, Olympus, Tokyo, Japan) and fluorescence microscope (BX50, Olympus, Tokyo, Japan) equipped with a CCD camera (DP80, Olympus, Tokyo, Japan).

2.5. Injection into the lateral ventricle

Mice were anesthetized with avertin (400 mg/kg, intraperitoneally injected) and placed on a stereotaxic surgery apparatus. To allow direct passage of the needle into the right ventricle of the brain, a hole was drilled through the right side of the skull, and 0.5 μl of solution containing condensed conditioned medium from mock- or Reelin-transfected cells was injected (A.P. −0.4 mm from bregma, L, −0.8 mm from the sagittal suture, and V −2.5 mm from the flat skull surface) through a stereotaxic injector at a rate of 1.0 μl/min. The needle was then removed, and after sealing the holes with dental cement, and the incision was closed.

2.6. PCP treatment

After the administration of Reelin, mice were injected with PCP dissolved in saline (1 mg/kg, subcutaneously) 30 min before the prepulse inhibition (PPI) test and the training session in the novel

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