Mechanisms of the spatial memory deficits induced by injection of okadaic acid into the Meynert nucleus basalis of rats

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Abstract We previously reported that the injection of okadaic acid (OA) into the Meynert nucleus basalis of rats induced spatial memory deficits. The present study was designed to further explore the underlying mechanisms. We found that the level of acetylcholine (Ach) in the hippocampus significantly decreased 24 h after injection of OA into the Meynert nucleus basalis of rats. Simultaneously, spatial memory deficit, PP-2A inhibition and tau hyperphosphorylation at Ser-198/Ser-199/Ser-202 (Tau-1 epitope) and Ser-396/Ser-404 (PHF-1 epitope) were observed. With the restoration of hippocampus Ach to normal levels at 48 and 72 h after the injection, the spatial memory deficits, PP-2A inhibition and tau hyperphosphorylation were reversed. It is suggested that injection of OA into the Meynert nucleus basalis of rats may impair the hippocampus-dependent spatial memory through damaging the cholinergic projection between the Meynert nucleus basalis and the hippocampus and the selective inhibition of PP-2A and tau hyperphosphorylation may be at least part of the underlying mechanisms.

Keywords protein phosphatase 2; tau proteins; phosphorylation; hippocampus; acetylcholine

1 Introduction Alzheimer’s disease (AD) is the most commonly seen neurodegenerative disease, characterized by the presence of neurofibrillary tangles (NFTs), senile plaques (SPs) and loss of cholinergic neurons in the brain. NFTs largely consist of insoluble fibrils of the microtubule-associated protein tau in its hyperphosphorylated forms. SPs are comprised mainly of aggregates of β-amyloid peptides of 40 and 42 amino acids, collectively denoted as Aβ.
2 Materials and methods

2.1 Experimental animals

3–5 months old Male Sprague-Dawley rats, (250 ± 20) g of body weight, were obtained from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All rats were housed in groups of two rats per cage with free access to food and water, and were maintained with a 12 hours light/12 hours dark cycle (lights on at 5:00 p.m., and off at 5:00 a.m.), so that behavioral tests were done during their active hours. Housing and testing were done at stable temperature (23–25°C) and humidity.

2.2 Chemicals and antibodies

OA was purchased from Calbiochem (La Jolla, CA, USA) and dissolved in dimethyl sulfoxide to 20 mmol/L as stock solution and further diluted to a final solvent of 0.2 μmol/L with 0.9% NaCl before use. Polyclonal antibody 92 e to total tau (1:5000) was a gift from Drs. Iqbal and Grundke-Iqbal from the New York State Institute for Basic Research, New York, USA. Monoclonal antibody Tau-1 to unphosphorylated tau (1:5000) at Ser-198/Ser-199/ Ser-202 and PHF-1 to phosphorylated tau (1:500) at Ser-396/Ser-404 were from Dr. Binder (Northwestern University, Chicago, Illinois, USA) and Dr. Davies (Albert Einstein College of Medicine, Bronx, New York, USA), respectively. Alkaline phosphatase-conjugated anti-mouse and anti-rabbit IgGs were from Amersham Pharmacia Biotech (Piscataway, NJ, USA).

2.3 Behavioral test

The rats were trained in a Morris water maze by an experimenter who was blind to the treatment schedule (double blind). The water maze consisted of a circular metal pool (diameter: 120 cm, height: 80 cm) in which a circular plexiglas platform (diameter: 10 cm, height: 40 cm) was hidden 1–2 cm below the surface of the water [(26 ± 1)°C]. The maze was located in an experimental room under stable conditions during the experiment. For post-training injection experiments, rats were trained in the hidden platform version of the Morris water maze task [16] with an inter-trial interval (iti) of 4 min until they could locate the platform three consecutive times in 10 sec. Rats which failed to reach this criterion by trial 12 were excluded from the study. Each trial was initiated by placing the animal in one of four randomly chosen locations facing the wall of the tank. The rats were allowed to search for the hidden platform in 60 sec. If it failed to find the platform, the rat was placed on the platform by the experimenter. Rats were allowed to remain on the platform for a period of 30 sec before being returned to their home cages. The training protocol consisted of 20 trials in 5 days (4 trials per day) with an inter-trial interval of 20 sec. Latencies and swim paths searching for the platform were monitored by a computerized tracking system. The last trial was recorded as pre-injection behavior.

Rats were divided into 3 groups randomly after training, namely group uninjected, group control-injected and group OA-injected. The rats were tested in the water maze again and the post-injection behavior was recorded 24, 48, 72 hours after injection.

2.4 Stereotaxic injection

Rats were anesthetized with 3% chloral hydrate (400 mg/kg) and placed in a Jiangwan-II stereotaxic instrument (Jiangwan Medical Instrument Co., Shanghai, China) at a rat-skull position, with the incisor bar set 2 mm below the earbars. After the scalp was incised (5–8 mm), the skull was cleaned and two holes (diameter 1.0 mm) were made for injection at the co-ordinates anterior-posterior (AP) −1.4, lateral (L/R) −2.5, vertical (V) −7.0 (in mm from bregma and dura, flat skull) according to the stereotaxic atlas of Franklin and Paxinos [17]. A sterilized needle connected to a 5 μL syringe was stereotaxically placed into the nucleus basalis of Meynert. The rats were injected bilaterally with 0.2 μmol/L OA or as a vehicle control with 0.9% NaCl (2 μL/side for each rat).

2.5 Western blot analyses and activity assay of protein phosphatase

After the post-injection water maze tests, the rats were decapitated while deeply anesthetized. The nucleus basalis of Meynert were removed immediately and homogenized at a ratio of 9.0 mL of buffer/1.0 g tissue in a buffer containing 50 mmol/L Tris-HCl (pH 7.0), 1.0 mmol/L EDTA, 0.1 mmol/L phenylmethyl sulfonyl fluoride, 1 mmol/L Benzamidine, and 2.0 μg/mL each of aprotinin, leupeptin and Pepstatin A. The homogenates were immediately centrifuged at 12500 r/min, 4°C for 15 min and the supernatant was divided into two parts. One was used for protein phosphatase activity assays. Into the other part, an equal volume of phosphate inhibitor mixture (20 mmol/L β-glycerophosphate, 2.0 mmol/L Na3VO4, 100 mmol/L NaF, pH 7.0) was added. Protein concentrations of all samples were quantitated using the BCA Protein Assay Reagent (Pierce, Rockford, IL, USA).

The protein bands were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The target proteins were probed with specific primary antibodies and corresponding alkaline phosphatase-labeled secondary antibodies. The immunoreactivity of the blots was developed with 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT) (Amersham Pharmacia Biotech,