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Research report

ERK and p38 inhibitors attenuate memory deficits and increase CREB phosphorylation and PGC-1α levels in Aβ-injected rats

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ABSTRACT

In this study, we investigated the effect of intracerebroventricular administration of ERK and p38 specific inhibitors, U0126 and PD169316, respectively, on learning and memory deficits induced by amyloid beta (Aβ) in rats. To investigate the effects of these compounds on learning and memory, we performed Morris water maze (MWM) test. U0126 and/or PD169316 improved spatial learning in MWM in Aβ-injected rats, 20 days after Aβ-injection. To determine the mechanisms of action of U0126 and PD169316, we studies their effect on some intracellular signaling pathways such as Ca^+/cAMP-response element binding protein (CREB), c-fos, and transcription factors that regulate mitochondrial biogenesis. Based on our data, CREB and c-fos levels decreased 7 days after Aβ-injection, while U0126 and/or PD169316 pretreatments significantly increased these levels. Moreover, U0126 and PD169316 activated peroxisome proliferator-activated receptor gamma coactivator-1a, nuclear respiratory factor 1, and mitochondrial transcription factor A, 7 days after Aβ-injection. Surprisingly, these factors were returned to vehicle level, 20 days after Aβ-injection. Our findings reinforce the potential neuroprotective effect of these inhibitors against the Aβ toxicity.

1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible loss of neurons and the loss of intellectual abilities, including memory and reasoning. Amyloid β protein (Aβ) is a 40 ± 42 amino acid peptide that is considered to be one of the major contributing factors to the development of AD. Nabeshima and co-workers [1] demonstrated that a continuous infusion of Aβ into the cerebral ventricle in rats results in learning and memory deficits that were accompanied by a reduction of choline acetyltransferase activity, suggesting that accumulation of Aβ is related to cognitive impairments in AD [1,2]. One of the most interesting pathways is mitogen-activated protein kinases (MAPKs) signaling, which is considered as an important regulator of a broad range of genes involved in cellular responses to pro-inflammatory and other stress signals. Three distinct groups of well characterized major MAPK subfamily members include extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK which are serine/threonine protein kinases [3]. A dynamic balance between JNK, ERK and p38-MAPK activation will determine the cellular response, either cell growth or death [4]. Recently, we have reported that inhibition of JNK phosphorylation reverses memory deficit induced by Aβ [5]. Moreover, several groups using human post-mortem tissues from control and Alzheimer's cases confirmed the observations that the p38 MAPK activation occurs at the very early stages in human AD brains [6,7]. Accumulating evidence has shown that the ERK signaling pathway has an important role in learning and memory. It has been reported that ERK affects the maintenance of long-term memory, but administration of U0126, had no significant effect on short-term memory in rats [8]. In hippocampus cells oxidative stress reduced ERK expression and ERK suppressed its downstream transcription factor, cAMP-response element binding protein (CREB) [9].CREB is a nuclear transcription factor essential for long-lasting changes in synaptic plasticity that mediates the conversion of short-term memory to long-term memory. Besides this transcription factor is a candidate sensor for energy insufficiency and has been implicated in support of mitochondrial biogenesis [10]. A role of CREB in mitochondrial biogenesis was highlighted by discovery that the CREB translational complex controls the expression of PGC-1α [11]. In the present study, we investigated the effects of U0126 and PD169316,

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ANOVA, Analysis of Variance; APP, amyloid precursor protein; ARE, antioxidant response element; CREB, Ca^+/cAMP-response element binding protein; ECL, electrochemiluminescence; ERK, extracellular signal-regulated protein kinase; i.c.v., intracerebroventricular; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MWM, Morris water maze; NRF-1, nuclear respiratory factor-1; PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1α; TFAM, mitochondrial transcription factor A.

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the Aβ-induced cognitive impairment in animal model of AD. Moreover, we studied their effect on CREB signaling, as well as some other transcription factors that regulate mitochondrial biogenesis.

2. Materials and methods

2.1. Materials

Antibodies directed against ERK, p38, p-ERK, p-p38, c-fos, CREB, p-CREB, and β-actin were obtained from Cell Signaling Technology (Beverly, MA, USA). Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) antibody was obtained from ABCAM (Cambridge, UK). Nuclear respiratory factor-1 (NRF-1) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mitochondrial transcription factor A (TFAM) antibody was obtained from BioVision (Palo Alto, CA). Electrochemiluminescence (ECL) kit was provided from Amersham Bioscience (Piscataway, NJ, USA). Aβ (1–42), U0126, and PD169316 were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Animals

Adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 210–280 g were used in these experiments. Animals were housed in groups of three per cage in a 12/12 h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.) with free access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for 1 week before the experimental procedure was started. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.3. Preparation of Aβ1–42 and fiber formation

The Aβ1–42 was dissolved in PBS, and aliquots were stored at −20 °C until use. Aliquots of Aβ1–42 at a concentration of 200 ng/μl prepared in Phosphate Buffer Saline (PBS, 0.1 M) were incubated for 5 days at 37 °C. On the test day, PBS was added to the solution to reach the final concentration of 10 ng/μl (see supplementary Fig. 1).

2.4. Stereotaxic surgery

Rats were anesthetized by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg), and placed into stereotaxic device (Stoelting, USA). An incision was made along the midline, the scalp was retracted, and the area surrounding bregma was cleaned and dried. In addition, lidocaine with epinephrine solution (0.2 ml) was injected in several locations around the incision. Microinjections were performed by 30-gauge injector cannula. Polyethylene tube (PE-10) was used to attach injector cannula to the 5-μl Hamilton syringe. For intracerebroventricular (i.c.v.) administration of ERK and p38 inhibitor solution, 30-gauge injector cannula was aimed at the lateral ventricle (stereotaxic coordinates: incisor bar −3.3 mm, 0.5 mm posterior to the bregma, 1.5 mm lateral to the sagittal suture and 4 mm down from top of the skull) [12]. Cannulae were secured with jewelers' screws and dental acrylic cement. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intra-muscular) was administered immediately after surgery. Animals received a total volume of 5 μl ERK/p38 inhibitor into the left or right ventricle, and 3 μl/side Aβ microinjection into the CA1. All microinjections were performed slowly over a period of 60 s, and injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs.

2.5. Behavioral test: Morris water maze (MWM)

2.5.1. Apparatus

The water maze that was used has been described extensively [13]. Briefly, it consisted of a dark circular pool (140 cm in diameter and 55 cm high) filled with water (20 ± 1 °C) to a depth of 25 cm. A transparent Plexiglas platform (11 cm diameter) was located 1 cm below the water surface in the center of one of the arbitrarily designed north-east (NE), south-east (SE), south-west (SW) or north-west (NW) orthogonal quadrants. The platform provided the only escape from the water. Many extra-maze cues such as racks, a window, a door, bookshelves and pictures on the walls surrounded the room where the water maze was performed. These were kept in fixed positions with respect to the swimming pool to allow the rat to locate the escape platform hidden below the water surface. The position of the animal was monitored by a camera that was mounted above the center of the pool. Animal displacement was recorded using a 3CCD camera (Panasonic Inc., Japan) placed 2 m above the MWM apparatus and locomotion tracking was measured by ethovision software (version XT7), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands).

In these series of experiments, time spent in the target quadrant and swimming speed was recorded during 60 s, in both probe and training trials.

2.5.2. Habituation

Twenty-four-hour prior to the start of training, the rats were habituated to the pool by allowing them to perform a 60 s swimming without the platform.

2.5.3. Procedure

Nineteen days after surgery, the behavioral tests were started. The single training session consisted of eight trials with four different starting positions that were equally distributed around the perimeter of the maze [14].

Each rat was placed in the water facing the wall of the tank at one of the four designated starting points (north, east, south and west) and was allowed to swim and find the hidden platform located in the SW quadrant (target quadrant) of the maze. Each of four starting positions was used twice in eight training sessions; their order was randomized. During each trial, each rat was given 60 s to find the hidden platform. After mounting the platform, the animals were allowed to remain there for 20 s, and were then placed in a holding cage for 30 s until the start of next trial. After completion of training, the animals were returned to their home cages until the probe trial 24 h later (on the test day). In the probe trial, the hidden platform was removed and the animals were released from the north location and allowed to swim freely for 60 s. After the probe trial, the platform was elevated above the water surface and placed in the different position (SE quadrant) and rats were allowed to swim freely for 120 s in order to test their visual ability. All of experiments were conducted between 9:00 and 13:00.

2.6. Western blot analysis

The hippocampi were homogenized in lysis buffer containing protease inhibitor cocktail. Then, the total proteins were electrophoresed in 12% SDS-PAGE gels, transferred to polyvinylidine fluoride membranes and probed with specific antibodies. Immunoreactive polypeptides were detected by chemiluminescence using enhanced ECL reagents and subsequent autoradiography. Quantification of the results was performed by densitometric scan of films. Data analysis was done by Image J, measuring integrated density of bands after background subtraction. Protein concentrations were determined according to Bradford's method [15]. Standard plot was generated using bovine serum albumin. Nuclear extracts were prepared as described by Rong and Baudry [16].

2.7. Experimental design

Animals were divided into six groups: (A) Aβ-injected group, which received bilateral intra-CA1 injection of Aβ (30 ng/3 μl PBS per side), 4 h after unilateral i.c.v. administration of DMSO (5 μl/rat), without receiving any treatment; (B) vehicle group, which only received carrier, DMSO in lateral ventricle and PBS (3 μl/side) in both CA1 regions; (C) ERK inhibitor group, which received i.c.v. infusion of U0126 (30 μg/5 μl 1% DMSO in PBS) with PBS injection (3 μl/side in CA1); (D) p38 inhibitor group, which received i.c.v. infusion of PD169316 (30 μg/5 μl 1% DMSO in PBS) with PBS injection (3 μl/side in CA1); and (E) treatment group which received i.c.v. administration of U0126 (30 μg/5 μl 1% DMSO in PBS), 4 h prior to intra-hippocampal Aβ (30 ng/3 μl PBS per side) injection; (F) treatment group which received i.c.v. administration of PD169316 (30 μg/5 μl 1% DMSO in PBS), 4 h prior to intra-hippocampal Aβ (30 ng/3 μl PBS per side) injection. The aforementioned groups entered two experimental protocols: behavioral experiments and molecular studies. All the groups of animals in molecular study were considered as 7 and 20-day experimental groups.

2.8. Statistics

Data were expressed as mean ± SEM (Standard Error of Mean) and processed by commercially available software GraphPad Prism® 5.0. Two-way analysis of variance (ANOVA) and randomized block model followed by post hoc analysis (Newman–Keuls test) were used for behavioral studies. The mean value of training data for the first and second four trials was compared by paired Student *t*-test. On the other hand, two-way ANOVA followed by post hoc analysis (Bonferroni test) was performed for molecular tests. *P*-value less than 0.05 ($P < 0.05$) was considered to be statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

3. Results

3.1. Behavioral results

3.1.1. U0126 and/or PD169316 improved spatial learning in MWM

Various lines of investigations revealed that for the observation of spatial learning and memory of rats, MWM is more useful than