Neuroscience Letters 552 (2013) 140–145

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Depletion of intracellular zinc induces apoptosis of cultured hippocampal neurons through suppression of ERK signaling pathway and activation of caspase-3

Wei Pang[a], Xue Leng[b], Hao Lu[a,c], Hongpeng Yang[a], Nan Song[a], Long Tan[a], Yugang Jiang[a,*,1], Changjiang Guo[a,**,1]

[a] Department of Nutrition, Institute of Hygiene & Environmental Medicine, Academy of Military Medical Science, Tianjin, China
[b] Institute of Medical Equipment, Academy of Military Medical Science, Tianjin, China
[c] Center for Disease Control and Prevention, PLA Chengdu Military Area Command, Chengdu, China

HIGHLIGHTS

- LDH activity and apoptosis in TPEN-incubated neurons were markedly increased.
- We confirmed that TPEN induces apoptosis of hippocampal neurons through inhibition of ERK pathway.
- We show for the first time that activation of caspase-3 induced by TPEN in was mediated by ERK pathway.

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 15 July 2013
Accepted 31 July 2013

Keywords:
Zinc depletion
Apoptosis
MEK/ERK
Caspase-3
Hippocampal neurons

ABSTRACT

Although Zinc depletion induces apoptosis in different cells and tissues, exact mechanism of this action of zinc depletion is not completely understood. In our previous study, the results suggested that the significant down-regulation of MEK/ERK signaling pathway was observed in zinc deficiency neurons. Here, we investigate whether, in hippocampal neurons, this increased rate of apoptosis induced by zinc depletion is the result of hypophosphorylation of ERK pathway. In this study, we found that NGF, ERK agonist, prevented neurons against TPEN-induced apoptosis, whereas TPEN-induced apoptosis was potentiated by U0126, inhibitors of ERK. Moreover, TPEN-induced caspase-3 activity was further increased by the pretreatment with U0126, but it was further decreased by the pretreatment with NGF. However, pretreatment of the cells with U0126 or NGF had no effect on the changes of Bcl-2 and Bax protein expression induced by zinc depletion. Thus, the results indicate that TPEN induces apoptosis of hippocampal neurons through inhibition of ERK and, in turn, activation of caspase-3

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Zinc (Zn) is a biofactor that plays essential roles in the nervous system, deficits of this nutrient can result in numerous diverse pathologies including apoptotic neuronal death [7,16]. Zinc depletion induces apoptosis in different cells and tissues [12,15,33]. *N*,*N*,*N*′,*N*′-Tetrakis (2-pyridylmethyl) ethylenediamine (TPEN) is a membrane-permeable metal ion chelator with structural similarity to EDTA, it has particularly high affinity for Zn^{2+} [stability constant (log K_i) values for Zn is 15.6] [6]. This property has led to the use of TPEN in a variety of settings, often as a tool to probe the functions of Zn^{2+}. For example, depletion of intracellular Zn^{2+} with TPEN causes human neuroblastoma IMR-32 cell apoptosis [34,37]. Though mechanisms involved in the induction of apoptosis secondary to zinc deficiency are multiple, and differ among different cell types, the mechanisms underlying the induction of hippocampal neuronal apoptosis by zinc deficiency are still unclear.

Extracellular signal-regulated kinase (ERK) signaling pathway plays a crucial role in many processes of cell life [24,29]. In our previous study, the results suggested that the significant down-regulation of MEK/ERK signaling pathway was observed in zinc deficiency neurons induced by TPEN-treated [26]. Furthermore, many studies shown that ERK pathway control a variety of pro- and anti-apoptotic mechanisms that determine cell viability. Zhang et al. reported that administration of U0126, an ERK inhibitor, to the cultured neuron stem cells largely eliminated anti-apoptotic effects of folate [38]. So we speculated that the apoptosis of hippocampal neurons induced by zinc deficiency was mediated by

* Corresponding author. Tel.: +86 22 84655333.
** Corresponding author. Tel.: +86 22 84655429.
E-mail addresses: jyg1967@126.com (Y. Jiang), guocjtj@yahoo.com.cn (C. Guo).
[1] These authors contributed equally to the supervision of this work.

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.07.057

hypophosphorylation of ERK pathway. In this paper, to get some insight into the hypothesis, we investigated the effects of various chemical factors, including U0126 (an inhibitor of ERK), NGF (an agonist of ERK) on the changes of apoptosis rate and the expression of apoptosis-related molecules induced by TPEN.

2. Materials and methods

2.1. Hippocampal cell cultures

Hippocampi were isolated from newborn (postnatal day 1) Wistar rats and cut into <0.5 mm^3 pieces in Ca^{2+} and Mg^{2+}-free Hank's solution (D-Hank's). After digesting at 37 °C for 20 min with 0.25% trypsin in Hanks' balanced salt solution, serum was added to stop the action of trypsin. Tissue pieces were resuspended in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 10% heat-inactivated horse serum (Hyclone), 2 mM L-glutamine (Sigma, St. Louis, MO, USA), 100 U/ml penicillin, and 100 μM streptomycin (Hyclone) and mechanically dissociated into single cells by trituration with fire polished Pasteur pipette at a concentration of 1×10^9 cells/l. The hippocampal cells were plated onto six-well plastic plates (2.0×10^6 cells per well) precoated with poly L-lysine (0.5 g/l in D-Hank's/phosphate-buffered saline (PBS)) (Sigma, St. Louis, MO, USA) and maintained at 37 °C in a humid atmosphere of 95% air and 5% CO_2. After 24 h, cells were changed into neurobasal medium (Gibco) containing 5% horse serum, 1% N2 (Gibco), 2% B27 (Gibco), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. N2 and B27 are nerve cell basal medium additives that can take place of serum. Cytosine arabinoside(5 mg/l) (Sigma) were added into neurobasal medium 5 days after plating to inhibit the proliferation of non-neuronal cells. Two days later, cultured cells were washed several times in warm DMEM and fed with serum-free DMEM supplemented with 1% N2, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Half of the medium was changed twice a week. The obtained cultures resulted in a population enriched in large pyramidal neurons. Seven days in vitro cells were used for the experiments. All procedures were performed under sterile conditions.

2.2. Cell treatments

Hippocampal neurons were exposed to either TPEN (2 μM) or TPEN plus $ZnSO_4$ (5 μM) for 24 h. Before the exposure, the pre-existing medium was washed out several times. Then, exposure to TPEN and zinc sulfate was accomplished by the addition of desired volumes of stock solutions to the serum-free exposure medium. TPEN was solubilized in DMSO. Additionally, some cultures were treated with the U0126 (10 μM) or NGF (100 ng/ml).

2.3. Assessment of cell injury

The overall neuronal cell injury was quantitatively assessed by the measurement of lactate dehydrogenase (LDH) by the LDH assay kit (Zhongsheng Biotech, China). The LDH was released by damaged or destroyed cells into the bathing medium after exposure to TPEN or plus zinc sulfate.

2.4. Assessment of nuclear morphology by Hoechst 33342 staining

In order to visually assess the apoptotic changes in neurons, Hoechst 33342 staining was applied after the treatment of neurons. Neurons grown on coverslips were labeled with Hoechst 33242 (5 μg/ml) for 20 min, washed in PBS, and fixed in 4% formaldehyde.

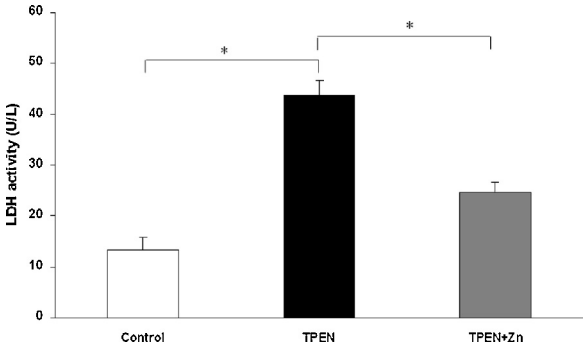

Fig. 1. The effect of zinc depletion with TPEN on neuronal injury in cultured hippocampal neurons. Bars denote LDH activity in non-treatment hippocampal cultures (Control), or in cultures after 24 h exposure to TPEN (2 μM) alone, TPEN plus $ZnSO_4$ (5 μM). Bars represent the mean ± SD; $^*P < 0.05$ ($n = 4$).

Fixed cells were washed and observed under a Leica DMI 3000B fluorescence microscope (Leica, Germany). Neurons with bright blue fragmented nuclei showing condensation of chromatin were identified as apoptotic cells, and about 500 neurons were counted in five random high-power fields and all experiments were performed in triplicate.

2.5. Western blot analysis

After 24 h exposure to TPEN and $ZnSO_4$, total protein was extracted from hippocampal neurons using protein lysis kit (Beyotime, Haimen, JS, China). After determining the amount of protein, each sample was diluted with sample buffer (5×) and then boiled for 5 min. Protein samples were separated by SDS-polyacrylamide gel electrophoresis on a 12% polyacryl-amide resolving gel and transferred to polyvinylidene difluoride membrane (Millipore, Grand Island, NY, USA). The membrane was blocked with 3% BSA in Tris-buffered saline containing 0.1% Tween 20 (TBST) overnight at 4 °C, followed by incubation with anti-bcl-2, anti-bax, anti-caspase-3, made in rabbit antibodies (1:1000 dilution, Cell Signaling, Danvers, MA, USA) for overnight at 4 °C and then an additional incubation with goat horseradish peroxidase-conjugated anti-rabbit IgG (1:2000 dilution, Santa Cruz, Santa Cruz, CA, USA) for 1 h at room temperature. Protein bands were visualized on a Kodak film using the ECL kit (Santa Cruz) and the film was subjected to Image Master R VDS (Pharmacia, Wikipedia, Sweden). The membranes were then re-probed and tested for polyclonal antibodies to GAPDH. The membranes were then washed and visualized as described above. The density of protein bands was quantified by Tatallab software/Quantity One software (Bio-Rad, USA).

2.6. Statistical analysis

The statistical analysis was performed using one-way analysis of variance with post hoc testing by Scheffe (SPSS 11.5, Bizinsight, Beijing, China). Values are expressed as means and standard deviations. All P values were two-tailed, and differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of intracellular zinc depletion with TPEN on the injury in cultured hippocampal neurons

The effect of zinc depletion with TPEN on neuronal injury was measured (Fig. 1). It was shown that LDH activity in TPEN-exposed

Download English Version:

https://daneshyari.com/en/article/6282830

Download Persian Version:

https://daneshyari.com/article/6282830

Daneshyari.com