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Phosphatidylethanolamine-binding protein 1 protects CA1 neurons against ischemic damage via ERK-CREB signaling in Mongolian gerbils

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ABSTRACT

In the present study, we made a PEP-1-phosphatidylethanolamine-binding protein 1 (PEP-1-PEBP1) fusion protein to facilitate the transduction of PEBP1 into cells and observed significant ameliorative effects of PEP-1-PEBP1 against H_2O_2-induced neuronal damage and the formation of reactive oxygen species in the HT22 hippocampal cells. In addition, administration of PEP-1-PEBP1 fusion protein ameliorated H_2O_2-induced phosphorylation of extracellular signal-regulated kinases (ERK1/2) and facilitated the phosphorylation of cyclic-AMP response element binding protein (CREB) in HT22 cells after exposure to H_2O_2. We also investigated the temporal and spatial changes of phosphorylated phosphatidylethanolamine-binding protein 1 (pPEBP1) in the hippocampus, after 5 min of transient forebrain ischemia in gerbils. In the sham-operated animals, pPEBP1 immunoreactivity was not detectable in the hippocampal CA1 region. pPEBP1 immunoreactivity was significantly increased in the hippocampal CA1 region, 1–2 days after ischemia, compared to that in the sham-operated group and pPEBP1 immunoreactivity was returned to levels in sham-operated group at 3–4 days after ischemia. pPEBP1 immunoreactivity significantly increased at day 7 after ischemia and decreased to sham-operated group levels by day 10 after ischemia/reperfusion. In addition, administration of PEP-1-PEBP1 fusion protein significantly reduced the ischemia-induced hyperactivity of locomotion, 1 day after ischemia and PEP-1-PEBP1 reduced neuronal damage and reactive gliosis (astrocytosis and microgliosis) in the gerbil hippocampal CA1 region, 4 days after ischemia. Administration of PEP-1-PEBP1 fusion protein ameliorated the ischemia-induced phosphorylation of ERK at 3 h and 6 h after ischemia/reperfusion and accelerated the phosphorylation of CREB in ischemic hippocampus at 6 h after ischemia. These results suggest that the increase in PEBP1 phosphorylation causes neuronal damage in the hippocampus and treatment with PEP-1-PEBP1 fusion protein provides neuroprotection from increasing phosphorylation of ERK-CREB pathways in the hippocampal CA1 region, during ischemic damage.

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1. Introduction

Cerebral infarction and cardiac arrest cause deprivation of oxygen and glucose in the brain, resulting in neuronal damage in many brain regions, including the hippocampus. The hippocampus is one of the key brain regions implicated in memory, cognition, and emotion. In addition, the neural network in the hippocampus has been extensively studied due to the simple and clear projection of axons such as mossy fibers and the Schaffer collateral pathway (Bannerman et al., 2014; Derkach et al., 2007). Transient forebrain ischemia induces neuronal damage in the hippocampal CA1 region (Pulsinelli and Brierley, 1979). Several lines of evidence demonstrate the possible pathophysiological mechanisms of delayed neuronal death induced by ischemia, including free radical damage and inflammation (Jeong et al., 2014; Julio-Amilpas et al., 2015; Yan et al., 2012; Zhou et al., 2016).

The central nervous system has a unique structure, the blood-brain barrier, to prevent cytotoxic damage from blood toxic substances. However, this barrier system also shows poor delivery of bioactive compounds from blood to the brain. Many researchers attempt to facilitate the penetration of bioactive compounds and drugs into the brain via the protein transduction domain (PTD). Among PTDs, PEP-1 peptide can transport full-length proteins into the cells as it consists of a hydrophobic tryptophan-rich domain and a hydrophilic lysine-rich domain separated by a spacer (Morris et al., 2001). It has been reported that PEP-1 does not colocalize with different endocytotic markers such as dextran, caveolin-1, and cathepsin D (Henriques and Castanho, 2008; Henriques et al., 2005) and this result suggests that PEP-1 transverses membrane by an endocytosis-independent mechanism. In contrast, PEP-1 has a high affinity to lipid membrane, it is able to insert and induce local destabilization in the lipid bilayer in the presence of anionic phospholipids without any pore formation (Henriques and Castanho, 2008). In previous studies, we demonstrated that the PEP-1 fusion protein efficiently transduced into the hippocampal CA1 region and protected neurons from ischemic damage (Cho et al., 2008; Kim et al., 2014). In addition, administration of PEP-1-GFP showed GFP positive fluorescence in the hippocampal CA1 region at 6 h after ischemia/reperfusion and peaked at 12 h after ischemia/reperfusion and thereafter decreased with time after ischemia (Cho et al., 2008).

Phosphatidylethanolamine-binding protein 1 (PEBP1), also called to Raf 1 kinase inhibitory protein (RKIP), is a highly conserved protein in various animals, including human (Banfield et al., 1998; Bernier and Jollès, 1984). PEBP1 is expressed in the cytoplasm, nucleus, synapse, and plasma membrane (Al-Mulla et al., 2006; Hagan et al., 2005; Seddiqi et al., 1996). PEBP1 plays a diverse role via many cellular signaling pathways including the promotion of differentiation in neurons and oligodendrocytes (Hellmann et al., 2010; Sagisaka et al., 2010) and autophagy (Noh et al., 2016). Overexpression of PEBP1 increases neuronal differentiation with or without retinoic acid treatment (Hellmann et al., 2010) and decreases autophagy-related cell death in starved cells (Noh et al., 2016).

PEBP1 is considered as a potential biomarker for the prognosis of cancer (Chatterjee et al., 2004; Yu et al., 2014) and facilitates differentiation of adult hippocampal progenitor cells (Sagisaka et al., 2010) or neuroblasts (Hellmann et al., 2010). In the brains of Tg2576 mice, PEBP1 levels decreased with age, starting at 11 months, compared to age-matched control mice (George et al., 2006; Mullan et al., 1992). This is also the time period when amyloid-beta (Aβ) plaques accumulate in the brains of Tg2576 mice (George et al., 2006). Chronic treatment with corticosterone decreases hippocampal PEBP1 expression and impairs cognitive function in rats (Feldmann et al., 2008). PEBP1 mRNA decreases after oxygen-glucose deprivation and overexpression of PEBP1 ameliorates focal ischemic damage and ischemia-induced activation of microglia in the brain (Su et al., 2017). In addition, levels of PEBP1 and phosphorylated PEBP1 at Ser 153 (pPEBP1) are elevated penumbra tissue at 6 h after focal ischemia (Wang et al., 2017). However, this study does not show the chronological changes in PEBP1 expression after ischemic damage and instead, uses lentivirus-mediated overexpression of PEBP1 against ischemic damage.

In a previous study, we observed the significant reduction in PEBP1 protein levels in the spinal cord at 3 h after ischemia (Yoo et al., 2017b). We hypothesized that supplementation of PEBP1 could be one of therapeutic targets to ameliorate the neuronal damage in ischemic tissue. Therefore, we investigated the spatial and temporal changes of pPEBP1 in the hippocampus 5 min of transient forebrain ischemia at various time points after ischemia in gerbils because PEBP1 as a good protein may switch into bad protein (pPEBP1) following ischemia/reperfusion (Wang et al., 2017). In addition, we observed effects of PEP-1-PEBP1 fusion protein on H_2O_2-induced neuronal damage in the HT22 cell line and ischemia-induced neuronal damage in the gerbil hippocampus.

2. Materials and methods

2.1. In vitro efficiency to penetrate into HT22 cells

2.1.1. Cell preparation

HT22, murine hippocampal neuronal cells, were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics at 37 °C under humidified conditions of 95% air and 5% CO_2.

2.1.2. Construction of expression vectors

Preparation of the PEP-1 expression vector has been described in a previous study. Human PEBP1 was amplified by PCR with two primers. The sense primer 5′-CTCGAGATGCCGGTGGACC-3′ contained an *Xho*I restriction site. The antisense primer 5′-GGATCCCTACTTCCCAGAC AGC-3′ contained a *Bam*HI restriction site. The resulting PCR products were ligated into the TA vector and cut with *Xho*I and *Bam*HI. Fragments were then ligated into the PEP-1 expression vector to generate PEP-1-PEBP1. Control PEBP1 was manufactured without the PEP-1 peptide. Recombinant PEP-1-PEBP1 plasmid was transformed into *Escherichia coli* BL21 (DE3) and cultured in 0.5 mM isopropyl-β-D-thiogalactoside (Duchefa, Haarlem, the Netherlands) at 18 °C for over 24 h. Harvested cells were lysed by sonication and PEP-1-PEBP1 protein was purified using a Ni^{b+}- → Ni^{2+}- nitrilotriacetic acid Sepharose affinity column and PD-10 column chromatography to generate PEP-1-PEBP1 protein. Bovine serum albumin was used as a standard and protein concentration was measured by Bradford assay (Bradford, 1976).

2.1.3. Transduction of PEP-1-PEBP1 proteins into HT22 cells

HT22 cells were treated with different concentrations (0.5–3 μM) of PEP-1-PEBP1 protein and PEBP1 protein for 1 h and with 3 μM of both proteins for various periods (15–60 min) to examine the concentration-dependent and time-dependent transduction abilities of PEP-1-PEBP1 protein, respectively. The cells were then treated with trypsin-EDTA for 10 min and washed with PBS to eliminate proteins attached to the cellular membranes. Cells were lysed with ice-cold RIPA buffer (Thermo Scientific, IL, USA) and the lysates were centrifuged at 13,000 × *g* for 20 min at 4 °C. The protein concentration of the supernatant was quantified by the Bradford assay. Equal amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by western blot analysis using a rabbit anti-polyhistidine primary antibody (1:2,000, His-probe, SantaCruz Biotechnology, Santa Cruz, CA, USA) and a goat anti-rabbit secondary antibody (1:5000, Santa Cruz Biotechnology).

2.1.4. Confocal fluorescence microscopy

The intracellular distribution of transduced PEP-1-PEBP1 protein in HT22 cells was observed by confocal fluorescence microscopy, as described previously (Shin et al., 2014). Culture media were grown on coverslips and treated with 3 μM PEP-1-PEBP1 protein. After 1 h of incubation at 37 °C, cells were washed twice with PBS and fixed with 4% paraformaldehyde for 5 min. The cells were treated with PBS