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CDK5 inhibitors prevent astroglial apoptosis and reactive astrogliosis by regulating PKA and DRP1 phosphorylations in the rat hippocampus

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ABSTRACT

Status epilepticus (SE) results in the unique pattern of dynamin-related protein 1 (DRP1)-mediated mitochondrial dynamics, which is associated with astroglial apoptosis and reactive astrogliosis in the regional-specific pattern representing the differential astroglial properties. However, less defined are the epiphenomena/upstream effecters for DRP1 phosphorylation in this process. Since cyclin-dependent kinase 5 (CDK5) is involved in reactive astrogliosis, CDK5 is one of the possible upstream regulators for DRP1 phosphorylation. In the present study, both olomoucine and roscovitine (CDK5 inhibitors) effectively ameliorated SE-induced astroglial apoptosis in the dentate gyrus without changed seizure susceptibility. In addition, they inhibited reactive astrogliosis in the CA1 region independent of neuronal death induced by SE. These effects of CDK5 inhibitors were relevant to abrogation of altered DRP1 phosphorylation ratio and mitochondrial length induced by SE. CDK5 inhibitors also negatively regulated protein kinase A (PKA) activity in astrocytes. Therefore, our findings suggest that CDK5 inhibitors may mitigate astroglial apoptosis and reactive astrogliosis accompanied by modulations of DRP1-mediated mitochondrial dynamics.

1. Introduction

Astrocytes are the major glial cell type in the brain that performs various functions to regulate brain homeostasis (Chen and Swanson, 2003; Wang and Bordey, 2008). In response to brain damage, astrocytes acquire characteristic functional and morphological features, referred to as reactive astrogliosis (Aono et al., 1988; Eddleston and Mucke, 1993; Eng et al., 1992; Ridet et al., 1997). Furthermore, a number of studies have demonstrated regional-specific astroglial damage/death following brain injury (Kang et al., 2006; Kim et al., 2008; Kim et al., 2011; Kim et al., 2014). Briefly, apoptotic astroglial death is observed in the molecular layer (not the hilus) of the dentate gyrus, while clasmatodendrosis (a TUNEL negative lysosome-derived autophagic astroglial death) is detected within the stratum radiatum of the CA1 region (Kim et al., 2008; Kim et al., 2009; Kim et al., 2011; Sugawara et al., 2002).

Mitochondria are morphologically dynamic organelles that regulate cell function, growth, and survival (MacAskill et al., 2010; Sheng and Cai, 2012; Birsa et al., 2013). Mitochondrial dynamics (fission and fusion) play critical roles in mitochondrial functions, activity-dependent regulation of mitochondrial distribution and cell death mechanisms (Benard and Rossignol, 2008; Chen and Chan, 2009; Rintoul and Reynolds, 2010; Li et al., 2004; Sung et al., 2008; Kim et al., 2014). Recently, we have reported that the unique pattern of mitochondrial dynamics is associated with apoptotic astroglial death and autophagic astroglial damage in the regional-specific pattern. Briefly, status epilepticus (a prolonged seizure activity, SE) induces astroglial apoptosis in the dentate gyrus accompanied by decreased mitochondrial length, and results in autophagic astroglial death in the CA1 region with mitochondrial elongation. Indeed, enhanced mitochondrial fission aggravates astroglial apoptosis. In contrast, inhibition of mitochondrial fission effectively attenuates astroglial apoptosis, but accelerates autophagic astroglial death (Ko et al., 2016).

Mitochondrial dynamics are regulated by dynamin-related GTPases (Chen and Chan, 2005). The fusion-associated GTPases,

Abbreviations: SE, status epilepticus; MFN1, mitofusin 1; MFN2, mitofusin 2; OPA1, optic atrophy 1; DRP1, dynamin-related protein 1; Fis-1, fission related protein-1; MFF, mitochondrial fission factor; CDK5, cyclin-dependent kinase 5; PKA, protein kinase A; AIF, apoptotic inducing factor; pCDK5-Y15, phospho-CDK5 tyrosine 15; PB, phosphate buffer.

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mitofusin 1 (MFN1), mitofusin 2 (MFN2) and optic atrophy 1 (OPA1) increase mitochondrial length (Chen et al., 2003; Rambold et al., 2011). Dynamin-related protein 1 (DRP1) involves mitochondrial fission by interacting with fission related protein-1 (Fis-1) and mitochondrial fission factor (MFF), which is differently regulated by phosphorylation of serine (S) residues: S616 phosphorylation activates mitochondrial fission, while S637 phosphorylation inhibits fission by the detachment of DRP1 from mitochondria (Kashatus et al., 2011; Wang et al., 2012). Interestingly, alterations in DRP1 S616/S637 phosphorylation ratio or interferences of the DRP1 activity/expression are involved in acute programmed neuronal necrosis (Kim et al., 2014; Ko et al., 2016; Hyun et al., 2016). Furthermore, regional specific astroglial death is closely correlated with mitochondrial dynamics by DRP1 phosphorylation, not OPA1 (Ko et al., 2016), although less defined are the epiphenomena/upstream effecters for DRP1 activity induced by SE.

Cyclin-dependent kinase 5 (CDK5) is a unique CDK that plays a role in various neuronal activities unrelated to the cell cycle (Dhavan and Tsai, 2001; Lai and Ip, 2009; Su and Tsai, 2011). CDK5 is also involved in reactive astrogliosis (He et al., 2007) as well as the formation of toxic intracellular aggregates (Castro-Alvarez et al., 2014a; Castro-Alvarez et al., 2014b; García-Matas et al., 2008). Unlike other CDKs, activation of CDK5 results from binding with CDK5-specific activators including p35 or its truncated form, p25 (Lew et al., 1994; Tsai et al., 1994) and p39 (Tang et al., 1995). CDK5 also requires its phosphorylation for maximal rates of activation (Sharma et al., 1999). With respect to these reports, implications of CDK5 in astroglial response to stress would be one of the possible mechanisms for regional specific astroglial death induced by SE. Since CDK5 phosphorylates DRP1-S616 to promote mitochondrial fission (Taguchi et al., 2007; Liesa et al., 2009), CDK5 activation would facilitate mitochondrial fission in astrocytes. However, the roles of CDK5 in the molecular mechanisms underlying regional specific astroglial damage have not been examined, and this issue is the focus of the present study.

Here, we demonstrate, for the first time, that both olomoucine and roscovitine (CDK5 inhibitors) effectively ameliorated astroglial apoptosis and reactive astrogliosis by maintaining DRP1-S616/S637 phosphorylation ratio and mitochondrial length following SE. In these processes, CDK5 inhibitors also negatively regulated protein kinase A (PKA) activity. These findings indicate that CDK5 inhibitors may attenuate SE-induced astroglial apoptosis and reactive astrogliosis accompanied by modulations of DRP1-mediated mitochondrial dynamics.

2. Materials and methods

2.1. Experimental animals and chemicals

Male Sprague-Dawley rats (7 weeks old, Daehan Biolink, South Korea) were used in the present study. Rats were housed under 12 h light dark cycle condition and fed standard rat chow with free access to water. Animal protocols were approved by the Institutional Animal Care and Use Committee of Hallym University (Chuncheon, South Korea). All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), except as noted.

2.2. Surgery and drug infusion

Surgery for drug infusion were performed as previously described (Ko et al., 2016). Briefly, rats were anesthetized with isoflurane anesthesia (3% induction, 1.5–2% for surgery and 1.5% maintenance in a 65:35 mixture of N_2O:O_2) and placed in a stereotaxic frame. A brain infusion kit 1 (Alzet, USA) was implanted into the right lateral ventricle (1 mm posterior; 1.5 mm lateral; 3.5 mm depth), and connected to an osmotic pump (1007D, Alzet, USA) containing: (1) vehicle; (2) olomoucine (100 μM); or (3) roscovitine (100 μM). The pump was placed in a subcutaneous pocket in the interscapular region. Each compound were infused over 7 days. Some animals were also implanted with a stainless steel electrode (Plastics One, USA) into the left hippocampus.

2.3. SE induction and EEG recording

Three days after surgery, rats were treated with pilocarpine (380 mg/kg, i.p.) as previously described (Ko et al., 2016; Kim et al., 2014; Kim et al., 2015). Atropine methylbromide (5 mg/kg, i.p.) was injected 20 min before a single dose of pilocarpine in order to avoid the peripheral muscarinic effect. In some animals, EEG signals were recorded with a DAM 80 differential amplifier (World Precision Instruments, USA) and analyzed using LabChart Pro v7 (AD Instruments, Australia). Spectrograms were automatically calculated using a Hanning sliding window. Two h after SE, diazepam (10 mg/kg, i.p.) was administered to terminate seizure activity, and repeated, as needed. As controls, age-matched normal rats were treated with saline instead of pilocarpine.

2.4. Tissue processing and immunohistochemistry

Under urethane anesthesia (1.5 g/kg, i.p.), animals were subjected to transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Brains were removed immediately following infusion and kept in a solution of 4% paraformaldehyde for 48 h. Thereafter, the brains were transferred to a solution of 30% sucrose in PBS (pH = 7.4) at 4 °C. After 72 h in the sucrose solution, the brains were sliced in coronal sections of 30 μm using a cryostat. Thereafter, sections were incubated overnight at room temperature in a mixture of each primary antisera (Table 1) in PBS containing 0.3% Triton X-100. Next, sections were placed in the solution containing a mixture of FITC- and Cy3-conjugated IgG (Amersham, NJ, USA). For negative control, the hippocampal tissues obtained from non-SE and post-SE animals were incubated with pre-immune serum instead of primary antibody. Images were captured using an Axiocam HRc camera and AxioVision Rel. 4.8 software. After regions were outlined, areas of interest (500 μm^2/area) were selected from the stratum radiatum of the CA1 field and the molecular layer of the dentate gyrus. Each image was normalized by adjusting the black and white range of the image using AxioVision Rel. 4.8 Software, as previously described (Ko et al., 2016).

2.5. Cell count and measurement of mitochondrial length

The number of cells and mitochondrial length were measured, as previously described (Ko et al., 2016; Kim et al., 2014; Kim et al., 2010). Briefly, areas of interest (1×10^5 μm^2) were selected from the stratum radiatum of the CA1 field and the molecular layer of the dentate. Cells were counted on 20× images using

Table 1
Primary antibodies used in the present study.

Antigen	Host	Manufacturer (catalog number)	Dilution used
GFAP	Mouse	Millipore (MAB3402)	1:5000 (IF)
AIF	Rabbit	Millipore (AB16501)	1:500 (IF)
CDK5	Rabbit	Abcam (ab40773)	1:200 (IF)
pDRP1-S616	Rabbit	Cell signaling (4494)	1:3000 (IF)
pDRP1-S637	Rabbit	Cell signaling (4867)	1:500 (IF)
Mito	Mouse	Abcam (ab14705)	1:500 (IF)
pPKA-T197	Rabbit	Assay Biotech (A0548)	1:500 (IF)

IF, Immunofluorescence.
WB, Western blot.
DRP1, Actin 추가하고, WB 농도 추가할 것.