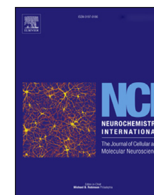
Neurochemistry International 93 (2016) 103–112

Contents lists available at ScienceDirect

Neurochemistry International

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



The mechanisms regulating cyclin-dependent kinase 5 in hippocampus during systemic inflammatory response: The effect on inflammatory gene expression

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A R T I C L E I N F O

Article history:
Received 15 September 2015
Received in revised form
15 January 2016
Accepted 20 January 2016
Available online 21 January 2016

Keywords:
Cyclin-dependent kinase 5
Hippocampus
Lipopolysaccharide
Systemic inflammation
Neuroinflammation

A B S T R A C T

Cyclin-dependent kinase 5 (Cdk5) is critical for nervous system's development and function, and its aberrant activation contributes to pathomechanism of Alzheimer's disease and other neurodegenerative disorders. It was recently suggested that Cdk5 may participate in regulation of inflammatory signalling. The aim of this study was to analyse the mechanisms involved in regulating Cdk5 activity in the brain during systemic inflammatory response (SIR) as well as the involvement of Cdk5 in controlling the expression of inflammatory genes. Genetic and biochemical alterations in hippocampus were analysed 3 and 12 h after intraperitoneal injection of lipopolysaccharide. We observed an increase in both Cdk5 gene expression and protein level. Moreover, phosphorylation of Cdk5 on Ser159 was significantly enhanced. Also transcription of Cdk5-regulatory protein (p35/Cdk5r1) was augmented, and the level of p25, calpain-dependent cleavage product of p35, was increased. All these results demonstrated rapid activation of Cdk5 in the brain during SIR. Hyperactivity of Cdk5 contributed to enhanced phosphorylation of tau and glycogen synthase kinase 3β. Inhibition of Cdk5 with Roscovitine reduced activation of NF-κB and expression of inflammation-related genes, demonstrating the critical role of Cdk5 in regulation of gene transcription during SIR.

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1. Introduction

Cyclin-dependent kinase 5 (Cdk5) is proline-directed serine–-threonine kinase that was implicated in several physiological processes, including development of the central nervous system (CNS), synaptic function and apoptosis. Moreover, deregulation of Cdk5 is a crucial component of the pathomechanism of many neurodegenerative disorders, including Alzheimer's disease (AD) (Lopes and Agostinho, 2011).

Till now, four basic mechanisms of Cdk5 deregulation were proposed. Phosphorylation of Cdk5 on Tyr15 or Ser159, that occurs mainly in pathological conditions, leads to the increase in Cdk5 activity (Czapski et al., 2011, 2013b; Morigaki et al., 2011; Yamamura et al., 2013; Zukerberg et al., 2000). S-nitrosylation of cysteines at positions 83 and 157 as well as acetylation of Lys33 may also upregulate Cdk5 (Lee et al., 2014; Qu et al., 2011, 2012). However, the main mechanism responsible for pathological overactivation of Cdk5 involves calcium-induced, calpain-mediated cleavage of activator protein p35 and formation of p25 (Kusakawa et al., 2000). The complex of Cdk5 with truncated p25 peptide is not catalytically more active than Cdk5/p35, but the significantly longer half-life, due to greater stability of p25, prolongs Cdk5 activation (Patrick et al., 1998, 1999; Peterson et al., 2010). Additionally, p25 is devoid of myristoylation site that is responsible for change in cellular localization of Cdk5/p25 complex. These alterations of Cdk5 could activate cell death signalling, therefore Cdk5

Abbreviations: AIF, apoptosis inducing factor; Cdk5, cyclin-dependent kinase 5; COX-2, cyclooxygenase 2; cPLA2, calcium-dependent phospholipase A2; Egr-1, early growth response protein 1; ERK1/2, extracellular-signal-regulated kinase 1/2; Gsk-3β, glycogen synthase kinase 3β; IκB, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor beta; IKKβ, IκB kinase β; iNOS/Nos2, inducible isoform of nitric oxide synthase; LOX, lipoxygenase; LPS, lipopolysaccharide; MEK, mitogen-activated protein kinase kinase 1; NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B cells; NFT, neurofibrillary tangles; PARP-1, poly(ADP-ribose) polymerase 1; ROS, reactive oxygen species; SIR, systemic inflammatory response; TNF-α, tumor necrosis factor α.

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http://dx.doi.org/10.1016/j.neuint.2016.01.005
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was proposed, as a target for new therapeutic strategy in preventing neurodegeneration (Castro-Alvarez et al., 2015; Crews et al., 2011; Kanungo et al., 2009; Lopez-Tobon et al., 2011). One of the most important consequences of deregulation of Cdk5 is hyperphosphorylation of microtubule-associated protein Tau (Tau) leading to aggregation of Tau filaments and subsequent formation of the intracellular deposits known as neurofibrillary tangles (NFT) (Kimura et al., 2014). In parallel, hyperphosphorylation of Tau evokes loss of its homoeostatic functions and leads to destabilisation of cytoskeleton.

Opposite to early reports, it was demonstrated that the active form of Cdk5 is present not only in post-mitotic neurons, but also in other types of cells (Arif, 2012). Activity of Cdk5 was noticed i.a. in macrophages and astrocytes. A growing body of evidence demonstrates that Cdk5 is involved in regulation of peripheral inflammatory reaction (Arif et al., 2011; Berberich et al., 2011; Du et al., 2009; Jhou et al., 2009; Lee et al., 2012). However, the role of Cdk5 in neuroinflammatory processes is poorly understood. It was previously demonstrated that many stressors, including bacterial lipopolysaccharide (LPS), may enhance phosphorylation of Tau in mice brain (Bhaskar et al., 2010; Kitazawa et al., 2005; Nikkel et al., 2012; Roe et al., 2011), that might suggest Cdk5 overactivation. Also experiments on transgenic mouse model of AD suggested interplay between inflammatory processes, Cdk5 and neurodegeneration (Kitazawa et al., 2006, 2005).

The aim of this study was to analyse the mechanisms responsible for deregulating Cdk5 activity in the hippocampus during LPS-evoked systemic inflammation in mice. Moreover, we investigated the involvement of Cdk5 in controlling inflammatory gene expression. Our previous studies demonstrated that peripheral administration of LPS evoked characteristic pathological changes known as systemic inflammatory response (SIR). These alterations were accompanied by rapid increase in expression of inflammation-related genes in the brain (iNOS, cPLA2, COX-2, LOXs, and NADPH subunits) which was followed by elevation of reactive oxygen species (ROS) level, oxidative modifications of macromolecules and activation of the PARP-1/AIF-related apoptotic pathway in the brain (Czapski et al., 2013a, 2007, 2006, 2010; Jacewicz et al., 2009). Our present study demonstrated for the first time that systemic administration of LPS evokes rapid molecular response affecting Cdk5 activity, leading in consequence to altered phosphorylation of Cdk5 substrates and NF-κB activation. Moreover, those changes were shown to be responsible for the increase in inflammatory gene expression.

2. Materials and methods

2.1. Materials

LPS (from *E. coli* serotype 055: B5; toxicity 15×10^6 U/mg), DMSO, dithiothreitol, Tri-zol and DNAse I were obtained from Sigma–Aldrich (St. Louis, MO, USA). Roscovitine was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies anti-phospho-Tau(Ser396), anti-phospho-Gsk-3β(Ser9), anti-Gsk-3β, anti-ERK1/2, anti-phospho-ERK1/2(Thr202/Tyr204), anti-p35/p25, anti-phospho-p65(Ser536) and anti-nitro-Tyrosine were obtained from Cell Signalling (Beverly, MA, USA), anti-Cdk5, anti-phospho-Cdk5(Tyr15), anti-phospho-Cdk5(Ser159), anti-Tau, anti-α-spectrin and anti-p65 were obtained from Santa Cruz Biotechnology Inc., anti-phospho-Gsk-3β(Tyr216) antibody was from BD Biosciences Pharmingen (NJ, Franklin Lakes, USA), anti-S-nitroso-Cysteine (SNO-Cys), anti-GAPDH and anti-rabbit IgG were from Sigma–Aldrich (St. Louis, MO, USA), anti-mouse IgG was from GE Health Care UK (Little Chalfont, Buckinghamshire, UK). Chemiluminescent reagent Clarity Western ECL Substrate was from Bio-Rad Laboratories (Hercules, CA, USA). Protease inhibitors cocktail Complete was from Roche Diagnostics GmbH (Mannheim, Germany). NF-κB (p65) Transcription Factor Assay Kit was from Cayman Chemical (Ann Arbor, MI, USA). The High Capacity cDNA Reverse Transcription Kit, Power SYBR Green PCR Master Mix, TaqMan Gene Expression Master Mix and TaqMan Gene Expression Assays were from Applied Biosystems (Foster City, CA, USA). Dynabeads M–280 Sheep Anti-Mouse IgG were obtained from Life Technologies (Carlsbad, CA, USA).

2.2. Animals

All of the experiments were carried out on male, 2-month-old (20–25 g), healthy C57BL6J mice supplied by the Animal House of Mossakowski Medical Research Centre PAS (Warsaw, Poland), which runs breeding of small rodents in SPF standard. The animals were maintained under controlled temperature and humidity conditions on a 12-h light/dark cycle. All of the experiments conducted on the animals were approved by the IV Local Ethics Committee for Animal Experimentation in Warsaw and were carried out in accordance with the EC Council Directive of November 24, 1986 (86/609/EEC) following the ARRIVE guidelines and guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. All efforts were made to minimize animal suffering and to reduce the number of animals used. Injections were performed between 9 a.m. and 11 a.m. All manipulations were performed gently and quickly to avoid stress-induced alterations.

Animals were weighed and injection volume was adjusted according to body weight with 0.1 ml/30 g. LPS was administered intraperitoneally (i.p.) in a dose of 1 mg/kg b.w. Control animals received an i.p. injection of appropriate volume of the solvent (0.9% NaCl). Separate groups of mice received additional intraperitoneal injection of potent and selective Cdk5 inhibitor, Roscovitine (seliciclib, CYC202). Roscovitine was dissolved in DMSO, diluted to the desired concentration with saline and administered intraperitoneally at dose of 50 mg/kg b.w. Roscovitine was studied in a variety of cellular and animal models, and numerous reports demonstrated its ability to inhibit Cdk5 *in vitro* and *in vivo* (Chagniel et al., 2012; Crews et al., 2011; Czapski et al., 2013b; Lopes et al., 2009; Song et al., 2007). The dose of Roscovitine was based on earlier studies that demonstrated neuroprotective efficacy and lack of effects on physiologic parameters, such as heart rate, oxygen saturation levels, and breathing rate (Kabadi et al., 2012). It was previously shown that Roscovitine was able to cross brain barrier and to rapidly accumulate in the brain leading to transient inhibition of Cdk5 (Hassan et al., 2011; Menn et al., 2010; Sallam et al., 2008; Vita et al., 2005). Animals from respective experimental groups received an appropriate volume of the solvent. Roscovitine was injected directly before injection of LPS. Then, after 3–12 h the hippocampi were collected. Every effort has been made to minimize the number of animals used and reduce the amount of pain, distress, and/or discomfort. Twenty-four animals were used in whole project; at least three mice were used for each group.

2.3. Preparation of brain structures for genetic analysis

Directly after decapitation, mice brains were dissected and hippocampi were isolated on ice-cold Petri dish. The tissue was used immediately or was frozen in liquid nitrogen and stored in −80 °C until analysis.

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