Journal of Neuroimmunology 313 (2017) 1–9

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

LPS-induced cortical kynurenic acid and neurogranin-NFAT signaling is associated with deficits in stimulus processing during Pavlovian conditioning

Oliveros A.[a], Wininger K.[c], Sens J.[c], Larsson M.K.[d], Liu X.C.[d], Choi S.[a], Faka A.[d], Schwieler L.[d], Engberg G.[d], Erhardt S.[d], Choi D.S.[a,b,c,*]

[a] Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
[b] Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
[c] Neurobiology of Disease Program, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
[d] Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

ARTICLE INFO

Keywords:
Kynurenic-acid
Neurogranin
Ventral-hippocampus
Medial-prefrontal cortex
Lipopolysaccharide
NFAT

ABSTRACT

The *N*-Methyl-D-Aspartate receptor (NMDAR) antagonist kynurenic acid (KYNA) and the post-synaptic calmodulin binding protein neurogranin (Nrgn) have been implicated in neurological and neuropsychiatric conditions including Alzheimer's disease and schizophrenia. This study indicates that systemic dual-lipopolysaccharide (LPS) injections increases KYNA in the medial prefrontal cortex (mPFC), which is accompanied with increased phosphorylation of nuclear factor kappa chain of activated B cells (NFκB) and activation of the nuclear factor of activated T- cells (NFAT). Our results also indicate that dual-LPS increases Nrgn phosphorylation and concomitantly reduces phosphorylation of calmodulin kinase-II (CaMKII). We confirmed that systemic blockade of kynurenine-3 monooxygenase in conjunction with kynurenine administration results in significant increases in Nrgn phosphorylation and a significant reduction of CaMKII phosphorylation in the mPFC. Consequently, dual-LPS administration induced significant impairments in stimulus processing during Pavlovian conditioning. Taken together, our study indicates that elevations in KYNA in the mPFC can directly regulate NMDA-Nrgn-CaMKII signaling, suggesting that neuroinflammatory conditions affecting this pathway may be associated with cognitive dysfunction.

1. Introduction

Although the molecular underpinnings of neuropsychiatric pathophysiology remain elusive, recent studies have implicated pro-inflammatory cytokines in schizophrenia (SCZ), bipolar disorder, and depression (Beumer et al., 2012; Khandaker and Dantzer, 2016; Soderlund et al., 2011, 2009). In microglia and astrocytes, pro-inflammatory cytokines induce indoleamine 2, 3 dioxygenase (IDO-1) and tryptophan 2, 3 dioxygenase (TDO2), thus stimulating tryptophan degradation along the kynurenine pathway (Campbell et al., 2014; Sellgren et al., 2015). Activation of this pathway increases formation of kynurenic acid (KYNA), a terminal metabolite shown to be elevated in the cerebrospinal fluid (CSF) and post mortem brain of patients with bipolar disorder and schizophrenia (Erhardt et al., 2001; Schwarcz et al., 2001; Sellgren et al., 2015). Interestingly, the kynurenine pathway also produces the excitotoxic *N*-Methyl-D-Aspartate receptor (NMDAR) agonist quinolinic acid (QUIN), which has been shown to be elevated in the CSF of suicide attempters (Brundin et al., 2016; Erhardt et al., 2013; Lugo-Huitron et al., 2013). KYNA blocks the glycine site of the NMDAR as well as the cholinergic α7 nicotinic receptor (Hilmas et al., 2001; Schwarcz et al., 2012). Investigation of NMDAR dependent signaling has recently identified associations between patients with SCZ and risk variants on the neurogranin (Nrgn) gene, which codes for a calmodulin binding protein functionally situated upstream of calcineurin and downstream of the NMDAR (Broadbelt et al., 2006; Ruano et al., 2008; Thong et al., 2013). Additional studies have shown that Nrgn variants are clinically associated with deficits in hippocampal activity during contextual learning (Pohlack et al., 2011), while constitutive deletion of this gene in mice indicate that it is necessary for hippocampal spatial memory function (Huang et al., 2006; Pak et al., 2000). However, the role of Nrgn in neuropsychiatric conditions remains unclear, as this protein has been proposed to be a

* Corresponding author at: Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, Minnesota 55905, USA.
E-mail address: choids@mayo.edu (D.S. Choi).

http://dx.doi.org/10.1016/j.jneuroim.2017.09.010
Received 15 December 2016; Received in revised form 4 May 2017; Accepted 27 September 2017

neurodegenerative biomarker which accumulates in the CSF of patients with Alzheimer's disease (AD) (Portelius et al., 2015).

Here we investigate the effects of dual-lipopolysaccharide (dual-LPS) administration on KYNA generation and signaling alterations downstream of the NMDAR in the cortex and hippocampus, two critical brain regions associated with neurodegenerative and neuropsychiatric disease, including SCZ and AD (Campbell et al., 2014; Lipska, 2004). Compared to a single LPS injection, dual-LPS administration significantly elevates kynurenine and KYNA in whole brain (Larsson et al., 2016). Using this paradigm, we examined KYNA in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHip) and detected significant elevations of this metabolite. We investigated effectors downstream of the NMDAR and found that dual-LPS differentially affected calmodulin associated signaling molecules including Nrgn, calmodulin kinase-II (CaMKII) and the neuroinflammatory mediator nuclear factor of activated T- cells (NFAT) in the mPFC without significantly affecting the vHip. We also demonstrate that pharmacological inhibition of kynurenine-3 monooxygenase (KMO) by Ro-61-8048, which is known to increase KYNA, similarly affected Nrgn and CaMKII phosphorylation in the mPFC. We found that dual-LPS induced stimulus processing deficits during Pavlovian conditioning, which is consistent with studies showing that KYNA elevations impair conditioned stimulus (CS +) processing in rodents (Chess and Bucci, 2006), and patients with SCZ (Vaitl et al., 2002). Taken together, the dysregulation of kynurenine-mediated NMDAR signaling on Nrgn and CaMKII provides novel biological mechanisms underlying neurocognitive disorders.

2. Materials and methods

2.1. Animals

Male C57BL/6N and C57BL/6J mice utilized for this study were bred at the Karolinska Institutet (Stockholm, Sweden) Department of Physiology & Pharmacology, or were acquired from Jackson Laboratories (Bar Harbor, ME USA), respectively. All mice utilized in this study were age matched at 2-to-4 months of age and were cared for as previously described (Larsson et al., 2016; Nam et al., 2013). Water and food was available ad libitum, although food was restricted only in control mice that underwent Pavlovian conditioning. Animals were housed in groups of 3–5 individuals on a 12-h lights on/off cycle (lights on at 06:00 h). Temperature was maintained at 25 °C and humidity between 40 and 60%. All efforts were made to minimize the number of animals used. Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden, and the Mayo Clinic Institutional Animal Care and Use Committee in accordance with NIH guidelines.

2.2. Experimental design

Mice utilized for this study weighed between 25 and 30 g and were weighed immediately prior to administration (*i.p.*) of saline vehicle (0.9%) or lipopolysaccharide (LPS; *Escherichia coli*, Sigma-Aldrich, St. Louis MO, USA) prepared immediately prior to T = 0 injection in 0.9% saline. Adult C57BL/6N mice were divided into four groups that separately received either vehicle or dual-LPS (serotype O111:B4) at 0.83 mg/kg (0.1 ml/10 g body weight). As shown in Supplementary Fig. 1A, vehicle and LPS were initially administered at T = 0, with a second injection given 16 h later (T = 16) as previously described (Larsson et al., 2016). This dose was selected because single administration of LPS has been reported to induce depressive-like symptomatology as well as have an effect on conditioning behavior (Mormede et al., 2004; O'Connor et al., 2009). Following dual-LPS injections, C57BL/6N mice were sacrificed at 24 h for High Performance Liquid Chromatography (HPLC) analysis. A separate cohort of C57BL/6 J mice were used for western blot and behavior testing at 24 h, 48 h, or 72 h following T = 0 LPS (serotype O55:B5) or vehicle administration (Supplementary Fig. 1A). Animals decreasing > 15% of body weight or showed excess signs of sickness due to LPS administration were excluded from the study. To determine the relationship between KYNA and Nrgn signaling, C57BL/6J mice were administered vehicle alone, L-Kynurenine alone (100 mg/kg, *i.p.*, Sigma), or the kynurenine monooxygenase inhibitor (KMO-I) Ro-61-8048 (100 mg/kg, *i.p.*, EMD Millipore, MA USA) plus L-Kynurenine (100 mg/kg, *i.p.*). L-Kynurenine was administered 30 min post vehicle or Ro-61-8048 injections (Supplementary Fig. 1B). Injection volumes were delivered at 0.1 ml/10 g body weight. L-Kynurenine was dissolved in 15% DMSO (Sigma) in 0.9% saline, while Ro-61-8048 was dissolved with 15% DMSO and 15% Cremophor (Sigma) in 0.9% saline.

2.3. Tissue extraction

All mice were subjected to rapid isofluorane inhalation to induce unconsciousness, followed by decapitation and subsequent harvesting of brain for manual isolation of the mPFC (AP: + 1.70 mm from Bregma) and vHip (AP: − 2.92 mm from Bregma) from both hemispheres under a surgical microscope. The extracted tissue was weighed and snap-frozen on dry ice for storage at − 80 °C until processing for HPLC or western blot.

2.4. HPLC analysis of L-Kynurenine and kynurenic acid

The mPFC and vHip (separately from each mouse) was combined with a volume (1: 5) of 0.4 M perchloric acid (PCA), 0.1% sodium metabisulfite ($Na_2S_2O_5$) and 0.05% EDTA. Each brain sample was homogenized in a Storm 24 magnetic Bullet Blender for 3 min at a speed setting of 4 (Next Advance Inc., Averill Park NY, USA), with 0.5 mm zirconium oxide beads. The samples were then centrifuged (21,000 × *g*) for 5 min at 4 °C and the supernatants were collected. The supernatants were mixed with 10% volume of 70% PCA and centrifuged (21,000 x g) for an additional 5 min at 4 °C. The resulting supernatant was transferred to a new Eppendorf tube and stored at − 20 °C until ready for HPLC analysis.

Using an isocratic reversed-phase HPLC system coupled to a UV detector (Shimadzu SPD-10A, 360 nm wavelength) followed by a fluorescence detector (FP-2020 Plus, Jasco Ltd., Hachioji, Japan; 344 nm excitation wavelength, 398 nm emission wavelength, 18 nm bandwidth), samples (50 µl) were injected to measure kynurenine and KYNA concentrations in the mPFC of mice treated with vehicle (n = 5) or dual-LPS at 0.83 mg/kg (n = 5). Similarly, we determined kynurenine and KYNA concentrations in the vHip of mice treated with vehicle (n = 6) or dual-LPS at 0.83 mg/kg (n = 5). A mobile phase consisting of 50 mM sodium acetate and 7% acetonitrile (pH set to 6.2 using acetic acid) was pumped through a ReproSil-Pur C18 column (4 × 150 mm, Dr. Maisch GmbH, Ammerbuch, Germany). A second mobile phase containing 0.5 M Zinc acetate in dH_2O was delivered post-columnar, before the fluorescence detector, by a Pharmacia P-500 (GE Healthcare; Uppsala, Sweden) at a flow rate of 10 ml/h. Signals from the detectors were transferred to a computer for analysis with Datalys Azur (Grenoble, France). Retention times for kynurenine and KYNA were 4 and 7 min, respectively.

2.5. Sample preparation for cytosolic proteins and enrichment of synaptosome fractions

2.5.1. Sample preparation for non-synaptosome enriched cytosolic proteins

To confirm whether systemic dual-LPS administration induced signaling alterations in the mPFC and vHip, protein expression of inflammatory mediator NFκB was examined. This tissue preparation method differed from sample preparation for synaptosome enriched fractions (described below). In this non-behavior tested cohort, the mPFC (n = 4/treatment) and vHip (n = 3/treatment) of C57BL/6J