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Quantitative profiling of neurotransmitter abnormalities in the hippocampus of rats treated with lipopolysaccharide: Focusing on kynurenine pathway and implications for depression

Yujin Guo [a,1], Hualin Cai [b,1], Lei Chen [b], Donglou Liang [a], Ranyao Yang [a], Ruili Dang [a,*], Pei Jiang [a,*]

[a] Institute of Clinical Pharmacy & Pharmacology, Jining First People's Hospital, Jining Medical University, Jining 272000, China
[b] Department of pharmacy, Second Xiangya Hospital, Central South University, Changsha 410010, China

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ABSTRACT

Peripheral administration of lipopolysaccharide (LPS) can induce the rodents to a depression-like state accompanied with remarkable changes of neurotransmitter systems. In this study, the effect of an intraperitoneal LPS injection (3 mg/kg) on the concentrations of neurotransmitters was investigated by in vivo microdialysis in rat hippocampus. To further explore dysregulation pattern of the neurotransmitters following continuous inflammatory process, we then analyzed the neurotransmitters in the hippocampus of rats after 2-week LPS exposure (500 μg/kg every other day). Acute treatment of LPS quickly enhanced glutamate release and increased the extracellular levels of dopamine, serotonin and their metabolites. Elevated glutamate status was also found in the chronic inflamed hippocampus, whereas dopamine and serotonin was decreased following prolonged LPS exposure. Interestingly, both acute and chronic treatment of LPS significantly elevated hippocampal kynurenine concentrations and altered the balance between the serotonin and kynurenine branches of tryptophan metabolism—increasing kynurenine/tryptophan ratio, but decreasing serotonin/tryptophan ratio. Additionally, kynurenic acid, the endogenous NMDA receptor antagonist, and the ratio of kynurenic acid/kynurenine were significantly decreased by acute treatment of LPS, which may further strengthen NMDA receptor activation. Since that NMDA activation can exacerbate inflammatory and neurodegenerative process, the enhanced glutamate release and dysregulated kynurenine pathway might constitute a vicious cycle playing a pivotal role in the neuropsychiatric disorders associated with inflammation, such as depression.

1. Introduction

Accumulating evidence indicates a close relationship between inflammation and depression (Dantzer et al., 2011). An immunological model of major depression is "sickness behavior" which is the non-specific reaction of the organism to various infectious stimuli. Immune system activation, such as exposure to the bacterial endotoxin lipopolysaccharide (LPS), induces sickness behavior in animals, which resembles depressive-like symptoms, including fatigue and anhedonia (Biesmans et al., 2013).

The switch from sickness to depression is associated with dysregulated neurotransmission, especially the imbalance between serotonin (5-HT) and kynurenine (KYN) branches of tryptophan metabolism (Salazar et al., 2012). One important consequence of LPS-induced secretion of proinflammatory cytokines, which is relevant to the progression of depression, is stimulation of indolamine 2, 3 dioxygenase (IDO). When activated, IDO breaks down tryptophan (TRP), the primary amino acid precursor of 5-HT, into KYN (Park et al., 2011). Microglia cells express kynurenine-3-monooxygenase (KMO) which is responsible for the conversion of KYN into quinolinic acid (QA), an agonist of N-Methyl-D-Aspartate (NMDA) glutamate receptor, whereas astrocytes express kynurenine aminotransferase (KAT) that metabolizes KYN into kynurenic acid (KA) which has NMDA receptor antagonism properties (Corona et al., 2010). Recent evidence suggests that LPS-induced systematic inflammation cannot only induce KYN formation, but also disturb the downstream balance between QA and KA, shunting toward the NMDA receptor agonistic QA arm and resulting in exacerbated neurotoxicity (Zheng et al., 2012). In line with these findings, disturbed glutamatergic and GABAergic neurotransmission also have been observed in the postmortem brain of depression patients and in the brain tissues of animal models of depression (Abdallah et al., 2015, Kaut et al., 2015). Additionally, pretreatment with ketamine, a NMDA receptor antagonist, can effectively ameliorate LPS-induced behavioral changes (Walker et al., 2013). Taken together, these findings highlight that the interaction between KYN pathway and NMDA mediated glutamatergic signaling is critical to the depressive-like behaviors caused by systematic immune activation.

* Corresponding authors at: Institute of Clinical Pharmacy & Pharmacology, Jining First People's Hospital, Jining Medical University, 6# Jiankang Road, Jining 272000, China.
E-mail addresses: ruilidang@gmail.com (R. Dang), jiangpeicsu@sina.com (P. Jiang).
[1] Authors contributed equally to this work.

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Aside from amino acids and TRP metabolites, the catecholamines also actively participate in the mood regulation. For example, anhedonia, the core clinical feature of depression, is most likely related to abnormities in dopamine (DA)-reward pathway (Shimamoto et al., 2015). In this context, it becomes insufficient and potentially unreliable to focus solely on a small portion of the active neurochemical components. While the perturbations of neurochemistry have been repeatedly documented in the experimentally induced inflammed brain, comprehensive determination of these neurotransmitters and their metabolites is essential and urgently needed for monitoring global neurochemical responses to the environmental stimuli to gain further insight into the interrelationship between inflammation and neurotransmission, which is essential for not only the progression of depression, but also a variety of other brain diseases and neurodegenerative disorders. Therefore, in the present study, we simultaneously analyzed 14 neurochemical metabolites spanning amino acids, DA, noradrenaline (NE), 5-HT and KYN metabolic pathways in the hippocampus of rats following either acute treatment of a large dose of LPS or prolonged moderate LPS exposure.

2. Materials and methods

2.1. Animals

Male, Sprague–Dawley rats (230–280 g), supplied by the Experimental Animal Center of the Hunan Provincial Hospital, were housed under standard conditions of temperature (23 ± 2 °C) and light (12:12 h light/dark cycle), with free access to food and water. All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in our university.

2.2. Experiment 1: in vivo microdialysis and acute administration of LPS

The rats were anesthetized by an intraperitoneal injection of chloral hydrate solution (4 mg/kg). The body temperature of the rat was controlled by a microwaveable heating pad and maintained at 37 °C using a temperature controller (CMA, Stockholm, Sweden). An intracerebral guide cannula was stereotaxically inserted into the hippocampus through a cranial burr hole, using the following coordinates: 4.3 mm anterior, 4.6 mm lateral and 5.8 mm ventral, according to the stereotaxic atlas of Paxinos and Watson (Swanson, 2004). After 24 h of the surgery, the guide cannula was replaced by the brain microdialysis probes (CMA/12) with 4 mm membranes (CMA, Stockholm, Sweden). The probes were perfused with Ringer's solution at a flow rate of 2 ml/min using a perfusion pump (CMA, Solna, Sweden). The fractions were collected every 30 min. After 2 h of sample collection for baseline, 3 mg/kg of LPS or same volume of saline was given intraperitoneally. The dose was chosen based on previous findings to induce the rats to a depression-like state (Quan et al., 1998, Sekio and Seki, 2015) and the samples were collected for the next 8 h.

2.3. Experiment 2: prolonged LPS treatment and behavioral tests

Taking into account the underlying continuous subclinical inflammatory process in depression, we then measured the neurotransmitters in the hippocampus homogenates of rats after prolonged exposure to LPS. The animals received intraperitoneal injection of 500 μg/kg of LPS or same volume of saline between 8:00 a.m. and 8:40 a.m. every other day for 2 weeks. The dose was chosen according to previous findings showing that repeated stimulation by low doses of LPS is effective to provoke depressive-like behaviors with better face validity than acute LPS-induced behavioral models (Elgarf et al., 2014, Kubera et al., 2013).

2.3.1. Sucrose preference test (SPT)

SPT is widely used for the measurement of stress-induced anhedonia state, a key depressive-like behavior in rats (Jiang et al., 2013). Prior to SPT, all the rats were housed individually and habituated to 48 h of forced 1% sucrose solution consumption in two bottles on each side. Then after 14 h water deprivation, we placed two pre-weighed bottles, one containing 1% sucrose solution and another containing tap water to each rat. The side (left and right) of the two bottles was randomly placed in order to avoid spatial bias. The bottles were weighed again after 1 h and the weight difference was considered to be the rat intake from each bottle. The preference for sucrose was measured as a percentage of the consumed 1% sucrose solution relative to the total amount of liquid intake.

2.3.2. Forced swim test (FST)

The paradigm is based upon the evaluation of immobility, as a measure of behavioral despair in stressful and inescapable situations (Jiang et al., 2014a). In brief, each rat was placed in a Plexiglas cylinder (45 cm height, 25 cm diameter) containing approximately 35 cm of water (24 ± 1 °C) for 15 min. The rats were then dried and removed to their home cage. They were placed again in the cylinders 24 h later, and a 5 min swim test was conducted. Each test session was videotaped and the duration of immobility, which defined as floating passively and only making slight movements to keep the head above water, was scored by an experienced observer blind to the experiment design. One day after FST, rats were anesthetized with 10% chloralhydrate (4 ml/kg), and the tissues were rapidly collected and stored at −80 °C until analysis.

2.4. Neurochemistry analysis

Following our previous established method (Jiang et al., 2014b, Zhang et al., 2015), neurotransmitters and their metabolites in rat brain microdialysates and homogenates were determined by using high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Briefly, 1 ml of 85% acetonitrile-water and 10 μl of mixed internal standard solution (containing 0.12 μg/ml 3,4-dihydroxybenzylamine, 0.19 μg/ml 5-hydroxyindole-2-carboxylic acid and 1.41 μg/ml L-aspartic acid-$^{13}C_4$,^{15}N) were added to the samples. After vortex mixing for 5 min, the mixture was centrifuged at 4 °C for 5 min at 15,000 rpm. The supernatant (500 μL) was subsequently evaporated to dryness under vacuum. For derivatization, 150 μl of dansyl chloride solution (4 mg/ml in acetonitrile) and 50 μl of 0.1 M Na_2CO_3-$NaHCO_3$ buffer (pH 11.0) were added to the residue and reacted at 35 °C for 30 min. After the reaction, the pH of the mixture was adjusted to approximately 7.0 by adding 5 μl of 15% formic acid-water solution. LC–MS/MS analyses were carried out on a Waters Acquity ultra-performance liquid chromatography system (Waters, USA) with a Micromass Quattro Premier XE tandem quadruple mass spectrometer (Waters, USA) equipped with ESI source. The analytes were separated on an Ultimate XB-C8 column, 2.1 mm × 50 mm, 3.0 μm particle size (Welch, China) with the column temperature at 40 °C. The mobile phase for elution was a gradient established between solvent A (water with 20 mM ammonium acetate and 0.1% formic acid) and solvent B (acetonitrile) at a flow rate of 0.25 ml/min. The source operated in positive ion mode, and the working parameters were set as follows: capillary voltage, 3.00 kV; extractor voltage, 3.00 V; source temperature, 120 °C; desolvation temperature, 450 °C; desolvation gas flow, 750 L/h; cone gas flow, 50 L/h. Argon used as the collision gas was introduced into the collision cell at a flow rate of 0.16 ml/min. Neurotransmitters were quantified relative to the internal standard areas and calibrated using standard curves. Probably due to the low concentration and non-specific binding effect during sample preparation, we failed to determine QA in the matrixes and we only successfully measured KA concentration in the microdialysates.