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Positive modulation of α5 $GABA_A$ receptors in preadolescence prevents reduced locomotor response to amphetamine in adult female but not male rats prenatally exposed to lipopolysaccharide

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ABSTRACT

We previously demonstrated that lipopolysaccharide (LPS) administered intraperitoneally (i.p.) to pregnant Wistar rat dams, at embryonic days 15 and 16 (E15/16), induced a decrease of baseline locomotor activity and diminished reactivity to amphetamine in adult female offspring. In the present study we aimed to assess the duration of LPS-induced maternal immune activation (MIA) and investigate possible changes in levels of main neurotransmitters in fetal brain during MIA. We hypothesized that the observed behavioral changes may be linked with MIA-induced disturbance of prenatal GABAergic system development, especially with α5 $GABA_A$ receptors (α5$GABA_A$Rs), expression of which takes place between E14 and E17. Thereafter, we set to investigate if later potentiation of α5$GABA_A$Rs in offspring's preadolescence (from postnatal day 22–28) could prevent the deficit in locomotor reactivity to amphetamine observed in adulthood, at postnatal day P60. The elevation of IL-6 in amniotic fluid 6 h after LPS treatment (100 μg/kg, i.p.) at E15 was concurrent with a significant increase of GABA and decrease of glutamate concentration in fetal brain. Moreover, repeated administration of MP-III-022, a selective positive allosteric modulator of α5$GABA_A$Rs, at a dose (2 mg/kg daily, i.p.) derived from a separate pharmacokinetic study, prevented the LPS-induced decrease in locomotor reactivity to amphetamine (0.5 mg/kg, i.p.) in adult females. These results were not mirrored in the parallel set of experiments with male offspring from LPS-treated rats. The results suggest that pharmacological potentiation of α5$GABA_A$Rs activity in preadolescence may ameliorate at least some of adverse consequences of exposure to MIA *in utero*.

1. Introduction

Maternal immune activation (MIA) is one of the most investigated prenatal influences with potentially harmful effects on brain development. Epidemiological studies have linked the emergence of schizophrenia and several other neurodevelopmental disorders with an increased incidence of MIA caused by bacterial or viral infections during pregnancy (Brown and Derkits, 2010). MIA is suggested to lead to a disbalance between pro- and anti-inflammatory cytokines in the fetal brain, which may ultimately impact *in utero* neurodevelopment and result in sustained long-lasting alterations in postnatal brain (Meyer et al., 2009, 2011). Several animal models of prenatal exposure to MIA, designed to mimic behavioral symptoms and elucidate pathophysiological mechanisms underlying the disorders related to abnormal fetal brain development, differ with respect to the challenging agents and the time of exposure (Harvey and Boksa, 2012).

Lipopolysaccharide (LPS), a cell wall component of gram negative bacteria, induces febrile response and cytokine release when administered intraperitoneally to pregnant rodents. Due to a significant variability in LPS exposure protocols, a diversity of findings was reported, describing acute changes in fetal compartment, as well as behavioral symptoms and morphological alterations in offspring. Nevertheless, the changes observed in a number of studies on LPS-treated rodents are consistent with those seen in schizophrenia patients, including

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behavioral changes or postmortem findings in brain tissue (reviewed in Boksa, 2010). A growing body of evidence supports the concept that development of GABAergic system may be a major convergence point for both genetic and environmental susceptibility factors for schizophrenia, as reviewed by Schmidt and Mirnics (2015). Oskvig et al. (2012) showed that a substantial number of GABA-related genes are acutely dysregulated by LPS treatment at E15, such as genes for GAD (glutamate decarboxylase) 67 and GAD65, as well as those which expression enables the hippocampal and cortical migration (Dlx1, 2, 5, and 6) (Cobos et al., 2005). In line with reports on reduction in mRNA and protein for GAD67 (e.g. Benes et al., 2007) and reelin (e.g. Eastwood and Harrison, 2006) in various brain regions, including the hippocampus, of schizophrenia subjects, Nouel et al. (2012) found that LPS (100 μg/kg) treatment of pregnant rat dams at embryonic day (E) 15 and 16 led to a reduction of GAD67-immunoreactive and reelin-immunoreactive hippocampal neurons in preadolescent rat offspring. Moreover, an identical protocol of exposure to LPS resulted in a decreased prepulse inhibition of acoustic startle in adult offspring, indicating the deficits in sensorimotor gating, which is a finding consistent with schizophrenia (Fortier et al., 2007). Nouel et al. (2012) suggested that the changes in prepulse inhibition, as a form of behavior partially modulated by the hippocampus, may be influenced by alterations in levels of GABA or reelin, as key neurochemical components of hippocampal function. The postnatal persistence of developmental MIA-induced changes in GABAergic system is still to be unequivocally assessed. For example, in the hippocampus, LPS treatment on E15/16 has not only immediate effects on dentate cells, but also results in a decreased proliferation and survival of dentate cells generated at postnatal day (P) 14, whereas adult hippocampal neurogenesis at P60 was not affected (Cui et al., 2009).

We have previously shown that LPS (100 μg/kg) administered to pregnant Wistar dams at E15/16 resulted in a significantly diminished baseline locomotor activity and reduced locomotor response to amphetamine in adult female offspring (P60), while the changes in adult males were less pronounced (Batinić et al., 2016); the reduction in spontaneous and amphetamine-induced locomotor activity was also observed in studies using the similar protocol of LPS exposure in rats (Harvey and Boksa, 2014a, 2014b). Importantly, the time of LPS treatment in our protocol (E15/16) correlates with the emergence of α5 $GABA_A$ receptors (α5$GABA_A$Rs) and migration of interneurons (Lauri et al., 1992). In the period between E14 and E17, the α5 subunit of $GABA_A$ receptor is the only one among all six α subunits that is in the process of expression in important brain structures: fetal cortex, fetal thalamus and fetal hippocampus (Lauri et al., 1992). Thus, we hypothesized that behavioral alterations observed in our model may at least partly derive from LPS-mediated disturbance of α5$GABA_A$Rs function, possibly occurring in the developing brain at E15/16. We aimed to investigate whether the onset of behavioral symptoms in adulthood may be affected by preadolescent modulation of α5$GABA_A$Rs, accomplished after GABA excitatory/inhibitory shift (Ben-Ari, 2002) and relocalization from the cell bodies to the dendritic layers have taken place (Ramos et al., 2004). To potentiate the activity of α5$GABA_A$Rs in the settings of decreased GABA-ergic neurotransmission demonstrated in the hippocampus of LPS-exposed offspring at P14, and especially P28 (Nouel et al., 2012), we repeatedly administered MP-III-022, a thoroughly profiled selective positive allosteric modulator (PAM) (Stamenić et al., 2016), during the fourth week of postnatal development.

2. Materials and methods

2.1. Animals

Male and female Wistar rats were obtained from Military Farm (Belgrade, Serbia) at the age of two months, weighting 180–220 g, and were housed separately in male and female animal rooms. The rats were housed in transparent plastic cages and had free access to food pellets and tap water. The temperature of the animal room was 22 ± 1°C, the relative humidity 40–70%, the illumination 120 Lx, and the 12/12 h light/dark period (light on at 6:00 h). All handling and testing took place during the light phase of the diurnal cycle. All procedures in the study conformed to EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Faculty of Pharmacy in Belgrade.

2.2. LPS treatment

The procedure of handling and treatment of animals was replicated from our previous study (Batinic et al., 2016). After joining a male and female rat in a single cage, vaginal smears were taken daily at 9 am and if found positive for spermatozoa females were separated from the males. To avoid a social isolation context, pregnant dams were housed three per cage until gestation day 19 and then individually until delivery. On gestation (embryonic) days 15 and 16 (E15/16) pregnant dams were treated intraperitoneally either with LPS (from Escherichia coli, serotype 0111:B4, Sigma L2630) at a dose of 100 g/kg per day, or with 0.9% saline (2 mL/kg per day). After delivery, offspring were left with their mothers undisturbed until postnatal day 21, and then were weaned, separated by gender and housed up to five and not less than three per cage. The pups from different litters were not mixed. In the further text, offspring rats born to dams treated with LPS will be referred to as LPS offspring or LPS males or LPS females.

2.3. Cytokine determination

For the determination of cytokines at both E15 and E16, pregnant dams of separate cohorts were sacrificed 2 h and 6 h after LPS or SAL treatment, as suggested by Bell et al. (2004). The following samples were harvested: dam's blood, placenta, amniotic fluid, and fetal brain. To determine possible cytokine induction in adult offspring of both genders, their blood and brains were taken at P60. Blood was collected in heparinized tubes, centrifuged at 1000*g* for 10 min and plasma was collected. The placenta and brain tissue samples were placed in tubes with buffer (50 mM Tris-HCl, 0,6 M NaCl, 0,2% Triton X-100, 0,5% BSA and protease inhibitors), then homogenized and centrifuged at 14000*g* at 4 °C, and supernatants were collected. Plasma, amniotic fluid and supernatants were stored at − 80 °C until analysis. Cytokine assays using enzyme-linked immunosorbent assay (ELISA) kits (Rat TNF-alpha ELISA Ready-SET-Go!®, eBioscence, USA and IL-6 ELISA Kit, Rat, Life Technologies, USA) were processed according to the manufacturer's instructions. The detection limits were 16 pg/mL for TNF-α and 23.5 pg/mL for IL-6.

2.4. Neurotransmiters quantification

Five neurotransmitters: glutamate (GLU), gamma-Aminobutyric acid (GABA), dopamine (DA), noradrenaline (NA) and serotonin (5-HT) were determined using the protocol described in Huang et al. (2014) with additional determination of acetylcholine (Ach), in fetal brain in total and selected parts of adult brain. Calibration solutions for acetylcholine were the same concentration range as for dopamine, noradrenaline and serotonin.

Whole fetal brains were collected 2 h and 6 h after LPS or SAL treatment at E15 and E16, while adult brain tissues (prefrontal cortex, dorsal striatum, nucleus accumbens and ventral hippocampus) were sampled from experimentally naive rats decapitated at P60. Brains of adult animals were instantly removed from the skull, washed with ice-cold saline and put into the cold brain matrices (Zivic Instruments). Brain sections were cut according to Paxinos-Watson atlas, and removed from the matrix onto ice-cold pad to isolate the analyzed structures. The whole hippocampus was separated from the rest of its brain section and ventral two-fifths of the structure were cut. Samples

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