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Prenatal stress affects viability, activation, and chemokine signaling in astroglial cultures

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ABSTRACT

CXCL12/SDF-1α and CX3CL1/fractalkine are constitutively expressed in the brain, which indicates their significant functions. Emerging evidence highlights the role of astrocytes and the immune system in the pathophysiology of stress-related disorders. The aim of this study was to assess whether prenatal stress affects chemokine signaling, cell viability/activation, and the iNOS pathway in astroglial cultures. Our results showed that prenatal stress lowered astrocyte viability and simultaneously increased GFAP expression. Furthermore, CX3CL1 production and the CXCL12/CXCR4-7 axis were also altered by prenatal stress. Taken together, malfunctions caused by prenatal stress may adversely influence brain development, leading to long-term effects on adult brain function and behavior.

1. Introduction

A growing body of evidence supports the contention that stress is an important risk factor for many neuropsychiatric disorders, including depression (Baram and Joels, 2009). Among others, stressful events during the prenatal period, which is critical for the development of the central nervous system (CNS), may result in long-lasting neuroanatomic, metabolic and functional changes (Brunton, 2015; Budziszewska et al., 2010; Darnaudéry and Maccari, 2008). Epidemiological studies have confirmed that stressful events experienced during pregnancy are associated with disturbances in neurodevelopment, and they lead to cognitive dysfunction, emotional problems, increased negative temperament, attention deficit/hyperactivity disorder (ADHD), and neuro-immuno-endocrine disturbances in the offspring (Blair et al., 2011; King et al., 2012; Pereira and Ferreira, 2015; Schuurmans and Kurrasch, 2013). Considering these data, the molecular mechanisms underlying these changes have become the subject of many studies in recent years. Therefore, animal models based on stress are widely used.

The prenatal stress procedure in rats is a well-documented animal model of depression. It has been shown that prenatally-stressed rats exhibit long-lasting behavioral changes, such as increased immobility time in the forced swim test (Basta-Kaim et al., 2014; Maccari et al., 2003; Szczesny et al., 2014), enhanced anxiety-like behavior (Patin et al., 2005; Szczesny et al., 2014) and cognitive dysfunction (Abdul Aziz et al., 2012). Stress during pregnancy in rats also leads to prolonged neurobiological changes, such as hypothalamic-pituitary-adrenal (HPA) axis disturbance (Budziszewska et al., 2010; Maccari et al., 2003; Szymańska et al., 2009), neuroplasticity (Grigoryan and Segal, 2016; Lemaire et al., 2000; Sowa et al., 2015), and neurotransmitter network dysfunction (Fine et al., 2014). Moreover, unfavorable events that occur early in life lead to immune system malfunction not only in the peripheral but also in the central nervous system (Diz-Chaves et al., 2013; Ślusarczyk et al., 2015). In line with these data, we demonstrated that prenatal stress impairs the release of insulin-like growth factor 1 (IGF-1), which regulates immune cell function by influencing the ratio of pro-inflammatory cytokines in the brain (Basta-Kaim et al., 2014; Szczesny et al., 2014; Trojan et al., 2016).

Recently, chemokines, which are chemoattractant cytokines shed new light on the immune malfunction associated with stress-related disorders, and they have become a promising area of research. Chemokines are a diverse family of small (7–11 kDa) proteins. They were originally identified as serving chemotactic functions on immune cells; however, recent evidence has elucidated novel, brain-specific functions of these proteins. In fact, chemokines may act as neuromodulators and regulate phenomena such as neurodevelopment, synaptic transmission and neuroendocrine functions, such as thermoregulation, drinking and feeding (Adler et al., 2005; Rostène et al., 2011).

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Moreover, chemokines are present in virtually all brain inflammation pathologies, including neurodegenerative and neuroinflammatory diseases. Under such conditions, activated glial cells express high levels of chemokines and chemokine receptors, suggesting the involvement of these proteins in brain defense mechanisms (Rostène et al., 2011). Interestingly, fractalkine (CX3CL1) and stromal-derived factor 1α (SDF-1α, CXCL12) are two chemokines secreted not only in pathological conditions but they are also constitutively expressed and distributed in many brain areas (Banisadr et al., 2002; Hesselgesser and Horuk, 1999; Schönemeier et al., 2008), which may indicate their significant functions in brain homeostatic processes (Stuart and Baune, 2014). Unlike other chemokines, levels of fractalkine in the brain are higher than those in the periphery (Bajetto et al., 2001), which may suggest its specific role in the CNS. Recent data indicate that neurons are the main source of CX3CL1, although in some cases, astroglia may also express this chemokine, while CX3CR1 is localized mainly on microglia (Lindia et al., 2005).

On the other hand, SDF-1α/CXCL12 has attracted a lot of attention because of its extensive expression by both neurons and glial cells in the CNS (Banisadr et al., 2003; Heinisch and Kirby, 2010; Stumm et al., 2002). SDF-1α/CXCL12 exerts its biological function through two receptors, CXCR4 and CXCR7. Recent data have demonstrated CXCR4 and CXCR7 expression in astroglia, microglial cells, Schwann cells, distinct neuronal populations, and endothelial cells (Levoye et al., 2009; Li and Ransohoff, 2008). Both the abovementioned chemokines are widely distributed in the brain and constitutively expressed under physiological conditions, while their expression is strongly upregulated during inflammation.

Our results show that prenatal stress changes the morphological and biochemical profile of microglia as well as modulates the CX3CL1/CX3CR1 axis in distinct brain areas (Ślusarczyk et al., 2016, 2015). Astroglia regulate synaptic transmission and influence the external environment. Astroglia are also trophic and metabolic support for neurons (Perea et al., 2009). It is speculated that prenatal stress leads not only to biological activity malfunction but also to chemokine-chemokine receptor expression in astroglia (Rajkowska and Miguel-Hidalgo, 2007; Sanacora and Banasr, 2013).

To the best of our knowledge, there are currently no reports regarding the impact of prenatal stress on astroglia. Therefore, the present study was designed to investigate not only the effects of prenatal stress procedure on viability/death parameters and glial fibrillary acidic protein (GFAP) expression but also nitric oxide (NO) release and inducible nitric oxide synthase (iNOS) levels in astroglial cultures. Moreover, to determine the effects of prenatal stress on the chemokines that are constitutively expressed in the brain, we evaluated the mRNA and protein levels of fractalkine (CX3CL1) and SDF-1α/CXCL12, as well as those of both of its receptors, CXCR4 and CXCR7, in primary astroglial cultures.

2. Materials and methods

2.1. Animals

All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee in Krakow, Poland. Sprague-Dawley rats (200–250 g upon arrival) were obtained from Charles-River Laboratories (Germany) and housed under standard conditions (a room maintained at 23 °C in a 12/12 h light/dark cycle, with the lights on at 6:00) with food and water available ad libitum. One week after the rats arrived, vaginal smears

were obtained from the female rats daily to determine the phase of their estrous cycle. During proestrus, the females were placed with males for 12 h. Then, the vaginal smears were evaluated for the presence of sperm. On approximately the 10th day of pregnancy, the females were randomly assigned to either a control group or a stress group.

2.2. Stress procedure

Prenatal stress was performed as previously described (Maccari et al., 1995; Ślusarczyk et al., 2016). Briefly, the pregnant female rats were subjected to three stress sessions at 9:00 a.m., 12:00 p.m., and 5:00 p.m. daily from the 14th day of pregnancy until delivery. During this time, the rats were placed in plastic cylinders (d = 7 cm; l = 19 cm) and exposed to a bright light (150 W; 1800–2000 lx) for 45 min. The control pregnant females remained undisturbed in their home cages.

2.3. Cell culture

The primary astroglia cultures were prepared from the cortices of 1–2-day-old Sprague-Dawley rats as previously described (Piotrowska et al., 2016; Zawadzka and Kaminska, 2005). Briefly, cells isolated from the cerebral cortex were plated in a poly-L-coated 75 cm^2 culture bottle at a density of 3×10^5 cells/cm^2 in DMEM/GlutaMAX/high glucose (Gibco, USA) culture medium supplemented with heat-inactivated 10% fetal bovine serum, 0.1 mg/ml streptomycin and 100 U/ml penicillin (Gibco, USA). Cultures were maintained at 37 °C and 5% CO_2. After 3 days, the culture medium was changed. On the 9th day, the cultures were shaken gently by a horizontal shaker (80 rpm for 1 h and 100 rpm for 15 min) to recover loosely adherent cells, specifically microglial cells. Then, on the 12th day, the non-adherent cells were once again removed, the culture medium was changed, and the culture was left for 2 additional days. Next, the cultures were shaken gently for 3 h (80 rpm). The culture medium was removed, and the astroglial cells were trypsinized (0.05% trypsin EDTA solution, Sigma-Aldrich, USA). The cells were plated in culture medium at a final density of 1.2×10^6 cells/well in 6-well plates for protein analysis via Western blotting, 2×10^5 cells/well in 24-well plates for mRNA and protein analysis via ELISA, or 4×10^4 cells/well in 96-well plates for the NO, MTT and LDH assays and then incubated for 48 h. Two days after plating, the cells were used in experiments. The cultures from both groups of animals (control animals and animals subjected to the prenatal stress procedure) were obtained according to the method described above and simultaneously grown under the same conditions.

2.4. Immunofluorescence staining

To assess the purity of astroglial cell cultures, astroglia obtained from control rats were cultured on sterile cover slips in 6-well plates (1.2×10^6 cells/well). The cells were rinsed with PBS and fixed with 4% paraformaldehyde (Sigma Aldrich, USA) for 20 min at room temperature and washed twice more with PBS solution. The fixed cells were then permeabilized with 0.1% Triton™ X-100 (Sigma Aldrich, USA) in PBS for 30 min at room temperature, washed with PBS and blocked with 5% goat serum in PBS. The astroglia were stained overnight at 4 °C with an antibody against GFAP (an astroglia marker; sc-33673, Santa Cruz Biotechnology Inc., USA) and an anti-Iba1 antibody (a microglial marker, sc-32725, Santa Cruz Biotechnology Inc., USA) After being washed with PBS, the cells were incubated for 2 h at room temperature with the appropriate fluorescent-conjugated secondary antibody (Alexa Fluor, Jackson ImmunoResearch, USA). Images were captured using a fluorescence microscope (Zeiss, Germany). We obtained a highly homogeneous astroglia population (greater than 95% GFAP positivity) (Fig. 1).

2.5. Cell viability test

The cell viability was determined by the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Germany) assay. At 48 h after the astroglial cells were plated,