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Modulation of schizophrenia-related genes in the forebrain of adolescent and adult rats exposed to maternal immune activation

Ann M. Hemmerle [a,b], Rebecca Ahlbrand [c], Stefanie L. Bronson [b,c,1], Kerstin H. Lundgren [a], Neil M. Richtand [b,c,d,e], Kim B. Seroogy *[,a,b]

[a] Department of Neurology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA
[b] Neuroscience Graduate Program, University of Cincinnati, Cincinnati, OH 45267, USA
[c] Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA
[d] San Diego Veterans Affairs Healthcare System, San Diego, CA 92161, USA
[e] Department of Psychiatry, University of California, San Diego School of Medicine, La Jolla, CA 92093, USA

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ABSTRACT

Maternal immune activation (MIA) is an environmental risk factor for schizophrenia, and may contribute to other developmental disorders including autism and epilepsy. Activation of pro-inflammatory cytokine systems by injection of the synthetic double-stranded RNA polyriboinosinic-polyribocytidilic acid (Poly I:C) mediates important neurochemical and behavioral corollaries of MIA, which have relevance to deficits observed in schizophrenia. We examined the consequences of MIA on forebrain expression of neuregulin-1 (NRG-1), brain-derived neurotrophic factor (BDNF) and their receptors, ErbB4 and trkB, respectively, genes associated with schizophrenia. On gestational day 14, pregnant rats were injected with Poly I:C or vehicle. Utilizing *in situ* hybridization, expression of NRG-1, ErbB4, BDNF, and trkB was examined in male rat offspring at postnatal day (P) 14, P30 and P60. ErbB4 mRNA expression was significantly increased at P30 in the anterior cingulate (AC Ctx), frontal, and parietal cortices, with increases in AC Ctx expression continuing through P60. ErbB4 expression was also elevated in the prefrontal cortex (PFC) at P14. In contrast, NRG-1 mRNA was decreased in the PFC at P60. Expression of BDNF mRNA was significantly upregulated in the PFC at P60 and decreased in the AC Ctx at P14. Expression of trkB was increased in two regions, the piriform cortex at P14 and the striatum at P60. These findings demonstrate developmentally and regionally selective alterations in the expression of schizophrenia-related genes as a consequence of MIA. Further study is needed to determine contributions of these effects to the development of alterations of relevance to neuropsychiatric diseases.

1. Introduction

Maternal infections during pregnancy are implicated in elevated risk for several mental disorders, including schizophrenia, autism, and epilepsy (Patterson, 2002; Brown, 2006; Brown and Patterson, 2011). The strongest evidence for this linkage is in schizophrenia, a neuropsychiatric disorder affecting 1% of the population resulting from the combined influence of genetic and environmental factors. Compelling evidence demonstrates that maternal immune activation (MIA), *i.e.* maternal exposure to infection during pregnancy, accounts for up to one third of attributable environmental risk for schizophrenia (Brown and Derkits, 2010). Evidence demonstrates that the immune response itself, rather than a specific infectious agent, is responsible for elevated risk for schizophrenia (Smith et al., 2007; Brown and Derkits, 2010).

In animal models attempting to replicate this environmental risk, prenatal exposure to infectious agents, including influenza, lipopolysaccharide and the viral mimetic polyriboinosinic-polyribocytidilic acid (Poly I:C), results in cellular, neurochemical, and behavioral alterations relevant to schizophrenia (Meyer et al., 2009; Brown and Derkits, 2010). Key alterations stem from the maternal inflammatory response rather than the virus itself, as behavioral abnormalities were induced in the absence of viral infection by injection of Poly I:C, which stimulates maternal cytokine expression (Shi et al., 2003). The synthetic double-stranded Poly I:C RNA is commonly used to create a strong, acute, non-specific immune reaction via the Toll-like receptor 3, resulting in cytokine release (Fortier et al., 2004). Cytokines are polypeptides involved in the inflammatory response and whose prenatal elevation is linked to increased risk of brain damage in offspring (Yoon et al., 1997). Cytokine levels are elevated in mothers of offspring later diagnosed with schizophrenia (Brown et al., 2004). In the MIA model, both behavioral

* Corresponding author at: The Selma Schottenstein Harris Laboratory for Research in Parkinson's, Gardner Family Center for Parkinson's Disease and Movement Disorders, Department of Neurology, University of Cincinnati College of Medicine, Medical Sciences Building, ML0536, 231 Albert Sabin Way, Cincinnati, OH 45267-0536, USA.
E-mail address: kim.seroogy@uc.edu (K.B. Seroogy).
[1] Department of Veterinary Animal Biology, University of Pennsylvania, Philadelphia, PA 19104, USA.

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and transcriptional alterations observed in MIA offspring were also inducible through maternal exposure to the cytokine interleukin-6 (IL-6) (Smith et al., 2007). In contrast to the alterations of cytokine levels, viral RNA is not detected in the tissue of animals exposed to infections prenatally despite later adult abnormalities (Shi et al., 2005). These observations suggest that the stimulation of maternal pro-inflammatory cytokine systems by injection of Poly I:C mediates important neurochemical and behavioral consequences of MIA (Ozawa et al., 2006; Smith et al., 2007; Pratt et al., 2013).

Maternal immune activation offspring display behavioral abnormalities into adulthood of relevance to schizophrenia. These behavioral abnormalities include altered locomotor responsiveness to stress, amphetamine and novel environment, increased anxiety and depressive symptomatology, deficits in prepulse inhibition of startle (PPI) and latent inhibition, and increased responsiveness to stimulant drugs (Shi et al., 2005; Meyer et al., 2005; Brown and Patterson, 2011; Missault et al., 2014). Animals also display impaired working memory, place preference, and novel object recognition (Meyer et al., 2009; Richtand et al., 2012; Lukasz et al., 2013). Neuroanatomically, MIA elicits many brain alterations, including increases in γ-aminobutyric (GABA) receptor expression in the hippocampus and cerebellum, and widely reported developmental and functional abnormalities of the dopaminergic mesotelencephalic system (Bakos et al., 2004; Ozawa et al., 2006; Romero et al., 2010; Vuillermot et al., 2010). Prefrontal cortical abnormalities observed in MIA offspring include elevated basal extracellular glutamate (Roenker et al., 2011) and altered synaptophysin expression (Romero et al., 2010). Within the hippocampus, MIA offspring exhibit glutamate system defects including decreased N-methyl-D-aspartate (NMDA) receptor-dependent synaptic current and plasticity (Lante et al., 2007; Escobar et al., 2011) and elevated basal extracellular glutamate (Ibi et al., 2009). Imaging studies of this animal model consistently observe increased ventricular size in MIA offspring, with volume reductions in the hippocampus, prefrontal cortex (PFC), and striatum (Meyer et al., 2009; Piontkewitz et al., 2011b). Striatal and hippocampal volume reductions preceded the onset of behavioral abnormalities in both sexes, with structural abnormalities appearing developmentally earlier in males (Piontkewitz et al., 2009, 2011a,b). Of interest, antipsychotic medications impact some of these behavioral alterations, including the increased responsiveness to amphetamine, PPI, latent inhibition and alterations in the volume of brain regions (Meyer and Feldon, 2010; Piontkewitz et al., 2011b; Richtand et al., 2011).

Exposure to maternal infection during development may interact with expression of genes mediating schizophrenia risk. Neurotrophic factors are signaling molecules important during many stages of neurodevelopment including proliferation, differentiation, and migration and continue to support neuronal health and synaptic maintenance into adulthood (Knusel et al., 1991; Poo, 2001; Yarden and Sliwkowski, 2001; Buonanno and Fischbach, 2001; Seroogy et al., 2013). Neuregulin-1 (NRG-1) and its receptor ErbB4, as well as brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, are associated with the development of schizophrenia (Stefansson et al., 2002; Chong et al., 2008; Rybakowski, 2008; Ray et al., 2014). ErbB4 and NRG-1 are associated with familial inheritance of schizophrenia and the interaction between the two genes increases the risk of schizophrenia in some studies (Stefansson et al., 2003; Nicodemus et al., 2006; Norton et al., 2006; Silberberg et al., 2006). Dysregulation of NRG-1 signaling may also contribute to the development of positive schizophrenic symptoms (Shamir et al., 2012). Whereas studies directly linking BDNF gene mutations to schizophrenia are conflicting, multiple reports find that BDNF (both mRNA and protein), as well as its high-affinity receptor trkB, are reduced in cortical regions of schizophrenia patients (Durany et al., 2001; Weickert et al., 2003, 2005; Pandya et al., 2013). We therefore sought to examine the effects of MIA on mRNA expression of these genes throughout the postnatal period in the rat forebrain, testing the hypothesis that MIA will result in aberrant regulation of NRG-1, BDNF and their respective receptors. The regions and time points examined were chosen for their relevance to the manifestation of behavioral symptoms in the MIA model.

2. Methods

2.1. Animals

Nulliparous female Sprague Dawley rats, aged 3–5 months, were obtained from Harlan Laboratories (Indianapolis, IN). Male breeders were produced within the animal facility. All animals were housed under standard conditions with food and water available *ad libitum* and a 12:12 light/dark cycle. All procedures were in accordance with the Guide for Care and Use of Laboratory Animals, with approval by our Institutional Animal Care and Use Committee.

2.2. Prenatal Poly I:C treatment

The female rats were acclimated for at least two weeks and then placed in the same cage overnight with a male Sprague Dawley rat. Males were removed the next morning, considered gestational day (G) 0 (Taylor, 1986). The female rats were singly housed throughout the pregnancy. On G14, pregnant dams were weighed (weight gain >40 g) and injected with Poly I:C (Sigma, St. Louis, MO) (8 mg/kg, i.p.) (Bronson et al., 2011) dissolved in saline or with saline vehicle control (1 ml/kg, i.p.). Dams were weighed again 24 h following the Poly I:C or saline injection to identify anorexia and weight loss resulting from an inflammatory response (Fortier et al., 2004). The MIA dams without weight loss and saline dams with weight loss were removed from study as the offspring of these animals have a different phenotypic response (Bronson et al., 2011). On postnatal day (P) 1, litters were culled to eight. Pups were weaned on P21 and housed 2–3 per cage by sex and litter. No more than 2 rats per litter were used in each experimental group to avoid litter effect confounds. On P14, P30 and P60 male MIA offspring were sacrificed and brains were collected and frozen in dry ice to be subsequently processed for *in situ* hybridization.

2.3. In situ hybridization

Fresh-frozen brains (n = 5–6 per condition) were serially sectioned (at 10-μm thickness) throughout the PFC and striatal levels using a cryostat, thaw-mounted onto Superfrost plus microslides (VWR, Batavia, IL), and stored at −20 °C until hybridization. Semi-adjacent sections were processed for the *in situ* hybridization localization of BDNF, trkB, NRG-1 and ErbB4 mRNAs using linearized cDNA plasmids and were labeled using the proper polymerase (T3 for BDNF and T7 for NRG-1, ErbB4 and trkB) and ^{35}S-UTP (PerkinElmer, Boston, MA), as previously described (Seroogy and Herman, 1997; Numan et al., 2005; Dickerson et al., 2009; Hemmerle et al., 2012). The BDNF cRNA (a gift from Drs. Christine Gall and Julie Lauterborn, University of California-Irvine) included 384 bases complementary to the rat BDNF mRNA coding region (nucleotides 388–771; Isackson et al., 1991; Gall et al., 1992). The trkB riboprobe detects the kinase-specific, full-length form of the rat trkB receptor (nucleotides 1358–1558; Middlemas et al., 1991; Goodness et al., 1997). The pan-NRG-1 plasmid was contained in a pCR-TOPO vector and consisted of 500 bp. The ErbB4 cRNA (kindly provided by Dr. Harley Kornblum, UCLA) was transcribed from a PCR 2.1 vector containing a 1.8 kb fragment complementary to a region of the ErbB4 receptor extracellular domain (Kornblum et al., 2000).

For hybridization pretreatment, slides were brought to room temperature and placed in 4% paraformaldehyde (pH 7.4) for 10 min to fix tissue. The slides were then placed in a series of five-minute washes involving phosphate-buffered saline (PBS) (twice), followed by 0.1 M PBS/0.2% glycine (twice), and again 0.1 M PBS (twice). All washes were made with diethyl pyrocarbonate (DEPC)-treated water. Triethanolamine (pH 8.0) containing 0.25% acetic anhydride was then used for 10 min for acetylation of the slides. Finally, slides were

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