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Maternal immune activation alters glutamic acid decarboxylase-67 expression in the brains of adult rat offspring

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ABSTRACT

Activation of the maternal innate immune system, termed "maternal immune activation" (MIA), represents a common environmental risk factor for schizophrenia. Whereas evidence suggests dysregulation of GABA systems may underlie the pathophysiology of schizophrenia, a role for MIA in alteration of GABAergic systems is less clear. Here, pregnant rats received either the viral mimetic polyriboinosinic–polyribocytidilic acid or vehicle injection on gestational day 14. Glutamic acid decarboxylase-67 (GAD_{67}) mRNA expression was examined in male offspring at postnatal day (P)14, P30 and P60. At P60, GAD_{67} mRNA was elevated in hippocampus and thalamus and decreased in prefrontal cortex of MIA offspring. MIA-induced alterations in GAD expression could contribute to the pathophysiology of schizophrenia.

1. Introduction

Schizophrenia is a neurodevelopmental disorder affecting 1% of the population (Knapp et al., 2004). The etiology of schizophrenia requires a combination of genetic and environmental factors acting in concert. Epidemiological evidence demonstrates that maternal bacterial and viral infection during pregnancy is associated with increased risk of schizophrenia (Mednick et al., 1988; Brown, 2006; Clarke et al., 2009).

In animal models, prenatal exposure to maternal immune activation (MIA) is used to recapitulate this effect (Shi et al., 2003). One such model utilizes the synthetic double-stranded RNA polyriboinosinic–polyribocytidilic acid (Poly I:C). Injection of Poly I:C to pregnant dams activates the maternal innate immune response, stimulating pro-inflammatory cytokine systems (Fortier et al., 2004; Smith et al., 2007). This produces cellular, neurochemical, and behavioral alterations in the offspring attributable to the MIA rather than the virus itself (Zuckerman et al., 2003; Shi et al., 2005; Richtand et al., 2012; Missault et al., 2014; Vorhees et al., 2015) and of relevance to schizophrenia (Meyer et al., 2005, 2009; Brown and Derkits, 2010).

MIA offspring exhibit alterations in several neurotransmitter systems of relevance to schizophrenia including dopamine, glutamate, and γ-aminobutyric acid (GABA) systems (Samuelsson et al., 2006; Lanté et al., 2007; Meyer et al., 2008, 2009; Ibi et al., 2009; Bitanihirwe et al., 2010; Escobar et al., 2011; Roenker et al., 2011; Richetto et al., 2014). As the chief inhibitory neurotransmitter in the central nervous system, GABA is found in numerous locations including neocortical regions, the hippocampus and the thalamus. Imbalance between excitatory glutamate and inhibitory GABA function has been implicated in the pathophysiology of schizophrenia (Roberts, 1972; Huguenard and Prince, 1992; Hashimoto et al., 2003; Lewis et al., 2005; Woo et al., 2008; Chiang et al., 2012; Nakazawa et al., 2012). GABAergic systems are also critical in proper brain development and, therefore, a point of convergence and target in the study of schizophrenia (Wassef et al., 2003; Cellot and Cherubini, 2013; Schmidt and Mirnics, 2015). Modulation of GABA transmission in the brain is often monitored via glutamate decarboxylase isoform 67 kDa (GAD_{67}), the rate-limiting enzyme in

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GABA synthesis. Here, we examined the consequences of MIA via Poly I:C exposure on GAD_{67} mRNA expression at multiple postnatal time points of the developing rat brain.

2. Methods

2.1. Poly I:C treatment

Female Sprague Dawley rats from Harlan Laboratories (Indianapolis, IN), aged 3–5 months, and males produced within the animal facility were paired for breeding. Animals were housed under standard conditions with access to food and water ad libitum. The Poly I:C treatment protocol was performed as previously described (Bronson et al., 2011; Hemmerle et al., 2015). Briefly, on gestational day 14 pregnant dams were injected with Poly I:C (8 mg/kg i.p.; Sigma, St. Louis, MO) dissolved in saline or with saline vehicle (1 ml/kg). On postnatal day (P) 14, P30 and P60 male offspring were sacrificed and their brains were subsequently processed for in situ hybridization. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

2.2. In situ hybridization

Fresh-frozen brains (n = 6/condition) were serially sectioned (at 10-μm thickness) throughout the forebrain 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 GAD_{67} mRNA using a ^{35}S-labeled cDNA probe, as previously described (Seroogy and Herman, 1997; Hemmerle et al., 2012, 2015; Makinson et al., 2015). Briefly, slides were pretreated, dehydrated and delipidated prior to hybridization. The hybridization probe was prepared from a linearized cDNA plasmid using T3 RNA polymerase and labeled with ^{35}S-UTP (PerkinElmer, Boston, MA). The GAD_{67} plasmid (a generous gift from Dr. James Herman, University of Cincinnati) was contained in a Bluescript SK vector that consisted of 3086 bases (GenBank Gene ID: 24379). Sections were hybridized overnight, washed, treated with RNase, rinsed, air-dried, and exposed to BioMax MR film (Kodak, Rochester, NY) for 7 days. The films were developed with Kodak GPX developer and fixer.

2.3. Analysis

Analysis of the film autoradiograms was performed by taking densitometry measurements utilizing Scion Image software (NIH) as described previously (Numan et al., 2005; Hemmerle et al., 2012, 2015). At least six sections per area per animal were measured from the following regions: prefrontal cortex (PFC, including prelimbic, infralimbic and anterior cingulate cortex subdivisions), frontal, parietal and piriform cortices, striatum, hippocampus, and thalamus. Brain regions were selected for study based upon their participation in circuitry implicated in schizophrenia abnormalities modeled by MIA (Volk and Lewis, 2013). Boundaries of brain regions analyzed were determined using the Paxinos and Watson (2007) rat brain atlas. Background measurements were taken from an unlabeled region of each section and subtracted from each optical density (OD) value to give a corrected OD value. The experimental data are shown as a percentage of the control group. Graph Pad Prism was used to determine group differences via t-test and results were considered significant when $p < 0.05$.

3. Results

3.1. Increased GAD_{67} mRNA expression in adult MIA offspring

Hybridization for GAD_{67} mRNA was increased in multiple regions of adult (P60) MIA compared to control offspring, but not in young or adolescent offspring (P14 and P30, respectively). In the hippocampus,

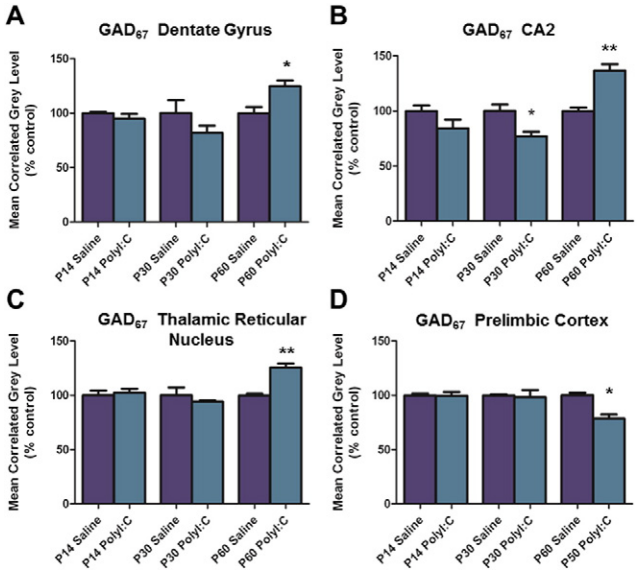

Fig. 1. A. Quantification of GAD_{67} mRNA hybridization signal demonstrates increased levels in the dentate gyrus granule cell layer of Poly I:C (maternal immune activation) animals at P60. B. Quantification of GAD_{67} mRNA labeling in the CA2 region of the hippocampus revealed decreased expression at P30 and, in contrast, increased expression at P60 in Poly I:C offspring. C. Measurement of GAD_{67} mRNA expression in the thalamic reticular nucleus revealed an increase in hybridization signal at P60. D. Decreased cRNA-labeling for GAD_{67} mRNA was found in the prelimbic region of the medial prefrontal cortex of Poly I:C-treated animals at P60. Data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to respective control values.

levels of GAD_{67} mRNA were elevated in the granule cell layer of the dentate gyrus (DG) ($t(4) = 3.169$, $p < 0.05$) and in region CA2 of the pyramidal cell layer ($t(4) = 5.546$, $p < 0.01$) (Figs. 1A–B, 2). A trend towards an increase in expression was detected in hippocampal region

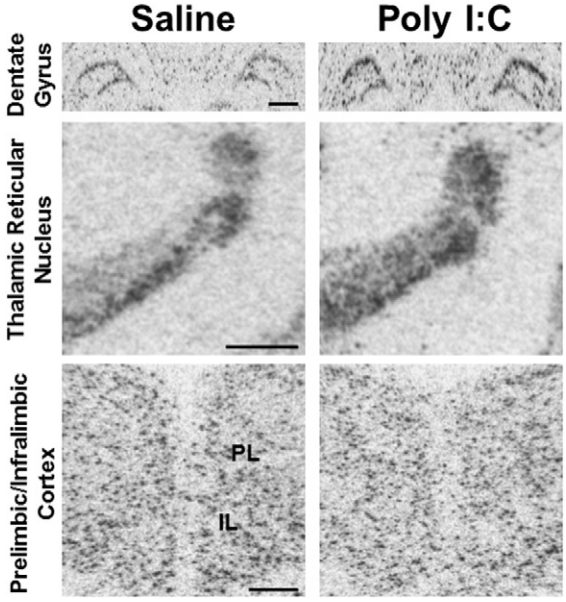

Fig. 2. Representative autoradiograms of GAD_{67} mRNA labeling for several forebrain regions in saline (control) versus Poly I:C (maternal immune activation) conditions at P60. Top row: cRNA-labeling of GAD_{67} mRNA is increased in the dentate gyrus granule cell layer in Poly I:C animals compared to saline controls. Middle row: increased hybridization signal for GAD67 mRNA is observed in the thalamic reticular nucleus under Poly I:C conditions. Bottom row: decreased levels of GAD_{67} mRNA expression are present in the prelimbic (PL) region of the medial prefrontal cortex in Poly I:C animals. Note that levels in the infralimbic (IL) cortex also appear decreased, but this did not reach significance when quantified. Scale bar in top row and middle row = 1000 μm; scale bar in bottom row = 500 μm.