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Dopamine and serotonin levels following prenatal viral infection in mouse—Implications for psychiatric disorders such as schizophrenia and autism

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Abstract

Prenatal viral infection has been associated with neurodevelopmental disorders such as schizophrenia and autism. It has previously been demonstrated that viral infection causes deleterious effects on brain structure and function in mouse offspring following late first trimester (E9) and middle-late second trimester (E18) administration of influenza virus. Neurochemical analysis following infection on E18 using this model has revealed significantly altered levels of serotonin, 5-hydroxyindoleacetic acid, and taurine, but not dopamine. In order to monitor these different patterns of monoamine expression in exposed offspring in more detail and to see if there are changes in the dopamine system at another time point, pregnant C57BL6J mice were infected with a sublethal dose of human influenza virus or sham-infected using vehicle solution on E16. Male offspring of the infected mice were collected at P0, P14, and P56, their brains removed and cerebellum dissected and flash frozen. Dopamine and serotonin levels were then measured using HPLC-ED technique. When compared to controls, there was a significant decrease in serotonin levels in the cerebella of offspring of virally exposed mice at P14. No differences in levels of dopamine were observed in exposed and control mice, although there was a significant decrease in dopamine at P14 and P56 when compared to P0. The present study shows that the serotonergic system is disrupted following prenatal viral infection, potentially modelling disruptions that occur in patients with schizophrenia and autism.

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1. Introduction

According to the neurodevelopmental hypothesis of schizophrenia, pathophysiologically relevant brain developmental genes are perturbed in the second trimester of pregnancy, leading to subsequent disturbances in brain maturation, limbic disorganization, neurochemical alterations, and dysfunction of the monaminergic system (Weinberger, 1995). The efficacy of dopamine D2 receptor blocking drugs in the treatment of schizophrenia, as well as SPECT studies on neuroleptic naïve patients, suggest that dopamine hyperfunction in the ventral striatum and dopamine hypofunction in the prefontal cortex may be responsible for the positive symptomology of schizophrenia (Abi-Dargham et al., 2000, 2002). Additionally, electrophysiological studies have suggested increased serotonergic function in schizophrenia (Juckel et al., 2003, 2008).

Recent serologic evidence points to prenatal exposures to a number of viruses as causative factors in the rise of births leading to schizophrenia (Mednick et al., 1988; Brown et al., 2004; Dalman et al., 2008) and autism (Barak et al., 1995; Singh et al., 1997; Connolly et al., 1999).

Furthermore, recent findings from *in vitro* and *in vivo* studies emphasize the influence of enhanced anti-inflammatory cytokine signaling on early brain development (Meyer et al., 2008). Several groups have shown evidence for viral infections and/or immune challenges being responsible for production of abnormal brain structure and function in rodents whose mothers were exposed to viral insults throughout pregnancy (Fatemi et al., 2005; Meyer et al., 2006a,b). Furthermore, the immune systems of patients with schizophrenic disorders show clear signs of over-activation, and anti-inflammatory treatment leads to improvement of schizophrenic symptomatology (Rothermundt et al., 2001). A promising animal model of the inflammatory genesis of schizophrenia is that of prenatal viral infection of mice (Fatemi et al., 1999, 2002; Shi et al., 2003). Offspring of female mice that were exposed to a mouse-adapted influenza virus in the middle of pregnancy display several changes in brain morphology, physiology, and behavior that are comparable to those of patients with schizophrenia (Fatemi et al., 2002, 2005, 2008).

In the current study, we examined post-mortem levels of dopamine and serotonin in the cerebella of exposed mouse progeny following maternal infection at E16. The cerebellum is one of the key structures in the early course of schizophrenia, since dysdiadochokinesia, a neurological sign specific to cerebellar dysfunction, often presents at the beginning of this disease (Boks et al., 2000). Furthermore, there is growing evidence that the cerebellum plays a role in higher cortical functions in schizophrenia (Andreasen and Pierson, 2008). While the neurochemistry is cerebella in unknown in patients with schizophrenia, previous experiments using this animal model have revealed altered levels of serotonin (5-HT), its metabolite 5-hydroxy indole acetic acid (5-HIAA), and taurine, but not dopamine in the progeny of mice exposed to virus on E18 (Fatemi et al., 2008). In order to monitor these different patterns of monoamine expression in exposed offspring in more detail and to see if there are changes in the dopamine and serotonin systems at E16, levels of monoamines were measured at three different postnatal days (P0, P14 and P56) using high performance liquid chromatography with electrochemical detection (HPLC-ED). Our results again suggest that the serotonergic, but not the dopaminergic, system is impacted by maternal prenatal infection.

2. Experimental procedures

2.1. Viral infection and brain collection and dissection

All experimental protocols used in this study were approved by the Institute for Animal Care and Use and Institutional Biosafety Committees at the University of Minnesota. Influenza A/NWS/33 (H1N1) virus was obtained from R.W. Cochran, University of Michigan (Ann Arbor). A virus pool was prepared in Maden Darby canine kidney (MDCK) cells; the virus was ampuled and frozen at −80 °C until used. Data were expressed as $\log_{10}$ cell culture infectious doses ($CCID_{50}$)/ml by the method of Reed and Muench (1938). By this titration, it was determined that at a dilution of $10^{-4.5}$, none of the mice died of the infection but displayed a mean lung consolidation scores and mean lung weights similar to those obtained by Fatemi et al. (2002) and had a mean virus titer of $10^{5.25}$ $CCID_{50}$/ml, indicating that a moderate but sublethal infection had been induced. This was the virus dose selected for use in the pregnant mouse study. On day 16 of pregnancy, C57BL6J mice (Charles River, Wilmington, MA) were anesthetized using 200 μl isoflurane, and intranasally (i.n.) administered a dilution of $10^{-4.5}$ of 6.5 log 10 ($CCID_{50}$) per 0.1 ml human influenza virus A/NWS/33 in 90 μl of minimum essential medium (MEM). Sham infected mothers were treated identically but administered i.n. 90 μl MEM. After being infected, their drinking water contained 0.006% oxytetracycline (Pfizer, New York, NY) to control possible bacterial infections. Pregnant mice were allowed to deliver pups. The day of delivery was considered day 0. Groups of infected and sham-infected neonates were deeply anesthetized and killed on postnatal day (P) P0, P14, and P56. Offspring were weaned from mothers at P21, and males and females were caged separately in groups of 2–4 littermates. Groups of infected ($N=3$) and sham-infected male neonates ($N=3$) were deeply anesthetized. Brains were snap-frozen by

Table 1 Concentrations of monoamines in offspring of infected vs. control mice

Neurotransmitter (μmol/g protein)	Controls	VEA
Postnatal day 0		
DA	13.4±0.2##	14.1±4.1#
5-HT	32.7±3.1	28.9±4.6
Postnatal day 14		
DA	6.0±1.1	4.8±0.3
5-HT	37.2±2.0	23.8±2.5*
Postnatal day 56		
DA	2.9±0.5	3.1±0.8
5-HT	34.8±4.7	29.4±4.6

DA, dopamine; 5-HT, serotonin; * $p<0.05$ (t-test); # $p<0.05$ and ## $p<0.01$ (one way ANOVA); VEA, virally exposed animals.