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Research Report

Adulthood olanzapine treatment fails to alleviate decreases of ChAT and BDNF RNA expression in rats quinpirole-primed as neonates

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ABSTRACT

Neonatal quinpirole (dopamine D_2/D_3 agonist) treatment to rats has been shown to increase dopamine D_2 receptor sensitivity throughout the animal's lifetime. Male and female Sprague–Dawley rats were neonatalally treated with quinpirole (1 mg/kg) from postnatal days (P) 1–21 and raised to adulthood. Beginning on P62, rats were administered the atypical antipsychotic olanzapine (2.5 mg/kg) twice daily for 28 days. Starting 1 day after the end of olanzapine treatment, animals were behaviorally tested on the place and match-to-place version of the Morris water maze (MWM) over seven consecutive days, and a yawning behavioral test was also performed to test for sensitivity of the D_2 receptor 1 day following MWM testing. Similar to results from a past study, olanzapine alleviated cognitive impairment on the MWM place version and increases in yawning produced by neonatal quinpirole treatment. Brain tissue analyses showed that neonatal quinpirole treatment resulted in a significant decrease of hipppocampal ChAT and BDNF RNA expression that were unaffected by adulthood olanzapine treatment, although adulthood olanzapine treatment produced a significant increase in cerebellar ChAT RNA expression. There were no significant effects of drug treatment on NGF RNA expression in any brain area. These results show that neonatal quinpirole treatment produced significant decreases of protein RNA expression that is specific to the hippocampus. Although olanzapine alleviated cognitive deficits produced by neonatal quinpirole treatment, it did not affect expression of proteins known to be important in cognitive performance.

1. Introduction

Previous work from this laboratory has shown that neonatal quinpirole treatment to rats given during the first 3 weeks of development results in long-term increases in sensitivity of the dopamine D_2-like receptor, a phenomenon called 'priming' (Brown et al., 2004a,b). Interestingly, this increase in dopamine D_2-like sensitivity is not accompanied by an increase in receptor proliferation (Kostrzewa et al., 1993, 2004; Kostrzewa and Brus, 1991; Kostrzewa, 1995). Increased activation of the dopamine D_2 receptor is relevant to number of clinical conditions, but is most commonly observed in schizophrenia (Crow et al., 1979;

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Castaneda et al., 1988, Davis et al., 1991; Kokkinidis and Anisman, 1980). We have recently demonstrated that neonatal quinpirole treatment results in a significant decrease of the genetic transcript for a regulator of G-protein signaling (RGS), *Rgs9*, in the striatum, nucleus accumbens, and frontal cortex (Maple et al., 2007). *Rgs9* is co-localized with D_2-like receptors, and significant decreases in *Rgs9* have been shown when dopaminergic activity is increased (for review, see Seeman et al., 2006). Additionally, significant decreases in *Rgs9* expression have also been shown in post-mortem analyses of the striatum in schizophrenics (Seeman et al., 2007).

Several past studies from our laboratory have shown that neonatal quinpirole treatment resulted in cognitive impairment in rats behaviorally tested on the Morris water maze (MWM) (Brown et al., 2002, 2004a,b) as well as significant decreases of the proteins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and choline acetyltransferase (ChAT) in the hippocampus, and decreased ChAT in the frontal cortex (Brown et al., 2004b; Thacker et al., 2006). Both NGF and BDNF are neurotrophic factors important in the development and maintenance of neurons and synaptic connectivity, and ChAT is the enzyme that catalyzes acetylcholine formation. All of these proteins have been shown to be important in cognition (Berger-Sweeney et al., 2001; Zou et al., 2002; Lukoyanov et al., 2003), and NGF and BDNF have been shown to be especially important in the maintenance of the septohippocampal pathway (Dekker et al., 1992; Van der Zee et al., 1995; Schaaf et al., 2001). Studies have shown decreases in hippocampal neurotrophic factors and cholinergic activity in disorders in which the D_2 receptor is increased in its activity (Durany et al., 2001; Toyooka et al., 2002), and cognitive deficits are common in disorders in which D_2 receptor activation is increased (Elvevag and Goldberg, 2000).

Olanzapine is a drug in the atypical antipsychotic class commonly prescribed in cases of psychosis that is primarily a D_2, 5-HT_{2a}, and 5-HT_{2c} receptor antagonist (Sanchez and Arnt, 2000; Horacek et al., 2006). We have recently shown that adulthood olanzapine treatment alleviated cognitive performance deficits on the MWM as well as significant decreases of hippocampal NGF, BDNF, and ChAT protein produced by ontogentic quinpirole treatment (Thacker et al., 2006). In the frontal cortex, the significant decrease in ChAT produced by neonatal quinpirole treatment was unaffected by adulthood olanzapine treatment. Therefore, it appears that D_2-like receptor priming produces effects on neurotrophins and the cholinergic system in the hippocampus and frontal cortex, and although olanazapine alleviates MWM performance deficits produced by D_2-like priming, its effects on NGF, BDNF, and ChAT appear to be relatively selective to the hippocampus.

The present study was primarily designed to investigate the effects of D_2 priming on RNA expression of NGF, BDNF, and ChAT in brain areas known to be involved in cognitive performance using the quantifiable PCR technique. Additionally, we investigated whether the atypical antipsychotic olanzapine, which has been prevalently used as a pharmacological treatment for schizophrenia, would alleviate changes in RNA expression produced by neonatal quinpirole treatment. Finally, cognitive performance was assessed by behaviorally testing rats on two versions of the MWM using the identical behavioral method and design as Thacker et al. (2006). There has been relatively little information as to the effects of dopamine D_2-like receptor activation on RNA expression of NGF, BDNF or ChAT, and although acute or chronic quinpirole has been shown to modulate the cholinergic system (Day and Fibiger, 1994; Nava et al., 2000; Pisani et al., 2000), there is no information on the effects of D_2 activation on genetic expression of ChAT or acetylcholine. Olanzapine has been shown to increase BDNF mRNA as analyzed through *in situ* hybridization in the CA1, CA3, and dentate gyrus regions of rats (Bai et al., 2003) and has been shown to protect PC12 cells against oxidative stress, suggesting it has neuroprotective potential (Wei et al., 2003). Therefore, we hypothesized that olanzapine may alleviate decreases in RNA expression produced by increases in sensitivity of the dopamine D_2 receptor. There is no information regarding olanzapine treatment and neurotrophic factor gene expression utilizing the quantifiable PCR technique, a more accurate and comprehensive analysis of RNA within the cell.

2. Results

2.1. *Swim speed*

To verify that neonatal nor adulthood drug treatment did not affect overall motor performance on the MWM, which could confound the results, swim speeds were analyzed on acquisition of the place and match-to-place versions as well as the probe trial given after the place version of the task. There were no significant effects of sex, neonatal, or adulthood drug condition, nor any significant interactions involving these variables. Thus, neither neonatal quinpirole nor adulthood olanzapine treatment produced any significant change in swim speed.

2.2. *Acquisition latency*

Acquisition latency is presented in Fig. 1(A). First, a 2×2×2 three-way ANOVA did not reveal a significant main effect or interaction involving sex, so this was factor was dropped to simplify the analysis. A 2×2 two-way ANOVA revealed only a significant main effect of trial block $F(5,41)=82.7$, $p<.001$, indicating a significant change over trial blocks occurred across all drug conditions. However, neonatal nor adulthood drug treatment produced any significant effects. This is in contrast to our recent study that showed adulthood olanzapine produced a slight but significant deficit in females on trial block 6 (Thacker et al., 2006) that was not replicated in this study, demonstrating that the acquisition latency deficit produced by olanzapine in this past study was not consistent in females.

2.3. *Probe trial measures*

MSD scores are presented as function of drug treatment in Fig. 1(B) and MZD scores are presented as a function of drug treatment in Fig. 1(C). As with the acquisition latency analysis, a 2×2×2 three-way ANOVA did not reveal a significant main effect or interaction involving sex, so this factor was dropped to simplify the analyses of these measures. For the MSD score, A 2×2 two-way ANOVA revealed a significant neonatal drug treatment main effect $F(1,45)=5.38$, $p<.02$ and a significant two-way interaction of Neonatal Drug Treatment × Adulthood Drug Treatment $F(1,45)=6.92$, $p<.01$. For the MZD score, presented in Fig. 1(C), a