Atypical antipsychotics clozapine and quetiapine attenuate prepulse inhibition deficits in dopamine transporter knockout mice

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Abstract

Sensorimotor gating disruptions are seen in various psychiatric illnesses with putatively different pathologies, including schizophrenia and bipolar disorder. Interestingly, mice lacking the dopamine (DA) transporter (DAT) gene display markedly increased levels of DA, deficits in sensorimotor gating, and hyperactivity relative to wild-type mice. Atypical antipsychotics are effective treatments of schizophrenia and manic symptoms, presumably in part by antagonizing DA receptors. Here we report that treatment with clozapine (3 mg/kg) or quetiapine (2.5 mg/kg) attenuated prepulse inhibition deficits in male DAT knockout mice. Thus male DAT knockout mice may provide a useful animal model for predicting the efficacy of novel drugs in treating psychiatric illnesses characterized by a dysregulated DA system.

Keywords

antipsychotics; bipolar disorder; clozapine; dopamine transporter; knockout; mouse; prepulse inhibition; quetiapine; schizophrenia

Introduction

Hyperdopaminergia has been implicated in numerous neuropsychiatric disorders such as schizophrenia and bipolar disorder. Dopaminergic homeostasis is maintained by the dopamine (DA) transporter (DAT), a Na+/Cl− dependent transmembrane transporter containing 12 putative transmembrane domains (Volz and Schenk, 2005), which functions to take up released DA from the synaptic cleft (Zhuang et al., 2001). DAT abnormalities have been implicated in schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder (Cook et al., 1995; Inada et al., 1996; Kelsoe et al., 1996; Fujiwara et al., 1997; Greenwood et al., 2001; Friedel et al., 2007).

DAT knockout (KO) mice lack the gene coding for the DAT and exhibit chronic hyperdopaminergia compared with wild-type (WT) mice (Giros et al., 1996). Despite compensatory changes in DA release and receptor expression, DAT KO mice are dramatically hyperactive in a novel environment (Giros et al., 1996; Gainetdinov et al., 1999; Spielewoy et al., 2001), have impairments in spatial cognitive function (Weiss et al., 2007a), and display
deficits in prepulse inhibition (PPI) of startle (Ralph et al., 2001; Barr et al., 2004; Yamashita et al., 2006).

PPI is a form of startle plasticity in which presentation of a weak stimulus (prepulse) preceding an intense startling stimulus (pulse) by 30–500 ms reduces the startle response (Graham et al., 1975). Deficits in PPI have been reported repeatedly in patients with schizophrenia (Brann et al., 2001), as well as in patients with bipolar disorder during the manic phase (Perry et al., 2001), and in a PPI attendance task in attention deficit hyperactivity disorder sufferers (Hawk et al., 2003). PPI has also shown good predictive validity as a screen for antipsychotic drugs (Swerdlow et al., 1994; Geyer et al., 2001).

Second-generation atypical antipsychotic drugs are the main line of treatment for both schizophrenia and bipolar disorder. In fact, 77–89% of bipolar patients are on antipsychotics at time of discharge (Yatham, 2003). Atypical antipsychotics may be more effective at normalizing PPI deficits in patients with schizophrenia than are typical antipsychotics (Kumari et al., 1999). Although both DA D2 and 5-HT2A antagonists reverse the PPI deficit in DAT KO mice (Ralph et al., 2001; Barr et al., 2004), there have been no reports as to whether atypical antipsychotic drugs reverse the deficient PPI phenotype in DAT KO mice. To further assess the validity of this model to screen putative antipsychotic medications, we examined whether or not two clinically effective atypical antipsychotic drugs, clozapine and quetiapine, reverse PPI deficits in DAT KO mice.

Methods

Subjects

The DAT mutant mice used in these experiments were derived from a breeding colony from parental DAT (+/−) mice on a C57BL/6 × 129SvJ hybrid background, originally received from Duke University (Giros et al., 1996). Mice from each strain were group housed (n = 4/cage) in a climate-controlled animal colony with a 12-h reversed day/night cycle (lights on at 20.00 h). Food and water were freely available, except during behavioral testing. Animal facilities were AAALAC-approved, and protocols were in accordance with the ‘Guiding Principles in the Care and Use of Animals’ from the American Physiological Society and guidelines from the National Institutes of Health.

Apparatus and procedure

Startle and PPI testing were performed in SR-LAB startle chambers (San Diego Instruments, San Diego, California, USA), using an experimental session [background noise level (65 dB), prepulse trials (69, 73, and 81 dB), pulse alone trials (120 dB), etc.] that has been specified previously (Barr et al., 2004). Mice (3–6 months old) were first tested in a characterization session to confirm PPI deficits (data not shown) in DAT KO mice reported earlier by our group (Ralph et al., 2001). Four weeks later, mice were tested with clozapine in a within-subjects crossover design, with 1 week between drug treatments. Following a 5-week washout period, the same mice were retested with quetiapine or vehicle in a similar crossover design. Mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging 65 one-ms readings taken from the onset of the startle P120 stimulus.

Drugs

Clozapine (3.0 mg/kg, intraperitoneally; Tocris, Ellisville, Missouri, USA) was dissolved in tartaric acid (5% volume) and 0.9% saline and brought to a pH of about 5–6 with 0.1N NaOH. Quetiapine (2.5 mg/kg; subcutaneously; gift from Astra Zeneca, Wilmington, Delaware, USA) was dissolved in a small amount of 0.1N HCl and distilled water and brought to a pH of about 5–6 with 0.1N NaOH. All injections were given 20 min before behavioral testing at a volume
of 5-ml/kg body weight. The doses of antipsychotics chosen were based on preliminary dose–response studies in vendor-supplied C57BL/6 and 129SvEv mice.

**Statistical analysis**

Startle reactivity, mean startle magnitudes within the test session, and percentage of PPI were each analyzed using two-factor analyses of variance (ANOVAs) with drug as a within-subjects factor and genotype as between-subjects factor.

**Results**

In the clozapine experiment, there was a significant overall interaction between genotype and clozapine treatment on PPI \[F(1,34) = 5.59, P < 0.05\] as well as a significant main effect of genotype \[F(1,34) = 9.13, P < 0.01\]; Fig. 1a]. Similarly, in the quetiapine study there was a significant overall quetiapine × genotype interaction on PPI \[F(1,33) = 8.23, P < 0.01\] as well as a significant main effect of genotype \[F(1,33) = 21.38, P < 0.001\]; Fig. 1b]. On the basis of an inconsistent PPI deficit in female DAT KO mice (data not shown), only data from male mice are presented and included in subsequent statistical analyses.

Separate post-hoc ANOVAs were conducted in male vehicle-treated and clozapine-treated mice to determine the nature of the genotype × drug interaction. Male KO mice administered vehicle had significantly lower PPI levels compared with their WT counterparts administered vehicle [genotype; \(F(1,14) = 9.89, P < 0.01\); Fig. 1a]. The percentage of PPI of the DAT KO mice administered clozapine, however, did not differ significantly from that of the DAT WT mice administered clozapine, suggesting that clozapine attenuated the PPI deficit in male DAT KO mice. Pairwise ANOVAs revealed that clozapine did not increase PPI significantly in either DAT KO or DAT WT mice.

Separate post-hoc ANOVAs conducted in male vehicle-treated and quetiapine-treated mice revealed that male KO mice administered vehicle had significantly lower PPI levels compared with WT littermates administered vehicle [genotype; \(F(1,13) = 23.11, P < 0.001\); Fig. 1b]. In contrast, the percentage of PPI of male DAT KO mice administered quetiapine did not differ significantly from that of WT mice administered quetiapine, suggesting that quetiapine restored PPI to normal levels in DAT KO mice. Pairwise ANOVAs revealed that in DAT KO mice, quetiapine increased PPI [drug; \(F(1,5) = 10.49, P < 0.025\)]; whereas, in DAT WT mice, quetiapine had no effect on PPI.

No significant main effect of genotype on startle magnitude in either the clozapine or quetiapine experiments (Table 1) was observed. Clozapine decreased startle magnitude [main effect of drug; \(F(1,14) = 10.09, P < 0.01\)] and did not interact with genotype. A significant interaction, however, between quetiapine and genotype \[F(1,13) = 7.37, P < 0.025\] on startle magnitude was observed. Pairwise ANOVA revealed that quetiapine decreased startle magnitude in DAT WT mice \[F(1,8) = 5.70, P < 0.05\].

**Discussion**

In these studies, DAT KO mice displayed PPI deficits, corroborating previous findings from our group (Ralph et al., 2001; Barr et al., 2004) and others (Yamashita et al., 2006). The present data demonstrate that the PPI deficits in male DAT KO mice were attenuated by acute administration of the atypical antipsychotic drugs clozapine and quetiapine. Thus, greater predictive validity is afforded to the use of these mice as a model for the prediction of antipsychotic drugs that are effective in treating schizophrenia and bipolar disorder.
Despite compensatory changes in neurotransmission in these mice (Trinh et al., 2003; Weiss et al., 2007b), the PPI deficits observed are likely to be a consequence of their lack of DATs (Giros et al., 1996). Pharmacological blockade of the DAT with the selective DAT inhibitor GBR-12909 produces similar deficits in PPI in mice (Young et al., unpublished observations). Pharmacological or genetically reduced function of the DAT results in a hyperdopaminergic state (Giros et al., 1996), and thus DA receptors are activated in these mice at a greater rate than in WT mice (Trinh et al., 2003). Both clozapine and quetiapine exhibit high affinities for the serotonin 5-HT$_{2A}$ receptor as well as the DA D$_2$ receptor (Schotte et al., 1996; Richelson and Souder, 2000). Consistent with these studies, both selective D$_2$ receptor and 5-HT$_{2A}$ receptor antagonists have been shown to attenuate PPI deficits in DAT KO mice (Ralph et al., 2001; Barr et al., 2004). Antipsychotics are not the only drug class that has been shown to reverse or attenuate PPI deficits in DAT KO mice, however. For example, the norepinephrine transporter inhibitor nisoxetine, the serotonin transporter inhibitor fluoxetine, the monoamine transporter inhibitor methylphenidate, and cocaine all reversed PPI deficits in DAT KO mice, whereas citalopram did not (Yamashita et al., 2006). Hence, general monoamine transporter inhibition seems to attenuate PPI deficits in DAT KO mice, and as such this model may produce false positives when using it as a screen for antipsychotic medications.

In summary, we describe the reversal of PPI deficits in male DAT KO mice by the atypical antipsychotics clozapine and quetiapine. Quetiapine was more effective than clozapine at reversing the PPI deficit in DAT KO mice. These studies support the use of DAT KO mice as a model for assessing the efficacy of putative antipsychotics.

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**References**


Fig. 1.
(a) Effect of clozapine (3.0 mg/kg, intraperitoneally) on average prepulse inhibition (PPI) in male dopamine transporter (DAT) wild-type (WT) and knockout (KO) mice. Vehicle-treated DAT KO mice (n=7) showed PPI deficits compared with vehicle-treated DAT WT mice (n=9), **P<0.01. PPI deficits in DAT KO mice were attenuated by clozapine treatment, as indicated by a lack of a significant genotype effect in clozapine-treated mice. (b) Effect of quetiapine (2.5 mg/kg, subcutaneously) on average PPI in male DAT WT and KO mice. Vehicle-treated DAT KO mice (n=6) showed PPI deficits compared with vehicle-treated DAT WT mice (n=9), **P<0.01. PPI deficits in DAT KO mice were attenuated by quetiapine treatment, as indicated by a lack of a significant effect of genotype in quetiapine-treated mice.
and a significant effect of quetiapine in DAT KO mice, \( P < 0.05 \). Data were expressed as mean ± SEM.
Table 1
Mean (SEM) startle magnitude in DAT WT and KO mice treated with vehicle and clozapine or quetiapine

<table>
<thead>
<tr>
<th>Startle magnitude</th>
<th>DAT WT</th>
<th>DAT KO</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>81.2 (28.1)</td>
<td>147.4 (58.4)</td>
</tr>
<tr>
<td>Clozapine *</td>
<td>41.0 (12.5)</td>
<td>66.1 (20.3)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>111.9 (31.4)</td>
<td>91.4 (21.9)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>61.0 (26.4)*</td>
<td>134.3 (48.9)</td>
</tr>
</tbody>
</table>

DAT KO mice, dopamine transporter knockout mice; DAT WT mice, dopamine transporter wild-type mice

* P < 0.05 vs. vehicle.