Chronic antipsychotic drug administration alters the expression of neuregulin 1β, ErbB2, ErbB3, and ErbB4 in the rat prefrontal cortex and hippocampus

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Abstract
Neuregulin 1 (NRG1) has been identified as a susceptibility gene for schizophrenia, and dysregulation of NRG1 and its ErbB receptors is implicated in the pathophysiology of the disorder. The present study examined the protein expression levels of NRG1β, ErbB2, ErbB3, and ErbB4 in the rat prefrontal cortex and hippocampus following a 4-wk administration of haloperidol (1 mg/kg i.p.), clozapine (10 mg/kg i.p.), or risperidone (1 mg/kg i.p.) by using immunohistochemistry and Western blot. The results showed that haloperidol promoted the expression of NRG1β and ErbB4, whereas clozapine inhibited NRG1β expression in the rat prefrontal cortex. Both haloperidol and clozapine significantly increased the protein levels of NRG1β and ErbB receptors in the rat hippocampus. Repeated administration of risperidone only increased the expression of NRG1β and ErbB4 in the hippocampus. Our findings demonstrate that antipsychotic drugs differentially regulate the expression of NRG1 and ErbB receptors in the rat brain, which may provide insight into the molecular basis of the pharmacological profile of antipsychotic drugs.

Key words: Antipsychotic drugs, ErbB, hippocampus, neuregulin 1, prefrontal cortex.

Introduction
Neuregulin 1 (NRG1) is a family of soluble or membrane anchored proteins containing an epidermal growth factor (EGF)-like domain that activates members of ErbB receptor tyrosine kinases, including ErbB2, ErbB3, and ErbB4. NRG1-ErbB signalling serves multiple roles in the development, maintenance, and plasticity of the central nervous system (Buonanno and Fischbach, 2001; Falls, 2003). Recent molecular genetics studies point to NRG1 as a schizophrenia susceptibility gene (see Harrison and Law, 2006), and one of the NRG1 variants is found to be associated with impaired cortical function, psychotic symptoms, and cognitive deficits (Hall et al., 2006). Mutant mice heterozygous for different NRG1 isoforms and ErbB4 have been reported to display behavioural abnormalities that resemble the mouse models for schizophrenia (Gerlai et al., 2000; Rimer et al., 2005; Stefansson et al., 2002). Moreover, clinical findings from post-mortem brains of schizophrenia subjects reveal abnormal expression of NRG1, ErbB3 and ErbB4, although the results are relatively inconsistent (Bertram et al., 2007; Hahn et al., 2006; Hakak et al., 2001; Hashimoto et al., 2004; Law et al., 2006; Silberberg et al., 2006).

Recent findings have shown that chronic haloperidol treatment significantly reduces NRG1-mediated ErbB4 activation in mice (Hahn et al., 2006) and that clozapine can suppress the schizophrenia-like behaviour of NRG1 and ErbB4 mutant mice (Rimer et al., 2005; Stefansson et al., 2002). It is possible that the protein expression levels of NRG1 and ErbB receptors are also modified by chronic antipsychotic drug treatment, and these alterations might contribute to the pharmacological action of antipsychotic drugs.

In this study, we utilized an immunohistochemical approach and Western blot to evaluate the effect of chronic antipsychotic drug treatment on the
expression of NRG1β, ErbB2, ErbB3, and ErbB4 proteins in rat brain regions closely related to the pathophysiology of schizophrenia. NRG1 isoforms with a β-type EGF-like domain were exclusively selected because they are 10–100 times more potent than isoforms with an α-type EGF-like domain, which are predominantly involved in breast development (Falls, 2003).

Materials and methods

Animals

Adult male Wistar rats (Vital River, Beijing, China) weighing 185–225 g were individually housed under a 12 h light/dark cycle (lights on at 08:00 hours) with ad libitum access to both food and water. All procedures were performed in accordance with the National Institute of Health’s Guide for the Use and Care of Laboratory Animals and were approved by the Peking University Committee on Animal Care and Use.

Drug treatment

Haloperidol (Ortho-McNeil Pharmaceutical Inc., Titusville, NJ, USA), clozapine (Huanghe Pharmaceutical Inc., Shanghai, China), and risperidone (Janssen Research Foundation, Beerse, Belgium) were acidified by several drops of acetic acid and dissolved with normal saline. After a 1-wk acclimatization, the rats were randomly divided into four groups, and each rat received intraperitoneal injections of either haloperidol (1 mg/kg, n = 9), clozapine (10 mg/kg, n = 9), risperidone (1 mg/kg, n = 9), or normal saline (vehicle, n = 9) for 4 wk. The dosages of the antipsychotic drugs used in this study were chosen on the basis of ex-vivo dopamine D2 and serotonin 5-HT1 receptor occupancies in the rat brain (Schotte et al., 1993) and other previous studies (Bai et al., 2003; Chen and Chen, 2005). All rats received single, daily injections between 09:00 and 12:00 hours. The rats were sacrificed 24 h after the last injection.

Immunohistochemistry

The rats (n = 4 per group) were deeply anaesthetized with chloral hydrate (300 mg/kg) and transcardially perfused with 250 ml cool saline solution followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Brains were immediately removed, post-fixed in the same fixative overnight and cryoprotected in 30% sucrose at 4 °C for 5 d. After quenching by immersing them in cold N-hexane on dry ice, the brains were stored at −20 °C until required.

Serial coronal sections through the prefrontal cortex (bregma 3.72–2.52) and hippocampus (bregma −2.76 to −3.96) (Paxinos and Watson, 2005) were cut at 20 μm on a cryostat (Leica, Wetzlar, Germany) and slide-mounted. After blocking in 10% normal goat serum in 0.1 M PBS containing 0.3% Triton X-100 for 1 h at room temperature (RT), sections were incubated with NRG1β, ErbB2, ErbB3 or ErbB4 specific antibodies (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) diluted 1:100 in 0.1 M PBS overnight at 4 °C. The next morning, sections were incubated with primary antibodies for an additional 1 h at RT. Following PBS rinses, sections were incubated with biotinylated secondary antibodies (Santa Cruz Biotechnology Inc.) for 3 h at RT, again rinsed, and then incubated with avidin-biotin-horseradish peroxidase (Santa Cruz Biotechnology Inc.) for 3 h at RT. Finally, the sections were developed with a solution of 50% 3,3′-diaminobenzidine (DAB) and 3% hydrogen peroxide in 0.1 M PBS, dehydrated, and coverslipped.

Immunoreactive staining was observed and photographed at ×40 magnification using an Olympus BX51 microscope fitted with a CoolSNAP MP5 CCD camera (Roper Scientific, Tucson, AZ, USA). Images were converted to grey scale and analysed with Image Pro-Plus software (Media Cybernetics, Silver Spring, MD, USA). Immunoreactivity of the prefrontal cortex was calculated using an approach modified from previous studies (Kodama et al., 2004; Mura et al., 2004). A 1×1 mm² cursor was placed on the prelimbic cortex (PrL) or the cingulate cortex area 1 (Cg1) plus secondary motor cortex (M2) (Paxinos and Watson, 2005). Constant background intensity across different sections from each animal was set so that positive-labelled cells were selected only if they reached a defined threshold above background, then the integrated optical density (IOD = immunoreactive area × average optical density) values of PrL and Cg1 plus M2 were measured and designated as optical density (OD) (Figure 1a). For densitometry of staining in the hippocampus, the optical densities of CA1, CA3, dentate gyrus, and corpus callosum were measured according to a previous protocol (Li et al., 2002). The differences of value between the target and background (corpus callosum) were calculated and designated as OD (Figure 2a). All results were normalized by taking the value of the vehicle group as 100%.

Western blot

Following treatment, the rats (n = 5 per group) were sacrificed, decapitated, and the brains rapidly
removed and dissected to obtain the prefrontal cortex and hippocampus, as previously described (Gearhart et al., 2006). Tissue from individual rats was immediately homogenized on ice in ice-cold lysis buffer [137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP-40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate], sonicated, and centrifuged. The supernatants were stored at −80°C until required.

Protein concentrations were determined spectrophotometrically at A_{280} nm using the BCA protein assay kit (Pierce, Rockford, IL, USA). Samples containing 20–40 μg of protein were resolved by 7.5% (for detection of ErbB receptors) or 10% (for NRG1β isoforms) SDS–PAGE gels, and then transferred electrophoretically to a polyvinylidene difluoride (PVDF)
membrane (Millipore, Bedford, MA, USA). To detect the proteins of interest, PVDF membranes were blocked for 1 h at RT in Tris-buffered saline-Tween (TBST) [150 mM NaCl, 10 mM Tris–HCl (pH 7.5), and 0.1% Tween] containing 5% non-fat milk, and incubated overnight at 4°C in primary antibodies (NRG1, 1:1000; ErbB2, 1:750; ErbB3, 1:750; ErbB4, 1:1000) diluted in TBST containing 1% non-fat milk. The next day, membranes were rinsed three times with TBST for 8 min each and incubated for 2 h at RT with horseradish peroxidase-conjugated goat anti-rabbit (1:2000, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) or anti-mouse (1:2000, Sigma-Aldrich, St Louis, MO, USA) secondary antibodies diluted in TBST containing 1% non-fat milk. After another three TBST rinses, proteins were visualized using an ECL system (Pierce, Rockford, IL, USA) and Kodak XBT-1 film. The membranes were then stripped and reprobed with rabbit anti-actin polyclonal antibody (Santa Cruz Biotechnology Inc.) and reacted with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories Inc.). The immunoreactive signals were quantified by densitometry and the values corrected based on their corresponding actin levels. All results were normalized by taking the value of the vehicle group as 100%. At least three independent experiments were performed.

Antibody specificity

The specificity of antibodies was examined by Western blot analysis. NRG1β was identified as five main bands of approximately 35, 65, 80, 85, and 95 kDa representing the β-isofoms of NRG1. ErbB4 was detected as a 60 kDa fragment possibly representing the ErbB4 transmembrane and cytoplasmic domains, a 120 kDa fragment representing the ErbB4 shed ectodomain, and a 185 kDa intact molecule (Carpenter, 2003). The signal of the 65 kDa NRG1β-isofom was reduced and labelling of the other NRG1β-isofoms and ErbB4 isofoms eliminated by preabsorption with corresponding antigen peptides (Santa Cruz Biotechnology Inc.). Antibodies against ErbB2, ErbB3 or actin generated one main band.

Statistical analysis

All data were expressed as means ± S.E. Statistical differences between groups were determined by one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test. Differences of \( p < 0.05 \) were considered to be significant.

Results

Effect of chronic antipsychotic drug administration on NRG1β, ErbB2, ErbB3, and ErbB4 expression in the prefrontal cortex

NRG1, ErbB2, ErbB3, and ErbB4 have a widespread expression throughout the adult rat cerebral cortex (Corfas et al., 1995; Gerecke et al., 2001). Immunostaining of NRG1β (Figure 1) and ErbB receptors (not shown) was associated with most neurons in the prefrontal cortex. Following 4 wk administration, 1 mg/kg haloperidol significantly increased the immunoreactivity of NRG1β in the Cg1 and M2 regions.

Table 1. Immunoreactivity of NRG1β, ErbB2, ErbB3, and ErbB4 in the rat prefrontal cortex following chronic antipsychotic drug treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NRG1β</th>
<th>ErbB2</th>
<th>ErbB3</th>
<th>ErbB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cg1 and M2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>100 ± 6.60</td>
<td>100 ± 2.94</td>
<td>100 ± 2.88</td>
<td>100 ± 4.15</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>130.00 ± 7.83*</td>
<td>87.72 ± 3.65</td>
<td>84.58 ± 3.65</td>
<td>115.29 ± 4.76*</td>
</tr>
<tr>
<td>Clozapine</td>
<td>81.61 ± 3.56</td>
<td>92.50 ± 1.95</td>
<td>86.20 ± 4.57</td>
<td>97.22 ± 2.03*</td>
</tr>
<tr>
<td>Risperidone</td>
<td>77.02 ± 4.75</td>
<td>94.79 ± 2.77</td>
<td>95.73 ± 3.30</td>
<td>91.72 ± 2.76</td>
</tr>
<tr>
<td>Prelimbic cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>100 ± 5.64</td>
<td>100 ± 2.40</td>
<td>100 ± 3.43</td>
<td>100 ± 5.53</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>106.85 ± 5.12</td>
<td>93.86 ± 3.54</td>
<td>89.97 ± 5.87</td>
<td>117.63 ± 3.51*</td>
</tr>
<tr>
<td>Clozapine</td>
<td>65.05 ± 4.01**</td>
<td>91.45 ± 1.50</td>
<td>84.50 ± 3.20</td>
<td>96.27 ± 5.01</td>
</tr>
<tr>
<td>Risperidone</td>
<td>82.09 ± 1.10</td>
<td>90.28 ± 2.21</td>
<td>93.81 ± 2.76</td>
<td>89.69 ± 1.17</td>
</tr>
</tbody>
</table>

Cg1, Cingulate cortex area 1; M2, secondary motor cortex.

\* \( p < 0.05 \) compared to vehicle group; \** \( p < 0.01 \) compared to vehicle group.
and of ErbB4 in PrL, Cg1 and M2 regions. In contrast, 10 mg/kg clozapine decreased NRG1β immunoreactivity in the PrL region, while risperidone had no effect on the expression of NRG1β in the prefrontal cortex. Moreover, immunoreactivity of ErbB2 and ErbB3 was not significantly different between the control and treatment groups (Table 1).

To determine whether antipsychotic drugs change the relative quantity of NRG1β isoforms and ErbB receptors, rat prefrontal cortex extracts were analysed by immunoblot. Levels of certain NRG1β isoforms were significantly altered between the haloperidol or clozapine treatment groups and the vehicle group. Haloperidol induced a significant increase in the levels of the 65 kDa and 80 kDa NRG1β isoforms (124% and 133%, respectively), while clozapine downregulated the level of the 65 kDa NRG1β isoform (74%). Consistent with the findings of immunohistochemistry, risperidone did not affect the expression of NRG1β isoforms (Figure 3a, b). None of the drugs tested had an appreciable effect on the expression of ErbB receptors in the prefrontal cortex (Figure 3c–f).

Figure 3. Expression of NRG1β, ErbB2, ErbB3, and ErbB4 proteins in the rat prefrontal cortex following chronic antipsychotic drug treatment. (a, c, e) Representative Western blots of (a) NRG1β isoforms, (c) ErbB2 and ErbB3, and (e) ErbB4 proteins in the prefrontal cortex after rats received a 4-wk treatment of either vehicle (Veh; normal saline), haloperidol (Hal; 1 mg/kg), clozapine (Clo; 10 mg/kg), or risperidone (Ris; 1 mg/kg). (b, d, f) Quantitative analysis of (b) NRG1β isoforms, (d) ErbB2 and ErbB3, and (f) ErbB4 immunoblots. The data were normalized by taking the value of vehicle group as 100% and expressed as means ± s.e. (n = 5). *p < 0.05 compared to vehicle group.

Chronic antipsychotic drug administration increases NRG1β, ErbB2, ErbB3, and ErbB4 protein levels in the hippocampus

Neurons stained by immunohistochemistry showed moderate labelling of NRG1β in CA1 and dense
labelling of NRG1β in CA3 and dentate gyrus of the hippocampus. Chronic administration of haloperidol (1 mg/kg), clozapine (10 mg/kg), or risperidone (1 mg/kg) significantly increased NRG1β immunoreactivity in the pyramidal cell layer of CA3 (Figure 2). Clozapine also increased NRG1β staining in the granule cell layer of the dentate gyrus (Table 2).

ErbB2, ErbB3 and ErbB4 are differentially expressed in the hippocampus of the adult rat brain (Gerecke et al., 2001). Similar to the results for NRG1β, chronic treatment of antipsychotic drugs mainly increased the expression of ErbB receptors in the CA3 subregion (not shown). After repeated administration, haloperidol and clozapine caused a significant increase in the immunoreactivity of all three ErbB receptors in CA3, whereas risperidone only increased the expression of ErbB4 in the CA3 subregion (Table 2).

Consistent with immunohistochemical results, immunoblot analysis revealed that all three drugs significantly increased the expression levels of NRG1β isoforms in the hippocampus (Figure 4a, b). Haloperidol and clozapine had a more potent effect on the levels of NRG1β isoforms than risperidone, and both drugs significantly increased the protein levels of ErbB2, ErbB3 (Figure 4c, d), and the 60 kDa fragment of ErbB4 (Figure 4e, f). Compared with the robust influence of haloperidol and clozapine on the expression of ErbB receptors, risperidone did not up-regulate the protein levels of ErbB receptors except for the 120 kDa fragment of ErbB4 (Figure 4e, f). Taken together, these results suggest that antipsychotic drugs regulate the expression of NRG1β and ErbB receptors in a type of drug- and region-specific manner.

**Discussion**

In the present study, we demonstrate that NRG1β expression in the rat prefrontal cortex is increased by chronic administration of haloperidol (1 mg/kg) and decreased by clozapine (10 mg/kg); whereas haloperidol, clozapine, and risperidone (1 mg/kg) each up-regulate NRG1-ErbB expression in the rat hippocampus. This study is relevant to previous animal studies highlighting the functional importance of NRG1-ErbB. As revealed by mouse models with heterozygous deletion of the EGF-like domain, the transmembrane domain, or the immunoglobulin-like domain of NRG1 or ErbB receptors, impaired expression and function of NRG1 or ErbB4 results in behavioural abnormalities resembling the phenotype of schizophrenia (Gerlai et al., 2000; Rimer et al., 2005; Stefansson et al., 2002). Interestingly, the behavioural deficits of NRG1 hypomorphs can be partially reversed or suppressed by clozapine (Rimer et al., 2005; Stefansson et al., 2002). This reversal effect may be indirectly explained by our preliminary findings that clozapine induced a robust up-regulation of NRG1-ErbB in the hippocampus. Although it is unclear whether a single administration could alter NRG1-ErbB expression, clozapine might normalize

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**Table 2. Immunoreactivity of NRG1β, ErbB2, ErbB3, and ErbB4 in rat hippocampal subregions following chronic antipsychotic drug treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NRG1β</th>
<th>ErbB2</th>
<th>ErbB3</th>
<th>ErbB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>100 ± 3.39</td>
<td>100 ± 22.54</td>
<td>100 ± 10.39</td>
<td>100 ± 8.84</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>112.33 ± 7.82</td>
<td>108.63 ± 13.49</td>
<td>98.60 ± 10.23</td>
<td>129.54 ± 14.58</td>
</tr>
<tr>
<td>Clozapine</td>
<td>124.11 ± 7.23</td>
<td>106.80 ± 11.62</td>
<td>110.21 ± 14.41</td>
<td>113.25 ± 9.95</td>
</tr>
<tr>
<td>Risperidone</td>
<td>92.07 ± 4.87</td>
<td>81.63 ± 5.29</td>
<td>120.14 ± 9.32</td>
<td>114.28 ± 19.48</td>
</tr>
<tr>
<td>CA3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>100 ± 2.85</td>
<td>100 ± 6.14</td>
<td>100 ± 6.04</td>
<td>100 ± 5.29</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>129.18 ± 2.59**</td>
<td>129.68 ± 3.72*</td>
<td>133.23 ± 8.11*</td>
<td>133.45 ± 9.51*</td>
</tr>
<tr>
<td>Clozapine</td>
<td>129.82 ± 1.81**</td>
<td>151.60 ± 7.15**</td>
<td>159.83 ± 7.64**</td>
<td>141.99 ± 4.18*</td>
</tr>
<tr>
<td>Risperidone</td>
<td>117.51 ± 3.52**</td>
<td>119.09 ± 6.71</td>
<td>112.91 ± 7.29</td>
<td>136.71 ± 10.43*</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>100 ± 1.32</td>
<td>100 ± 15.58</td>
<td>100 ± 3.31</td>
<td>100 ± 3.64</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>110.99 ± 3.06</td>
<td>74.92 ± 10.61</td>
<td>112.85 ± 12.35</td>
<td>86.56 ± 9.18</td>
</tr>
<tr>
<td>Clozapine</td>
<td>128.56 ± 1.60**</td>
<td>84.93 ± 11.88</td>
<td>121.23 ± 14.16</td>
<td>102.36 ± 7.62</td>
</tr>
<tr>
<td>Risperidone</td>
<td>107.55 ± 8.26</td>
<td>70.92 ± 4.63</td>
<td>107.14 ± 8.45</td>
<td>99.58 ± 3.81</td>
</tr>
</tbody>
</table>

* $p < 0.05$ compared to vehicle group; ** $p < 0.01$ compared to vehicle group.
the decreased protein levels and function of NRG1 and ErbB in mutant mice.

Clinical studies have identified abnormal expression and function of NRG1-ErbB in post-mortem brain of schizophrenia patients (see Harrison and Law, 2006). In the prefrontal cortex of schizophrenia patients, the ErbB3 gene is found to be downregulated (Hakak et al., 2001; Tkachev et al., 2003), type I NRG1 mRNA is increased (Hashimoto et al., 2004), and ErbB4 isoforms containing the cytoplasmic tail (CYT)-1 domain are overexpressed (Silberberg et al., 2006). Protein levels of NRG1 and ErbB4 remain unchanged (Hahn et al., 2006); whereas, the NRG1α isoform is reduced (Bertram et al., 2007). In the hippocampus, the type I NRG1 mRNA is significantly increased in schizophrenia subjects (Law et al., 2006). These findings are inconsistent and even contradictory; however, a plausible explanation for this discrepancy is the potential difference in lifetime usage of antipsychotic drugs, such as type of drug or dose selected. One study has found a positive correlation between NRG1 mRNA expression level and the daily dose of medications (Hashimoto et al., 2004). As indicated in the present study, different drugs lead to different alterations of NRG1 and ErbB receptors, which could be responsible for these variable clinical findings.

Antipsychotic drugs can effectively alleviate the symptoms of schizophrenia, but they take several weeks to exhibit full therapeutic effects. It has been

Figure 4. Chronic antipsychotic drug treatment increases NRG1/β, ErbB2, ErbB3, and ErbB4 protein levels in rat hippocampus. (a, c, e) Representative Western blots of (a) NRG1/β isoforms, (c) ErbB2 and ErbB3, and (e) ErbB4 proteins in the hippocampus after rats received a 4-wk treatment of either vehicle (Veh; normal saline), haloperidol (Hal; 1 mg/kg), clozapine (Clo; 10 mg/kg), or risperidone (Ris; 1 mg/kg). (b, d, f) Quantitative analysis of (b) NRG1/β isoforms, (d) ErbB2 and ErbB3, and (f) ErbB4 immunoblots. The data were normalized by taking the value of vehicle group as 100% and expressed as means±S.E. (n=5). *p<0.05 compared to vehicle group; **p<0.01 compared to vehicle group.
suggested that, after repeated drug administration, molecular and cellular adaptations of the brain, such as alterations of genes involved in neurotransmission and synaptic plasticity (Chen and Chen, 2005; MacDonald et al., 2005) contribute to the therapeutic properties of antipsychotic drugs (Hyman and Nestler, 1996). Our finding that antipsychotic drugs regulate the expression of NRG1-ErbB in a type of drug- and region-specific manner adds a new line of evidence to the molecular adaptations induced by chronic antipsychotic drug treatment. Although its pathophysiological role in schizophrenia is poorly understood, perturbation of NRG1-ErbB signalling is implicated in the glutamatergic hypofunction and developmental abnormalities of schizophrenia (Hahn et al., 2006; Li et al., 2007). Antipsychotic drugs may normalize NRG1-ErbB signalling in schizophrenia by modulating NRG1-ErbB expression, which could be relevant to their clinical efficacy. Nevertheless, it should be noted that, because the half-life of antipsychotic drugs in rodents is much shorter than in humans, our treatment regimen does not lead to clinically comparable D₂ receptor occupancy in rats, which is unrepresentative of the clinical condition (Kapur et al., 2003). Thus, one should be cautious in extrapolating our results to clinical findings.

Our data reveal inconsistent alterations among the drugs tested and the brain regions selected, indicating that antipsychotic drugs differentially regulate the expression of NRG1-ErbB in both the prefrontal cortex and hippocampus. This difference may lie in the distinct D₂ and 5-HT₂ receptor occupancies of antipsychotic drugs. Haloperidol (1 mg/kg) occupies ~90% of D₂ receptors and 20–30% of 5-HT₂ receptors. At the dose selected, clozapine (10 mg/kg) and risperidone (1 mg/kg) have lower levels of D₂ receptor occupancy (50–60% for both), but higher levels of 5-HT₂ receptor occupancy (70–80% and >80%, respectively) (Schotte et al., 1993). It is possible that a higher affinity to D₂ receptor and a relatively lower affinity to 5-HT₂ receptor correlate with the up-regulation of NRG1-ErbB by antipsychotic drugs. This could partially explain the differential influences between typical and atypical antipsychotic drugs, although the discrepancies between clozapine and risperidone do not fit such a hypothesis. Since atypical antipsychotic drugs, particularly clozapine, have multiple sites of action other than D₂ and 5-HT₂ receptors, other neuroreceptors or molecular mechanisms might contribute to these differential effects. In addition, the prefrontal cortex and hippocampus showed different responses in NRG1-ErbB expression to antipsychotic drugs, which might reflect the features of the neural circuits in these two brain regions. Finally, protein levels of several NRG1β isoforms appear to be selectively altered, raising the possibility that antipsychotic drugs might specifically regulate the expression of certain NRG1 transcripts. Further studies are needed to characterize the potential changes in NRG1 mRNA transcription, splicing, translation or proteolytic cleavage of precursor proteins induced by antipsychotic drugs.

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Statement of Interest
None.

References


