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Effects of antipsychotic drugs on the expression of synapse-associated proteins in the frontal cortex of rats subjected to immobilization stress

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ABSTRACT

The present study examined the effects of antipsychotic drugs on the expression of synapse-associated proteins in the frontal cortex of rats with and without immobilization stress. Rats were subjected to immobilization stress 6 h/day for 3 weeks. The effects of atypical antipsychotic drugs, olanzapine and aripiprazole, on expression of serine^{9}-phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin were determined by Western blotting. A typical antipsychotic drug, haloperidol, was used for comparison. Immobilization stress significantly decreased the expression of these proteins in the frontal cortex. Chronic administration of olanzapine and aripiprazole significantly attenuated the immobilization stress-induced decrease in the levels of these proteins, whereas haloperidol had no such effect. Additionally, olanzapine and aripiprazole significantly increased levels of phosphorylated GSK-3β under normal conditions without stress, and aripiprazole also increased BDNF levels under this condition. These results indicate that olanzapine and aripiprazole, and, haloperidol, differentially regulate the levels of synapse-associated proteins in the rat frontal cortex. These findings may contribute to explain the neurobiological basis of how olanzapine and aripiprazole up-regulated synapse-associated proteins.

1. Introduction

Schizophrenia is a severe psychiatric disorder characterized by positive (hallucinations and delusions), negative (affective and social dysfunction), and cognitive (impairments in learning and memory, information processing, and executive function) symptoms (Stahl, 2008). Although the underlying cause of the disorder remains largely unknown, converging lines of evidences propose that disrupted cortical synaptic circuitry, including reduced gray matter volume, synaptic markers, and neuropil, is a main deficit in schizophrenia (Lewis and Lieberman, 2000; Glantz et al., 2006). These neuronal alterations may underpin the cognitive deficits observed in patients with schizophrenia.

Two classes of drugs used in the treatment of schizophrenia, typical and atypical antipsychotic medications, differ in their efficacy for treating the symptoms of this disorder. Although the underlying mechanisms of these actions are not fully understood, it has recently been suggested that atypical antipsychotic drugs up-regulate dendritic spine formation, dendritic outgrowth, and synaptic protein levels in rat hippocampal neurons, whereas the typical antipsychotic drug, haloperidol, down-regulates them or has no effect (Critchlow et al. 2006; Park et al., 2013). Therefore, it can be suggested that the positive effects of atypical antipsychotic drugs on synaptic plasticity reflect a significant difference between typical and atypical antipsychotic medications.

The canonical *wnt* signaling pathway regulates various aspects of neural circuit formation, including neural polarity, axon guidance, synapse formation, and synaptic plasticity, in vertebrate and invertebrate nervous systems (Park and Shen, 2012). Glycogen synthase kinase-3β (GSK-3β) is normally inhibited in *wnt* signaling, where its primary target is β-catenin. As a result of the inactivation of GSK-3β, intracellular levels of β-catenin increase, allowing its nuclear translocation to activate the expression of *wnt* target genes in concert with the TCF/LEF family of transcription factors, whereas active GSK-3β phosphorylates β-catenin, leading to its ubiquitin-dependent degradation (Logan and Nusse, 2004). Additionally, stable β-catenin is a critical regulator of synaptogenesis and synaptic plasticity, acting as a link between cadherins in the plasma membrane (Arikkath and Reichardt, 2008).

Postsynaptic brain-derived neurotrophic factor (BDNF), the

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most abundant neurotrophin in the brain, contributes to axonal branching, dendritic differentiation, and connectivity among neurons (Poo, 2001; Lessmann et al., 2003; Ji et al., 2005). Increased levels of BDNF are associated with improved learning and memory, and a reduction in BDNF plays a role in age-related memory deficits (Bimonte et al., 2003). Postsynaptic density protein PSD-95 is a scaffolding protein that anchors receptors, including glutamate receptors (Han and Kim, 2008). It is located preferentially in dendritic spines and plays a critical role in regulating dendritic spine size and shape (Ehrlich et al., 2007; Han and Kim, 2008). Thus, PSD-95 is widely used as a postsynaptic marker (Okabe et al., 1999). Synaptophysin is the major integral membrane protein of presynaptic vesicles required for vesicle formation and exocytosis (Valtorta et al., 2004). It is widely used as a presynaptic marker for synapse activity, and an increase in synaptophysin is generally correlated with synaptogenesis (Eastwood and Harrison, 2001). In this context, increased BDNF, PSD-95, or synaptophysin levels may reflect increased synaptic density, activity, and vesicles, indicating improved functioning of synapses.

Decreased levels of GSK-3β phosphorylation, β-catenin, BDNF, PSD-95, or synaptophysin have been reported in the postmortem brains of patients with schizophrenia (Cotter et al., 1998; Karson et al. 1999; Vawter et al., 1999; Durany and Thome, 2004; Kozlovsky et al., 2004; Nadri et al., 2004; Funk et al., 2012). Changes in GSK-3β phosphorylation, β-catenin, or BDNF levels have been identified in rat brains following chronic administration of antipsychotic drugs (Bai et al., 2003; Alimohamad et al., 2005a, 2005b; Park et al., 2006, 2009a, 2011). However, the effects of antipsychotic drugs on the regulation of presynaptic and postsynaptic proteins, PSD-95, and synaptophysin have not been explored in any detail under in vivo conditions. Atypical antipsychotic drugs may exert beneficial effects by reversing deficits in the levels of these synapse-associated proteins. To test this hypothesis, we used an established immobilization stress model in which levels of GSK-3β phosphorylation, β-catenin, BDNF expression, and synaptic proteins are decreased (Park et al., 2006, 2009a, 2011; Fang et al., 2013). Moreover, immobilization stress for 21 days decreases the neurogenesis in the dentat gyrus of rat hippocampus (Park et al., 2007). Another common observation is that this stress model causes atrophy of the apical dendrite and dendritic spine loss in rat prefrontal cortex, as well as working memory impairment (Hains et al., 2009). These cellular and molecular changes in response to stress have been also observed in the studies for the investigation of pathophysiology of schizophrenia (Cotter et al., 1998; Durany and Thome, 2004; Kozlovsky et al., 2004; Nadri et al., 2004; Glantz et al., 2006; Benarroch, 2013; Eich et al., 2014).

The present study investigated the effects of haloperidol and the atypical antipsychotic drugs olanzapine and aripiprazole on phosphorylaed-GSK-3β, β-catenin, BNDF, PSD-95, and synaptophysin expression in the frontal cortex, a brain region known to be involved in the pathophysiology of schizophrenia and in key cognitive functions.

2. Materials and methods

2.1. Drugs and reagents

Olanzapine was supplied by Eli Lilly Research Laboratories (Indianapolis, IN, USA), aripiprazole was supplied by Otsuka Pharmaceuticals (Tokushima, Japan), and haloperidol was purchased from Sigma (St. Louis, MO, USA). Antibodies used for Western blot analysis were obtained from the following sources: anti-BDNF (sc-546) and anti-GSK-3 (sc-7291) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-phosphorylated-GSK-3β (Ser 9; 9336) from Cell Signaling Technology (Beverly, MA, USA); anti-PSD95 (AB9634) from Millipore (Temecula, CA, USA); anti-synaptophysin (ab52636) from Abcam (Cambridge, UK); and anti-β-catenin (C2206) and anti-α-tubulin (T9026) from Sigma. Anti-mouse IgG peroxidase conjugates were obtained from Sigma, and goat-anti-rabbit IgG-horseradish-peroxide conjugates were from Santa Cruz Biotechnology.

2.2. Animals and drug administration

The procedures used in the present study complied with the animal care guidelines in the "Principles of Laboratory Animal Care" (NIH publication no. 23-85, 1996). All experiments involving animals were approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical College (Approval no. 2012-010).

Male Sprague–Dawley rats (Orient Bio, GyeongGi-Do, Korea) weighing 200–250 g were housed two or three per cage with food and water available ad libitum; they were maintained at 21 °C on a 12/12 h light/dark cycle. After 7 days of acclimatization, the rats were randomly divided into eight groups of five rats each. All drugs were dissolved in vehicle (0.8% glacial acetic acid in 0.9% saline) and injected intraperitoneally (i.p.) into the animals. The first group (vehicle) received vehicle (1 mL/kg, i.p.) without immobilization stress. The second (olanzapine), third (aripiprazole), and fourth (haloperidol) groups received olanzapine (2 mg/kg, i.p.), aripiprazole (1.5 mg/kg, i.p.), and haloperidol (1 mg/kg, i.p.), without immobilization stress. The fifth group (vehicle+stress) received the vehicle at 10:00. Then, 1 h later, the rats were completely immobilized for 6 h (from 11:00 to 17:00) in specially designed plastic restraint tubes (dimensions: 20 cm high, 7-cm diameter). The rats in the sixth (olanzapine+stress), seventh (aripiprazole+stress), and eighth (haloperidol+stress) groups received olanzapine (2 mg/kg, i.p.), aripiprazole (1.5 mg/kg, i.p.), and haloperidol (1 mg/kg, i.p.), respectively, and were then immobilized in the same way as were the rats in the fifth group. These procedures were repeated once daily for 3 weeks.

The clinical effect of many antipsychotic drugs are reflected in dopamine D2 receptor occupancy of 60–70% (Farde et al., 1988; Kapur et al., 2000). Drug doses were calculated based on rat studies that investigated D2 receptor occupancy (Barth et al., 2006; Natesan et al., 2006) and had plasma levels well within the therapeutic range of the doses used for treatment of patients with schizophrenia (Andersson et al., 2000).

2.3. Protein extraction and Western blot

Rats were sacrificed 24 h after the last immobilization session. The brain was removed, and the frontal cortex was dissected out. Whole frontal cortex samples were homogenized in ice-cold lysis buffer containing 20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet p-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 2 mM EDTA, one tablet of complete protease inhibitor (Roche, Laval, Quebec, Canada), 20 mM NaF, and 1 mM Na_3VO_4. The tissue homogenate solutions were centrifuged (13,000 rpm, 30 min, 4 °C), and the supernatants were collected and used to quantify the total protein. Equal amounts of protein (30 μg) from tissue extracts under each treatment condition were separated by SDS-polyacrylamide gel electrophoresis and transferred electrophoretically onto polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were blocked by incubation in 5% (w/v) nonfat milk in Tris-buffered saline (TBS) with 0.15% Tween 20 (TBS-T) for 1 h. After incubation with a primary antibody (anti-phosphor-ser^9-GSK-3β, 1:1000; anti-GSK-3, 1:1000; anti-β-catenin, 1:1000; anti-BDNF, 1:1000; anti-PSD-95, 1:1000; anti-synaptophysin, 1:1000; anti-α-tubulin, 1:2000) in TBS-T at 4 °C overnight, the membranes were washed three times in TBS-T for 10 min. The membranes were then incubated for 1 h in TBS-T containing horseradish peroxidase-conjugated secondary antibody (goat-anti-rabbit IgG for anti-phospho-ser^9-GSK-3β, 1:2000; anti-β-catenin, 1: 10,000; anti-BDNF, 1:2000; anti-PSD-95, 1:2000; anti-synaptophysin, 1:2000; anti-mouse IgG for anti-GSK-3, 1:2000; anti-α-tubulin, 1: 10,000). Immunoreactive bands were visualized and quantified using ECL Western blotting reagents (Bio-Rad, Hercules, CA), and chemifluorescence was detected with the Las-3000 Image Reader software (Fuji Film, Tokyo, Japan). Protein levels were normalized to the housekeeping protein α-tubulin to adjust for variability in protein loading. Data are expressed as percentages of the vehicle control (deemed to be 100%).

2.4. Statistical analysis

To determine the individual and interactive effects of drug administration and immobilization stress on protein levels, two-way ANOVA was performed. Scheffe's test was used for the post hoc comparison. A p-value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Effects of olanzapine, aripiprazole, and haloperidol on levels of serine^9-phosphorylated GSK-3β and β-catenin in the frontal cortex

Immobilization stress significantly reduced the levels of GSK-3β phosphorylation and β-catenin in the frontal cortex of