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Protective effects of olanzapine and haloperidol on serum withdrawal-induced apoptosis in SH-SY5Y cells

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Abstract

Purpose: Recent clinical studies have suggested that treatment with second generation antipsychotic drugs such as olanzapine may prevent progressive alterations of brain structure in patients with schizophrenia. However, the molecular mechanisms underlying these different effects remain to be determined. We investigated the mechanisms of action of olanzapine and haloperidol, on serum withdrawal apoptosis in human neuroblastoma SH-SY5Y cells.

Methods: SH-SY5Y cells were cultured with olanzapine and haloperidol in medium with or without serum. We determined the effects of the drugs on cell viability against serum withdrawal by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Additionally, to explore the drugs’ actions, Western blot was performed to examine the expression of key genes involved in GSK-3β-mediated signaling, notably GSK-3β, β-catenin, and Bcl-2.

Results: SH-SY5Y cells suffered about a 38% loss in cell number under serum-free conditions for 48 h. Olanzapine (10–200 μM) up to 100 μM significantly attenuated serum withdrawal-induced cell loss (p<0.01), and a dose of 100 μM also increased cell viability (p<0.05). In contrast, haloperidol (0.01–10 μM) did not affect cell viability but exacerbated cell death at 10 μM under serum-free conditions (p<0.01). Western blot analysis showed that olanzapine, but not haloperidol, prevented the serum withdrawal-induced decrease in levels of neuroprotective proteins such as p-GSK-3β, β-catenin, and Bcl-2 (p<0.01), whereas haloperidol robustly reduced the levels of these proteins at a 10 μM dose in serum-starved cells (p<0.05). Moreover, olanzapine alone significantly increased phosphorylation of GSK-3β under normal conditions (p<0.05).

Conclusions: This study showed that olanzapine may have neuroprotective effects, whereas haloperidol was apparently neurotoxic. The actions of signaling systems associated with GSK-3β may be key targets for olanzapine and haloperidol, but their effects are distinct. These differences suggest different therapeutic effects of first and second generation antipsychotic drugs in patients with schizophrenia.

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Keywords: Bcl-2; GSK-3β; Haloperidol; Neuroprotection; Olanzapine; β-catenin

1. Introduction

Recent clinical and neuroimaging studies have suggested an association between schizophrenia and abnormalities of brain morphology, such as ventricular enlargement, reduction in gray matter volume, and differences in thalamic volume (Konick and Friedman, 2001; Wright et al., 2000). In addition, post-mortem studies in patients with schizophrenia have provided evidence for these structural alterations in the brain that originated from problems in neurodevelopment (Heckers, 1997). These studies also showed changes in neural proliferation, differentiation, and migration in patients with schizophrenia. Anatomical changes in the brain and neuronal plasticity may have significance in the neurobiology of schizophrenia, and it has been suggested that antipsychotic drugs may enhance neuronal plasticity (Anderson et al., 2002; Konradi and Heckers, 2001).

Compared with first generation antipsychotics (FGAs), second generation antipsychotic drugs (SGAs) have superior effectiveness...
therapeutic efficacy for negative symptoms and cognitive deficits, and lower propensity to induce extrapyramidal side effects (EPS) (Kinon and Lieberman, 1996). Growing evidence clearly showed that SGAs might be neuroprotective and involved in neuroplasticity, including neuronal cell growth and survival. It has also been reported that SGAs trigger neurogenesis in the adult rat brain under normal state and repeated restraint stress, and particularly, olanzapine showed protective effects in a rat model of permanent focal cerebral ischemia (Wang et al., 2004; Yulug et al., 2006). In vitro studies showed that SGAs offered protection in cytotoxicity models using hydrogen peroxide, β-amyloid peptide, and 1-methyl-4-phenylpyridinium (MPP+) which cause cell apoptosis in pheochromocytoma (PC12) cell line (Qing et al., 2003; Wei et al., 2003a,b). On the other hand, haloperidol has been reported to cause necrotic and apoptotic cell death (Behl et al., 1995; Noh et al., 2000). Thus, SGAs may have neuroprotective effects in patients with schizophrenia. However, the molecular mechanisms of SGAs are not fully understood, and little has been done to discover changes in intracellular signaling related to the neuroprotective effects.

Glycogen synthase kinase-3β (GSK-3β), a component of diverse signaling pathways, including insulin/insulin-like growth factor signaling, Wnt signaling, and the neurotrophic signaling pathway, is important for cell survival and is related to the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (Doble and Woodgett, 2003; Gould and Manji, 2005). In particular, dysregulation of Wnt signaling and the neurotrophic signaling pathway contribute to the pathophysiology of neurodegenerative disorders (Caricasole et al., 2004; Dunany et al., 2001). Therefore the activation of these pathways can be neuroprotective (De Ferrari et al., 2003). The activity of GSK-3β is modulated by phosphorylation at Ser-9 (Wang et al., 1994), and the inhibition of GSK-3β exerted protective effects and increased neuroplasticity (Gould and Manji, 2005). GSK-3β is normally inhibited in Wnt pathway, where its primary target is β-catenin. β-catenin acts as a transcription factor in concert with T-cell-specific transcription factor/lymphoid enhancer factor (TCF/LEF) in Wnt pathway. Normally, the inhibition of GSK-3β results in nonphosphorylated β-catenin, allowing for its interaction with TCF/LEF, whereas active GSK-3β phosphorylates β-catenin, leading to its ubiquitin-dependent degradation (Logan Nusse, 2004). Thus, the activation of GSK-3β may have neurodegenerative effects through the degradation of β-catenin. In neurotrophic pathways, the activation of phosphatidylinositol 3-kinase (PI3K)-Akt through neurotrophins, such as brain-derived neurotrophic factor (BDNF), phosphorylates GSK-3β. The precise downstream mechanisms that mediate the actions of GSK-3β in this pathway are not fully understood but are believed to include effectors such as c-jun, p53, and cyclic AMP response-element-binding protein (CREB). The pro-apoptotic bax and the anti-apoptotic Bcl-2 (B-cell lymphoma 2) (Grimes and Jope, 2001; Linseman et al., 2004; Pap and Cooper, 1998; Watcharasit et al., 2002) hold key positions in neurotrophic signaling pathways. Among these effectors, Bcl-2 exhibits a neuroprotective effect by promoting dendrite branching, producing regeneration of damaged CNS neurons, and enhancing neurite outgrowth (Allsopp et al., 1993; Chen et al., 1997). It is known that the Bcl-2 protein promotes regeneration of axons in the CNS (Chen et al., 1997).

In this study, we investigated the effects of two antipsychotic drugs, olanzapine and haloperidol, on cell viability and on the levels of GSK-3β, β-catenin, and Bcl-2 in SH-SY5Y cells after serum withdrawal.

2. Methods

2.1. Materials

Olanzapine was purchased from Eli Lilly Research Laboratories (Indianapolis, IN, USA). Haloperidol and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were from Sigma (St. Louis, MO, USA).

Antibodies used for Western blot analysis were obtained from the following sources: anti-phosphorylated GSK-3β, anti-GSK-3β, and anti-Bcl-2 antibodies from Santa Cruz Biotechnology (Santa Cruz, CA, USA), β-catenin and α-tubulin antibodies from Sigma; and goat anti-mouse and anti-rabbit IgG-horse-radish peroxide conjugates from Amersham-Pharmacia (Little Chalfont, England) and Santa Cruz Biotechnology. All other chemicals were purchased from commercial sources.

2.2. Cell culture

The SH-SY5Y human neuroblastoma cell line was obtained from the American Type Tissue Culture Collection (Rockville, MD, USA) and was cultured in minimum essential medium (MEM)/nutrient mixture Ham’s F-12 (1:1) medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 Ag/ml), and l-glutamine (2 mM). The cultures were maintained in a humidified atmosphere of 5% CO2 at 37 °C. The culture medium was changed every 2–3 days.

2.3. Cell viability

SH-SY5Y cells were seeded on 96-well plates at a density of 2 × 104 cells/well and incubated under one of the following conditions: 1) medium with serum (control), 2) medium with drugs, 3) medium without serum (positive control), and 4) medium without serum plus drugs. The drugs were tested over a range of concentrations, 10–200 μM for olanzapine and 0.1–10 μM for haloperidol, as outlined in results. 200 μM of olanzapine and 10 μM of haloperidol were completely dissolved in phosphate-buffered saline (PBS) and 0.5% dimethyl sulfoxide (DMSO), respectively. Immediately before use, the solutions were diluted in to various concentrations with MEM medium. After 48 h of incubation, cell viability was determined by colorimetric measurement of the reduction product of MTT. DMSO (100 μl) was added to each well, and the plates were incubated at 37 °C for 4 h to dissolve the formazan that had formed. Reduced MTT was measured using a kinetic microplate reader (Molecular Devices, Palo Alto, CA) at a wavelength of 570 nm. The value for each treatment group was converted to a percentage of the control.
2.4. Western blot analysis

Cells were plated at a density of $2 \times 10^6$ cells per 100-mm dish. After 24 h of incubation, the cells were treated with various concentrations of olanzapine (10–200 μM) or haloperidol (0.1–10 μM) for 96 h.

For whole cell lysates, the cells were washed twice with ice-cold phosphate-buffered saline (PBS). Lysis buffer [1% Triton, 20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 tablet complete protease inhibitor (Roche, QC, Canada), and phosphatase inhibitor cocktail (Sigma)] was added, and the lysates were centrifuged (1000 × g, 10 min, 4 °C). Equal amounts of protein (10 μg) from the cell extracts for each treatment condition were separated in 12% sodium dodecyl sulfate-polyacrylamide gels and transferred electrophoretically onto polyvinylidene fluoride membranes. The blots were blocked by incubation in 5% (w/v) non-fat milk in PBS with 0.1% Tween 20 (PBS-T) for 1 h. After incubation with a primary antibody (anti-phosphorylated GSK-3β, 1:1000; anti-β-catenin, 1:2000; anti-Bcl-2, 1:500; anti-α-tubulin, 1:2000, in PBS-T) at 4 °C overnight, the membranes were washed three times in PBS-T for 5 min. Subsequently, the membranes were incubated for 1 h in PBS-T containing the appropriate horseradish peroxidase-conjugated secondary antibody (anti-mouse IgG, 1:1000; anti-rabbit IgG, 1:1000; respectively). The immunoreactive bands were visualized and quantitated using ECL+ Western blotting reagents, with chemifluorescence detected by Las-3000 Image Reader (Fuji Film, Tokyo, Japan) software.

To normalize the amount of phosphorylated protein against the total amount of protein, the membrane was stripped after being blotted with anti-phospho-GSK3-β antibody and was then re-blotted with anti-GSK-3β antibody (1:1500). To adjust for protein loading variation, the amounts of β-catenin or Bcl-2 were normalized based on α-tubulin, which was not affected by the drug treatments, and expressed as the percentage of vehicle control, which was deemed to be 100%.

2.5. Statistical analysis

All statistical analyses were carried out using repeated measures one-way ANOVA followed by Scheffe’s test, to determine statistical differences between groups. p values ≤0.05 were deemed to be statistically significant. All data are presented as means ± S.E.M. of three independent experiments.

3. Results

3.1. Effect of olanzapine and haloperidol on viability of SH-SY5Y cells with serum withdrawal

To evaluate the viability of SH-SY5Y cells after serum withdrawal, cells were treated with serum-free medium for 0, 24, 48, 72, and 96 h, and then assessed using an MTT assay. As shown in Fig. 1, the viable cell count decreased in a time-dependent pattern (0 h = 100%, 24 h = 75%, 48 h = 63%, 72 h = 59%, and 96 h = 31%). As an optimal experimental condition, 48 h was selected for subsequent experiments, because cell viability was 62–65% at that time.

To assess the effects of olanzapine and haloperidol on cytotoxicity after serum withdrawal, we investigated viability after exposure to these drugs, in a dose-dependent manner in serum-free medium. Serum withdrawal reduced cell viability to about 40%, compared with the control ($p<0.01$, Fig. 2). When SH-SY5Y cells were treated with 0, 10, 50, 100, and 200 μM olanzapine in serum-free medium, the values for cell viability were 61, 70, 79, 88, and 72%, respectively (Fig. 2A). Olanzapine up to a dose of 100 μM significantly reduced the cell loss induced by serum withdrawal (all $p<0.01$). In addition, olanzapine at 100 μM effectively increased cell viability ($p<0.05$). In contrast, haloperidol did not have this effect (Fig. 2B). Under serum-starved conditions, the values for cell viability at 0, 0.01, 0.1, 1, and 10 μM haloperidol were 64, 60, 58, 56, and 42%, respectively (Fig. 2B). These results were not statistically significant up to a dose of 1 μM. However, treatment with 10 μM haloperidol potentiated cell death in serum-starved conditions (Fig. 2B, $p<0.01$), while haloperidol itself at 10 μM was not toxic (0 μM=100%, 1 μM=104%, and 10 μM=101%).

3.2. Effects of olanzapine and haloperidol on the level of GSK-3β phosphorylation

The neuroprotective effect of olanzapine on cell growth suggested that it might affect PI3K-Akt signaling, one of the neurotrophic pathways (Lu and Dwyer, 2005). Thus, we evaluated phosphorylation of GSK-3β, a downstream target of PI3K-Akt, by Western blot analysis.

SH-SY5Y cells were maintained separately in media with serum and without serum. Olanzapine or haloperidol was added at 10–200 μM or 0.01–10 μM, respectively, for 96 h. Serum withdrawal reduced the phosphorylation of GSK-3β to approximately 30% compared with control levels ($p<0.01$, Fig. 3). Olanzapine up to dose of 200 μM significantly increased the level of p-GSK3β in serum-free medium (0 μM = 74%, 10 μM = 98%, 50 μM = 107%, 100 μM = 124%, and 200 μM = 111%, all $p<0.01$). The most pronounced change
was observed at a dose of 100 μM. Moreover, treatment with olanzapine alone at 100 μM increased the level of p-GSK3β by 20%, compared with control levels (0 μM=100%, 50 μM=111%, and 100 μM=121%, p<0.05, Fig. 3A). However, haloperidol reduced the expression of p-GSK-3β at 10 μM under serum-free conditions (medium without serum: 0 μM=75%, 0.01 μM=70%, 0.1 μM=68%, 1 μM=68%, and 10 μM=62%; medium with serum: 0 μM=100%, 1 μM=100%, and 10 μM=100%; p<0.01, Fig. 4B).

3.3. Effects of olanzapine and haloperidol on the level of β-catenin

Both β-catenin and GSK-3β are key proteins in Wnt pathway. In this study, we examined the level of β-catenin, a target protein of GSK-3β.

In SH-SY5H cells without serum, the levels of β-catenin decreased by 35-45%, compared with control levels (p<0.01). A positive effect was observed at up to 100 μM olanzapine in serum-free medium (p<0.01) and was not detected in cells with the drug alone (medium without serum: 0 μM=78%, 10 μM=88%, 50 μM=100%, 100 μM=111%, and 200 μM=87%; medium with serum: 0 μM=100%, 50 μM=106%, and 100 μM=112%; Fig. 4A). In contrast, 10 μM haloperidol significantly decreased the β-catenin level in SH-SY5H cells without serum (p<0.05, Fig. 4B) (medium without serum: 0 μM=57%, 0.01 μM=59%, 0.1 μM=53%, 1 μM=55%, and 10 μM=37%; medium with serum: 0 μM=100%, 1 μM=95%, and 10 μM=93%; Fig. 4B).

3.4. Effects of olanzapine and haloperidol on the level of Bcl-2

We investigated whether olanzapine or haloperidol influenced regulation of the expression of Bcl-2, a downstream target of GSK-3β, mediated through PI3K-Akt signaling.
The level of Bcl-2 was significantly reduced by about 36% in serum-starved cells, compared with control levels \( (p<0.01) \). Cells treated with olanzapine had increased protein levels at all doses \((10-200 \mu M)\) under serum-free conditions \( (p<0.01) \). However, no significant increase was observed in cells treated with olanzapine alone in medium with serum (medium without serum: \(0 \mu M=68\%, 10 \mu M=104\%, 50 \mu M=125\%, 100 \mu M=142\%, \) and \(200 \mu M=116\%;\) medium with serum: \(0 \mu M=100\%, 50 \mu M=108\%, \) and \(100 \mu M=118\%;\) Fig. 5A). Haloperidol had little or no effect on Bcl-2 expression (medium without serum: \(0 \mu M=62\%, 0.01 \mu M=58\%, 0.1 \mu M=65\%, 1 \mu M=56\%, \) and \(10 \mu M=48\%;\) medium with

Fig. 3. Effects of olanzapine and haloperidol on the levels of phosphorylated GSK-3β in SH-SY5Y cells. Cells were treated with different doses of olanzapine (A) or haloperidol (B) for 96 h with (+serum) or without serum (-serum). Cell lysates were analyzed by SDS-PAGE and Western blotting with a phospho-Ser9-GSK-3β specific antibody. A representative image and quantitative analysis, normalized to the GSK-3 (upper bands: GSK-3α, lower bands: GSK-3β) and α-tubulin band, are shown. Values represent a percentage of the control, in which the cells were treated with neither serum starvation nor drugs, and are expressed as means±S.E.M. from three independent experiments. *\(p<0.05\) vs. untreated control, **\(p<0.01\) vs. untreated control, *\(p<0.01\) vs. serum starved-alone, ***\(p<0.05\) vs. serum starved-alone.
serum: 0 μM=100%, 1 μM=102%, and 10 μM=102%), but reduced Bcl-2 levels were seen with 10 μM haloperidol in serum-starved cells (p<0.05, Fig. 5B).

4. Discussion

Cell death induced by serum withdrawal usually manifests as the hallmark features of apoptosis, including DNA laddering, activation of caspases 3 and 9, cytochrome c release, increased levels of bax, and decreased Bcl-2 levels, NF-kappa B (NF-κB) binding activity, and cellular glutathione levels (Charles et al., 2005). According to the present study, not only cell viability but also levels of the neuroprotective proteins p-GSK-3β, β-catenin, and Bcl-2 were reduced in serum withdrawal-induced apoptotic conditions. Moreover, treatment with olanzapine, but not haloperidol, attenuated the decreases in these proteins. Thus, the neuroprotective effect of olanzapine against neuronal cell death was more prominent than that of haloperidol (Lu et al., 2004). Interestingly, olanzapine showed more protective effects when the cells were in serum-starved conditions. This suggests

Fig. 4. Effects of olanzapine and haloperidol on the levels of β-catenin in SH-SY5Y cells. Cells were treated with different doses of olanzapine (A) or haloperidol (B) for 96 h with (+serum) or without serum (−serum). Cell lysates were analyzed by SDS-PAGE and Western blotting with an anti-β-catenin antibody. A representative image and quantitative analysis, normalized to the α-tubulin band, are shown. Values represent a percentage of the control, in which the cells were treated with neither serum starvation nor drugs, and are expressed as means±S.E.M. from three independent experiments. *p<0.01 vs. untreated control, **p<0.01 vs. serum starved-alone, ***p<0.05 vs. serum starved-alone.
that olanzapine may increase the levels of protective proteins when the cells are in stressful conditions. Further studies are needed to examine this.

Several signal transduction pathways have been suggested to be involved in the actions of antipsychotics. They include the PI3K-Akt, extracellular signal-regulated kinase (ERK), protein kinase A (PKA), Ca$^{2+}$-calmodulin-dependent protein kinase (Ca$^{2+}$/CaM), and Wnt pathways (Chen et al., 1997; Kang et al., 2004; Yang et al., 2004). GSK-3β's role has been emphasized in these mechanisms. It is known that GSK-3β directly affects myc, heat shock transcription factor-1, tau, CREB, β-catenin, p53, bax, and Bcl-2, as well as the release of cytochrome c and caspase 3, and finally induces neuronal cell death (Bijur and Jope, 2000; Grimes and Jope, 2001; Linseman et al., 2004; Pap and Cooper, 1998; Takadera and Ohyashiki, 2004; Watcharasit et al., 2002).

Postmortem studies have found decreases in protein and mRNA levels of p-GSK3β in brains of schizophrenic patients (Kozlovsky et al., 2004; Nadri et al., 2004). In a similar pattern, another study showed decreased β-catenin staining in the
hippocampus of post-mortem schizophrenic brains (Cotter et al., 1998). In Wnt pathway, β-catenin functions as a transcription factor, regulating gene expression. Kang et al. (2004) found that clozapine administration increased the phosphorylation of GSK-3β and the levels of β-catenin through Wnt pathway, but not through the PI3K-Akt pathway, in cell culture. Administration of SGAs, including clozapine, olanzapine, quetiapine, and ziprasidone, rapidly increased the level of p-GSK3β in the cortex, hippocampus, striatum, and cerebellum of mice (Li et al., 2007). However, it was found that a single injection of haloperidol in mice did not increase GSK-3β phosphorylation in the frontal cortex at 1 h following administration (Li et al., 2004). Another study showed that chronic treatment of mice with haloperidol increased Ser-9 phosphorylation in GSK-3β (Emanian et al., 2004).

Thus, the neuroprotective effect of olanzapine is apparently mediated through the inhibition of GSK-3β and subsequently by increased β-catenin. Haloperidol appears to have the opposite effect. Although the effects of haloperidol on GSK-3β have been controversial (Li et al., 2004; Emanian et al., 2004), we showed that olanzapine and haloperidol exert different effects, consistent with previous studies (Li et al., 2004, 2007).

Another finding of the present study was the difference in Bcl-2 expression in response to olanzapine and haloperidol. The increase in Bcl-2 expression was more pronounced in response to olanzapine than haloperidol. Administration of olanzapine accelerated the recovery of decreased Bcl-2 levels from the serum-starved conditions in SH-SY5Y cells, but haloperidol did not. This is consistent with postmortem evidence showing a 25% reduction of the Bcl-2 level in the temporal cortices of schizophrenic subjects, compared with control subjects, and a 96% elevation of Bcl-2 in antipsychotic-treated subjects, compared with neuroleptic-naïve subjects (Jarskog et al., 2000). Chronic administration of olanzapine also upregulated the expression of Bcl-2 mRNA in rat hippocampus (Bai et al., 2004) and prevented the down-regulation of Bcl-2 by methamphetamine or restraint stresses (He et al., 2004; Luo et al., 2004). In contrast, administration of haloperidol significantly aggravated the levels of Bcl-2 in mouse clonal hippocampal HT22 cells (Lezoüale'h et al., 1996). On the other hand, chronic haloperidol-treated animals showed that haloperidol increases the expression of Bcl-2 in the substantia nigra, suggesting that the upregulation of Bcl-2 may indicate a compensatory mechanism of remaining neurons then protected them from the cell damage (Saldaña et al., 2007). This result was inconsistent with our study. This discrepancy might be attributed to the differences between the experiment methods of the two studies, such as in vitro vs. in vivo and human neuroblastoma cell line vs. rat substantia nigra.

It is known that haloperidol causes oxidative stress, which is implicated in tardive dyskinesia (TD) (Reinke et al., 2004). The Bcl-2 protein has an ability to protect cells from oxidative stress such as serum withdrawal and hydrogen peroxide (Kane et al., 1993). One study also showed that the toxic effects of haloperidol were prevented by vitamin E, both in vitro and in vivo (Behl et al., 1995; Egan et al., 1992). In another study, olanzapine increased superoxide dismutase enzyme (SOD) activity in PC12 cells, and the decreased in SOD-1 mRNA level induced by hydrogen peroxide was blocked by pretreatment with olanzapine (Wei et al., 2003a,b). Thus, these results may reflect the different effects of olanzapine and haloperidol in the pathogenesis of TD through differential regulation of Bcl-2.

We cannot draw conclusions about the clinical effects of olanzapine and haloperidol from our in vitro data, because it is not clear whether the drugs reach concentrations in the clinical situation that are sufficient to activate the signaling pathways discussed here. For example, the concentrations of olanzapine used here (10–200 μM) to produce positive effects are higher than the concentrations normally observed in plasma, which are in a range of 0.1 to 0.3 μM (Olesen and Linnet, 1999). This is because the olanzapine is accumulated at 10-to 30-fold higher concentrations in brain tissue (Aravagiri et al., 1999). This is equally applicable to haloperidol. (Korpi et al., 1984; Kornhuber et al., 1999). At physiological concentration of olanzapine (5 μM), these three proteins were clearly activated under our experimental conditions (data not shown), although the values are lower than those of high concentrations (100 or 200 μM).

To our knowledge, this is the first study to demonstrate that olanzapine and haloperidol could lead to different profiles of neuroprotective proteins, via GSK-3β-related pathways. Furthermore, we propose that olanzapine might exert protective effects, that is, the inhibition of GSK-3β (Kang et al., 2004; Lu and Dwyer, 2005; Sinha et al., 2005), and the regulation of downstream targets of GSK-3β, including p53, Bcl-2, bax, and β-catenin (Bai et al., 2004; Chen and Chuang, 1999; He et al., 2004; Li et al., 2007; Luo et al., 2004; Sinha et al., 2005).

5. Conclusions

A key finding of this study is that actions on signaling systems associated with GSK-3β may be an important target for antipsychotic drugs. Significant differences in actions between olanzapine and haloperidol suggest that the mechanisms of the drugs may explain their differential effects. Further studies are needed to evaluate whether other antipsychotic drugs affect the activation of the signaling pathways.

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