Differential effects of amisulpride and haloperidol on dopamine D2 receptor-mediated signaling in SH-SY5Y cells

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**Abstract**

Dopamine D2 receptors (D2R) are the primary target of antipsychotic drugs and have been shown to regulate Akt/glycogen synthase kinase-3β (GSK-3β) signaling through scaffolding protein β-arrestin 2. Amisulpride, an atypical antipsychotic drug, and haloperidol, a typical antipsychotic drug, are both potent D2R antagonists, but their therapeutic effects differ. In the present study, we compared the effects of amisulpride and haloperidol on the β-arrestin 2-mediated Akt/GSK-3β pathway in SH-SY5Y cells. To determine whether these drugs affected neuronal morphology in SH-SY5Y cells, we investigated the effects of amisulpride and haloperidol on neurite outgrowth using immunostaining. We examined the effects of these drugs on Akt and GSK-3β and its well-known downstream regulators, cAMP response element-binding protein (CREB), brain-derived neurotrophic factor (BDNF), and Bcl-2 levels using Western blot analysis. Amisulpride, but not haloperidol, was found to enhance neurite outgrowth. Small interfering RNA (siRNA) for β-arrestin 2 knockdown blocked the increase in amisulpride-induced neurite outgrowth. Furthermore, amisulpride increased the levels of Akt and GSK-3β phosphorylation, while haloperidol had no effect. The elevation of Akt phosphorylation induced by amisulpride was reduced by β-arrestin 2 siRNA. Moreover, amisulpride effectively increased the levels of phospho-CREB, BDNF, and Bcl-2. However, haloperidol had no effect on the levels of these proteins. Additionally, wortmannin, a phosphatidylinositol 3-kinase (PI3 K) inhibitor, blocked the stimulatory effect of amisulpride on phosphorylated Akt. Together, these results suggest that regulation of the β-arrestin 2-dependent pathway via blockade of the D2R in SH-SY5Y cells is one mechanism underlying the neuroprotective effect of amisulpride, but not haloperidol.

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1. Introduction

Antipsychotic drugs are classified as typical and atypical based on their efficacy in alleviating schizophrenic symptoms and the incidence of extrapyramidal side effects (Andreasen, 1994; Meltzer, 1995). A growing body of evidence indicates that several atypical antipsychotic drugs have a neuroprotective effect against a variety of toxins (Bai et al., 2002; Luo et al., 2005; Wang et al., 2004; Wei et al., 2003a,b; Yulug et al., 2006). In contrast, the typical antipsychotic drug haloperidol lacks this effect (Behl et al., 1995; Noh et al., 2000). However, the molecular mechanisms underlying the differing effects of typical and atypical antipsychotic drugs have not been fully determined.

Both classes of antipsychotic drugs have an affinity for dopamine D2 receptors (D2R), but their therapeutic effects differ (Creese et al., 1996). D2R belongs to the G protein-coupled receptor (GPCR) family. It couples with Gi/0 proteins to negatively regulate cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signaling, G protein-dependent mechanism (Missale et al., 1998). In addition to this canonical action, Beaulieu et al. (2004, 2005, 2006, 2007a, 2007b, 2008) recently suggested that the D2R regulates Akt/glycogen synthase kinase-3β (GSK-3β) signaling by a G-protein-independent mechanism, involving the multifunctional scaffolding protein β-arrestin 2. D2R stimulation induces receptor phosphorylation via the GPCR kinases (GRKs). Receptors phosphorylated by GRKs are then bound to β-arrestin 2. Subsequent β-arrestin 2 binding terminates G-protein-dependent signaling by blocking the receptor–G protein interaction causing the formation of a signaling complex, composed of...
β-arrestin 2, Akt, and the protein phosphatase2A (PP2A), which dephosphorylates (inactivates) Akt (Beaulieu et al., 2005). The formation of this complex modulates GSK-3β-mediated signaling. Thus, β-arrestin 2 may be an important mediator of Akt and GSK-3β regulation by D2R (Marsil et al., 2008). In this context, regulation of the Akt/GSK-3β pathway through β-arrestin 2 may be a key molecular mechanism modulating the action of antipsychotic drugs. However, this pathway has not been investigated. Recent studies indicate that Akt/GSK-3β signaling is a potential target for antipsychotic drugs, and alterations in this pathway have been implicated in the pathogenesis of schizophrenia (Emamian et al., 2004; Roh et al., 2007). Furthermore, we recently demonstrated that the neuroprotective effect of the atypical antipsychotic aripiprazole on human neuroblasts SH-SY5Y cells involved inactivation of GSK-3β through brain-derived neurotrophic factor (BDNF)-mediated activation of phosphatidylinositol-3 kinase (PI3 K/Akt signaling. The subsequent increase in transcription factor CAMP response element-binding protein (CREB) activity enhanced expression of its survival transcriptional target, Bcl-2, but the typical antipsychotic drug haloperidol did not show these effects (Park et al., 2009a).

Amisulpride, an atypical antipsychotic drug, selectively blocks dopamine D2/D3 receptors, but has no affinity for adrenergic, serotonergic, histaminergic, or muscarinic receptor systems (Perrault et al., 1997). This makes it unique among atypical antipsychotic drugs. Moreover, it has a dual effect that selectively blocks presynaptic D2/D3 dopamine autoreceptors at a low dose, enhancing dopamine transmission, and blocks postsynaptic D2/D3 dopamine receptors at a higher dose, inhibiting dopaminergic hyperactivity. This dual dopamine receptor-blocking effect contributes to the improvement of negative symptoms of schizophrenia at low doses and of positive symptoms at high doses (Rosenzweig et al., 2002). In contrast, haloperidol blocks D2 receptors reduce only the positive symptoms of schizophrenia (Andreasen, 1994; Meltzer, 1995). To specifically investigate D2R signaling, we studied amisulpride and haloperidol, which have a high affinity and selectivity for the D2R.

Atypical antipsychotic drugs have a positive effect on neuronal viability compared with typical antipsychotic drugs (Lu and Dwyer, 2005). In the present study, we sought to investigate whether amisulpride and haloperidol affected neurite outgrowth in SH-SY5Y human neuroblastoma cells via the β-arrestin 2-dependent pathway. Next, we compared the effect of these drugs on the phosphorylation of Akt, GSK-3β, and CREB and the expression of BDNF and Bcl-2, downstream effectors of CREB, to detect changes in downstream signaling associated with the β-arrestin 2/Akt/GSK-3β pathway.

2. Methods

2.1. Drugs and reagents

Amisulpride was purchased from Sanofi-Aventis (Paris, France) and haloperidol was from Sigma (St. Louis, MO, USA). Antibodies used for immunostaining were purchased from the following sources: anti-microtubule associated protein 2 (MAP-2) from Millipore (Temecula, CA, USA); Alexa Fluor 568 goat anti-mouse IgG and Hoechst 33258 from Invitrogen (Carlsbad, CA, USA). Antibodies used for Western blotting were purchased from the following sources: anti-phospho-ser473β-Akt, anti-Akt, and anti-CREB from Cell Signaling Technology (Beverly, MA, USA), anti-phospho-ser133-CREB from Upstate Biotech (Lake Placid, NY, USA); anti-BDNF, anti-phospho-ser72-GSK-3β, anti-GSK-3β (Santa Cruz, CA, USA); anti-α-tubulin from Sigma, ECL anti-mouse IgG, horseradish peroxidase linked species-specific whole antibody from Amersham Pharmacia (Little Chalfont, Buckinghamshire, UK), and anti-goat and anti-rabbit IgG-horseradish peroxidase conjugates from Santa Cruz Biotechnology. The PI3 K inhibitor, wortmannin, was purchased from Invitrogen.

2.2. Cell culture

SH-SY5Y human neuroblastoma cells, obtained from the American Type Culture Collection (Rockville, MD, USA), were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic–antimycotic solution. The cells were maintained in a humidified atmosphere of 5% CO2 at 37 °C. For the neurite assay, cells were plated at a density of 2 × 104 cells/well in a 12-well plate. For Western blot analysis, cells were plated at a density of 2 × 105 cells per 100 mm dish. After incubation for 24 h, the cells were treated with amisulpride (1, 10, 100 μM) or haloperidol (10, 1 μM) for 96 h. The culture media and drugs were changed every 2 days. No cell death was observed at the drug doses tested. Amisulpride and haloperidol were dissolved in dimethyl sulfoxide (DMSO) and diluted with DMEM medium to a concentration of 50 mM amisulpride and 10 mM haloperidol (final 0.5% DMSO).

2.3. Small interfering RNA-mediated knockdown of β-arrestin 2

β-arrestin 2 knockdown using small interfering RNAs (siRNAs) was performed as follows. Chemically synthesized double-stranded siRNAs were purchased from Invitrogen. The catalog numbers of the two sets of human β-arrestin 2-specific siRNA duplexes were ARRB2-HSS180981 and ARRB2-HSS180982. A nonsilencing RNA duplex (5′-AAUUCGUCCAAUGUCGCUU-3′) was synthesized at Samchully Pharmaceucicals (Seoul, Korea) as a control (Shenoy et al., 2006). SH-SY5Y cells that were 50–60% confluent in 12-well and 6-well plates were transfected with 100 pmol and 150 pmol of siRNA (combination set), respectively, for the neurite assay and Western blot analysis, using the Lipofectamine RNAiMAX transfection reagent (Invitrogen) according to the manufacturer's protocol. The growth medium was changed 4 h later, and amisulpride and haloperidol were treated simultaneously. The immunofluorescence assay and Western blot analysis to detect neurite outgrowth and Akt phosphorylation levels, respectively, were performed at least 3 days following siRNA transfection.

2.4. Neurite outgrowth assay

Neurites were visualized using immunostaining with a MAP2 antibody as follows. Cells were fixed for 20 min at room temperature using 4% paraformaldehyde, then permeabilized with 0.1% Triton X-100, and blocked with 4% bovine serum albumin in phosphate-buffered saline (PBS) for 2 h to reduce non-specific binding. Cells were incubated with anti-MAP2 antibody diluted 1/200 in PBS for 2 h. Alexa Fluor 568 goat anti-mouse IgG was used as a secondary antibody and Hoechst 33258 was used for nuclear staining. Stained cells on the cover glass were observed using a fluorescence microscope (Olympus, Tokyo, Japan). Five fields were randomly selected from each sample, and their images were captured using a digital camera by a person blinded to their identities. The entire procedure from neurite differentiation to image capture was performed twice. All the cells in the ten fields, 300–400 in total, were included in the neurite analysis using MetaMorph, an automated image analysis program (Molecular Devices, Downingtown, PA, USA) (Klimaschevski, 2002).

2.5. Western blot analysis

Whole cell lysate preparation was conducted as follows. The cells were washed twice with ice-cold PBS. Lysis buffer (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, and 1 tablet complete protease inhibitor (Roche, Laval, Quebec, Canada)) were added, and the lysates were centrifuged (1000 × g, 10 min, 4 °C). Equal amounts of protein (20 μg) from the cell extracts for each treatment condition were separated on SDS-polyacrylamide gels and transferred electrophoretically onto polyvinylidene fluoride membranes. The blots were blocked by incubation in 5% (w/v) non-fat milk in Tris-buffered saline (TBS) with 0.15% Triton X-100, and blocked with 4% bovine serum albumin in phosphate-buffered saline (PBS) for 2 h to reduce non-specific binding. Cells were incubated with anti-MAP2 antibody diluted 1/200 in PBS for 2 h. Alexa Fluor 568 goat anti-mouse IgG was used as a secondary antibody and Hoechst 33258 was used for nuclear staining. Stained cells on the cover glass were observed using a fluorescent microscope (Olympus, Tokyo, Japan). Five fields were randomly selected from each sample, and their images were captured using a digital camera by a person blinded to their identities. The entire procedure from neurite differentiation to image capture was performed twice. All the cells in the ten fields, 300–400 in total, were included in the neurite analysis using MetaMorph, an automated image analysis program (Molecular Devices, Downingtown, PA, USA) (Klimaschevski, 2002).

2.6. Statistical analysis

Data are expressed as means ± SEM of three independent experiments. One-way analyses of variance (ANOVA) were performed and followed with post-hoc comparisons using Duncan's test. Values were deemed to be statistically significant at p < 0.05.
3. Results

3.1. Effect of amisulpride and haloperidol on neurite outgrowth

We performed a neurite outgrowth assay to investigate whether amisulpride and haloperidol differentially regulated neuronal morphology in SH-SY5Y cells. SH-SY5Y cells were incubated with amisulpride (1, 10, 100 μM) or haloperidol (1, 10 μM) for 4 days. After day 4, cells treated with amisulpride began to show fiber-like processes, such as increased neurite length (Fig. 1B). Amisulpride produced a dose-dependent increase in neurite outgrowth up to a dose of 100 μM (p < 0.01, Fig. 1B, D). In contrast, no change in morphology was observed in haloperidol-treated cells (Fig. 1C, E).

We performed β-arrestin 2 knockdown using siRNA to explore the possible relationship between β-arrestin 2 and amisulpride-induced morphological changes. β-arrestin 2 expression was suppressed by about 80% in β-arrestin 2 siRNA-transfected cells compared with mock-transfected cells (no RNA; control) and nonsilencing RNA-transfected cells (control siRNA; Fig. 2A). Total neurite outgrowth was unchanged in cells transfected with β-arrestin 2 siRNA under normal conditions (Fig. 2D, F). However, the amisulpride-induced increase in neurite length was completely abolished in cells transfected with β-arrestin 2 siRNA, indicating that amisulpride stimulated neurite outgrowth by regulating D2R-mediated β-arrestin 2 signaling (p < 0.01; Fig. 2E, F).

3.2. Effect of amisulpride and haloperidol on Akt and GSK-3β phosphorylation

Amisulpride (1, 10, 100 μM) produced a dose-dependent increase in levels of ser473-phosphorylated Akt, and ser9-phosphorylated GSK-3β. Amisulpride (10, 100 μM) significantly increased the percentage of phosphorylated Akt and GSK-3β to 41% and 60%, respectively (p < 0.01; Figs. 3A and 4A). No difference in phosphorylated Akt or GSK-3β levels was observed at either dose of haloperidol (1, 10 μM; Figs. 3B and 4B).

To determine the role of β-arrestin 2 in amisulpride-induced Akt activation, β-arrestin 2 siRNA was transfected into SH-SY5Y cells. β-arrestin 2 knockdown suppressed Akt phosphorylation (p < 0.01; Fig. 5). Up-regulation of amisulpride-induced Akt phosphorylation was completely blocked by the inhibition of β-arrestin 2 (p < 0.01; Fig. 5), suggesting that β-arrestin 2 is involved in the amisulpride-induced change in Akt/GSK-3β signaling.

3.3. Effect of amisulpride and haloperidol on CREB phosphorylation and BDNF and Bcl-2 expression

Levels of ser133-phosphorylated CREB were elevated by 22%, 37%, and 69% in cells treated with 1, 10, and 100 μM amisulpride, respectively (Fig. 6A; p < 0.01). No change in the expression of phosphorylated CREB was observed in haloperidol-treated cells (Fig. 6B).

To test the possibility that CREB activation stimulated the upregulation of BDNF and Bcl-2, we examined the effect of amisulpride and haloperidol on BDNF and Bcl-2 expression. Amisulpride treatment produced a dose-dependent increase, of 144% and 65% in BDNF and Bcl-2 expression, respectively (p < 0.01; Figs. 7A and 8A), whereas haloperidol had no effect (Figs. 7B and 8B).

3.4. Effects of PI3 K inhibitor on amisulpride-induced Akt phosphorylation

Amisulpride, but not haloperidol, significantly increased expression of the neuroprotective protein BDNF in SH-SY5Y cells (Fig. 7A).
An experiment using wortmannin, a PI3 K inhibitor, was carried out to investigate whether this upregulation was associated with activation of the PI3 K/Akt pathway via the high-affinity TrkB receptor of BDNF. We found that amisulpride-induced upregulation of Akt was reversed by wortmannin (10 μM) in SH-SY5Y cells (p < 0.01; Fig. 9).

4. Discussion

We previously reported that several atypical, but not typical, antipsychotic drugs, produced neuroprotective effects in human and rat neuronal cell lines and the rat hippocampus (Kim et al., 2008; Park et al., 2009a, 2009b, in press). Most atypical antipsychotic drugs block both dopamine D_2 and serotonin 2A receptors, and typical antipsychotic drugs block dopamine D_2 receptors (Andreasen, 1994; Meltzer, 1995). Thus, dopamine D_2 receptors may play an important role in antipsychotic drug-induced neuroprotection; however, the role of D_2R has not been examined in detail. Both amisulpride and haloperidol are pure D_2R blockers. The present study is the first reported to demonstrate that amisulpride, but not haloperidol, increased total neurite outgrowth and the level of proteins associated with the Akt/GSK-3β pathway in SH-SY5Y cells. The positive effects of amisulpride appear to involve D_2R-induced β-arrestin 2-dependent signaling.

To our knowledge, this is the first report to demonstrate that amisulpride increases neurite outgrowth in human neuroblastoma cell lines. Furthermore, amisulpride-induced neurite outgrowth was blocked by β-arrestin 2 siRNA. However, neurite outgrowth was not inhibited by β-arrestin 2 siRNA alone under normal conditions. Thus, our results indicate that β-arrestin 2 is required for the amisulpride-induced potentiation of neurite outgrowth. This response may involve D_2R-mediated activation of the Akt/GSK-3β pathway.

The present study revealed different effects of amisulpride and haloperidol on the phosphorylation of Akt and GSK-3β, although both drugs are antipsychotics with potent D_2R antagonism. Regulation of Akt/GSK-3β by D_2R action is mediated by β-arrestin 2 (Beaulieu et al., 2005). Moreover, amisulpride-induced Akt phosphorylation was attenuated by β-arrestin 2 knockdown, suggesting a role for β-arrestin 2 in this response. Reports of the effect of antipsychotic drugs on Akt and GSK-3β expression are conflicting. Lu and Dwyer (2005) reported that atypical antipsychotic drugs, such as the 5-HT_2A receptor blockers olanzapine, quetiapine, and clozapine, stimulated Akt phosphorylation, whereas typical antipsychotic drugs, such as chlorpromazine, fluphenazine, and haloperidol, which are D_2R antagonists, decreased Akt phosphorylation stimulated by nerve growth factor in PC12 cells. In other studies, haloperidol treatment has been reported to increase the level of Akt phosphorylation, thus inhibiting GSK-3β in normal animals (Emamian et al., 2004; Roh et al., 2007). Li et al. (2007) reported that administration of atypical antipsychotics, including clozapine, olanzapine, quetiapine, and ziprasidone, rapidly increased the level of GSK3β phosphorylation in the cortex, hippocampus, striatum, and cerebellum of mice. These differences may be the result of different modes of drug action on multiple neurotransmitter receptors, particularly dopamine D_2 and 5-HT_2A receptors. Haloperidol has a higher antagonist affinity for the D_2R than does amisulpride (Schoemaker et al., 1997). Thus, we hypothesized that...
this property may contribute to the difference in the regulation of the β-arrestin 2-dependent Akt/GSK-3β pathway.

The differential effect of amisulpride and haloperidol on β-arrestin 2-dependent signaling may be explained by their effect on GRKs, upstream of β-arrestin 2. Haloperidol and clozapine have been reported to differentially affect the expression of arrestins and GRKs, which may play a role in determining the clinical profile of these drugs (Ahmed et al., 2008). In this study, Ahmed and colleagues reported that haloperidol did not affect the expression of the arrestin subtype in any region of the rat brain. In contrast, clozapine increased the expression of arrestin 2 and GRK2 in specific brain regions. Thus, it is conceivable that amisulpride and haloperidol may differentially affect the regulation of GRKs activity.

Furthermore, our results suggest that β-arrestin 2-dependent Akt/GSK-3β signaling plays a significant role in the neuroprotective effect of amisulpride. In the present study, amisulpride significantly increased the level of CREB phosphorylation and BDNF and Bcl-2 expression. Extensive cross-talk occurs between CREB and cAMP-dependent kinase (PKA), mitogen-activated protein kinase (MAPK), and the Akt/GSK-3β pathway (Lonze and Ginty, 2002). In particular, CREB is a key transcription factor responsible for GSK-3β gene regulation, and CREB activity is generally increased...
by inhibition of GSK-3β (Liang and Chuang, 2006). CREB-mediated gene expression is necessary for the survival of multiple neuronal subtypes and is involved in differentiation, synaptic plasticity, and memory (Giachino et al., 2005; Lonze and Ginty, 2002). Moreover, BDNF and Bcl-2 upregulation require CREB activation (Finkbeiner, 2000). In a recent animal study, Hammonds and Shim (2009) showed that olanzapine treatment for 4 weeks increased the level of phosphorylated CREB, BDNF, and Bcl-2 in the hippocampus. Additionally, previous reports have shown that long-term olanzapine treatment prevented BDNF and Bcl-2 downregulation in the rat hippocampus in response to restraint stress (Luo et al., 2004) and reversed a methamphetamine-induced decrease in Bcl-2 in the striatum (He et al., 2004). Thus, our results suggest that the CREB-activated upregulation of BDNF and Bcl-2 may be one mechanism underlying the neuroprotective effect of amisulpride, and neurite outgrowth may be promoted, in part, by regulation through β-arrestin 2/Akt/GSK-3β signaling.

An increase in BDNF has been associated with the expression of its high-affinity receptor, TrkB (Tsai, 2007). BDNF-induced TrkB activation has been reported to stimulate MAPK and PI3 K signaling pathways (Pizzorusso et al., 2000; Rodgers and Theibert, 2002). PI3 K/Akt signaling plays a primary role in regulating the balance between cell survival and apoptosis (Franke et al., 1997; Hetman et al., 1999). Akt activation depends upon its phosphorylation on ser473 and PI3 K activity is important for promoting phosphorylation of Akt Ser473 (Chan et al., 1999; Scheid et al., 2002). GSK-3β is inactivated by phosphorylation on ser9 by Akt activation (Cross et al., 1995). Phosphorylation (ser9) of GSK-3β by Akt promotes cell survival by inhibiting apoptosis, whereas dephosphorylated (active) GSK-3β activates the apoptosis pathway by inhibiting Akt (Crowder and Freeman, 2000) and impairs activation of CREB (Grimes and Jope, 2001). BDNF leads to the increased phosphorylation of CREB ser133 in a MAPK-dependent manner (Bonni et al., 1999). The phosphorylation and activation of CREB contributes to the enhancement of neuronal survival (Bonni et al., 1999). In the present study, we found that amisulpride-induced Akt phosphorylation was prevented by the addition of specific PI3 K inhibitors, suggesting that PI3 K/Akt is involved in the positive effect of amisulpride in SH-SYSY cells. Thus,

![Fig. 5. Effect of β-arrestin 2 knockdown on amisulpride-induced Akt phosphorylation in β-arrestin 2 siRNA-transfected SH-SYSY cells.](image)

![Fig. 6. Effects of amisulpride and haloperidol on CREB phosphorylation (ser133) in SH-SYSY cells.](image)
we suggest that amisulpride-induced upregulation of BDNF expression may be linked to enhanced neurite outgrowth via the PI3 K/Akt signaling pathway, and that GSK-3β-induced CREB activation may be partially responsible for the efficacy of amisulpride.

We cannot draw conclusions about the clinical effects of amisulpride and haloperidol from our in vitro data because it is unclear whether these drugs reached sufficient therapeutic concentrations to activate neurite outgrowth and the signaling pathways discussed here. Although the doses of amisulpride and haloperidol we used were generally higher than those normally found in brain tissue, such high doses are routinely used in in vitro studies. The difference between complex brain structures and cell lines may explain the need for higher drug concentrations in in vitro studies. In our preliminary experiment, no change in neurite outgrowth or protein levels was observed at a drug concentration less than 1 μM, and the number of nonviable cells increased by 30–40% at amisulpride doses over 120 μM and haloperidol doses over 20 μM. The dose ranges of 1–100 μM for amisulpride and 1–10 μM for haloperidol were based on previous studies demonstrating the action of antipsychotic drugs on signal transduction pathways in PC12 cells (Lu et al., 2004; Lu and Dwyer, 2005).

A key finding of our study was that the β-arrestin 2-mediated Akt/GSK-3β pathway is a potential target for amisulpride via D2R blockade. The differential actions of amisulpride and haloperidol at

Fig. 7. Effect of amisulpride and haloperidol on BDNF levels in SH-SY5Y cells. Cells were treated with amisulpride (1, 10, 100 μM; A) and haloperidol (1 and 10 μM; B) for 96 h. Cell lysates were analyzed using SDS-PAGE and Western blotting with an anti-BDNF antibody. A representative image and quantitative analysis normalized to the α-tubulin band are shown. Values represent the percentage of the vehicle-treated cells (control) and are expressed as means ± SEM from the three independent experiments. **p < 0.01 vs. control.

Fig. 8. Effect of amisulpride and haloperidol on Bcl-2 levels in SH-SY5Y cells. Cells were treated with amisulpride (1, 10, 100 μM; A) and haloperidol (1 and 10 μM; B) for 96 h. Cell lysates were analyzed using SDS-PAGE and Western blotting with an anti-Bcl-2 antibody. A representative image and quantitative analysis normalized to the α-tubulin band are shown. Values represent a percentage of the vehicle-treated cells (control) and are expressed as means ± SEM from the three independent experiments. **p < 0.01 vs. control.
5. Conclusions

The molecular level may explain their differing therapeutic effects and side effects. Further studies are needed to evaluate whether the present findings can be extended to other atypical antipsychotic drugs because serotonin receptor activity may also affect β-arrestin 2.

5. Conclusions

To our knowledge, the present in vitro data are the first showing a neuroprotective effect of amisulpride via a β-arrestin 2-dependent signaling system. Our results suggest that the protective effect of amisulpride in human neuroblastoma SH-SY5Y cells involves activation of Akt/GSK-3β through the D2R/β-arrestin 2-dependent pathway.

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