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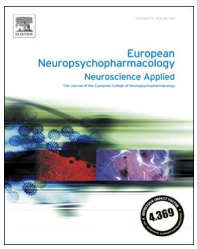

# Effects of the antipsychotics haloperidol, clozapine, and aripiprazole on the dendritic spine

**Manabu Takaki*, Masafumi Kodama, Yutaka Mizuki, Hiroki Kawai, Bunta Yoshimura, Makiko Kishimoto, Shinji Sakamoto, Yuko Okahisa, Norihito Yamada**

*Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan*

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**Abstract**
Three types of antipsychotics, typical (e.g. haloperidol), atypical (e.g. clozapine), and dopamine partial agonist (e.g. aripiprazole), are administered for treatment of schizophrenia. These antipsychotics have different efficacy and side-effect profiles. We investigated whether aripiprazole, clozapine, and haloperidol differentially regulate the dendritic spine through the AKT-GSK-3 beta cascade. Dissociated cortical neurons from Sprague-Dawley rats were prepared and cultured for 28 days. Aripiprazole, clozapine, or haloperidol was administered to the rat cortical neurons. The levels of PSD95 protein and AKT-GSK-3 beta cascade-related proteins were investigated by Western blot. The number of spines and PSD95 puncta were investigated by immunofluorescence cell staining. Aripiprazole (1 μM or 10 μM) and clozapine (1 μM) increased the levels of PSD95 protein, the number of spines, phosphorylated Akt Thr308 and Ser473, and phosphorylated GSK-3 beta Ser9. On the other hand, haloperidol (1 μM or 10 μM) or an inappropriate concentration of clozapine (10 μM) decreased them. A GSK inhibitor also increased the levels of PSD-95 protein and caused the same morphology. Aripiprazole, clozapine, and haloperidol differentially regulate the dendritic spine, and this effect may occur through the AKT-GSK-3 beta cascade. Selection and appropriate dose of these antipsychotics may be important for the protection of dendritic spines in patients with schizophrenia.


*Corresponding author. Fax: +81 86 235 7246.
*E-mail address:* manabuta@cc.okayama-u.ac.jp (M. Takaki).

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## 1. Introduction

Schizophrenia is a neurodevelopmental disorder and one of the most severe psychiatric disorders, with a lifetime risk of about 1% of the population in all cultures and a refractory clinical course (Mueser and McGurk, 2004). For treatment of schizophrenia, three types of antipsychotics, typical (e.g. haloperidol; HPD), atypical (e.g. clozapine; CZP), and a dopamine partial agonist (e.g. aripiprazole; APZ) are administered. These antipsychotics differ in efficacy and side-effect profiles (Leucht et al., 2013). Clozapine is regarded as the most effective drug for the treatment of schizophrenia but has complex adverse effects associated with hyperglycemia, seizure, and diabetes mellitus (Leucht et al., 2013). APZ is as effective as and better tolerated than HPD (Marder et al., 2003). Atypical antipsychotics and a dopamine partial agonist are reported to have neuroprotective effects. CZP but not HPD inhibits apoptosis (Qing et al., 2003; Abekawa et al., 2011), increases brain-derived neurotrophic factor (BDNF) (Bai et al., 2003), nerve growth factor (Parikh et al., 2004), and neurogenesis (Halim et al., 2004). APZ also inhibits apoptosis (Abekawa et al., 2011) and increases BDNF (Bai et al., 2003; Park et al., 2009). In patients with schizophrenia, greater intensity of antipsychotic treatment was reported to be associated with smaller gray matter volumes (Ho et al., 2011). Because HPD was associated with significant reductions in gray matter volume, whereas an atypical antipsychotic, olanzapine, was not, there may be a difference between typical and atypical antipsychotics (Lieberman et al., 2005).

Dendritic spine pathology is thought to be related to the pathology of schizophrenia (Glausier and Lewis, 2013). Many studies have reported that dendritic spine density was decreased in multiple brain regions such as the neocortex (Garey et al., 1998; Glantz and Lewis, 2000), striatum (Roberts et al., 1996), and hippocampal formation (Rosoklija et al., 2000) of patients with schizophrenia. A candidate susceptibility gene for schizophrenia, disrupted in schizophrenia 1 (DISC1) (Chubb et al., 2008), is involved in mitochondrial trafficking (Atkin et al., 2011) and regulates spine formation via Kalirin-7 and Rac1 (Hayashi-Takagi et al., 2010). Neuregulin-1 and ErbB4 are also candidate susceptibility genes for schizophrenia (Mei and Xiong, 2008; Walsh et al., 2008), and their activity is associated with changes in spine density (Penzes et al., 2011). Recently, our group reported that human Rho guanine nucleotide exchange factor 11 (ARHGEF11), a specific GEF for RhoA, is associated with a higher risk for the onset of schizophrenia in a Japanese population (Mizuki et al., 2014) and regulates dendritic morphogenesis (Mizuki et al., 2016).

Glycogen synthase kinase-3 (GSK-3) beta and the GSK-3 beta gene have been related to mood disorder and schizophrenia (Jope et al., 2006; Lachman et al., 2007; Souza et al., 2008). Antipsychotics are reported to phosphorylate GSK-3 beta to Ser9-GSK-3 beta and inactivate it (Roh et al., 2007; Mohammad et al., 2008; Sutton and Rushlow, 2011). GSK-3 beta also related to synaptic function (Nelson et al., 2013). CZP and HPD differentially regulate dendritic spines, and CZP increases spines, but HPD decreases them in hippocampal neurons (Critchlow et al., 2006). In SH-SY5Y cells, APZ increased the phosphorylation of GSK-3 beta but HPD did not (Park et al., 2009).

Based on these observations, we attempted to establish whether these three types of antipsychotics, APZ, CZP, and HPD, regulate the dendritic spine differentially through the AKT-GSK-3 beta cascade in the rat cortical neuron (Table 1).

## 2. Experimental procedures

### 2.1. Neuron culture and antipsychotic administration

These experiments were approved by the Animal Care and Use Committee, Okayama University. Dissociated cortical neuron cultures from Sprague-Dawley rats were prepared as described previously (Penzes et al., 2001). We followed the 'Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions'. In summary, Sprague-Dawley rat brains were digested at 18 d of gestation (E18). Holding the brain stem with one forceps, the hemispheres were separated with another forceps; then the hippocampus was removed, the cortex was cut and the cortical tissue was carefully peeled off the meninges.

Dissociated neurons were plated at a density of $2 \times 10^6$ cell/ml on poly-L-lysine coated cover glasses and incubated in a humidified atmosphere of 5% $CO_2$ at 37°C for 28 d. One half of the medium was exchanged twice every week. One or 10 μM HPD, CZP (Sigma), or APZ (gift from Otsuka Pharmaceutical Co.), 1, 5, or 10 μM GSK-3 inhibitor (Sigma; SB 216763) in DMSO, or 0.02% v/v DMSO as a control was added at 26 d *in vitro* (D.I.V.). Samples were collected

**Table 1** Summary of results.

| | | PSD95 | SYN | AKT | p-AKT | | GSK3β | p-GSK3β | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Thr 308 | Ser 473 | | Ser 9 | Y 216 |
| HPD | 1μM | ↓ | → | → | ↓ | ↓ | → | ↓ | → |
| | 10μM | ↓ | → | → | ↓ | ↓ | → | ↓ | → |
| CZP | 1μM | ↑ | → | → | ↑ | ↑ | → | ↑ | → |
| | 10μM | ↓ | → | → | ↓ | ↓ | → | ↓ | → |
| APZ | 1μM | ↑ | → | → | ↑ | ↑ | → | ↑ | → |
| | 10μM | ↑ | → | → | ↑ | ↑ | → | ↑ | → |

SYN: synaptophysin.

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