Neuropharmacology 114 (2017) 1–11

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

The atypical antipsychotic olanzapine disturbs depotentiation by modulating mAChRs and impairs reversal learning

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ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form
13 October 2016
Accepted 16 November 2016
Available online 17 November 2016

Keywords:
Olanzapine
Antipsychotics
Synaptic plasticity
Depotentiation
mAChR
Reversal learning

ABSTRACT

Antipsychotic medication is an essential component for treating schizophrenia, which is a serious mental disorder that affects approximately 1% of the global population. Olanzapine (Olz), one of the most frequently prescribed atypical antipsychotics, is generally considered a first-line drug for treating schizophrenia. In contrast to psychotic symptoms, the effects of Olz on cognitive symptoms of schizophrenia are still unclear. In addition, the mechanisms by which Olz affects the neural circuits associated with cognitive function are unknown. Here we show that Olz interrupts depotentiation (reversal of long-term potentiation) without disturbing *de novo* LTP (long-term potentiation) and LTD (long-term depression). At hippocampal SC-CA1 synapses, inhibition of NMDARs (*N*-methyl-D-aspartate receptors), mGluRs (metabotropic glutamate receptors), or mAChRs (muscarinic acetylcholine receptors) disrupted depotentiation. In addition, co-activation of NMDARs, mGluRs, and mAChRs reversed stably expressed LTP. Olz inhibits the activation of mAChRs, which amplifies glutamate signaling through enhanced NMDAR opening and Gq (Gq class of G protein)-mediated signal transduction. Behaviorally, Olz impairs spatial reversal learning of mice in the Morris water maze test. Our results uncover a novel mechanism underpinning the cognitive modulation of Olz and show that the anticholinergic property of Olz affects glutamate signaling and synaptic plasticity.

1. Introduction

Schizophrenia is a devastating brain disorder that affects one's thoughts, feeling, and behaviors. Although the etiology of schizophrenia is unknown, approximately 1% of the population develops schizophrenia during their lifetime (Miyamoto et al., 2012). The symptoms of schizophrenia usually include positive (psychotic), negative (affective), and cognitive symptoms (Kim et al., 2009). In schizophrenia, antipsychotic medication is believed to be essential for achieving therapeutic outcomes and usually reduces the psychotic symptoms. Blockade of dopamine D_2 receptor is believed to be associated with the therapeutic effects of antipsychotics (Kim et al., 2009). Thus, conventional antipsychotics, which are also known as first-generation or typical antipsychotics, have a high affinity for dopamine D_2 receptors. In addition to dopamine (D_1–D_4) receptors, atypical antipsychotics, also termed second-generation antipsychotics, bind multiple sites, including serotonin, muscarinic acetylcholine, and histamine receptors (Miyamoto et al., 2005).

Olanzapine (Olz), an atypical antipsychotic, is frequently used as a first-line treatment for schizophrenia and inhibits all dopamine receptors in the nanomolar range. Because Olz has a higher affinity for 5-HT_{2a} receptors than D_2 receptors (Bymaster et al., 1997), antagonism of dopamine and serotonin receptors is assumed to be involved in the antipsychotic effects of Olz. Olz also exhibits antagonist activity at muscarinic acetylcholine and histamine receptors (Bymaster et al., 1999). In contrast to its efficacy on psychotic symptoms, the effect of Olz on cognitive symptom is still poorly understood. Olz has been reported to improve some domains of cognitive function, such as verbal learning and executive function, in schizophrenic patients. However, Olz had no effect on

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http://dx.doi.org/10.1016/j.neuropharm.2016.11.012
0028-3908/

visual learning and working memory in schizophrenic patients (Cuesta et al., 2001; Meltzer and McGurk, 1999). Cognitive dysfunction is a core symptom of schizophrenia, which usually persists even when the psychotic symptoms are improved by antipsychotics (Keefe et al., 1999). Because cognitive dysfunction hampers independent living, the dysfunction is the strong predictor of functional disability in schizophrenia (Bowie and Harvey, 2006). However, importantly, the neural mechanisms underlying the modulation of cognition by Olz, as well as by other antipsychotics, are unknown. To date, most studies have been focused on comparing the relative efficacy of existing antipsychotics on cognitive improvement in schizophrenic patients (Cuesta et al., 2001; Keefe et al., 1999; Meltzer and McGurk, 1999). Recently, antipsychotic medication with adjunctive pharmacological agents has been used to improve the cognitive dysfunction in schizophrenia (Kim et al., 2009; Miyamoto et al., 2012). Thus, an understanding of the neural circuits that are modulated by individual antipsychotics is essential for this strategy.

To understand effects of Olz on neural circuits and cognitive function, we investigated synaptic plasticity and learning behaviors in mice. Activity-dependent strengthening and weakening of synaptic function are widely believed to be the cellular basis of learning and memory (Malinow and Malenka, 2002; Martin et al., 2000). Compared with clinical studies, electrophysiological investigations of synaptic plasticity provide direct experimental evidences and information about the pharmacological mechanisms of the cognitive effects of Olz. In clinical studies, the improvement of psychotic symptoms or attention in schizophrenic patients is a potential confounding variable (Keefe et al., 1999). In addition, most atypical antipsychotics have multiple binding sites, and schizophrenic patients exhibit genetic and phenotypic heterogeneity (Cuesta et al., 2001). These properties hinder the identification of the pharmacological mechanism for the cognitive effects observed in a clinical study.

The present study shows that the anticholinergic property of Olz interrupts synaptic depotentiation (the reversal of long-term potentiation). We also provide experimental evidence that Olz affects behavioral flexibility.

2. Methods

2.1. Animals

All experiments were performed with C57BL/6J mice. The animals were housed under SPF and temperature-controlled conditions with free access to food and water. All animals were maintained on a diurnal cycle of 12:12 with the light on at 7:00. Animal maintenance and all animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at SNU.

2.2. Electrophysiology

2.2.1. Hippocampal slice preparation

Parasagittal hippocampal slices (400 μm thick) were prepared from the brains of 4–6-week-old mice of both sexes using a vibratome (Leica, Germany) in ice-cold dissection buffer (in mM: sucrose 213; $NaHCO_3$ 26; KCl 2.5; NaH_2PO_4 1.25; D-glucose 10; Na-ascorbate 1.3; $MgCl_2$ 3.5; $CaCl_2$ 0.5 bubbled with 95% O_2/5% CO_2). The CA3 region was surgically removed immediately after sectioning. The slices were allowed to recover at 36 °C for 1 h in normal artificial cerebrospinal fluid (ACSF), and then maintained at room temperature. The ACSF contained (in mM): NaCl 125; $NaHCO_3$ 26; KCl 2.5; NaH_2PO_4 1.25; D-glucose 10; $MgCl_2$ 1.3; $CaCl_2$ 2.5.

2.2.2. Instrumentation

All electrophysiological recordings were performed in a submerged recording chamber, which was perfused with heated (29–30 °C) ACSF. The signals were filtered at 2.8 kHz and digitized at 10 kHz using a MultiClamp 700B amplifier and a Digidata 1440A interface (Molecular Devices, CA, USA). The data were analyzed by using custom macros written in Igor Pro (WaveMetrics, OR, USA).

2.2.3. Field excitatory postsynaptic potential (fEPSP) recording

For fEPSP recording (Figs. 1–3, 4A, 4B, 4E, 4F, and 5), hippocampal slices were placed in a submerged recording chamber and synaptic responses were evoked at 0.05 Hz with an ACSF-filled broken glass pipette (0.3–0.5 MΩ) placed in the stratum radiatum. Stimulus intensity was adjusted to yield one-thirds of the maximal response. After stable baseline of at least 20 min, LTP (long-term potentiation) or LTD (long-term depression) was induced. LTP was induced by high-frequency stimulation (HFS). HFS consists of 2 trains of 100 stimuli at 100 Hz with a 10 s inter-train interval. Low-frequency stimulation (LFS) consists of 900 stimuli (1 Hz) and paired-pulse low-frequency stimulation (PP-LFS) consists of 900 pairs of stimuli at 1 Hz with a 50 ms paired-pulse interval. To determine the induction and expression magnitude of LTP, LTD, or depotentiation, fEPSP slopes during the last 5 min of recording were compared with those of baseline (20 min). Slices displaying an unstable baseline (10%) or changes in the fiber volley were discarded.

2.2.4. Whole-cell patch clamp recording

To measure AMPA/NMDA ratios (Fig. 4C and D), whole-cell voltage clamp recordings were made using patch pipettes (3–4 MΩ) filled with solution containing (in mM) 100 $CsMeSO_4$, 10 TEA-Cl, 20 CsCl, 8 NaCl, 10 HEPES, 5 QX-314-Cl, 4 Mg-ATP, 0.3 Na-GTP, and 0.5 EGTA, adjusted to pH 7.25 and 290 mOsm/kg. The $GABA_A$ receptor antagonist picrotoxin (50 μM) was added to the ACSF to inhibit inhibitory post-synaptic currents (IPSCs). The mean AMPAR (AMPA receptor)-mediated currents were obtained by averaging 30–40 traces recorded at −70 mV. Stimulation intensity was adjusted to yield a 100–250 pA EPSC (excitatory post-synaptic current) peak amplitude. To isolate the NMDAR-mediated currents, 30–40 traces of EPSCs were recorded at +40 mV in the presence of the AMPAR antagonist NBQX (10 μM) in ACSF. To compare the NMDAR-EPSCs at −65 mV and −58 mV (Fig. 6B–D), NBQX and picrotoxin were added to the ACSF, and the NMDAR-EPSCs were evoked by paired-pulse stimulation (50 ms interval). The series resistance and seal resistance were monitored, and the data were discarded if they changed by more than 20% during the whole-cell voltage clamp recording.

For membrane potential recording (Fig. 6A), whole-cell current clamp recordings were made using a pipette solution containing (in mM) 110 K-gluconate, 20 KCl, 8 NaCl, 10 HEPES, 1 QX-314-Cl, 4 Mg-ATP, 0.3 Na-GTP, and 0.5 EGTA, adjusted to pH 7.25 and 290 mOsm/kg. NBQX and picrotoxin were added to the ACSF. Neurons displaying an unstable resting potential at the beginning or during the whole-cell current clamp recording were discarded.

2.2.5. Drugs

Olz, DHPG (a group I mGluR agonist), MPEP (an mGluR5 antagonist), AP-5 (an NMDAR antagonist), NBQX, picrotoxin, atropine (a muscarinic receptor antagonist), SKF83566 (a D1-like dopamine receptor antagonist), L-741,626 (a D2-like dopamine receptor antagonist), L-745,870 (a dopamine D4 receptor antagonist), anisomycin (a protein synthesis inhibitor), and cycloheximide (a protein synthesis inhibitor) were purchased from Tocris Cookson (Bristol, UK). All other reagents and chemicals were purchased from Sigma-Aldrich (MO, USA). All the drugs used in the