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Olanzapine augments the effect of selective serotonin reuptake inhibitors by suppressing GABAergic inhibition via antagonism of 5-HT_6 receptors in the dorsal raphe nucleus

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ABSTRACT

The combination of the selective serotonin reuptake inhibitors (SSRIs) and atypical antipsychotic drugs shows better therapeutic efficacy than SSRI monotherapy in the treatment of depression. However, the underlying mechanisms responsible for the augmenting effects of olanzapine are not fully understood. Here, we report that olanzapine enhances the SSRI-induced increase in extracellular serotonin (5-HT) levels and antidepressant-like effects by inhibiting GABAergic neurons through 5-HT_6 receptor antagonism in the dorsal raphe nucleus (DRN). In organotypic raphe slice cultures, treatment with olanzapine (1–100 μM) enhanced the increase in extracellular 5-HT levels in the presence of fluoxetine (10 μM) or citalopram (1 μM). The enhancing effect of olanzapine was not further augmented by the $GABA_A$ receptor antagonist bicuculline. Electrophysiological analysis revealed that olanzapine (50 μM) decreased the firing frequency of GABAergic neurons in acute DRN slices. Among many serotonergic agents, the 5-HT_6 receptor antagonist SB399885 (1–100 μM) mimicked the effects of olanzapine by enhancing the SSRI-induced increase in extracellular 5-HT levels, which was not further augmented by bicuculline or olanzapine. SB399885 (50 μM) also decreased the firing frequency of GABAergic neurons in the DRN. In addition, an intraperitoneal administration of SB399885 (10 mg/kg) to mice significantly enhanced the antidepressant-like effect of a subeffective dose of citalopram (3 mg/kg) in the tail-suspension test. These results suggest that olanzapine decreases local inhibitory GABAergic tone in the DRN through antagonism of 5-HT_6 receptors, thereby increasing the activity of at least part of serotonergic neurons, which may contribute to the augmentation of the efficacy of SSRIs.

1. Introduction

Major depressive disorder (MDD), one of the most common psychiatric disorders worldwide, is characterized by persistent depressed mood, loss of interest and anhedonia. Considering the high prevalence of MDD and risk for suicide, development of more effective treatments are needed (Belmaker and Agam, 2008). Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are one of the first-line drug treatments for MDD, although approximately 30% of patients do not respond to SSRI monotherapy (Corya et al., 2006). To overcome treatment resistance in depression, many types of augmentation therapies have been

Abbreviations: ANOVA, analysis of variance; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; DMSO, dimethyl sulfoxide; DRN, dorsal raphe nucleus; KRH, Krebs-Ringer-Henseleit; MDD, major depressive disorder; PBS, phosphate-buffered saline; RT-PCR, reverse transcription polymerase chain reaction; SSRI, selective serotonin reuptake inhibitor; 5-HT, serotonin; TPH2, tryptophan hydroxylase 2.

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developed to enhance treatment efficacy of conventional antidepressants, such as tricyclics, SSRIs and serotonin-noradrenaline reuptake inhibitors (Nelson and Papakostas., 2009; Whale et al., 2010; Khan et al., 2011). The olanzapine-fluoxetine drug combination, one of the most common augmentation therapies, shows more rapid and higher efficacy than SSRI monotherapy (Tohen et al., 2003; Corya et al., 2006; Dubé et al., 2007). Despite its frequent use in clinical practice, the underlying mechanisms by which olanzapine potentiates the antidepressant effect of SSRIs are not fully understood.

Olanzapine is classified as an atypical antipsychotic, acting as a potent antagonist on a variety of neurotransmitter receptors. Similar to other atypical antipsychotics, olanzapine antagonizes dopamine D_2 and 5-HT_2 receptors (Bymaster et al., 1999; Theisen et al., 2007). Although evidence suggests that 5-HT_{2A} receptor antagonism may play an important role in the antidepressant-like effect of olanzapine (Marek et al., 2003, 2005), the involvement of other potential sites of action of olanzapine have not been elucidated.

Almost all conventional antidepressants increase extracellular 5-HT levels by blocking the 5-HT transporter; therefore, the enhancement of the serotonergic system is thought to play an important role in the therapeutic effect of antidepressants (Wegener et al., 2000; Bosker et al., 2001; Kamińska et al., 2013). Among the serotonergic nuclei, the dorsal raphe nucleus (DRN) has been implicated in a variety of psychiatric disorders, including MDD (Michelsen et al., 2008; Hammack et al., 2012). In the DRN, γ-aminobutyric acidergic (GABAergic) neurons modulate 5-HT neuronal activity (Liu et al., 2000; Gartside et al., 2007; Challis et al., 2013), which may affect extracellular 5-HT levels, although noradrenergic, glutamatergic and serotonergic neurons can affect 5-HT neuronal activity (Corradetti et al., 2005; Nagayasu et al., 2013; Nishitani et al., 2014). However, it is unclear whether olanzapine affects extracellular 5-HT levels and the neuronal activity of these neurons in the DRN.

The aim of the present study was to determine whether and how olanzapine augments the effects of SSRIs. Therefore, we examined the effects of olanzapine on extracellular 5-HT levels in the organotypic raphe slice cultures that we had previously established (Higuchi et al., 2008; Nagayasu et al., 2010a). We also investigated the involvement of DRN GABAergic neurons and 5-HT receptors in the effects of olanzapine using organotypic raphe slice cultures and electrophysiological analysis in an acute raphe slice preparation. Finally, we evaluated the antidepressant-like effect in the tail-suspension test. Our data demonstrate that olanzapine enhances the effects of SSRIs by inhibiting GABAergic neurons through 5-HT_6 receptor antagonism in the DRN.

2. Materials and Methods

2.1. Animals

All animal care and experimental procedures were conducted in accordance with the ethical guidelines of the Kyoto University Animal Research Committee. The protocol was approved by the Kyoto University Animal Research Committee (Permission Number: 2014-48). All experiments were designed to minimize the use of animals and number of experiments. Pregnant Wistar/ST rats and male C57BL/6 mice were purchased from Nihon SLC (Shizuoka, Japan) and housed at a constant ambient temperature of 24 ± 1 °C on a 12 h light–dark cycle with food and water freely available.

2.2. Drugs

Citalopram was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). Fluoxetine and ketanserin were purchased from Sigma–Aldrich (Saint-Louis, MO, USA). SB242084 was purchased from Tocris Bioscience (Bristol, UK). Olanzapine was purchased from Wako Pure Chemical Industries (Osaka, Japan). Raclopride and SB399885 were purchased from Abcam Biochemicals (Cambridge, UK). For slice culture experiments and electrophysiological experiments, drugs were dissolved in stock solutions of phosphate-buffered saline (PBS), water or dimethyl sulfoxide (DMSO) and stored at −20 °C until use. On the day of treatment, stock solutions were thawed and diluted in PBS. The final concentration of DMSO in the vehicle was 1% for slice culture experiments and 0.05% for electrophysiological experiments.

2.3. Preparation of rat organotypic raphe slice cultures

The rat organotypic raphe slice cultures were prepared as previously described (Higuchi et al., 2008; Nagayasu et al., 2010b, 2013). Briefly, Wistar/ST rat pups on postnatal days 2–3 were deeply anesthetized using hypothermia. After decapitation, the brain was immediately isolated and coronal slices (350 μm thick) containing dorsal and median raphe nuclei were prepared with a tissue chopper (Narishige, Tokyo, Japan). Four consecutive slices from each pup were obtained and placed on 30 mm Millicell-CM inserts (pore size 0.4 μm; Millipore, Billerica, MA, USA), and the inserts were transferred to a six-well plate. Slices were maintained at the liquid/air interface for 14–16 days at 37 °C under 5% CO_2, and were subsequently used in the experiments. Culture medium was replaced with fresh medium every other day.

2.4. Measurement of extracellular 5-HT levels

Extracellular 5-HT levels were analyzed as previously described (Nagayasu et al., 2010b, 2013). Briefly, culture inserts were washed in 0.7 mL of Krebs-Ringer-Henseleit (KRH) buffer (146 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM D-glucose, 15 mM HEPES, 5 mM HEPES-Na, 0.2 mM ascorbic acid, pH 7.4) for 15 min and subsequently incubated in 0.7 mL of drug-containing KRH buffer for 30 min. The conditioned KRH buffer was collected, and 1 M acetic acid (50 μL) was immediately added to prevent possible 5-HT degradation. The 5-HT concentration was measured using high performance liquid chromatography with an electrochemical detector (Eicom, Kyoto, Japan).

2.5. Electrophysiological analysis of acute raphe slices

Electrophysiological analysis was performed as described in a previous report (Mitsumori et al., 2011). Male C57BL/6J mice aged 8–13 weeks old (Nihon SLC) were deeply anesthetized by isoflurane and decapitated. The brain was collected in ice-cold cutting solution (120 mM NMDG-Cl, 2.5 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 15 mM D-glucose, 1.3 mM ascorbic acid, pH 7.2). Coronal midbrain slices (200 μm thick) were prepared with a vibratome (VT1000S, Leica, Wetzlar, Germany), and recovered in oxygenated artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM D-glucose, pH 7.3) at 32 °C for at least 1 h before recording. After recovery, individual slices were transferred to a recording chamber with continuous perfusion of oxygenated aCSF at 32 °C at a flow rate of 2–3 mL/min.

Whole-cell current clamp recordings were performed with an EPC9 amplifier (HEKA, Pfalz, Germany), and the data were recorded using Patchmaster software. The resistance of the electrodes was 4–7 MΩ when filled with the internal solution (140 mM K-gluconate, 5 mM KCl, 10 mM HEPES, 2 mM Na-ATP, 2 mM $MgCl_2$, 0.2 mM EGTA, pH 7.3 adjusted with KOH). Individual neurons were visualized with a microscope equipped with a 40× water-immersion objective lens (Carl Zeiss, Jena, Germany) and a CCD camera.

Neurons that met all of the following criteria were considered as GABAergic neurons; located at the lateral wing of the DRN, possessing a small cell body, spontaneously firing at more than 1.5 Hz, and not hyperpolarized by bath application of 5-HT (50 μM). Examining the presumed GABAergic neurons using single-cell reverse transcription polymerase chain reaction (RT-PCR) demonstrated that all of the these neurons were tryptophan hydroxylase 2 (*Tph2*)-negative and glutamate decarboxylase 2 (*Gad2*)-positive (Supplementary Fig. 1), defining GABAergic neurons. Similarly, serotonergic neurons were identified using following criteria; located at the dorsomedial or ventromedial DRN, possessing a large cell body and hyperpolarized by bath application of 5-HT (50 μM). Single-cell RT-PCR demonstrated that all of these neurons were *Tph2*-positive, *Gad1*-negativie and *Gad2*-negative, defining serotonergic neurons. After stabilization, spontaneous firing was recorded for 60 s, and the mean firing rate (spikes/10 s) during this period was considered the basal firing rate. Then, vehicle, olanzapine or SB399885 was perfused.

2.6. Single-cell RT-PCR

Single-cell PCR was performed as previously described (Shikanai et al., 2012). Briefly, after whole-cell recording, the contents of the cell were aspirated into the recording electrode and harvested in a sampling tube. The collected samples were reverse-transcribed using a ReverTra Ace RT kit (TOYOBO, Tokyo, Japan) and amplified with Blend Taq (TOYOBO, Tokyo, Japan) using the following primers: *Tph2* (forward 5′-GAAAAACCTCCCCCTGCTGA-3′, reverse 5′-GTCTCTTGGGCTCAGGTAGC-3′), *Gad1* (forward 5′-GGCCTGAAGATCTGTGGCTT-3′, reverse 5′-CAGAACCTTGGTGGAGCGAT-3′), *Gad2* (forward 5′-ATGCAGAGCTGCAACCAGAT-3′, reverse 5′-GCCTCAAACCCAGTAGTCCC-3′), *Htr6* (forward 5′-TCGATGTCCTCACATGGCTG-3′, reverse 5′-CCTCTTGAAGTCCCGCATGA-3′), and *Eno2* (forward 5′-CCGCTGATCCTTCCCGATAC-3′, reverse 5′-

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