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Potent antagonism of 5-HT_3 and 5-HT_6 receptors by olanzapine

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Abstract

The interaction of the psychotropic agent olanzapine with serotonin 5-HT_3 and 5-HT_6 receptors was investigated. Olanzapine did not contract the isolated guinea pig ileum, but blocked contractions induced by the 5-HT_3 receptor agonist 2-methyl serotonin (2-CH_3 5-HT) with a pK_B value of 6.38 ± 0.03, close to the affinity of the 5-HT_3 receptor antagonist ondansetron. The atypical antipsychotic risperidone (1 μM) did not significantly inhibit 2-CH_3 5-HT-induced contractions. Olanzapine had high affinity ($pK_i = 8.30 \pm 0.06$) for human 5-HT_6 receptors in radioligand binding studies. Olanzapine did not stimulate [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate ([^{35}S]GTPγS) binding to the G protein G_s in cells containing human 5-HT_6 receptors, but inhibited 5-HT-stimulated [^{35}S]GTPγS binding ($pK_B = 7.38 \pm 0.16$). Among other antipsychotics investigated, clozapine antagonized 5-HT_6 receptors with a $pK_B = 7.42 \pm 0.15$, ziprasidone was three-fold less potent, and risperidone, quetiapine and haloperidol were weak antagonists. Thus, olanzapine was not an agonist, but was a potent antagonist at 5-HT_6 receptors and had marked antagonism at 5-HT_3 receptors.

Keywords: Olanzapine; Risperidone; Quetiapine; Ziprasidone; 5-HT_3 receptor; 5-HT_6 receptor

1. Introduction

Olanzapine is a psychotropic agent that has been shown to be therapeutically useful for the treatment of schizophrenia, bipolar disorder-associated mania, and the behavioral symptoms of Alzheimer's disease (Beasley et al., 1996; Tohen et al., 1999, 2000; Street et al., 2000). Olanzapine has relatively high affinity for many neuronal receptors including dopamine D_1–D_5, α_1-adrenoceptors, histamine H_1 receptors, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3 and 5-HT_6 receptors (Bymaster et al., 1996, 1999c; Roth et al., 1994). Olanzapine also has moderate affinity for muscarinic M_{1-5} receptors (Bymaster and Falcone, 2000). Olanzapine is an antagonist at dopamine D_1, D_2, and D_4 receptors (Bymaster et al., 1999b; Gilliland and Alper, 2000; Newman-Tancredi et al., 1997), 5-HT_2-type receptors, muscarinic M_1–M_5 receptors, α_1-adrenoceptors, and histamine H_1 receptors (Bymaster et al., 1999b). The functional blockade of these multiple receptors may contribute to the wide range of pharmacologic and therapeutic activities of olanzapine, which have been described as multi-acting-receptor-targeted-agent (MARTA) (Bymaster et al., 1999a).

Although olanzapine possesses marked 5-HT_3 and 5-HT_6 affinity, its functional activity at these receptors has not been determined. Whether olanzapine acts as an agonist or antagonist at these receptors may impact the spectrum of olanzapine's potential therapeutic effects. The 5-HT_3 receptor is a ligand-gated ion channel and is found in smooth muscle including the small intestine and colon in the periphery and brain regions such as the hippocampus, striatum, and area postrema (Morales et al., 1998; Miyake et al., 1995). Clinically, antagonists of the 5-HT_3 receptor are useful for treatment of chemotherapy-related nausea (Slaby et al., 2000; Martin et al., 1998) and irritable bowel syndrome (Farthing, 1999; Mertz, 1999). In addition, 5-HT_3 receptor antagonists are potentially useful for treatment of neuroleptic-resistant Tourette's syndrome (Toren et al., 1999), neuroleptic-induced tardive dyskinesia (Sirota et al., 2000), and fibromyalgia syndrome (Farber et al., 2000). Antagonists of 5-HT_3 receptors may also have a potential role in treatment of cognitive disorders and anxiety symp-

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toms (Wolf, 2000) characterized by reduced release of central nervous system acetylcholine (Ramirez et al., 1996; Wolf, 2000).

The 5-HT_6 receptors are coupled to the G protein G_s and stimulate adenylate cyclase activity. They are found in brain regions including the striatum, nucleus accumbens, cortex, and hippocampus (Monsma et al., 1993; Kohen et al., 1996). Antagonism of the 5-HT_6 receptor has been suggested to produce antipsychotic, anxiolytic, and anticonvulsant properties, and particularly enhancement of cognitive performance (improved visual–spatial performance and enhanced consolidation of new learning) in animal models (Bourson et al., 1998; Yoshioka et al., 1998; Routledge et al., 2000; Rogers et al., 1999; Meneses, 2001).

Because of the potential importance of these two receptors to the action of antipsychotic agents, it was of interest to characterize the interaction of olanzapine with these two receptors and to compare olanzapine to several typical and atypical antipsychotic agents. We explored the 5-HT_3 interactions of olanzapine using the guinea pig ileum in vitro, a model established to possess 5-HT_3 receptors (Cohen et al., 1989). The binding of a nonhydrolyzable analog of GTP, [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate ([^{35}S]GTPγS), to the heterotrimeric GTP binding protein G_s using an antibody capture technique was used to determine the functional effects of olanzapine at clonal 5-HT_6 receptors.

2. Materials and methods

2.1. 2-Methyl serotonin (2-CH_3 5-HT)-induced contraction of the guinea pig ileum

The interaction of compounds with 5-HT_3 receptors was determined in the guinea pig ileum according to published methods (Cohen et al., 1989). Hartley male guinea pigs (300–350 g) (Harlan Sprague–Dawley, Indianapolis, IN) were killed by cervical dislocation and longitudinal sections of the ilea (2–3 cm long) were used. To stabilize the base-line contraction, segments of ilea were placed between two electrodes consisting of a stainless steel rod (bottom) and a circular platinum wire (top). Square-wave impulses (0.1 Hz) at 40 V and 0.7-ms duration (twitch) were provided by a Grass S44 stimulator (Grass Instruments, Quincy, MA).

Tissues were mounted in organ baths containing 10 ml of modified Krebs' solution of the following composition (in mM): 118.2 NaCl, 4.6 KCl, 1.6 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 dextrose, 24.8 $NaHCO_3$. Tissue bath solutions were maintained at 37 °C and equilibrated with 95% O_2–5% CO_2. Each tissue was placed under optimal resting force and allowed to equilibrate for approximately 1 h before exposure to drugs. Isometric contractions were recorded as changes in grams of force with MP100 data acquisition software (BIOPAC Systems, Santa Barbara, CA) connected to Statham UC-3 transducers (Statham Medical Instruments, Los Angeles, CA) and a Compaq Deskpro computer.

To determine the agonist activity of drugs, risperidone (1000 nM) and olanzapine (300 and 1000 nM) were added noncumulatively to stabilized guinea pig ileum. Contraction from baseline was measured 5 min after drug or vehicle administration and calculated as a percent of KCl (67 mM) contraction obtained prior to agonist administration in each tissue and compared to the contraction produced by the 5-HT_3 receptor agonist, 2-CH_3 5-HT.

For antagonist activity of drugs, noncumulative contractile concentration response curves were generated for 2-CH_3 5-HT, a selective 5-HT_3 receptor agonist (Richardson et al., 1985), by a stepwise increase in concentration. After control responses to 2-CH_3 5-HT were obtained, ilea were incubated with appropriate concentrations of antagonist or vehicle for 1 h. Noncumulative responses to 2-CH_3 5-HT (tissues were washed between 2-CH_3 5-HT additions) were then repeated in the presence of the antagonist that was added to the bath between 2-CH_3 5-HT additions. In each tissue, only one concentration of antagonist was examined. Tissues not treated with the antagonist served as a control to correct for time-related changes in sensitivity.

2.2. Binding to 5-HT_6 receptors

Inhibition of binding to 5-HT_6 receptors was determined according to a published method (Boess et al., 1997). HeLa cells expressing the human 5-HT_6 receptor were obtained from Dr. David Sibley of the National Institute of Health. The cells were cultured in monolayer in defined medium. After growing to about 80% confluency, the cells were harvested, counted, centrifuged, and stored at −80 °C until use. On the day of use, the cells were homogenized in 50 mM Tris Cl buffer, pH 7.5, for 30 s with a polytron at setting 7 and centrifuged at 17,000 × *g* for 10 min. The pelleted broken cells were resuspended in buffer at the equivalent of 2 million/ml.

The binding of [^{3}H]lysergic acid diethylamide (LSD, 2 nM final concentration, 68.5 Ci/mmol) was determined by adding 50 μl of radioligand, 200 μl of membrane preparation, and 0.75 ml of buffer to a final concentration of 50 mM Tris Cl, pH 7.5, 10 mM $MgSO_4$, and 0.5 mM EDTA. The samples were incubated for 90 min at 37 °C, and bound radioactivity was determined by vacuum filtration with a cell harvester using GF/c glass fiber filters (Whatman, Maidstone, England) soaked in 0.1% polyethyleneimine. The filters were rinsed three times with 1 ml of cold buffer and then placed in scintillation vials containing scintillation fluid (Ready Protein$^+$, Beckman Coulter, Fullerton, CA). Radioactivity was determined using liquid scintillation spectrometry with about 40% counting effi-

ciency. Nonspecific binding was determined using 1 μM clozapine.

2.3. [^{35}S]GTPγS binding to 5-HT$_6$ clonal cells

Binding of GTPγS to membranes containing clonal human 5-HT$_6$ receptors was determined according to a modification of the antibody capture method of DeLapp et al. (1999). Membrane preparations of human embryonic kidney (HEK)-293 cells transfected with human 5-HT$_6$ receptors were obtained from Receptor Biology (Wilmington, DE). Membranes from one vial were homogenized in cold 20 mM HEPES buffer, pH 7.0, containing 100 mM NaCl, 5 mM $MgCl_2$, 1.7 mM ascorbic acid, and 163 μM pargyline by five strokes in a glass teflon homogenizer. The membranes were then brought up to a final volume of 24 ml with buffer and kept on ice until addition to the assay.

The binding of [^{35}S]GTPγS to membranes was determined in the above HEPES buffer in the presence of 0.01 mg/ml saponin in a final volume of 200 μl in a 96-well Costar plate (Fisher Scientific, Springfield, NJ) at room temperature. Aliquots of 50 μl of appropriate concentrations of drug in buffer, 50 μl of [^{35}S]GTPγS (1000–1200 Ci/mmol) to give a final concentration of 0.5 nM, and a final addition of 100 μl of membrane preparation (50 μg protein/well) were added to initiate the binding. For antagonist studies, the concentration of 5-HT was 1000 nM.

After 30 min of incubation, the labeled membranes were solubilized for 30 min at room temperature with 0.27% NP40 (20 μl/well of a solution containing 1.5 ml of 10% Nonidet P-40 for every 3.5 ml of buffer) followed by a 1-h incubation with 20 μl of 1:500 diluted anti-Gs/olf. An aliquot of 50 μl of anti-rabbit immunoglobulin G (IgG)-coated scintillation proximity assay (SPA) beads was added, and the membranes were allowed to bind to the beads for 3 h. After centrifugation at $1000 \times g$ for 15 min, radioactivity was determined using a plate counter (PerkinElmer Wallac, Gaithersburg, MD).

2.4. Data analysis

In the isolated tissue studies, apparent antagonist dissociation constants (K_B) were determined according to the following equation for competitive agonist activity (Furchgott, 1972):

$$K_B = [B]/(\text{Dose ratio} - 1)$$

where [B] is the concentration of antagonist and the dose ratio is EC_{50} of the agonist in the presence of the antagonist divided by control EC_{50}. The EC_{50} was calculated as the concentration of 2-CH_3 5-HT required to produce half-maximal contraction. These results were then expressed as the negative logarithm of K_B (i.e., pK_B).

In addition, for apparent noncompetitive kinetics as occurred with olanzapine (10^{-6} M), antagonist equilibrium dissociation constants were estimated according to the following equations:

$$K_B = [B]/\text{Slope} - 1$$

where [B] equals the antagonist concentration and slope is determined from a double reciprocal plot of $1/x^1$ vs. $1/x$ where x^1 and x are equieffective concentrations of serotonin in the presence (x^1) and absence (x) of inhibitor (Kenakin, 1993; Steinberg et al., 1994).

In the 5-HT$_6$ receptor binding studies, inhibition constants (K_i) values were calculated from the Cheng–Prusoff equation ($K_i = IC_{50}/[1 + \text{Conc}/K_d$ (1.6 nM) of radioligand]) (Cheng and Prusoff, 1973) where IC_{50} is the concentration required to inhibit the activity 50%. For the [^{35}S]GTPγS binding studies, concentration response curves were fitted using sigmoidal nonlinear regression with variable slope in Graphpad Prism. Equilibrium K_B values for antagonists were calculated in the presence of a fixed concentration of agonist from the Cheng–Prusoff equation ($K_B = IC_{50}/(1 + \text{concentration of 5-HT}/EC_{50}$ of 5-HT)).

2.5. Materials

Anti-rabbit-IgG and wheat germ agglutin (WGA)-coated SPA beads were obtained from Amersham (Arlington Heights, IL). The radioligands [^{3}H]LSD and [^{35}S]GTPγS were obtained from Dupont NEN (Beltsville, MD). Nonidet P-40 (10%) was obtained from Boehringer Mannheim (Indianapolis, IN) and anti-Gs/olf was obtained from Santa

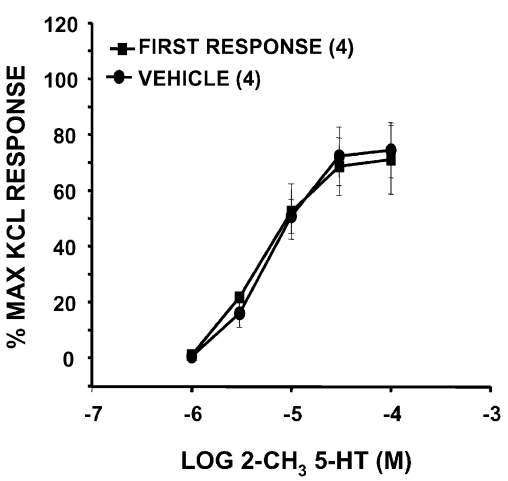

Fig. 1. Noncumulative concentration-dependent increase of contractile response in isolated guinea pig ileum by 2-CH_3 5-HT. The response is compared between first addition stepwise of receptor agonist followed by several washes and a second stepwise addition. The response is expressed as percent of maximal (Max) KCl response ± standard error of the mean (S.E.M.) at 67 mM KCl. Numbers in parenthesis indicate number of replicates.

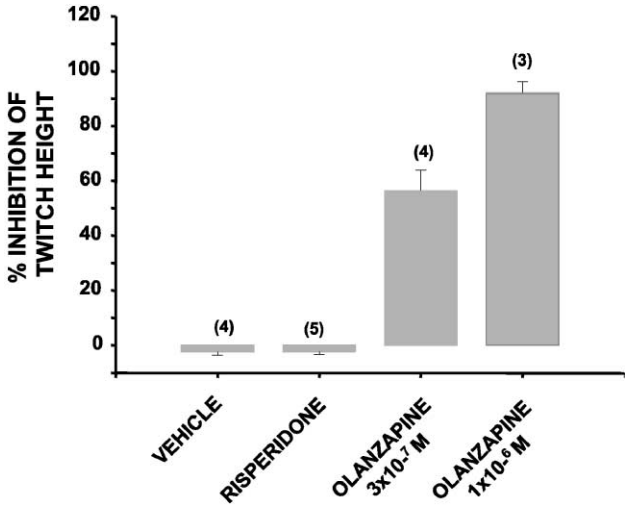

Fig. 2. Inhibition of electrically stimulated contractile response of isolated guinea pig ileum by vehicle, risperidone (1×10^{-6} M) or olanzapine at 3×10^{-7} and 1×10^{-6} M. Data are expressed as percent of inhibition of contractile response ± S.E.M. Numbers in parenthesis indicate number of replicates.

Cruz Biotechnology (Santa Cruz, CA). Olanzapine, quetiapine, ondansetron, and ziprasidone were provided by the Lilly Research Laboratories. Haloperidol, clozapine, tropisetron, 2-CH_3 5-HT and risperidone were obtained from RBI (Natick, MA).

3. Results

3.1. Agonist and antagonist activity at 5-HT_3 receptors

The 5-HT_3 receptor agonist 2-CH_3 5-HT produced a marked, concentration-dependent contraction of guinea pig ileum (Fig. 1) as previously reported (Cohen et al., 1989). The second response to 2-CH_3 5-HT resulted in comparable contractions to each concentration as observed with the first response, indicating that two concentration response curves to 2-CH_3 5-HT may be studied in each tissue.

Table 1
Affinity of olanzapine, risperidone, ondansetron and tropisetron for 5-HT_3 receptors and inhibition of contractile response to 2-CH_3 5-HT in guinea pig ileum

Compound	Binding affinity, $pK_i \pm$ S.E.M., M ($K_i \pm$ S.E.M., nM)	Inhibition of contractions, $pK_B \pm$ S.E.M., M (K_B, nM)
Olanzapine	7.24 ± 0.04[a] (57 ± 6)[a]	6.38 ± 0.03 (417)
Risperidone	< 5[a] ($> 10{,}000$)[a,b]	< 6[b] (> 1000)
Ondansetron	8.4 ± 0.1[c] (3.98)[c]	6.95 ± 0.01 (112)
Tropisetron	8.3 ± 0.4[c] (5.01)[c]	7.74 ± 0.11 (18)

[a] Data from Bymaster et al., 1996.
[b] Effect at highest concentration tested.
[c] Data from Eglen et al., 1993.

Olanzapine did not contract the guinea pig ileum in concentrations up to 10^{-6} M (data not shown). However, olanzapine (300 and 1000 nM) inhibited the twitch response in the guinea pig ileum, but risperidone (1000 nM) did not significantly alter the twitch height (Fig. 2). Olanzapine (300 and 1000 nM) significantly inhibited the contractile response of 2-CH_3 5-HT with moderate affinity ($pK_B = 6.38 \pm 0.03$) (Table 1), whereas risperidone (1000 nM) did not significantly inhibit contraction to 2-CH_3 5-HT in guinea pig ileum (Fig. 3). The 5-HT_3 receptor antagonists, ondansetron (1000 nM) and tropisetron (1000 nM), dextrally shifted the contractile response to 2-CH_3

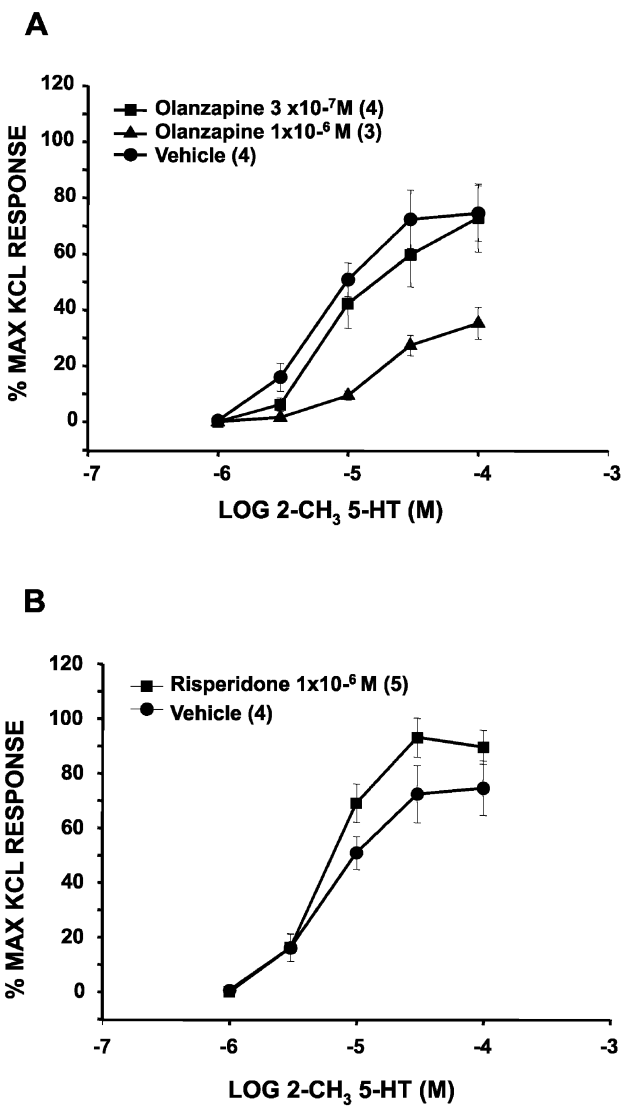

Fig. 3. Inhibition of 2-CH_3 5-HT-induced contractile response in isolated guinea pig ileum by olanzapine (A) (3×10^{-7} and 1×10^{-6} M) and risperidone (B) (1×10^{-6} M). The response is compared between first addition stepwise of receptor agonist in the presence of vehicle followed by several washes and a second stepwise addition in the presence of receptor antagonist. The response is expressed as percent of maximal (Max) KCl response ± S.E.M. at 67 mM KCl. Numbers in parenthesis indicate number of replicates.

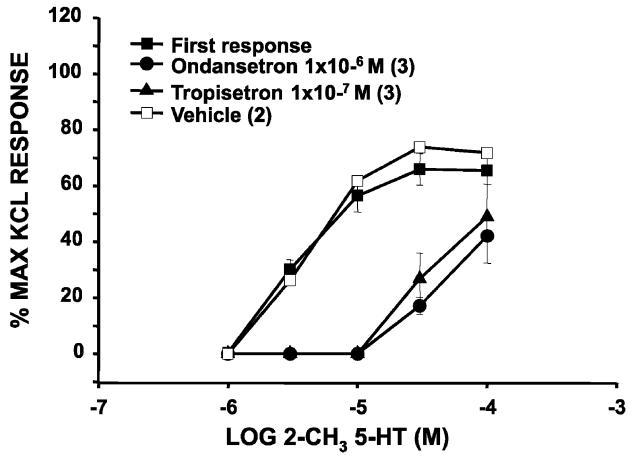

Fig. 4. Inhibition of 2-CH_3 5-HT-induced contractile response in isolated guinea pig ileum by ondansetron (1×10^{-6} M) and tropisetron (1×10^{-7} M). The response is compared between first additions stepwise of receptor agonist in the presence of vehicle followed by several washes and a second stepwise addition in the presence of receptor antagonist. The response is expressed as percent of maximal (Max) KCl response ± S.E.M. at 67 mM KCl. Numbers in parenthesis indicate number of replicates.

5-HT with pK_B values of 6.95 ± 0.01 and 7.74 ± 0.11, respectively (Fig. 4, Table 1). These antagonist dissociation constants are in general agreement with previously generated values for ondansetron and tropisetron (Cohen et al., 1989; Eglen et al., 1993). Overall, the 5-HT_3 receptor antagonists had higher 5-HT_3 receptor affinity in the binding studies than in the functional assays, consistent with previous studies and suggesting a guinea pig variant of the receptor (Eglen et al., 1993; Butler et al., 1990).

3.2. Inhibition of binding to 5-HT_6 receptors

Olanzapine, clozapine and ziprasidone potently inhibited binding of [^{3}H]LSD to human 5-HT_6 receptors with pK_i values of 8.30, 8.28, and 7.52, respectively (Fig. 5, Table 2). Risperidone, quetiapine and haloperidol had low affinity for human 5-HT_6 receptors with pK_i values of < 6. The affinities reported here are in general agreement with previously reported values (Roth et al., 1994; Kohen et al., 1996).

Table 2
Inhibition of binding of [^{3}H]LSD and serotonin-stimulated [^{35}S]GTPγS to human 5-HT_6 receptors by olanzapine and antipsychotic drugs

Compound	[^{3}H]LSD, $pK_i \pm$S.E.M., M ($K_i \pm$S.E.M., nM)	[^{35}S]GTPγS, $pK_B \pm$S.E.M., M ($K_B \pm$S.E.M, nM)
Olanzapine	8.30 ± 0.06 (5.0 ± 0.8)	7.38 ± 0.16 (42 ± 15)
Clozapine	8.28 ± 0.06 (5.2 ± 0.8)	7.42 ± 0.15 (38 ± 12)
Risperidone	5.59 ± 0.09 (2586 ± 570)	< 5 ($> 10{,}000$)[a]
Quetiapine	5.65 ± 0.03 (2241 ± 151)	< 5 ($> 10{,}000$)[a]
Ziprasidone	7.52 ± 0.03 (30 ± 2)	6.94 ± 0.11 (114 ± 27)
Haloperidol	5.35 ± 0.06 (4475 ± 570)	< 5 ($> 10{,}000$)[a]

Drugs were evaluated in triplicate from six to nine concentrations as described in Materials and methods.
[a] Less than 50% inhibition at 10,000 nM.

3.3. Effects on [^{35}S]GTPγS binding to 5-HT_6 receptors

The binding of [^{35}S]GTPγS to cell membranes possessing the human 5-HT_6 receptor was determined using an antibody capture method for the G protein G_s (DeLapp et al., 1999). 5-HT stimulated binding of [^{35}S]GTPγS to membranes from cells transfected with human 5-HT_6 receptors with an EC_{50} value of 43 ± 20 nM and increased binding from the basal level of 4004 ± 850 to a maximal level of 9076 ± 1623 cpm in a representative experiment (Fig. 6). In contrast, olanzapine and clozapine did not stimulate [^{35}S]GTPγS binding, indicating a lack of agonist activity. However, olanzapine and clozapine dose-dependently decreased 5-HT (1000 nM)-induced increases in

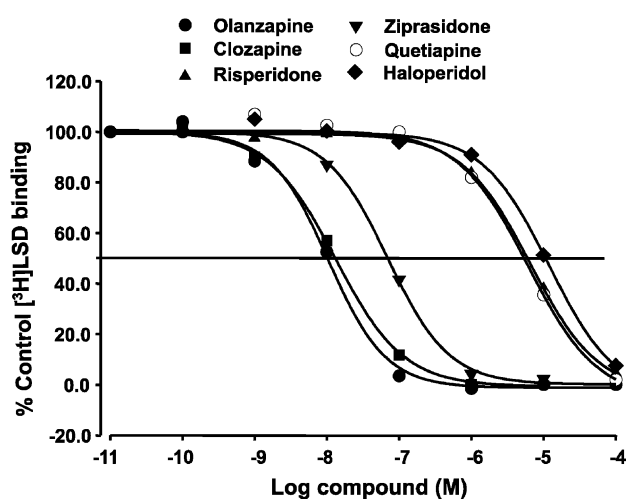

Fig. 5. Concentration-dependent inhibition of [^{3}H]LSD binding to clonal 5-HT_6 receptors by drugs. Data are expressed as percent control specific binding of 2 nM [^{3}H]LSD with triplicate samples.

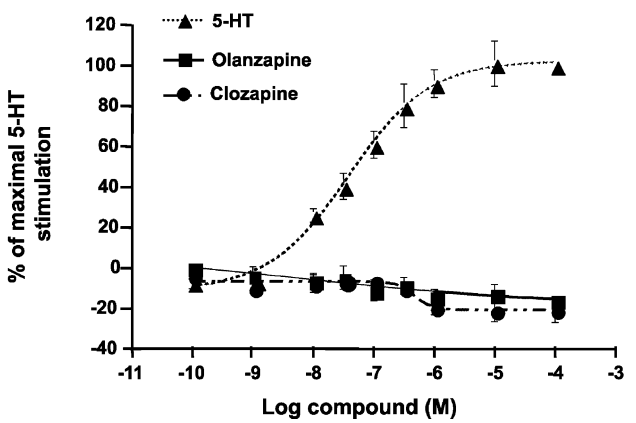

Fig. 6. Stimulation of [^{35}S]GTPγS binding to clonal human 5-HT_6 receptors by serotonin (5-HT), olanzapine and clozapine. The binding of [^{35}S]GTPγS to membranes containing 5-HT_6 receptors was determined with four replicates using an antibody capture technique for the G protein G_s. Data are expressed as percent maximal stimulation ± S.E.M. of [^{35}S]GTPγS binding by 1×10^{-4} M 5-HT.

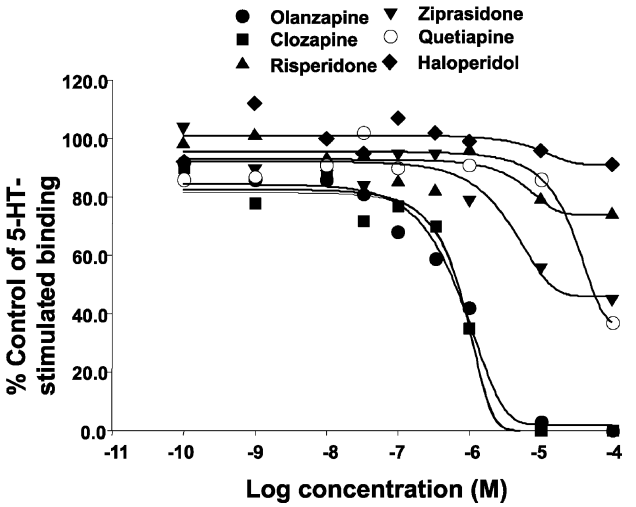

Fig. 7. Concentration-dependent inhibition of 5-HT-stimulated [^{35}S]GTPγS binding to clonal human 5-HT$_6$ receptors by drugs. The binding of [^{35}S]GTPγS to membranes containing 5-HT$_6$ receptors was determined with four replicates using an antibody capture technique for the G protein G_s. Data are expressed as percent maximal stimulation ± S.E.M. of [^{35}S]GTPγS binding by 1×10^{-6} M 5-HT.

[^{35}S]GTPγS binding to 5-HT$_6$ receptors with IC_{50} values of approximately 600 nM and calculated pK_B values of 7.38 ± 0.16 and 7.42 ± 0.15, respectively (Fig. 7, Table 2). Ziprasidone decreased 5-HT-induced increases in [^{35}S]GTPγS binding with a pK_B value of 6.94 ± 0.11 but did not completely inhibit [^{35}S]GTPγS binding. Ziprasidone alone did not significantly stimulate [^{35}S]GTPγS binding indicating no agonist activity (data not shown), but its activity may be limited by poor solubility in the buffer. Risperidone, quetiapine and haloperidol did not block 5-HT-induced increases in [^{35}S]GTPγS binding greater than 50% at a concentration of 10 μM.

4. Discussion

Olanzapine possessed marked affinity for the 5-HT$_3$ receptor in binding assays (Bymaster et al., 1996), and has antagonist activity at 5-HT$_3$ receptors. In contrast, the atypical antipsychotic risperidone had lower affinity for 5-HT$_3$ receptors (Bymaster et al., 1996) and did not significantly block 2-CH_3 5-HT-induced contraction in the guinea pig ileum. Clinically available antagonists of 5-HT$_3$ receptors such as ondansetron have been shown to block emesis induced by cancer therapeutic agents including cisplatin (Perez et al., 1991; Cubeddu et al., 1990), possibly by blocking 5-HT$_3$ receptors highly localized in the gastrointestinal tract as well as the chemoreceptor trigger zone in the area postrema (Morales et al., 1998; Miyake et al., 1995). However, these antagonists are quite expensive and also are known as a class to produce prolonged cardiac (QTc) conduction changes (Kuryshev et al., 2000; Benedict et al., 1996; De Lorenzi et al., 1994), which, in some cases, may increase the risk of arrhythmias and potential fatal outcomes (Haverkamp et al., 2000). Therefore, the breadth of their clinical application is limited.

Olanzapine is only about three-fold less potent as an antagonist of 5-HT$_3$ receptors as ondansetron and unlike ondansetron, has affinity for dopamine D_2 receptors (Bymaster et al., 1996). Dopamine D_2 receptor antagonism is also used clinically to reduce some forms of nausea as is seen in the broad use of the nonantipsychotic phenothiazine compounds (Alberts et al., 1996). Therefore, olanzapine's combination of two potential antiemetic properties, antagonism of 5-HT$_3$ and dopamine D_2 receptors, could produce potent antiemetic activities. Indeed, olanzapine has been clinically used as an antiemetic in patients with chronic morphine-induced nausea (Passik et al., 2000). Opioid-induced nausea is a condition which is resistant to either 5-HT$_3$ or dopamine D_2 receptor blockade alone and frequently requires combined 5-HT$_3$ plus dopamine D_2 receptor antagonist therapy (Shoji et al., 1999; Danzer et al., 1997; Pitkanen et al., 1987; Cole et al., 1994).

Antagonists of 5-HT$_3$ receptors have also been shown to be useful for treatment of irritable bowel syndrome (Steadman et al., 1992; Prior and Read, 1993; Mangel and Northcutt, 1999). Olanzapine's 5-HT$_3$ receptor antagonism, along with its novel anxiolytic-like activity in the punished responding model in animals (Moore et al., 1994; Benvenga and Leander, 1995) and reduction of anxiety symptoms in patients with schizophrenia (Tollefson et al., 1998), suggest that olanzapine could affect both smooth muscle abnormalities and stress symptoms associated with the disorder. Furthermore, the finding that olanzapine inhibited guinea pig ileum motility induced by field stimulation makes it a particularly attractive candidate for irritable bowel syndrome, diarrhea prominent. The inhibitory effect of olanzapine on the twitch response is probably not due to 5-HT$_3$ receptor antagonism since ondansetron and tropisetron did not inhibit the twitch in guinea pig ileum, but more likely may be attributed to the relatively weak antimuscarinic activity of olanzapine (Bymaster and Falcone, 2000). Antagonists of 5-HT$_3$ receptors have also been suggested to be potentially useful in the treatment of fibromyalgia, a chronic pain syndrome which is considered to be difficult to treat (Farber et al., 2000), and also have been suggested to have a potential role in the treatment of movement disorder syndromes such as Tourette's syndrome (Toren et al., 1999) and neuroleptic-induced tardive dyskinesia (Sirota et al., 2000).

Behavioral studies have also suggested that 5-HT$_3$ receptor antagonists may have an anxiolytic and an antipsychotic property (Higgins and Kilpatrick, 1999). Recently, an open study of ondansetron provided preliminary clinical evidence that 5-HT$_3$ receptor antagonists may diminish psychosis in patients with schizophrenia (Sirota et al., 2000); however, thus far, these agents have not proven therapeutically useful for those indications (for review, see Higgins and Kilpatrick, 1999). It is not clear what impact, if any, 5-HT$_3$ receptor antagonism has on the clinically

reported anxiolytic (Tollefson et al., 1998) and antipsychotic properties of olanzapine (Beasley et al., 1996). Clinical trials of olanzapine in a range of patients, including patients with schizophrenia or bipolar disorder as well as elderly, frail, female patient with Alzheimer's disease, did not identify a clinically significant effect on the QTc interval at therapeutic doses (Czekalla et al., 2001; Tohen et al., 2000; Street et al., 2000). Therefore, olanzapine should be evaluated as a clinical treatment for patients who currently receive selective 5-HT_3 receptor antagonist therapy.

Olanzapine also had high affinity for 5-HT_6 receptors and was without agonist activity at this receptor. However, olanzapine and clozapine were potent antagonists of 5-HT-induced increases in [^{35}S]GTPγS binding in membranes transfected with human 5-HT_6 receptors. Ziprasidone blocked 5-HT-stimulated [^{35}S]GTPγS binding with about three-fold less potency than olanzapine and clozapine, whereas the other antipsychotic agents including risperidone, quetiapine, and haloperidol were only weak antagonists of 5-HT_6 receptors. Animal studies have suggested that 5-HT_6 receptor inhibition may play a role in several neuropsychiatric disorders. For example, antisense oligonucleotides for the 5-HT_6 receptor abolished the enhancement of 5-HT release in the prefrontal cortex produced by condition fear stress, suggesting a role for 5-HT_6 receptor antagonists in anxiety disorders (Yoshioka et al., 1998). The 5-HT_6 receptor antagonist, 4-amino-*N*-(2,4 bis-methylamino-pyrimidin-4-yl) benzene sulphonamide (Ro 04-6790), inhibited rotational behavior induced by muscarinic receptor antagonists in unilaterally lesioned 6-hydroxydopamine-treated rats, indicating a role in control of cholinergic neurotransmission (Bourson et al., 1998). Compound Ro 04-6790 has also been shown to promote learning consolidation under normal and dysfunctional memory conditions in rats (Meneses, 2001). The 5-HT_6 receptor antagonist 5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfon-amide (SB-271046) enhanced cognition in two models of learning and memory in rats. Retention of a previously learned position in a water maze task in rats and performance of a delayed alternation task by aged rats was enhanced by SB-271046 (Rogers et al., 1999). Thus, 5-HT_6 receptor antagonists may improve cognition, possibly by enhancement of cholinergic neurotransmission.

The potential therapeutic roles for 5-HT_6 receptor antagonist drugs have been recently reviewed (Branchek and Blackburn, 2000). In addition, recent genetic studies of humans suggest the presence of abnormalities of the 5-HT_6 receptor gene in schizophrenia, bipolar disorder, and Alzheimer's disease (Vogt et al., 2000; Tsai et al., 1999a,b). Consistent with these studies, olanzapine has been shown to improve psychotic and nonpsychotic symptoms in patients with schizophrenia (Beasley et al., 1996; Tollefson et al., 1998), bipolar disorder (Tohen et al., 1999), and Alzheimer's disease (Street et al., 2000), as well as cognitive dysfunction in patients with early phase schizophrenia (Purdon et al., 2000). This potential broad-spectrum therapeutic activity may be due, at least in part, to antagonism of 5-HT_6 receptors. Furthermore, because 5-HT_6 receptor antagonism has been suggested to enhance the functioning of the central nervous system's cholinergic pathways (Bourson et al., 1998), any central anticholinergic adverse events potentially attributable to olanzapine's moderate to weak acetylcholine receptor antagonism (Bymaster and Falcone, 2000) might be mitigated by 5-HT_6 receptor antagonism. In this regard, a recent double-blind clinical study of 206 patients with Alzheimer's disease found no significant difference between patients treated with olanzapine or placebo on 30 central or peripheral potential anticholinergic adverse events (Street et al., 2000), although the sum of all peripheral potential anticholinergic adverse events was significantly greater in the olanzapine 15 mg/day group compared to placebo.

In summary, the present study documents relatively potent 5-HT_3 and 5-HT_6 receptor antagonist activity of olanzapine, properties that may contribute to the antipsychotic effects of olanzapine and that warrant evaluation of olanzapine as a treatment for other illnesses such as chemotherapy-induced emesis, irritable bowel syndrome and certain cognitive disorders.

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