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Radioreceptor Binding Profile of the Atypical Antipsychotic Olanzapine

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The affinities of olanzapine, clozapine, haloperidol, and four potential antipsychotics were compared on binding to the neuronal receptors of a number of neurotransmitters. In both rat tissues and cell lines transfected with human receptors olanzapine had high affinity for dopamine D_1, D_2, D_4, serotonin $(5HT)_{2A}$, $5HT_{2C}$, $5HT_3$, α_1-adrenergic, histamine H_1, and five muscarinic receptor subtypes. Olanzapine had lower affinity for α_2-adrenergic receptors and relatively low affinity for $5HT_1$ subtypes, $GABA_A$, β-adrenergic receptors, and benzodiazepine binding sites. The receptor binding affinities for olanzapine was quite similar in tissues from rat and human brain. The binding profile of olanzapine was comparable to the atypical antipsychotic clozapine, while the binding profiles for haloperidol, resperidone, remoxipride, Org 5222, and seroquel were substantially different from that of clozapine. The receptor binding profile of olanzapine is consistent with the antidopaminergic, antiserotonergic, and antimuscarinic activity observed in animal models and predicts atypical antipsychotic activity in man.

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KEY WORDS: *Olanzapine; Clozapine; Haloperidol; Antipsychotic; Binding profile; Dopamine; Risperidone; Seroquel; Org 5222*

Neuroleptics have been used for several decades to treat the positive symptoms of schizophrenia such as delusions and hallucinations (Davis and Casper 1974). However, classical neuroleptics like haloperidol produce movement disorders referred to as extrapyramidal side effects (EPSs) and tardive dyskinesia (Tarsy 1983). Antipsychotics are believed to act via blockade of dopamine D_2 receptors (Seeman et al. 1976), and the EPSs have been attributed to a high degree of occupation of dopamine D_2 receptors in the striatum of neuroleptic-treated schizophrenics (Farde et al. 1989).

Unlike typical antipsychotics, clozapine, a dibenzodiazepine, has been found to be an effective antipsychotic that rarely produces EPSs or tardive dyskinesia (Casey 1989; Claghorn et al. 1987) and thus was termed atypical. Further, clozapine uniquely reduced the negative symptoms as well as the positive symptoms of schizophrenia and was active in a portion of treatment-resistant patients (Kane et al. 1988). Explanations of the atypical nature of clozapine have focusd on its interaction with several neuronal receptors other than dopamine D_2, including dopamine D_1 (Andersen et al. 1986), dopamine D_4 (Van Tol et al. 1991), serotonin $(5HT)_{2A}$ (Meltzer et al. 1989), $5HT_{2C}$ (Canton et al. 1990; Roth et al. 1992), and muscarinic subtypes (Miller and Hiley 1974). In addition, clozapine was recently reported to have high affinity for the cloned $5HT_6$ and $5HT_7$ receptors (Roth et al. 1994). Although clozapine is an efficacious antipsychotic and may be considered the prototype for atypical antipsychotics, its use has

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Table 1. Experimental Conditions for Radioligand Binding to Subtypes of Dopamine and Serotonin Receptors

Receptor Subtype	[^{3}H]ligand Concentration (K_d, nM)	Membrane Source	Buffer	Incubation Time (min) temp °C	Nonspecific Binding Compound (μM)	Reference
Dopamine D_1	SCH23390 0.2, 0.39	Striatum	Tris Cl[a]	30, 22	SCH23390 0.03	Seeman et al. 1979
Dopamine D_2	Raclopride 0.8, 1.26	Striatum	Tris Cl[a]	30, 22	Spiperone 0.03	Hall et al. 1988
Dopamine D_4	Spiperone 0.25, 0.07	COS-7 cells	Tris Cl[b]	120, 22	Dopamine 30	Seeman and Van Tol 1993a
$5HT_{1A}$	8-OHDPAT 0.4, 2.9	Rat cortex	Tris Cl[c]	30, 22	Spiperone 10	Wong et al. 1991
$5HT_{1B}$	5HT 1, 2.2	Rat cortex	Tris Cl[c,d]	15, 37	5HT 10	Wong et al. 1991
$5HT_{1D}$	5HT 1, 4.4	Beef striatum	Tris Cl[c,d]	15, 37	5HT 10	Wong et al. 1991
$5HT_{2A}$	Ketanserin 0.4, 1.4	Cortex	Tris Cl[c]	30, 37	Spiperone 10	Wong et al. 1991
$5HT_{2C}$	Mesulergine 2	Human cortex	Tris Cl[c,e]	30, 37	Mianserin 10	Wong et al. 1991
$5HT_{2C}$	Mesulergine 2, 1.0	Beef choroid plexus	Tris Cl[c]	30, 37	Mianserin 10	Wong et al. 1991
$5HT_3$	LY278584 1, 0.7	Rat cortex	Tris Cl[c]	30, 25	5HT 10	Wong et al. 1991

The concentration of Tris Cl buffer was 50 mM at pH 7.4
[a] Salts added: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$.
[b] Salts added: 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA.
[c] Added 10 μM pargyline and 0.1 mg/ml ascorbic acid.
[d] Added SCH23390 and 8-OHDPAT at 100 nM each as masking agents.
[e] Added ketanserin and 8-OHDPAT at 100 nM each as masking agents.

been curtailed because of occurrence of agranulocytosis in 1% to 3% of patients (Krupp and Barnes 1992).

Recently, a series of thienobenzodiazepines have been synthesized (Chakrabarti et al. 1980). One member of the series, olanzapine [LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-B][1,5]benzodiazepine], has been shown to have a pharmacological profile in animals similar to that of clozapine (Moore et al. 1992). For example, in an animal model of antipsychotic activity, conditioned avoidance responding, olanzapine had a favorable ratio of potency versus occurrence of catalepsy, a result that may be predictive of a low incidence of EPSs in man (Moore et al. 1992). In addition, like clozapine, olanzapine had potent antiserotonergic activity in vivo as evidenced by the inhibition of 5-hydroxytrytophan-induced head twitches in mice (Moore et al. 1992) and quipazine-induced increases in serum corticosterone in rats (Fuller and Snoddy 1992). Olanzapine had high affinity for cloned $5HT_6$ receptors as was found with clozapine, but had only moderate affinity for cloned $5HT_7$ receptors (Roth et al. 1994). Further, as with clozapine, chronic treatment of rats with olanzapine reduced the number of spontaneously active dopamine cells in the ventral tegmental area, but not in the substantia nigra (Stockton and Rasmussen 1996). Moreover, in clinical trials, olanzapine has been shown to have antipsychotic activity and did not produce appreciable EPSs (Beasley et al. 1996). We report here that olanzapine has a binding profile similar to that of clozapine and exhibits high affinity for dopamine D_1, D_2, D_4, $5HT_{2A}$, $5HT_{2C}$, $5HT_3$, muscarinic, α_1-adrenergic, and histamine H_1 receptors in rat and human tissue and in cell lines transfected with human receptors. The binding profile of olanzapine was compared to clozapine, haloperidol, and the new potential antipsychotics risperidone (Roose et al. 1988), remoxipride (Lewander et al. 1990), Org 5222 (Siten and Vrijmoed-de Vries 1992) and seroquel (Migler et al. 1993). Portions of these data have been presented in preliminary form (Moore et al. 1993; Wong et al. 1993).

MATERIALS AND METHODS

For serotonergic and muscarinic receptor binding assays, male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 100–150 g were sacrificed by decapitation, the brains quickly removed and either whole brain was obtained or cerebral cortex and striatum were dissected on ice. Beef brain was removed immediately after slaughter and striatum and choroid plexus were dissected over ice. Membranes were prepared according to previously described methods (Wong et al. 1991). For dopamine D_1, D_2, α_1-, α_2-, β-adrenergic, histamine H_1, and benzodiazepine receptor binding, the rat brain tissues were obtained from Pel-Freeze Biologicals (Rogers, AR), and membranes were prepared according to appropriate methods as detailed in Tables 1 and 2.

Hearts were removed from rats, blotted, and homogenized in 50 volumes of 50 mM Tris-Cl buffer, pH 7.4, for 30 seconds with a polytron. Cardiac membranes were isolated by centrifugation at 50,000 × g for 10 minutes, resuspension of the pellet in fresh buffer,

Table 2. Experimental Conditions for Radioligand Binding to Subtypes of Muscarinic and Other Receptors

Receptor Subtype	[^{3}H]ligand Concentration (K_d, nM)	Membrane Source	Buffer	Incubation Time (min temp °C)	Nonspecific Binding Compound (μM)	Reference
Muscarinic m_1	Pirenzepine 1, 3	Cortex	Tris Cl 20 mM pH 7.4[a]	60, 25	Atropine 1	Potter et al. 1988
Muscarinic m_2	NMS 0.24, 0.3	Rat heart	NaPi 50 mM pH 7.4[b]	120, 25	Atropine 1	Waelbroeck et al. 1990
Muscarinic m_3	NMS 0.24, 0.08	Salivary gland	NaPi 50 mM pH 7.4[b,c]	120, 25	Atropine 1	Lazareno et al. 1990
Muscarinic m_4	NMS 0.24, 0.05	Rat striatum	NaPi 50 mM pH 7.4[d]	120 + 45 dissociation, 25	Atropine 1	Waelbroeck et al. 1990
Musarinic m_1	NMS 0.24, 0.06	CHO-K1	NaPi 50 mM pH 7.4[b]	120, 25	Atropine 1	Dorje et al. 1991
Muscarinic m_3	NMS 0.24, 0.06	CHO-K1	NaPi 50 mM pH 7.4[b]	120, 25	Atropine 1	Dorje et al. 1991
Muscarinic m_4	NMS 0.24, 0.05	CHO-K1	NaPi 50 mM pH 7.4[b]	120, 25	Atropine 1	Dorje et al. 1991
Muscarinic m_5	NMS 0.24, 0.22	CHO-K1	NaPi 50 mM pH 7.4[b]	120, 25	Atropine 1	Dorje et al. 1991
Adrenergic α_1	Prazosin 0.2, 0.05	Whole brain	Tris Cl 50 mM pH 7.7	30, 25	WB4101 0.1	Greengrass and Bremner 1979
Adrenergic α_2	Rauwoscine 0.4, 0.6	Whole brain	Tris Cl 50 mM pH 7.7[d]	15, 22	Mianserin 10	Boyajian and Leslie, 1987
Adrenergic β	DHA 0.2, 0.16	Whole brain	Tris Cl 50 mM pH 7.7	15, 23	(−) Propanolol 1	Bylund and Snyder 1976
Histamine H_1	Pyrilamine 2, 4.0	Whole brain	NaPi 50 mM pH 7.5	30, 25	Promethazine 10	Tran et al. 1978
$GABA_A$	Muscimol 2, 0.84	Cortex	Tris Cl 50 mM pH 7.4	30, 37	GABA 10	Williams and Risley 1979
Benzodiazepine	Flunitrazepam 2, 1.85	Whole brain	Tris Cl 50 mM pH 7.4	20, 37	Clonazepam 10	Braestrup and Squires 1977

[a] Added 1 mM $MnCl_2$.
[b] Added 2 mM $MgCl_2$.
[c] Added 100 mM NaCl.
[d] Salts added: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$.

and centrifugation again. The cardiac membranes were resuspended at 0.5 g/3 ml buffer and frozen at −70°C until used. Submaxillary salivary glands from rats were homogenized in 50 volumes of 50 mM Na phosphate, pH 7.4, containing 100 mM NaCl, and membranes were isolated by centrifugation at 50,000 × g for 10 minutes, resuspension of the pellet in fresh buffer, and centrifugation again. Large pieces of tissue were removed from the homogenate by filtering through cheesecloth. Binding in salivary glands was determined in tissue that had not been frozen.

Chinese hamster ovary cell lines (CHO-K1) transfected with muscarinic receptor subtypes (Dorje et al. 1991) were obtained from Dr. Mark Brann at the University of Vermont. The cells were grown in a monolayer at 37°C in a humidified atmosphere containing 5% CO_2 and were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), 100 units of penicillin G/l, 100 μg streptomycin/l, 4 mM L-glutamine and 100 μM MEM nonessential amino acids. After growing to about 80% confluency, the cells were harvested with 0.25% trypsin in Ca^{++}-free medium, centrifuged, and frozen until used. After vigorous suspension with a polytron, the cells were washed two times with 20 mM Tris-Cl buffer, pH 7.4, followed by centrifugation. The number of cells/tube were adjusted to bind 5% to 8% of the radioligand. For dopamine D_4 binding COS-7 cells were transiently transfected with the human D_4 receptor, and binding to receptors was determined as described in Seeman and Van Tol (1993a).

Autopsy samples of human frontal cortex and corpus striatum (Analytical Biological Services, Inc., Wilmington, DE) were processed identically to the method for rat brain tissue.

The binding assay methods are summarized in Tables 1 and 2. After incubation for the specified period, the homogenates were filtered through glass filters (Whatman, GF/c or GF/b, Maidstone, England) with vacuum. The filters were washed several times with cold buffer and placed in scintillation vials containing 10 ml of scintillation fluid (Ready Protein$^+$, Beckman, Fullerton, CA). Filters were presoaked in either 0.05% or 0.1% polyethylenimine for several hours. Radioactivity trapped on the filters was determined by liquid scintillation spectrometry at approximately 40% efficiency.

The mean IC_{50} values were generally obtained

Table 3. The K_i Values for Olanzapine, Clozapine, and Other Antipsychotic Compounds for Dopamine Receptor Subtypes

Compound	K_i (nM)		
	D_1	D_2	D_4
Olanzapine	31 ± 0.7	11 ± 2	27 ± 3
Clozapine	85 ± 0.7	125 ± 20	9 ± 1[b], 21 ± 2[c]
Risperidone	75 ± 8	3 ± 0.1	7 ± 1[c]
Remoxipride	>10,000	275 ± 180	3690 ± 360[b]
Seroquel	455 ± 105	160 ± 15	–
Org 5222	5 ± 0.1	1 ± 0.1	–
Haloperidol	25 ± 7	1 ± 0.04	5 ± 0.5[b]

[a] The K_i ± SE values for the dopamine receptor subtypes were determined as described in Materials and Methods. All compounds were tested in at least three independent experiments at each receptor subtype.
[b] Data from Van Tol et al. 1991.
[c] Data from Seeman and Van Tol 1993b.

from at least three separate experiments performed in duplicate or triplicate with at least 6 to 11 concentrations of drugs. The data were analyzed and IC_{50} values determined using either Allfit (De Lean et al. 1978) or Ligand (Munson and Rodbard 1980) software programs, and inhibition constants (K_i) were calculated utilizing the Cheng-Prusoff equation (Cheng and Prusoff 1973). The Hill coefficients of the antipsychotics for the various neuronal receptors were not significantly different from unity.

Olanzapine was synthesized in the Lilly Research Laboratories and the other antipsychotics were from the following sources: clozapine (Sandoz), risiperidone (Janssen), remoxipride (Astra), seroquel (Zeneca), Org 5222 (Organon), and haloperidol (Research Biochemicals, Inc., Natick, MA). The following radioligands were used for the binding studies: [^{3}H]pirenzepine (87.0 Ci/mmol), [^{3}H]n-methylscopolamine ([^{3}H]NMS, 79.5 Ci/mmol), [^{3}H]8-OHDPAT (142.9 Ci/mmol), [^{3}H]5HT (25.4 Ci/mmol), [^{3}H]ketanserin (60 Ci/mmol), [^{3}H]-prazosin (70 Ci/mmol), [^{3}H]rauwolscine (70 Ci/mmol), [^{3}H]dihydroalprenolol ([^{3}H]DHA, 70 Ci/mmol), [^{3}H]-SCH23390 (79 Ci/mmol), [^{3}H]raclopride (60 Ci/mmol), [^{3}H]flunitrazepam (60 Ci/mmol), [^{3}H]pyrilamine (20 Ci/mmol), and [^{3}H]muscimol (20 Ci/mmol) were purchased from New England Nuclear Corp.; and [^{3}H]mesulergine (85 Ci/mmol) and [^{3}H]LY278584 (80.5 Ci/mmol) were supplied by Amersham Laboratories. All other chemicals used were reagent grade and were obtained from Sigma Chemical Company (St. Louis, MO).

RESULTS

Olanzapine had high affinity for dopamine receptor subtypes (Table 3, Fig. 1). Olanzapine had inhibition constants (K_i) of 31 and 11 nM for D_1 and D_2 receptors in rat striatum, respectively. Moreover, olanzapine inhibited binding to human D_4 receptors transfected into COS-7 cells with a K_i of 27 nM. Olanzapine had higher affinity than clozapine for D_1 and D_2 receptors, but clozapine had slightly higher affinity for D_4 receptors (Table 3; Seeman and Van Tol 1993b; Van Tol et al. 1991). Haloperidol, risperidone, and Org 5222 had high affinity for D_2 receptors and were considerably less potent on D_1 receptors. In addition, haloperidol and risperidone had high affinity for D_4 receptors (Seeman and Van Tol 1993b; Van Tol et al. 1991). Remoxipride and seroquel had relatively low affinity for D_1 and D_2

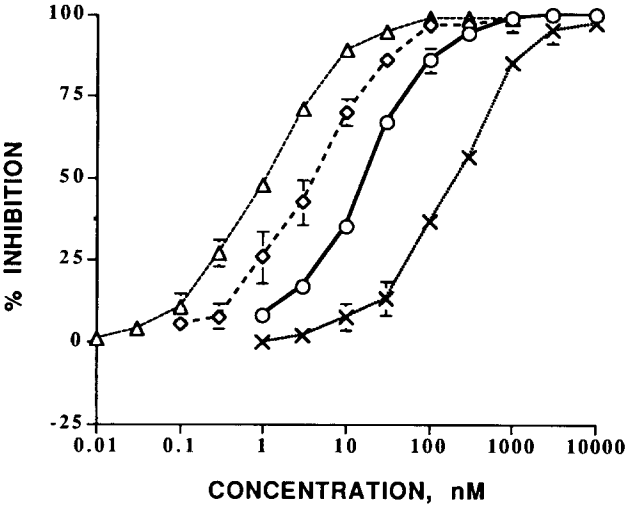

Figure 1. Inhibition of binding to dopamine D_2 receptors by haloperidol, risperidone, olanzapine, and clozapine. The concentration-dependent inhibition of ^{3}H-spiperone binding to dopamine D_2 receptors by haloperidol (△), risperidone (◇) olanzapine (○) and clozapine (×) was determined in rat striatal membranes. Vertical lines represent ± 1 SE and are absent when less than the size of the point.

Table 4. Affinity Constants (K_i [nM]) for Olanzapine, Clozapine, and Other Antipsychotic Compounds for Serotonin Receptor Subtypes[a]

	K_i (nM)					
Compound	**5HT$_{1A}$**	**5HT$_{1B}$**	**5HT$_{1D}$**	**5HT$_{2A}$**	**5HT$_{2C}$**	**5HT$_3$**
Olanzapine	>1000	1355 ± 380	800 ± 190	4 ± 0.4	11 ± 1	57 ± 6
Clozapine	770 ± 220	1200 ± 170	980 ± 115	12 ± 3	8 ± 0.8	69 ± 8
Risperidone	490 ± 10	1325 ± 130	100 ± 11	0.6 ± 0.2	26 ± 5	N[b]
Remoxipride	N[b]	N[b]	6150	N[b]	N[b]	N[b]
Seroquel	2450 ± 500	5400 ± 350	6220	220 ± 4	615 ± 110	170 ± 15
Org 5222	19 ± 4	68 ± 4	18	0.4 ± 0.3	0.2 ± 0.05	3000
Haloperidol	7930 ± 500	N[b]	6950 ± 950	78 ± 22	3085	>1000

[a] The K_i values ± SE for the serotonin receptor subtypes were determined as described in Materials and Methods. All compounds were tested in duplicate in at least two or three (with SE) independent experiments at each receptor site.
[b] N = Inhibition of binding < 50% at 10,000-nM concentration.

receptors (Table 3), and remoxipride had low affinity for D_4 receptors (Van Tol et al. 1991).

Olanzapine was a potent inhibitor of radioligand binding to 5HT$_{2A}$ and 5HT$_{2C}$ receptor subtypes with K_i values of 4 and 11 nM, respectively, and had moderate affinity for 5HT$_3$ receptors (Table 4). Olanzapine had lower affinity for 5HT$_{1A}$, 5HT$_{1B}$, and 5HT$_{1D}$ receptor subtypes. Clozapine had a similar radioligand binding profile for 5HT receptor subtypes. Risperidone was a potent inhibitor of [^{3}H]ketanserin binding to 5HT$_{2A}$ receptors with a K_i value of 0.6 nM, had moderate affinity for 5HT$_{2C}$ and 5HT$_{1D}$ receptors, and low affinity for 5HT$_{1A}$, 5HT$_{1B}$, and 5HT$_3$ receptors. Org 5222 had very high affinity for 5HT$_{2A}$ and 5HT$_{2C}$ receptors with K_i values less than 1 nM and high affinity for 5HT$_{1A}$ and 5HT$_{1D}$ receptors but did not have appreciable affinity for 5HT$_3$ receptors. Seroquel had moderate affinity for 5HT$_{2A}$ and 5HT$_3$ receptors and relatively low affinity for the other serotonin receptor subtypes. Haloperidol interacted with 5HT$_{2A}$ receptors with moderate affinity and had very low affinity for other serotonin receptor subtypes. On the other hand, remoxipride did not have appreciable affinity for any of the serotonin receptor subtypes examined.

Table 5. The K_i Values (nM) for Olanzapine, Clozapine, and Other Antipsychotic Compounds for Muscarinic Receptor Subtypes[a]

	K_i (nM)			
Compound	**m$_1$**	**m$_2$**	**m$_3$**	**m$_4$**
Rat tissue				
Olanzapine	1.9 ± 0.1	18 ± 5	25 ± 2	13 ± 2
Clozapine	1.9 ± 0.4	10 ± 1	14 ± 1	18 ± 5
Risperidone	N[b]	N[b]	N[b]	N[b]
Remoxipride	N[b]	N[b]	N[b]	N[b]
Seroquel	120 ± 35	630 ± 230	1320 ± 80	660 ± 100
Org 5222	N[b]	N[b]	N[b]	N[b]
Haloperidol	1475 ± 300	1200 ± 180	1600 ± 305	N[b]
	m$_1$	**m$_3$**	**m$_4$**	**m$_5$**
Cell lines transfected with muscarinic receptors				
Olanzapine	2.5 ± 0.3	13 ± 0.8	10 ± 0.6	6 ± 0.8
Clozapine	1.4 ± 0.3	7 ± 1	6 ± 0.5	5 ± 1.2
Risperidone	N[b]	N[b]	N[b]	N[b]
Remoxipride	N[b]	N[b]	N[b]	N[b]
Seroquel	135 ± 30	705 ± 45	225 ± 40	2990 ± 670
Org 5222	N[b]	N[b]	N[b]	N[b]

[a] The K_i ± SE values (nM) for the muscarinic receptor subtypes were determined using [^{3}H]pirenzepine binding to m$_1$ receptors in cerebral cortex, [^{3}H]NMS binding to heart tissue for m$_2$ receptors, [^{3}H]NMS binding to submaxillary salivary glands for m$_3$ receptors, and [^{3}H]NMS binding to striatum with dissociation for m$_4$ receptors. Muscarinic receptor binding in CHO-K1 cell lines was determined with [^{3}H]NMS binding to muscarinic receptors as described in Materials and Methods. All compounds were tested in at least three independent experiments at each receptor site.
[b] N = Inhibition of binding <50% at 10,000-nM concentration.

Table 6. The K_i Values for Olanzapine, Clozapine, and Other Antipsychotic Compounds for Adrenergic, Histaminergic, GABAergic, and Benzodiazepine Receptors[a]

	K_i (nM)[b]		
Compound	α_1	α_2	H_1
Olanzapine	19 ± 1	230 ± 40	7 ± 0.3
Clozapine	7 ± 4	8 ± 3	6 ± 2
Risperidone	2 ± 0.1	3 ± 0.7	155 ± 35
Remoxipride	>10,000	2900 ± 125	>10,000
Seroquel	7 ± 0.2	87 ± 4	11 ± 12
Org 5222	1 ± 0.3	4 ± 0.7	2 ± 2.0
Haloperidol	46 ± 6	360 ± 100	3630 ± 85

[a] The K_i values ± SE for α_1-adrenergic, α_2-adrenergic, histamine H_1, β-adrenergic, $GABA_A$, and benzodiazepine receptors were determined in rat tissues as described in Materials and Methods. All compounds were tested in at least 3 independent experiments at each receptor site.

[b] The compounds did not inhibit 50% of the binding to $GABA_A$, β-adrenergic, and benzodiazepine receptors at 10-μM concentration.

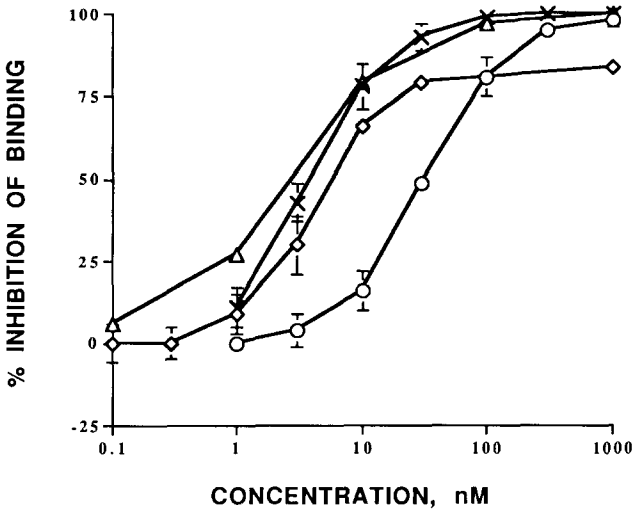

Figure 2. Inhibition of binding to various receptors from human tissue by olanzapine. The concentration-dependent inhibition of binding to dopamine D_1 (○) and dopamine D_2 (×) receptors by olanzapine was determined in human striatum. The inhibition of binding to $5HT_{2A}$ (◇) and muscarinic M_1 (△) receptors by olanzapine was determined in human frontal cortex. Vertical lines represent ±1 SE and are absent when less than the size of the point.

Binding of radioligands to muscarinic receptor subtypes in rat tissue and in CHO-K1 cell lines transfected with muscarinic receptor subtypes was potently inhibited by olanzapine (Table 5). Olanzapine and clozapine had highest affinity for m_1 receptors among all the neuronal receptors examined with K_i values in cell lines of 2.5 and 1.4 nM, respectively, and in rat cortex the K_i value was 1.9 nM for both compounds. On the other hand, risperidone, remoxipride, seroquel, Org 5222, and haloperidol had moderate to low affinity for muscarinic receptors.

The binding to α_1- and α_2-adrenergic receptors was inhibited by olanzapine with K_i values of 19 and 230 nM, respectively; in contrast, clozapine had higher affinity for α_1-adrenergic receptors and in particular for α_2-adrenergic receptors (Table 6). Risperidone, Org 5222, and seroquel also had high affinity for α_1-adrenergic and α_2-adrenergic receptors. None of the antipsychotic compounds had affinity for β-adrenergic receptors (Table 6).

Olanzapine, clozapine, seroquel, and Org 5222 had high affinity for histamine H_1 receptors (Table 6). None of the compounds evaluated had appreciable affinity for $GABA_A$ or benzodiazepine receptors.

The inhibition of radioligand binding to receptors in human brain tissue was examined (Table 7, Figure 2). Olanzapine had high affinity for dopamine D_1 and D_2 receptors in human striatal tissue and for $5HT_{2A}$, $5HT_{2C}$, m_1, and α_1-adrenergic receptors in human frontal cortex. We were unable to determine specific binding of [^{3}H]pyrilamine to histamine H_1 receptors in human frontal cortex.

Table 7. Inhibition of Radioligand Binding by Olanzapine to Human Neuronal Receptors[a]

Receptor	**IC_{50} (nM)**
Dopamine D_1	25 ± 4
Dopamine D_2	10 ± 2
$5HT_{2A}$	7 ± 2
$5HT_{2C}$	71 ± 8
Muscarinic m_1	2 ± 0.1
α_1-adrenergic	70 ± 14
α_2-adrenergic	280 ± 20
β-adrenergic	>10,000
$GABA_A$	>10,000
Benzodiazepine	>10,000

[a] The affinity of olanzapine for D_1 and D_2 receptors was determined in human corpus striatum. The inhibition of binding by olanzapine was determined in membranes from human frontal cortex for $5HT_{2A}$, $5HT_{2C}$, m_1, α_1-adrenergic, α_2-adrenergic, β-adrenergic, $GABA_A$, and benzodiazepine receptors as described in Materials and Methods. The IC_{50} values ± SE are the mean of at least three independent experiments at each receptor site.

DISCUSSION

Olanzapine exhibited high affinity for dopamine D_1, D_2, D_4, $5HT_{2A}$, $5HT_{2C}$, $5HT_3$, α_1-adrenergic, histamine H_1, and five subtypes of muscarinic receptors in animal tissues and cell lines transfected with neuronal receptors. The receptor binding profile of olanzapine was compared to the atypical antipsychotic clozapine, the widely used typical antipsychotic haloperidol and the new antipsychotics risperidone (Leysen et al. 1988;

Roose et al. 1988), remoxipride (Lewander et al. 1990), seroquel (Migler et al. 1993; Saller and Salama 1993), and Org 5222 (Siten and Vrijmoed-de Vries 1992). Only olanzapine had a broad radioreceptor binding profile that mirrored the profile of the prototype clozapine. For example, haloperidol had high affinity for dopamine D_2 and D_4 receptors, moderate affinity for dopamine D_1 and α_1-adrenergic receptors, and relatively low affinity for the other receptors examined. Risperidone had high affinity for $5HT_{2A}$, dopamine D_1, D_2, D_4, α_1-adrenergic, α_2-adrenergic receptors ($K_i < 100$ nM), but had moderate affinity (K_i between 100 and 1000 nM) for histamine H_1 receptors and was devoid of activity at muscarinic receptors. Seroquel had high affinity for α_1-, α_2-adrenergic and histamine H_1 receptors and moderate affinity for dopamine D_1, D_2, $5HT_{2A}$, $5HT_3$, and m_1 receptors. Remoxipride had moderate affinity for dopamine D_2 receptors but had low affinity for the other receptors examined. Org 5222 had high affinity for the $5HT_1$ and $5HT_2$ subtypes, dopamine D_1, D_2, α_1-adrenergic, α_2-adrenergic, and histamine H_1 receptors, but had low affinity for the muscarinic receptor subtypes. Thus, high affinity for muscarinic receptors subtypes is unique for olanzapine and clozapine among the antipsychotics tested. Interestingly, all the antipsychotic compounds tested, with the exception of remoxipride, had high affinity for the α_1-adrenergic receptor and a number had high affinity for the histamine H_1 receptor. In general, the binding results presented here for antipsychotic compounds are in agreement with those of previous studies (Bolden et al. 1992; Leysen et al. 1988; Meltzer et al. 1989; Saller and Salama 1993; Seeman et al. 1976).

The blockade of dopamine D_2 receptors in the mesolimbic area has been hypothesized to play an important role in the efficacy of antipsychotic drugs (Creese et al. 1976; Seeman et al. 1976). However, interaction with other dopamine receptor subtypes may be critical to produce the atypical profile of clozapine. For example, clozapine was a potent inhibitor of the binding of radioligands to dopamine D_1, D_2, and particularly D_4 receptors. Olanzapine also had high affinity for dopamine D_1, D_2, and D_4 receptors, although olanzapine had higher affinity for D_1 and D_2 receptors and clozapine was slightly more potent at D_4 receptors. High potency at D_1 receptors relative to D_2 receptors has been suggested to reduce EPS liability (Andersen et al. 1986). Dopamine D_4 receptors have been implicated in schizophrenia by the finding that the number of D_4 receptors are elevated in schizophrenic patients (Seeman et al. 1993), and clozapine has about 10 times higher affinity for D_4 than D_2 receptors (Van Tol et al. 1991).

The atypical nature of clozapine may also involve interaction with nondopaminergic receptors. For example, high affinity for $5HT_{2A}$ receptors relative to D_2 receptors has been postulated to be involved in low EPS potential (Altar et al. 1986; Meltzer et al. 1989; Rasmussen and Aghajanian 1988). In this regard olanzapine, as well as clozapine, was more potent in inhibiting binding of radioligands to $5HT_{2A}$ than dopamine D_2 receptors. In addition to $5HT_{2A}$ receptors, olanzapine and clozapine (Canton et al. 1990; Roth et al. 1992) also have high affinity for $5HT_{2C}$ receptors. Clozapine has high affinity for cloned $5HT_6$ and $5HT_7$ receptor subtypes, whereas olanzapine only has high affinity for $5HT_6$ receptors (Roth et al. 1994). Moreover, olanzapine and clozapine have moderate affinity for $5HT_3$ receptors, and $5HT_3$ antagonists have been hypothesized to have antipsychotic potential through interaction with the dopamine system (Costall et al. 1987; Rasmussen et al. 1991). Thus, olanzapine and clozapine have high affinity for a number of the 5HT receptor subtypes, and this, along with interaction with other receptors, may be a key factor in their atypical nature.

Only olanzapine and clozapine among the compounds tested have high affinity for the m_1 receptor subtype, and it has been proposed that m_1 selectivity may contribute to the atypical profile of antipsychotics (Bolden et al. 1992). In addition, interaction with muscarinic receptors reduce the cataleptogenic response of antipsychotic compounds (Jenner and Marsden 1983). Olanzapine, clozapine, and antipsychotics in general have a high affinity for α_1-adrenergic receptors that may contribute to their antipsychotic activity (Cohen and Lipinski 1986).

Although the overall binding profile of olanzapine is quite comparable to that of clozapine, the radioreceptor binding affinity of olanzapine for α-adrenergic receptors is significantly different from that of clozapine. The affinity of olanzapine for α_1-adrenergic receptors was about one-half of the dopamine D_2 affinity, while the affinity of clozapine for α_1-adrenergic receptors was about 18 times higher than the dopamine D_2 affinity. In addition, the affinity of clozapine for α_2-adrenergic receptors was 28 times higher than that of olanzapine. The decreased affinity of olanzapine for α-adrenergic receptors suggests that this compound may be less likely to produce sedation and hypotension, which are side effects closely related to the α-adrenergic blockade produced by certain antipsychotics (Peroutka and Snyder 1980).

The binding profile of olanzapine in human brain tissue was consistent with results obtained in animal tissue. In human tissue olanzapine had high affinity for dopamine D_1, D_2, $5HT_{2A}$, $5HT_{2C}$, m_1, and α_1-adrenergic receptors, as found in rat tissues. Olanzapine had very low affinity for β-adrenergic, benzodiazepine, and $GABA_A$ receptors in both human and rat tissues.

The similar broad radioreceptor binding profiles of olanzapine and clozapine in rat and human tissues suggest that olanzapine would also have a broad pharmaco-

logical profile in vivo. In fact, olanzapine has potent antidopaminergic activity in vivo as demonstrated by the blockade of apomorphine-induced climbing behavior in mice (Moore et al. 1992) and the pergolide-induced increases in serum corticosterone in rats (Fuller and Snoddy 1992). In neurochemical studies, olanzapine increased the levels of dopamine metabolites in the striatum and nucleus accumbens and lowered the levels of striatal acetylcholine in rats, consistent with antagonism of D_2 receptors (Hemrick-Luecke et al. 1993). Olanzapine not only was a potent dopamine antagonist in vivo but also had activity in vivo at other neuronal receptors. The antiserotonergic and anticholinergic activity of olanzapine was demonstrated by potent blockade of 5-hydroxytrytophan-induced head twitches and oxotremorine-induced tremors in mice, respectively (Moore et al. 1992). Further, olanzapine blocked quipazine-induced elevation of serum corticosterone in rats, indicative of $5HT_2$ antagonism (Fuller and Snoddy 1992).

Olanzapine is also active in animal models that have been used to predict antipsychotic and anxiolytic activity. For example, olanzapine was active in conditioned avoidance responding (Moore et al. 1992) that has been used as test for predicting antipsychotic activity (Arnt 1982). Moreover, olanzapine produced catalepsy only at doses fourfold higher than those required to block conditioned avoidance responding (Moore et al. 1992). The induction of catalepsy by antipsychotic compounds has been associated with EPS production in man (Worms et al. 1983). Furthermore, olanzapine substituted for the discriminative effects of clozapine in rats and had activity similar to clozapine in a conflict schedule (Moore et al. 1994) despite no appreciable affinity for benzodiazepine receptors. In addition, electrophysiological studies demonstrated that chronic olanzapine treatment selectively reduced the number of spontaneously firing mesolimbic dopamine cells (A10) without altering the number of spontaneously firing nigrostriatal dopamine (A9) cells (Stockton and Rasmussen 1996). Clozapine, but not haloperidol, displayed a similar selectivity for decreasing A10 dopamine cell activity (Chiodo and Bunney 1983; White and Wang 1983). Therefore, olanzapine has a broad pharmacological profile in animals similar to that clozapine and consistent with that of an atypical antipsychotic. Indeed, in preliminary clinical trails olanzapine has been demonstrated to have efficacy in reducing both the positive and negative symptoms of schizophrenia, coupled with a favorable adverse event profile, including a low level of EPS and minimal elevation of prolactin levels (Beasley et al. 1996).

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