In Vitro and In Vivo Biochemistry of Olanzapine: A Novel, Atypical Antipsychotic Drug

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Background: Classical (typical) antipsychotic drugs are in wide use clinically, but some patients do not respond at all to treatment, while in others, negative symptoms and cognitive deficits fail to respond. Also, these drugs often cause serious motor disturbances. Clozapine, an atypical antipsychotic, appears to correct many of these deficiencies, but has a significant incidence of potentially fatal agranulocytosis. Accordingly, we attempted to develop a prototype of a new generation of antipsychotics that is both more efficacious and safe. Our strategy was to create a compound that is not only active in behavioral tests that predict antipsychotic action but also shares the rich, multifaceted receptor pharmacology of clozapine without its side effects. To this end, Eli Lilly and Co. developed olanzapine. In this article we characterize the in vitro and in vivo receptor pharmacology of olanzapine. ***Method:*** We evaluated olanzapine interactions with neuronal receptors using standard assays of radioreceptor binding in vitro and well-established in vivo (functional) assays. ***Results:*** Binding studies showed that olanzapine interacts with key receptors of interest in schizophrenia, having a nanomolar affinity for dopaminergic, serotonergic, α_1-adrenergic, and muscarinic receptors. In vivo olanzapine is a potent antagonist at DA receptors (DOPAC levels; pergolide-stimulated increases in plasma corticosterone) and 5-HT receptors (quipazine-stimulated increases in corticosterone), but is weaker at α-adrenergic and muscarinic receptors. Olanzapine has little or no effect at other receptors, enzymes, or key proteins in neuronal function. Olanzapine has a receptor profile that is similar to that of clozapine: it is relatively nonselective at dopamine receptor subtypes and it shows selectivity for mesolimbic and mesocortical over striatal dopamine tracts (electrophysiology; Fos). ***Conclusion:*** The binding and functional profile of olanzapine (1) is similar to that of clozapine, (2) indicates that olanzapine is an atypical antipsychotic drug, and (3) is consistent with clinical efficacy. If olanzapine also proves to be safe, then it will have high potential to become a more ideal antipsychotic drug.

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Although the biological basis of schizophrenia is not well understood,[1] it is clear from clinical experience since the 1960s that drugs that are antagonists at dopamine receptors can ameliorate many of the symptoms of this major psychosis.[2] These agents, which are often referred to as neuroleptics, include phenothiazines, butyrophenones, benzamides, and agents of other chemical classes as well as some atypical agents.

One major problem with the use of these agents is that some patients (up to 15%–30%) do not respond to treatment[3,4] and in others, only positive symptoms of schizophrenia (delusions, hallucinations, etc.) are amenable to drug therapy while negative symptoms (blunted affect, social isolation, etc.) are not affected.[2,5] Even more difficult are the extrapyramidal side effects associated with the use of antipsychotics.[6] These include acute side effects (e.g., dystonia, parkinsonism, akathisia) and chronic side effects—particularly, tardive dyskinesia which can become chronic and, occasionally, irreversible, especially in the elderly.

Clozapine, an atypical antipsychotic,[7] appears to correct many of these deficiencies[8–12] without producing extrapyramidal side effects,[13] but has a significant incidence of agranulocytosis.[10,14,15]

There is a need, therefore, for a new generation of antipsychotic agents that are more efficacious and safe. This report discusses one such new agent, olanzapine, and its multifaceted receptor binding profile, a profile that was a

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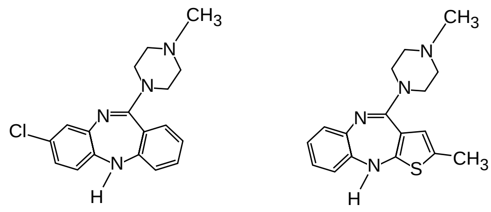
Figure 1. Structural Similarity of Olanzapine and Clozapine



Clozapine   Olanzapine

Table 1. Binding Affinities of Olanzapine, Clozapine, and Haloperidol for Dopamine Receptor Subtypes*

	K_i (nM)		
Receptor	Olanzapine	Clozapine	Haloperidol
Dopamine D_1	31 ± 0.7	85 ± 0.7	25 ± 7
Dopamine D_2	11 ± 2	125 ± 20	1 ± 0.04
Dopamine D_3	16 ± 3	84 ± 12	5 ± 1
Dopamine $D_{4.2}$	26 ± 1	47 ± 4	1.6 ± 0.2
Dopamine D_5	51 ± 2	85 ± 21	12 ± 0.7

*Dopamine D_1 and D_2 data from reference 18. Binding to rat D_3, human $D_{4.2}$, and human D_5 receptors were from transfected cells.

major criterion in our search for a safer, more efficacious antipsychotic drug and is, perhaps, a major mechanism underlying the advantages of this compound.

Olanzapine (Figure 1) was originally discovered and developed at Erl Wood, the Lilly Research Center in Surrey, England.[16] The project was based on the premise that it was possible to develop a safe antipsychotic drug that not only is as efficacious as all current antipsychotics are in the treatment of schizophrenia, but also (1) can treat the negative symptoms and (2) has a better profile of side effects than existing compounds. Clozapine, which was already known, well characterized, and in clinical use,[12] was and is considered an atypical antipsychotic because it elicits fewer, if any, extrapyramidal side effects and even appeared to be somewhat more efficacious in treating the negative symptoms of schizophrenia. However, about 1% of long-term clozapine users develop agranulocytosis, which can be fatal if not caught early enough and clozapine is not withdrawn.

Having adopted this broad strategy, we chose to focus our search on similar but safer clozapine-like agents. In particular, we sought agents (1) that had antipsychotic potential based on classical tests in behavioral pharmacology and (2) that had a receptor binding profile that was similar to that of clozapine. The available evidence suggested that the atypical profile of clozapine cannot be attributed to a single action, but rather is more likely to be due to its nonspecificity and interactions at a number of different neurotransmitter receptors. These receptors include dopamine receptors (D_1, D_2, D_4), serotonin receptors (5-HT_{2A}, 5-HT_{2C}), histamine (H_1), α-adrenergic (α_1), and muscarinic cholinergic receptors. Thus, while others have suggested that the next generation of antipsychotic agents should have mesolimbic selectivity, or should bind selectively to a subset of clozapine binding sites or to a subset of dopamine receptors, our notion was that the key to the increased efficacy and reduced extrapyramidal side effects of clozapine might lie in its broad, multi-neurotransmitter spectrum of activity.

Based on a battery of tests using well-known assays from behavioral pharmacology (see the article by Moore et al.[57] in this Supplement), olanzapine was selected as the most promising candidate for further investigation. Results from in vitro receptor binding studies, which are discussed in this article (see below), indicated that olanzapine had the desired biochemical profile (pattern of interactions with various neurotransmitter receptors), and olanzapine was chosen to be further tested in clinical trials (reference 17; also see the articles by Tollefson[58] and by Beasley et al.[59] in this Supplement).

METHOD

Details of the various receptor binding assays were described previously.[18]

RESULTS AND DISCUSSION

Interactions of Olanzapine With Dopamine Receptors

As noted above, antagonism at dopamine receptors, in particular dopamine D_2 receptors, appears to be one of the keys to the therapeutic efficacy of antipsychotic agents (it is less clear whether abnormalities in brain dopamine biochemistry are part of the etiology of schizophrenia). We therefore investigated the ability of olanzapine to interact with dopamine receptors. Accordingly, radioreceptor binding studies were carried out in which we labeled each dopamine receptor subtype with a radioactive ligand and then measured the ability of antipsychotic drugs to inhibit the binding of the radioactive probe. Either brain tissues rich in dopamine receptors or membranes from cell lines transfected with individual dopamine receptor subtypes were used in the binding studies. Our experiments showed that, unlike haloperidol, which has substantial selectivity (25 to 1) for the D_2-like dopamine receptors (D_2, D_3, D_4) over the D_1-like dopamine receptors (D_1, D_5), olanzapine (D_2/D_1 ratio of about 3 to 1), like clozapine (0.7 to 1), interacts more nonselectively with the dopamine receptors (Table 1). Thus, the *pattern* of binding affinities of olanzapine is more similar to that of clozapine than to that of haloperidol. Finally, olanzapine has greater affinity than clozapine at each of these dopamine receptors, particularly at the D_2, the ratio of affinities being 2.7, 11.4, 5.3, 1.8, and 1.7, respectively, at the D_1 to D_5 dopamine receptors.

Interactions With Serotonin Receptors

While the dopamine hypothesis of schizophrenia appears to be supported by the strongest evidence, theories

Table 2. Binding Affinities of Olanzapine, Clozapine, and Haloperidol for 5-HT Receptor Subtypes*

	K_i (nM)		
Receptor	Olanzapine	Clozapine	Haloperidol
$5\text{-}HT_{1A}$	> 1000	770 ± 220	7930 ± 500
$5\text{-}HT_{1B}$	1355 ± 380	1200 ± 170	> 10,000
$5\text{-}HT_{1D}$	800 ± 190	980 ± 115	6950 ± 950
$5\text{-}HT_{2A}$	2.5 ± 0.4	6.5 ± 0.4	58 ± 16
$5\text{-}HT_{2B}$	12 ± 2	8 ± 1	1450 ± 140
$5\text{-}HT_{2C}$	29 ± 4	36 ± 1	12,375 ± 2650
$5\text{-}HT_3$	57 ± 6	69 ± 8	> 1000
$5\text{-}HT_4$	> 1000	> 1000	…
$5\text{-}HT_6$	2.5 ± 9	4 ± 0.7	> 5000
$5\text{-}HT_7$	104 ± 12	6.3 ± 3.7	263 ± 41

*$5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$, and $5\text{-}HT_3$ data from reference 18.
$5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_{2C}$ data from reference 53.
$5\text{-}HT_6$ and $5\text{-}HT_7$ data from reference 24; $5\text{-}HT_4$ unpublished data from Bymaster FP, 1996.

of schizophrenia implicating serotonin and serotonin receptors have also been put forward.[19,20] Evidence also exists that serotonin receptors may play a role in the antipsychotic effects of neuroleptics[3,7,20–23] and olanzapine and clozapine interact with several subtypes of the serotonin (5-HT) receptor. It can be seen in Table 2 that olanzapine is a potent inhibitor of binding to $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, $5\text{-}HT_{2C}$, and $5\text{-}HT_6$ subtypes (range, 2.5–29 mM), has lower affinity for $5\text{-}HT_3$ and $5\text{-}HT_7$ subtypes, and low affinity for $5\text{-}HT_1$ and $5\text{-}HT_4$ serotonin receptor subtypes. It can also be seen that the affinity of olanzapine for each 5-HT receptor is remarkably similar to the affinity of clozapine for the same receptor. An exception is the relatively low affinity (16-fold) of olanzapine, compared to clozapine, for the $5\text{-}HT_7$ receptor. Both agents appear to be more potent than haloperidol at each of these serotonin receptors. This greater potency ranges from about 2.5 to 1 for the $5\text{-}HT_7$ receptor to 2000 to 1 for the $5\text{-}HT_{2C}$ receptor. Clearly, the ideal ratio for antipsychotic drugs of serotonergic antagonism to dopaminergic antagonism remains to be established, but clozapine may be used as a model.

Interactions With Muscarinic Cholinergic Receptors

Muscarinic receptors appear to be important in the treatment of schizophrenia because they are thought to establish a balance in the extrapyramidal circuit between dopamine and acetylcholine, a balance that is critical to normal motor functions and movements.[25] These receptors, which include m1 through m5 subtypes, may therefore be critical to the extrapyramidal side effects of antipsychotic drugs. Our investigation into the effects of olanzapine on muscarinic receptor subtypes (Table 3) revealed that olanzapine is a potent inhibitor of binding to all five subtypes of the muscarinic cholinergic receptor (range, 1.9 to 25×10^{-9} M). Moreover, the affinities of olanzapine for the subtypes of the muscarinic receptor are quite similar to the affinities of clozapine for these same receptors. This contrasts with the affinities of haloperidol for these receptor subtypes, the values for which are considerably lower (60- to 1300-fold). It should be noted that other neuroleptics, particularly the phenothiazines, often have affinities for the muscarinic cholinergic receptors that are considerably higher than that of haloperidol.

Table 3. Binding Affinities of Olanzapine, Clozapine, and Haloperidol for Muscarinic Receptor Subtypes*

	K_i (nM)		
Receptor	Olanzapine	Clozapine	Haloperidol
Muscarinic m1	1.9 ± 0.1	1.9 ± 0.4	1475 ± 300
Muscarinic m2	18 ± 5	10 ± 1	1200 ± 180
Muscarinic m3	25 ± 2	14 ± 1	1600 ± 305
Muscarinic m4	10 ± 0.6	6 ± 0.5	> 1500
Muscarinic m5	6 ± 0.8	5 ± 1.2	8120 ± 912

*Data from reference 18.

Table 4. Affinities of Olanzapine, Clozapine, and Haloperidol for Adrenergic and Histamine Receptor Subtypes*

	K_i (nM)		
Receptor	Olanzapine	Clozapine	Haloperidol
α_1-Adrenegric	19 ± 1	7 ± 4	46 ± 6
α_2-Adrenegric	230 ± 40	8 ± 3	360 ± 100
β-Adrenergic	> 10,000	> 10,000	> 10,000
Histamine H_1	7 ± 0.3	6 ± 2	3630 ± 85
Histamine H_2	> 1000	> 1000	…
Histamine H_3	> 1000	708	…

*Data from reference 18 and histamine H_3 data from reference 26.

Interactions With Adrenergic Receptors

Antipsychotic drugs are known to act at receptors of various other neurotransmitters. While these interactions may not necessarily contribute to the therapeutic effect of these drugs, they may contribute to the side effects. For example, antagonism of the α-adrenergic or histamine H_1 receptors may mediate the sedating effect of neuroleptics. We therefore determined the affinities of olanzapine for the families of α-adrenergic, β-adrenergic, and histamine receptors. It can be seen (Table 4) that olanzapine has high nanomolar affinity for the α_1-adrenergic receptor, less affinity for the α_2 receptor, and only weakly interacts with the β-adrenergic receptor. Olanzapine appears to be slightly less potent than clozapine at the α_1 receptor, but slightly more potent than haloperidol at this same receptor. The pattern is different for the α_2 receptor where olanzapine is over 25 times less potent than clozapine, although it is still more potent than haloperidol (1.6-fold). The lower affinity of olanzapine for α_2-adrenergic receptors may result in reduced cardiovascular side effects compared to clozapine. All three agents only weakly interact with the β-adrenergic receptor. Since antipsychotic-induced sedation and hypotension are thought to be related to antagonism at α-adrenergic receptors, olanzapine may be less likely to produce these side effects, especially because of the much lower clinical dose.

In the same table, it can be seen that both olanzapine and clozapine are about 500 times more potent than halo-

Table 5. Olanzapine Has Low Affinity for Other Receptors, Enzymes, and Key Neuronal Proteins*

Receptors
- Adenosine, purinergic
- $GABA_A$ and $GABA_B$, benzodiazepine
- Glutamate (AMPA, kainate, NMDA)
- Glycine
- Nicotinic (neuronal)
- Sigma (non-selective)
- Opiate (mu, kappa, delta)
- Peptide (CCK_A, CCK_B, NK1, NK2, NPY, NT)

Ion channel receptors
- Ca^{++} (L and N)
- Cl^-
- Glutamate (MK-801, PCP)
- Na^+ (site 2)
- K^+ (ATP, Ca, Volt + and –)

Uptake transporters
- Choline, GABA, norepinephrine, 5-HT, dopamine

Enzymes
- Acetylcholinesterase
- Choline acetyltransferase
- MAO-A and MAO-B

*Olanzapine was either inactive or had an IC_{50} > 1 μM for the above receptors, binding sites, and enzymes.

Table 6. Inhibition of Binding to Human and Rat Neuronal Receptors by Olanzapine*

	IC_{50}, (nM)	
Receptor	Rat Tissue	Human Tissue
Dopamine D_1	180 ± 30	25 ± 4
Dopamine D_2	30 ± 12	10 ± 2
$5\text{-}HT_{2A}$	5 ± 0.7	7 ± 2
$5\text{-}HT_{2C}$	23 ± 3	71 ± 8
Muscarinic m1	2.5 ± 0.3	2 ± 0.1
α_1-Adrenergic	26 ± 5	70 ± 14
α_2-Adrenergic	475 ± 20	280 ± 20
β-Adrenergic	> 10,000	> 10,000
$GABA_A$	> 10,000	> 10,000
Benzodiazapine	> 10,000	> 10,000

*Data from reference 18.

Table 7. Receptor Binding Profile and Clinical Dose Range of Olanzapine and Comparator Compounds*

	K_i (nM)						
Compound	D_1	D_2	$5\text{-}HT_{2A}$	m1	α_1	H_1	Clinical Dose (mg)
Olanzapine	31	11	4	1.9	19	7	10–20
Clozapine	85	125	12	1.9	7	6	200–400
Risperidone	75	3	0.6	> 1000	2	155	4–8
Sertindole	210	7	0.8	> 5000	1.8	570	12–24
Quetiapine	455	160	220	120	7	11	50–750
Ziprasidone	330	10	0.3	> 5000	12	5	> 60
Haloperidol	25	1	78	1475	46	3630	10–20

*Binding data from references 18 and 54.

peridol at the histamine H_1 receptor. All three agents show low affinity for the histamine H_3 receptor[26] and the H_2 receptor.

Lack of Interaction With Other Key Neuronal Receptors, Enzymes, and Proteins

In addition to the above studies, we looked at olanzapine in a range of other assays, mostly to exclude the possibility that olanzapine might be acting pharmacologically at these other targets. It can be seen (Table 5) that olanzapine has little or no affinity (> 1000 nM) at other neurotransmitter receptors including receptors for adenosine, GABA, glutamate, glycine, the nicotinic cholinergic receptor, the sigma receptor, opioid receptors, and various peptide receptors including cholecystokinin (CCK), neuropeptide Y (NPY), and neurotensin. Similarly, olanzapine had little or no affinity for binding sites on ion channels including those for MK-801, phencyclidine, calcium, chloride, sodium, and potassium. Again, olanzapine did not affect uptake transporters for choline, GABA, norepinephrine, 5-HT, or dopamine and did not alter the activity of enzymes that metabolize or synthesize neurotransmitters including acetylcholinesterase, choline acetyltransferase, and monoamine oxidase.

Comparison With Human Receptor Interactions

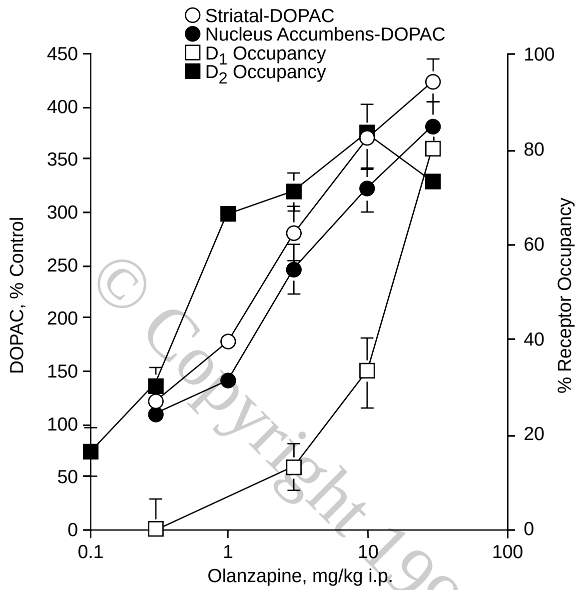
Since most of the in vitro studies discussed above were done using either rat brain tissues or membranes from cells expressing cloned receptors, it was worthwhile to compare binding data obtained from rat brain versus human brain tissues. As expected, we found (Table 6) that the data are quite similar for the interaction of olanzapine with receptors found in the human brain and rat brain.[18] Notably, we found in human brain studies a similar nonselectivity of olanzapine for D_2 versus D_1 receptors.

Comparison With Other, Newer, Antipsychotic Agents

Other interesting comparisons that we can make are between olanzapine and other antipsychotic agents or drug candidates (Table 7) such as sertindole,[27,28] quetiapine,[29,30] risperidone,[31,32] remoxipride,[33] and ziprasidone.[34] The table shows that there are many differences in the receptor binding profiles of these agents, some subtle, some substantial. It will therefore be interesting to see what differences turn up in clinical experience with these compounds. For example, quetiapine is relatively weak at dopamine, 5-HT, and muscarinic receptors but has high affinity for the α_1 receptor and the histamine H_1 receptor. Ziprasidone, in contrast, has its highest affinity at $5\text{-}HT_{2A}$, histamine H_1, and dopamine D_2 receptors, whereas sertindole has high affinity for $5\text{-}HT_{2A}$ and dopamine D_2 receptors and significantly weaker affinity for histamine H_1 receptors. Risperidone, sertindole, and ziprasidone, in contrast to olanzapine and clozapine, have low affinity for muscarinic receptors.

The concentration of a drug present at the receptor and the receptor affinities or relative receptor affinity of the drug are parameters affecting the interaction of drug with receptor. It is thus particularly important to consider these relative receptor affinities of a drug in light of the dose range used clinically for each drug. For example, although

Figure 2. In Vivo Occupancy of Dopamine D_1 and D_2 Receptors and Increases in Striatal and Nucleus Accumbens DOPAC Levels Induced by Olanzapine*



*The concentration of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), was determined in neostriatum and nucleus accumbens 1 h after administration of vehicle or various doses of olanzapine, i.p., according to our previously described method.[36] The inhibition of in vivo binding by olanzapine was determined in neostriatum using [^{3}H]SCH23390 for D_1 and [^{3}H]raclopride for D_2 receptors (unpublished data from Bymaster FP).

olanzapine has about the same affinity (K_i = 19 nM) for α_1 receptors as does ziprasidone (K_i = 12 nM), the overall effect (receptor occupancy) of olanzapine at α_1 receptors is likely to be less because it is clinically used at a lower dose (10 to 20 mg) than ziprasidone (> 60 mg). Thus, the fact that olanzapine is used at much lower absolute doses than clozapine presumably would lead to a lower occupancy at α-adrenergic receptors and might explain why olanzapine does not elicit much sedation.

Functional Assays for Receptor Interactions

In addition to determining the receptor binding profile of a new antipsychotic agent, it can be useful to measure the functional consequences of interacting with these receptors, that is, to measure potential agonism or antagonism at neurotransmitter receptors using functional assays that are done either in vitro or in vivo.

An assay that can be used as an in vivo functional assay for dopamine receptor antagonism involves increases in the levels of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) induced in rodent brain by administration of dopamine receptor antagonists. The large increases in dopamine metabolites are presumably the result of blockade of dopamine D_2 autoreceptors located on dopamine nerve terminals. The effects of olanzapine on DOPAC levels in two dopamine-rich brain areas—corpus striatum and nucleus accumbens—can be seen in Figure 2.

Table 8. Antagonism of Pergolide (D_2)- and Quipazine ($5\text{-}HT_{2A}$)-Induced Increases in Serum Corticosterone by Antipsychotic Agents*

	ED_{50} (mg/kg i.p.)	
Compound	Pergolide-Induced	Quipazine-Induced
Olanzapine	3	0.6
Clozapine	> 10	2.6
Haloperidol	0.18	> 1

*From reference 55 and unpublished observations of Fuller R, 1992. Pergolide mesylate (0.3 mg/kg i.p.) or quipazine maleate (2.5 mg/kg s.c.) was injected 1 h before killing and 1 h after antipsychotics.

In vivo receptor occupancy of D_1 and D_2 receptors is shown, for comparison, in the same figure. The results show that occupancy of the D_2 dopamine receptor by olanzapine parallels the increase in DOPAC and occurs at a lower olanzapine dose than occupancy of D_1 dopamine receptors. Because the D_1 dopamine receptors are only about 20% occupied at a point where the effect on DOPAC is greater than half-maximal, these data further suggest that the change in DOPAC primarily reflects blockade of D_2 dopamine receptors. So, clearly, olanzapine exerts its antagonistic effect in vivo as well as in vitro. These observations are consistent with a recently published report[36] showing that olanzapine increased the concentrations of both DOPAC and homovanillic acid (HVA) in rat striatum and nucleus accumbens.

Another assay that measures antagonism in vivo at dopamine receptors and serotonin receptors involves the ability of these antipsychotic agents to block increases in serum corticosterone levels induced by drugs (pergolide for D_2; quipazine for $5\text{-}HT_2$). Using these assays, we observed (Table 8) that olanzapine is a potent antagonist (ED_{50} = 3 mg/kg) of pergolide-induced increases in corticosterone levels and is more potent than clozapine, although not quite as potent as haloperidol. The increase in serum corticosterone levels was similarly antagonized with olanzapine (ED_{50} = 0.6 mg/kg) when quipazine was used as a stimulant of $5\text{-}HT_2$ receptors. Since pergolide-stimulated increases in corticosterone levels are mediated by activation of dopamine D_2 receptors, and quipazine-induced increases in corticosterone levels are mediated via $5\text{-}HT_2$ receptors, these data suggest that olanzapine antagonizes both dopamine and $5\text{-}HT_2$ receptors. These findings are also consistent with the data shown above (Tables 1 and 2), which indicate that olanzapine has high affinity for both the dopamine D_2 and $5\text{-}HT_2$ receptors. These data demonstrate that olanzapine is over five times more potent (on a mg/kg basis) in these assays against $5\text{-}HT_2$ than against dopamine D_2 receptors. The quipazine-induced increases in serum corticosterone may be predominantly mediated by $5\text{-}HT_{2A}$ receptors; thus, the higher potency of olanzapine for blocking 5-HT effects versus dopamine effects is consistent with higher affinity for $5\text{-}HT_{2A}$ than dopamine D_2 receptors.

Table 9. Functional Effect of Olanzapine at Neuronal Receptors*

Receptor	In Vitro	Potency	In Vivo	Potency
Dopamine D_1	Antagonist	+ +	Antagonist	+ +
Dopamine D_2	ND	ND	Antagonist	+ + +
$5\text{-}HT_{2A}$	Antagonist	+ +	Antagonist	+ + +
Muscarinic m1	Antagonist	+	ND	ND
Muscarinic m3	Antagonist	+	Inactive	–
Muscarinic m4	Antagonist	+	ND	ND
α_1-Adrenergic	ND	ND	Antagonist	+

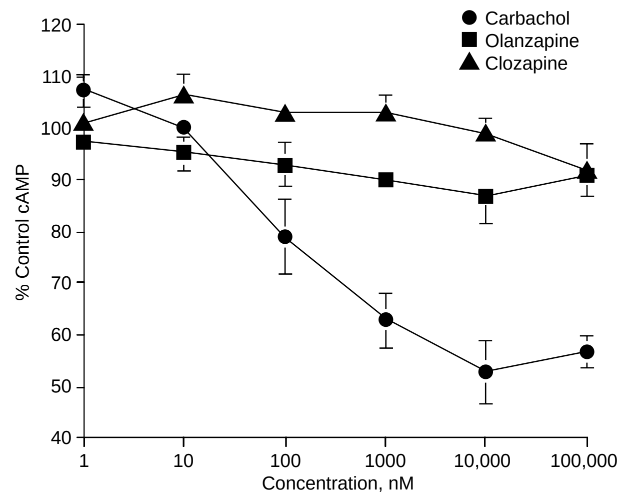
*The in vitro effects of olanzapine were determined in functional assays in tissue and cell lines and the in vivo effects of olanzapine were determined in neurochemical assays (reference 36 and unpublished observations of Bymaster FP, 1996).
In vitro potency + + = 1–50 nM IC_{50}; + = 100–1000 nM IC_{50}.
In vivo potency + + + = 0.1–1 mg/kg i.p.; + + = 1–10 mg/kg i.p.; + = 10–30 mg/kg i.p.; – ≥ 30 mg/kg i.p.
ND = not determined.

These findings are consistent with previous reports using in vivo assays of dopamine receptor and serotonin receptor antagonism. Thus, olanzapine was reported to block apomorphine-induced climbing in mice[35] and lowered the levels of striatal acetylcholine in rats, which is probably mediated by dopamine receptors.[36] And, it was previously shown that olanzapine potently inhibits 5-hydroxytryptophan-induced head twitches in mice.[35] More recently, Bymaster et al.[36] reported that olanzapine treatment inhibited the ex vivo binding of the $5\text{-}HT_2$ radioligand [^{3}H]ketanserin and inhibited quipazine-induced increases in brain MHPG-SO_4, which is further evidence that olanzapine antagonizes $5\text{-}HT_2$ receptors in vivo.

A similar line of evidence indicates that olanzapine has functional effects against the dopamine D_1 receptor (Table 9). We found (data not shown) that olanzapine inhibits dopamine-stimulated adenylate cyclase in vitro, an enzymatic activity known to be mediated by the dopamine D_1 receptor, with an IC_{50} of 0.3 μM (Truex L. Unpublished observations). This is consistent with our recent finding[36] that inactivation of dopamine D_1 and D_2 receptors by the receptor alkylating agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) is antagonized by olanzapine and olanzapine more potently blocked the inactivation of D_2 receptors.

Evidence exists that olanzapine is active in vivo against muscarinic cholinergic receptors. For example, it was previously shown that olanzapine inhibits oxotremorine-induced tremors in mice.[35] In our laboratory, olanzapine was also tested in a functional assay for muscarinic cholinergic receptors in vitro, an assay in which we measured release of arachidonic acid. Olanzapine had no agonist activity at muscarinic m1, m3, or m5 receptors, but did antagonize agonist-induced release of arachidonic acid from muscarinic m1, m3, and m5 receptors with modest IC_{50} values of 680, 970, and 995 nM, respectively. Nonselective muscarinic agonists such as carbachol inhibit cyclic AMP formation in cell lines transfected with muscarinic m2 or m4 receptors, which are negatively coupled to the adenylate cyclase second messenger system (Figure 3). Although others have suggested that clozapine may have agonist activity at the muscarinic m4 receptor,[37] we have not found agonist activity with either clozapine or olanzapine in the cell lines used in our studies (Figure 3). However, olanzapine and clozapine modestly antagonized the muscarinic agonist-induced inhibition of cyclic AMP formation (data not shown).

Figure 3. Lack of Inhibition by Olanzapine and Clozapine of Forskolin-Stimulated Adenylate Cyclase Activity in Muscarinic m4 CHO Cells*



*The inhibition of cAMP formation by the muscarinic full agonist carbachol, olanzapine, or clozapine was determined in a muscarinic m4 CHO cell line. Adenylate cyclase activity was stimulated by addition of forskolin (unpublished data from Bymaster FP).

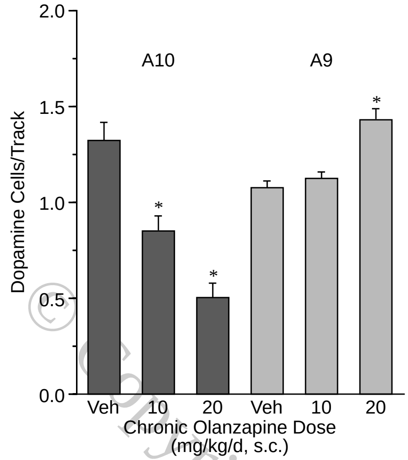
Table 9 also summarizes the data on the effects of olanzapine on the key neuronal receptors of interest in schizophrenia. The main point, of course, is that olanzapine acts as a potent antagonist at dopamine and serotonin receptors and possibly as a less potent antagonist at muscarinic receptors. These interactions of olanzapine with muscarinic receptors are consistent with observations we recently reported[36] that olanzapine ex vivo inhibits the binding of the muscarinic radioligand [^{3}H]pirenzepine and lowers concentrations of striatal but not hippocampal acetylcholine levels.

Some evidence also exists[36] that olanzapine antagonizes α_1-adrenergic receptors in vivo. Bymaster et al.[36] recently showed that olanzapine, at higher doses than required to interact with dopamine and $5\text{-}HT_2$ receptors, increased hypothalamic concentrations of the norepinephrine metabolite MHPG-SO_4.

Mesolimbic Selectivity and Atypicalness of Antipsychotics

The expression of the immediate-early gene c-*fos* has been shown to be an early marker of neuronal activation due to rapid induction by stimuli such as stress or pharma-

Figure 4. The Effect of Chronic Olanzapine Treatment on Spontaneously Firing A9 and A10 Dopamine Cells†



†The number of spontaneously firing A10 (dark bars) and A9 (light bars) dopamine cells was determined electrophysiologically after chronic administration of olanzapine. Olanzapine was administered for 21 days in an osmotic minipump at the indicated dose.[47]
*$p < .001$.

Table 10. Inhibition of Firing of Nigrostriatal (A9) and Mesolimbic (A10) Dopamine Cells by Chronic Treatment With Antipsychotics*

	Inhibition	
Compound	Nigrostriatal (A9)	Mesolimbic (A10)
Olanzapine	…	√
Clozapine	…	√
Risperidone	√?	√
Sertindole	…	√
Quetiapine	√?	√
Ziprasidone	√?	√
Haloperidol	√	√
Chlorpromazine	√	√

*Data from references 47 and 56. √ = inhibition of spontaneously firing dopamine cells after chronic treatment with antipsychotics, … = no effect.

cologic treatments. Typical and atypical antipsychotics have been shown to increase Fos protein expression[38–40] in dopamine-rich brain regions. Expression of Fos in different brain regions may be useful to differentiate typical and atypical antipsychotics.[39,41] Thus far, all antipsychotics have induced Fos expression in the nucleus accumbens, and typical antipsychotics also induce Fos in the dorsolateral striatum. The induction of Fos expression in the nucleus accumbens is consistent with efficacy for positive symptoms, whereas induction of dorsolateral striatal Fos expression may be related to production of EPS.[41] The atypical antipsychotic clozapine induced Fos expression in the nucleus accumbens and uniquely induced Fos expression in the prefrontal cortex, consistent with activity against negative symptoms, which have been postulated to involve the prefrontal cortex.[42] In addition, clozapine did not induce Fos expression in the dorsolateral striatum, consistent with its lack of catalepsy and EPS.[41]

Recently, olanzapine has been shown to induce Fos expression in nucleus accumbens and prefrontal cortex,[43] in agreement with its atypical nature and efficacy against positive and negative symptoms of schizophrenia. Olanzapine at higher doses also induced some Fos expression in dorsolateral striatum, consistent with induction of catalepsy at higher doses, suggesting the possibility of EPS at very high clinical doses. Robertson et al.[44] have proposed that the "atypical index" be used to predict if antipsychotics are atypical by subtracting the number of Fos positive neurons in the nucleus accumbens from the number of Fos positive neurons in the dorsolateral striatum. Compounds that are positive in index meet the criterion for atypicality, and olanzapine had values of +19 and +30 for 5 and 10 mg/kg doses, respectively. Thus, these data are suggestive that olanzapine activates CNS neurons in a similar pattern to clozapine and has selectivity for mesolimbic and mesocortical brain areas. Consistent with induction of Fos expression in prefrontal cortex, we have recently found using microdialysis techniques that olanzapine, like clozapine, and unlike haloperidol, increases extracellular levels of dopamine in the prefrontal cortex (Bymaster FP, Perry KW. Unpublished observations).

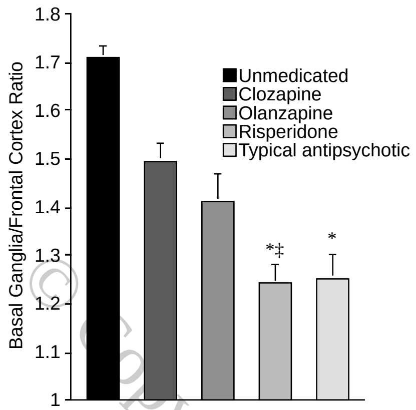
Yet another way to determine whether an antipsychotic drug is atypical is to determine whether chronic dosing reduces the number of spontaneously active dopaminergic neurons in both the mesolimbic and nigrostriatal tracts.[45,46] It can be seen (Figure 4) that chronic treatment with olanzapine affected only the mesolimbic (A10) dopaminergic tract, not the nigrostriatal (A9) dopaminergic tract.[47] Thus, olanzapine, like clozapine and sertindole (Table 10), appears to exhibit mesolimbic selectivity. Typical antipsychotics, in contrast, affect both tracts. This mesolimbic selectivity may be a reason that clozapine and olanzapine have antipsychotic efficacy but elicit fewer extrapyramidal side effects than the other, more typical antipsychotics such as chlorpromazine and haloperidol.

Finally, an indication that the biochemical uniqueness of olanzapine is being translated into in vivo consequences is the observation[35] that olanzapine produced catalepsy only at doses fourfold higher than those required to block conditioned avoidance (an assay that has been classically used to predict antipsychotic activity). The lack of catalepsy suggests that olanzapine has a lower propensity to induce EPS.

In Vivo Imaging Studies

Imaging techniques in living monkeys and humans have become an important tool to determine receptor interaction of drugs in vivo. Positron emission tomography (PET) and single photon emission tomography (SPET) studies in humans have shown that antipsychotics occupy central dopamine receptors[48,49] and some occupy central $5\text{-}HT_2$ receptors as well. At therapeutic doses in schizo-

Figure 5. Basal Ganglia/Frontal Cortex Ratios of ^{123}I-Iodobenzamide SPET Binding to Brain Regions of Schizophrenic Patients†



†Data from references 49 and 50. Single photon emission tomography (SPET) was used to determine the binding of ^{123}I-iodobenzamide to human brain regions. Binding in the frontal cortex was used as a reference background region.
*p < .05 clozapine alone.
‡p < .05 olanzapine alone.

phrenic patients, typical antipsychotics have uniformly high occupancy of dopamine D_2 receptors (70%–90%), and this occupancy is near the threshold (about 80%) for induction of EPS.[48] However, the dopamine D_2 receptor occupancy at therapeutic doses of clozapine in schizophrenic patients is much lower (20%–60%), and clozapine presumably does not block D_2 receptors to the extent necessary to produce EPS.[48]

The occupancy of central receptors by olanzapine has been investigated in PET and SPET studies and compared to typical antipsychotics and clozapine. It has been shown,[49,50] using SPET analysis of ^{123}I-iodobenzamide binding to dopamine D_2 receptors in the striatum of schizophrenic patients, that olanzapine as well as clozapine occupies D_2 receptors to a lesser extent than does risperidone or typical antipsychotics (Figure 5). Additionally, using PET analysis in three normal subjects, olanzapine (10 mg) occupied 59% to 63% and 74% to 92% of D_2 and 5-HT_2 receptors, respectively.[51] Thus, olanzapine penetrates into the human brain and occupies dopamine D_2 and 5-HT_2 receptors. These data also suggest that therapeutic doses of olanzapine, like clozapine,[52] occupy 5-HT_2 receptors to a greater extent than dopamine D_2 receptors, and, in addition, will not excessively occupy dopamine D_2 receptors to the degree necessary to produce EPS.

SUMMARY

On the basis of the biochemistry and electrophysiology presented in this article, along with previous olanzapine studies, we can conclude that in vitro olanzapine is, like clozapine, a nonselective dopamine receptor antagonist with high affinity for all dopamine receptors. But it also has high affinity for 5-HT receptors, muscarinic receptors, α_1-adrenergic receptors, and histamine H_1 receptors. In vivo, we can confirm that olanzapine has antagonist activity at dopamine and 5-HT_2 receptors and somewhat weaker activity at α_1-adrenergic receptors. Olanzapine appears to have weak muscarinic cholinergic antagonist activity in vivo. Olanzapine also shows the mesolimbic and mesocortical selectivity that one sees with clozapine. Thus, on the basis of receptor binding and functional data, we can conclude that olanzapine, like clozapine, has a pharmacologic receptor profile of an atypical antipsychotic. This is further supported by data from behavioral pharmacology (reference 35; also see the article by Moore et al.[57] in this Supplement).

This rather novel receptor profile may indeed explain the unique (atypical) behavioral and clinical actions of clozapine and olanzapine. Thus, in clinical trials, olanzapine appeared to be efficacious (reference 17; also see articles by Beasley et al.[59] and Tollefson et al.[58] in this issue) in reducing both the positive and negative symptoms of schizophrenia, coupled with a favorable adverse event profile, including a low level of extrapyramidal symptoms and minimum elevation of prolactin levels.

Olanzapine may also be safer than clozapine since there have been no reports thus far of agranulocytosis. Olanzapine may thus represent an important step forward in the development of the next generation of antipsychotic drugs. Future studies may now lead to a greater understanding of the molecular mechanisms underlying the increased efficacy and safety of this agent.

Drug names: chlorpromazine (Thorazine and others), clozapine (Clozaril), haloperidol (Haldol and others), olanzapine (Zyprexa), pergolide (Permax), quetiapine (Seroquel), risperidone (Risperdal).

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