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In Vivo Olanzapine Occupancy of Muscarinic Acetylcholine Receptors in Patients with Schizophrenia

Thomas J. Raedler, M.D., Michael B. Knable, D.O., Douglas W. Jones, Ph.D., Todd Lafargue, M.D., Richard A. Urbina, B.A., Michael F. Egan, M.D., David Pickar, M.D. , and Daniel R. Weinberger, M.D.

Olanzapine is an atypical antipsychotic with potent antimuscarinic properties in vitro *(K_i = 2–25 nM). We studied* in vivo *muscarinic receptor occupancy by olanzapine at both low dose (5 mg/dy) and high dose (20 mg/dy) in several regions of cortex, striatum, thalamus and pons by analyzing [I-123]IQNB SPECT images of seven schizophrenia patients. Both low-dose and high-dose olanzapine studies revealed significantly lower [I-123]IQNB binding than that of drug-free schizophrenia patients (N = 12) in all regions except striatum. [I-123]IQNB binding was significantly lower at high-dose than low-dose in the same regions. Muscarinic occupancy by olanzapine ranged from 13% to 57% at 5 mg/dy and 26% to 79% at 20 mg/dy with an anatomical pattern indicating M_2 subtype selectivity. The [I-123]IQNB data indicate that olanzapine is a potent and subtype-selective muscarinic antagonist* in vivo, *perhaps explaining its low extrapyramidal side effect profile and low incidence of anticholinergic side effects.* ***[Neuropsychopharmacology 23:56–68, 2000]*** *Published by Elsevier Science Inc. on behalf of the American College of Neuropsychopharmacology*

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Muscarinic acetylcholine receptors are found throughout the brain and play a role in motor function, cognition and mood. Five different subtypes, M_1–M_5, of muscarinic receptors have been distinguished pharmacologically (Watling et al. 1995) and correspond to genes (respectively, *m1–m5*) that have been cloned (Bonner et al. 1987; Bonner et al. 1988). All five subtypes are found in the human brain, albeit in regionally varying concentrations (Levey et al. 1991). For example, the basal ganglia have predominantly M_1 and M_4 receptors as does the cortex, though in different proportions, while the thalamus and brainstem have predominantly M_2 receptors (Flynn and Mash 1993; Li et al. 1991; Vilaro et al. 1991; Wall et al. 1991a,b; Yasuda et al. 1993). Though all muscarinic receptor subtypes occur postsynaptically, the M_2 receptor is also found on presynaptic neurons as an autoreceptor.

Anticholinergic drugs have long been used for the treatment of idiopathic Parkinson's Disease. These drugs exert their effects by blocking cholinergic interneurons in the basal ganglia, and antagonism of the muscarinic M_1 receptor may be of particular benefit given the M_1 selectivity of biperiden, a commonly used

From the Clinical Brain Disorders Branch (TJR, MBK, DWJ, RAU, MFE, DRW) , DIRP, NIMH, Bethesda, MD 20892; Stanley Research Foundation (MBK,), 5430 Grosvenor Lane, Suite 200, Bethesda, MD 20814; Experimental Therapeutics Branch (TL, DP), DIRP, NIMH, Bethesda, MD 20892

Address correspondence to: Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, NIMH, National Institutes of Health 10 Center Drive, 4S-235 (MSC 1379), Bethesda, MD 20892-1379, Tel.: (301) 402-7956, Fax: (301) 480-7795 E-mail: Weinberd@intra.nimh.nih.gov

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antiparkinson drug (Bolden et al. 1992). Anticholinergics have also found widespread use for prophylaxis and treatment of neuroleptic induced extrapyramidal side effects (EPS) including acute dystonia, akathisia, parkinsonism and tardive dyskinesia. While the clinical potency of typical antipsychotic drugs may be predicted by their affinity to the dopamine D_2 receptor (Seeman et al. 1976; Creese et al. 1976), the propensity of dopamine D_2 receptor antagonists to induce EPS tends to be mitigated by their ability to antagonize muscarinic receptors (Snyder et al. 1974). Serum anticholinergic activity of antipsychotics and anticholinergics are inversely correlated with the emergence of EPS (Tune and Coyle 1980). In addition to their beneficial effects on motor side effects, anticholinergic drugs have also been associated with a reduction in negative symptoms as well as an increase in positive symptoms in schizophrenia (Tandon et al. 1991), and there have suggestions that muscarinic receptors may be implicated in schizophrenia, per se (Dean et al. 1996; Yeomans 1995).

Clozapine was the first of a new group of "atypical antipsychotics" that combine superior clinical efficacy with a reduced risk of EPS. Compared to typical antipsychotics, clozapine has a unique receptor binding profile. In addition to blocking the dopamine D_2 receptor, clozapine also antagonizes other dopamine, serotonin, histamine, norepinephrine and muscarinic receptors.

Olanzapine, another atypical antipsychotic, is structurally related to clozapine. In clinical studies olanzapine has proven effective for the treatment of positive and negative symptoms of schizophrenia (Beasley et al. 1996a; Beasley et al. 1997; Tollefson et al. 1997a) with relatively few side effects and minimal EPS (Tollefson et al. 1997b; Tran et al. 1997). *In vitro*, olanzapine antagonizes various dopamine, serotonin, histamine, norepinephrine and muscarinic receptors. The *in vitro* K_i values for the muscarinic receptor subtypes range between 2 and 25 nM, similar to the K_i value of 11 nM for olanzapine at the dopamine D_2 receptor (Bymaster et al. 1996b).

Using [I-123]IBZM single photon emission computed tomography (SPECT), we have previously reported a dose dependent, moderate to high degree of dopamine D_2 receptor occupancy in subjects treated with olanzapine *in vivo* (Raedler et al. 1999a). These data are consistent with several other reports (Kapur et al. 1998; Tauscher et al. 1999). *In vivo* studies of the muscarinic receptor availability in subjects treated with olanzapine or other antipsychotics have thus far not been performed.

Quinuclidinyl benzilate (QNB), a very potent muscarinic antagonist, binds specifically and with subnanomolar affinity to all five muscarinic receptor subtypes (Bolden et al. 1992) and has been used in studies as a marker for muscarinic receptors *in vitro.* Iodoquinuclidinyl benzilate (IQNB) has similar binding properties, and, when iodinated with radioactive iodine-123, [I-123]IQNB can be used as a SPECT ligand to study muscarinic receptors *in vivo* (Eckelman et al. 1984; Weinberger et al. 1991; Sunderland et al. 1995). Both *in vitro* and *in vivo*, (R,S)-[I-123]IQNB binding has been shown to be distributed in accordance with total muscarinic receptor concentration with an absence of subtype selectivity in rat brain (Boulay et al. 1995; McRee et al. 1995). In this study we utilized (R,S)-[I-123]IQNB (henceforth referred to as [I-123]IQNB) *in vivo* to determine muscarinic receptor availability in seven schizophrenic patients who were treated with both a low dose (5 mg/dy) and a high dose (20 mg/dy) of olanzapine for a minimum of two weeks, and, for comparison, in twelve drug-free schizophrenic patients.

METHODS

Subjects

Seven schizophrenic inpatients (5 male, 2 female; mean age: 38.4 ± 12.6 yr; illness duration: 17.1 ± 10.1 yr) were recruited for the olanzapine treatment group from the inpatient service at the National Institute of Mental Health (NIMH) Neuropsychiatric Research Hospital (NRH) at St. Elizabeths in Washington, DC, and the Experimental Therapeutics Branch of the NIMH in Bethesda, MD. A matched group of 12 schizophrenic inpatients (8 male, 4 female; mean age: 34.6 ± 6.8 yr; illness duration: 12.2 ± 8.0 yr) at the NRH were recruited as a drug-free comparison group (median drug-period: 17.5 dy, range: 7 dy–180 dy). All subjects in the olanzapine treatment group also participated in a previously reported [I-123]IBZM study of the dopamine D_2 receptor occupancy by olanzapine (Raedler et al. 1999a). Each subject gave written informed consent to participate in this study according to protocols approved by the Institutional Review Board of the NIMH and by the Radiation Safety Committee and the Radioactive Drug Research Committee of the NIMH Neuroscience Center at St. Elizabeths. Prior to enrollment, all subjects received a thorough medical, neurological and psychiatric evaluation. The clinical work-up included a brain MRI to rule out structural lesions as well as for coregistration with the SPECT scans. All subjects were chronically ill and were diagnosed with schizophrenia according to DSM-IV (American Psychiatric Association, 1994). Subjects were free of active medical problems or substance abuse during the six months preceding this study. Two patients participated in both the olanzapine treatment group and the drug-free comparison group by virtue of having had a [I-123]IQNB SPECT scan during a drug-free period prior to the beginning of the olanzapine study. All drug-free patients were carefully monitored for the duration of the study as inpatients at the NRH.

Pharmacological Treatment

All patients in both groups had been treated with antipsychotics prior to the beginning of this study. Other antipsychotics and anticholinergic drugs were tapered before the start of this study. Patients in the treatment group were maintained on monotherapy with both a low dose (5 mg at night) and a high dose (20 mg at night) of olanzapine daily for a minimum of two weeks at each dose (5 mg/dy mean: 20.7 ± 3.2 dy, range: 14 dy–24 dy; 20 mg/dy mean: 24.0 ± 9.2 dy, range: 14 dy–35 dy) before each [I-123]IQNB SPECT scan. Five subjects started this study on olanzapine 5 mg/dy, while the remaining two subjects were first studied on olanzapine 20 mg/dy. Concomitant medications were reviewed carefully and were limited to ibuprofen as needed for pain and, in one subject, atenolol for tachycardia.

Clinical and Neurological Ratings

Clinical and neurological ratings were obtained on all subjects on the day of the [I-123]IQNB SPECT scan. Clinical ratings included the Positive and Negative Symptom Scale (PANSS) (Kay et al. 1986) and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962). Extrapyramidal signs, parkinsonism and dyskinesia were assessed with the Simpson Angus Rating Scale (Simpson and Angus 1970) and the Modified Abnormal Involuntary Movement Scale (AIMS) (Wyatt 1993).

[I-123]IQNB SPECT Procedure

All subjects received five drops of a nonradioactive iodine solution (Lugol's solution) on the evening before the injection of [I-123]IQNB and for three evenings following the study to minimize thyroid uptake of radioactive iodine. Isomerically pure (R,S)-[I-123]IQNB was prepared as previously described by Lee et al. (1996). Each subject received an intravenous injection of approximately 7 mCi (mean: 7.0 ± 1.4 mCi) of [I-123] IQNB at approximately 1 p.m. on the day prior to SPECT imaging and returned to the SPECT lab the following day for a 60 min SPECT scan 21 hr after the injection. We have previously shown that at this time point non-specific binding of [I-123]IQNB is reduced to background levels of radiation and the distribution of specific binding reflects the distribution of muscarinic receptors in the human brain (Lee et al. 1996; Sunderland et al. 1995; Weinberger et al. 1991). The injected mass of IQNB is a trace amount, generally much less than 10μg. Based on previous [I-123]IQNB imaging studies and a typical specific activity of about 8000 Ci/mmol (Lee et al. 1996), the peak concentration of IQNB bound to receptor at the dose used in this study is estimated to be less than 200 pM, which is less than 0.1% of the expected total muscarinic receptor concentration in these tissues (McRee et al. 1995) and corresponds to a concentration of free (unbound) IQNB on the order of 20 fM. Thus, the injected IQNB does not occupy an appreciably number of muscarinic receptors and does not affect the percent occupancy by olanzapine in these studies.

During imaging, subjects reclined comfortably in the chair of the CERASPECT camera (Digital Scintigraphics, Waltham, MA). SPECT data were acquired with a high-resolution collimator (7.5 mm FWHM) in 120-projection step-and-shoot mode. Windows below (127–145 keV) and above (175–191 keV) the photopeak window (145–175 keV) were acquired and subtracted from the photopeak window to correct for scatter and penetration. The data were reconstructed by filtered backprojection with a Butterworth filter (cutoff = 1 cm, power factor = 10) and stored as a 64 × 128 × 128 matrix composed of isotropic 1.67 mm voxels.

Image Analysis

In order to accurately demarcate regions of interest (ROIs) according to each individuals unique brain anatomy, the [I-123]IQNB image volumes were coregistered with volume magnetic resonance imaging (MRI) data sets. The T1-weighted MRI volumes were acquired on a 1.5T GE Sigma (General Electric Medical Systems, Milwaukee, WI) with a spoiled gradient recalled acquisition in the steady state (SPGR) sequence (TR = 24 msec, TE = 5 msec). The MRI image volume consisted of 124 contiguous sagittal slices with a slice thickness of 1.5 mm and an in-plane field of view of 240 mm across a 256 × 256 matrix. Using the NIH Image computer program (public domain), each MRI volume was reoriented such that a line connecting the anterior and posterior commissures in a midsagittal slice was aligned horizontally, and rescaled to the dimensions of the SPECT image volumes. These transformed MRI volumes were imported into the CERASPECT console where all subsequent processing took place.

Analyses of all [I-123]IQNB SPECT images were done by a single investigator (RAU), who was blind to the clinical status of subjects. First, the SPECT image volumes were coregistered with the MRI volumes by semi-automatically drawing alignment ROIs on the SPECT images that delineated gross anatomical features (e.g. cortical surface, ventricles, etc.) in three orthogonal planes. These alignment ROIs were then superimposed onto the MRI image volume and reoriented visually to best conform with corresponding features of the MRI. Finally, the reoriented ROIs were superimposed back onto the SPECT image volume, and the SPECT image volume was reoriented to fit back into the ROI shell created from it. In cases of severe initial misalignment, this process was repeated iteratively to obtain the best fit.

With the aid of standard anatomical atlases (Aquilonius and Eckernas 1980; Talairach and Tournoux 1988; Duvernoy 1991), measurement ROIs were drawn on five consecutive transverse slices of MRI for the following brain regions: caudate, putamen, thalamus, pons, medial frontal cortex, lateral frontal cortex, posterior temporal cortex, occipital cortex, and cerebellum. These measurement ROIs were transferred onto the corresponding slices of the SPECT volumes for analysis of [I-123]IQNB binding (Figure 1). Raw ROI data were measured as counts per minute per milliliter of tissue (cpm/ml). After subtracting cerebellar (background) activity, the data were corrected for decay, and converted to absolute units (nCi/ml) based on calibration data acquired with a uniform flood phantom on the day of SPECT imaging. Lastly the ROI data were normalized to the injected dose of [I-123]IQNB to yield nCi/ml per mCi injected dose for subsequent statistical analysis.

In an effort to better quantify individual scan data as effective binding potential, BP′, we attempted to measure levels of free [I-123]IQNB in blood plasma by using thin layer chromatography (TLC) to analyze in ultrafiltrates (cutoff: 30 kDa) of plasma (Jones et al. 1997). However, free parent [I-123]IQNB in plasma at 21 hr post-injection proved to have an exceedingly low concentration of approximately 0.14% of the total radioactivity in plasma. Further, approximately 93% of the ultrafiltrate radioactivity was in the form of polar metabolite(s). The polar metabolite(s) constituted only about 2.3% of total plasma radioactivity, however; total plasma radioactivity was dominated by species, including parent [I-123]IQNB, that were bound to plasma proteins. In the case of [I-123]IQNB, it would appear that a largely protein-bound concentration of radioligand in blood plasma is in equilibrium with a very small concentration of free ligand (on the order of 10–20 fM) that, in turn, maintains the persistent [I-123]IQNB binding observed in brain at extended times after injection. Unfortunately, the very low concentrations of free parent [I-123]IQNB in plasma and the large variance associated with measuring these small values, severely restricted the utility of these measurements and made ac-

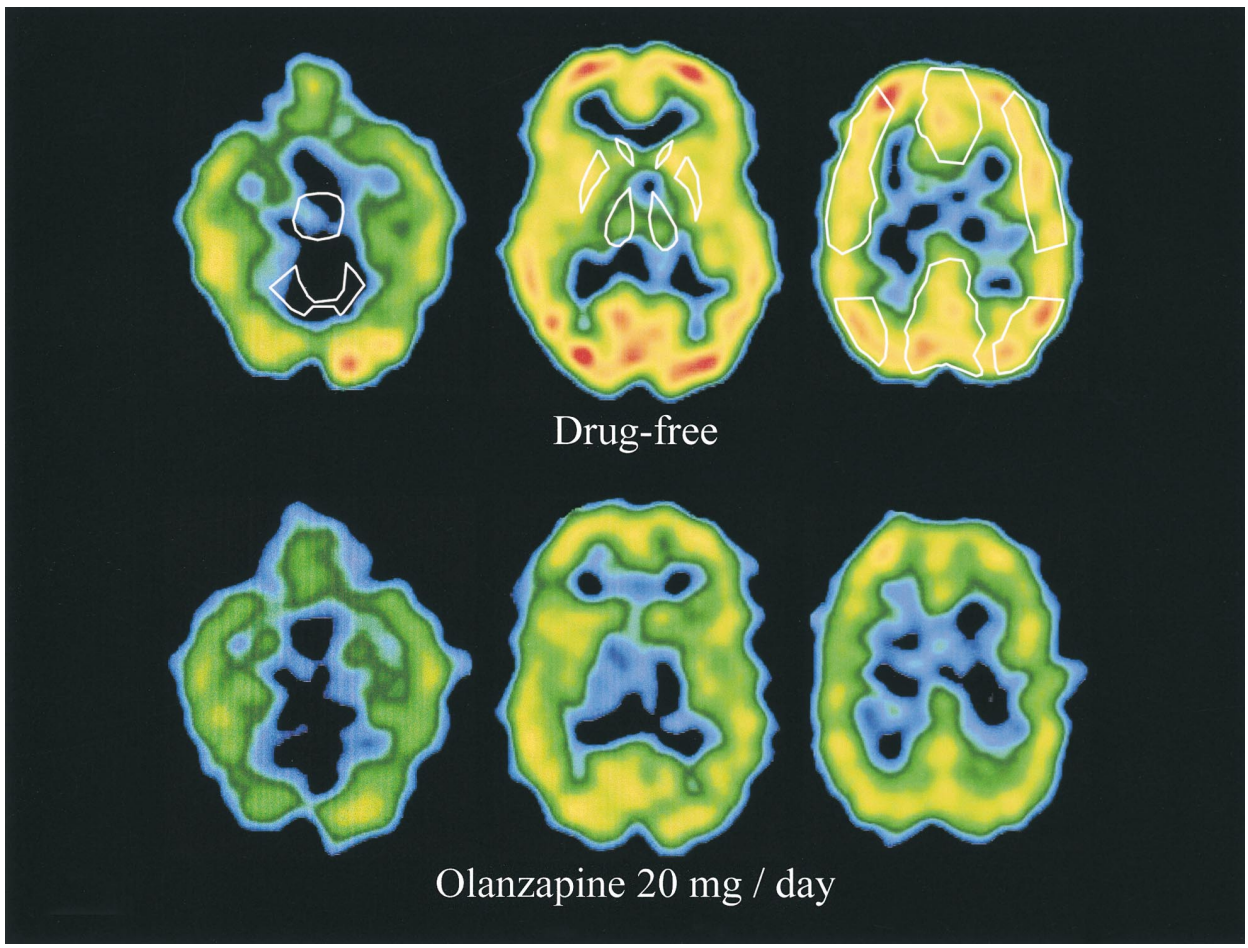

Figure 1. Typical [I-123]IQNB SPECT scans of a medication-free patient (upper) and a patient treated with olanzapine 20 mg/dy (lower) at the levels of pons (left), the basal ganglia and thalamus (center), and dorsolateral cortex (right). Regions of interest (ROI) are shown for the medication-free SPECT images.

curate determination of the effective binding potential, BP′, impossible in this data set. In what follows, only analyses of ROI data normalized to injected dose are presented.

Occupancy Estimation

The percent occupancy of muscarinic receptors by olanzapine at each of the two dosage levels was estimated by the following expression:

$$\text{Percent Occupancy (ROI)} = \left[1 - \frac{B_s(ROI)}{B_{df}(ROI)}\right] \times 100$$

where $B_s(ROI)$ is the [I-123]IQNB binding observed in a ROI for an individual subject on olanzapine $B_{df}(ROI)$ is the mean value of binding in the drug-free group of patients for the same ROI. Binding was expressed as decay and background corrected nCi/ml per mCi injected dose of [I-123]IQNB throughout as noted above. Often occupancy is estimated by comparison to binding data from a cohort of normal control subjects, but this is only valid if it has previously been established that normal control subjects and drug-free patients do not differ significantly with respect to binding of the ligand being considered. Our preliminary data comparing drug-free schizophrenic patients and normal control subjects indicate that this assumption may not be valid for [I-123] IQNB binding to muscarinic receptors (Raedler et al. 1999b). Thus, comparison to drug-free schizophrenic patients was chosen as the appropriate estimator.

Equilibrium saturation binding analysis of percent occupancy data was performed using the formula (Yamamura et al. 1985):

$$\text{Percent Occupancy (ROI)} = \left[\frac{F_o}{F_o + K_d'}\right] \times O_{\max}(ROI)$$

where F_O is the concentration of free olanzapine in brain, K_d' is an effective *in vivo* dissociation constant for olanzapine binding at muscarinic receptors, and $O_{\max}(ROI$ is the maximum percent occupancy (saturation) level. Using normative data on olanzapine (Aravagiri et al. 1996; Eli Lilly and Company 1999), we have estimated that the concentration of free olanzapine in blood plasma is approximately given by 0.5 nM/(mg/dy) at steady-state with daily dosing Further, assuming the free concentration of olanzapine in the extracellular brain compartment to be in equilibrium with the free concentation in blood plasma, this approximate factor was used to estimate F_O from the daily olanzapine dose, This permitted fitted values of K_d' to be expressed in familiar, though very approximate, absolute units (nM) rather than in mg/dy. The maximum percent occupancy, $O_{\max}(ROI)$, may be less that 100% if olanzapine shows subtype selectivity since [I-123]IQNB is not subtype selective. Nonlinear curve fitting using observed percent occupancy and F_O as input was used to determine best-fit values for $K_{d'}$ and $O_{\max}(ROI)$ in each of four types of brain tissue: 1) striatum (caudate and putamen), 2) thalamus, 3) pons, and 4) cortex (medial frontal, lateral frontal, temporal,and occipital). Each of these four types of tissue has distinct proportions of subtypes of muscarinic receptors (McRee et al.; Levey et al. 1991).

Statistical Analysis

Statistical analysis of the data was performed with the aid of Statistica for Window 5.1 (StatSoft, Inc., Tulsa, OK) and Microsoft Excel 97 (Microsoft Corp., Redmond, WA). To compare data from patients on olanzapine with that from drug-free patients Hotelling's T^2 and Student's t-test for independent samples was applied. Within-subject comparisons of effect of olanzapine dosage were accomplished with paired t-tests. Associations with [I-123]IQNB binding and occupancy data were assessed with Pearson's product moment correlation coefficient (r) and Spearman's rank order correlation coefficient (R) with the requirement of significance on both tests to reduce the influence of outliers and spurious associations. Nonlinear curve fitting and parameter estimation for equilibrium analysis of the olanzapine occupancy data were carried out with Solver in Excel 97.

RESULTS

Clinical and Neurological Ratings

All patients tolerated olanzapine and the drug-free period well and were able to complete the study. One subject developed tachycardia on olanzapine at 20 mg/dy and required treatment with atenolol. Overall, clinical ratings (BPRS, PANSS, and PANSS subscores) were significantly different for patients on olanzapine than for drug-free patients ($T^2 = 19.9$, $F(5,16) = 3.19$, $p = .035$); significantly lower scores were noted for BPRS (52 ± 10 vs. 66 ± 12, $t = 2.90$, $df = 20$, $p = .009$) and the general symptom subscale of the PANSS (34 ± 8 vs. 41 ± 9, $t = 2.21$, $df = 24$, $p = .037$). A similar analysis of EPS measures revealed no significant differences ($T^2 = 0.30$, $F(3,18) = 0.09$, $p = .96$; all $t < 0.69$, $p > .49$). Patients taking 5 mg/dy of olanzapine had significantly lower BPRS scores only ($t = 2.30$, $df = 13$, $p < .038$), whereas at 20 mg/dy both BPRS and the PANSS general symptom subscale were significantly reduced ($t = 2.44$, $df = 13$, $p < .030$ and $t = 2.38$, $df = 17$, $p < .029$, respectively). Paired t-tests did not reveal significant within-subject differences between low (5 mg/dy) and high (20 mg/dy) dose on any clinical or EPS rating ($t < 1.45$, $df = 6$, $p > .22$) with the exception of a weakly significant increase in the AIMS total symptom rating (3.1 vs. 6.6, $t = 2.40$, $p = .053$) at high dose. All observed extrapyramidal side effects were mild and did not require dose-adjustment or concomitant medications during the study.

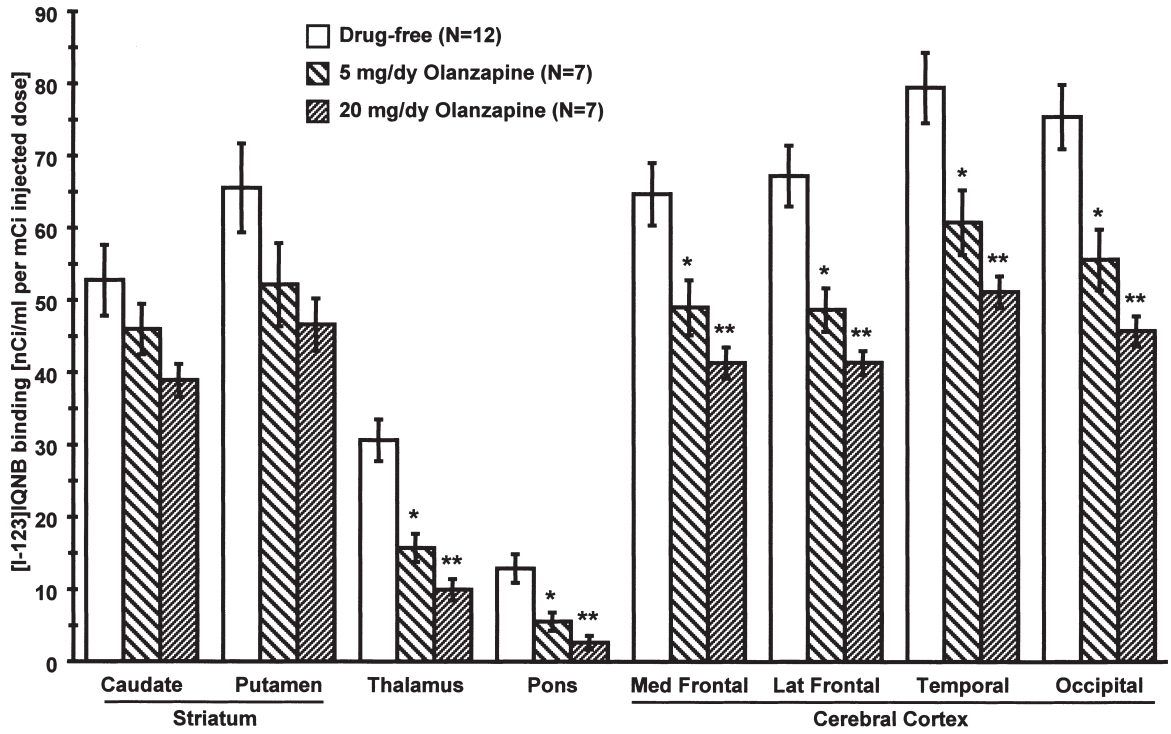

Figure 2. [I-123]IQNB binding to muscarinic receptors in schizophrenic patients that were drug-free (open bars), administered 5 mg/dy olanzapine (coarse hatching), and administered 20 mg/dy olanzapine (fine hatching). Single asterisks (*) indicate data where binding at 5 mg/dy is significantly less ($p < .05$) than drug-free binding; double asterisks (**) indicate significantly lower binding at 20 mg/dy as compared to 5 mg/dy.

Muscarinic Receptor Binding and Occupancy

All subjects showed a similar qualitative pattern of [I-123]IQNB binding with highest binding in the cortical ROIs, followed by somewhat lower binding in the basal ganglia and substantially lower binding in thalamus and pons. This regional pattern of distribution is consistent with previously reported data (Eckelman et al. 1984; Weinberger et al. 1991; Sunderland et al. 1995; Lee et al. 1996). Mean values for [I-123]IQNB binding in nCi/ml per mCi injected dose and for percent occupancy of muscarinic receptors by olanzapine are given in Table 1. [I-123]IQNB binding was significantly reduced relative to drug-free patients both by the 5 mg/dy dose (Hotelling's $T^2 = 42.9$, $F(8,10) = 3.15$, $p = .046$) and by the 20 mg/dy dose (Hotelling's $T^2 = 83.5$, $F(8,10) = 6.14$, $p = .005$) of olanzapine. However, as show in the table, [I-123]IQNB binding was not significantly diminished relative to drug-free patients in the caudate at either dose (all $t < 1.91$, $df = 17$, $p > .06$), and in the putamen the reduction was only significant at the higher dose ($t = 2.19$, $df = 17$, $p = .042$). In thalamus and pons and in the cerebral cortex significant reductions in [I-123]IQNB binding are apparent in all regions at both 5 mg/dy and 20 mg/dy (all $t > 2.46$, $df = 17$, $p < .025$; Table 1). Similarly, paired t-tests comparing the 5 mg/dy treatment with the 20 mg/dy treatment revealed a significant dose dependent difference in all regions (all $t > 2.44$, $df = 6$, $p < .050$) except striatum (all $t < 2.14$, $df = 6$, $p > .08$; Table 1). Figure 2 illustrates these findings.

Mean percent occupancy of muscarinic receptors by olazanpine at 5 mg/dy and 20 mg/dy are listed also in Table 1. Muscarinic receptor occupancy values at 5 mg/dy are moderate and range from 13% (caudate) to 57% (pons); at 20 mg/dy, percent occupancy values are correspondingly higher ranging from 26% (caudate) to 79% (pons). The high occupancy values for thalamus and pons is a strong indication that olanzapine has high affinity *in vivo* for M_2 subtype receptors since both these regions are have predominantly M_2 subtype receptors. Of total muscarinic receptors in pons 82% are of the M_2 subtype, and in thalamus 50% are M_2 (McRee et al. 1995). However, binding to M_2 subtype receptors alone cannot account for the occupancy levels observed in the cortex and striatum, and some degree of olanzapine binding to other muscarinic subtypes must be introduced in order to satisfactorily explain these data. M_1 and M_4 subtypes occur prominently in striatum and cortex (McRee et al. 1995) and are the most likely loci of olanzapine binding in these regions. On the other hand, the relatively lower occupancy observed in these regions argues that olanzapine has lower affinity for these subtypes *in vivo.* The status of olanzapine occu-

Table 1. [I-123]IQNB Binding in nCi/ml Per mCi Injected Dose (Mean ± S.E.M) and Percent Occupancy by Olanzapine

		Striatum				Cortex			
		Caudate	Putamen	Thalamus	Pons	Med Frontal	Lat Frontal	Temporal	Occipital
Drug-free (N = 12)		52.8 ± 4.9	65.6 ± 6.2	30.6 ± 2.9	12.9 ± 2.0	64.7 ± 4.3	67.3 ± 4.2	79.5 ± 4.9	75.5 ± 4.5
Olanzapine (N = 7)	5 mg/dy	46.0 ± 3.5	52.2 ± 5.8	15.7 ± 2.0	5.6 ± 1.3	49.0 ± 3.8	48.7 ± 3.0	60.8 ± 4.5	55.7 ± 4.2
Occupancy		13%	20%	49%	57%	24%	28%	23%	26%
	20 mg/dy	38.9 ± 2.3	46.7 ± 3.6	9.9 ± 1.5	2.7 ± 1.0	41.4 ± 1.7	41.4 ± 1.7	51.2 ± 2.2	45.8 ± 2.1
Occupancy		26%	29%	68%	79%	36%	38%	36%	39%
Drug-free vs 5 mg/dy	t	0.96	1.45	3.63	2.63	2.46	3.06	2.56	2.95
df = 17 (independent)	p	0.35	0.17	0.002	0.018	0.025	0.007	0.020	0.009
Drug-free vs 20 mg/dy	t	2.06	2.19	5.19	3.76	3.92	4.50	4.24	4.85
df = 17 (independent)	p	0.06	0.042	0.0001	0.0016	0.0011	0.0003	0.0005	0.0001
20 mg/dy vs 5 mg/dy	t	2.14	1.16	3.35	3.16	2.44	2.61	2.55	2.84
df = 6 (paired)	p	0.08	0.29	0.015	0.020	0.050	0.040	0.043	0.030

pancy at the much less ubiquitous M_3 and M_5 subtypes is unclear from these data. The results of the nonlinear curve fitting to determine values for the effective *in vivo* dissociation constant, K_d', and maximum projected occupancy, O_{max}, are summarized in Figure 3. Values of K_d' fell into a reasonable range from 1.5 nM (thalamus and pons) to 2.8 nM (striatum) indicating that the estimation from normative data of approximate free olan-

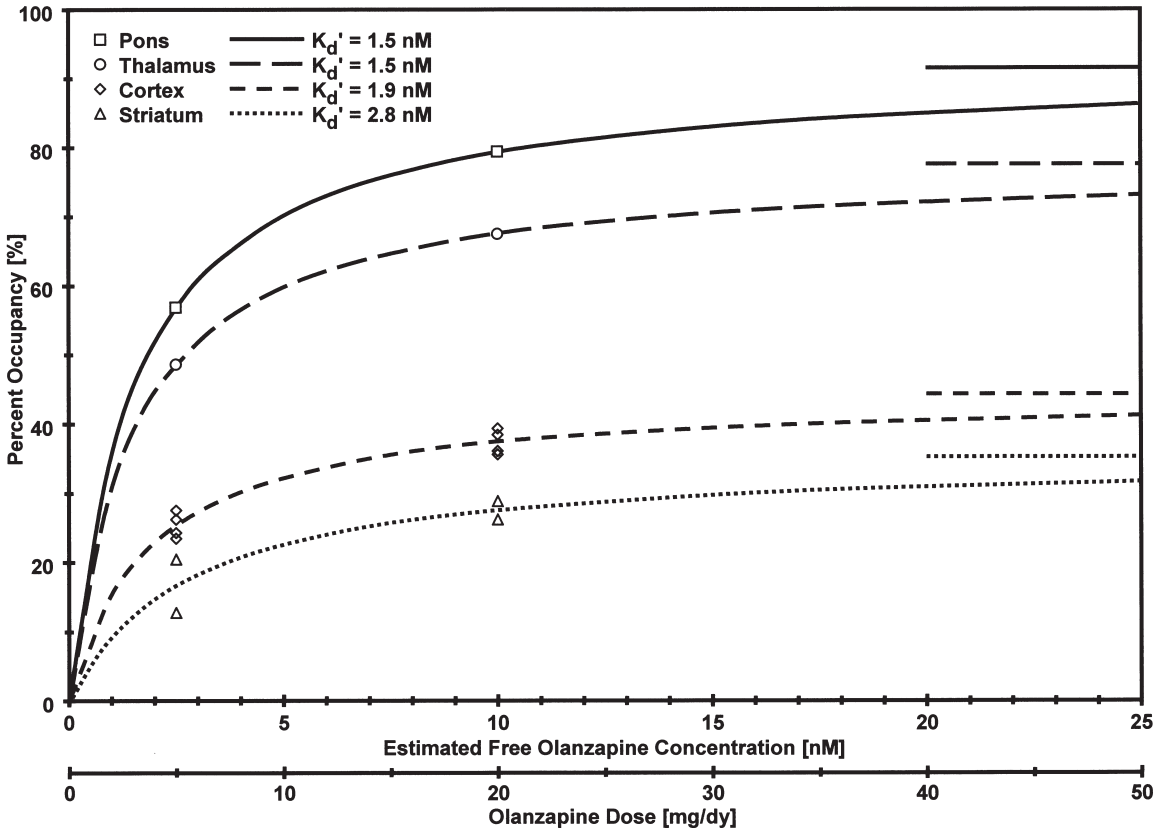

Figure 3. Saturation binding curves obtained by nonlinear curve fitting to observed olanzapine occupancy data measured with [I-123]IQNB *in vivo.* A single curve and its corresponding K_d' value from the fit is shown for each of the four tissue types that are distinct with respect to muscarinic receptor subtypes: pons, thalamus, cortex, and striatum. Each point represents the mean value of occupancy data for the seven patients. Multiple points for the cortex data correspond to the four cortical regions analyzed (medial frontal, lateral frontal, temporal and occipital) while those for the striatum correspond to caudate and putamen regions. Short horizontal lines at the left edge indicate maximum occupancies predicted by data at 5 mg/dy and 20 mg/dy. Free olanzapine units along the x-axis were estimated by scaling the oral dosages to normative data taken from the literature (Aravagiri et al. 1996). As such, the units along this scale may be of limited absolute accuracy; however, they retain value as approximate, relative measures in convenient units.

zapine concentrations in brain tissue was not unreasonable. Presumably, absolute accuracy of K_d' values determined by this method might be improved substantially by careful measurements of the concentration of free olanzapine in blood plasma for each individual patient. But, be that as it may, the *relative* values and the rank ordering of the *in vivo* affinities are unaffected by the choice of absolute units, and these ratios might ultimately be the most clinically useful measures. Care, however, must be taken in interpreting the fitted parameters, relative or absolute. Since olanzapine clearly shows subtype selectivity for muscarinic receptors *in vivo*, the true equilibrium binding curves will not exactly follow the simple, single-component model employed here; at higher doses of olanzapine where binding to subtypes with low affinity becomes significant one would anticipate substantial deviations form these curves. But over the range of clinically relevant doses, as employed here, the curves and the parameters may still have meaning.

Finally, the four types of brain tissue, i.e. striatum, thalamus, pons and cortex, studied here are distinct from one another with respect to muscarinic subtype proportions that have been tabulated by McRee et al. (1995). Using these proportions and assuming the percent occupancy of each individual subtype by olanzapine to be constant throughout the brain, it was possible to perform a least squares fit to estimate the relative proportions of olanzapine occupancy for the three most prevalent subtypes, M_1, M_2, and M_4. In other words, relative occupancies for each individual subtype were determined that, when summed, provided the best matched to the overall pattern of net occupancy levels observed at a single olanzapine dose. The results of this analysis are summarized in Figure 4. Shown in the figure are the olanzapine occupancy values for each region at 20 mg/dy (open bars). The leftmost hatched bars in the figure that extend to 100% show the proportions of the muscarinic receptor subtypes in each type of tissue. The lower hatched bars to the right indicate the predicted binding from the least squares fit that found the proportions of olanzapine binding to muscarinic subtypes, M_2:M_1:M_4, to be 20:9:3. This suggests that olanzapine may be roughly twice as potent at M_2 than at M_1 and almost 7-fold more potent at M_2 than at M_4 *in vivo.*

The muscarinic receptor occupancy values were compared to previously reported striatal dopamine D_2 receptor occupancy values measured with [I-123]IBZM SPECT in the same subjects (Raedler et al. 1999a). At 5 mg/dy olanzapine, D_2 occupancy in striatum significantly exceed muscarinic occupancy (all $t > 4.38$, $df = 6$, $p < .005$) in all ROIs except thalamus (58% vs 49%, t =

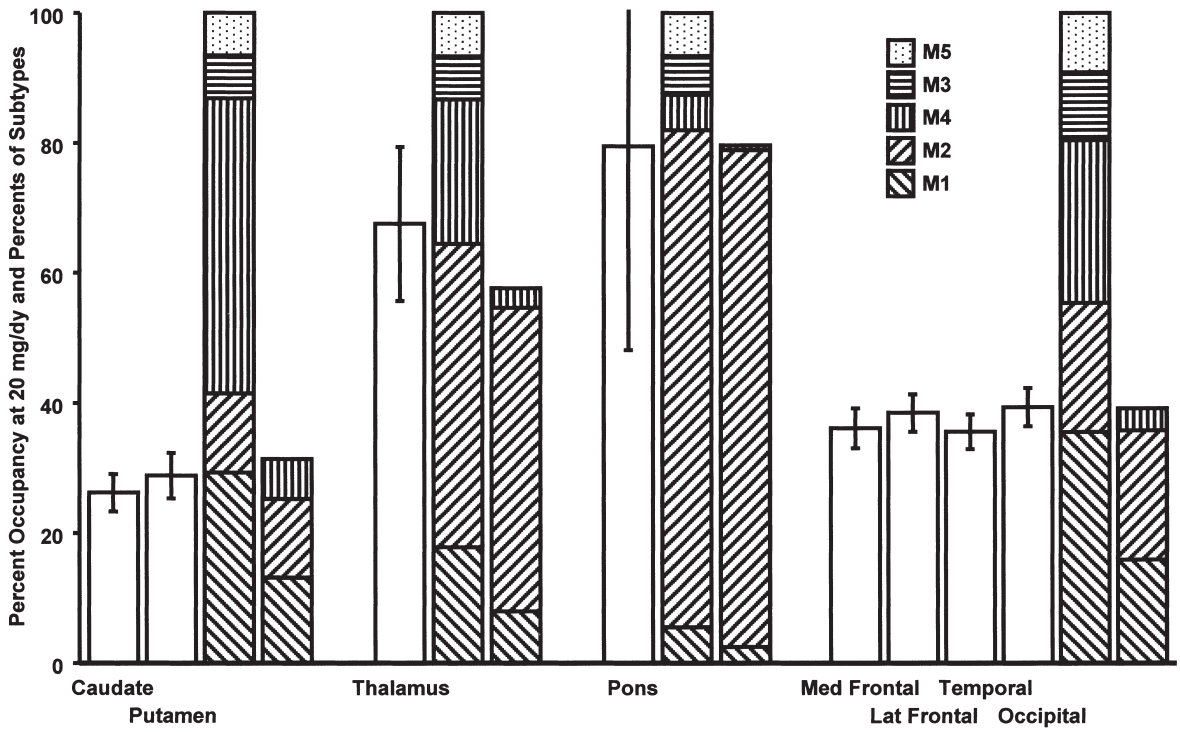

Figure 4. Olanzapine occupancies at 20 mg/dy compared with relative concentrations of muscarinic receptor subtypes, M_1 through M_5, *in vitro* (McRee et al. 1995; Levey et al. 1991; leftmost hatched bars). The rightmost hatched bars indicate the predicted occupancy if olanzapine occupies muscarinic subtypes in the proportions 20:9:3 = M_2:M_1:M_4 as predicted by a least-squares fit to the observed data utilizing only these three most prevalent subtypes (see text).

1.71, df = 6, $p = .14$) and pons (58% vs 57%, t = 0.12, df = 6, $p = .91$). No significant correlation between striatal D_2 occupancy and muscarinic occupancy were observed at this dose. At 20 mg/dy olanzapine, a similar pattern was observed with D_2 occupancy in the striatum once again significantly exceeding muscarinic occupancy (all $t > 2.48$, df = 6, $p < .047$, thalamus) in all ROIs except pons (79% vs 79%, t = 0.18, df = 6, $p = .86$). These findings at both dose levels appear to indicate that potency of olanzapine at M_2 subtype muscarinic receptors is roughly comparable to its potency at striatal D_2 dopamine receptors. At 20 mg/dy olanzapine, significant correlations between striatal D_2 occupancy by olanzapine and muscarinic occupancy appeared in three ROIs: putamen ($r = 0.80$, $p = .03$; $R = 0.82$, $p = .02$), pons ($r = 0.85$, $p = .02$; $R = 0.75$, $p = .05$), and medial frontal cortex ($r = 0.90$, $p = .006$; $R = 0.86$, $p = .01$).

Several significant associations between changes in PANSS ratings from 5 mg/dy to 20mg/dy of olanzapine and the corresponding change in muscarinic occupancy as measured by [I-123]IQNB SPECT were found. Change in the total PANSS ratings correlated positively at a significant level with the change in occupancy in caudate ($r = 0.84$, $p = .018$; $R = 0.86$, $p = .014$) and thalamus ($r = 0.78$, $p = .041$; $R = 0.82$, $p = .023$); change in the general symptom PANSS ratings also correlated significantly with occupancy change in caudate ($r = 0.89$, $p = .008$; $R = 0.86$, $p = .012$). The strongest positive correlations, however, were between changes in negative symptom PANSS ratings and changes in muscarinic occupancy by olanzapine in caudate ($r = 0.91$, $p = .004$; $R = 0.95$, $p = .001$), putamen ($r = 0.90$, $p = .005$; $R = 0.88$, $p = .008$), and occipital cortex ($r = 0.80$, $p = .032$; $R = 0.77$, $p = .041$). The strong correlations between increased muscarinic occupancy by olanzapine in the striatum and increased negative symptoms suggests that antagonism of non-M_2 muscarinic receptors in the striatum at the higher (20 mg/dy) dose may be exacerbating negative symptoms of schizophrenia. The [I-123]IQNB measurements of increased olanzapine occupancy in the striatum accounts for over 80% of the variance in negative symptom increase.

DISCUSSION

This is the first study to examine the effects of any antipsychotic drug on muscarinic receptor availability *in vivo* and the first to image [I-123]IQNB binding in schizophrenic patients on and off medication. We compared muscarinic receptor occupancy by the atypical neuroleptic, olanzapine, in patients treated taking both a low dose (5 mg/dy) and a high dose (20 mg/dy) with a control group of drug-free schizophrenic patients. [I-123]IQNB binding was significantly lower at both low dose and high dose of olanzapine in all regions of interest except the striatum, though changes in the striatum were in the same direction as in other region. Comparing the low dose with the high dose of olanzapine, there was a further dose-dependent reduction of [I-123]IQNB binding at the higher dose. This reduction of [I-123]IQNB binding, even on a low dose of olanzapine, indicates that olanzapine binds potently to muscarinic acetylcholine receptors *in vivo*, particularly those receptors of the M_2 subtype.

We estimated muscarinic receptor occupancy levels by comparing [I-123]IQNB binding for patients treated with olanzapine with a group of drug-free schizophrenic patients to avoid the potential confound of an effect in binding due to diagnosis, per se. We obtained moderate mean muscarinic receptor occupancy levels in the cortex and striatum ranging between 13% and 28% (olanzapine 5 mg/dy) and 26% and 39% (olanzapine 20 mg/dy). The four-fold increase in the dose of olanzapine resulted in muscarinic receptor occupancy values that conform reasonably to a simple equilibrium binding model for olanzapine over this dose range.

The muscarinic receptor occupancy values as measured with [I-123]IQNB SPECT correlated positively with striatal dopamine D_2 receptor occupancy as determined with [I-123]IBZM SPECT in the putamen, pons, and medial frontal cortex at 20 mg/dy olanzapine. However, the mean muscarinic receptor occupancy values at either dose were significantly lower than comparable mean striatal dopamine D_2 receptor occupancy in all regions except thalamus and pons. In our prior study in schizophrenic patients we found mean striatal dopamine D_2 receptor occupancy values of 58% on 5 mg/dy olanzapine and 79% on 20 mg/dy olanzapine (Raedler et al. 1999a). Other groups have reported similar levels of dopamine D_2 receptor occupancy at comparable doses (Kapur et al. 1998; Tauscher et al. 1999). These results suggest that at least *in vivo*, olanzapine is a relatively less potent muscarinic antagonist than D_2 antagonist except at the M_2 subtype of muscarinic receptor where the potencies may be comparable. In the M_2-rich pons, occupancy of muscarinic receptors by olanzapine roughly equaled that of striatal D_2 receptors, being 57% on 5 mg/dy olanzapine and 79% on 20 mg/dy olanzapine.

Our comparison of muscarinic receptor availability on olanzapine to medications with clinically evident antimuscarinic activity may help shed light on the relationship between *in vivo* muscarinic receptor availability and frequency of extrapyramidal side effects or anticholinergic side effects. Using the analogy of dopamine D_2 receptor occupancy, a threshold of 60%–70% receptor occupancy has been suggested for therapeutic efficacy on the symptoms of psychosis (Nordström et al. 1993; Heinz et al. 1996). A similar threshold has not been established for anticholinergic properties. However, the strong correlation ($r > 0.90$) between increased muscarinic occupancy in the striatum and increased

negative symptom PANSS ratings in going from low dose to high dose may be an indication that antimuscarinic side effects are coming into play at any overall muscarinic occupancy of only 30%; this appears to be due to the subtype selectivity we have observed for olanzapine that leads to some subtypes being close to saturation binding while others subtypes appear to be relatively unaffected.

Our results are not fully consistent with *in vitro* assays of the receptor binding profile of olanzapine. In particular, our data clear indicates that olanzapine is most potent *in vivo* at the M_2 subtype of muscarinic receptor as shown by very high occupancy values in the M_2-rich pons and thalamus, whereas the findings of Bymaster et al. (1996a) indicate that olanzapine has nearly ten-fold higher affinity for M_1 than for M_2 in rat tissue *in vitro.* However, the estimates we have made of the effective *in vivo* dissociation constant K_d' (1.5 nM to 2.8 nM) agree fairly well with the low range of K_i values (1.9 nM to 2.5 nM) found by Bymaster et al. (1996a). In another study by the same group, treatment with olanzapine also resulted in an inhibition of *ex vivo* binding of [H-3]pirenzepine, (Bymaster et al. 1996b). Yet, unpublished experiments by this group suggest that the previous study may have overestimated the affinity of olanzapine to the muscarinic receptor and that the true affinity may be 10-fold weaker (Bymaster et al. 1999). The apparent discrepancy between the *in vitro* binding assays and our *in vivo* observations remains unresolved. However, the *in vitro* assays were performed using a different radioligand ([H-3]pirenzepine) for M_1 receptors in rat tissue than for other subtypes ([H-3]N-methylscopolamine) and non-brain tissues were used to assay M_2 (heart tissue) and M_3 (salivary gland tissue) (Bymaster et al. 1996a). Several animal experiments suggest a higher affinity of olanzapine to dopamine D_2 receptors than to muscarinic receptors. Using *in vitro* receptor binding in rat striatum, Schotte et al. (1996) determined a nanomolar affinity of olanzapine to the muscarinic cholinergic receptors (K_i = 26 nM). In *ex vivo* receptor autoradiography, however, olanzapine had a ten times higher potency for dopamine D_2 receptors than for muscarinic receptors (Schotte et al. 1996). Using an *in vivo* assay based on the blockade of phosphoinositide (PI) hydrolysis, olanzapine was also a less potent inhibitor of muscarinic M_1, M_3 and M_5 receptor binding than serotonin 5-HT_2 as well as dopamine D_2 and D_3 receptor binding (Zhang and Bymaster 1999). These observations also are consistent with our finding of relatively greater dopamine D_2 than muscarinic M_1 occupancy *in vivo.* It should also noted that *in vitro* assays are prone to several adverse effects to which *in vivo* assays are immune. These include the effects of pH, ion concentrations, temperature, and generally the difficulty of simulating closely both the intra- and extra-cellular environment to which the transmembranic receptor is naturally exposed *in vivo.* Many subtle factors can strongly influence *in vitro* binding assays and in particular affinity values (Yamamura 1985), and perhaps the extent to which *in vitro* values can be carried over into the *in vivo* realm is questionable. This is certainly a topic that warrants further investigation. Also, it must be stressed that the mathematical methodologies we have used are an exploratory approach aimed at obtaining more quantitative and detailed information regarding *in vivo* drug occupancy. Though relatively simple and straightforward, the methods, as presented here, suffer from a number of limitations including the minimal number of data points used to determine saturation binding parameters, the use of normative data rather than individual assays to estimate levels of free olanzapine in blood plasma and the desirability of more reliable data on the proportions of receptor subtypes in various brain regions. Further studies are clearly needed to validate these methods and to determine both their reliability and the extent to which they might be improved and individualized to each subject.

Although clinical studies have shown the superiority of higher doses of olanzapine (e.g. Beasley et al. 1996a, 1996b, 1997; Tollefson et al. 1997a), this study failed to find differences in clinical ratings between the low dose and high dose of olanzapine. We did not design this study to examine the clinical efficacy of olanzapine and the small sample size and the short duration of treatment preclude any conclusions about clinical efficacy of olanzapine from our data.

The prevalence of extrapyramidal side effects in this study was small and did not differ significantly between the low and high dose of olanzapine. Clinical studies in larger patient samples have shown lower incidence of extrapyramidal side effects (Tran et al. 1997) as well as reduced risk of tardive dyskinesia (Tollefson et al. 1997b) in subjects treated with olanzapine. We previously demonstrated a dose dependent, moderate to high degree of Dopamine D_2 receptor occupancy in subjects treated with olanzapine (Raedler et al. 1999a). For typical antipsychotics, dopamine D_2 receptor occupancy levels of more than 75%–80% have been associated with increased incidence of extrapyramidal side effects (Farde et al. 1992; Nordström et al. 1993). The lack of obvious extrapyramidal side effects, despite relatively high levels of D_2 receptor occupancy, suggests that other mechanisms may mitigate the development of these side effects in patients treated with olanzapine.

Several factors may play a role in reduced EPS liability with olanzapine. In animal studies the decrease in number of spontaneously active A10 but not A9 dopamine cells after chronic administration of olanzapine suggests a reduced propensity of extrapyramidal side-effects (Stockton and Rasmussen 1996). At higher doses, however, the increase in the number of Fos-positive neurons in the dorsolateral striatum may be consis-

tent with the potential of olanzapine to produce extrapyramidal side effects (Robertson and Fibiger 1996). With regards to the receptor binding properties of olanzapine, additional protection from extrapyramidal side effects may come from serotonin 5-HT2 receptor antagonistic properties (Kapur 1996). Both *in vitro* (Bymaster et al. 1996a) and *in vivo* studies (Nyberg et al. 1997; Kapur et al. 1998) have shown that olanzapine has a high affinity to the serotonin 5-HT_2 receptor and that treatment with olanzapine results in high degree of blockade of this receptor even at low doses. However, these effects on dopamine cell firing patterns and on 5-HT_2 binding do not differentiate olanzapine from other "atypical" antipsychotics with relatively high dopamine D_2 affinity (e.g. risperidone, ziprasidone). An additional factor that may explain the reduced EPS liability of olanzapine may be its antimuscarinic properties, which we have demonstrated *in vivo* in this report.

In conclusion, we describe the effects of the atypical antipsychotic olanzapine on muscarinic receptor availability *in vivo* as measured by [I-123]IQNB SPECT. We demonstrate that treatment with olanzapine (5 mg/dy–20 mg/dy) results in a subtype selective, dose dependent reduction of muscarinic receptor availability that ranges from moderate (striatum and cortex) to high (thalamus and pons) with a pattern indicating highest affinity at M_2 receptors. The results may implicate anticholinergic mechanisms in the low EPS liability of this compound. Also, the associations with clinical ratings that we have observed appear to implicate muscarinic mechanisms in the incidence of positive symptoms of schizophrenia, and possibly negative symptom increases arising from high doses of olanzapine. These results should be confirmed in a larger sample of subjects and compared with results for other antipsychotic and anticholinergic medications.

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