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Electrophysiological Effects of Olanzapine, a Novel Atypical Antipsychotic, on A9 and A10 Dopamine Neurons

Marsha E. Stockton, B.S., and Kurt Rasmussen, Ph.D.

This study examined the effects of the novel atypical antipsychotic olanzapine (LY170053) on the activity of substantia nigra pars compacta (A9) and ventral tegmental area (A10) dopamine cells in anesthetized rats. Acute administration of olanzapine (10, 20 mg/kg sc) increased the number of spontaneously active A10, but not A9, dopamine cells. Chronic administration of olanzapine (10, 20 mg/kg/day × 21 days) decreased the number of spontaneously active A10, but not A9, dopamine cells. Administration of the dopamine agonist apomorphine reversed the effects of chronic olanzapine on A10 cells, indicating a possible depolarization-inactivation mechanism. In conclusion, olanzapine has selective effects on A10 versus A9 dopamine cells following acute and chronic administration. These effects of olanzapine on dopamine cells are similar to the effects observed with clozapine and may play an important role in the atypical antipsychotic profile of olanzapine. ***[Neuropsychopharmacology 14:97–104, 1996]***

KEY WORDS: *Schizophrenia; Olanzapine; Electrophysiology; Dopamine; Atypical antipsychotics*

Schizophrenia is a debilitating disorder of the central nervous system whose symptoms have been divided into two classes: positive symptoms, including hallucinations, delusions, and conceptual disorganization; and negative symptoms, including social withdrawal, blunted affect, and poverty of speech. Traditionally, classical antipsychotics like haloperidol have been used to treat the positive symptoms of schizophrenia. However, classical antipsychotics have much lower efficacy in treating the negative symptoms of schizophrenia and can also cause extrapyramidal motor side effects (EPS), such as tremor and rigidity. Clozapine has been described as an atypical antipsychotic because it is effective in the treatment of both positive and negative symptoms of schizophrenia and has a low EPS liability (Claghorn et al. 1987; Juul Povlsen et al. 1985). Clozapine also has superior efficacy compared to classical antipsychotics in treating positive and negative symptoms in treatment-responsive (Claghorn et al. 1987) and treatment-refractory (Kane et al. 1988; Meltzer et al. 1989; Miller et al. 1994) schizophrenic patients. In addition, clozapine has been shown to improve cognitive function in treatment-refractory schizophrenic patients (Hagger et al. 1993). The use of clozapine, however, has been severely limited by the occurrence of agranulocytosis (a potentially fatal condition marked by depression of the granulocyte-producing bone marrow) in a small percentage (1–3%) of the patient population (De la Chapelle et al. 1977; Griffith and Saameli 1975; Lieberman et al. 1988).

One proposal for the etiology of schizophrenia is the dopamine hypothesis. It postulates that hyperactivity of the brain's dopamine system results in schizophrenic symptoms (see Losonczy et al. 1987 for review), although modifications of this hypothesis have recently

From Lilly Research Laboratories, Indianapolis, IN.
Address correspondence to: Kurt Rasmussen, Ph.D., Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.
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been proposed (Davis et al. 1991; Deutch 1993; Grace 1991; Joyce 1993; Weinberger 1987). The dopamine hypothesis is supported in part by studies demonstrating that antipsychotic drugs have dopamine antagonist properties in vitro (Creese et al. 1976; Malmberg et al. 1993; Seeman et al. 1975) and in vivo (Farde et al. 1992; Nordstrom et al. 1993; see Seeman 1992, 1993 for review). However, it has recently been shown that many atypical antipsychotics show high in vivo affinity for 5-HT_{2A} receptors but do not show appreciable in vivo affinity for D_2 receptors (Matsubara et al. 1993; Stockmeier et al. 1993). Additional support for the dopamine hypothesis has come from electrophysiological studies which have shown that chronic treatment with classical antipsychotics (e.g., haloperidol) decreased the number of spontaneously active dopamine cells in both the ventral tegmental area (A10, which project primarily to the limbic and cortical regions) and the substantia nigra pars compacta (A9, which project primarily to the striatum) (Bunney and Grace 1978; Chiodo and Bunney 1983; White and Wang 1983). In addition, the time course for the decrease in A9 and A10 activity paralleled the time course of the emergence of some of the therapeutic and EPS of these drugs. However, chronic treatment with atypical antipsychotics (e.g., clozapine) selectively decreased the number of spontaneously active A10, but not A9, dopamine cells (Chiodo and Bunney 1983; White and Wang 1983; see Bunney 1992 for review). Therefore, since atypical antipsychotics have a greatly reduced propensity for producing EPS, and because the time course required to decrease dopamine neuronal activity closely parallels the onset of the clinical effects of these drugs, it has been hypothesized that the decrease in A10 activity may underlie the therapeutic effects of antipsychotics, while the decrease in A9 activity may underlie the EPS associated with classical neuroleptic treatment.

Olanzapine, a thienobenzodiazepine [LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-B][1,5] benzodiazepine)] (Figure 1) is a novel atypical antipsychotic. Olanzapine has a binding profile similar to that of clozapine; each displays high affinity for D_1, D_2, and D_4 dopamine, serotonin 5-HT_{2A} (formerly 5-HT_2), 5-HT_{2C} (formerly 5-HT_{1C}), 5-HT_3, and 5-HT_6, α_1-adrenergic, histamine$_1$, and muscarinic receptors (Bymaster et al. 1996; Roth et al. 1994). The effects of olanzapine in several behavioral tests (including conditioned avoidance responding, apomorphine induced climbing, 5-HTP-induced head twitch, punished responding and drug discrimination studies—(Moore et al. 1992) and in neuroendocrine assays (Fuller and Snoddy 1992) suggest that olanzapine and clozapine have similar pharmacological profiles and might therefore have similar atypical antipsychotic profiles in man. Indeed, in clinical studies of schizophrenic and schizophreniform patients, olanzapine effectively treated both positive and negative symptoms of schizophrenia and had a low incidence of EPS (Beasley et al. 1996). In order to explore the electrophysiological profile of olanzapine, we examined the effects of acute and chronic administration of olanzapine on A9 and A10 dopamine neuronal activity using extracellular single-unit recording techniques in anesthetized rats. These studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

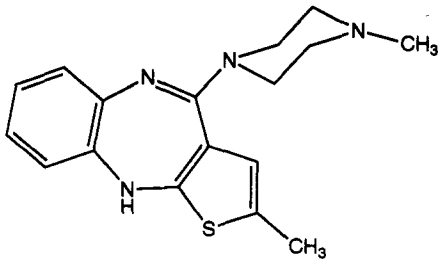

Figure 1. The chemical structure of olanzapine (LY170053), 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-B][1,5] benzodiazepine.

METHODS

Electrophysiological Recordings

Male Sprague-Dawley rats (280–330 g; Charles River) were anesthetized with chloral hydrate (400 mg/kg IP); supplemental doses of anesthetic were administered through the lateral tail vein as needed. Body temperature was maintained at 35°C by a heating pad (K-Module, American Pharmaseal Co., Valencia, CA). The anesthetized rats were mounted in a stereotaxic apparatus (Kopf Instruments), the skull exposed, and a cisternal drain performed to prevent tissue swelling. A burr hole was made in the skull over the A9 and A10 areas. To construct recording electrodes, single-barrel glass micropipettes (Radnoti, starbore glass) were pulled (Narishige PE-2 vertical puller); the resulting fine tips broken back and the barrels were backfilled with 2M NaCl. Electrode impedances were 1.8 to 2.6 MΩ (in vivo measurements with a Dagan 2400 preamplifier utilizing a 2-Hz, 100-nA peak-to-peak square wave).

For recording neuronal activity the bandpass filter on the preamplifier was set from 0.3 to 3 kHz. The tip of the recording electrode was lowered to the dorsal border of either A9 or A10 and then advanced, using a micropositioning device (Burleigh, Inchworm Motor Controller), in 5-μm increments through the nucleus. The electrode was passed through nine tracks (each track was separated by 0.2 mm) in a stereotaxically defined block of tissue (5.0–5.4 mm posterior, 2.0–2.4 mm lateral to bregma and 6.0–8.5 mm ventral to the cortical surface for A9; and 5.0–5.4 mm posterior and 0.5–0.9 mm lateral to bregma and 6.0–8.5 mm ventral to the cortical surface for A10), and the number of spon-

taneously active dopamine cells was counted. The electrode tracks were made in a preset sequence that was kept constant from animal to animal. For animals acutely treated, proper anatomical positioning of the recording electrode was ensured by scoring three control tracks prior to drug treatment. Six additional tracks were recorded 1 hour following SC injection of olanzapine or vehicle. Only one area, either A9 or A10, was recorded in each acutely treated animal. For chronically treated animals, nine tracks were scored in both A9 and A10; the first site examined (either A9 or A10) was alternated to control for order effects. Spontaneously active dopamine cells were recorded as previously reported (Chiodo and Bunney 1983; Rasmussen et al. 1991a, b; White and Wang 1983). Briefly, cells were considered dopaminergic if they possessed the following characteristics: (1) action potential duration of 2.5 to 4.5 ms; (2) triphasic waveform containing a notch in the initial rising phase of the first positive peak; and (3) slow, slightly irregular firing pattern, with a rate of 2 to 10 Hz. Previously, these characteristics have been demonstrated to be shown only by dopaminergic neurons (Bunney et al. 1973). A digital oscilloscope (Gould 1604) was used to analyze the spike waveforms; 8 waveforms were captured, averaged, and then displayed for analysis. The firing rate of each cell was monitored for 1 to 2 minutes to ensure that the cells had not been mechanically excited.

Drug Treatment

Olanzapine was dissolved in a minimal amount of 1N HCl; the solution was pH-adjusted to 5.1–5.5 with 0.1N NaOH and brought to volume with dH_2O. The vehicle was dH_2O, pH-adjusted to 5.1–5.5 with 0.1N HCl.

For acute treatment olanzapine or the vehicle was administered by a single SC injection. For chronic treatment, a constant rate of drug delivery was maintained by SC implantation of Alzet 2ML4 osmotic minipumps (Alza, Palo Alto, CA) containing olanzapine or the vehicle into 150-g male Sprague-Dawley rats. Because of solubility limitations, animals receiving 10 mg/kg/day olanzapine had one pump implanted, while those receiving 20 mg/kg/day olanzapine had two pumps implanted. Implant surgeries were carried out while the animals were lightly anesthetized with halothane. Electrophysiological recordings were performed 21 days postimplantation; pumps were not removed prior to recording. The volume of fluid in the pump was measured before and after treatment to verify that the proper amount of olanzapine was delivered.

Data Analysis

Results were analyzed using either paired *t*-tests, with each animal serving as its own control, or one-way analysis of variance (ANOVA) coupled with Fisher PLSD tests.

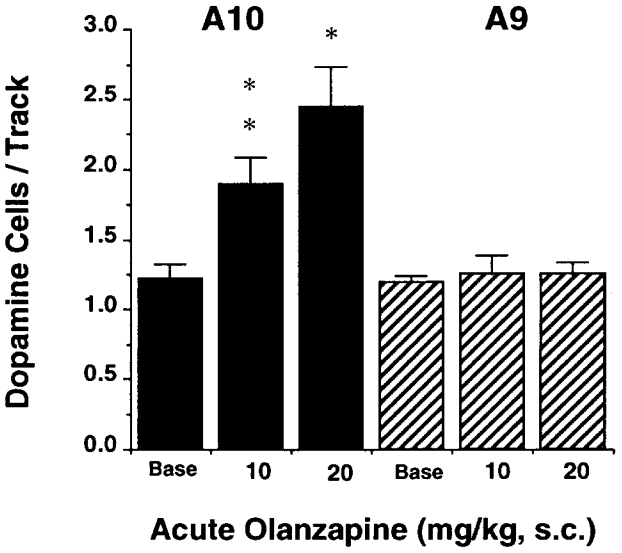

Figure 2. The number of spontaneously active A10 (*solid bars*) and A9 (*striped bars*) dopamine cells encountered 1 hour after a single SC administration of olanzapine (mean ± SE, n = 4–6). Asterisks indicate values that are significantly different (paired *t*-tests) from baseline (base; average number of dopamine cells encountered prior to drug administration; see Methods): $^{*}\ p < .05$; $^{**}\ p < .01$.

RESULTS

Acute administration of olanzapine (10 and 20 mg/kg SC) significantly increased ($p < .01$, $p < .05$, respectively) the number of spontaneously active A10 dopamine cells (n = 4 per group), but it did not change the number of spontaneously active A9 dopamine cells (n = 4 and 6 per group, respectively) (Figure 2). Following acute administration, the lower dose of olanzapine (10 mg/kg SC) significantly decreased the mean firing rate of spontaneously active A10 dopamine cells (n = 32), while the higher dose of olanzapine (20 mg/kg SC) did not change the mean firing rate of A10 dopamine cells (n = 22) (Figure 3). Neither dose of olanzapine (10 or 20 mg/kg) had any significant effect on the mean firing rate of A9 dopamine cells (n = 17 and 27 per group, respectively) (Figure 3).

Following chronic administration (10 and 20 mg/kg/day for 21 days), olanzapine dose-dependently decreased the number of spontaneously active A10 dopamine cells (n = 5 and 14 animals per group, respectively) compared to vehicle treatment (n = 5) and increased the number of spontaneously active A9 dopamine cells only at the higher (20-mg/kg/day SC) dose (n = 24 animals) compared to vehicle treatment (n = 7) (Figure 4). Chronic administration of olanzapine (10 and 20

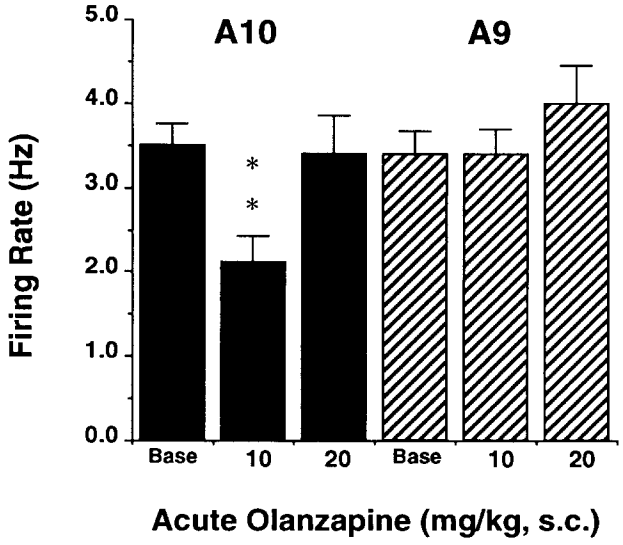

Figure 3. Firing rate (Hz; number of cell-firings/s) of spontaneously active A10 (*solid bars*) and A9 (*striped bars*) dopamine cells 1 hour after a single SC administration of olanzapine (mean ± SE, n = 17–48). Asterisks indicate values that are significantly different (ANOVA) from baseline (base; average firing rate of spontaneously active A9 or A10 dopamine cells prior to drug administration): ** $p < .01$.

mg/kg/day SC) did not significantly increase the mean firing rate of spontaneously active A9 (n = 47 and 39, respectively) or A10 (n = 38 and 15, respectively) dopamine cells compared to vehicle treatment (n = 48 for A9 and 43 for A10) (Figure 5).

Administration of apomorphine (10 and 63 µg/kg IV) dose-dependently reversed the decrease in the number of spontaneously active A10 dopamine cells encountered following chronic administration of olanzapine (20 mg/kg/day). These same doses of apomorphine decreased the number of spontaneously active A9 dopamine cells encountered following chronic administration of olanzapine (20 mg/kg/day); (n = 3 and 4 animals per group, respectively) (Figure 6).

DISCUSSION

Acute administration of olanzapine (10, 20 mg/kg SC) produced an increase in the number of spontaneously active A10 dopamine cells but no change in the number of spontaneously active A9 dopamine cells (Figure 2). Acute administration of clozapine and other atypical antipsychotics has also been reported to selectively increase the number of spontaneously active A10 (relative to A9) dopamine cells (Goldstein et al. 1993; White and Wang 1983; but see Chiodo and Bunney 1983). Acute administration of antipsychotics has been shown to produce this increase in the number of spontaneously active dopamine cells by activating a subpopulation of dopamine cells that were previously inactive due to

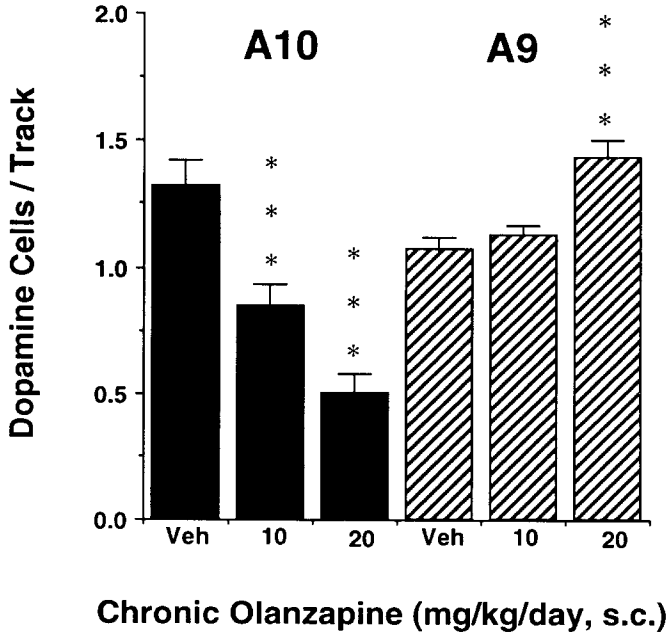

Figure 4. The number of spontaneously active A10 (*solid bars*) and A9 (*striped bars*) dopamine cells following chronic administration (21 days via osmotic minipump) of olanzapine or vehicle (*Veh*) (mean ± SE, n = 5–9). Asterisks indicate values that are significantly different (ANOVA) from vehicle controls: *** $p < .001$.

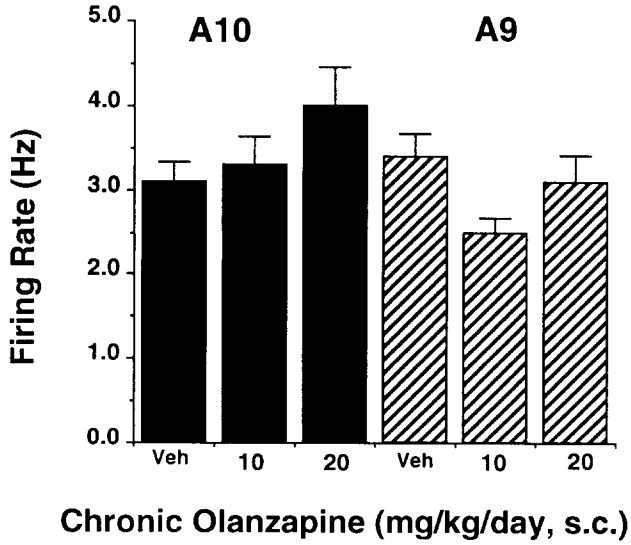

Figure 5. Firing rate (Hz; number of cell-firings/s) of spontaneously active A10 (*solid bars*) and A9 (*striped bars*) dopamine cells following chronic administration (21 days via osmotic minipump) of olanzapine or vehicle (Veh) (mean ± SE, n = 15–48).

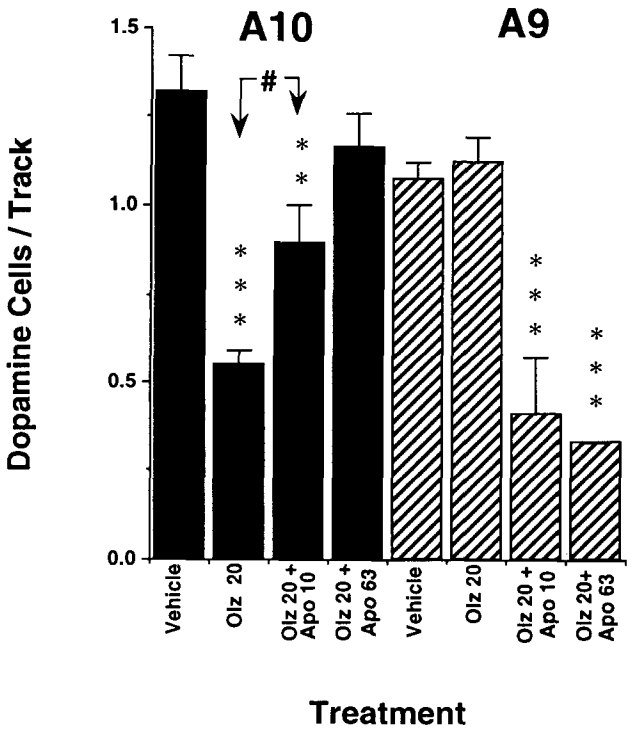

Figure 6. Effect of administration of apomorphine (Apo; 10 or 63 μg/kg IV) on the number of spontaneously active A10 (*solid bars*) and A9 (*striped bars*) dopamine cells following chronic administration (21 days via osmotic minipump) of either olanzapine (Olz; 20 mg/kg/day) or vehicle (Veh) (mean ± SE, n = 3–7). Asterisks indicate values that are significantly different (ANOVA) from vehicle controls: ** $p <$ .01; *** $p <$ .001. Pound-sign indicates values that are significantly different between dose groups: # $p <$ .05.

hyperpolarization (Bunney and Grace 1978; Chiodo and Bunney 1983). A selective increase in the number of spontaneously active A10 dopamine cells following acute administration of antipsychotics has been hypothesized to underlie the subsequent selective decrease in the number of spontaneously active A10 dopamine cells seen following chronic administration of antipsychotics via depolarization inactivation—see later (Chiodo and Bunney 1983; Goldstein et al. 1993). Furthermore, the selective increase in the number of spontaneously active A10 dopamine cells seen following acute administration of antipsychotics has been hypothesized to be predictive of atypical antipsychotic activity in man (Bunney and Grace 1978; Chiodo and Bunney 1983; Goldstein et al. 1993). In preliminary clinical trials olanzapine was efficacious in treating both the positive and negative symptoms of schizophrenia and had a low incidence of EPS (Beasley et al. 1996). Thus, clinical results obtained with olanzapine support the predictive validity of the selective increase in the number of spontaneously active A10 dopamine cells following acute administration as a preclinical test for atypical antipsychotic effects in man.

Although acute administration of olanzapine selectively increased the number of spontaneously active A10 dopamine cells, only modest changes in the mean firing rate of the spontaneously active A9 and A10 dopamine cells were observed. In A10, 20 mg/kg olanzapine did not change the mean firing rate of the dopamine cells encountered. However, a lower dose (10 mg/kg) resulted in a significant decrease in the mean firing rate of the A10 dopamine cells (Figure 3). In A9, the firing rate of the spontaneously active dopamine neurons was not different from control following either dose of olanzapine (Figure 3). Previous studies report that acute administration of atypical antipsychotics did not significantly alter the firing rate of A9 or A10 dopamine cells that were already active prior to drug administration (Bunney and Aghajanian 1974, 1975). Preliminary results from our laboratory also indicate that olanzapine does not significantly alter the firing rate of A9 (n = 3) or A10 (n = 5) dopamine cells that are active prior to drug administration. Thus, one possible explanation for the effects of olanzapine on the mean firing rates of A10 dopamine cells is that 10 mg/kg may be a sufficient dose of olanzapine to cause previously inactive cells to fire, but only at rates below control levels. However, 20 mg/kg of olanzapine may be enough to induce previously inactive cells to fire at control rates. Single-unit recordings examining the activity of both spontaneously active and inactive (i.e., hyperpolarized) A10 dopamine cells in response to increasing doses of olanzapine would help to test this hypothesis.

Chronic administration of olanzapine resulted in a decrease in the number of spontaneously active A10 dopamine neurons (Figure 4). In A9, chronic administration of 10 mg/kg olanzapine produced no change in the number of spontaneously active dopamine cells, while chronic administration of 20 mg/kg produced a significant increase in the number of spontaneously active dopamine cells (Figure 4). These results with olanzapine were similar to those seen following the chronic administration of clozapine and other proposed atypical antipsychotics (Chiodo and Bunney 1983; Skarsfeldt 1988b; White and Wang 1983). This selective decrease of A10 (relative to A9) dopamine activity has been hypothesized to be predictive of atypical antipsychotic activity (Chiodo and Bunney 1983; White and Wang 1983); clinical results obtained with olanzapine (Beasley et al. 1996) support this hypothesis. Following chronic administration of olanzapine, the firing rates of the A9 and A10 dopamine cells encountered were not significantly different from those of vehicle-treated controls. However, in the high-dose group there was a trend toward significantly higher firing rates for A10 dopamine cells. Chronic administration of clozapine has previously been reported to result in increased firing rates of the remaining active A10 dopamine cells (Chiodo and Bunney 1983). Whether higher doses of olanzapine would produce a greater increase in the firing rates of the remaining active A10 dopamine cells remains to be determined.

The selective effects of olanzapine in A10 relative to A9 dopamine cells following both acute and chronic administration could be accounted for by its complex pharmacological profile. Previous reports indicated that compounds which are relatively selective for particular receptor subtypes decreased the number of spontaneously active A9 and/or A10 dopamine cells following chronic administration. For example, following chronic administration, compounds selective for 5-HT_3 (Minabe et al. 1991; Sorensen et al. 1989), 5-$HT_{3/4}$ (Skarsfeldt 1993), dopamine D_2 (Chiodo and Bunney 1983; Skarsfeldt 1993; White and Wang 1983); dopamine D_1/5-$HT_{2A/2C}$ (Skarsfeldt 1988a; but see Esposito and Bunney 1988), and CCK-B (Rasmussen et al. 1991b) receptors have been reported to decrease the number of both spontaneously active A9 and A10 dopamine cells. However, compounds selective for 5-HT_{2A} (Palfreyman et al. 1993), 5-$HT_{2A/2C}$ (Goldstein et al. 1989), 5-$HT_{2A/2B/2C/1D}$ (Goldstein and Litwin 1988b), 5-HT_3 (Minabe et al. 1992; Prisco et al. 1991; Rasmussen et al. 1991a); dopamine D_2 (Skarsfeldt 1993; White and Wang 1983), dopamine D_1/5-$HT_{2A/2C}$ (Goldstein and Litwin 1988a; Wachtel and White 1992), and sigma/5-HT_{1A} (Watchel and White 1988) receptors have been reported selectively to decrease only the number of spontaneously active A10 dopamine cells. In addition, compounds with affinity for several receptor subtypes also decreased the number of spontaneously active A9 and/or A10 dopamine cells following chronic administration. For example, following chronic administration, haloperidol, chlorpromazine (Chiodo and Bunney 1983; White and Wang 1983) and tefludazine (Skarsfeldt 1988b) have been reported to decrease the number of both spontaneously active A9 and A10 dopamine cells, while clozapine (Chiodo and Bunney 1983; Skarsfeldt 1988b, White and Wang 1983), molindone (White and Wang 1983), sertindole (Skarsfeldt 1992), and seroquel (Goldstein et al. 1993) have been reported selectively to decrease only the number of spontaneously active A10 dopamine cells. Olanzapine has a high binding affinity for a variety of dopaminergic (D_1, D_2, D_3), serotonergic (5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6), noradrenergic (α_1), histaminic (H_1), and muscarinic (m_{1-5}) receptors (Roth et al. 1994; Bymaster et al. this issue) and has antagonist properties in vivo at most of these same receptors (Fuller and Snoddy 1992; Moore et al. 1992). Whether activity at any one receptor or a specific combination of these receptors accounts for the selective effects of olanzapine on A10 dopamine cells remains to be determined.

The decreased activity of A9 and A10 dopamine neurons produced by chronic administration of antipsychotic drugs has been postulated to arise from a chronic state of strong depolarization (i.e, depolarization inactivation or depolarization block) (Bunney and Grace 1978; Chiodo and Bunney 1983; see Grace 1992 for review). The depolarization inactivation hypothesis is supported in part by studies showing that systemic administration of apomorphine, a dopamine agonist which hyperpolarizes dopamine cells in control animals, reverses the effects of chronic administration of antipsychotic drugs by causing previously nonfiring dopamine cells to become active (Bunney and Grace 1978). In the present study systemic administration of apomorphine dose-dependently reversed the effects of chronic administration of olanzapine on A10 dopamine cells (Figure 6). In contrast, these same doses of apomorphine inhibited the activity of A9 dopamine cells in animals chronically treated with olanzapine. A previous study reported that apomorphine did not decrease the number of spontaneously active A9 dopamine cells in animals chronically treated with clozapine, thioridazine, or d,l-sulpiride (White and Wang 1983). However, another study reported that apomorphine was able to decrease the number of spontaneously active A9 dopamine cells in animals chronically treated with 5-HT_3 antagonist DAU 6215 (Prisco et al. 1991). Whether chronic administration of olanzapine has different effects from clozapine, thioridazine, or d,l-sulpiride on A9 dopamine cells remains to be determined. Apomorphine-induced reversal of the effects of chronically administered olanzapine on A10 dopamine cells suggests that olanzapine, similarly to other antipsychotic drugs, affects the activity of A10 dopamine cells through depolarization inactivation. Intracellular recordings and iontophoretic studies using hyperpolarizing and depolarizing agents would help to confirm this hypothesis. Whether depolarization inactivation of dopamine neurons actually occurs in man and, if it does, what role it plays in the therapeutic effects of antipsychotic drugs is not clear (Bunney 1992; Grace 1992; Meltzer 1991; Moghaddam and Bunney 1993). Whatever the ultimate role for depolarization inactivation in the mechanism of action of antipsychotic drugs, the present results support the predictive validity of the selective decrease of A10 dopamine activity, through a depolarization inactivation mechanism, as a preclinical predictor of antipsychotic efficacy and decreased motor side effect liability in man. However, it is important to note that selective inactivation of A10 versus A9 neurons is not a perfect predictor of atypical antipsychotic behavior in man. For example, BMY 14802 selectively inactivates A10 neurons following chronic administration in rats (Wachtel and White 1988) but does not have antipsychotic activity in man (Gewirtz et al. 1994).

In conclusion, olanzapine selectively increased the number of spontaneously active A10 (relative to A9) dopamine cells following acute administration and selectively decreased the number of spontaneously active A10 (relative to A9) dopamine cells following chronic administration. These results are similar to those obtained for clozapine and other proposed atyp-

ical antipsychotic drugs (Chiodo and Bunney 1983; Skarsfeldt 1988b; White and Wang 1983). Since olanzapine has been demonstrated to have an atypical antipsychotic profile in clinical trials (Beasley et al. in press), these findings support the hypothesis that a decrease in the number of spontaneously active A10 dopamine neurons may play a role in the therapeutic effects of antipsychotic drugs, while a decrease in the number of spontaneously active A9 dopamine neurons may play a role in the motor side effects of antipsychotic drugs.

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