

Effect of Olanzapine on Functional Responses from Sensitized D₁-Dopamine Receptors in Rats with Neonatal Dopamine Loss

Sheryl S. Moy, Ph.D., Darin J. Knapp, Ph.D., and George R. Breese, Ph.D.

Previous work has suggested that the therapeutic efficacy of olanzapine might be partially dependent on action at the D₁-dopamine (DA) receptor site. Because early DA loss can lead to supersensitive D₁-DA receptors, effects of olanzapine were investigated in adult rats given lesions to DA-containing neurons as neonates. In these animals, locomotor effects of SKF-38393 (a D₁-DA agonist) were attenuated by olanzapine, but at doses (5 and 10 mg/kg) that decreased activity when given alone. Olanzapine prevented induction of striatal Fos protein by SKF-38393 and partially attenuated the long-term "priming" effect of repeated SKF-38393 treatment. Olanzapine also

antagonized the stimulant effects of quinpirole (a D₂-type DA agonist) in animals lesioned as young adults, at doses lower than those necessary to antagonize SKF-38393-induced activity. In addition, olanzapine antagonized apomorphine-induced self-injurious behavior in neonate-lesioned rats in a dose-related fashion. Attenuation of self-injury in this animal model suggests that olanzapine should be tested against this symptom in patient populations.
[*Neuropsychopharmacology* 25:224–233]

© 2001 American College of Neuropsychopharmacology.
Published by Elsevier Science Inc.

KEY WORDS: Antipsychotic drugs; D₁-dopamine receptor; Fos; Olanzapine; Self-injurious behavior; Sensitization

Rats given selective lesions of dopamine (DA)-containing neurons as neonates later show a unique profile of behavioral responses to D₁-DA receptor activation (Breese et al. 1985a,b). For example, neonate-lesioned animals have increased locomotion following treatment with SKF-38393, a D₁-DA receptor agonist, at doses that have minimal impact on control rats (Breese et al. 1985a,b). Repeated treatment with D₁-DA agonists can

lead to extremely high rates of activity in lesioned rats, and this "priming" effect can still be observed 6 months after the chronic drug treatment (Breese et al. 1985b; Criswell et al. 1989b). Supersensitivity of D₁-DA receptors in neonatal-6-OHDA rats has also been linked to stereotyped responses and self-injurious behavior (SIB) following treatment with L-Dopa or apomorphine, suggesting that these animals can provide a valuable model for the self-injury and DA loss associated with Lesch–Nyhan disease (Breese et al. 1984a,b), a metabolic disorder primarily observed in male children (Lesch and Nyhan 1964).

Paradoxically, although the aberrant responses in the neonate-lesioned rats can be completely blocked with selective D₁-DA receptor antagonists (Breese et al. 1985a), these animals are remarkably subsensitive to haloperidol, even at doses which induce catalepsy in normal controls and in rats given 6-OHDA as young adults (Bruno et al. 1985; Duncan et al. 1987). Similarly, treatment with typical antipsychotic agents does not prevent the painful self-injury that occurs in Lesch–

From the Departments of Psychiatry (SSM, DJK, GRB), Anesthesiology (GRB) and Pharmacology (GRB), Skipper Bowles Center For Alcohol Studies (SSM, DJK, GRB), University of North Carolina Neuroscience Center (GRB), and Neurodevelopmental Disorders Research Center (GRB), University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

Address correspondence to: Dr. S.S. Moy, Skipper Bowles Center for Alcohol Studies, UNC School of Medicine, CB # 7178, University of North Carolina, Chapel Hill, NC 27599-7178. Tel.: (919) 966-3082; Fax: (919) 966-7006. E-mail: sheryl.moy@css.unc.edu

Received August 9, 2000; revised December 19, 2000; accepted January 3, 2001.

Nyhan patients (Watts et al. 1982). However, clozapine, an atypical neuroleptic, prevented SIB in the animal model (Criswell et al. 1989a), which led to testing of related compounds for similar effects on SIB in rodents (Davis et al. 1997; Allen et al. 1998). One promising candidate for further investigation is olanzapine, an atypical antipsychotic drug that has a pharmacological profile comparable, to some extent, with that of clozapine, but without the deleterious tendency to promote agranulocytosis (Meltzer and Fibiger 1996). Binding investigations of the action of olanzapine have demonstrated a significant affinity for the D_2 -DA receptor site, as well as for the D_1 -DA, D_4 -DA, and serotonin (5-HT_{2A} , 5-HT_{2C} , and 5-HT_3 receptor sites (Bymaster et al. 1996; Arnt and Skardfeldt 1998). Previous work has suggested that the unique antipsychotic efficacy of atypical neuroleptics is based, at least in part, on significant blockade of D_1 -DA receptor function (Miller et al. 1990; Nordstrom et al. 1995; Josselyn et al. 1997).

Given the past reports of atypical antipsychotic effects on functions associated with D_1 -DA receptors in neonate-lesioned rats (Criswell et al. 1989a; Davis et al. 1997; Allen et al. 1998), the present studies were designed to systematically test olanzapine in this animal model. The first experiments investigated the action of olanzapine on measures linked to sensitized D_1 -DA receptors in lesioned animals: locomotor activity and the induction of Fos protein in brain following SKF-38393 treatment, and the development of behavioral sensitization (priming) with repeated treatment with this D_1 -DA agonist. In addition, olanzapine was tested in rats given 6-OHDA-lesions as young adults, because previous work has demonstrated that animals lesioned in adulthood show enhanced locomotor responses to selective D_2 -DA receptor agonists, in comparison to both neonate-lesioned animals and controls (Breese et al. 1985b). Differential effects on sensitized D_2 -DA, versus D_1 -DA, receptor function were investigated by testing olanzapine against quinpirole-induced activity in adult-lesioned rats. Finally, the action of olanzapine on induction of SIB following apomorphine administration to neonate-lesioned rats was evaluated, because self-biting and self-injury has been linked to D_1 -DA receptor activation (Breese et al. 1984a,b).

METHODS

Subjects

Subjects given treatment with 6-OHDA as neonates were offspring of Sprague-Dawley breeding stock obtained from Charles River Laboratories, Inc. (Raleigh, NC). Both males and females were included in the experiments, and subjects were distributed into groups as evenly as possible on the basis of gender and litter. Subjects given 6-OHDA treatment as young adults were male animals directly ordered from Charles River Laboratories, Inc.

The animals were housed in groups of two or three, with free access to water and food (Purina Rat Chow, supplemented with apples), and maintained on a 12-h light-dark cycle in a temperature controlled environment. All procedures involved in this work were in strict compliance with the policies on animal welfare of the National Institutes of Health and the University of North Carolina (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition).

Lesioning of Dopamine-Containing Neurons in Neonate Rats

On day 3 after birth, male and female rat pups were anesthetized with ether and injected intracisternally with 100 μg (free base) 6-OHDA in 10 μl saline (0.5% ascorbic acid). Subjects also received a 1-hour pretreatment with desipramine (20 mg/kg, given IP), to protect norepinephrine-containing neurons (Breese and Traylor 1972). Previous studies have demonstrated that striatal dopamine is reduced by greater than 90% with this treatment, with no reduction in brain levels of norepinephrine or serotonin (Breese et al. 1984a). Pups were returned to their dams following recovery from the ether. All pups in a given litter were treated with 6-OHDA, and litter size was restricted to a maximum of 10 pups.

Lesioning of Dopamine-Containing Neurons in Young Adult Rats

At approximately 40 days of age, male rats were anesthetized with ether and injected intracisternally with 200 μg (free base) 6-OHDA in 10 μl saline (0.5% ascorbic acid). Subjects received a 1-hour pretreatment with desipramine (20 mg/kg, given IP), to protect norepinephrine-containing neurons. In addition, each rat was given pargyline (35 mg/kg), an MAO inhibitor, one-half hour before the 6-OHDA treatment to prolong the neurotoxicant action. This treatment has been shown in previous work to reduce dopamine by greater than 90%, with no reduction in brain levels of norepinephrine or serotonin (Breese et al. 1984a). One week later, the animals were again treated with 6-OHDA and desipramine, as described above, but were not given the pargyline pretreatment. For 2 to 3 weeks following the second treatment with 6-OHDA, the subjects were hand fed either a nutritionally complete liquid diet (Frye et al. 1981) or softened rat chow mixed with flavored yogurt to promote recovery from the dopamine loss. Testing began 8 weeks following the second 6-OHDA treatment.

Testing of Locomotor Activity

Neonate-Lesioned Subjects. For the studies on acute olanzapine effects on sensitized responses, only rats

primed by repeated administration of DA agonists were used. The priming procedure began with one dose of SKF-38393 (6 mg/kg), administered to experimentally naive rats. Following the injection, each animal was returned to its home cage. One week later, the animals were treated with a lower dose of SKF-38393 (3 mg/kg) and observed in home cages for stereotyped responses or repeated locomotion. Any subject not demonstrating a supersensitive reaction was tested again, 1 week later, with SKF-38393 (3 mg/kg). Animals still not showing supersensitization were not used in the subsequent studies on olanzapine effects.

Tests for locomotor counts began 3 to 5 weeks following the priming procedure for D₁-DA receptor sensitization. Subjects were measured for activity in circular locomotor chambers, each with six photocells arranged around the periphery. Scores were derived from breaks in the photobeams crossing the chambers. Each test session began with a 50-min habituation period, followed by a 150-min testing period. The first two experiments investigated the effects of olanzapine on locomotion in adult rats treated with 6-OHDA as neonates. For the first experiment, olanzapine (3, 5, or 10 mg/kg) or vehicle was administered immediately following the habituation period. In the second experiment, SKF-38393 (3 mg/kg) was administered immediately following the habituation period. Pretreatment with olanzapine (3, 5, or 10 mg/kg) or vehicle occurred 20 min before the administration of SKF-38393. There were at least 5 days between each test session.

Young Adult-Lesioned Subjects. For the experiment on the effects of olanzapine in adult rats lesioned with 6-OHDA in early adulthood, only subjects showing supersensitized responses to quinpirole (0.3 mg/kg) were used. The procedure for inducing sensitization was the same as described above, with an initial injection of 0.6 mg/kg quinpirole followed, 1 week later, with a dose of 0.3 mg/kg. Subjects were tested with a third dose of quinpirole (0.3 mg/kg) only if sensitized responses were not observed after the second treatment.

Tests for locomotor counts began 3 to 5 weeks following the priming procedure. Subjects were measured for activity as described above. For this experiment, quinpirole (0.3 mg/kg) was administered immediately following the habituation period. Pretreatment with olanzapine (1, 2, or 3 mg/kg) or vehicle occurred 20 min before the administration of quinpirole. There were at least 5 days between each test session.

Testing for Effects of Olanzapine on Sensitization to a D₁-DA Receptor Agonist in Neonate-Lesioned Rats

For this experiment, only experimentally naive animals were used. Locomotor scores were determined from

photocell counts during a 150-min testing session, as previously described. All subjects were first assessed for baseline locomotor scores following vehicle injection. Following this first session, one group of subjects ($n = 14$) was measured for locomotor activity after SKF-38393 (3 mg/kg), with only vehicle pretreatment, once per week for 5 weeks. A second group ($n = 14$) underwent the same procedure, except with olanzapine (10 mg/kg) pretreatment before the first three exposures to SKF-38393. The olanzapine or vehicle pretreatment occurred 20 min before the administration of SKF-38393. Sensitization to the D₁-DA agonist was determined on the last two sessions, test days 1 and 2, when all subjects received only SKF-38393.

Testing for Self-Injurious Behavior (SIB)

Experimentally naive subjects between 3 and 4 months of age were first tested for supersensitivity to a D₁-dopamine agonist (SKF 38393, 3 mg/kg), as described in the preceding section. Only animals showing high levels of locomotor activity following repeated treatment (at 1-week intervals) of the D₁-DA receptor agonist were used in the later experiment. Next, the animals were screened for SIB. Each subject was given the mixed D₁/D₂-type DA receptor agonist, apomorphine (10 mg/kg), and then placed in a clear plastic cage. Behavior was recorded for 1 min, at 10-min intervals, for 2 1/2 hours following drug administration. This treatment typically leads to vigorous self-biting of the forepaws, forelimbs, or (less often) the chest area. In the present study, as soon as a lesion was observed, the animal was marked positive for SIB and given a D₁-DA receptor antagonist (SCH-23390, 0.5 mg/kg) to suppress the self-biting. Only animals identified as SIB positive were used as subjects for the subsequent experiment on olanzapine blockade of self-injury. These subjects were taken from six litters, and were distributed into groups based on litter and gender. Testing with olanzapine in the neonate-lesioned rats did not proceed for at least 3 weeks after initial screening for self-injury. Olanzapine (10 or 20 mg/kg) or vehicle pretreatment was given 10 min before the administration of apomorphine (10 mg/kg) to the neonate-lesioned rats, and behavioral responses were recorded as described above.

Drugs

Olanzapine (a gift from Lilly Research Laboratories, Indianapolis, IN) was dissolved in a minimal amount of 1.0 N HCl, then diluted in saline, with the pH adjusted between 5.0 and 6.0 with 1.0 N NaOH. SKF-38393 (3 mg/kg; Research Biochemicals Inc., Natick, MA) was dissolved in heated saline. Apomorphine (10 mg/kg; Research Biochemicals Inc., Natick, MA) was dissolved in a saline solution with 0.1% ascorbic acid. Quinpirole

(LY171555; 0.3 mg/kg; a gift from Lilly Research Laboratories) was dissolved in saline. All drugs were administered by intraperitoneal injection.

Measurement of Fos-Like Immunoreactivity (Fos-LI)

Anesthetized animals were perfused transcardially with 0.1 M phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.1 M phosphate-buffer solution. Brains were then placed in paraformaldehyde and kept in cold storage until cut with a vibratome. Sections (50 μ m thick) were taken for selected brain regions, and a standard avidin-biotin/horseradish peroxidase method was used for the Fos-LI assay, as previously described (Duncan et al. 1996). Briefly, Fos-LI was detected using an affinity-purified sheep polyclonal antibody (1:10,000) raised to a synthetic peptide of fos (Cambridge Research Biochemicals, Inc., Wilmington, DE; current source is Biogenesis, Brentwood, NH), and visualized with Vectastain ABC kits (Vector Laboratories, Inc., Burlingame, CA) containing rabbit anti-goat IgG, and 3,3'-diamino-benzidine tetrahydrochloride (DAB; Polysciences, Inc., Warrington, PA). Cells demonstrating nuclear Fos-LI were counted at a total magnification of 200 X for each striatal quadrant.

Data Analysis

Locomotor measures were first analyzed using a repeated measures analysis of variance (ANOVA). Levels of Fos-LI were also analyzed using a repeated measures ANOVA, with striatal region serving as the repeated measure. Fisher's protected least-significant difference (PLSD) tests were conducted on group means only when a significant F value was found. For the experiment on olanzapine pretreatment during a chronic priming regimen, Student's *t*-tests were used for data collected on test days 1 and 2. Significance was set at $p < .05$.

RESULTS

Effect of Acute Olanzapine Pretreatment on Baseline and SKF-38393-Induced Locomotion

The effects of olanzapine on baseline levels of activity, as well as on sensitized locomotor responses to D_1 -DA receptor stimulation, were determined for animals with neonatal loss of DA-containing neurons. Figure 1 demonstrates that olanzapine, by itself, significantly decreased locomotion in the lesioned animals at the two higher doses tested (5 and 10 mg/kg) [$F(3,42) = 10.37, p = .0001$]. Treatment with the D_1 -DA receptor agonist SKF-38393 markedly enhanced activity levels in the lesioned rats (Figure 2). It is notable that published reports have

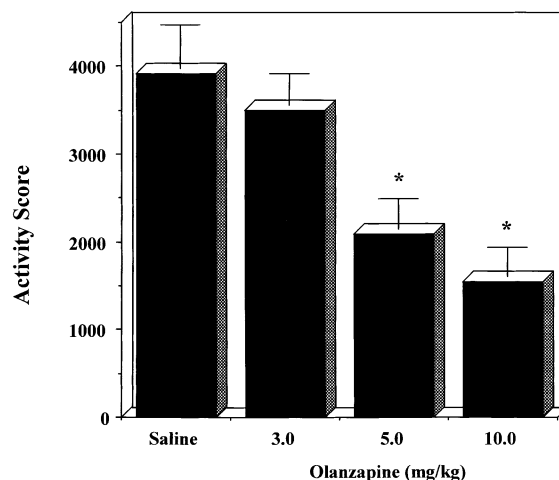


Figure 1. Effect of olanzapine on locomotor activity in neonatal 6-OHDA-lesioned rats. Data shown are mean (\pm SEM) activity scores taken across a 150-min session, with at least 5 days between each session. Results are from male rats ($n = 6$) and female rats ($n = 9$), taken from six litters, and previously primed with repeated doses of SKF-38393 (3 mg/kg). * $p < .05$, olanzapine treatment versus saline treatment.

consistently documented that control animals (rats given sham lesions via neonatal vehicle treatment) typically show no response to single or repeated treatment with SKF-38393 (Breese et al. 1985a,b). In the present

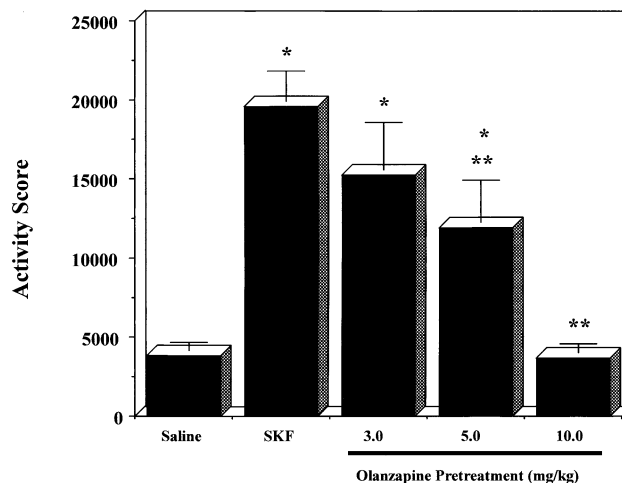


Figure 2. Effect of olanzapine on locomotor activity following SKF-38393 (SKF; 3 mg/kg) in neonatal 6-OHDA-lesioned rats. Data shown are mean (\pm SEM) activity scores taken across a 150-min session, with at least 5 days between each session. Results are from male rats ($n = 6$) and female rats ($n = 9$), taken from six litters, previously primed with repeated doses of SKF-38393 (3 mg/kg). * $p < .05$, SKF-38393 treatment versus saline treatment. ** $p < .05$, olanzapine pretreatment versus SKF-38393 alone.

study, olanzapine significantly blocked the stimulant impact of SKF-38393 within a moderate dose range [$F(4,52) = 17.95, p < .001$]. Analysis of the data for possible gender effects indicated that there were no significant differences between the means for male and female subjects under any of the experimental conditions. In fact, the very high rates of activity induced by SKF-38393 were almost identical for the two groups (males, mean \pm SEM = $20,396 \pm 3437$; females, $20,070 \pm 2250$). Therefore, the data from male and female animals have been combined in the experiments using neonate-lesioned subjects.

Effect of Olanzapine on SKF-38393-Induced Fos-Like Immunoreactivity (Fos-LI)

The antagonistic power of olanzapine against D_1 -DA receptor function was also determined for the potent Fos-LI induction that occurs in the striatal area of neonate-lesioned rats following treatment with SKF-38393 (Johnson et al. 1992; Criswell et al. 1993). As depicted in Figure 3, and the photomicrographs in Figure 4, D_1 -DA receptor activation led to a marked increase in levels of Fos-LI in each striatal quadrant (significant main effect

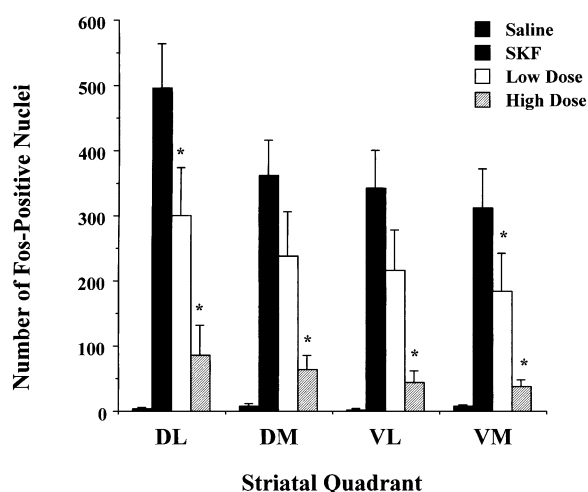


Figure 3. Olanzapine antagonism of striatal Fos-LI induction by SKF-38393 in neonatal 6-OHDA-lesioned rats. Data shown are mean (\pm SEM) number of Fos-positive nuclei counted within each striatal quadrant. On the test day, all animals received either saline or a low dose (3 mg/kg) or high dose (10 mg/kg) of olanzapine. Twenty minutes later, subjects received either saline (saline group only) or SKF-38393 (3 mg/kg). After each injection, rats were returned to their home cages. Two hours following the second injection, animals were sacrificed and brains collected for analysis. For group sizes, $n = 5$ for saline, $n = 6$ for SKF-38393, and $n = 7$ each for the low dose and high dose olanzapine pretreatment groups. Overall, there were 10 male and 15 female animals, taken from 11 litters. DL: dorsal-lateral; DM: dorsal-medial; VL: ventral-lateral; VM: ventral-medial. * $p < .05$, olanzapine pretreatment versus SKF-38393 alone.

of treatment [$F(3,21) = 12.955, p = .0001$] and striatal region (the repeated measure), [$F(3,63) = 14.155, p = .0001$]). Further analysis showed that pretreatment with the high dose of olanzapine (10 mg/kg) significantly reduced these effects of SKF-38393 in each striatal quadrant. The remaining low levels of Fos-LI still apparent after pretreatment with the higher dose of olanzapine were not significantly different from saline levels in any quadrant ($p > .05$). The effect of the low dose of olanzapine (5 mg/kg) was significant in only the dorsal-lateral and ventral-medial areas (significant interaction between treatment and striatal region [$F(9,63) = 2.768, p < .009$]).

Effect of Olanzapine Pretreatment on Sensitization to a D_1 -DA Receptor Agonist in Neonate-Lesion Rats

In confirmation of previous work (Breese et al. 1985b; Criswell et al. 1989b), repeated treatment with SKF-38393 resulted in a clear priming effect, demonstrated by an increase in locomotor scores across time (Figure 5; significant effect of the repeated measure [$F(3,78) = 37.84, p = .0001$]). Pretreatment with olanzapine (10 mg/kg) significantly attenuated the stimulant impact of SKF-38393 for the first three sessions of the priming regimen (significant treatment effect [$F(1,26) = 24.49, p = .0001$]), with the extent of the blockade increasing across time (significant interaction between treatment and repeated measure [$F(3,78) = 14.03, p = .0001$]). On the first test day following the priming regimen, all sub-

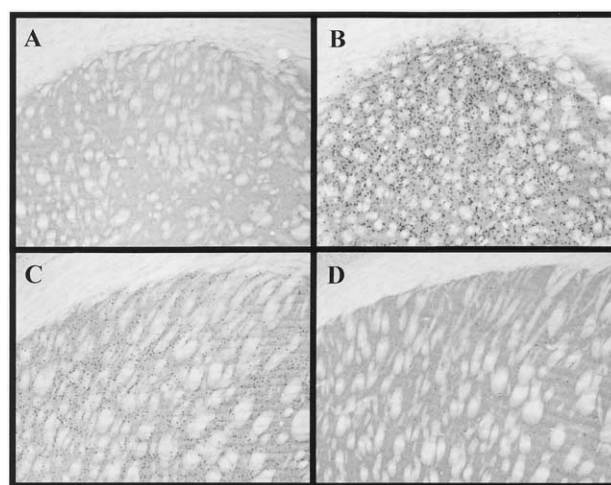


Figure 4. Photomicrographs showing Fos-LI induction in the dorsal striatal region of neonatal 6-OHDA-lesioned rats. A. Lack of Fos-LI following saline treatment. B. Robust induction of Fos-LI following SKF-38393 (3 mg/kg). C. Partial blockade of SKF-38393 effects by pretreatment with a low dose (3 mg/kg) olanzapine. D. Full blockade of SKF-38393 effects by pretreatment with a high dose (10 mg/kg) olanzapine. Magnification level was X 40. See Figure 3 for group data and procedural details.

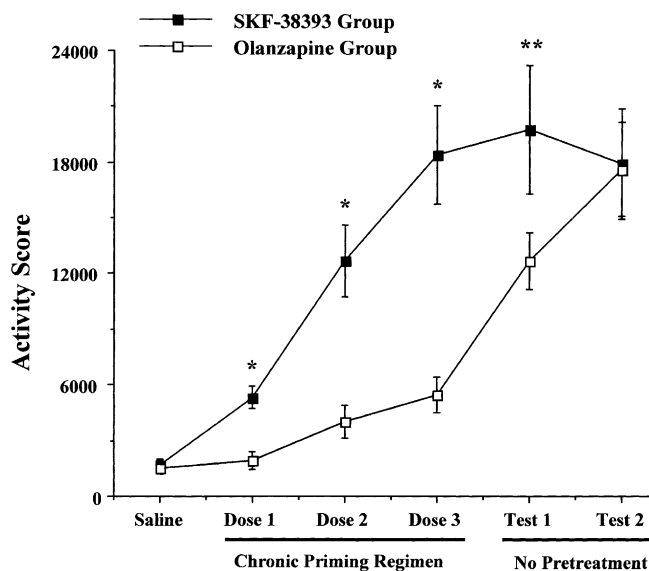


Figure 5. Effect of olanzapine on priming in neonatal 6-OHDA-lesioned rats. Data shown are mean (\pm SEM) activity scores taken across a 150-min session, with at least 5 days between each session. Rats in the SKF-38393 group ($n = 5$ males and 9 females) were given five treatments with the D1-agonist (3 mg/kg), with vehicle pretreatment before dose 1, dose 2, and dose 3. Animals in the olanzapine group ($n = 6$ males and 8 females) were given the same treatment with SKF-38393 (3 mg/kg), but with olanzapine pretreatment (10 mg/kg) before dose 1 through dose 3. There were no pretreatments before SKF-38393 administration on test days 1 and 2. Subjects were taken from 7 litters. * $p < .05$, SKF-38393 group versus olanzapine group, Fisher's PLSD test. ** $p < .05$, SKF-38393 group versus olanzapine group, Student's t -test.

jects received a dose of SKF-38393, to assess if the sensitization process had been blocked by the olanzapine pretreatment. A small, but significant, difference was found between activity levels for the group given olanzapine, in comparison to the vehicle-treated animals [$t(26) = 1.84$, $p < .04$]. By the second test day, the groups had virtually identical average scores, suggesting that the maximal level of sensitivity in the two groups was comparable (Figure 5).

Effect of Olanzapine Against Sensitized Responses to a D₂-DA Receptor Agonist in Adult-Lesion Animals

Rats given selective dopamine lesions as young adults typically show markedly high rates of activity in response to treatment with quinpirole, a D₂-type DA receptor agonist (Breese et al. 1985b). As presented in Figure 6, olanzapine was able to block this locomotor enhancement by quinpirole in a dose-related fashion [$F(4,40) = 13.69$, $p = .0001$]. This antagonism occurred

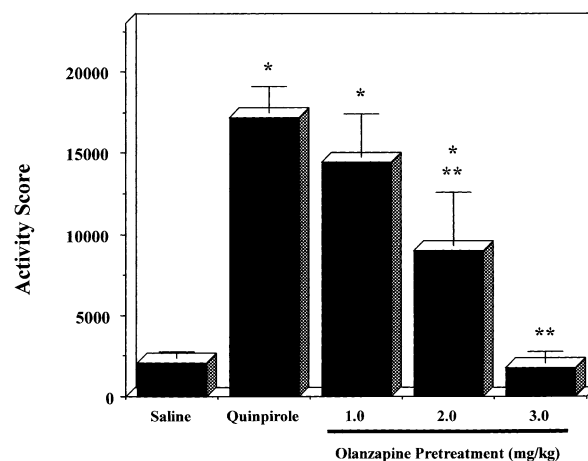


Figure 6. Effect of olanzapine on locomotor activity following quinpirole (0.3 mg/kg) in adult rats treated with 6-OHDA as young adults. Data shown are mean (\pm SEM) activity scores taken across a 150-min session, with at least 5 days between each session. Results are from male rats ($n = 11$), previously assessed for supersensitivity for quinpirole. * $p < .05$, quinpirole treatment versus saline treatment. ** $p < .05$, olanzapine pretreatment versus quinpirole alone.

over a much narrower dose range than observed with olanzapine against D₁-DA receptor-mediated responses in the animals given lesions as neonates, suggesting a greater potency for olanzapine against D₂-DA receptor-mediated events. It is notable that a dose of 3 mg/kg olanzapine fully prevented the high levels of activity induced by quinpirole, but did not significantly decrease the comparable activation observed with SKF-38393.

Effect of Olanzapine Against Self-Injurious Behavior (SIB) Induced by Apomorphine

One of the most striking aberrant responses found in rats given selective dopamine lesions as neonates is the self-injury observed following treatment with DA receptor agonists (Breese et al. 1984a,b). In agreement with these earlier reports, a high dose of apomorphine (10 mg/kg) elicited a characteristic behavioral profile that included head-bobbing, taffy-pulling (forepaws drawn repeatedly up to the mouth or face), and self-biting, as well as self-injury, in the neonate-lesioned rats. As shown in Table 1, pretreatment with olanzapine attenuated the observed SIB in a dose-dependent fashion, with an almost complete blockade of self-injury at the highest dose (20 mg/kg). Examination of the behavioral records for the animals given the highest dose of olanzapine indicates that these subjects exhibited some type of overt behavioral response during 70% of the recording interval, including high rates of sniffing (62% of the time) and lower rates of rearing (8%) and locomotion (7%). It is notable that one of the subjects that did not show SIB still demonstrated

Table 1. Effect of Olanzapine on SIB Following Treatment with Apomorphine in Neonatal 6-OHDA-Lesioned Rats.

Dose of Olanzapine ^a	Number of SIB	
	Positive Animals/ Total Number Tested	% SIB ^b
0 mg/kg	4 SIB+ / 4 total	100%
10 mg/kg	2 SIB+ / 4 total	50%
20 mg/kg	1 SIB+ / 6 total	17%

^aAnimals were pretreated with olanzapine 10 min before the administration of apomorphine (10 mg/kg).

^bSIB (self-injurious behavior) was defined as a break in the skin attributable to self-biting.

repeated paw licks (31% of the time), which were not blocked by the high dose of olanzapine. Similarly, a second subject still evidenced taffy-pulling (19% of the time) following treatment with the higher olanzapine dose. While further comparisons between the behavioral data from all of the experimental groups is confounded by the immediate use of a D₁-DA receptor antagonist following the first observation of SIB (a treatment that might have altered all subsequent behavioral responses), these data still provide an indication that the animals given the highest dose of olanzapine were still capable of emitting some stereotyped responses.

DISCUSSION

The main purpose of the present investigation was to determine whether olanzapine would antagonize functional responses mediated by sensitized D₁-DA receptors in rats given early 6-OHDA treatment. Previous work had demonstrated that the binding profile for olanzapine includes a high level of affinity for the D₁-DA receptor site (Bymaster et al. 1996; Schotte et al. 1996; Arnt and Skardfeldt 1998). In line with these reports, olanzapine was found to act as a potent antagonist against several measures of selective D₁-DA agonist effects in rats with sensitized D₁-DA receptors. In particular, olanzapine attenuated the increase in locomotion observed after the acute administration of SKF-38393, in contrast to previous findings with clozapine, which did not fully block SKF-38393-induced activity in neonate-lesioned rats, even at a maximal dose of 100 mg/kg (Criswell et al. 1989a). It is notable that, although olanzapine and clozapine have been characterized as having comparable effects in behavioral tests (Moore et al. 1992, 1997; Devaney and Waddington 1996) and similar therapeutic profiles for the treatment of schizophrenia (Beasley et al. 1996), radioreceptor binding assays show that olanzapine has a greater affinity for both D₁-DA and D₂-DA receptors, but lower affinity for D₄-DA receptors, in comparison to clozapine (Bymaster et al. 1996).

In addition to suppressing the high rates of locomotion observed with SKF-38393, olanzapine also dose-dependently reduced the increase in striatal Fos protein levels, measured as Fos-LI (Fos-like immunoreactivity). In normal animals, the administration of selective D₁-DA receptor agonists does not induce Fos-LI in brain, yet these same compounds, when given to rats with neonatal 6-OHDA treatment, elicit a pronounced expression of Fos-LI in striatum and cortex (Johnson et al. 1992; Criswell et al. 1993).

Sensitized D₁-DA receptors may also be necessary to observe an antagonistic effect of olanzapine against striatal Fos protein expression, because treatment with this antipsychotic alone can induce significant levels of Fos-LI in the striatum and other brain areas of normal rats (Robertson and Fibiger 1996; Sebens et al. 1998). Interestingly, treatment with quinpirole, a selective D₂-type DA receptor agonist, invokes high rates of activity in lesioned rats, but does not have a comparable impact on Fos-LI (Johnson et al. 1992). Pretreatment with MK-801 (1.0 mg/kg), an antagonist for the NMDA receptor, can significantly reduce the striatal Fos-LI observed following SKF-38393 (Criswell et al. 1993), indicating that a component of this neuronal response might be attributable to glutamatergic neurotransmission.

The impact of olanzapine on SKF-38393-induced locomotion suggests that its action at the D₁-DA receptor site could be a mechanism for the observed antagonistic power against the induction of Fos-LI, but other mechanisms will have to be explored to establish this possibility. In fact, work examining *ex vivo* receptor binding in rat brain indicates that the dose range used in the present study was at or above the ED₅₀ values for high receptor occupancy of specific 5-HT receptor subtypes, in particular the 5-HT_{2A} subtype, as well as adrenergic α_1 and α_2 receptors, cholinergic muscarinic receptors, and histamine H₁ receptors (Schotte et al. 1996). Although high potency at the α_1 and H₁ receptors might be linked to sedative effects (Schotte et al. 1996), the failure of a markedly high dose of clozapine (100 mg/kg) to block locomotion induced by a DA-D1 receptor agonist fully (Criswell et al. 1989a) suggests that a comparable action of olanzapine on these receptors did not mediate the decreases in locomotion and the induction of Fos-LI observed in the present study. On the other hand, previous work using binding assays has shown that olanzapine has a very low affinity for sites on the NMDA receptor complex (see Bymaster et al. 1999, for review); however, other researchers have found that olanzapine can block behavioral responses induced by treatment with NMDA antagonists (e.g., Ninan and Kulkarni 1999), indicating that olanzapine might have a significant impact on glutamate receptor function.

Previous work has shown that repeated treatment with selective D₁-DA receptor agonists can induce pro-

gressive increases in activity (priming) in animals given neonatal 6-OHDA treatment, but not in animals with intact dopaminergic function (Breese et al. 1985b; Criswell et al. 1989b). Pretreatment with SCH-23390, a selective D_1 -DA receptor antagonist, or MK-801, can block priming in the lesioned rats (Criswell et al. 1989b, 1990). In the present study, olanzapine (10 mg/kg) had a small, but significant, effect on the emergence of priming in the lesioned animals. At this same dose, olanzapine could fully block the acute effects of SKF-38393-induced locomotion, but attenuated SIB in only 50% of the animals tested for self-injury following apomorphine (see discussion below). It is possible that a higher dose of olanzapine might be necessary to prevent sensitization of D_1 -DA receptors completely.

Past research has demonstrated that the self-injury observed in neonate-lesioned rats following treatment with L-Dopa or apomorphine can be prevented by SCH-23390 (Breese et al. 1985a; Allen et al. 1998). Therefore, given the ability of olanzapine to block D_1 -DA receptors, we tested whether olanzapine would also be effective against this aberrant behavior linked to sensitized dopaminergic receptors. The successful blockade of self-injury with olanzapine observed in the present study corresponds with previous work on the efficacy of atypical antipsychotics against SIB (Criswell et al. 1989a; Davis et al. 1997; Allen et al. 1998). Although the effective dose of olanzapine was high enough to reduce spontaneous activity, this dose is still below the dose range associated with catalepsy in rats (Moore et al. 1992). Examination of the behavioral records indicated that the animals had low activity levels, but were not immobilized. The observation of repeated stereotyped responses in two of the subjects that did not exhibit SIB provides some evidence that the action of olanzapine at the higher dose was not based on sedation of the rats.

Although the effect of olanzapine on SIB could be attributed to an action on D_1 -DA receptor function, other evidence suggests that serotonergic neurotransmission might also influence self-injury induced by apomorphine. Mianserin, a selective 5-HT_{2C} receptor antagonist, can prevent SIB (Allen et al. 1997; Davis et al. 1997), and m-CPP, a serotonergic agonist for the same receptor site, can induce self-biting (Allen et al. 1997) in neonatal-6-OHDA-treated rats. Both clozapine and olanzapine have been shown to have a high affinity for the 5-HT_{2C} receptor, as well as the 5-HT_{2A} and 5-HT₃ receptors (Kuoppamäki et al. 1995; Bymaster et al. 1996; Schotte et al. 1996). Further *in vitro* work using functional responses from receptor-transfected cells has demonstrated that olanzapine is especially effective as an antagonist of 5-HT_{2A} and 5-HT_{2C} receptors (Bymaster et al. 1999). The potency of olanzapine on these serotonergic receptors is considerably greater than for its action on the D_1 -DA receptor (Zhang and Bymaster, 1999). Allen et al. (1998) suggested that the high affinity

of risperidone (another atypical antipsychotic) for the 5-HT_{2C} receptor is the basis for the successful amelioration of SIB in neonate-lesioned rats. Consequently, questions remain whether influences on D_1 -DA or 5-HT receptors or collectively on both receptor systems account for the therapeutic efficacy of olanzapine and other atypical antipsychotic agents that reduce the self-injury induced in the neonate-lesioned rats.

The present study also assessed the ability of olanzapine to block locomotor responses mediated by sensitized D_2 -DA receptors. In this case, rats were given lesions of DA-containing as young adults, because previous work has indicated that DA loss in older animals leads to a greater sensitivity to selective D_2 -DA receptor agonists, in comparison to both neonate-lesioned rats and normal animals (Breese et al. 1985b). Olanzapine proved to have a much more potent impact against quinpirole-induced activity in the adult-lesioned animals, with a full blockade of locomotor effects at a dose (3.0 mg/kg) that failed to reduce SKF-38393-induced activity by a significant amount in the neonate-lesioned rats. This finding is in line with some past reports showing a greater impact for olanzapine on D_2 -DA, in contrast to D_1 -DA, receptor binding (Bymaster et al. 1996; Schotte et al. 1996; Arnt and Skarfeldt 1998; Zhang and Bymaster 1999), but also may be reflective of a greater sensitivity of adult-lesioned animals, in comparison to neonate-lesioned animals, to dopamine antagonists acting on D_2 -DA receptors (Bruno et al. 1985; Duncan et al. 1987).

In summary, the present study provides evidence that olanzapine is a potent antagonist of DA receptor function in animals with supersensitive responses to DA agonists. However, the possibility remains that alterations in serotonergic or other neurotransmitter function, and not antagonism of DA receptor function, by olanzapine underlie the observed efficacy against self-injury in neonate-lesioned animals. Overall, the results support the use of olanzapine in clinical syndromes that may involve aberrant behaviors, such as those observed in Lesch-Nyhan disease and some types of mental retardation (Lloyd et al. 1981; Breese et al. 1986).

ACKNOWLEDGMENTS

This research was funded by a Young Investigator Award from the National Alliance for Research on Schizophrenia and Affective Disorders (NARSAD), and by NIAAA grant AA00214.

REFERENCES

- Allen SM, Davis WM, Freeman JN (1997): Interaction between dopaminergic and serotonergic systems after neonatal 6-OHDA lesions. *Soc Neurosci Abstr* 23:412

- Allen SM, Freeman JN, Davis WM (1998): Evaluation of risperidone in the neonatal 6-hydroxydopamine model of Lesch-Nyhan syndrome. *Pharmacol Biochem Behav* 59:327–330
- Arnt J, Skardfeldt M (1998): Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* 18:63–101
- Beasley CM, Tollefson GD, Tran P, Satterlee W, Sanger T, Holman S (1996): Olanzapine versus placebo and haloperidol: Acute phase results of the North American double blind olanzapine trial. *Neuropsychopharmacology* 14:111–123
- Breese GR, Baumeister AA, McCown TJ, Emerick SG, Frye GD, Crotty K, Mueller RA (1984a): Behavioral differences between neonatal and young adult 6-hydroxydopamine-treated rats to dopamine agonists: Relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *J Pharmacol Exper Therap* 231:343–354
- Breese GR, Baumeister AA, McCown TJ, Emerick SG, Frye GD, Mueller RA (1984b): Neonatal-6-hydroxydopamine treatment: Model of susceptibility for self-mutilation in the Lesch-Nyhan syndrome. *Pharmacol Biochem Behav* 21:459–461
- Breese GR, Baumeister AA, Napier TC, Frye GD, Mueller RA (1985a): Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *J Pharmacol Exper Therap* 235:287–295
- Breese GR, Mueller RA, Napier TC, Duncan GE (1986): Neurobiology of D1 dopamine receptors after neonatal-6-OHDA treatment: Relevance to Lesch-Nyhan disease. *Adv Exper Med Biol* 204:197–215
- Breese GR, Napier TC, Mueller RA (1985b): Dopamine agonist-induced locomotor activity in rats treated with 6-hydroxydopamine at differing ages: Functional supersensitivity of D-1 dopamine receptors in neonatally lesioned rats. *J Pharmacol Exper Therap* 234:447–455
- Breese GR, Traylor TD (1972): Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *Brit J Pharmacol* 44:210–222
- Bruno JP, Stricker EM, Zigmond MJ (1985): Rats given dopamine-depleting brain lesions as neonates are subsensitive to dopaminergic antagonists as adults. *Behav Neurosci* 99:771–775
- Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P, Wong DT (1996): Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology* 14:87–96
- Bymaster F, Perry KW, Nelson DL, Wong DT, Rasmussen K, Moore NA, Calligaro DO (1999): Olanzapine: A basic science update. *Br J Psychiat* 174 (suppl 37):36–40
- Criswell HE, Johnson KB, Mueller RA, Breese GR (1993): Evidence for involvement of brain dopamine and other mechanisms in the behavioral action of the N-Methyl-D-aspartic acid antagonist MK-801 in control and 6-hydroxydopamine-lesioned rats. *J Pharmacol Exper Therap* 265:1001–1010
- Criswell HE, Mueller RA, Breese GR (1989a): Clozapine antagonism of D-1 and D-2 dopamine receptor-mediated behaviors. *Euro J Pharmacol* 159:141–147
- Criswell HE, Mueller RA, Breese GR (1989b): Priming of D₁-dopamine receptor responses: Long-lasting behavioral supersensitivity to a D₁-dopamine agonist following repeated administration to neonatal 6-OHDA-lesioned rats. *J Neurosci* 9:125–133
- Criswell HE, Mueller RA, Breese GR (1990): Long-term D₁-dopamine receptor sensitization in neonatal 6-OHDA-lesioned rats is blocked by an NMDA antagonist. *Brain Res* 512:284–290
- Davis WM, Allen SM, Freeman JN (1997): Pharmacological evaluation of atypical neuroleptics in the 6-OHDA model of Lesch-Nyhan syndrome. *Soc Neurosci Abstr* 23:412
- Devaney AM, Waddington JL (1996): Comparison of the new atypical antipsychotics olanzapine and ICI 204,636 with clozapine on behavioral responses to the selective "D₁-like" dopamine receptor agonist A 68930 and selective "D₂-like" agonist RU 24213. *Psychopharmacology* 124:40–49
- Duncan GE, Criswell HE, McCown TJ, Paul IA, Mueller RA, Breese GR (1987): Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. *J Pharmacol Exper Therap* 243:1027–1034
- Duncan GE, Knapp DJ, Breese GR (1996): Neuronal anatomical characterization of Fos induction in rat behavioral models of anxiety. *Brain Res* 713:79–91
- Frye GD, Chapin RE, Vogel RA, Mailman RB, Kilts CD, Mueller RA, Breese GR (1981): Effects of acute and chronic 1,3-butanediol treatment on central nervous system function: A comparison with ethanol. *J Pharmacol Exper Therap* 216:306–314
- Johnson KR, Criswell HE, Jensen KF, Simson PE, Mueller RA, Breese GR (1992): Comparison of the D₁-dopamine agonists SKF-38393 and A-68930 in neonatal 6-hydroxydopamine-lesioned rats: Behavioral effects and induction of c-fos-like immunoreactivity. *J Pharmacol Exper Therap* 262:855–865
- Josselyn SA, Miller R, Beninger RJ (1997): Behavioral effects of clozapine and dopamine receptor subtypes. *Neurosci Biobehav Rev* 21:531–558
- Kuoppamaki M, Palvimaki E-P, Hietala J, Syvalahti E (1995): Differential regulation of rat 5-HT_{2A} and 5-HT_{2C} receptors after chronic treatment with clozapine, chlorpromazine, and three putative atypical antipsychotic drugs. *Neuropsychopharmacology* 13:139–150
- Lesch M, Nyhan WL (1964): A familial disorder of uric acid metabolism and central nervous system function. *Am J Med* 36:561–570
- Lloyd KG, Hornykiewicz O, Davidson L, Shannak K, Farley I, Goldstein M, Shibuta M, Kelley WN, Fox IH (1981): Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. *New Engl J Med* 305:1106–1111
- Meltzer HY, Fibiger HC (1996): Olanzapine: A new atypical antipsychotic drug. *Neuropsychopharmacology* 14:83–85
- Miller R, Wickens JR, Beninger RJ (1990): Dopamine D-1 and D-2 receptors in relation to reward and performance: A

- case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. *Prog Neurobiol* 34:143–183
- Moore NA, Leander JD, Benvenista MJ, Gleason SD, Shannon H (1997): Behavioral pharmacology of olanzapine: A novel antipsychotic drug. *J Clin Psychiat* 58(Suppl 10):37–44
- Moore NA, Tye NC, Axton MS, Risius FC (1992): The behavioral pharmacology of olanzapine, a novel “atypical” antipsychotic agent. *J Pharmacol Exper Therap* 262:545–551
- Ninan I, Kulkarni, SK (1999): Preferential inhibition of dizocilpine-induced hyperlocomotion by olanzapine. *Eur J Pharmacol* 368:1–7
- Nordstrom A-L, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G (1995): D₁, D₂, and 5-HT₂ receptor occupancy in relation to clozapine serum concentration: A PET study of schizophrenic patients. *Am J Psychiat* 152: 1444–1449
- Robertson GS, Fibiger HC (1996): Effects of olanzapine on regional C-Fos expression in rat forebrain. *Neuropsychopharmacology* 14:105–110
- Schotte A, Jannssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loore K, Leysen JE (1996): Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. *Psychopharmacology* 124:57–73
- Sebens JB, Koch T, Ter Horst GJ, Korf J (1998): Olanzapine-induced Fos expression in the rat forebrain; cross-tolerance with haloperidol and clozapine. *Eur J Pharmacol* 353:13–21
- Watts RWE, Spellacy E, Gibbs DA, Allsop J, McKernan RO, Slavin GE (1982): Clinical, post-mortem, biochemical, and therapeutic observations on the Lesch–Nyhan syndrome with particular reference to the neurological manifestations. *Quart J Med* 51:43–78
- Zhang W, Bymaster FP (1999): The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D₁, D₂, D₃, 5HT_{2A}, and muscarinic receptors. *Psychopharmacology* 141: 267–278