PNU-96415E, a Potential Antipsychotic Agent with Clozapine-Like Pharmacological Properties


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ABSTRACT

The atypical antipsychotic drug clozapine interacts with multiple transmitter systems, among them the D₂ subtype of dopamine receptors. PNU-96415E is chemically unrelated to clozapine and has its highest binding affinity for the D₂ and 5-HT₂A receptors. In comparison to clozapine, PNU-96415E is weaker in binding to D₁, D₃, α₁, and muscarinic receptors. PNU-96415E inhibited exploratory locomotor activity in mice and rats, and antagonized d-amphetamine-induced locomotor stimulation in rats. It antagonized apomorphine-induced cage climbing, and blocked head and body twitch produced by 5-HTP in mice. Like clozapine, but unlike haloperidol, PNU-96415E did not antagonize stereotypic behaviors produced by a high dose of d-amphetamine or methylphenidate in rats and mice. PNU-96415E blocked conditioned avoidance in rats but produced no catalepsy, a pattern similar to clozapine but different from haloperidol. In rats trained to discriminate clozapine from saline injections, the stimulus effect generalized completely with PNU-96415E, but not haloperidol. This profile of pharmacological activities is consistent with that of an atypical antipsychotic and, as in the case with clozapine, the behavioral effects of PNU-96415E cannot be ascribed to a single receptor mechanism.

Clozapine is an antipsychotic agent with an atypical profile of clinical efficacy. It was effective in some schizophrenic patients unresponsive to the neuroleptic haloperidol, and it was able to reduce both positive and negative symptoms of the disease (Kane et al., 1988). Another advantage of clozapine over the neuroleptics is a lower incidence of extrapyramidal side effects, which is the major drawback of conventional antipsychotic drug therapy. However, the usefulness of clozapine is limited by toxicity in the form of agranulocytosis (Krupp and Barnes, 1992). The challenge of developing a better clozapine-like atypical antipsychotic has stimulated much research. Neuroleptics share a common property as effective dopamine D₂ receptor antagonists. However, clozapine is a weak D₂ antagonist based on its receptor binding affinity on other systems, including the dopamine D₁ receptor subtype, 5-HT, muscarinic, histamine and adrenergic receptors (Creese et al., 1976; Leysen et al., 1993). Several hypotheses have been proposed to explain the unique antipsychotic efficacy of clozapine and the low incidence of extrapyramidal side effects (Meltzer, 1989; Baldessarini et al., 1993; Van Tol et al., 1991). Some investigators favor a combined-receptor mechanism (e.g., D₂ and muscarinic antagonism, D₂ and 5-HT₂ antagonism). The rationale for the specific combination and optimal balance of receptor interactions is a matter of continued investigation. More recently, the discovery of the D₄ dopamine receptor and ligands with selective affinity for that receptor presented new opportunities for clozapine-like antipsychotic compounds (Kulagowski et al., 1996). However, clinical results are not yet available to support a D₄ mechanism of antipsychotic efficacy. In the search for atypical antipsychotics, we have screened compounds with radioligand receptor binding in vitro to obtain a profile of receptor affinity, followed by a behavioral test (clozapine stimulus discrimination) to identify clozapine-like compounds. PNU-96415E (fig. 1) has an interesting combination of receptor affinities, including a high affinity for the D₄ receptor, and shares the discriminative effect of clozapine in rats. We describe the antipsychotic-like pharmacological activities of PNU-96415E.

Materials and Methods

Animals. Male rats (Sprague-Dawley or Fischer 344) and male mice (B6C3F1) obtained from Harlan Laboratories (Indianapolis, IN) were given free access to food and water (except as indicated in the clozapine discrimination experiment) and maintained on a 12-hr day-night cycle (lights on at 0600).

Receptor-binding in vitro. The binding profiles of PNU-96415E and clozapine were evaluated in radioligand competition binding assays employing 11 half-log dilutions of drugs run in duplicate. The radioligands (all tritiated) were SCH 23390 (D₁, 71 Ci/mmol, 0.3 nM), raclopride (D₂, 80 Ci/mmol, 1.1 nM), spiperone (D₃ and D₄, 97 Ci/mmol, 0.7 nM), 8-hydroxy-2-(di-n-propylamino)tetralin (5-HT₁A).
85 Ci/mmol, 1.2 nM), ketanserin (5-HT2A, 62 Ci/mmol, 0.8 nM), prazosin (α1, 76 Ci/mmol, 1.2 nM), clonidine (α2, 60 Ci/mmol, 3.8 nM) and oxotremorine-M (muscarinic, 86 Ci/mmol, 0.5 nM). Non-specific binding (5–20% of total) was determined with 3 μM of the following cold compounds (listed in the same order as the radioligands above): SCH 23390, haloperidol (D2, D3 and D4), lisuride, spiperone, phenothiazines, clonidine and atropine.

The sources of binding sites were as follows: homogenized rat striatum (D1); Chinese hamster ovary cell membranes prepared from cells expressing the rat D2 dopamine receptor (Chio et al., 1990), the rat D3, dopamine receptor (Chio et al., 1994a), the human 5-HT1A receptor (Fargin et al., 1988), or the rat 5-HT2A receptor (Julius et al., 1990); HEK 293 cell membranes from cells expressing the human D2, receptor (Chio et al., 1994b) and homogenized rat cortex (α1, α2 and muscarinic sites). Buffers used were 50 mM Tris, 5 mM MgCl2, pH 7.4 (α1, α2, 5-HT1A, 5-HT2A and muscarinic assays), 50 mM Tris, 120 mM NaCl, 5 mM KCl, 5 mM CaCl2, 1 mM MgCl2, pH 7.4 (D1 assay), 20 mM HEPES, 10 mM MgSO4, pH 7.4 (D2 assay), 20 mM HEPES, 10 mM MgSO4, 150 mM NaCl, and 1 mM EDTA, pH 7.4 (D3 and D4 assays).

Incubation of the 0.9 ml binding reactions was for 1 hour at room temperature. Reactions were stopped by vacuum filtration using ice cold 50 mM Tris, 5 mM MgCl2, pH 7.4. IC50 values were estimated by fitting the data to a one-site model by nonlinear least squares minimization using GraphPad Prism. K values were calculated according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Locomotor activity. Male B6.CF1 mice or male F344 rats were used in this study. The apparatus was a symmetrical Y-shaped chamber, with each arm having the following dimensions: 21 × 21 × 12 cm. Photopeams and detectors positioned at mid-point of each arm recorded entry into an arm. Repeated activation of the same detector was not counted as an arm-entry until the detector in another arm was activated. Spontaneous alternation was defined as entry to an arm that was least recently occupied. Each Y-maze was housed in a sound-attenuated enclosure with a dimly lit overhead light and masking noise. Thirty min before the experimental session, mice or rats were pretreated with PNU-96415E s.c., clozapine i.p., or haloperidol i.p., and then placed in the Y-maze for 30 min. For each compound, four or five doses were tested in parallel with an accompanying saline group (n = 6/group). Behavioral effects for each compound were evaluated by a one-way analysis of variance followed by Dunnett’s t test comparing to the contemporaneous saline group.

Antagonism of d-amphetamine sulfate (1 mg/kg, s.c.) was studied in rats. Three doses of an antagonist were tested in combination with amphetamine, with both compounds injected 30 min before the experimental session. For each antagonist, a saline-only and an amphetamine-only group was tested in parallel (n = 6/group). Significant antagonism of amphetamine-induced ambulation and reduction of spontaneous alternation were tested by one-way analysis of variance among the groups receiving amphetamine, followed by Dunnett’s t test comparing to amphetamine-only group.

Aptomorphine-induced cage climbing. The procedure was similar to that described by Protais et al. (1976). Male CF1 mice weighing 18 to 20 g were used. Aptomorphine HCl (1 mg/kg) was injected s.c., and the mouse was immediately placed inside a cylindrical cage. The cage was 14 cm high and had a diameter of 12 cm, with vertical metal bars, 2 mm in diameter, 1 cm apart. The top of the cage was made of smooth sheet metal and the floor was covered by a piece of corrugated paper. The animals were observed for climbing behavior at 10-, 20- and 30-min time periods after being in the cage, with the following scoring system: 0 = all four feet on the floor, 1 = one or two feet on the wall and 2 = all four feet on the wall. The sum of the three scores for each animal was used for statistical evaluation. The antagonists were injected 30 min before the test, with six mice per dose. Comparison was made against an amphetamine-only group using one-way analysis of variance within each antagonist dose group.

Methylphenidate-induced gnawing. Male B6.CF1 mice were injected s.c. with methylphenidate HCl at 60 mg/kg and placed individually in a transparent plexiglass cubic (6 × 6 × 6 cm) for observation. The floor of the cubic was covered with a piece of corrugated paper. Gnawing at the paper was observed continuously for 30 min and scored every 10 min: 0 = no gnawing, 1 = intermittent and 2 = continuous gnawing. An animal with a total score of 2 or more (maximum = 6) was considered a responder. Test compounds were injected 30 min before methylphenidate with 6 animals for each dose. The ED50 for antagonism was determined by the method of Spearman-Karber with a half-log dose interval (Finney, 1952).

d-Amphetamine-induced stereotypy. Male F344 rats received s.c. injections with d-amphetamine SO4 (30 mg/kg) and placed in individual observation boxes with a ventilated top (30 × 17 × 12 cm). The floor of the box was covered with a piece of corrugated paper. Stereotyped sniffing and chewing of the paper was observed for 1 min at 30, 60 and 90 min after injection. The intensity of stereotypy was scored 0 to 2, and an animal with a score of 2 at any one observation period constituted a positive responder. Drugs tested for amphetamine antagonism were injected 30 min before amphetamine, and ED50 values for protection were estimated by the method of Spearman-Karber with a half-log dose interval and six rats for each dose group (Finney, 1952).

5-HTP-induced head and body twitch. Male B6.CF1 mice were given the following treatment: nialamide (50 mg/kg i.p., -60 min), test compound (-30 min), 5-HTP (30 mg/kg i.p.), then observed for head- and body-twitch for the next 30 min. The characteristic motor effect, as described in the literature (Peroutka et al., 1981), was scored 0 to 2 every 10 min. The total score (maximum = 6) for each animal was used to compare between different dose groups of an antagonist and a parallel group (N = 6/group) receiving the agonists only. Statistical significance was evaluated by one-way analysis of variance followed by Dunnett’s t test.

Catalepsy. Male F344 rats were used. Catalepsy was evaluated by gently placing the forepaws of a rat on a smooth steel bar, 1 cm in diameter and 10 cm above the table top. If the rat removed both paws from the bar, it was immediately placed back on the bar. This process repeated five times, or until a total elapsed time of 5 min was reached. The total time from the 5 trials (maximum = 300 sec) was compared between vehicle- and drug-treated animals (N = 6/group) using the Mann-Whitney U test with the group medians presented.

Unsanguled (Sidman) avoidance. Male F344 rats were trained to avoid electric shock in a shuttle box (Coulbourn Instruments Co.). The chamber was partitioned in the middle with a 6.5 × 7.5 cm opening at the floor level. During a 30-min experimental session, electric shock (0.5 mA) was applied to the grid floor every 20 sec in the absence of movement from one side to another (a shuttle). Each shuttle postoned shock for 20 sec (an avoidance). A shuttle during shock immediately terminated the shock and restarted the 20-sec interval to the next shock (an escape). In the absence of escape.
response, shock terminated after 2.5 sec. Rats that performed with a high efficiency (less than 10 shocks per session) were used for drug testing. Performance on a drug-treatment day was compared to that of the immediately preceding vehicle-control day using Student’s paired t test with $N = 6$ dose group.

**Discriminative stimulus effect of clozapine.** Male Sprague-Dawley rats were trained to discriminate an i.p. injection of clozapine (3.2 mg/kg) from saline (Franklin and Tang, 1994). A two-lever, food-reinforced, FR-10 schedule was used for training after initial bar press training over a 3- to 5-day period. Rats were deprived of food for 23 h before each session and supplemented with 15 to 20 g of lab food after each session. The daily session (5 days/wk) terminated after 75 reinforcements or 15 min, whichever occurred first. A rat’s responding was considered under drug stimulus control when there were fewer than five responses on the incorrect lever before the first reinforcement in a session. After extensive training (mean = 50 ± 3 sessions; range = 28–78 sessions), a total of 24 rats reached the criterion of correct discrimination in 10 consecutive training sessions. For testing of stimulus generalization, the drug was injected i.p. 30 min before the experimental session where 10 responses on either lever produced food reinforcement. The ratio of clozapine-appropriate responses before the first reinforcement was a measure of clozapine-like stimulus effects, and responses on both levers during a 15-min session were used for overall response rate. Five to eight animals were randomly selected to test for each dose of a drug and, between drug test sessions, rats were retrained to correct discrimination in at least one saline and one clozapine session each. Statistical evaluation of the degree of generalization to the clozapine cue was accomplished by using two-tailed binomial test upon the frequency of drug responders at each dose compared to 100% accuracy on either training condition. Rate effects were evaluated by analysis of variance followed by Dunnett’s t test compared to a saline-injected test session.

**Drugs.** PNU-96415 was synthesized in the laboratory of R. Ten-Brink (Pharmacia & Upjohn, Inc., Kalamazoo, MI). It was used in this study either as the dihydrochloride salt (PNU-96415E) or the succinate salt (PNU-96415F), injected s.c. Clozapine was purchased from Research Biochemicals International (RBI) (Natick, MA). It was dissolved in water with a small amount of citric acid and injected s.c., except in the drug discrimination study where it was injected i.p. Haloperidol was also purchased from RBI. It was suspended in a 0.9% cellulose solution and injected i.p. Other drugs used in the study (d-amphetamine SO4, methylphenidate HCl, 5-hydroxytryptophan and Nialamid) were also from commercial sources. All doses are expressed as the respective salts.

**Results**

**Receptor binding.** In radioligand receptor binding assays using rat brain homogenates or cell lines expressing selective cloned receptors, clozapine has highest affinities for 5-HT2, α1 adrenergic and muscarinic receptors (table 1). It also has considerable affinities for dopamine D1 and D2 receptors. In comparison, PNU-96415E binds selectively to D4 and 5-HT2 receptors with an affinity greater than, or comparable to, that of clozapine. The affinities for α1 and D2 receptors are less than clozapine, and the affinity for the muscarinic receptors is extremely low. Both compounds have relatively lower affinities for D1, D2, D3, 5-HT1A and α2 receptors. PNU-96415E is therefore different from the conventional neuroleptics in being a weak D2 antagonist, but shares some receptor selectivity (e.g., 5-HT2A and D4) with clozapine.

**Spontaneous locomotor activity.** Pretreatment with PNU-96415E suppressed exploratory locomotion of mice and rats in an automated Y-maze, with mice being more sensitive (fig. 2). In both species, PNU-96415E was approximately $\frac{1}{2}$ and $\frac{1}{10}$ less potent than clozapine and haloperidol, respectively.

**Antagonism of locomotor stimulation from d-amphetamine.** Pretreatment with d-amphetamine (1 mg/kg) stimulated locomotion in rats at least 2-fold and reduced spontaneous alternation significantly. The locomotor stimulation was reversed by haloperidol, clozapine and PNU-96415F (fig. 3, upper graph). The effective dose of amphetamine antagonism only partially reduced locomotion when given by itself. At the dose required to reverse locomotor stimulation, the reduction in spontaneous alternation induced by d-amphetamine was also completely reversed after PNU-96415F or haloperidol (fig. 3, lower graph). Pretreatment with clozapine also produced a dose-related reversal of amphetamine’s effect on spontaneous alternation, although the effect was not statistically significant.

**Antagonism of apomorphine-induced cage climbing.** The cage-climbing behavior produced by apomorphine (1 mg/kg) in mice was dose dependently reversed by haloperidol, clozapine, and PNU-96415E, with the latter compound approximately $\frac{1}{10}$ as potent as clozapine (fig. 4).

**Antagonism of 5-HTP-induced head twitch.** Clozapine and ketanserin antagonized the overt behavioral effects of 5-HTP in mice with approximately equal potencies (fig. 5). PNU-96415F also antagonized 5-HTP, with about one-third the potency of clozapine. Haloperidol was much weaker, antagonizing the behavioral effects only at a dose that produced gross motor depression by itself.

**Antagonism of stereotypy.** Although haloperidol was potent in blocking the compulsive gnawing produced by methylphenidate in mice, neither clozapine nor PNU-96415E were effective at doses up to 30 mg/kg (table 2). Haloperidol

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**TABLE 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Inhibition Constants, $K_i$ (nM)</th>
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<tr>
<td></td>
<td></td>
<td>Clozapine</td>
</tr>
<tr>
<td>Dopamine D2</td>
<td>$[^3]H$Raclopride</td>
<td>82 (73–92)</td>
</tr>
<tr>
<td>Dopamine D4</td>
<td>$[^3]H$Spiperone</td>
<td>32 (23–44)</td>
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<tr>
<td>5-HT-2A</td>
<td>$[^3]H$Ketanserin</td>
<td>3.4 (2.0–5.9)</td>
</tr>
<tr>
<td>Adrenergic-$\alpha$1</td>
<td>$[^3]H$Prazosin</td>
<td>3.6 (2.7–4.8)</td>
</tr>
</tbody>
</table>

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was also effective in blocking stereotypic movements produced by a high dose of d-amphetamine in rats. Again, clozapine and PNU-96415E had no effect in amphetamine-induced stereotypy.

**Sidman avoidance.** In rats trained to avoid unsignaled shocks by shuttling between two compartments of the chamber, pretreatment with PNU-96415E suppressed the avoidance response and increased the number of shocks taken (fig. 6). The potency of PNU-96415E was weaker than clozapine, and much weaker than haloperidol or the D1 antagonist, SCH 23390.

**Catalepsy in rats.** Rats treated with haloperidol or SCH 23390 exhibited cataleptic posture when placed with forepaws on an elevated bar (fig. 7). Very little catalepsy was observed with clozapine or U-96415E at doses that reduced locomotion or impaired conditioned avoidance in rats.

**Discriminative effect of clozapine.** In rats trained to discriminate an injection of clozapine (3.2 mg/kg i.p.) from saline injections, PNU-96415E produced dose-related drug-appropriate responses with a full generalization after 10 mg/kg (table 3). Both compounds also suppressed response rate with comparable potencies. From the same pool of animals trained to discriminate clozapine, ketanserin produced a partial generalization and haloperidol only suppressed responses with no generalization to clozapine.

**Discussion**

The unique antipsychotic properties of clozapine in the treatment of schizophrenia have stimulated much research and development of atypical antipsychotic agents (Lieberman, 1993). Because clozapine is a relatively weak dopamine D2 receptor antagonist, which distinguishes it from traditional neuroleptics, most of the newer compounds attempt to mimic clozapine by combining antagonism of several receptor systems. For instance, risperidone has a higher affinity for the 5-HT2 than the D2 receptor (Schotte et al., 1993). ICI 204636 has affinities for α1 and 5-HT2 receptors (Goldstein and Arvanitis, 1995), and olanzapine is similar to clozapine...
in binding to D₂, D₄, 5-HT₂ and muscarinic receptors (Bymaster et al., 1996). Most intriguing is the relatively high affinity of clozapine for the dopamine D₄ receptor (Van Tol et al., 1991). The mRNA of this receptor subtype was found in monkey cerebral cortex, midbrain, amygdala and the medulla, but lower levels were found in the basal ganglia. It was suggested that this pattern of anatomical distribution, as compared to that of the D₂ receptor, may explain the lack of extrapyramidal side effects for clozapine. PNU-96415E has an affinity for the D₄ receptor 10-fold more than that of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methylphenidate Gnawing (Mice)</th>
<th>d-Amphetamine Stereotypy (Rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>0.13 (0.08–0.22)</td>
<td>2.5 (1.5–4.2)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>PNU-96415E</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

Fig. 4. Antagonism of apomorphine-induced cage-climbing in mice. Haloperidol (○), clozapine (●) or PNU-96415E (▲) were injected 30 min before the apomorphine (1 mg/kg s.c.) challenge. *P < .05; **P < .01; compared to the apomorphine-alone group (□) (N = 6/dose group) tested in parallel.

Fig. 5. Antagonism of head- and body-twitch in mice produced by 5-HTP (30 mg/kg i.p.) after pretreatment with nialamide (50 mg/kg i.p.). Haloperidol (○), clozapine (●), PNU-96415F (▲) and ketanserin (▲) were injected 30 min before 5-HTP with six mice per dose. *P < .05; **P < .01; compared to the group receiving (5-HTP + nialamide) (□) (N = 6/group).

Fig. 6. Performance of conditioned avoidance in rats. Inhibition of avoidance is indicated by a reduction in number of shuttles (upper graph) and an increase in shocks taken (lower graph). Data points represent means (± S.E.M.) after haloperidol (○), clozapine (●), PNU-96415E (▲) and SCH 23390 (▲). *P < .05; **P < .01; compared to the saline control (□) (N = 6/group).

Fig. 7. Cataleptic effect measured in rats. Haloperidol (○), clozapine (●), U-96415E (▲), SCH 23390 (▲), and vehicle (□). *P < .05; **P < .01; compared to the parallel vehicle-treated group (N = 6/group).
clozapine, and an affinity for the 5-HT<sub>2A</sub> receptor that is similar to clozapine. It is considerably weaker than clozapine in binding to D<sub>2</sub>, α<sub>1</sub>, and muscarinic receptors. This pattern of receptor selectivity for PNU-96415E represents both an overlap of, and significant differences from, that of clozapine. However, the results of the in vivo studies are consistent with a clozapine-like pharmacological profile that differs from that of the neuroleptic, haloperidol.

Inhibition of exploratory locomotor activity in mice and rats showed that there is approximately a 3-fold difference in potency between PNU-96415E and clozapine, and between clozapine and haloperidol. This nonspecific behavioral depressant effect could be due to one of several receptor mechanisms (e.g., D<sub>2</sub>, α<sub>1</sub>, etc.). More importantly, all three compounds reversed the locomotor stimulation produced by d-amphetamine in rats at doses that did not completely suppress locomotion when given by themselves. In addition to locomotor stimulation, d-amphetamine produces sensory perseveration as indicated by a loss of spontaneous alternation when a mouse or rat is exploring a symmetrical Y-maze (Kokkinidis and Anisman, 1977). Spontaneous alternation in a Y-maze was also impaired in rats after pretreatment with psychotomimetic agents (Drew et al., 1973). This effect of d-amphetamine was reversed by haloperidol and PNU-96415E, with clozapine having a similar, but weaker, effect. This normalizing effect on spontaneous alternation is important in that it involves more than the motor system and may be a model for some aspects of the pathology in psychosis (Anisman et al., 1985; Hahn et al., 1986; Yadin et al., 1991).

At doses higher than those required to increase locomotor activity, dopamine agonists produce stereotypic motor effects. The compulsive oral movements produced by apomorphine in rats for the unsignaled (Sidman) avoidance procedure in rats, Kuribara and Tadokoro (1981) showed that the dose to increase shocks correlated closely with clinical daily dose of both neuroleptics and atypical antipsychotics. These investigators found that, unlike all other antipsychotic agents, the shock rate increase from clozapine was not accompanied by a corresponding decrease in response rate. We used a shuttle response instead of lever press in rats for the unsignaled (Sidman) avoidance procedure. As with clozapine, PNU-96415E increased shock rate. However, both compounds were relatively weak in suppressing response rate when compared to haloperidol or the D<sub>1</sub> antagonist, SCH 23390. This result suggests that the suppression of avoidance by PNU-96415E was not a direct result of motor retardation. A parallel comparison is found in the induction of catalepsy, where clozapine and PNU-96415E produced little or no catalepsy at doses that increased shock rate in the shuttle avoidance task. However, the similarity of haloperidol and SCH 23390 in the conditioned avoidance and catalepsy tests suggests that D<sub>1</sub> antagonism is an important

Clozapine and olanzapine antagonized apomorphine-induced cage climbing and 5-HTP-induced head twitch, consistent with their dopamine and 5-HT antagonist properties (Moore et al., 1992). Cage climbing behavior in mice is a D<sub>2</sub>-mediated effect, with the D<sub>1</sub> receptor playing a permissive role (Moore and Axton, 1988). Costall et al. (1980) demonstrated that the nucleus accumbens is important for the apomorphine-induced climbing behavior. PNU-96415E is effective in this test, with a potency about one-tenth of clozapine. In comparison, PNU-96415F is considerably more potent than PNU-96415E at antagonizing 5-HTP-induced head twitch in mice, which has been shown to be mediated by the 5-HT<sub>2A</sub> receptor (Peroutka et al., 1981; Ortmann et al., 1982). In this test, clozapine and ketanserin were more potent than U-96415F, which in turn was more potent than haloperidol. On a molar basis, U-96415F is more potent at antagonizing the behavioral effects of 5-HTP than PNU-96415E at antagonizing apomorphine. This potency difference is consistent with the fact that PNU-96415E has a greater in vitro binding affinity for 5-HT<sub>2A</sub> than D<sub>2</sub> receptors.

Conditioned avoidance has long been a standard animal model for evaluating antipsychotic efficacy (Janssen and Niemegeers, 1961). Using an unsignaled, lever-press (Sidman) avoidance procedure in rats, Kuribara and Tadokoro (1981) showed that the dose to increase shocks correlated closely with clinical daily dose of both neuroleptics and atypical antipsychotics. These investigators found that, unlike all other antipsychotic agents, the shock rate increase from clozapine was not accompanied by a corresponding decrease in response rate. We used a shuttle response instead of lever press in rats for the unsignaled (Sidman) avoidance procedure. As with clozapine, PNU-96415E increased shock rate. However, both compounds were relatively weak in suppressing response rate when compared to haloperidol or the D<sub>1</sub> antagonist, SCH 23390. This result suggests that the suppression of avoidance by PNU-96415E was not a direct result of motor retardation. A parallel comparison is found in the induction of catalepsy, where clozapine and PNU-96415E produced little or no catalepsy at doses that increased shock rate in the shuttle avoidance task. However, the similarity of haloperidol and SCH 23390 in the conditioned avoidance and catalepsy tests suggests that D<sub>1</sub> antagonism is an important

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Clozapine Lever Response (% of Total)</th>
<th>No. of Drug Responders/Total</th>
<th>Response Rate (per Min)</th>
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<tr>
<td>Saline</td>
<td>—</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/24</td>
<td>104.7 ± 4.3</td>
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<tr>
<td>Clozapine</td>
<td>1</td>
<td>19.0 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>106.3 ± 5.2</td>
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<td></td>
<td>3</td>
<td>94.5 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24/24</td>
<td>88.3 ± 5.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>PNU-96415E</td>
<td>0.3</td>
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<td>1/6</td>
<td>124.0 ± 7.7</td>
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<td>Ketanserin</td>
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Clozapine lever response (mean ± S.E.M.) is taken from the response distribution before the first reinforcement (N = 5–8); response rate (mean ± S.E.M.) is averaged over the 15-min session.

<sup>a</sup> P < .01, compared to clozapine training data. <sup>b</sup> P < .01, <sup>c</sup> P < .05 compared to saline training data.
of the neuroleptic-like effects. Taken together, the pattern of behavioral effects in rodents predicts that PNU-96415E has antipsychotic efficacy with minimal extrapyramidal side effects.

Clozapine has an interesting DS property. Browne and Koe (1982) reported that the DS effect of clozapine (3.2 mg/kg) in rats did not generalize with a close analog, loxapine or haloperidol. Using a slightly higher training dose (5.76 mg/kg i.p.), Nielsen (1988) showed that the DS effect of clozapine generalized with muscarinic antagonists, but not to the 5-HT antagonist, ketanserin or the adrenergic antagonist, prazosin. However, the DS effect of clozapine in pigeons was clearly demonstrated to have 5-HT1C- and 5-HT2- (or 5-HT2A- and 5-HT2C-) antagonist properties (Hoenicke et al., 1992). We have reported that the DS effect of clozapine (3.2 mg/kg) in rats has the characteristics of a stimulus complex comprised of multiple receptor antagonist (Franklin and Tang, 1994). For instance, cyproheptadine (H1 and 5-HT2 antagonist), scopolamine (antimuscarinic) and SCH 23390 (D1 antagonist) all occasioned significant, but incomplete, generalization by themselves, but generalized completely when combinations of two different antagonists were given. However, haloperidol (primarily a D2 antagonist) produced primarily vehicle-appropriate responses. The DS effect of clozapine generalized fully with PNU-96415E, which was not found with any of the selective antagonists tested. Because PNU-96415E binds to the D2 receptor with 10-fold greater affinity than does clozapine, the potency for the DS effect correlates better with 5-HT2A than D2 binding affinity. The more selective 5-HT2A antagonist, ketanserin, did not fully substitute for clozapine, unlike PNU-96415E. The DS effect of PNU-96415E in the clozapine-trained rats is best explained by a combination of effects at the 5-HT2A and D4 receptors, perhaps with contribution also from α1 and 5-HT1A antagonism.

In summary, PNU-96415E has a profile of behavioral effects in rodents very similar to that of clozapine, including complete discriminative stimulus generalization. The favorable antipsychotic to side effect separation of both PNU-96415E and clozapine may depend on multiple receptor interactions.

References


