Title: WAY-163909 ((7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole): A Novel 5-HT$_{2C}$ Receptor Selective Agonist with Preclinical Antipsychotic-like Activity


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a) **Running Title:** Antipsychotic-like Effects of WAY-163909

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PPI, prepulse inhibition of startle; CAR, conditioned avoidance response test; SNC, substantia nigra pars compacta; HPLC, high performance liquid chromatography; mPFC, medial prefrontal cortex; LCEC, liquid chromatography/electrochemistry; IMER, immobilized enzyme reactors; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; GABA, gamma amino butyric acid; NMDA, N-methyl-D-aspartate; SEM, standard error of the mean
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Abstract

Serotonin-2C (5-HT$_{2C}$) receptor antagonists and agonists have been shown to affect dopamine (DA) neurotransmission, with agonists selectively decreasing mesolimbic DA. As antipsychotic efficacy is proposed to be associated with decreased mesolimbic DA neurotransmission by virtue of DA D$_2$ receptor antagonism, the 5-HT$_{2C}$ selective receptor agonist, WAY-163909, was evaluated in animal models of schizophrenia and in vivo microdialysis and electrophysiology to determine the effects on mesolimbic and nigrostriatal DA neurotransmission. Similar to clozapine, WAY-163909 (1.7 - 30 mg/kg, ip) decreased apomorphine-induced climbing with little effect on stereotypy and no significant induction of catalepsy. WAY-163909 (0.3 - 3 mg/kg, sc) more potently reduced phencyclidine-induced locomotor activity compared to d-amphetamine with no effect on spontaneous activity. WAY-163909 (1.7 - 17 mg/kg, ip) reversed MK-801 and DOI disrupted prepulse inhibition of startle (PPI) and improved PPI in DBA/2N mice. In conditioned avoidance responding, WAY-163909 (0.3 - 3 mg/kg, ip; 1 - 17 mg/kg, po) reduced avoidance responding, an effect blocked by the 5-HT$_{2B/2C}$ receptor antagonist SB 206553. WAY-163909 (10 mg/kg, sc) selectively decreased extracellular levels of DA levels in the nucleus accumbens without affecting the striatum. Similarly, in vivo electrophysiological recordings showed a decrease in the number of spontaneously firing DA neurons in the ventral tegmental area, but not in the substantia nigra, with both acute and chronic (21 day) administration of WAY-163909 (1 - 10 mg/kg, ip). Thus, the 5-HT$_{2C}$ selective receptor agonist WAY-163909 profiles similarly to an atypical antipsychotic and additionally may have rapid onset properties.
Introduction

The serotonin receptor family is currently composed of fourteen subtypes (Hoyer et al., 2002). The three 5-HT2 receptors (5-HT2A, 5-HT2B and 5-HT2C) display high sequence homology and are Gq-linked receptors which signal primarily through the IP3 pathway and activation of phospholipase C (Conn and Sanders-Bush, 1987; Saltzman et al., 1991). The wide distribution of 5-HT2C receptors and mRNA in brain (Pompeiano et al., 1994; Abramowski et al., 1995), coupled with pharmacological studies, have suggested a role for 5-HT2C receptors in mediating a broad range of effects of 5-HT in the CNS. Studies have implicated the 5-HT2C receptor in schizophrenia, depression, obsessive-compulsive disorder, anxiety, and obesity (Bos et al., 1997; Cryan and Lucki, 2000; Dunlop et al., 2005; Siuciak et al., 2005; Rosenzweig-Lipson et al., 2006).

At present, the most frequently used treatments for schizophrenia are the ‘atypical’ antipsychotics, which combine dopamine D2 receptor antagonism with 5-HT2A receptor antagonism. Despite the reported advances in efficacy and extrapyramidal side effect liability of atypical antipsychotics, these compounds do not treat all of the symptoms of schizophrenia and are accompanied by problematic side effects, including weight gain and diabetogenesis (Bottai et al., 2005). Novel antipsychotics that are effective in treating mood disorders or the cognitive impairments in schizophrenia without producing weight gain and diabetogenesis would represent a significant advance in the treatment of schizophrenia.

Studies with 5-HT2C antagonists report increased synaptic levels of dopamine (Di Matteo et al., 1998), suggesting that 5-HT2C agonists should decrease dopamine neurotransmission. Since all current antipsychotic medications reduce dopamine neurotransmission via the blockade of postsynaptic dopamine receptors, mechanisms like 5-HT2C agonism that reduce dopamine
neurotransmission without stimulating postsynaptic dopamine receptors could have antipsychotic potential. Indeed, recent studies have demonstrated that 5-HT$_{2C}$ receptor agonists decrease levels of dopamine in the nucleus accumbens (Millan et al., 1998; Di Giovanni et al., 2000), the limbic brain region thought to mediate antipsychotic effects of drugs while not affecting dopamine levels in the striatum (Millan et al., 1998), the brain region associated with extrapyramidal side effects. In addition, studies have demonstrated that 5-HT$_{2C}$ receptor agonists decrease firing in the ventral tegmental area (VTA), but not in substantia nigra pars compacta (SNC) (Di Giovanni et al., 2000). The selective effects of 5-HT$_{2C}$ receptor agonists on the mesolimbic dopamine pathway suggests that 5-HT$_{2C}$ receptor agonists should have antipsychotic efficacy without the extrapyramidal side effects associated with typical antipsychotics.

Many atypical antipsychotics bind with high affinity to 5-HT$_{2C}$ receptors and function as 5-HT$_{2C}$ receptor antagonists or inverse agonists (Leysen, 2004). The antagonism of 5-HT$_{2C}$ receptors by atypical antipsychotics is a potential mechanism contributing to the significant, and problematic, weight gain often observed with atypical antipsychotics, such as clozapine and olanzapine. Conversely, stimulation of the 5-HT$_{2C}$ receptor is known to result in decreased food intake and body weight (Cowen et al., 1995; Dunlop et al., 2005; Rosenzweig-Lipson et al., 2006). As a result, 5-HT$_{2C}$ receptor agonists may be less likely to produce an increase in body weight.

Taken together, these studies suggest the potential for 5-HT$_{2C}$ receptor agonists to possess antipsychotic-like neurochemical, electrophysiological and behavioral effects. Coupled with the potential to treat mood disorder symptoms and reduced risk for extrapyramidal side effects, weight gain or diabetogenesis, 5-HT$_{2C}$ selective receptor agonists represent a novel treatment approach for schizophrenia. To date, WAY-163909 may be the most selective 5-HT$_{2C}$ agonist.
identified, compared to other compounds reported in the literature (see (Dunlop et al., 2005)), providing distinct advantages over other 5-HT2C agonists with respect to both binding affinity (Ki = 11 nM) and selectivity (20 and 46 fold respectively for 5-HT2A and 5-HT2B); functional selectivity (EC50 = 8 nM; Emax = 90%) and decreased intrinsic activity at the 5-HT2A (no agonist activity) and 5-HT2B (Emax = 40%) receptors. Thus, the current study was conducted to explore the effects of WAY-163909 in several animal models predictive of antipsychotic efficacy.
Methods

Subjects. Male CF-1 mice (20 – 28 g, Charles River, Wilmington, MA) were used in the antagonism of apomorphine-induced behaviors, cataleptogenic potential and locomotor activity assays. Male DBA/2N mice (20 – 25 g, Taconic Farms, Germantown, NY) were used in the potentiation of prepulse inhibition of startle response assay. Male Sprague-Dawley rats were used for conditioned avoidance test (350 – 450 g, Charles River, Wilmington, MA), prepulse inhibition of startle test (250 – 350 g, Charles River, Wilmington, MA), in vivo electrophysiology experiments (150 – 175 g upon arrival, Taconic Farms, Germantown, NY), in vivo microdialysis experiments in nucleus accumbens and striatum (280 – 350 g, Charles River, Wilmington, MA) and in vivo microdialysis experiments in medial prefrontal cortex (250 – 350 g, Zivic-Miller Laboratories, Porterville, PA). All animals were group housed (except for conditioned avoidance response test subjects) in an AAALAC-accredited facility that was maintained on a 12-h light / dark cycle (lights on at 0600 h). Food and water were available ad libitum, except where noted. All studies were previously approved by the Institutional Animal Care and Use Committee and were performed in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Pub. 85-23, 1985).

Drugs. WAY-163909 ((7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole) was prepared by Wyeth Research (Princeton, NJ). Haloperidol, apomorphine, d-amphetamine, DOI, MK-801, phencyclidine, Tween80, hydroxypropyl-β-cyclodextrin and Methylcellulose were obtained from Sigma (St. Louis, MO). Clozapine was purchased from Research Biochemicals International (Natick, MA). Drugs were
dissolved in saline (WAY-163909, haloperidol, apomorphine, d-amphetamine, DOI, MK-801, phencyclidine), 2% Tween80:0.5% Methylcellulose (clozapine for behavioral assays, SB 206533) or 45% hydroxypropyl-β-cyclodextrin (clozapine for electrophysiology) and solutions were administered at a volume of 10 ml/kg to mice and 1 ml/kg to rats unless otherwise noted. Dose calculations were based on active moiety. All other materials were analytical grade and were purchased from Aldrich & Sigma chemicals (Milwaukee, WI).

Procedures.

Antagonism of Apomorphine-Induced Climbing and Stereotypy: Drugs were administered ip to 6 mice per dose level. A control group, run simultaneously with drug-treated groups, received saline at equal volumes. Thirty minutes later, experimental and control animals were challenged with 1 mg/kg sc apomorphine. Five minutes after the apomorphine injection, the sniffing-licking-gnawing (0 = absent, 1 = present) syndrome (stereotyped behavior) and climbing behavior (0 = all 4 feet on ground, 1 = 2 feet up on wire cage, 2 = all 4 feet on wire cage) induced by apomorphine were scored and recorded for each animal. Readings were repeated every 5 minutes during a 30-minute test session. Scores for each animal were totaled over the 30-minute test session for each syndrome (stereotyped behavior and climbing). One-way ANOVA analysis followed by the least significance difference test was used to determine the minimal effective dose (MED) and if an effect reached a minimum of 50% inhibition, an ID$_{50}$ value (95% confidence interval) was calculated using a nonlinear least squares calculation with inverse prediction. Mean climbing and stereotypy scores were then expressed as a percent of control values observed in vehicle-treated mice that received apomorphine.
Cataleptogenic Potential in Mice: Drugs were administered ip to 6 mice per treatment group. Every 30 minutes for 2 hours post dosing, the animal's forelegs were draped over a thin horizontal rod 1 3/4" high. The amount of time (in seconds) for which the animal maintained this awkward position was recorded (60 second maximum). Maintenance of this position was considered catalepsy. Mean seconds spent in the catalepsy position for each dose at each time point was calculated. The time point at which the peak catalepsy was exhibited was analyzed with a one-way analysis of variance with post-hoc least significant difference test (p < 0.05) and expressed graphically.

Atypical Antipsychotic Locomotor Activity Profile: Drugs were administered sc to 10 mice per treatment group concurrently with d-amphetamine (3 mg/kg, sc), phencyclidine (3 mg/kg, sc) or vehicle. Mice were then immediately placed in the locomotor chambers for a 70-minute test. Locomotor activity data was recorded under room light and white noise using Accuscan infrared beam activity monitors with enclosed Plexiglas chambers (8 in. x 8 in.). Accuscan Versamax and Versadat software (Columbus, OH) was used to convert the infrared beam breaks into horizontal activity counts in 5-minute bins. Total horizontal activity counts collected in a 70-minute test period and the last 40 minutes of the test period were subjected to one-way analyses of variance with subsequent least significant difference test (p < 0.05).

Prepulse Inhibition of Startle Response: Each testing chamber (SR-LAB system, San Diego Instruments, San Diego, CA) consisted of a Plexiglas cylinder (8.8 cm in diameter) mounted on a frame and held in position by four metal pins to a base unit. Movement of the rat or mouse within the cylinder was detected by a piezoelectric accelerometer attached below the frame. A loudspeaker mounted 24 cm above the cylinder provided background white noise, acoustic noise bursts and acoustic prepulses. The entire apparatus was housed in a ventilated enclosure (39 x
38 x 56 cm). Presentation of acoustic pulse and prepulse stimuli were controlled by the SR-LAB software and interface system, which also digitized, rectified and recorded the responses from the accelerometer. Mean startle amplitude was determined by averaging 100, 1 ms readings taken from the beginning of the pulse stimulus onset. For calibration purposes, sound levels were measured with a Quest sound level meter, scale “A”, with the microphone placed inside the Plexiglas cylinder.

**Antagonism of Pharmacologically Induced Disruption of PPI:** Subjects were male Sprague Dawley rats. Test session consisted of 60 total trials with a 15 sec inter-trial interval. Following a 5 min acclimation to a 64 dB background noise, four trial types (120 dB pulse, or a 69, 74, or 79 dB prepulse paired with a 120 dB pulse) were presented in a pseudorandom order. WAY-163909, clozapine and haloperidol were administered 30 min prior to test. Disrupting agents, d-Amphetamine (4 mg/kg, sc), DOI (3 mg/kg, sc) and MK-801 (0.15 mg/kg, ip), were administered 15-20 min after WAY-163909. Prepulse inhibition was defined as 100-[(startle amplitude on prepulse trials/startle amplitude on pulse alone trials) x 100]. Data from the pulse alone trials and average PPI values was analyzed using one-way ANOVA followed by a least significant difference post-hoc test (p < 0.05).

**PPI in DBA mice:** Subjects were male DBA/2N mice. Test sessions consisted of 50 total trials with a 15 sec inter-trial interval. Following a 5 min acclimation to a 64 dB background noise, five trial types (a no stimulus trial, a 118 dB pulse, or a 66, 68 or 72 dB prepulse paired with a 118 dB pulse) were presented in pseudorandom order. WAY-163909, clozapine and haloperidol were administered 30 min prior to test. Prepulse inhibition was defined as 100-[(startle amplitude on prepulse trials/startle amplitude on pulse alone trials) x 100]. Data from the pulse
alone trials and PPI was analyzed using one-way ANOVA followed by a least significant difference post-hoc test (p < 0.05).

**Conditioned Avoidance Responding:** Rats were individually housed and maintained on a food-restricted schedule (15 g of standard rodent feed each day after training/testing). Four shuttlebox test chambers (MED Associates, St Albans, VT) were used (divided into two compartments by an archway). Each chamber floor half was composed of thirteen 3/16" diameter stainless steel grid rods placed on 1/2" centers wired for the presentation of an electric foot shock (0.5 mA). In addition, each side of the chamber is equipped with a stimulus light and tone (sonalert) and two infrared beam source/detectors used to locate the rat within the chamber. Rats trained to avoid the foot shock were placed in the experimental chambers for a 4-minute habituation period followed by fifty trials presented on a 15-second variable interval schedule (range = 7.5 - 22.5 seconds). Each trial consisted of a 10-second warning tone and stimulus light (conditioned stimulus) followed by a 10-second shock (unconditioned stimulus), presented through the grid floor on the side where the rat was located, in the presence of the tone and light. If an animal crossed through the archway during the initial 10 seconds of the trial, the tone and light were terminated and the response was considered an avoidance response. If an animal crossed through the archway after a foot shock was initiated, the tone, light and shock were terminated and the response was considered an escape response. If a response was made during an inter-trial interval (ITI), the animal was punished with a 0.5-second shock (0.5 mA). A MED Associates computer with MEDSTATE NOTATION™ software controlled the test session and counted the number of trials in which the animal avoided shock, escaped shock and did not respond. Only animals displaying stable performance (approximately 90% avoidance responding on the training session prior to test day) were considered “trained” and included on test day. Training was maintained
by at least one non-drug test session each week. On test days, drugs were administered ip, sc or po (2 ml/kg) 30 (WAY-163909) or 60 (SB 206553) minutes prior to testing. Eight animals received each dose of test drug. For the time course study, 8 animals received WAY-163909 at 3mg/kg ip and were tested at 30 minutes, 1 hr, 2 hr and 24 hrs. Avoidance response and response failure data were subjected to repeated-measures analyses of variance with post-hoc least significant difference tests (p < 0.05). Data were also subjected to a nonlinear regression analysis to determine doses that produce 50% reduction in avoidance trials (ED$_{50}$).

**In Vivo Electrophysiology Studies:** Rats were anesthetized with chloral hydrate (400 mg/kg, ip) and mounted in a stereotaxic instrument. A lateral tail vein was cannulated with a 25-gauge needle for the administration of additional anesthetic or drug solution. The animals were placed on a heating pad to maintain a constant body temperature of 37-38°C. A hole was drilled over the SNC (anterior 3.0-3.5 mm to the lambda, lateral 1.8-2.5 mm to the midline and 6.0-8.5 mm ventral to the cortical surface) and VTA (anterior 3.0-3.5 mm, lateral 0.5-1.0 mm and ventral 6.0-8.5 mm (Paxinos and Watson, 1986), and the dura retracted. Single barrel microelectrodes were used for recording single cell DA activity. Glass micropipettes, which were pulled with an electrode puller (Narishige PE-2) and the tip broken back under a light microscope, were filled with a solution of 2M NaCl saturated with 1% Fast Green dye. The impedance of the electrodes was usually 0.8-1.2 megaohm (MΩ) measured at 135 Hz **in vitro** and 1.5-2.0 MΩ **in vivo**.

During the recording sessions, a neuron encountered in the SNC or VTA area was considered dopaminergic if it possessed the following characteristics: 1) a wide action potential (> 2.5 msec), with a distinct initial segment and late positive component; 2) a characteristic low-pitch sound when monitored through an audioamplifier; 3) a slow, regular or bursting firing pattern,
and 4) a spontaneous firing rate of 2-9 Hz. The number of spontaneously active DA neurons were determined in ten stereotaxic electrode descents or tracks as previously described (White and Wang, 1983). Briefly, ten electrode tracks (separated from each other by 200 µm), whose sequence was constant from animal to animal, were made in the SNC and VTA areas. Each electrode descent was made in a slow (1-3 µm/sec), uniform speed using a hydraulic microdrive. Only the cells whose electrophysiological profile matched those previously established for midbrain DA cells were counted. Rats were randomly allocated to receive one ip injection (acute regimen) or one ip injection per day for 21 consecutive days (chronic regiment) of vehicle (1 ml/kg of deionized, distilled water), 20 mg/kg of clozapine (placed in 45% hydroxypropyl-β-cyclodextrin, w/v) or 1, 3 or 10 mg/kg of WAY-163909 (dissolved in deionized, distilled water).

The animals were prepared for the recording of SNC and VTA DA cells two hours after the injection of vehicle, clozapine or WAY-163909. The experimenter was "blind" as to the treatment of each animal. In addition, for half of the rats in each group, the order of recording was SNC-VTA and the order was reversed for the other rats. At the end of each experiment, a 25 µA cathodal current was passed through the electrode, a procedure that leads to the deposition of a discrete spot of Fast Green dye in the brain. The animals were overdosed with chloral hydrate and perfused transcardially with 10% buffered formalin for 10 minutes. The brains were removed and serial coronal sections were cut at 50 µm intervals, stained with cresyl violet and counterstained with neutral red. The dye spot was viewed under a light microscope and served as a reference point for the location of each cell. The SNC and VTA DA cells/track data were analyzed using an analysis of variance (ANOVA) and post hoc tests were performed using the Student-Newman-Keuls test.

In vivo Microdialysis Studies:
Nucleus Accumbens and Striatal Microdialysis Procedure: Following induction of anesthesia, with gaseous administration of halothane (3%) (Fluothane, Zeneca, Cheshire, UK), the animals were secured in a stereotaxic frame with ear and incisor bars. Anesthesia was maintained by continuous administration of halothane (1 - 2%). A microdialysis probe guide cannula (CMA/Microdialysis, Stockholm, Sweden) was implanted into the striatum (AP +0.2, L -3, V -3) or nucleus accumbens (AP +2.2, L -1.4, V -6.0). Coordinates were taken according to Paxinos and Watson (Paxinos and Watson, 1986) with reference points taken from bregma and vertical from the skull. A subcutaneous cannula (sc) was also implanted at this time between the animal’s shoulders. Both cannula were secured to the skull using dental acrylic (Plastics One, Roanoke, VA) and two stainless steel screws. Immediately following surgery, animals were individually housed in Plexiglass cages (45 cm sq.) and were provided food and water ad libitum. Animals were allowed approximately 24 hours of post-operative recovery time. A pre-equilibrated microdialysis probe was inserted into the guide cannula, in the striatum (O.D. 0.5 mm, membrane length 4 mm; CMA/Microdialysis, Sweden) or nucleus accumbens (O.D. 0.5 mm, membrane length 2 mm; CMA/Microdialysis, Sweden) of the unrestrained rat, post surgery. The probe was perfused with artificial cerebrospinal fluid (aCSF; NaCl 125 mM, KCl 3.0 mM, MgSO\textsubscript{4} 0.75 mM, CaCl\textsubscript{2} 1.2 mM and 0.1 M phosphate buffer pH 7.4) at a flow rate of 1.0 µl/minute. A 3-hour stabilization period was allowed following probe implantation, after which time microdialysis sampling began in the awake, freely moving rat. Four baseline samples were taken prior to drug injection to achieve a steady baseline. These four samples were averaged and all subsequent values were expressed as a percentage of this preinjection value. WAY-163909 (3 and 10 mg/kg) or vehicle was administered in a single bolus dose via the sc cannula (N = 6-8 rats per group). A 20-minute sampling regime was used throughout the experimental period. At
the end of the experiment, probe placement was verified histologically and data from animals with incorrect probe placement were discarded. Dopamine was separated by reverse phase high performance liquid chromatography (HPLC) (C18 ODS2 column, 100 x 3.0 mm, Metachem, Torrance, CA) and detected using an ANTEC electrochemical detector (ANTEC, Netherlands) set at a potential of 0.7V vs. an Ag/AgCl reference electrode. Mobile phase was delivered by a Jasco PU980 HPLC pump (Jasco Ltd, Essex, UK) at 0.5 ml/minute and contained 0.15 M NaH$_2$PO$_4$ buffer at pH 4.3, 0.25 mM EDTA, 1.5 mM 1-octane sodium sulphonic acid and 5% isopropanol. All data were acquired using the Atlas software package (Thermo Labsystems, Gulph Mills, PA) for the PC. The fmol values of dopamine for the first four baseline samples were averaged and this value denoted as 100%. Subsequent sample values were expressed as a percentage of this preinjection control value. Results were analyzed by analysis of variance with repeated measures followed by pairwise comparisons using Bonferroni adjustment for multiple comparisons using the Statview software application (Abacus Concepts Inc., Berkeley, CA) for the PC.

Cortical Microdialysis Procedure: Rats were anesthetized with a combination (ip) of xylazine (13 mg/kg, Rompun; Shawnee Mission, KS) and ketamine hydrochloride (87 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IO) and mounted in a stereotaxic frame (Stoetling, Wood Dale, IL). Two stainless 21G guide cannula with a dummy probe were placed and fixed by cranioplastc cement (Plastic One, Roanoke, VA) onto the cortex dorsal to the mPFC. Stereotaxic coordinate of probe, when implanted, is A +3.2, L -0.8, V -5.5 mm (incline 80°), relative to bregma; incision bar level: -3.0 mm, according to the atlas (Paxinos and Watson, 1986). Three to five days following cannulation, a probe with a 2-mm dialyzing membrane was implanted into the mPFC under slight anesthesia with isoflurane (AErrane; Fort Dodge Animal Health, Fort
Dodge, IO). A catheter constructed from microbore Tygon tubing (TGY-010, 0.03 in. O.D.×0.01 in. I.D.; Small Parts Inc., Miami Lakes, FL) was also implanted subcutaneously in the intrascapular space of the rats, in order to avoid injection artifacts when drugs or vehicle were systemically administered to rats. After a 3-hour perfusion (1.5 µl/minute) of the probe, microdialysate samples (45 µl) were collected every 30 minutes in awake freely moving rats. The perfusion medium was Dulbecco’s phosphate buffered saline solution (Sigma, St. Louis, MO) including Ca²⁺ (138 mM NaCl, 8.1 mM Na₂HPO₄, 2.7 mM KCl, 1.5 mM KH₂PO₄, 0.5 mM MgCl₂, 1.2 mM CaCl₂, pH = 7.4). After stable baseline values in the dialysate samples were obtained, WAY-163909 was administered subcutaneously in a single bolus dose. The location of the microdialysis probes was verified at the end of each experiment with 100 µm brain slices (OTS-4000; FHC, Bowdoinham, ME). The procedure for the assessment of DA, which has been published, has been modified slightly. Immediately after collection, microdialysate samples (45 µl/30 min) were split (20 µl each) to assay both DA and ACh in the same sample, and directly applied onto the liquid chromatography/electrochemistry (LCEC) system with a 10 µl sample loop and analyzed for DA with a Millennium chromatogram manager (Waters, Milford, MA). DA was separated on a stainless steel, reversed phase column (Xterra RP-18, 3.5 µm C₁₈, 1.0×100 mm; Waters) at 36 °C maintained by column oven (831 Temperature Controller; Gilson, Middleton, WI) or by column heater (LC-22C Temperature Controller; BAS, West Lafayette, IN). The mobile phase consisted of 24 mM anhydrous citric acid and 48 mM sodium acetate trihydrate containing 0.5 mM EDTA-Na₂, 10 mM NaCl, 2 mM dodecyl sulfate sodium salt (Fluka, Ronkonkoma, NY) and 15% (v/v) acetonitrile, adjusted to pH 4.8 with concentrated NaOH, and was pumped at a flow rate of 0.05 ml/min by LC-10AD (Shimadzu, Kyoto, Japan). Dopamine was detected by a 2-mm glassy carbon unijet working electrode (MF-1017, BAS) set
at +0.54 V (LC-4C, BAS) vs. an Ag/AgCl reference electrode. A highly sensitive method was utilized to measure endogenous ACh in extracellular space in the absence of AChesterase inhibition. Dialysate samples (20 µl) were directly injected onto the LCEC system, assisted by a chromatography manager (Millennium; Waters). ACh was separated on a coiled cation-exchanger ACh column (analytical column) (Sepstik 10 µm I.D. 530×1.0 mm; BAS), followed by the post-IMER (immobilized enzyme reactor) (BAS) which consisted of choline oxidase (ChO)/AChesterase. ACh was hydrolyzed by AChesterase to form acetate and choline in the post IMER, and then choline was oxidized by ChO to produce betaine and hydrogen peroxide (H2O2). H2O2 was detected and reduced to H2O on a unijet amperometric detector cell with a peroxidase-redox-coated (MF-2098; BAS) glassy carbon electrode (MF-1003; BAS), set at +100 mV (LC-4C; BAS) vs. Ag/AgCl reference electrode. This reduction was analyzed with the detector (LC-4C; BAS) as signal indicating ACh in the chromatogram. A pre-IMER (BAS), which consisted of ChO and peroxidase, was added prior to the analytical column. Choline in the microdialysate samples was oxidized by ChO to form betaine and H2O2 by the pre-IMER. The H2O2 was then reduced by the peroxidase to form H2O before entering the analytical column. The mobile phase (Na2HPO4 50 mM, pH 8.2) including ProClin (BAS), a microbiocide, was pumped at 0.14 ml/min by a LC-10AD pump (Shimadzu). Reagents used were analytical or high-performance liquid chromatography (HPLC)-grade. Only results derived from healthy rats with correctly positioned dialysis probes were included in the data analysis. Mean pre-drug baseline levels (time -90, time -60, time -30 and time 0) were designated as 100%. Repeated measure ANOVA followed by Fisher’s protected least significant difference post-hoc pairwise comparison procedure and one-way ANOVA were used to determine group differences (StatView 4.5 for the
Macintosh). A \( p < 0.05 \) was considered significant in this study. All results are given as mean ± standard error (S.E.).
Results

Antagonism of Apomorphine-Induced Climbing and Stereotypy in Mice: Apomorphine induced consistent levels of climbing and stereotypy in the vehicle treated mice across the three studies [WAY-163909 study climbing = 9.33 ± 0.56 and stereotypy = 6.0 ± 0; clozapine study climbing = 9.80 ± 0.66 and stereotypy = 6.0 ± 0; haloperidol study climbing = 11.67 ± 0.21 and stereotypy = 6.0 ± 0]. WAY-163909 (1.7 - 30 mg/kg, ip) produced a dose dependent decrease in climbing behavior induced by apomorphine (Fig.1A) \([\text{ID}_{50} = 10.69 \text{ mg/kg; 95\% CI, 7.5 - 15.2; MED = 5.4 mg/kg}]\) at doses that had negligible effects on stereotypy \([\text{ID}_{50} > 30 \text{ mg/kg; MED = 10 mg/kg}]\). This profile is similar to that produced by the atypical antipsychotic clozapine (Fig.1B) \([\text{climbing ID}_{50} = 8.2 \text{ mg/kg; 95\% CI, 2.3 - 29.8; MED = 10 mg/kg}; \text{ stereotypy ID}_{50} = 55.83 \text{ mg/kg; 95\% CI, 41.2 - 75.7; MED = 30 mg/kg}]\) with a separation between the effective doses on climbing and the doses showing side effect liability measured with stereotypy. WAY-163909’s separation in doses affecting climbing and stereotypy contrasts with that of the typical antipsychotic haloperidol (Fig. 1C) \([\text{climbing ID}_{50} = 0.077 \text{ mg/kg; 95\% CI, 0.04 - 0.14; MED = 0.1 mg/kg}; \text{ stereotypy ID}_{50} = 0.11 \text{ mg/kg; 95\% CI, 0.1 - 0.2; MED = 0.1 mg/kg}]\) that had no separation between doses affecting climbing and stereotypy.

Cataleptogenic Potential in Mice: Peak catalepsy occurred at the 60 min test time for WAY-163909 and clozapine and at the 120 min test time for haloperidol. WAY-163909 (5.4 - 30 mg/kg, ip) produced negligible catalepsy up to 30 mg/kg (Fig. 2A) \([F(4,28) = 2.363; p > 0.05]\). The atypical antipsychotic clozapine (Fig. 2B) \([F(4,29) = 3.904; p < 0.05]\), only showed catalepsy at 60 mg/kg. The profiles for WAY-163909 and clozapine were vastly different from haloperidol (Fig. 2C) \([F(4,29) = 13.32; p < 0.05]\), which displayed nearly full catalepsy at 1 mg/kg. While haloperidol reached maximal catalepsy effect at doses 10-fold higher than the
MED for block of apomorphine-induced climbing, WAY-163909 and clozapine did not reach maximal catalepsy at doses up to 6-fold the MED for block of climbing. Higher doses of WAY-163909 and clozapine were not tested due to sedation.

**Locomotor Activity:** In these experiments, PCP typically increased locomotor activity ~206% while amphetamine increased locomotor activity ~288%. WAY-163909 (0.1 - 3 mg/kg, sc) (Fig. 3A) was more potent in reducing PCP stimulated locomotor activity, MED = 0.3 mg/kg [F(4,49) = 4.134, p < 0.05], compared to d-amphetamine stimulated locomotor activity [F(3,39) = 3.622, p < 0.05; MED = 3 mg/kg] while having no effect on spontaneous locomotor activity [F(3,39) = 1.298, p > 0.05]. Clozapine (0.03 - 1 mg/kg, sc) (Fig. 3B) was also more potent in reducing PCP stimulated locomotor activity, MED = 0.1 mg/kg [F(5,59) = 5.563, p < 0.05], compared to d-amphetamine stimulated locomotor activity [F(5,59) = 4.544, p < 0.05; MED = 1 mg/kg] while having no effect on spontaneous locomotor activity [F(4,49) = 0.482, p > 0.05]. Haloperidol (0.01 - 0.3 mg/kg, sc) (Fig. 3C), on the other hand, was more potent in reducing amphetamine stimulated locomotor activity, MED = 0.03 mg/kg [F(4,49) = 25.402, p < 0.05], compared to phencyclidine stimulated locomotor activity [F(4,49) = 6.102, p < 0.05; MED = 0.1 mg/kg] while spontaneous locomotor activity was decreased at an MED = 0.1 mg/kg [F(4,49) = 10.668, p < 0.05]; thus haloperidol only blocked PCP stimulated locomotor activity at doses which affected spontaneous motor activity.

**Prepulse Inhibition of Startle Response:**

**Antagonism of Pharmacologically Induced Disruption of PPI:** A repeated measures analysis of variance showed no significant prepulse by treatment interaction. So the PPI values for the three-decibel levels were averaged to obtain a single PPI score in order to simplify the graphical presentation of the drug effects. MK-801 (0.15 mg/kg) produced a 77% decrease in PPI and
WAY-163909 (1.7 - 17 mg/kg, ip) dose dependently reversed the MK-801 induced disruption of PPI (Fig. 4A; open circles) \([F(5,63) = 48.615, p < 0.05]\) with a MED = 5.4 mg/kg of WAY-163909. MK-801 also elevated the basal startle response to the pulse alone trial and WAY-163909 also normalized the startle response (Table 1) \([F(5,63) = 12.572, p < 0.05]\). DOI (3 mg/kg) produced a 47% decrease in PPI and WAY-163909 reversed the DOI induced disruption of PPI (Fig. 4A; filled diamonds) \([F(5,63) = 7.451, p < 0.05]\) with an MED = 5.4 mg/kg. WAY-163909 in conjunction with DOI decreased the baseline startle response (Table 1) \([F(5,63) = 3.414, p < 0.05]\) in the DOI experiment. d-Amphetamine (4 mg/kg) resulted in a 29% decrease in PPI, which WAY-163909 was ineffective at reversing and, there was an enhancement of the deficit at 17 mg/kg (Fig. 4A; filled squares) \([F(5,63) = 11.097, p < 0.05]\). WAY-163909 did normalize the baseline startle response (Table 1) \([F(5,63) = 6.841, p < 0.05]\), which was elevated by d-amphetamine. All PPI and baseline startle data for clozapine and haloperidol are listed in Table 2. Clozapine (3 - 10 mg/kg, ip) only reversed the MK-801 induced disruption of PPI \([F(4,39) = 11.777, p < 0.05]\) at the 5.4 mg/kg dose. Clozapine did normalize the MK-801 elevated baseline startle response \([F(4,39) = 8.730, p < 0.05]\) at all doses tested. Clozapine, at 5.4 and 10 mg/kg, also reversed the DOI induced disruption of PPI \([F(4,38) = 2.961, p < 0.05]\). Neither DOI nor clozapine had an effect on the baseline startle response \([F(4,38) = 1.095, p > 0.05]\). With the d-amphetamine induced disruption, clozapine reversed the deficit at the 10 mg/kg dose \([F(4,39) = 6.812, p < 0.05]\). Neither d-amphetamine nor clozapine altered the baseline startle response \([F(4,39) = 1.125, p > 0.05]\). Haloperidol (0.3 - 3 mg/kg, ip) failed to reverse the MK-801 induced disruption of PPI \([F(4,39) = 18.690, p < 0.05]\), but haloperidol did normalize the MK-801 elevated baseline startle response \([F(4,39) = 6.566, p < 0.05]\) at the two lowest doses tested. Haloperidol also failed to reverse the DOI induced disruption of PPI.
[F(4,39) = 2.961, p < 0.05] and neither DOI nor haloperidol had an effect on the baseline startle response [F(4,39) = 0.823, p > 0.05]. With the d-amphetamine induced disruption, haloperidol reversed the deficit at all doses tested [F(4,39) = 4.268, p < 0.05] and haloperidol also normalized the d-amphetamine increase in the baseline startle response at all doses tested [F(4,39) = 4.479, p < 0.05].

**PPI in DBA mice:** A repeated measures analysis of variance showed no significant prepulse by treatment interaction. So the PPI values for the three decibel levels were averaged to obtain a single PPI score in order to simplify the graphical presentation of the drug effects. WAY-163909 (10 - 30 mg/kg, ip) improved PPI in DBA mice (Fig. 4B) [F(3,77) = 7.019, p < 0.05; MED = 30 mg/kg] with no effect on baseline startle (Table 1) [F(3,38) = 1.984, p > 0.05]. Clozapine and haloperidol data in DBA mice is listed in Table 2. Clozapine failed to improve PPI [F(3,39) = 0.41, p > 0.05] and the 5 mg/kg dose significantly decreased baseline startle [F(3,39) = 4.493, p < 0.05]. Haloperidol did improve PPI [F(3,38) = 5.75, p < 0.05] with an MED < 1 mg/kg, while only decreasing the baseline startle at the 5 mg/kg dose [F(3,38) = 6.335, p < 0.05].

**Conditioned Avoidance Responding in Rats:** WAY-163909 (0.3 - 3 mg/kg, ip; 1.0 - 17 mg/kg, po) produced dose dependent decreases in avoidance responding in rats (Fig. 5A) [F(4,39) = 68.99, p < 0.05; ED50 = 1.27[1.1 - 1.5] mg/kg ip; F(5,47) = 21.02, p < 0.05; ED50 = 6.50[4.7 - 9.1] mg/kg po] at doses that had little or no effect on the number of trials in which escape failures occurred. The block of conditioned avoidance responding by WAY-163909 was challenged by pretreatment with the 5-HT2B/2C antagonist SB 206553 (10 mg/kg, po) resulting in an increase in the ED50 for WAY-163909 in the presence of SB 206553 to 3.4 mg/kg ip (Fig.5B)
[CI, 2.9 - 4.1 mg/kg] from 1.28 mg/kg ip [CI, 1.0 - 1.6 mg/kg]. In a separate study, there was a significant effect of pretreatment time \[F(4,28) = 15.3, p < 0.05\] with 3 mg/kg ip of WAY-163909 producing a 47% reduction in avoidance responses following a 30 minute pretreatment interval (\(p < 0.05\)) while avoidances were reduced by 16%, 29% and 0% at the 1 hr, 2 hr and 24 hr pretreatment times, respectively. Additionally, 1.7 and 3.0 mg/kg of WAY-163909 were administered via subcutaneous route and evaluated 30 minutes post-injection and produced a 31% and 34% block of avoidance responding, respectively \[F(2,14) = 11.6, p < 0.05\].

**In Vivo Electrophysiology:** Statistical analysis indicated that there was a significant treatment effect on the number of spontaneously active VTA DA neurons (Table 3) \[F(4,45) = 31.4, p < 0.05\]. Subsequent post hoc analysis indicated that a single ip administration of 1, 3 or 10 mg/kg of WAY-163909 significantly decreased the number of spontaneously active VTA DA neurons. In contrast, the acute ip administration of 20 mg/kg of clozapine significantly increased the number of spontaneously active VTA DA neurons. In the SNC, the acute ip administration of WAY-163909 (1 - 10 mg/kg) or 20 mg/kg of clozapine did not significantly alter the number of spontaneously active DA neurons compared to vehicle-treated animals (Table 3) \[F(4,45) = 0.37, p > 0.05\].

Following the repeated (21-day) ip administration of WAY-163909, statistical analysis revealed a treatment effect on the number of spontaneously active VTA DA neurons (Table 4) \[F(4,45) = 36.4, p < 0.01\]. Subsequent post hoc analysis indicated that the chronic administration of clozapine or 1, 3 or 10 mg/kg of WAY-163909 significantly decreased the number of spontaneously active VTA DA neurons compared to vehicle treated animals. In addition, the decrease in the number of spontaneously active VTA DA neurons produced by the 10 mg/kg dose of WAY-163909 and 20 mg/kg of clozapine were significantly greater than that
for the 1 and 3 mg/kg of WAY-163909. As with acute treatment, neither clozapine nor WAY-163909 significantly altered the number of spontaneously active SNC DA neurons compared to vehicle treated animals (Table 4) [F(4,45) = 1.42, p > 0.05] following 21 days of treatment.

In Vivo Microdialysis:

Nucleus Accumbens and Striatal Microdialysis: The basal dopamine level, reported as mean ± standard error of the mean fmol/10 µl microdialysate, in the nucleus accumbens was 33.8 ± 2.0 and the striatum was 38.2 ± 0.8. WAY-163909 (10 mg/kg, sc) produced a significant decrease in nucleus accumbens DA [F (1,10) = 10.66, p < 0.05] reaching a maximum of 48% ± 9.2% of preinjection baseline levels (Fig. 6A). In contrast, WAY-163909 (10 mg/kg, sc) produced no change in extracellular levels of DA in the striatum (Fig. 6B).

Cortical Microdialysis: Basal extracellular Ach levels in the dialysates obtained from rats used in this study were 7.47 ± 0.41 fmol/10 µl, reported as mean ± standard error of the mean. The basal dopamine level, reported as mean ± standard error of the mean fmol/10 µl microdialysate, was 1.2 ± 0.2. WAY-163909 (3 and 10 mg/kg sc) produced an increase in microdialysate ACh concentrations in the mPFC with significant effects at both doses [F (1,9) = 10.21, p < 0.01; F (1,11) = 6.22, p < 0.5 respectively], compared to vehicle controls (Fig. 6C). The high dose of WAY-163909 (10 mg/kg) produced a small, but significant increase in microdialysate DA concentrations in the mPFC [F (1,10) = 9.04, p < 0.05] compared to vehicle controls (Fig. 6D).
Discussion

Blockade of dopaminergic neurotransmission in the nucleus accumbens via D₂ receptor antagonism or partial agonism is considered the primary mechanism underlying antipsychotic efficacy for the positive symptoms (i.e., hallucinations, delusions and thought disorder) of schizophrenia. An alternate approach to blocking dopamine D₂ receptors may be to reduce the activity of the mesolimbic pathway. Ideally, the nigrostriatal pathway would not be affected, thus avoiding potential extrapyramidal side effect liabilities. To this end, 5-HT₂C receptor agonists have been shown to decrease dopamine levels in the nucleus accumbens and the firing rate of DA neurons in the VTA (Millan et al., 1998; Di Giovanni et al., 2000). Therefore, the present studies were conducted to evaluate the effects of WAY-163909 in animal models predictive of antipsychotic activity and to compare the antipsychotic efficacy of a 5-HT₂C receptor agonist with both typical and atypical antipsychotics.

WAY-163909 was evaluated in the conditioned avoidance model, a standard screening model for antipsychotic efficacy (Arnt, 1982). WAY-163909, administered intraperitoneally, subcutaneously or orally, dose-dependently decreased avoidance responding, without increasing escape failures; these effects are similar to those observed with the typical antipsychotic haloperidol and the atypical antipsychotic clozapine. A time course study indicated the effect of WAY-163909 was greatest at 30 min following intraperitoneal dosing. The 5-HT₂B/₂C receptor antagonist SB 206553 produced a rightward shift of the effect of WAY-163909 on avoidance responding, indicative of 5-HT₂C receptor mediation.

Additional data suggest an atypical antipsychotic-like profile for WAY-163909. WAY-163909 selectively reduced apomorphine-induced climbing behavior, with a negligible effect on apomorphine-induced stereotypy. Dopamine activity in the mesolimbic pathway may mediate...
climbing behavior (Costall et al., 1980), while stereotypy correlates with dopamine activity of the nigrostriatal pathway and extrapyramidal side effect liability (Costall et al., 1975). Consistent with this idea, haloperidol, a typical antipsychotic, blocked both apomorphine-induced climbing and stereotypy over a similar dose range whereas clozapine, an atypical antipsychotic, more potently decreased apomorphine-induced climbing relative to stereotypy. Parkinsonian-like extrapyramidal side effects can also be predicted by evaluating the potential for a compound to induce catalepsy in rodents (Hoffman and Donovan, 1995). WAY-163909 did not induce catalepsy in the dose range producing significant reductions in apomorphine-induced climbing. These results suggest mesolimbic selectivity and an atypical antipsychotic-like profile for WAY-163909.

The behavioral results suggesting mesolimbic selectivity are supported by both electrophysiological and neurochemical data. Repeated administration of atypical antipsychotics results in a depolarization induced blockade of neuronal firing in the VTA and thus, a decrease in the number of spontaneously active DA neurons; this phenomenon is proposed to underlie the delayed onset to full efficacy with current antipsychotic drugs (Grace et al., 1997). Acute and repeated (21-day) administration of WAY-163909 selectively decreased the number of spontaneously active dopamine neurons in the ventral tegmental area (VTA; A10) without affecting the number of spontaneously active dopamine neurons in the substantia nigra pars compacta (SNC; A9). The decrease in the number of spontaneously active VTA DA neurons after acute administration of WAY-163909 contrasts with the increase produced by typical and atypical antipsychotics, making WAY-163909’s acute effects unique amongst antipsychotic agents. Consistent with the electrophysiology results, WAY-163909 decreased levels of dopamine in the nucleus accumbens (the projection region of the VTA neurons) at a dose that did
not affect striatal DA (the projection region of the SNC). The electrophysiological and neurochemical data are consistent with reports that 5-HT$_{2C}$ receptor agonists are mesolimbic selective (Millan et al., 1998). To the extent that control of mesolimbic dopamine neurotransmission contributes to the control of positive symptoms of schizophrenia, the rapid onset of the electrophysiological effects, coupled with the lack of tolerance to the effects of WAY-163909 suggests that 5-HT$_{2C}$ receptor agonists may show rapid onset of atypical antipsychotic-like action.

The mechanism for the selective decrease in mesolimbic dopaminergic transmission relative to the nigrostriatal pathway remains to be elucidated and could be due to differences in 5-HT$_{2C}$ receptors within the VTA and SNC or to differences in other portions of the dopaminergic pathway. Although mRNA levels for 5-HT$_{2C}$ receptors in the VTA and SNC (Pasqualetti et al., 1999) appear similar, it is possible that differential levels of protein expression for 5-HT$_{2C}$ receptors in these regions which could contribute to the mesolimbic selectivity. The 5-HT$_{2C}$ receptors localized in the VTA have been shown to regulate mesocortical DA release (Pozzi et al., 2002). The 5-HT$_{2C}$ receptors in the DA projection terminal regions, the medial prefrontal cortex, nucleus accumbens and striatum (Pompeiano et al., 1994; Abramowski et al., 1995; Pasqualetti et al., 1999), may selectively affect dopamine levels within these regions by modulating projections from the VTA and SNC to these regions or by feedback from these regions to the VTA and SNC. Research has shown that 5-HT$_{2C}$ receptors localized in the dorsal striatum directly influence dopamine levels within the region (Alex et al., 2005). Alternatively, the selective functionality between the VTA and SNC could also be due to effects on GABAergic systems. 5-HT$_{2C}$ mRNA is expressed by GABAergic but not by dopamine neurons in the VTA and SNC (Eberle-Wang et al., 1997) and a role of 5-HT$_{2C}$ receptors in regulation of
impulse-mediated GABA levels in the VTA has been demonstrated (Bankson and Yamamoto, 2004). To the extent that differential effects on the GABAergic system differentially affect the dopaminergic system, these mechanisms may be involved. Clearly there are multiple possible mechanisms underlying the selective effect of 5-HT$_{2C}$ receptors in the VTA and SNC that remain to be fully explored.

In addition to the dopamine hypothesis of schizophrenia, considerable evidence supports a role for reduced glutamatergic neurotransmission in this disease (Coyle et al., 2003; Lewis et al., 2003). In preclinical studies, atypical antipsychotics have been shown to be more potent in normalizing the hyperactivity induced by NMDA antagonists in rodents (Gleason and Shannon, 1997) and to reverse the effects of NMDA antagonists on other measures known to be abnormal in schizophrenics, such as prepulse inhibition of the startle response, a measure of auditory sensorimotor gating (Geyer et al., 2001). As with clozapine, but not haloperidol, WAY-163909 attenuated NMDA antagonist mediated effects (locomotor activity and PPI deficit) at much lower doses than were needed to attenuate amphetamine LMA. Inexplicably, amphetamine PPI was not normalized by WAY-163909 despite the reversal of the amphetamine induced increase in the basal startle response and the other antidopaminergic effects of WAY-163909. WAY-163909 also dose dependently improved prepulse inhibition in the DBA2 strain of mouse, a mouse strain reported to be sensitive to antipsychotic-induced enhancement in prepulse inhibition (Browman et al., 2004). Together these data suggest that 5-HT$_{2C}$ receptor agonists have unique effects, relative to other atypical antipsychotics, on multiple neurotransmitter systems that are altered in schizophrenia.

Treating the cognitive deficits and negative symptoms of schizophrenia remains an area of unmet medical need. Prefrontal cortical function is thought to be abnormal in schizophrenia.
and that these abnormalities underlie these symptom domains (Goldman-Rakic and Selemon, 1997). Atypical antipsychotics, reported to improve cognitive and negative symptoms in schizophrenia, have been shown to increase dopamine and acetylcholine neurotransmitter levels in the medial prefrontal cortex of freely moving rats (Ichikawa et al., 2002). Like atypical antipsychotics, WAY-163909 increased both acetylcholine and dopamine levels in the medial prefrontal cortex thereby suggesting potential for WAY-163909 to affect cholinergic and dopaminergic neurotransmission in this brain region. The effect of WAY-163909 on cognitive measures related to prefrontal cortical function, as well as an examination of its effects on other neurotransmitters, such as glutamate, are a subject of further investigation.

Although preclinical data supports a role for 5-HT$_{2C}$ receptor agonists in the treatment of schizophrenia, challenge studies using a single intravenous or oral dose of mCPP in schizophrenic patients have resulted in variable outcomes from exacerbation to no effect to alleviation of psychotic symptoms (Iqbal et al., 1991; Kahn et al., 1992; Krystal et al., 1993; Maes and Meltzer, 1996; Koreen et al., 1997). In light of multiple receptor activities of mCPP and the anxiogenic effects of acute mCPP, it is difficult to understand the implications of these studies for the effects of 5-HT$_{2C}$ receptor agonists in schizophrenia. Longer trials involving multiple doses of a selective 5-HT$_{2C}$ receptor agonist will be needed to fully understand the therapeutic potential of this class of compounds in schizophrenia.

In summary, WAY-163909 produces behavioral, neurochemical and electrophysiological effects that are consistent with mesolimbic dopamine selectivity and atypical antipsychotic-like activity. Neurochemical and PPI data suggest that WAY-163909 may effectively treat the cognitive aspects or negative symptoms of schizophrenia. Additionally, WAY-163909 is effective in animal models of depression (Rosenzweig-Lipson et al., 2004), suggesting possible
effects on the mood disorders associated with schizophrenia. Moreover, since WAY-163909 decreases both food intake and body weight at high doses (Dunlop et al., 2005), it is unlikely to produce the weight gain or diabetogenesis commonly associated with atypical antipsychotics. Taken together, these results suggest that 5-HT$_{2C}$ receptor agonists may be effective antipsychotics and could improve the mood disorders and cognitive impairments associated with schizophrenia without producing extrapyramidal side effects, weight gain or diabetogenesis.
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References


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Ichikawa J, Dai J, O'Laughlin IA, Fowler WL and Meltzer HY (2002) Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. *Neuropsychopharmacology* 26:325-339.


Legends for Figures

Figure 1. WAY-163909, clozapine, and haloperidol evaluated in apomorphine-induced climbing and stereotypy assay in CF-1 mice. WAY-163909 (A), clozapine (B) and haloperidol (C) were administered ip 30 minutes prior to administration of apomorphine (1 mg/kg, sc). Data are expressed as percent of climbing (■) and stereotypy (○) demonstrated by the vehicle treated group and represent the mean percent of control ± S.E.M. (n = 6 mice per group). *Significantly different from vehicle treated mice (p < 0.05).

Figure 2. Catalepsy in CF-1 mice induced by WAY-163909, clozapine and haloperidol. WAY-163909 (A), clozapine (B) and haloperidol (C) were administered ip and then time spent on a horizontal bar was measured at 30, 60, 90 and 120 min post dose. Data are expressed as the time in seconds spent on the bar at the point of peak catalepsy (60 min for WAY-163909 and clozapine; 120 min for haloperidol) and represent mean seconds on bar ± S.E.M. (n = 6 mice per group). *Significant catalepsy (p < 0.05).

Figure 3. WAY-163909, clozapine, and haloperidol evaluated in locomotor activity in CF-1 mice. WAY-163909 (A), clozapine (B) and haloperidol (C) were administered sc concurrently with PCP (3 mg/kg; ○), amphetamine (3 mg/kg; ■) or vehicle (spontaneous; ▲) and then locomotor activity was assessed for 70 minutes. The activity counts for the last 40 minutes of the test period were analyzed and represent mean activity count ± S.E.M. (n = 10 mice per group). *Significant decrease relative to vehicle treated group for each condition (p < 0.05).
Figure 4. Effect of WAY-163909 on pharmacologically disrupted PPI in Sprague Dawley rats and low PPI in DBA/2N mice. (A) In rats, WAY-163909 was administered ip 30 minutes before testing while the disrupting agent, MK-801 (0.15 mg/kg, ip, ○), DOI (3 mg/kg, sc, ♦) or d-amphetamine (4 mg/kg, sc, ■) were administered 10-15 minutes before testing. *Significantly increased PPI relative to Vehicle-Disrupting agent group (p < 0.05); #Significantly decreased PPI relative to Vehicle-Amphetamine group. (B) In mice, WAY-163909 was administered ip 30 minutes before testing. *Significantly increased PPI relative to Vehicle treated mice (p < 0.05). Data are expressed as average percent of prepulse inhibition and represent mean ± S.E.M. (n = 8-10 subjects per group).

Figure 5. Effect of WAY-163909 on conditioned avoidance response in Sprague Dawley rats and a challenge by the 5-HT\textsubscript{2B/2C} antagonist, SB 206553. A) WAY-163909 was administered ip (■) or po (▼), 30 minutes before testing. Data are expressed as average avoidance responses (filled symbols) and average number of escape failures (open symbols) made over 50 trials and represent mean ± S.E.M. (n = 8 rats per group). B) Vehicle (■) or SB 206553 (●) was administered po 30 minutes prior to WAY-163909 which was administered ip 30 minutes before testing. Data are expressed as average avoidance responses made over 50 trials and represent mean ± S.E.M. (n = 8 rats per group).

Figure 6. Effect of WAY-163909 on dopamine and acetylcholine levels in awake, freely moving Sprague Dawley rats. WAY-163909 was administered in a single bolus via sc cannulae at T = 0 min (marked by arrow). Data are expressed as percent of baseline levels and represent mean ± S.E.M. (n = 6 - 8 animals per group). In nucleus accumbens (A), WAY-163909 (10 mg/kg; □)
significantly decreased dopamine levels at 120 minutes post-dose relative to vehicle treatment (●) while having no effect in striatum (B). In frontal cortex, WAY-163909 (■, 3 mg/kg; □, 10 mg/kg) produced an increase in acetylcholine at 30 and 60 minutes post-dose (C) relative to vehicle treatment (●) while only the 10 mg/kg dose of WAY-163909 significantly increased dopamine levels (D) at 60 minutes post-dose.
Table 1. WAY-163909 effects on the basal startle response following treatment with MK-801, DOI and Amphetamine in Sprague-Dawley rats and on the basal startle response in DBA/2N mice. Data are expressed as mean startle to the pulse alone. Values in parentheses are the standard error of the mean (n = 10 subjects per group). *Significantly different from disrupting agent.

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Table 2. Clozapine & haloperidol effects on the basal startle response and prepulse inhibition of startle disrupted by MK-801, DOI and Amphetamine in Sprague-Dawley rats and the naturally low PPI in DBA/2 mice. Data are expressed as the mean basal startle and the mean percent of prepulse inhibition. The values in parentheses are the standard error of the mean (n = 10 subjects per group). *Significantly different from disrupting agent in rats and vehicle treatment in DBA/2 mice.

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</tr>
<tr>
<td>Veh/Veh</td>
<td>70.8(6.9)</td>
<td>72.5(4.5)*</td>
</tr>
<tr>
<td>Veh/DOI</td>
<td>71.7(24.7)</td>
<td>56.3(3.8)</td>
</tr>
<tr>
<td>3 cloz/DOI</td>
<td>82.8(15.1)</td>
<td>59.5(2.1)</td>
</tr>
<tr>
<td>5.4 cloz/DOI</td>
<td>47.0(6.9)</td>
<td>68.3(5.0)*</td>
</tr>
<tr>
<td>10 cloz/DOI</td>
<td>53.8(12.3)</td>
<td>68.8(3.6)*</td>
</tr>
<tr>
<td><strong>AMPH</strong> (4 mg/kg, sc)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh/Veh</td>
<td>78.4(7.3)</td>
<td>78.2(2.9)*</td>
</tr>
<tr>
<td>Veh/AMPH</td>
<td>133.9(23.2)</td>
<td>55.3(4.3)</td>
</tr>
<tr>
<td>3 cloz/AMPH</td>
<td>145.8(36.9)</td>
<td>57.3(5.0)</td>
</tr>
<tr>
<td>5.4 cloz/AMPH</td>
<td>110.5(13.1)</td>
<td>50.3(5.1)</td>
</tr>
<tr>
<td>10 cloz/AMPH</td>
<td>118.6(28.6)</td>
<td>68.5(4.0)*</td>
</tr>
<tr>
<td><strong>DBA/2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>55.5(7.3)</td>
<td>14.3(7.1)</td>
</tr>
<tr>
<td>0.5 cloz</td>
<td>60.0(10.5)</td>
<td>12.1(7.2)</td>
</tr>
<tr>
<td>1 cloz</td>
<td>70.4(13.9)</td>
<td>6.2(7.6)</td>
</tr>
<tr>
<td>5 cloz</td>
<td>21.7(7.0)*</td>
<td>5.3(5.4)</td>
</tr>
</tbody>
</table>

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Table 3. The effect of a single injection of vehicle, WAY-163909 and clozapine on the number of spontaneously active SNC and VTA DA neurons in anesthetized male Sprague-Dawley rats. Data are expressed as the average number of spontaneously active DA neurons detected per stereotaxic descent ± S.E.M. (n = 10 subjects per treatment). *Significantly greater than Vehicle and all doses of WAY-163909 (p < 0.01), #Significantly less than vehicle (p < 0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, ip)</th>
<th>SNC</th>
<th>VTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>0.75 ± 0.07</td>
<td>1.16 ± 0.07</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>1</td>
<td>0.70 ± 0.06</td>
<td>0.97 ± 0.05#</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>3</td>
<td>0.70 ± 0.05</td>
<td>0.84 ± 0.05#</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>10</td>
<td>0.68 ± 0.06</td>
<td>0.71 ± 0.05#</td>
</tr>
<tr>
<td>Clozapine</td>
<td>20</td>
<td>0.74 ± 0.08</td>
<td>1.55 ± 0.07*</td>
</tr>
</tbody>
</table>
Table 4. The effect of the repeated administration of vehicle, WAY-163909 and clozapine on the number of spontaneously active SNC and VTA DA neurons in anesthetized male Sprague-Dawley rats. Data are expressed as the average number of spontaneously active DA neurons detected per stereotaxic descent ± S.E.M. (n = 10 subjects per treatment). #Significantly less than Vehicle (p < 0.05), *Significantly greater than the 10 mg/kg dose of WAY-163909 and clozapine (p < 0.01)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, ip)</th>
<th>SNC</th>
<th>VTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>0.71 ± 0.05</td>
<td>1.19 ± 0.07</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>1</td>
<td>0.66 ± 0.05</td>
<td>0.90 ± 0.04#*</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>3</td>
<td>0.60 ± 0.06</td>
<td>0.69 ± 0.05#</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>10</td>
<td>0.61 ± 0.06</td>
<td>0.41 ± 0.05#</td>
</tr>
<tr>
<td>Clozapine</td>
<td>20</td>
<td>0.76 ± 0.06</td>
<td>0.48 ± 0.05#</td>
</tr>
</tbody>
</table>
Figure 2

A

Time on Bar (sec)

WAY-163909 (mg/kg, ip)

B

Time on Bar (sec)

clozapine (mg/kg, ip)

C

Time on Bar (sec)

haloperidol (mg/kg, ip)
Figure 3

A

Activity Count (x 100)

180
120
60
0

Veh
WAY-163909 (mg/kg, ip)
0.1
1
10

B

Activity Count (x 100)

180
120
60
0

Veh
clozapine (mg/kg, ip)
0.1
1
10

PCP
Amphetamine
Spontaneous

C

Activity Count (x 100)

180
120
60
0

Veh
haloperidol (mg/kg, ip)
0.01
0.1
1

PCP
Amphetamine
Spontaneous
Figure 4

A

Percent PPI

Veh Disrupt

B

Percent PPI

Veh WAY-163909 (mg/kg, ip)
Figure 6