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-Hydroxytryptamine 2C Receptor-Selective Agonist with Preclinical Antipsychotic-Like Activity


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 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole], was evaluated in animal models of schizophrenia and the treatment of obesity, depression, and schizophrenia: lack of tolerance. The wide distributions of 5-HT_2C receptors and mRNA in brain (Pompeiano et al., 1994; Saltzman et al., 1991). The three 5-HT_2 receptors (5-HT_2A, 5-HT_2B, and 5-HT_2C) display high sequence homology and are Gq-linked receptors that signal primarily through the inositol 1,4,5-trisphosphate pathway and activation of phospholipase C (Conn and Sanders-Bush, 1987; Saltzman et al., 1991). The wide distributions of 5-HT_2C receptors and mRNA in brain (Pompeiano et al., 1994; Abramowski et al., 1995), coupled with pharmacological studies, have suggested a role for 5-HT_2C receptors in mediating a broad range of effects of 5-HT in the central nervous system. Studies have implicated the 5-HT_2C receptor in schizophrenia, depression, obsessive-compulsive disorder, anxiety, and obesity (Bos et al., 1997; Cryan and Lucki, 2000;


The serotonin receptor family is currently composed of 14 subtypes (Hoyer et al., 2002). The three 5-HT_2 receptors (5-HT_2A, 5-HT_2B, and 5-HT_2C) display high sequence homology and are Gq-linked receptors that signal primarily through the inositol 1,4,5-trisphosphate pathway and activation of phospholipase C (Conn and Sanders-Bush, 1987; Saltzman et al., 1991). The wide distributions of 5-HT_2C receptors and mRNA in brain (Pompeiano et al., 1994; Abramowski et al., 1995), coupled with pharmacological studies, have suggested a role for 5-HT_2C receptors in mediating a broad range of effects of 5-HT in the central nervous system. Studies have implicated the 5-HT_2C receptor in schizophrenia, depression, obsessive-compulsive disorder, anxiety, and obesity (Bos et al., 1997; Cryan and Lucki, 2000;

ABBRVIEVATIONS: 5-HT, 5-hydroxytryptamine, serotonin; VTA, ventral tegmental area; SNC, substantia nigra pars compacta; WAY-163909, [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]; DOI, [(2,5-dimethoxy-4-iodophenyl)-2-aminopropane]-dizocilpine maleate]; SB206553, 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydroxyprolo[2,3-f]indole]; WAY-163909 (10 mg/kg s.c.) selectively decreased extracellular levels of DA in the nucleus accumbens without affecting the striatum. Likewise, 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 i.p.). Thus, the profile of the 5-HT_2C selective receptor agonist WAY-163909 is similar to that of an atypical antipsychotic and additionally may have rapid onset properties.

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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.

In studies with 5-HT2C antagonists, increased synaptic levels of dopamine have been reported (Di Matteo et al., 1998), suggesting that 5-HT2C agonists should decrease dopamine neurotransmission. Because all current antipsychotic medications reduce dopamine neurotransmission via the blockade of postsynaptic dopamine receptors, mechanisms such as 5-HT2C agonism that reduce dopamine neurotransmission without stimulating postsynaptic dopamine receptors could have antipsychotic potential. Indeed, recent studies have demonstrated that 5-HT2C 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 the 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-HT2C receptor agonists decrease firing in the ventral tegmental area (VTA), but not in the substantia nigra pars compacta (SNC) (Di Giovanni et al., 2000). The selective effects of 5-HT2C receptor agonists on the mesolimbic dopamine pathway suggest that 5-HT2C receptor agonists should have antipsychotic efficacy without the extrapyramidal side effects associated with typical antipsychotics.

Many atypical antipsychotics bind with high affinity to 5-HT2C receptors and function as 5-HT2C receptor antagonists or inverse agonists (Leysen, 2004). The antagonism of 5-HT2C 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-HT2C receptor is known to result in decreased food intake and body weight (Cowan et al., 1995; Dunlop et al., 2005; Rosenzweig-Lipson et al., 2006). As a result, 5-HT2C receptor agonists may be less likely to produce an increase in body weight.

Taken together, these studies suggest the potential for 5-HT2C receptor agonists to possess antipsychotic-like neurochemical, electrophysiological, and behavioral effects. Coupled with the potential to treat mood disorder symptoms and the reduced risk for extrapyramidal side effects, weight gain, or diabetogenesis, 5-HT2A-selective receptor agonists represent a novel treatment approach for schizophrenia. To date, WAY-163909 may be the most selective 5-HT2C agonist identified compared with other compounds reported in the literature (Dunlop et al., 2005), providing distinct advantages over other 5-HT2C agonists with respect to both binding affinity (\(K_i = 11 \text{nM}\)) and selectivity (20- and 46-fold, respectively, for 5-HT2A and 5-HT2B), functional selectivity (\(E_{50} = 8 \text{nM}; E_{\text{max}} = 90\%\)), and decreased intrinsic activity at the 5-HT2A (no agonist activity) and 5-HT2B (\(E_{\text{max}} = 40\%\)) receptors. Thus, the current study was conducted to explore the effects of WAY-163909 in several animal models predictive of antipsychotic efficacy.

**Materials and Methods**

**Subjects**

Male CF-1 mice (20–28 g; Charles River Laboratories, Inc., Wilmington, MA) were used in the antagonism of apomorphine-induced behaviors, catecholaminergic potential, and locomotor activity assays. Male DBA2/N 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 the conditioned avoidance test (350–450 g; Charles River Laboratories, Inc.), prepulse inhibition of startle test (250–350 g; Charles River Laboratories, Inc.), in vivo electrophysiology experiments (150–175 g upon arrival; Taconic Farms), in vivo microdialysis experiments in nucleus accumbens and striatum (250–350 g; Charles River Laboratories, Inc.), 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 test subjects) in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility that was maintained on a 12-h light/dark cycle (lights on at 6:00 AM). 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 with the Principles of Laboratory Animal Care as adopted and promulgated by the National Institutes of Health (publication 85-23, 1985).

**Drugs**

WAY-163909 was prepared by Wyeth Research (Princeton, NJ). Haloperidol, apomorphine, d-amphetamine, DOI, MK-801, phencyclidine, Tween 80, hydroxypropyl-β-cyclodextrin, and methylcellulose were obtained from Sigma-Aldrich (St. Louis, MO). Clozapine was purchased from Research Biochemicals (Natick, MA). Drugs were dissolved in saline (WAY-163909, haloperidol, apomorphine, d-amphetamine, DOI, MK-801, and phencyclidine), 2% Tween 80–0.5% hydroxypropyl-β-cyclodextrin, and methylcellulose were obtained from Sigma-Aldrich (St. Louis, MO) as received saline at equal volumes. Thirty minutes later, experimental and control animals were challenged with 1 mg/kg s.c. apomorphine. Five minutes after the apomorphine injection, the sniffing-licking-gnawing (0 = absent and 1 = present) syndrome (stereotyped behavior) and climbing behavior (0 = all four feet on ground, 1 = two feet up on wire cage, and 2 = all four feet on wire cage) induced by apomorphine were scored and recorded for each animal. Readings were repeated every 5 min during a 30-min test session. Scores for each animal were totaled over the 30-min test session for each syndrome (stereotyped behavior and climbing). One-way analysis of variance (ANOVA) 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 ID50 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 percentage of control values observed in vehicle-treated mice that received apomorphine.

Cataleptogenic Potential in Mice. Drugs were administered i.p. to six mice per treatment group concurrently with d-amphetamine (3 mg/kg s.c.), phencyclidine (3 mg/kg s.c.), or vehicle. Mice were then immediately placed in the locomotor chambers for a 70-min test. Locomotor activity data were recorded under room light and white noise using Acuscan infrared beam activity monitors with enclosed Plexiglas chambers (8 inch × 8 inch). Acuscan VersaMax and VersaDat software (Acuscan Instruments, Inc., Columbus, OH) was used to convert the infrared beam breaks into horizontal activity counts in 5-min bins. Total horizontal activity counts collected in a 70-min test period and in the last 40 min of the test period were subjected to one-way analyses of variance with a subsequent least significant difference test (p < 0.05) and expressed graphically.

Atypical Antipsychotic Locomotor Activity Profile. Drugs were administered s.c. to 10 mice per treatment group concurrently with d-amphetamine (3 mg/kg s.c.), phencyclidine (3 mg/kg s.c.), or vehicle. Mice were then immediately placed in the locomotor chambers for a 70-min test. Locomotor activity data were recorded under room light and white noise using Acuscan infrared beam activity monitors with enclosed Plexiglas chambers (8 inch × 8 inch). Acuscan VersaMax and VersaDat software (Acuscan Instruments, Inc., Columbus, OH) was used to convert the infrared beam breaks into horizontal activity counts in 5-min bins. Total horizontal activity counts collected in a 70-min test period and in the last 40 min of the test period were subjected to one-way analyses of variance with a 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 × 38 × 56 cm). Presentations 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 sessions consisted of 60 total trials with a 15-s intertrial interval. After a 5-min acclimation to 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 before testing. Prepulse inhibition was defined as 100 − [(startle amplitude on prepulse trials/startle amplitude on pulse alone trials) × 100]. Data from the pulse-alone trials and PPI values were analyzed using one-way ANOVA followed by a least significant difference post hoc test (p < 0.05).

PPI in DBA/2N mice. Subjects were male DBA/2N mice. Test sessions consisted of 50 total trials with a 15-s intertrial interval. After a 5-min acclimation to 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 before testing. Prepulse inhibition was defined as 100 − [(startle amplitude on prepulse trials/startle amplitude on pulse alone trials) × 100]. Data from the pulse-alone trials and PPI were 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 13 ¼-inch-diameter stainless steel grid rods placed on ½-inch 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-min habituation period followed by 50 trials presented on a 15-s variable interval schedule (range = 7.5–22.5 s). Each trial consisted of a 10-s warning tone and stimulus light (conditioned stimulus) followed by a 10-s 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 s 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 intertrial interval, the animal was punished with a 0.5-s 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 (~90% avoidance responding on the training session before the test day) were considered “trained” and included on the test day. Training was maintained by at least one nondrug test session each week. On test days, drugs were administered i.p., s.c., or p.o. (2 ml/kg) 30 (WAY-163909) or 60 (SB 206553) min before testing. Eight animals received each dose of test drug. For the time course study, eight animals received WAY-163909 at 3 mg/kg i.p. and were tested at 30 min, 1 h, 2 h, and 24 h. 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 (ED50).

In Vivo Electrophysiology Studies. Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotoxic 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 to 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 ventral 6.0–8.5 mm 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 was 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 which had the tip broken back under a light microscope, were filled with a solution of 2 M NaCl saturated with 1% Fast Green dye. The impedance of the electrodes was usually 0.8 to 1.2 MΩ measured at 135 Hz in vitro and 1.5 to 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 ms), with a distinct initial segment and late positive component; 2) a characteristic low-pitch sound when monitored through an audiometer; 3) a slow, regular, or bursting firing pattern; and 4) a spontaneous firing rate of 2 to 9 Hz. The number of spontaneously active DA neurons was determined in 10 stereotoxic electrode descents or tracks as described previously (White and Wang, 1983). In brief, 10 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/s), 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 i.p. injection (acute regimen) or one i.p. injection per day for 21 consecutive days (chronic regimen) of vehicle (1 ml/kg deionized distilled water), 20 mg/kg clozapine (placed in 45% hydroxypropyl-β-cyclodextrin, w/v), or 1, 3, or 10 mg/kg WAY-163909 (dissolved in deionized, distilled water). The animals were prepared for the recording of SNC and VTA DA cells 2 h 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 min. 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/tracks data were analyzed using ANOVA, and post hoc tests were performed using the Student-Newman-Keuls test.

**In Vivo Microdialysis Studies.** *Nucleus Accumbens and Striatum Microdialysis Procedure.* After induction of anesthesia with gaseous administration of halothane (3%)/Fluthoane; 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 (anteroposterior +0.2, lateral –3, and ventral –3.7) or nucleus accumbens (anteroposterior +2.2, lateral –1.4, and ventral –2.5). Coordinates were taken according to Paxinos and Watson (1986) with reference points taken from bregma and vertical from the skull. A s.c. cannula was also implanted at this time between the animal’s shoulders. Both cannulas were secured to the skull using dental acrylic (Plastics One, Roanoke, VA) and two stainless steel screws. Immediately after surgery, animals were individually housed in Plexiglas cages (45 cm square) and were provided food and water ad libitum. Animals were allowed –24 h of postoperative recovery time. A preequilibrated microdialysis probe was inserted into the guide cannula in the striatum (o.d. 0.5 mm, membrane length 4 mm; CMA/Microdialysis) or nucleus accumbens (o.d. 0.5 mm, membrane length 2 mm; CMA/Microdialysis) of the unrestrained rat post-surgery. The probe was perfused with artificial cerebrospinal fluid (125 mM NaCl, 3.0 mM KCl, 0.75 mM MgSO4, 1.2 mM CaCl2, and 0.1 M phosphate buffer, pH 7.4) at a flow rate of 1.0 μl/min. A 3-h stabilization period was allowed post probe implantation, after which time microdialysis sampling began in the awake, freely moving rat. Four baseline samples were taken before drug injection to achieve a steady baseline. These four samples were averaged, and this value was denoted as 100%. Subsequent samples were expressed as a percentage of this preinjection value. Results were analyzed by analysis of variance with repeated measures followed by pairwise comparisons with Bonferroni’s adjustment for multiple comparisons using the Statview software application (Abacus Concepts, Berkeley, CA) for the PC.

**Cortical Microdialysis Procedure.** Rats were anesthetized with a combination (i.p.) of xylazine (13 mg/kg, Rompun; Bayer Corporation, Shawnee Mission, KS) and ketamine hydrochloride (87 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and mounted in a stereotaxic frame (Stoettling, Wood Dale, IL). Two stainless steel 21-gauge cannula with a dummy probe were placed and fixed by cranioplastic cement (Plastics One) onto the cortex dorsal to the mPFC. The stereotaxic coordinates of the probe, when implanted, are anterior +3.2, lateral –0.8, and ventral –5.5 mm (incline 80°), relative to bregma; the incision bar level is –3.0 mm, according to the atlas (Paxinos and Watson, 1986). Three to 5 days after cannulation, a probe with a 2-mm dialyzing membrane was implanted into the mPFC under slight anesthesia with isoflurane (Aerrane; Fort Dodge Animal Health). A catheter constructed from microbore Tygon tubing (TGY-010, 0.03 inch o.d. x 0.01 inch i.d.; Small Parts Inc., Miami Lakes, FL) was also implanted s.c. in the intrascapular space of the rats to avoid injection artifacts when vehicle or drugs were systemically administered to rats. After a 3-h perfusion (1.5 μl/min) of the probe, microdialysate samples (45 μl) were collected every 30 min in awake, freely moving rats. The perfusion medium was Dulbecco’s phosphate-buffered saline solution (Sigma-Aldrich) including Ca2+ (138 mM NaCl, 8.1 mM Na2HPO4, 2.7 mM KCl, 1.5 mM KH2PO4, 0.5 mM MgCl2, and 1.2 mM CaCl2, pH = 7.4). After stable baseline values in the dialysate samples were obtained, WAY-163909 was administered s.c. 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; FHG, Bowdoinham, ME). The procedure for the assessment of DA, which has been published, was 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, directly applied onto the liquid chromatography/electrochemistry 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, reverse phase column (Xterra RP-18, 3.5 μm C18, 1.0 x 100 mm; Waters) at 36°C maintained by a column oven (831 Temperature Controller; Gibson, Middleton, WI) or by a column heater (LC-22C Temperature Controller; BAS, West Lafayette, IN). The mobile phase consisted of 24 mM anhydro 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 an LC-10AD pump (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) versus an Ag-AgCl reference electrode. A highly sensitive method was used to measure endogenous ACh in the extracellular space in the absence of ACh esterase inhibition. Dialysate samples (20 μl) were directly injected onto the liquid chromatography/electrochemistry system, assisted by a chromatogram manager (Millennium; Waters). ACh was separated on a coiled cation-exchanger ACh column (analytical column) (SepStik 10 μm i.d. 530 x 1.0 mm; BAS), followed by the postimmobilized enzyme reactor (IMER) (BAS), which consisted of choline oxidase (ChO)-ACh esterase. ACh was hydrolyzed by ACh esterase 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 (SepStik 10 μm i.d. 530 x 1.0 mm; BAS), followed by the postimmobilized enzyme reactor (IMER) (BAS), which consisted of choline oxidase (ChO)-ACh esterase. ACh was hydrolyzed by ACh esterase 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-2008; BAS) glassy carbon electrode (MF-1002; BAS), set at +100 mV (LC-4C; BAS) versus Ag-AgCl reference electrode. This reduction was monitored 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 before 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 m/min by a LC-10AD pump (Shimadzu). Reagents used were analytical or HPLC grade. Only results derived from healthy rats with correctly positioned dialysis probes were included in the data analysis. Mean predrug baseline levels (time = 0; time = 30, and time = 0) were designated as 100%. Repeated-measures 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). p < 0.05 was considered significant in this study. All results are given as means ± S.E.M.

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, and haloperidol study climbing = 11.67 ± 0.21 and stereotypy = 6.0 ± 0). WAY-163909 (1.7–30 mg/kg i.p.) produced a dose-dependent decrease in climbing behavior induced by apomorphine (Fig. 1A) [ID₅₀ = 10.69 mg/kg; 95% confidence interval (CI), 7.5–15.2; MED = 5.4 mg/kg] at doses that had negligible effects on stereotypy [ID₅₀ > 30 mg/kg; MED = 10 mg/kg]. This profile is similar to that produced by the atypical antipsychotic clozapine (Fig. 1B) (climbing ID₅₀ = 8.2 mg/kg; 95% CI, 2.3–29.8; MED = 10 mg/kg; stereotypy ID₅₀ = 55.83 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. The separation of WAY-163909 in doses affecting climbing and stereotypy contrasts with that of the typical antipsychotic haloperidol (Fig. 1C) (climbing ID₅₀ = 0.077 mg/kg; 95% CI, 0.04–0.14; MED = 0.1 mg/kg; stereotypy ID₅₀ = 0.11 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 i.p.) produced negligible catalepsy up to 30 mg/kg (Fig. 2A) [F(4,29) = 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 that for haloperidol (Fig. 2C) [F(4,29) = 13.32; p < 0.05], which displayed nearly full catalepsy at 1 mg/kg. Although haloperidol reached a 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 because of sedation.

Locomotor Activity

In these experiments, PCP typically increased locomotor activity ~206%, whereas amphetamine increased locomotor activity ~288%. WAY-163909 (0.1–3 mg/kg s.c.) (Fig. 3A) was more potent in reducing PCP-stimulated locomotor activity, MED = 0.3 mg/kg [F(3,39) = 4.194, p < 0.05], compared with 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 s.c.) (Fig. 3B) was also more potent in reducing PCP-stimulated locomotor activity, MED = 0.1 mg/kg [F(4,49) = 5.563, p < 0.05], compared with d-amphetamine-stimulated locomotor activity [F(4,49) = 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 s.c.) (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 with PCP-stimulated locomotor activity [F(4,49) = 6.102, p < 0.05; MED = 0.1 mg/kg], whereas spontaneous locomotor activity was decreased at a MED = 0.1 mg/kg [F(4,49) = 10.668, p < 0.05]; thus, haloperidol only blocked PCP-stimulated locomotor activity at doses that affected spontaneous motor activity.
reversed the DOI-induced disruption of PPI (Fig. 4A; 3 mg/kg) produced a 47% decrease in PPI, and WAY-163909 also normalized the startle response (Table 1) [5,63] F6.841, p < 0.05]. With the d-amphetamine-induced disruption, clozapine reversed the deficit at the 10 mg/kg dose [F4,39] = 6.812, p < 0.05]. Neither DOI nor clozapine altered the baseline startle response [F4,39] = 1.095, p > 0.05]. The activity counts for the last 40 min 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).

**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 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 i.p.) dose-dependently reversed the MK-801-induced disruption of PPI (Table 1) [4,38] F6.566, p < 0.05] at the two lowest doses tested. Haloperidol also failed to reverse the DOI-induced disruption of PPI [F4,39] = 18.680, p < 0.05] but haloperidol did normalize the MK-801-induced elevated baseline startle response [F4,39] = 6.966, p < 0.05] at the two lowest doses tested. Haloperidol also failed to reverse the DOI-induced disruption of PPI [F4,39] = 2.961, p < 0.05] at all doses tested. Clozapine at 5.4 and 10 mg/kg also reversed the DOI-induced disruption of PPI [F4,38] = 2.961, p < 0.05]. Neither DOI nor clozapine had an effect on the baseline startle response [F4,38] = 1.095, p > 0.05]. With the d-amphetamine-induced disruption, clozapine reversed the deficit at the 10 mg/kg dose [F4,39] = 6.812, p < 0.05]. Neither d-amphetamine nor clozapine altered the baseline startle response [F4,39] = 1.095, p > 0.05]. The activity counts for the last 40 min 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).

ampholine. All PPI and baseline startle data for clozapine and haloperidol are listed in Table 2. Clozapine (3–10 mg/kg i.p.) only reversed the MK-801-induced disruption of PPI [4,39] = 11.777, p < 0.05] at the 5.4 mg/kg dose. Clozapine did normalize the MK-801-elevated baseline startle response [F4,39] = 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 [F4,38] = 2.961, p < 0.05]. Neither DOI nor clozapine had an effect on the baseline startle response [F4,38] = 1.095, p > 0.05]. The activity counts for the last 40 min 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).
increase in the baseline startle response at all of the 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 to simplify the graphical presentation of the drug effects. WAY-163909 (10–30 mg/kg i.p.) improved PPI in DBA mice (Fig. 4B) \(F(3,77) = 7.019, p < 0.05; \text{MED} = 30 \text{ mg/kg}\) with no effect on baseline startle (Table 1) \(F(3,38) = 1.984, p > 0.05\). Clozapine and haloperidol data in DBA/N2 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 i.p.; 1.0–17 mg/kg p.o.) produced dose-dependent decreases in avoidance responding in rats (Fig. 5A) \(F(4,39) = 68.99, p < 0.05; \text{ED}_{50} = 1.27 (1.1–1.5) \text{ mg/kg i.p.; } F(5,47) = 21.02, p < 0.05; \text{ED}_{50} = 6.50 (4.7–9.1) \text{ mg/kg p.o.}\) 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-HT_{2A/C} antagonist SB 206553 (10 mg/kg p.o.), resulting in an increase in the \text{ED}_{50} for WAY-163909 in the presence of SB 206553 to 3.4 mg/kg i.p. (Fig. 5B) (CI, 2.9–4.1 mg/kg) from 1.28 mg/kg i.p. (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 i.p. of WAY-163909 producing a 47% reduction in avoidance responses after a 30-min pretreatment interval \(p < 0.05\), whereas avoidances were reduced by 16, 29, and 0% at the 1-, 2-, and 24-h pretreatment times, respectively. In addition, 1.7 and 3.0 mg/kg WAY-163909 were administered via the s.c. route and evaluated 30 min postinjection and produced 31 and 34% blocks 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 i.p. administration of 1, 3, or 10 mg/kg WAY-163909 significantly decreased the number of spontaneously active VTA DA neurons. In contrast, the acute i.p. administration of 20 mg/kg clozapine significantly increased the number of spontaneously active VTA DA neurons. In the SNC, the acute i.p. administration of WAY-163909 (1–10 mg/kg) or 20 mg/kg clozapine did not significantly alter the number of spontaneously active DA neurons compared with vehicle-treated animals (Table 3) \(F(4,45) = 0.37, p > 0.05\). After the repeated (21-day) i.p. 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 WAY-163909 significantly decreased the number

### Table 1

WAY-163909 effects on the basal startle response after treatment with MK-801, DOI, and d-amphetamine in Sprague-Dawley rats and on the basal startle response in DBA/2N mice

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Vehicle</th>
<th>Vehicle Disruptor</th>
<th>1.7 mg/kg Disruptor</th>
<th>5.4 mg/kg Disruptor</th>
<th>10 mg/kg Disruptor</th>
<th>17 mg/kg Disruptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MK-801 (0.5 mg/kg)</strong></td>
<td>84.9 (9.9)*</td>
<td>247.2 (31.7)</td>
<td>154.3 (14.9)*</td>
<td>50.3 (12.5)*</td>
<td>79.7 (15.2)*</td>
<td>94.6 (15.6)*</td>
</tr>
<tr>
<td><strong>DOI (3 mg/kg)</strong></td>
<td>58.7 (6.2)</td>
<td>64.6 (10.9)</td>
<td>27.9 (5.6)*</td>
<td>33.6 (8.8)*</td>
<td>28.0 (8.6)*</td>
<td>33.2 (10.4)*</td>
</tr>
<tr>
<td><strong>Amphetamine (4 mg/kg)</strong></td>
<td>63.1 (8.1)*</td>
<td>144.5 (26.7)</td>
<td>63.9 (13.1)*</td>
<td>30.7 (8.3)*</td>
<td>20.5 (4.9)*</td>
<td>46.6 (14.7)*</td>
</tr>
</tbody>
</table>

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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DBA/2N</strong></td>
<td>60.9 (6.1)</td>
<td>84.9 (6.8)</td>
<td>73.3 (10.9)</td>
<td>60.0 (8.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from the disrupting agent.
of spontaneously active VTA DA neurons compared with vehicle-treated animals. In addition, the decreases in the number of spontaneously active VTA DA neurons produced by the 10 mg/kg dose of WAY-163909 and the 20 mg/kg dose of clozapine were significantly greater than those for the 1 and 3 mg/kg doses of WAY-163909. As with acute treatment, neither clozapine nor WAY-163909 significantly altered the number of spontaneously active SNC DA neurons compared with vehicle-treated animals (Table 4) \(F(4,45) = 1.42, p > 0.05\) after 21 days of treatment.

**In Vivo Microdialysis**

**Nucleus Accumbens and Striatal Microdialysis.** The basal dopamine level, reported as mean \pm S.E.M. femtomoles per 10 l of microdialysate, in the nucleus accumbens was 33.8 \pm 2.0 and in the striatum was 38.2 \pm 10.2 fmol/10 l of microdialysate, in the nucleus accumbens was 38.2 \pm 10.2 fmol/10 l of microdialysate. WAY-163909 (10 mg/kg s.c.) produced a significant increase in nucleus accumbens DA \(F(1,10) = 10.66, p < 0.05\), reaching a maximum of 48 \pm 9.2% of the preinjection baseline levels (Fig. 6A). In contrast, WAY-163909 (10 mg/kg s.c.) 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 \pm 0.41 fmol/10 l, reported as mean \pm S.E.M. The basal dopamine level was 1.2 \pm 0.1 fmol/10 l, reported as mean \pm S.E.M. WAY-163909 (3 and 10 mg/kg s.c.) produced an increase in microdialysate ACh concentrations in the mPFC with significant effects at both doses \(F(1,9) = 10.21, p < 0.01; F(1,11) = 6.22, p < 0.05\), respectively), compared with 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 with 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 i.p., s.c., or p.o., 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 that the effect of WAY-163909 was greatest at 30 min after i.p. 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 amphetamine-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), whereas 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 and stereotypy over a similar dose range, whereas clozapine, an atypical antipsychotic, more potently decreased apomorphine-induced climbing. 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:

**TABLE 3**

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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg i.p.)</th>
<th>DA Cells/Track</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SNC</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>1</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>3</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>WAY-163909</td>
<td>10</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>Clozapine</td>
<td>20</td>
<td>0.76 ± 0.06</td>
</tr>
</tbody>
</table>

* Significantly greater than the 10 mg/kg dose of WAY-163909 and clozapine (p < 0.01).
** Significantly less than vehicle (p < 0.05).

**TABLE 4**

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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg i.p.)</th>
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</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>0.71 ± 0.05</td>
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<tr>
<td>WAY-163909</td>
<td>1</td>
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</tr>
<tr>
<td>Clozapine</td>
<td>20</td>
<td>0.76 ± 0.06</td>
</tr>
</tbody>
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* Significantly greater than the 10 mg/kg dose of WAY-163909 and clozapine (p < 0.01).
** Significantly less than vehicle (p < 0.05).
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 VTA (A10) without affecting the number of spontaneously active dopamine neurons in the substantia nigra pars compacta (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 the acute effects of WAY-163909 unique among 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) seem similar, it is possible that differential levels of protein expression for 5-HT_{2C} receptors in these regions 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 (Banks 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 locomotor activity. 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 DBA/2 strain of mouse, a mouse strain reported to be sensitive to antipsychotic-induced enhancement in prepulse inhibition (Brownman 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 these abnormalities are thought to underlie this symptom domain (Goldman-Rakic and Selemom, 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 a 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 and an examination of its effects on other neurotransmitters, such on glutamate, are a subject of further investigation.

Although preclinical data support a role for 5-HT_{2C} receptor agonists in the treatment of schizophrenia, challenge studies using a single i.v. or p.o. 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 the 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. In addition,
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, because 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-HT₂C 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.

Acknowledgments

We thank Menelas Pangalos and Ron Magdola for their support of the 5-HT₂C program.

References


