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Neuropharmacological Profile of Novel and Selective 5-HT$_6$ Receptor Agonists: WAY-181187 and WAY-208466

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One of the most recently identified serotonin (5-hydroxytryptamine (5-HT)) receptor subtypes is the 5-HT$_6$ receptor. Although in-depth localization studies reveal an exclusive distribution of 5-HT$_6$ mRNA in the central nervous system, the precise biological role of this receptor still remains unknown. In the present series of experiments, we report the pharmacological and neurochemical characterization of two novel and selective 5-HT$_6$ receptor agonists. WAY-181187 and WAY-208466 possess high affinity binding (2.2 and 4.8 nM, respectively) at the human 5-HT$_6$ receptor and profile as full receptor agonists (WAY-181187: $EC_{50} = 6.6$ nM, $E_{max} = 93\%$; WAY-208466: $EC_{50} = 7.3$ nM; $E_{max} = 100\%$). In the rat frontal cortex, acute administration of WAY-181187 (3–30 mg/kg, subcutaneous (s.c.)) significantly increased extracellular GABA concentrations without altering the levels of glutamate or norepinephrine. Additionally, WAY-181187 (30 mg/kg, s.c.) produced modest yet significant decreases in cortical dopamine and 5-HT levels. Subsequent studies showed that the neurochemical effects of WAY-181187 in the frontal cortex could be blocked by pretreatment with the 5-HT$_6$ antagonist, SB-271046 (10 mg/kg, s.c.), implicating 5-HT$_6$ receptor mechanisms in mediating these responses. Moreover, the effects of WAY-181187 on catecholamines were attenuated by an intracortical infusion of the GABA$_A$ receptor antagonist, bicuculline (10 μM), confirming a local relationship between 5-HT$_6$ receptors and GABAergic systems in the frontal cortex. In the dorsal hippocampus, striatum, and amygdala, WAY-181187 (10–30 mg/kg, s.c.) elicited robust elevations in extracellular levels of GABA without producing similar effects on concentrations of norepinephrine, serotonin, dopamine, or glutamate. In contrast to these brain regions, WAY-181187 had no effect on the extracellular levels of GABA in the nucleus accumbens or thalamus. Additional studies showed that WAY-208466 (10 mg/kg, s.c.) preferentially elevated cortical GABA levels following both acute and chronic (14 day) administration, indicating that neurochemical tolerance does not develop following repeated 5-HT$_6$ receptor stimulation. In hippocampal slice preparations (*in vitro*), 5-HT$_6$ receptor agonism attenuated stimulated glutamate levels elicited by sodium azide and high KCl treatment. Furthermore, in the rat schedule-induced polydipsia model of obsessive compulsive disorder (OCD), acute administration of WAY-181187 (56–178 mg/kg, po) decreased adjunctive drinking behavior in a dose-dependent manner. In summary, WAY-181187 and WAY-208466 are novel, selective, and potent 5-HT$_6$ receptor agonists displaying a unique neurochemical signature *in vivo*. Moreover, these data highlight a previously undescribed role for 5-HT$_6$ receptors to modulate basal GABA and stimulated glutamate transmission, as well as reveal a potential therapeutic role for this receptor in the treatment of some types of anxiety-related disorders (eg OCD).
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INTRODUCTION

The serotonin (5-HT, 5-hydroxytryptamine) receptor family currently consists of 14 members separated into seven classes (5-HT$_{1-7}$). One of the most recently identified 5-HT receptor subtypes is the 5-HT$_6$ receptor. Initially cloned from striatal tissue (Monsma *et al*, 1993), the rat 5-HT$_6$ receptor gene encodes a protein of 438 amino acids and shares 89% homology to the human form (Kohen *et al*, 1996; Ruat *et al*, 1993). Similar to 5-HT$_4$ and 5-HT$_7$, the 5-HT$_6$ receptor has no known functional subtypes and is a G-protein-coupled receptor that positively stimulates adenylate cyclase activity (Schoeffter and Waeber, 1994; Sebben *et al*, 1994). A nonfunctional truncated splice variant of the 5-HT$_6$ receptor has been identified but appears not to have any physiological significance (Olsen *et al*, 1999). *In situ* hybridization and northern blot studies revealed an exclusive distribution of 5-HT$_6$ mRNA in the rat central

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nervous system (CNS) with the highest density found in the olfactory tubercle, followed by the frontal and entorhinal cortices, dorsal hippocampus (ie, dentate gyrus and CA1, CA2, and CA3 regions), nucleus accumbens, and striatum (Ward *et al*, 1995; Gerard *et al*, 1997). Lower levels were observed in the hypothalamus, amygdala, substantia nigra, and several diencephalic nuclei. These findings have been corroborated by immunolocalization studies and radioligand binding showing a similar distribution of 5-HT$_6$ receptor protein in the rat CNS (Gerard *et al*, 1996; Hamon *et al*, 1999; Roberts *et al*, 2002). Additionally, destruction of 5-HT cell bodies in the raphe nuclei were not found to alter 5-HT$_6$ mRNA, suggesting that 5-HT$_6$ receptors are not autoreceptors on 5-HT perikarya and are not upregulated in post-synaptic areas following degeneration (Gerard *et al*, 1996).

To date, several 5-HT$_6$ receptor antagonists have been described in the literature. Early behavioral studies report that administration of various 5-HT$_6$ receptor antagonists produced a yawning and stretching syndrome that was blocked by the muscarinic antagonist, atropine (Bentley *et al*, 1999; Bourson *et al*, 1998). These studies supported previous work carried out with 5-HT$_6$ antisense (Bourson *et al*, 1995) and were suggestive of an interaction between 5-HT$_6$ receptors and central cholinergic systems. *In vivo* microdialysis experiments supported this claim by showing that antagonism of the 5-HT$_6$ receptor increased acetylcholine concentrations in both the rat frontal cortex and hippocampus (Riemer *et al*, 2003; Sleight *et al*, 1999). Subsequent microdialysis studies actually showed that systemic administration of a 5-HT$_6$ antagonist produced a two- and three-fold increase in basal glutamate levels in the rat hippocampus and frontal cortex, respectively (Dawson *et al*, 2000, 2001; Woolley *et al*, 2000). Given the purported role of the cholinergic and glutamatergic system in cognitive function, 5-HT$_6$ receptor antagonists were also examined in various models of learning and memory where they were found to enhance cognitive performance (Rogers and Hagan, 2001; Woolley *et al*, 2001). Collectively, these positive behavioral and neurochemical effects strongly suggest that 5-HT$_6$ receptor antagonists may work to enhance cognitive processes (Mitchell and Neumaier, 2005).

Despite these early results, the precise function and therapeutic relevance of the 5-HT$_6$ receptor has yet to be completely determined. Further elucidation of the biological role of the 5-HT$_6$ receptor has been hampered to an extent by the lack of suitable and selective 5-HT$_6$ receptor agonists, which would allow the consequences of receptor stimulation to be studied. Herein, we report the *in vitro* and neurochemical characterization of two novel and potent full 5-HT$_6$ agonists, WAY-181187 and WAY-208466. The *in vitro* neuropharmacological profile indicated that both agents are selective and full receptor agonists. Furthermore, in-depth neurochemical studies were performed to ascertain the modulatory influence of 5-HT$_6$ receptor stimulation on various neurotransmitter systems. Taken together, the studies performed have further defined the biological basis for the 5-HT$_6$ receptor in terms of affecting inhibitory and excitatory neurotransmission and indirect modulation of monoaminergic systems. Furthermore, these data may indicate a potential role of this receptor in antidepressant drug action. This hypothesis, which addresses the long-standing question of which 5-HT receptor is involved in mechanism of action of antidepressants (eg SSRIs), will be discussed.

MATERIALS AND METHODS

5-HT$_6$ Receptor Preparation

In-house cultured HeLa cells expressing the human 5-HT$_6$ receptor were harvested and centrifuged at low speed (1000*g*) for 10 min to remove the culture media. The harvested cells were suspended in a half volume of fresh physiological phosphate-buffered saline solution and re-centrifuged at the same speed (repeated 2 $\times$). The collected cells were subsequently homogenized in 10 volumes of 50 mM Tris-HCl, pH 7.4, and 0.5 mM EDTA. The homogenate was centrifuged at 40 000*g* for 30.0 min and the precipitate was collected. The pellet was resuspended in 10 volumes of Tris-HCl buffer and recentrifuged at the same speed. The final pellet was suspended in a small volume of Tris-HCl buffer and the tissue protein content determined before making aliquots of 10–25 µl. Bovine serum albumin was used as the standard in the protein determination by the method of Lowry *et al* (1951). The volume of the suspended cell membranes was adjusted to give a tissue protein concentration of 1.0 mg/ml of suspension. The prepared membrane suspension (10 times concentrated) was aliquoted in 1.0 ml volumes and stored at -70°C until used in subsequent *in vitro* experiments.

5-HT$_6$ Receptor Binding

Binding experiments were conducted in 96-well microtiter plates in a total volume of 200 µl of buffer, which includes 80 µl of incubation buffer made in 50 mM Tris-HCl buffer, pH 7.4, and containing 10 mM $MgSO_4$ and 0.5 mM EDTA; 20 µl of [^{3}H] LSD (S.A., 86.0 Ci/mmol, Amersham Life Science). The dissociation constant (K_D) of [^{3}H]LSD at the human 5-HT$_6$ receptor is 2.9 nM as determined in saturation-binding analysis. The reaction was initiated by the final addition of 100 µl of tissue suspension. Nonspecific binding was measured in the presence of 10 µM methiothepin, added in a 20 µl volume. Test compounds were added in 20 µl volume. The reaction proceeded in the dark for 120 min at room temperature, at that time, the bound ligand-receptor complex was filtered off on a 96-well unifilter with a Packard Filtermate 196 Harvester. The bound complex caught on the filter disk was air-dried and the radioactivity measured in a Packard TopCount® equipped with six (6) photomultiplier detectors, after the addition of 40.0 µl of Microscint®-20 scintillant to each well. The unifilter plate was heat sealed and counted in a Packard TopCount® with a tritium efficiency of 31.0%.

5-HT$_6$ Receptor Camp Assay

HeLa cells were transfected with the human 5-HT$_6$ receptor as described in the 5-HT$_6$ receptor preparation section above. Cells were washed with Krebs buffer and incubated at 37°C in Krebs supplemented with 500 µM IBMX for 5 min at 37°C. Cells were then stimulated with either WAY-181187 or WAY-208466 in the concentration range

0.1–10 000 nM for an additional 10 min at 37°C. The assay was terminated with the addition of 0.5 M perchloric and intracellular cAMP levels were determined by radioimmunoassay with the cAMP Scintillation Proximity Assay System (Amersham).

In Vitro Selectivity Assays

Recombinant human protein was used for all receptors except for the α-1 adrenergic receptor, which was prepared by rat cortical tissue. Specific assay conditions for each receptor are shown in Table 1.

Evaluation of *In Vitro* Results

Radioligand binding results were analyzed by constructing log concentration response curves to generate IC_{50} estimates. K_i values were calculated from the equation described by Cheng and Prusoff (1973). Results for the EC_{50} values from the cAMP assays were estimated from log–concentration response curves by non-linear regression analysis. The E_{max} values are relative to a maximally effective concentration of 5-HT (100 nM). Data are mean values (±SEM) from two to three independent experiments.

In Vivo Microdialysis Studies

Animals. Adult male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 280–350 g at the time of surgery were used for all experiments. Before surgery, animals had free access to food and water and were group housed in an AAALAC-accredited facility that was maintained on a 12-h light–dark cycle (lights on at 0600 hours). All *in vivo* studies were previously approved by the Wyeth's internal animal care and use committee and were performed in accordance to the *Guide for the Care and Use of Laboratory Animals* as promulgated by the National Institutes of Health (Pub. 85–23, 1985).

Stereotaxic surgery. Following the induction of anesthesia with gaseous administration of 3% halothane (Fluothane; Zeneca, Cheshire, UK), animals were secured in a stereotaxic frame with ear and incisor bars (David Kopf, Tujunga, CA). Anesthesia was maintained by continuous administration of halothane (1–2%), whereas a microdialysis guide cannula (CMA/Microdialysis, Stockholm, Sweden) was directed toward the brain region of interest. The coordinates for guide cannula surgery are shown in Table 2 and were based on the rat brain atlas of Paxinos and Watson (1986). A subcutaneous cannula (s.c.) was also implanted at this time between the shoulders of the animals. Cannulae were secured to the skull using dental acrylic (Plastics One, Roanoke, VA, USA) and two stainless-steel screws. Immediately after the surgery, animals were individually housed in Plexiglas cages (45 cm^2) where they had food and water *ad libitum* during a 24-h postoperative recovery period.

Table 2 Coordinates for Microdialysis Guide Cannula Surgery

Brain region	Stereotaxic coordinates[a]	Membrane length (mm)
Frontal cortex	A/P+3.2 M/L−3.5 D/V−1.3	2
Nucleus accumbens	A/P+2.2 M/L−1.2 D/V−6.0	2
Striatum	A/P+0.2 M/L−3.0 D/V−3.2	4
Amygdala	A/P−2.7 M/L−4.6 D/V−7.2	1
Dorsal hippocampus	A/P−4.3 M/L−2.6 D/V−2.1	2
Thalamus	A/P−4.5 M/L−2.6 D/V−5.4	2

A/P, anterior/posterior from bregma with a flat skull; M/L, medial/lateral to midline; D/V, dorsal/ventral from dura.
[a]Stereotaxic coordinates taken from the rat brain atlas of Paxinos and Watson (1986).

Table 1 Receptor Binding Assay Conditions[a]

Receptor	Radioligand	Blank (nonspecific)	Buffer[b]	Incubation conditions
5-HT$_{1A}$	^{3}H-5-HT	10 μM 5-HT	50 mM Tris-HCl	37°C, 120 min
5-HT$_{1B}$	^{3}H-5-HT	10 μM 5-HT	50 mM Tris-HCl	37°C, 120 min
5-HT$_{1D}$	^{3}H-5-HT	10 μM 5-HT	50 mM Tris-HCl	37°C, 120 min
5-HT$_{1F}$	^{3}H-5-HT	10 μM 5-HT	50 mM Tris-HCl	37°C, 120 min
5-HT$_{2A}$	^{125}I-DOI	1 μM DOI	50 mM Tris-HCl/4 mM $CaCl_2$	25°C, 60 min
5-HT$_{2B}$	^{3}H-5-HT	10 μM 5-HT	50 mM Tris-HCl	37°C, 30 min
5-HT$_{2C}$ (Ant)	^{3}H-mesulergine	1 μM mianserin	50 mM Tris-HCl, 4 mM $CaCl_2$	25°C, 120 min
5-HT$_{2C}$ (Ago)	^{125}I-DOI	1 μM DOI	50 mM Tris-HCl, 4 mM $CaCl_2$	25°C, 60 min
5-HT$_7$	^{3}H-LSD	10 μM methiothepin	50 mM Tris-HCl, 10 mM $MgSO_4$, 0.5 mM EDTA	25°C, 120 min
D2	^{3}H-spiperone	10 μM *d*-butaclamol	50 mM Tris-HCl	25°C, 120 min
D3	^{3}H-spiperone	10 μM 7-OH-DPAT	50 mM Tris-HCl	25°C, 120 min
D4	^{3}H-spiperone	10 μM clozapine	50 mM Tris-HCl	25°C, 120 min
α1	^{3}H-prazosin	10 μM phentolamine	50 mM Tris-HCl	25°C, 30 min

[a]Recombinant human protein was used for all receptors except α-1 adrenergic receptors, which were prepared from rat cortical tissue.
[b]pH = 7.4 for all buffers.

Acute microdialysis procedures. Microdialysis probes were purchased from CMA Microdialysis (CMA/12, Stockholm, Sweden) and equilibrated according to the manufacturer's specifications. Initially, microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 125 mM NaCl, 3 mM KCl, 0.75 mM $MgSO_4$, and 1.2 mM $CaCl_2$, pH 7.4) *in situ* for at least 18-h before experimentation. The microdialysis probe was then implanted, via the guide cannula, into the brain region of interest and perfused with aCSF at a flow rate of 1 μl/min. The active membrane length of the microdialysis probes varied depending on the size of the brain region tested (Table 2). A 3-h stabilization period was allowed following probe implantation thereafter dialysate sampling was conducted according to previously described methods (Beyer *et al*, 2002). In brief, five control samples (fmol/20 μl sample) were taken before drug injection to demonstrate a steady baseline. These five samples were averaged and all subsequent values were expressed as a percent change from this preinjection value (% change from baseline). At the end of the fifth baseline sample, vehicle (2% Tween/0.5% Dextrose dissolved in water) or, in some cases when designated, 10 mg/kg, s.c. of the selective 5-HT$_6$ antagonist SB-271046, was administered. Approximately 20 min later, at the end of the sixth sample, the 5-HT$_6$ receptor agonist, WAY-181187 (3, 10, or 30 mg/kg, s.c.), or vehicle (water) was administered. For separate studies in the frontal cortex, the GABA$_A$ receptor antagonist, bicuculline (10 μM) was infused for 1-h at a rate of 1 μl/min through the microdialysis probe (starting immediately at the end of the fifth sample). Following injections, dialysis samples were collected every 20 min into plastic tubes and frozen on dry ice immediately. At the end of the experiment, animals were euthanized and probe placement was verified histologically. Data from animals with incorrect probe placement were discarded.

Chronic microdialysis procedures. Animals received an injection of either vehicle (water) or WAY-208466 (10 mg/kg, s.c.) for 14 days in their homecages (one injection per day). On day 14 of their treatment, animals underwent stereotaxic surgery to have a guide cannula placed above the frontal cortex (see surgery section above). The following day animals were placed in the microdialysis boxes and studies were conducted as described above (see acute microdialysis procedure section). This treatment paradigm resulted in three treatment groups: vehicle control, acute WAY-208466 (chronic vehicle + drug challenge), and chronic WAY-208466 (chronic drug + drug challenge).

In Vitro Superfusion Studies

Preparation of hippocampal slices. Bilateral hippocampal sections were dissected from male Sprague–Dawley rats (250–300 g). Sections were placed on a cold platform and suspended in ice-cold oxygenated Krebs buffer (NaCl 122 mM, KCl 3 mM, $NaHCO_3$ 24 mM, glucose 10 mM, H_2KPO_4 0.315 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 4 mM, pH 7.4). Next, hippocampal tissue was cross-cut at 350 μm on a McIlwain tissue chopper, then re-suspended, and washed three times with ice-cold Krebs solution. Next, 130 μl of hippocampal suspension (approx. 80 μg of tissue) was added to each well of a Brandel super-perfusion apparatus. The surrounding temperature was maintained at 37°C throughout the experiment.

Sodium azide-induced ischemia. For pretreatment studies, each tissue sample was perfused with oxygenated normal Krebs solution and was allowed to equilibrate for 60 min at a flow rate of 0.4 ml/min. Following equilibration, four 10-min baseline fractions were collected and followed by a 30-min wash with modified Krebs solution (NaCl 122 mM, KCl 3 mM, $NaHCO_3$ 24 mM, H_2KPO_4 0.315 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 4 mM, pH 7.4) or vehicle (normal Krebs solution). Next, ischemia was induced by the application of a modified Krebs solution containing sodium azide (10 mM) for a period of 30 min. WAY-181187 or vehicle was added in normal Krebs or an aglycemic solution 30 min before and 30 min during azide-ischemia. Following chemical ischemia, the tissue was allowed to re-equilibrate with normal Krebs solution for 30 min. All sample fractions were collected and analyzed for amino-acid concentrations using high performance liquid chromatography (HPLC) (see below).

KCL-induced ischemia (50 mM). Hippocampal tissue was collected as mentioned above. Samples were perfused with oxygenated normal Krebs solution and allowed to equilibrate for 60 min at a flow rate of 0.4 ml/min. Following equilibration, six 10-min baseline fractions were collected. The 50 mM high KCl-modified krebs (NaCl 75.12 mM, KCl 50 mM, $NaHCO_3$ 24 mM, H_2KPO_4 0.315 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 4 mM, pH 7.4) was applied for a period of 30 min. WAY-181187 was added in vehicle normal krebs or 50 mM high KCl solution. Following the 50 mM KCl, the tissue was allowed to re-equilibrate with normal Krebs solution for 30 min. Sample fractions were collected and then analyzed for amino-acid levels using HPLC.

HPLC Analysis

For all neurochemical studies, samples (20 μl) were split and analyzed for catecholamine (norepinephrine, dopamine, and 5-HT) and amino-acid (GABA and glutamate) concentrations. HPLC methods were conducted using the following methods:

(1) Dialysate (10 μl) containing norepinephrine, dopamine, and 5-HT was separated by HPLC (C18 ODS3 column, 150 × 3.0 mm, Metachem, Torrance, CA, USA) and detected using an ANTEC electrochemical detector (ANTEC, Netherlands) set at a potential of 0.65V *vs* a Ag/AgCl reference electrode. Mobile phase (0.15 M NaH_2PO_4, 0.25 mM EDTA, 1.75 mM 1-octane sulfonic acid, 2% isopropanol, and 4% methanol, pH = 4.8) was delivered by a Jasco PU1580 HPLC pump (Jasco Ltd, Essex, UK) at a flow rate of 0.5 ml/min. The minimum detection level of these neurotransmitters is approximately 2 fmol per 10 μl dialysate. Neurochemical data were compared with an external standard curve and all data were acquired using the Atlas software package (Thermo Labsystems, Beverley, MA) for the PC.
(2) Dialysate (10 μl) containing GABA and glutamate was separated by HPLC. These units consisted of two Jasco PU-980 pumps (Jasco Ltd, Essex, UK) as the gradient, a

BAS sentinel autosampler (BAS) and a Jasco PF-920 fluorometer with excitation wavelength of 448 nm. The emission wavelength was 485 nm. Mobile phase A was 0.05 M acetate buffer (pH 6.5) with 20% methanol (v : v) and mobile phase B was a 0.05 M acetate buffer (pH 6.5) with 80% methanol (v : v). The gradient consisted of a linear transition from 80% mobile phase A to 0% in 18 min. The column was allowed to re-equilibrate for 10 min before each injection. Each sample was diluted 1 : 1 with normal Krebs solution containing 2.5 μM α-aminoadipic acid (αAA; final concentration 1.25 μM; internal standard). Samples containing αAA were derivatized with naphthalene 2,3-dicarboxaldehyde (NDA). Samples or standards were mixed with 30 mM boric acid buffer (pH 9.5) containing 20 μM cyanide, and 30 μM NDA in methanol (1 : 1 : 0.25; sample : borate : NDA) and were allowed to react for 10 min at 10°C before fluorometric detection. Under these conditions, the minimum detection level of GABA and glutamate is approximately 10 and 100 nmol per 10 μl dialysate, respectively. Data were acquired using the Atlas software package (Labsystems, Gulph Mills, PA) for the PC.

Statistical analysis of neurochemical results. The fmol concentrations of all neurochemicals for the baseline samples were averaged and this value was denoted as 100%. Subsequent sample values were expressed as a percent change from this preinjection baseline value. Neurochemical data, excluding preinjection values, were analyzed by a two-way analysis of variance (ANOVA) with repeated measures (time). *Post hoc* analyses were made using the Bonferroni/Dunns adjustment for multiple comparisons. All statistical calculations were performed using the Statview software application (Abacus Concepts Inc., Berkeley, CA) for the PC.

Schedule-Induced Polydipsia (SIP)

Individually housed male Sprague–Dawley rats (300–400 g) were maintained at approximately 85% of free feeding body weight. Rats were placed into an operant chamber (Med Associates, Vermont) and given free access to water. One food pellet was delivered into the food bin every minute of the procedure. This schedule of food delivery causes a repetitive drinking behavior that results in the consumption of a large volume of water, generally 5- to 10-fold greater than baseline, during the 120 min test session. On test days, WAY-181187 (1–10 mg/kg, i.p.) was administered acutely to investigate its effect on the adjunctive drinking behavior.

RESULTS

WAY-181187 and WAY-208466 are Potent and Selective 5-HT$_6$ Receptor Agonists

WAY-181187 displayed high affinity binding at the cloned human 5-HT$_6$ receptor ($K_i = 2.2 \pm 0.3$ nM) using [^{3}H]LSD. In a clonal HeLa cell line expressing the human 5-HT$_6$ receptor, WAY-181187 acted as a full agonist ($EC_{50} = 6.6 \pm 0.8$; $E_{max} = 93.3\% \pm 2.1$) relative to the maximal effects of 5-HT to stimulate cAMP production (Table 3). Using the same assay conditions, WAY-208466 similarly profiled as a high affinity ligand ($K_i = 4.8 \pm 0.3$ nM) and induced 5-HT$_6$ receptor agonist activity ($EC_{50} = 7.3 \pm 1.6$; $E_{max} = 100\% \pm 0$; Table 3).

Additional binding studies indicated that both WAY-181187 and WAY-208466 were selective over other monoamine receptors (Table 4). Despite the affinity for the 5-HT$_{2C}$ agonist binding site, WAY-181187 retained approximately 60-fold selectivity for the primary target. Moreover, additional studies did not demonstrate activity at the 5-HT$_{2C}$ receptor at efficacious doses that stimulate 5-HT$_6$ receptors *in vivo* (data not shown).

5-HT$_6$ Receptor and GABAergic Interactions in the Rat Frontal Cortex

The acute neurochemical effects of WAY-181187 were evaluated by *in vivo* microdialysis techniques. In the dorsolateral frontal cortex, WAY-181187 (3–30 mg/kg, s.c.) significantly ($P < 0.001$ at 3–30 mg/kg) increased extracellular GABA concentrations relative to vehicle at all the doses examined (Figure 1a). Maximal percent increases from baseline were 142, 186, and 220% at doses of 3, 10, and 30 mg/kg, respectively. The GABAergic effects of WAY-181187 (10 mg/kg) were fully reversed by a 20-min

Table 3 WAY-181187 and WAY-208466 are Potent 5-HT$_6$ Receptor Agonists[a]

	K_i (nM)	EC_{50} (nM)	E_{max} (nM)
WAY-181187	2.2 ± 0.3	6.6 ± 0.8	93.3 ± 2.1%
WAY-208466	4.8 ± 0.3	7.3 ± 1.6	100 ± 0%

[a]Binding and cAMP functional data at human 5-HT$_6$ receptors.

Table 4 WAY-181187 and WAY-208466 are Selective over Other GPCRs[a]

	WAY-181187	WAY-208466
Receptor		
5-HT$_{1A}$	0%	0%
5-HT$_{1B}$	36%	30%
5-HT$_{1D}$	21%	16%
5-HT$_{1F}$	40%	22%
5-HT$_{2A}$	25%	351 nM
5-HT$_{2B}$	459 nM	313 nM
5-HT$_{2C}$ (Ant)	51%	217 nM
5-HT$_{2C}$ (Ago)	124 nM	644 nM
5-HT$_7$	1579 nM	4764 nM
D2	1%	0%
D3	1%	0%
D4	1%	0%
α1	1334 nM	0%

[a]% inhibition @ 1 μM drug concentration or K_i (nM) determination at respective receptor.

pretreatment with the 5-HT$_6$ receptor antagonist, SB-271046 (10 mg/kg, s.c.). In the same animals, acute treatment with WAY-181187 (3–30 mg/kg, s.c.) produced a modest yet significant ($P<0.0001$) decrease (27%) in cortical DA levels at the dose of 30 mg/kg (Figure 1b). This effect, however, was not observed at 3 or 10 mg/kg ($P=0.1423$ and $P=0.0990$, respectively) and could be effectively blocked by SB-271046 (10 mg/kg, s.c.). Similar to the dopaminergic effects, acute administration of WAY-181187 produced modest yet significant decreases in cortical 5-HT levels with a maximum decrease of 34 and 37% occurring at 10 ($P=0.0014$) and 30 ($P<0.0001$) mg/kg, respectively (Figure 1c). This effect was blocked by SB-271046 (10 mg/kg, s.c.) and was not observed at 3 mg/kg ($P=0.7842$). In contrast to these neurochemical effects, WAY-181187 (3–30 mg/kg, s.c.) did not alter the extracellular levels of glutamate or norepinephrine in the rat frontal cortex (Table 5).

In addition to the neurochemical effects of WAY-181187 being blocked by a 5-HT$_6$ receptor antagonist, WAY-181187-induced decreases in extracellular dopamine (Figure 1b) and 5-HT (Figure 1c) were reversed by an intracortical infusion of the GABA$_A$ receptor antagonist, bicuculline (10 μM delivered for 60 min through the microdialysis probes). These results, taken together with the data showing that WAY-181187 elicits robust changes in extracellular GABA levels, reveal an interaction between the 5-HT$_6$ receptor and GABAergic systems in the frontal cortex. The basal level of all measured neurotransmitters in this brain region were: GABA: 0.20 μM; glutamate: 0.40 μM; 5-HT: 379.15 fmol; dopamine: 71.27 fmol; norepinephrine: 36.98 fmol (per 10 μl sample).

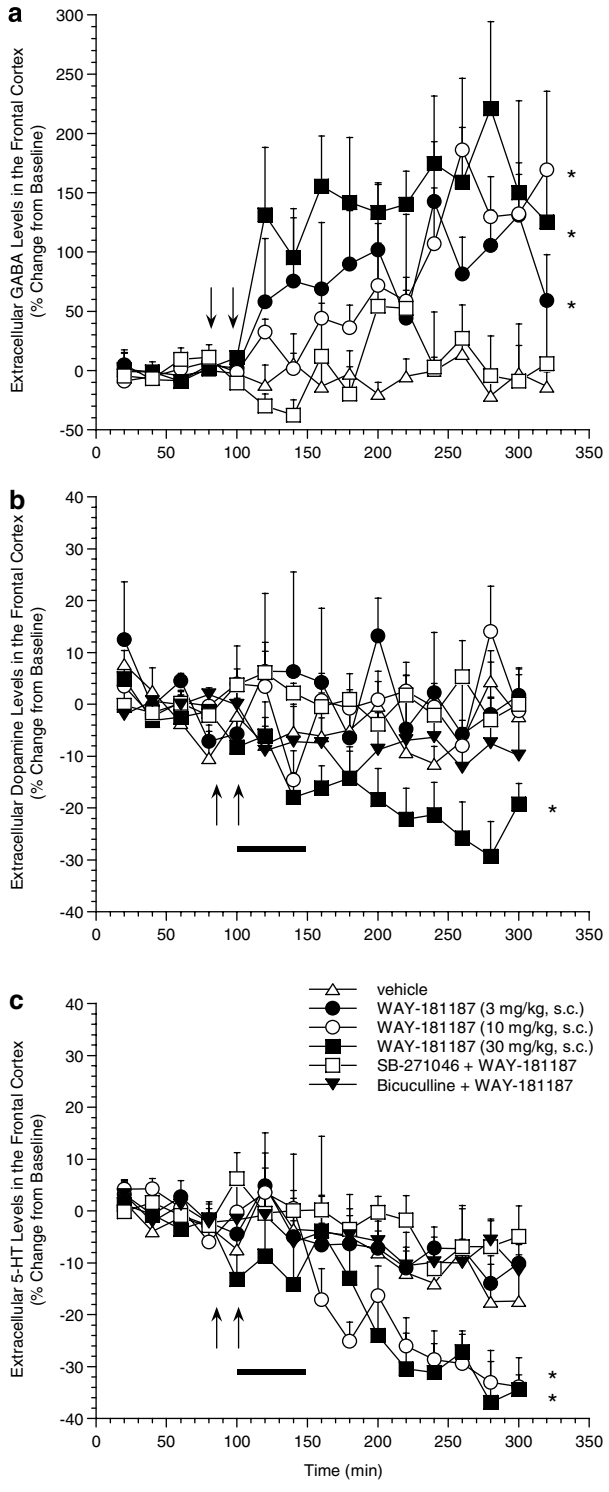

Figure 1 Neurochemical effects of WAY-181187 in the rat frontal cortex. (a) Acute administration of WAY-181187 (3–30 mg/kg, s.c.) significantly elevated the extracellular levels of GABA. The GABAergic effects of WAY-181187 (10 mg/kg) were blocked by a 20-min pretreatment with the 5-HT$_6$ receptor antagonist, SB-271046 (10 mg/kg, s.c.), confirming a role for the 5-HT$_6$ receptor in mediating this response. (b) Acute treatment with WAY-181187 produced modest yet significant decreases in cortical dopamine levels at the dose of 30 mg/kg, s.c. This effect was blocked by systemic SB-271046 treatment as well as an intracortical infusion of the GABA$_A$ receptor antagonist, bicuculline (10 μM for 60 min). (c) WAY-181187 (10 and 30 mg/kg, s.c.) significantly decreased 5-HT levels in the frontal cortex, an effect that was reversed by both 5-HT$_6$ (SB-271046, 10 mg/kg, s.c.) and GABA$_A$ (bicuculline, 10 μM local infusion) receptor antagonism. The arrows and black bar represent time of systemic and intracortical injections, respectively. $n=6$–10 rats per treatment group. A repeated measures ANOVA was performed and an asterisk denotes significant ($P<0.05$) treatment effect compared with vehicle treatment.

WAY-181187 Increases Extracellular GABA Levels in Corticolimbic Brain Regions

Figure 2a illustrates that acute treatment with WAY-181187 (10–30 mg/kg, s.c.) significantly elevated the extracellular levels of GABA in the rat striatum following both 10 ($P=0.0489$) and 30 ($P<0.001$) mg/kg. Maximal percent increases were 333 and 518% for doses of 10 and 30 mg/kg, respectively. Also in the striatum, a dose of 30 mg/kg, but not 10 mg/kg, significantly ($P=0.0005$ and $P=0.0992$, respectively) decreased the levels of dopamine (Figure 2b). At the 30 mg/kg dose, WAY-181187 produced a 34% decrease in striatal dopamine relative to vehicle-treated animals, an effect that was blocked by SB-271046 (10 mg/kg, s.c.). In contrast to these effects, acute treatment with WAY-181187 did not alter the extracellular levels of other neurotransmitters including norepinephrine, 5-HT, or glutamate in this brain region (data not shown). The

Table 5 Summary of the Acute Neurochemical Effects of WAY-181187[a]

	Dopamine	5-HT	Norepinephrine	Glutamate	GABA
Frontal cortex	27% decrease[b]	37% decrease[b]	NSE	NSE	220% increase[c]
Striatum	34% decrease	NSE	NSE	NSE	518% increase
Dorsal hippocampus	NSE	NSE	NSE	NSE	196% increase[c]
Amygdala	NSE	NSE	42% increase	NSE	215% increase
Nucleus accumbens	NSE	NSE	NSE	NSE	NSE
Thalamus	NSE	NSE	NSE	NSE	NSE

NSE, no significant effect observed.
[a]Maximal percent change from baseline at the dose of 30 mg/kg, s.c.
[b]Effects blocked by local infusion of the GABA$_A$ receptor antagonist, bicuculline (10 μM).
[c]Neurochemical effects blocked by the 5-HT$_6$ receptor antagonist, SB-271046 (10 mg/kg, s.c.).

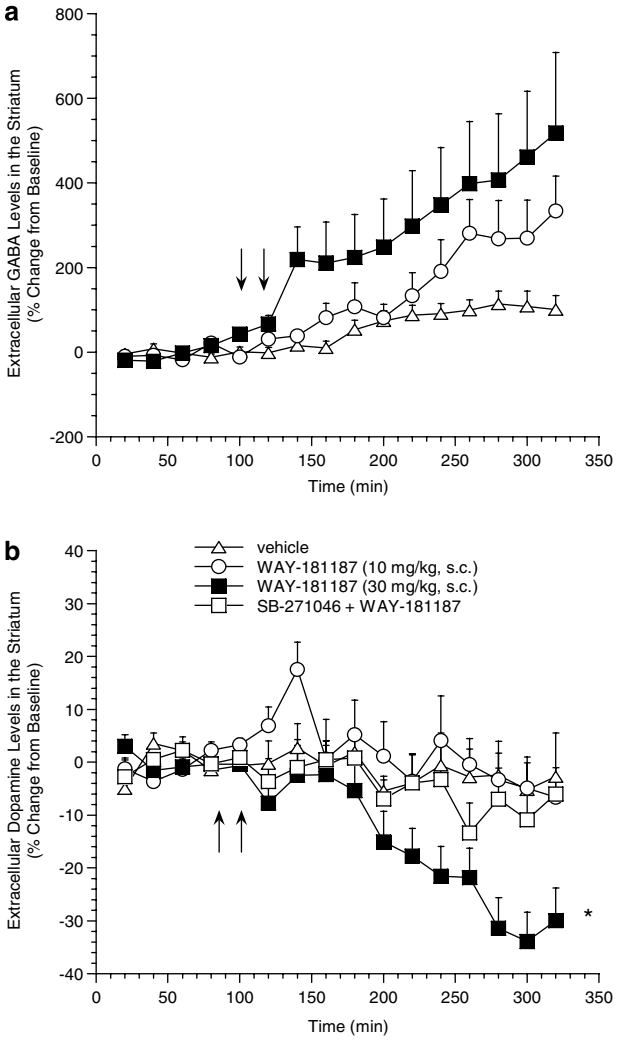

Figure 2 Neurochemical effects of WAY-181187 in the rat striatum. (a) Acute administration of WAY-181187 (10–30 mg/kg, s.c.) elevated the extracellular levels of GABA in the striatum. (b) Additionally, acute treatment with WAY-181187 significantly decreased striatal dopamine levels at the dose of 30 mg/kg, s.c., an effect blocked by systemic SB-271046 (10 mg/kg, s.c.) treatment. The arrows represent time of antagonist (or vehicle) and agonist (or vehicle) injection, respectively, drug injections. $n = 6$–10 rats per treatment group. A repeated measures ANOVA was performed and an asterisk denotes significant ($P < 0.05$) treatment effect compared with vehicle treatment.

basal level of all measured neurotransmitters in the striatum were: GABA: 0.03 μM; glutamate: 0.21 μM; 5-HT: 862.83 fmol; dopamine: 792.58 fmol; norepinephrine: 140.84 fmol (per 10 μl sample).

In addition to the frontal cortex and striatum, WAY-181187 (10–30 mg/kg, s.c.) significantly ($P < 0.05$) increased extracellular GABA concentrations in the dorsal hippocampus following the two doses tested (Figure 3). Maximal percent increases were 196 and 196% for doses of 10 and 30 mg/kg, respectively. The basal level of all measured neurotransmitters in the hippocampus were: GABA: 0.31 μM; glutamate: 0.98 μM; 5-HT: 311.05 fmol; dopamine: 185.86 fmol; norepinephrine: 8.61 fmol (per 10 μl sample).

Furthermore, in the rat amygdala, WAY-181187 (10 and 30 mg/kg, s.c.) produced robust increases in extracellular GABA concentrations (Figure 4a). The maximal increases in GABA were 188 and 215% above baseline at 10 and 30 mg/kg, respectively; however, because of variability in the vehicle-treated animals, the overall treatment effect of WAY-181187 in the amygdala did not reach statistical significance ($P = 0.3744$). Interestingly, acute treatment with WAY-181187 (10–30 mg/kg, s.c.) significantly ($P = 0.0046$) increased the extracellular concentrations of norepinephrine at 30 mg/kg (Figure 4b). The maximal effect at this dose was 42% above baseline. A concentration of 10 mg/kg resulted in a 29% increase in NE levels; however, this effect failed to reach statistical significance ($P = 0.0758$). The basal level of all measured neurotransmitters in the amygdala were: GABA: 51.96 μM; glutamate: 0.53 μM; 5-HT: 131.96 fmol; dopamine: 249.71 fmol; norepinephrine: 78.49 fmol (per 10 μl sample). A complete summary of the acute neurochemical effects of WAY-181187 is shown in Table 5.

Chronic 5-HT$_6$ Receptor Stimulation Increases GABA Levels in the Frontal Cortex

WAY-208466 was used to evaluate the neurochemical effects of chronic 5-HT$_6$ receptor stimulation. In the rat dorsolateral frontal cortex, 14-day treatment (10 mg/kg, s.c., q.d.) with WAY-208466 resulted in robust and significant ($P = 0.0175$) increases in extracellular GABA levels compared with animals that received chronic vehicle treatment (Figure 5a). In these studies, WAY-208466 elicited a maximal 745% increase in cortical GABA relative to

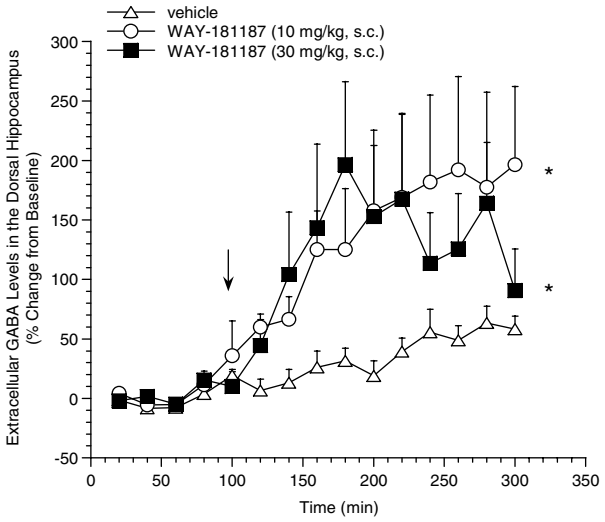

Figure 3 Acute treatment with WAY-181187 (10–30 mg/kg, s.c.) elevated the extracellular levels of GABA in the rat dorsal hippocampus. The arrow represents time of agonist (or vehicle) injection. $n = 6$–10 rats per treatment group. A repeated measures ANOVA was performed and an asterisk denotes significant ($P < 0.05$) treatment effect compared with vehicle treatment.

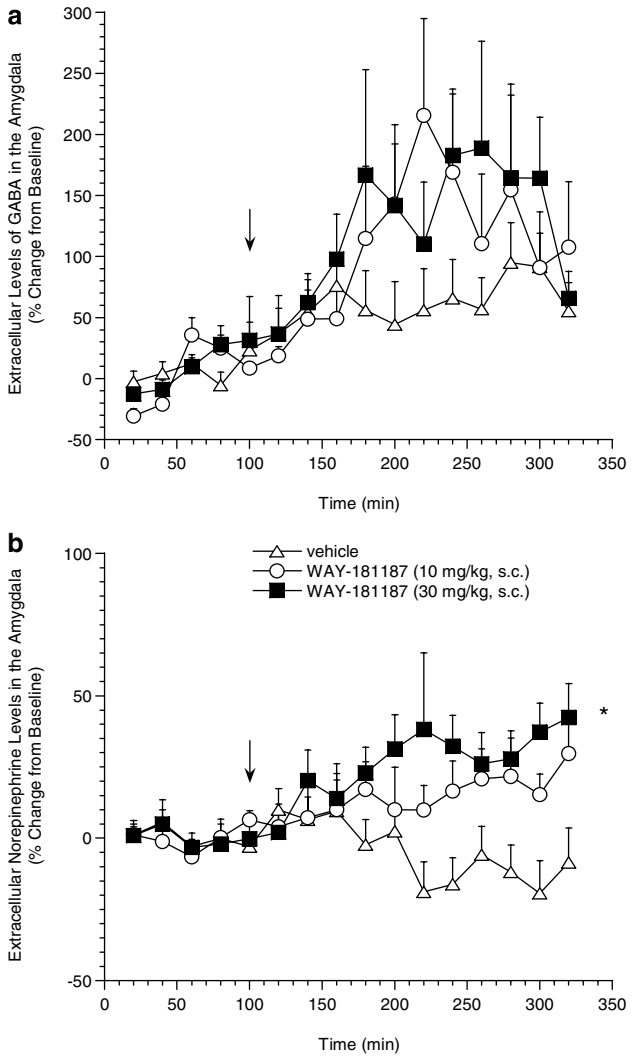

Figure 4 Neurochemical effects of WAY-181187 in the rat amygdala. Acute administration of WAY-181187 (10–30 mg/kg, s.c.) increased the extracellular levels of GABA (a) and norepinephrine (b) levels in the amygdala. The arrow represents time of agonist (or vehicle) injection. $n = 6$–10 rats per treatment group. A repeated measures ANOVA was performed and an asterisk denotes significant ($P < 0.05$) treatment effect compared with vehicle treatment.

baseline. This effect was greater than and significantly ($P = 0.0249$) different from the increase observed following the acute WAY-208466. Thus, in animals receiving chronic vehicle treatment, an acute challenge injection of WAY-208466 produced a maximal increase in GABA to 260% above baseline. However, due to variability in the vehicle-treated animals, the acute WAY-208466 treatment was not significantly different from vehicle ($P = 0.5539$). In contrast to the effects on GABA, the acute or the chronic treatment with WAY-208466 (10 mg/kg) did not significantly ($P > 0.05$) change extracellular levels of glutamate (Figure 5b) or norepinephrine, dopamine, or serotonin in these same animals (data not shown). In comparison, these overall neurochemical results, in particular, the magnitude and duration of the GABAergic effects of acute WAY-208446 treatment were similar to that induced by acute WAY-181187 (comparing Figure 1a with Figure 5a).

5-HT$_6$ Receptor Agonism Decreases Stimulated Glutamate Release

In hippocampal slice sections, sodium azide (10 mM) treatment significantly increased glutamate ($P = 0.0039$) release compared with vehicle treatment (Figure 6a). Pretreatment of WAY-181187 (0.1–10 μM) significantly and dose-dependently attenuated sodium azide-stimulated increases but did not alter basal amino-acid levels in this *in vitro* system. *P*-values comparing doses of WAY-181187 to sodium azide were as follows: glutamate—0.1 μM, $P = 0.1032$; 0.3 μM, $P < 0.0001$; 1 μM, $P = 0.0492$; 3 μM, $P < 0.0001$; 10 μM, $P < 0.0001$.

Figure 6b illustrates the effects of high KCl (50 mM) treatment on hippocampal glutamate release. 50 mM KCl significantly increased basal glutamate ($P = 0.0028$) release in hippocampal slices compared with vehicle treatment. Co-treatment of WAY-181187 (3–100 μM) significantly attenuated KCl-stimulated glutamate levels at several of the doses examined. *P*-values comparing doses of WAY-181187 to high KCl treatment were as follows: glutamate —3 μM, $P = 0.648$; 10 μM, $P = 0.6269$; 30 μM, $P = 0.0059$; 100 μM, $P = 0.0775$. It is worth pointing out that higher concentrations of these 5-HT$_6$ receptor agonists were used in the *in vitro* hippocampal studies. This is primarily the result of technical considerations in this assay, which requires higher doses of compounds to penetrate the hippocampal tissue slice; an observation that is similar to previously reported literature using the endogenous ligand, 5-HT, in a similar assay (Cayetanot *et al*, 2001).

5-HT$_6$ Receptor Agonism Displays Anti-Obsessive Compulsive Disorder-Like Activity in the Rat SIP Model

WAY-181187 dose-dependently decreased adjunctive drinking during a 1-h session. A dose of 178 mg/kg po WAY-181187 significantly decreased water intake

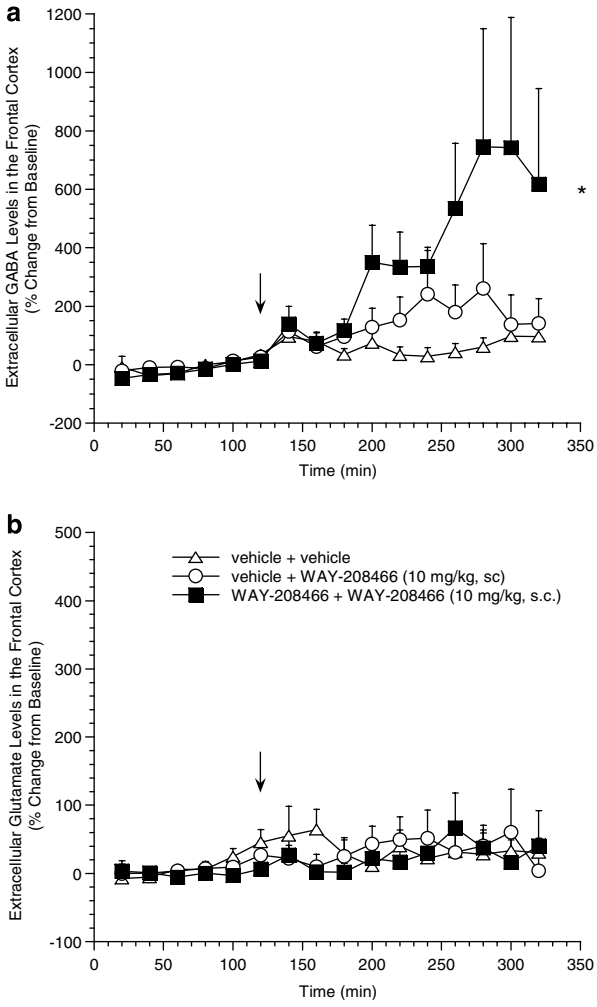

Figure 5 Neurochemical effects of WAY-208466 following acute and chronic treatment. (a) In the rat dorsolateral frontal cortex, 14-day treatment with WAY-208466 (10 mg/kg, s.c., q.d.) increased extracellular GABA levels compared with animals treated chronically with vehicle. Furthermore, in animals receiving chronic vehicle treatment, an acute challenge injection of WAY-208466 elicited a minimal increase in GABA in this brain region. (b) In contrast to the effects on GABA, acute or chronic treatment with WAY-208466 (10 mg/kg) did not significantly alter levels of glutamate in the rat frontal cortex. The arrows represent time of the agonist (or vehicle) challenge injection. $n = 6$–10 rats per treatment group. A repeated measures ANOVA was performed and an asterisk denotes significant ($P < 0.05$) treatment effect compared with vehicle treatment.

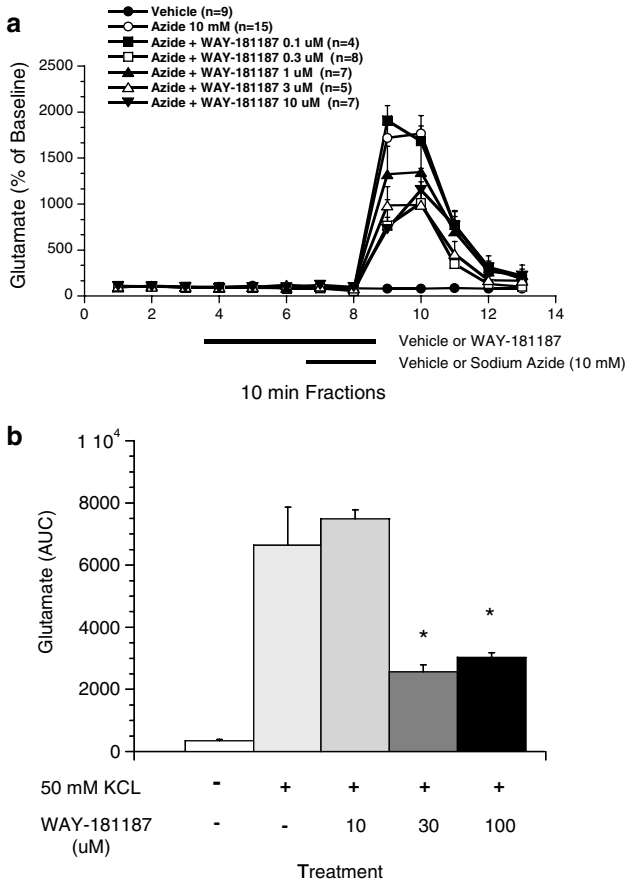

Figure 6 In hippocampal slice sections, sodium azide (10 mM) or high KCl (50 mM) treatment significantly increased release compared with vehicle treatment. (a) Pretreatment with WAY-181187 (0.1–10 μM) significantly and dose-dependently attenuated sodium azide-stimulated increases but did not alter basal amino-acid levels in this *in vitro* system. (b) Similarly, co-treatment with WAY-181187 (3–100 μM) significantly attenuated KCl-stimulated glutamate levels at several of the doses examined. An asterisk denotes significant ($P < 0.05$) treatment effect compared to KCl control.

(Figure 7), whereas lower doses (56 and 100 mg/kg, po) were not active in this model. These data indicate oral activity of this 5-HT$_6$ receptor agonist and were found to be similar to results observed after i.p. administration of either WAY-181187 or WAY-208466 (3–30 mg/kg, i.p.; data not shown). Importantly, under control conditions when all 60 pellets were given at the start of the session, WAY-181187 did not modify drinking (4.8 ± 0.67) compared with vehicle control (5.4 ± 1.15) indicating that nonspecific effects on drinking behavior were not observed (data not shown).

DISCUSSION

As the initial cloning of the 5-HT$_6$ receptor, there have been multiple investigations to suggest a role for this molecular protein in cognition. Early behavioral studies report that administration of various 5-HT$_6$ receptor antagonists produced a yawning and stretching syndrome that was blocked by muscarinic receptor antagonists (Bentley *et al*, 1999; Bourson *et al*, 1998). These studies supported previous work carried out with 5-HT$_6$ antisense (Bourson *et al*, 1995) and were suggestive of an interaction between 5-HT$_6$ receptors and central cholinergic systems. Given the purported role of the cholinergic system in cognitive function, 5-HT$_6$ receptor antagonists were subsequently examined in the Morris water maze, a test of spatial learning and memory. SB-271046, a competitive 5-HT$_6$ receptor antagonist, enhanced memory retention in the Morris water maze and reversed deficits in a novel object recognition task (Foley *et al*, 2004; King *et al*, 2004). These results were extended to other 5-HT$_6$ antagonists including SB-357134 in the water maze task and LY-483518 using the radial arm maze and two trial object recognition model (Rogers and Hagan, 2001; Woolley *et al*, 2001). Collectively, these positive behavioral effects strongly suggest that 5-HT$_6$ receptor antagonists may work to enhance cognitive processes, a hypothesis corroborated by neurochemical

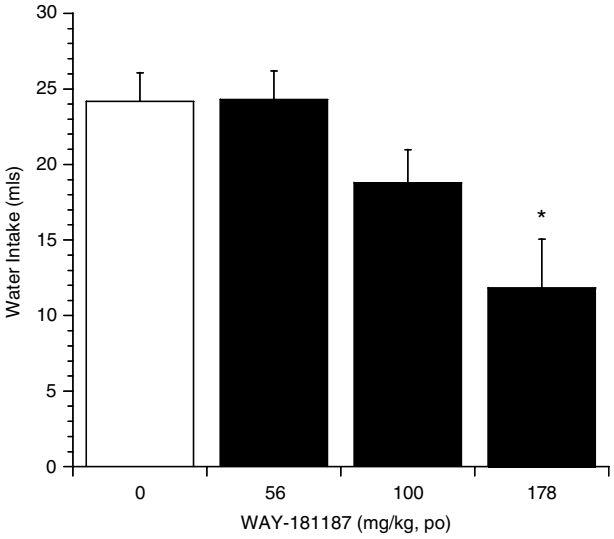

Figure 7 In the SIP model of obsessive-compulsive disorder, acute treatment with WAY-181187 dose-dependently decreased adjunctive drinking during a 2-h session. A dose of 178 mg/kg po WAY-181187 significantly decreased water intake, whereas lower doses (56 and 100 mg/kg, po) were not active in this model. Importantly, under control conditions when all 120 pellets were given at the start of the session, WAY-181187 did not modify drinking compared with vehicle control indicating that nonspecific effects on drinking behavior were not observed (data not shown). An asterisk denotes significant ($P<0.05$) compared to vehicle.

data showing that 5-HT$_6$ receptor antagonism increases levels of pro-cognitive neurotransmitters such as glutamate (Dawson *et al*, 2000) and acetylcholine (Riemer *et al*, 2003).

Whereas the data generated using selective 5-HT$_6$ antagonists suggests that the 5-HT$_6$ receptor can be involved in learning and memory, the lack of suitable 5-HT6 receptor agonists for *in vivo* study has hampered investigations to explore the biological significance of this 5-HT receptor subtype completely. Indeed, understanding the role of this receptor in regulating CNS activity is of considerable importance, but additionally, elucidating the biological significance of the 5-HT$_6$ receptor may also help to address the long-standing questions regarding which specific 5-HT receptor subtype, or subtypes, indirectly mediate the effects of antidepressants that target serotonergic receptor blockade as part of their mechanism of action. Herein, we report the pharmacological profile of two novel and selective 5-HT$_6$ receptor agonists: WAY-181187 and WAY-208466. *In vitro* studies revealed both compounds to be of high affinity and efficacy as 5-HT$_6$ receptor agonists relative to the pharmacological profile of the endogenous ligand (5-HT), while being at least 100-fold selective over a variety of other receptors, ion channels, and enzymes.

Employing *in vivo* microdialysis techniques, we found that acute administration of the selective 5-HT$_6$ agonist, WAY-181187, produced region-specific neurochemical changes in several areas of the brain associated with affective disorders. Thus, an acute systemic injection of WAY-181187 increased extracellular GABA concentrations in the rat frontal cortex, dorsal hippocampus, striatum, and amygdala, without altering levels of glutamate. In regions examined, we found that pretreatment with a 5-HT$_6$ receptor antagonist (SB-271046) blocked WAY-181187-induced elevations in GABA levels, indicating that 5-HT$_6$ receptor mechanisms mediate this response. In addition to these GABAergic effects, we observed modest, yet significant, decreases in cortical 5-HT and dopamine levels as well as decreases in striatal dopamine levels following acute WAY-181187 administration. These latter effects on cortical 5-HT and dopamine, in addition to be reversed by pretreatment with a 5-HT$_6$ receptor antagonist, were also found to be blocked by local infusion of a GABA$_A$ receptor antagonist. These results confirm a relationship between the 5-HT$_6$ receptor and GABAergic systems and are entirely consistent with a recent study showing dense colocalization of 5-HT$_6$ mRNA on GABAergic neurons in this brain region (Woolley *et al*, 2004; Ward and Dorsa, 1996).

In addition to the acute effects of 5-HT$_6$ receptor activation on GABA, the neurochemical effects of 5-HT$_6$ receptor agonism were studied following chronic treatment. Fourteen-day treatment with the 5-HT$_6$ receptor agonist, WAY-208466, resulted in robust elevations in the extracellular levels of GABA in the rat frontal cortex. In the same study, animals receiving an acute injection of WAY-208466 (ie injected chronically with vehicle) yielded robust increases in GABA concentrations. By comparison, acute treatment with WAY-208466 elicited similar elevations in GABA both in terms of magnitude and duration to that produced by WAY-181187 in the same brain region. It is also worth noting that the chronic neurochemical effects of this 5-HT$_6$ receptor agonist were found to be greater in magnitude to that elicited by acute treatment (800 *vs* 350%). Although these results were not found to be significantly different between the acute- and the chronic-treated animals, they do highlight the fact that 5-HT$_6$ receptor desensitization does not occur following chronic activation. This is in direct contrast to the desensitization observed in a previous *in vitro* study where rapid desensitization to the effects of 5-HT were observed in a HEK-293 cell line transfected with the human 5-HT$_6$ receptor (Max *et al*, 1995). Although these disparate results may be due to differences in comparing *in vitro vs in vivo* systems, the current microdialysis findings do suggest that neurochemical tolerance does not develop to the GABAergic effects of this 5-HT$_6$ receptor ligand *in vivo*. Furthermore, these results, consistent with data from WAY-181187, indicate that selective activation of the 5-HT$_6$ receptor preferentially elevates GABA levels in the corticolimbic brain regions.

The present results demonstrate that 5-HT$_6$ receptor stimulation by selective 5-HT$_6$ agonists increase extracellular levels of GABA. There is a compendium of data supporting the role of GABA in the treatment of mood disorder. Thus, impairment of GABAergic neurotransmission that is postulated to result from either deficits in levels of GABA in CSF and/or plasma or reduced GABA receptor sensitivity has been described in patients with a variety of anxiety disorders (Shiah and Yatham, 1998) or depressive illness (Leung and Xue, 2003; Petty, 1995; Sanacora *et al*, 2000). Moreover, current pharmacotherapy for the treatment of mood disorders includes benzodiazepines and a variety of antidepressants, which block monoaminergic reuptake and/or modulate monoaminergic neurotransmission. Despite the differential efficacy of these agents among the disorders, benzodiazepines and SSRIs or SNRIs, either directly or indirectly, modulate GABAergic neurotransmission.

Certainly, the strategic localization of serotonergic or noradrenergic neurons and their effects on GABAergic neurotransmission are well documented (Doze *et al*, 1991; Kawaguchi and Shindou, 1998; Smiley and Goldman-Rakic, 1996). Benzodiazepines act as positive allosteric modulators of $GABA_A$ receptors and have been shown to enhance signaling following receptor stimulation, whereas SSRI agents including fluoxetine or citalopram, appear to enhance levels of GABA as observed in recent imaging studies in humans using proton magnetic resonance spectroscopy (pMRS) (Bhagwagar *et al*, 2004; Sanacora *et al*, 2002; Shiah and Yatham, 1998). Notably, the clinical results in patients using pMRS demonstrate that increases in extracellular GABA is intriguing and one can hypothesize that this can be due to 5-HT6 receptor stimulation induced indirectly by fluoxetine or citalopram. Future experiments can address this from a preclinical setting but this may be more difficult to ascertain in the clinic. Indeed, although 5-HT_{2A} receptors are desensitized over a chronic treatment period with SSRIs (Goodnough and Baker, 1994; Klimek *et al*, 1994; Sanders-Bush *et al*, 1989), one can hypothesize that the 5-HT6 receptor may become a predominant signal mediating effects on GABA over the time period of chronic drug administration that coincides with the onset of efficacy. Taken together, it is interesting to speculate that the onset of efficacy in depression or anxiety, which are similar in the clinic following SSRI or SNRI administration is associated with 5-HT_6 receptor stimulation during long-term treatment.

The present *in vivo* results showing that WAY-181187 and WAY-208466 elevate GABA levels support the contention that 5-HT_6 receptors are colocalized with GABAergic neurons in the striatum (Ward and Dorsa, 1996) and with the GABA synthesizing enzyme, glutamic acid decarboxylase, in the rat cortex and hippocampus (Woolley *et al*, 2004). Based on the present neurochemical data, along with previous localization studies, it appears that 5-HT_6 receptors may tonically modulate the activity of GABAergic interneurons, which can indirectly modulate glutamatergic neurotransmission (Figure 8). These data, which demonstrate that 5-HT_6 agonists can increase extracellular GABA but not affect basal levels of glutamate, suggest that this system is tonically active. This hypothesis is further strengthened by the present report showing that when glutamate levels are stimulated by either KCl or Na-azide, 5-HT_6 receptor stimulation was able to decrease these levels. Thus, under conditions (ie due to stress) where levels of glutamate may be enhanced, 5-HT_6 receptors may be critically positioned to return the system to physiologically relevant levels. In this regard, it is important to note that Sanacora *et al* (2004) have observed higher glutamate levels in depressed patients compared with healthy aged match controls. Indeed, preclinical studies implicating glutamate in affective disorders, including depression, have shown that dampening glutamatergic transmission via NMDA receptor antagonists elicits antidepressant-like activity in preclinical behavioral models of depression (reviewed in Paul and Skolnick, 2003; Yilmaz *et al*, 2002). More recently, preliminary clinical trials with ketamine, a noncompetitive NMDA antagonist, have demonstrated that an acute dose induced a long-lasting antidepressant effect in a treatment resistant depressed patient population (Zarate *et al*, 2006), which supports the notion that attenuating glutamatergic

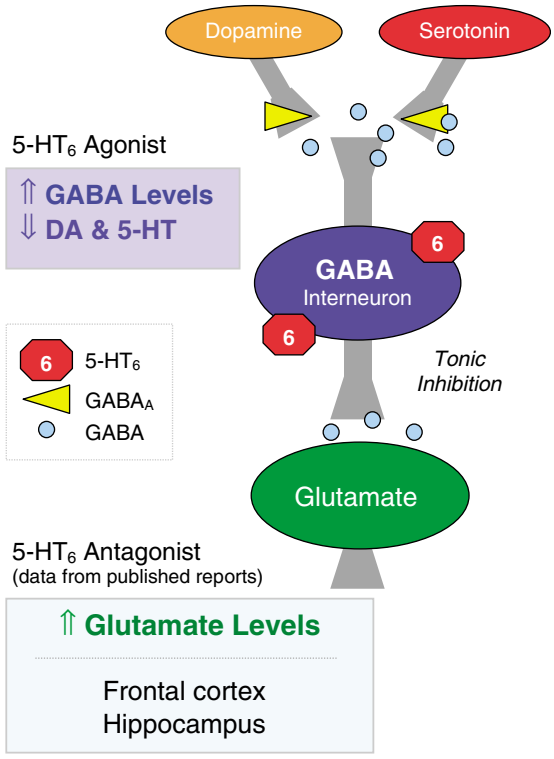

Figure 8 The proposed neurochemical circuitry for 5-HT_6 receptors to influence both GABA and glutamate transmission. The results of the present microdialysis studies show that WAY-181187 and WAY-208466 elevate GABA levels *in vivo*. These findings support the contention that 5-HT_6 receptors are colocalized with GABAergic neurons in a variety of brain corticolimbic brain regions. Based on these data, it is suggested that 5-HT_6 receptors tonically modulate the activity of GABAergic interneurons, which can indirectly modulate glutamatergic neurotransmission. These data, which demonstrate that 5-HT_6 agonists can increase extracellular GABA but not affect basal levels of glutamate, suggest that this system is tonically active. Moreover, the present results, taken together with previous published reports that 5-HT_6 receptor antagonism increases central glutamate levels, indicate that the 5-HT_6 receptor is critically positioned to modulate both inhibitory (GABA) and excitatory (glutamate) systems.

neurotransmission can be beneficial. Taken together, the ability of a 5-HT_6 agonist to enhance extracellular GABA levels and decrease stimulated glutamatergic neurotransmission support the hypothesis that 5-HT_6 receptor agonists may be effective agents for the treatment of depression or anxiety disorders, especially under such conditions when glutamate levels are enhanced under psychopathologic conditions.

In addition to the neurochemical results, behavioral investigations revealed an anxiolytic role for selective 5-HT_6 receptor agonists. Thus, WAY-181187 (Figure 7) and WAY-208466 (data not shown) effectively decreased adjunctive drinking behaviors in the SIP model. In comparison, both 5-HT_6 receptor agonists produced dose-related effects when administered acutely by various routes of administration (i.p. *vs* po). This model has been considered to be predictive for efficacy in obsessive compulsive disorder (Hogg and Dalvi, 2004), insomuch as the increased adjunctive drinking reflects an excessive manifestation of a normal behavior (ie drinking). Given that animals working in this model are not water deprived, this

excessive drinking cannot be easily explained by physiological mechanisms. Pharmacological studies demonstrate that SSRIs can decrease adjunctive drinking under SIP procedures; however, SSRIs typically have to be administered chronically and as such have a delayed onset, which corresponds to the clinical findings (Woods *et al*, 1993). These behavioral results suggest a novel therapeutic role for 5-HT$_6$ receptors in treating some types of anxiety disorders. Moreover, the results of the present microdialysis studies that show enhanced extracellular GABA levels following administration of WAY-181187 or WAY-208466 are consistent with a potential anxiolytic effect. This hypothesis is further strengthened by previous behavioral work showing that downregulation of 5-HT$_6$ receptor expression (via antisense oligonucleotides) is anxiogenic in two models of anxiety (Hamon *et al*, 1999; Otano *et al*, 1999).

Defining the biological role of the 5-HT$_6$ receptor is important from several standpoints. In the area of depression, it has been of interest to elucidate which of the multiple serotonin receptors are indirectly stimulated through the nonselective mechanism of SSRIs/SNRIs, which enhance extracellular 5-HT levels in multiple brain regions. Recent evidence implicates a potential role for various neurotrophins such as brain-derived neurotrophic factor or growth factors in mediating the therapeutic effects of antidepressants (Duman, 2004). Interestingly, a recent report suggest that the 5-HT$_6$ receptor agonist, LY586713, increased BDNF mRNA expression in the hippocampus following both acute and chronic administration; these effects were shown to be blocked by a 5-HT$_6$ receptor antagonist (De Foubert *et al*, 2004). We have extended these findings to show that WAY-208466 elicits marked elevations in BDNF protein expression (unpublished observation). Although growth factors levels were not studied in the present experiments, the findings by De Foubert *et al* (2004) suggest that 5-HT$_6$ receptor agonists may exhibit antidepressant activity via upregulating levels of trophic factors such as BDNF.

In summary, 5-HT$_6$ receptor localization, coupled to the neurochemical profile of WAY-181187 and WAY-208466, strongly suggests that activation of the 5-HT$_6$ receptor may be an effective treatment for some types of anxiety disorder. Moreover, a selective and potent 5-HT$_6$ agonist may have added advantages over currently available therapies including acute onset of action, which would be in marked contrast to the delayed onset observed for the SSRI antidepressants. The specificity in targeting the 5-HT$_6$ receptor may also have added therapeutic benefit because the stimulation of multiple serotonin receptors by SSRIs could possibly result in opposing mechanisms of action or deleterious side effects. In conclusion, the intriguing neurochemical profile of these novel and selective 5-HT$_6$ receptor agonists supports the rationale for investigating its potential therapeutic benefit in the clinical management of anxiety and depressive disorders.

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DISCLOSURE/CONFLICT OF INTEREST

The author(s) declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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