Pharmacological Properties and Procognitive Effects of ABT-288, a Potent and Selective Histamine H₃ Receptor Antagonist


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ABSTRACT

Blockade of the histamine H₃ receptor (H₃R) enhances central neurotransmitter release, making it an attractive target for the treatment of cognitive disorders. Here, we present in vitro and in vivo pharmacological profiles for the H₃R antagonist 2-[4-((3aR,6bR)-5-methyl-hexahydro-pyrrolo[3,4-aR]pyrrol-1-yl)-biphenyl-4-yl]-2H-pyridazin-3-one (ABT-288). ABT-288 is a competitive antagonist with high affinity and selectivity for human and rat H₃Rs (Kᵢ = 1.9 and 8.2 nM, respectively) that enhances the release of acetylcholine and dopamine in rat prefrontal cortex. In rat behavioral tests, ABT-288 improved acquisition of a five-trial inhibitory avoidance test in rat pups (0.001–0.03 mg/kg), social recognition memory in adult rats (0.03–0.1 mg/kg), and spatial learning and reference memory in a rat water maze test (0.1–1.0 mg/kg). ABT-288 attenuated methamphetamine-induced hyperactivity in mice. In vivo rat brain H₃R occupancy of ABT-288 was assessed in relation to rodent doses and exposure levels in behavioral tests. ABT-288 demonstrated a number of other favorable attributes, including good pharmacokinetics and oral bioavailability of 37 to 66%, with a wide central nervous system and cardiovascular safety margin. Thus, ABT-288 is a selective H₃R antagonist with broad procognitive efficacy in rodents and excellent drug-like properties that support its advancement to the clinical area.

Introduction

Histamine is a biogenic amine that is recognized as an important neurotransmitter involved in a number of physiological responses in humans. The variety of effects mediated by histamine is the result of the activation of H₁, H₂, H₃, and H₄ receptor subtypes. The specificity of histamine responses depends on the differential pharmacology, molecular biology, signal transduction characteristics, and localization exhibited by these four receptors (Hough, 2001; Bakker et al., 2002; Haas et al., 2008). H₃ and H₄ receptor antagonists have been very successful therapeutic agents in the treatment of allergic reactions and gastric ulcers, respectively.

Histamine is an important neurotransmitter in the CNS, regulating a variety of functions including attention, cognition, sleep, and weight homeostasis (Passani et al., 2004; Esbenshade et al., 2008; Brioni et al., 2011). Histamine is released from histaminergic neurons that originate in the hypothalamic tuberomammillary nucleus and project throughout most of the brain including cortex, striatum, thalamus, hippocampus, locus

ABBREVIATIONS: CNS, central nervous system; H₁R, H₁ receptor; AD, Alzheimer’s disease; SHR, spontaneously hypertensive rat; (R)-α-MeHA, (R)-α-methylhistamine; HEK, human embryonic kidney; EFS, electric field stimulation; GTPγS, guanosine 5′-O-(3-thio)triphosphate; ANOVA, analysis of variance; hERG, human ether-a-go-go-related gene; Veh, vehicle; ABT-288, 2-[4-((3aR,6aR)-5-methyl-hexahydro-pyrrolo[3,4-b]pyrrol-1-yl)-biphenyl-4-yl]-2H-pyridazin-3-one; BF2.649, 1-(3-(4-(chlorophenyl)propoxy)propyl)piperidine; GSK189254, 6-([3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl]oxy)-N-methyl-3-pyridinecarboxamide; GSK207040, 5-(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)oxy)-N-methyl-2-pyrazinylbenzonitrile; A-349821, 4-{3-[4-(3-pyrrolidin-1-ylpropoxy)phenyl]methoxy}benzonitrile; CEP-26401, 6-(4-(3-(2-methyl-pyrrolidin-1-yl)propoxy)phenyl)-2H-pyridazin-3-one; ABT-239, 4-[(2R,2S)-2-methyl-pyrrolidinyl]ethanol-benzofuran-5-ylbenzonitrile; A-349821, 4′-[(3aR,6aR)-5-dimethyl-pyrrolidin-1-yl]-propoxy)-biphenyl-4-yl-methanone.
coeruleus, and spinal cord. The H₃R was originally described as a presynaptic autoreceptor that, when stimulated, inhibits the release of histamine in the brain (Arrang et al., 1983). It also functions as a heteroreceptor that can inhibit the release of other neurotransmitters (Schlicker et al., 1988, 1989, 1993; Clapham and Kilpatrick, 1992; Blandina et al., 1996; Baldi et al., 2005). The H₃R is localized in rodent and human brain regions, with high expression in both species in the prefrontal cortex, hippocampus, and hypothalamus, regions that are associated with histaminergic innervation and responsible for learning, memory, and sleep (Martínez-Mir et al., 1990; Lovenberg et al., 1999; Drutel et al., 2001).

It is well established that histamine H₃R antagonists can enhance the release of multiple neurotransmitters involved in vigilance, alertness, and cognitive enhancement including histamine by blockade of H₃ autoreceptors (Arrang et al., 1983) as well as the release of acetylcholine, norpinephrine, dopamine, and serotonin via the blockade of H₃ heteroreceptors (Arrang et al., 1983; Blandina et al., 1998). These findings, in addition to the wide central nervous system projection of histaminergic neurons and localized expression of H₃Rs in cortical and limbic system areas, have made this receptor an attractive drug target for treating cognitive dysfunctions such as Alzheimer’s disease (AD), attention deficit hyperactivity disorder, and cognitive deficits of schizophrenia (Esbenshade et al., 2006, 2008; Brownman et al., 2009; Celanire et al., 2009; Passani et al., 2009; Brioni et al., 2011).

Considerable efforts have been expended to developing H₃R antagonists. First, imidazole-based H₃R antagonists such as thioperamide (Arrang et al., 1987), ciperoxan (Ligneau et al., 1998), clobenpropit (Barnes et al., 1993), and cipralisant (Tedford et al., 1998) were made, and although they were useful pharmacological tools, they suffered pharmacokinetic and metabolic issues that halted their development as human therapeutics (Esbenshade et al., 2008). More recently, novel selective nonimidazole H₃R antagonists (Esbenshade et al., 2008; Celanire et al., 2009; Leurs et al., 2011), including pitolisant [1-(3-(3-(4-chlorophenyl)propoxy)propyl)piperidine (BP2.649)], 6-(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-y1)oxy-N-methyl-3-pyrindinecarboxamide (GSK189254), 5-((3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-y1)oxy)-N-methyl-2-pyrazinecarboxamide (GSK207040), 2-methyl-3-(3-(3-pyrrolidin-1-yl)propoxy)phenyl)-5-trifluoromethyl-3H-quinazolin-4-one (MK-0249), 1-3-(4-(piperidin-1-ylmethyl)phenyl)propyl)piperidine (JNJ-17216498), and irudasant [6-(4-(3-(2-methylpyrrolidin-1-yl)propoxy)phenyl)-2H-pyridazin-3-one (CEP-26401)] have been advanced to early human trials for a variety of indications. Although preclinical data for some of these compounds are limited, pitolisant has been reported to increase acetylcholine and dopamine release in the rat cortex, reverse the cognitive deficits induced by scopolamine in the object recognition model, and exhibit antipsychotic potential in animal models (Ligneau et al., 2007a,b; Schwartz, 2011). Both GSK189254 and GSK207040 have been shown to enhance neurotransmitter release in rat cortex (Medhurst et al., 2007; Southam et al., 2009). GSK189254 reversed scopolamine-induced amnesia in an inhibitory avoidance assay and has shown efficacy in water maze and object recognition tests (Medhurst et al., 2007), whereas irudasant improved performance in the rat social recognition model of short-term memory (Raddatz et al., 2012). We reported previously that 4-(2-[2-[(2R)-

### Materials and Methods

#### Chemicals

- ABT-288 (Cowart et al.; WO2007/100990; Fig. 1), ABT-239, [³H]4-(3-((R,R)2,5-dimethyl-pyrrolidin-1-yl)-propoxy)-biphenyl-4-yl-morpholin-4-yl-methanone (A-349821) (24–36 Ci/mmol), and ciperoxan were synthesized at Abbott Laboratories. [³H]-N-α-methylhistamine (45–90 Ci/mmol), [³H]pyrilamine (20–30 Ci/mmol), [³H]tiotidine (70–90 Ci/mmol), [³H]histidine (40–60 Ci/mmol), and [³P]GTP·γ·S (1250 Ci/mmol) were obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA), and [³H]histamine (30–60 and 60–85 Ci/mmol) was from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). (R)-α-methylhistamine ([R]-α-MeHA) was purchased from Tocris Bioscience (Bristol, UK), and thioperamide was purchased from Sigma (St. Louis, MO).

#### Animals

Male spontaneously hypertensive rat (SHR) pups for repeated acquisition avoidance studies were obtained from Harlan (Indianapolis, IN) at postnatal days 7 and housed at Abbott Laboratories until their use on postnatal days 20 to 24 (body weights ranged from 35 to 50 g). Pups were housed up to 12 per cage (average of two litters) and fostered with Long-Evans lactating females (two per cage), largely to avoid the poor maternal care of SHR females and possible associated effects on brain and cognitive development (Fox et al., 2002). Adult (350–450 g) and juvenile (75–100 g) male Sprague-Dawley rats for social recognition studies, adult (250–350 g) male Sprague-Dawley rats for in vitro tissue assays and general observation studies, and adult Long-Evans rats for water maze studies (300–400 g) were obtained from Charles River Breeding Laboratories (Portage, MI). Sprague-Dawley rats for microdialysis studies (350–380 g) were purchased from Elevage Janvier (LeGenest Saint Isle, France). Male CD-1 mice were obtained from Charles River Breeding Laboratories at approximate 20 to 25 g body weight for methamphetamine hyperactivity studies. All rodents were housed in a quiet room under conditions of 12-h lights on/off (on at 6:00 AM), with food and water available ad libitum. Rats for EEG studies were housed singly. Male Hartley guinea pigs (weighing from 150–200 g on arrival) were supplied by Charles River Breeding Laboratories. All testing occurred during the light phase, and all experiments were conducted in accordance with guidelines from the Abbott Animal Care and Use

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**Fig. 1.** Chemical structure of ABT-288.
Committee and the National Institutes of Health’s Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. The animals were acclimated to laboratory conditions for at least 1 week before testing in a temperature-regulated environment with lights on between 6:00 AM and 6:00 PM.

**Radioligand Binding Assays**

For H₃R competition binding, membrane preparations were made from human embryonic kidney (HEK) cells expressing the full-length (445 amino acids) human histamine H₃R (Lovenberg et al., 1999) and the full-length rat histamine H₃R and brain cerebral cortices from humans (Analytical Biological Services, Wilmington, DE) and rats and mice (PelFreez, Rogers, AR) as described previously (Esbenshade et al., 2005). Membranes were incubated with [³H]N-α-methylhistamine (0.5–1.0 nM) in the presence or absence of increasing concentrations (from 5 to 11 concentrations over a five-log unit range) of competing ligands for 30 min at 25°C in a final volume of 0.5 ml of 50 mM Tris/5 mM EDTA, pH 7.4 buffer as described previously (Esbenshade et al., 2005; Strakhova and Esbenshade, 2007). Nonspecific binding was defined with 10 μM thioperamide. Cloned human histamine H₃, H₂, and H₁ receptor radioligand binding assays were performed as described previously (Esbenshade et al., 2005; Strakhova and Esbenshade, 2007) using [³H]يميپرامين, [³H]هیستامین, and [³H]هیستامین، respectively. All binding reactions were terminated by vacuum filtration onto polyethyleneimine (0.3%) presoaked Unifilter plates (PerkinElmer Life and Analytical Sciences) or Whatman GF/B filters (for human cortex H₂R and human H₂R receptor) followed by three brief washes with 2 ml of ice-cold 50 mM Tris/5 mM EDTA buffer. Liquid scintillation counting was used to determine bound ligand.

IC₅₀ values and Hill slopes were determined by Hill transformation for all of the radioligand competition binding data as described previously (Strakhova and Esbenshade, 2007). Kᵣ values were determined by the Cheng-Prusoff equation (Cheng and Prusoff, 1973), and the data are presented as the mean Kᵣ with 95% confidence intervals. The data were reanalyzed by using GraphPad Prism (GraphPad Software, Inc., San Diego, CA) and best-fit to a one-site binding curve.

**Receptor Occupancy Studies**

Receptor occupancy by ABT-288 was assessed by an established in vivo method using the H₃R antagonist ligand [³H]A-349821 as radiotracer (Miller et al., 2009). Various doses of unlabeled ABT-288 were administered intraperitoneally in a volume of 1 ml/kg, and [³H]A-349821 (1.5 μg/kg i.v.) was subsequently administered 30 min later. After an additional 30 min postdosing, the rats were anesthetized with CO₂ and decapitated, and blood and brain tissues were collected for quantification of unlabeled ABT-288 and [³H]A-349821 levels, respectively. The brain tissues were solubilized with Solvable and [³H]histamine, respectively. All binding reactions were terminated by vacuum filtration onto polyethyleneimine (0.3%) presoaked Unifilter plates (PerkinElmer Life and Analytical Sciences) or Whatman GF/B filters (for human cortex H₂R and human H₂R receptor) followed by three brief washes with 2 ml of ice-cold 50 mM Tris/5 mM EDTA buffer. Liquid scintillation counting was used to determine bound ligand.

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**[³⁵S]GTP·S Binding Assay**

HEK cells expressing the human H₁R or C6 cell membranes expressing the rat H₁R were prepared by homogenization in cold buffer containing 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 mM MgCl₂, and protease inhibitors. The homogenate was centrifuged twice at 20,000 g for 20 min at 4°C, and the resulting pellet was resuspended in buffer containing 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 10 mM MgCl₂. Glycerol and bovine serum albumin were added to a final concentration of 10% glycerol and 1% bovine serum albumin before freezing the membranes. The inverse agonist activity of H₁R antagonists was determined as described previously (Esbenshade et al., 2005). In brief, membranes were diluted in GTP·S assay buffer (25 mM HEPES, 2.5 mM MgCl₂, and 75 mM NaCl, pH 7.4), and 10 μg of membrane protein was incubated in a 96-well deep-well block in the presence of 0.5 μM unlabeled GDP, approximately 0.5 nM of [³⁵S]GTP·S, and various concentrations of H₁R antagonists. Samples were subsequently incubated at 37°C for 20 min. For assays to determine antagonist activity, (R)-α-MeHA (30 mM for human and 300 mM for rat H₁R) was added in addition to the assay components described above, and the samples were incubated at 37°C for 5 min. The assays were terminated by the addition of cold buffer (50 mM Tris-HCl, 75 mM NaCl, and 2.5 mM MgCl₂, pH 7.6) and subsequent harvesting by vacuum filtration onto a Packard Unifilter 96-well GF/B plate (PerkinElmer Life and Analytical Sciences). After extensive washing, the plates were dried, Microscint 20 was added to the samples, and the amount of bound [³⁵S]GTP·S was determined by using Topcount (PerkinElmer Life and Analytical Sciences). The bound [³⁵S]GTP·S in each sample was expressed as a percentage of that bound to control samples incubated in the absence of histamine H₁R ligands. Triplicate determinations were obtained at each concentration, and the data were analyzed by using GraphPad Prism to obtain EC₅₀ values for inverse agonism. The potency (pKᵣ) of antag-
onists to inhibit the (R)-α-MeHA response was calculated according to the method of Schild (1947). The mean ± S.E.M. of at least three independent experiments is reported.

**Microdialysis Levels of Acetylcholine, Dopamine, and Histamine**

For pain prophylaxis, animals were dosed preoperatively with rima-
dyl (5 mg/kg i.p.). Individual male Sprague-Dawley rats (290–320 g body weight) were anesthetized with pentobarbital (60 mg/kg i.p.; Narcoren, Rhone-Merieux, Lyon, France) were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and microdialysis guide can-
nulas (CMA/12; Axel Semrau GmbH, Sprockhöve, Germany) were im-
planted into the medial prefrontal cortex (anterior-posterior, 2.5; medi-
lateral, 0.6; tip of the 3-mm long active membrane, 3.2 mm below the
brain surface) according to Paxinos and Watson (1998). The guide
cannulas were secured with dental cement (Technovit powder, Tech-
notiv polymerization starter fluid, Kulzer GmbH, Dormagen, Ger-
many) and four anchor screws into the skull. The rats were allowed
to recover from surgery for 5 to 7 days. The day before the experi-
ment, each animal was transferred into a system allowing for free
movement (CMA/120; Axel Semrau GmbH), consisting of a plastic
bowl, wire attachment, counter balance arm, and swivel assembly
connecting in/outlet of the probe with the perfusion pump. Next, a
CMA/12 microdialysis probe (3-mm membrane length) was slowly
lowered into the final position. The probe was perfused with Ringer’s
solution (147 mM NaCl, 4.0 mM KCl, and 2.4 mM CaCl2, containing
0.1 μM neostigmine for the acetylcholine microdialysis study) for
approximately 1 h (CMA/102 microdialysis pump, Axel Semrau
GmbH; 1.5 μl/min). The probe was perfused again 24 h later for at
least 1 h before microdialysate fractions were collected every 20 min.
On the day of the experiment, ABT-288 or its vehicle (2 ml/kg) was
administered intraperitoneally. Six fractions before and six fractions
after the intraperitoneal administration of the test compound or
vehicle were analyzed for microdialysate levels of dopamine or ace-
tylosine by high-performance liquid chromatography with electro-
chemical detection.

**Assay of Microdialysate Acetylcholine Levels**

Ten microliters of each microdialysate fraction was injected onto a
reversed-phase column (MF 8908 Acetylcholine SepStik Kit; micro-
bore column, particle size 10 mm, 530 × 1.0 μm coupled to an
immobilized enzyme reactor 50 × 1.0 mm, particle size 10 μm,
containing acetylcholinesterase and choline oxidase; BAS Bioana-
litical Systems, West Lafayette, IN) using a refrigerated autosam-
pler (HTC PAL twin injector autosampler system; Axel Semrau
GmbH). The mobile phase consisted of 50 mM Na2HPO4, pH 6.5, and
5 ml/L Kathon (Dow, Midland, MI). Flow rate was 0.14 ml/min (Rheos
Flux pump; Axel Semrau GmbH), and the sample run time was less
than 15 min. Acetylcholine and choline were measured via an elec-
trochemical detector (LC-4C; BAS Bioanalytical Systems) with a
platinum working electrode set at +500 mV versus an Ag/AgCl
reference electrode. The system was calibrated by standard solutions
(acetylcholine and choline) containing 1 pmol/10 μl injection. Acetyl-
choline was identified by its retention time and peak height with an external standard method using chromatography software (Chrom
Perfect; Justice Laboratory Software, Denville, NJ). Microdialysis
data (area under curve for time 0–120 min was calculated by inte-
gration of the area by the trapezoidal rule) were evaluated for sig-
nificance by using one-way analysis of variance (ANOVA) followed by
Dunnett’s pair wise comparison post hoc test using GraphPad Prism
version 4.0 software.

**Assay of Microdialysate Dopamine Levels**

Ten microliters of each microdialysate fraction was injected onto a
reversed-phase column (Nucleosil C18; Macherey-Nagel, Düren,
Germany; 125 × 4.0 mm; 3-μm particle size) by using a refrigerated
autosampler (HTC PAL twin injector autosampler system; Axel Sem-
the current was slowly ramped up until the pup produced sustained vocalizations, after which the current was immediately ramped down until vocalizations ceased. These time points are referred to as iMAX and iMIN, respectively. To assess efficacy with repeated dosing, SHR pups were dosed for 5 days with an efficacious dose of ABT-288 (0.01 mg/kg s.c.). To further explore any effects of repeated dosing on efficacy, a second repeated-dosing study was conducted in which the maximally effective dose of ABT-288 (0.03 mg/kg s.c.) was administered for 4 days, and on day 5 the pups were dosed with 0.01 mg/kg s.c. ABT-288 and tested in the task 30 min later as described above in the acute assays. Data were analyzed by summing the responses from learning trials two to five. Statistical significance was determined by using two-tailed nonparametric Mann-Whitney U tests to compare drug treatment from vehicle (GraphPad Prism 4.0).

Adult Social Memory

Adult rats were trained in a social recognition memory test to recall prior exposure to a conspecific juvenile as described previously (Fox et al., 2005). In brief, adult rats (350–450 g) were separated into fresh test cages and allowed to habituate for 30 min. An unfamiliar juvenile was introduced, and overall investigation duration (grooming, sniffing, and close following) was recorded for a 5-min period. Both rats were then removed to their respective holding cages. After 90 min, the adult was replaced into the test cage, the same juvenile was reintroduced 30 min later, and overall investigation duration was again recorded during a second 5-min period. ABT-288 or saline vehicle was administered intraperitoneally to the adult rat immediately after the first exposure period. To control for nonspecific effects, immediately after the second investigation period, a new, unfamiliar juvenile was introduced to the same adult rat for a third 5-min period, and overall investigation duration was recorded once again. Social memory was quantified by determining, for each adult rat, the ratio of investigation duration of the second to the first investigation periods. Nonspecific effects were assessed by determining the ratio of investigation duration (third to the first investigation periods) for the unfamiliar juvenile. To assess efficacy with repeated dosing, rats were dosed for 5 days with ABT-288 (0.03 mg/kg i.p.) and tested according to the protocol described above for this task. Trial 2 testing took place 2 h after the last injection, which was immediately after trial 1 as described above. Group sizes were 15 to 16 animals. Data were analyzed by using one-way ANOVA with Dunnett’s post hoc analyses, comparing the response of drug-treated groups with the response of vehicle controls (GraphPad Prism 4.0).

Two-Choice Discrimination Water Maze

Adult male Long-Evans rats (Charles River Breeding Laboratories) were trained in a two-choice visual discrimination water maze test of spatial learning and reference memory as described previously (Komater et al., 2005). This version of the water maze is known to be sensitive to age, septal lesions, and scopolamine-induced impairments and is proposed as a model that may be pertinent to cognitive deficits associated with Alzheimer’s disease. Two daily habituation sessions were conducted in which each rat was trained to find a visible escape platform in a pool (180 cm diameter and 60 cm high) filled to a depth of 37 cm with water made opaque with powdered milk. Water temperature was maintained at 26°C. On the second day of habituation training, a latency to escape measure was obtained to assure that animals are assigned to groups without swim speed bias. After a 2-day rest period, spatial discrimination testing was conducted by using two visually similar platforms, each covered in aluminum foil. The platforms remained in the same position throughout 5 days of training. However, only one of the platforms provided escape; the other (incorrect), made of Styrofoam, floated and did not support the weight of the animal. Each rat received six trials/day from one of the two starting positions that were alternated from trial to trial, and the number of contacts with the incorrect platform (errors) was recorded for each trial and averaged per day. Drugs or vehicle control were administered intraperitoneally each day before training in a volume of 1.0 ml/kg. ABT-288 (0, 0.01, 0.1, or 1 mg/kg) or ABT-239 (1 mg/kg) was administered 15 min before scopolamine (0.3 mg/kg), and the animals were tested 15 min later. Data were compared over days by using a two-way, repeated-measures ANOVA. Subsequent one-way ANOVAs were used to evaluate errors for each of the 5 training days with Dunnett’s post hoc analyses (GraphPad Prism 4.0).

Methamphetamine-Induced Hyperactivity

To evaluate the efficacy of ABT-288 in reversing methamphetamine-induced hyperactivity, mice were injected with ABT-288 (0.1, 0.3, or 1 mg/kg i.p.) or vehicle and placed separately into one of 16 acrylic open-field environments [42 (length) × 42 (base) × 40 cm (height); Piper Plastics, Libertyville, IL] situated inside Versamax/Digiscan monitors, each equipped with 32 horizontal and 16 vertical infrared sensors (AccuScan Instruments, Inc., Columbus, OH) in a dimly illuminated test room. The mice were allowed to habituate while baseline activity data were recorded for 60 min at which point they were injected with methamphetamine (1.0 mg/kg i.p.) and monitored for another 90 min. Data were analyzed by using one-way ANOVA and Dunnett’s post hoc test (GraphPad Prism 4.0).

Electroencephalogram Slow-Wave Activity

EEG recording electrodes were bilaterally implanted under pentobarbital anesthesia (50 mg/kg i.p.; Abbott Laboratories) over the parietal cortex (−2.0 mm anterior-posterior; 4.0 mm lateral) in 400-g male rats (Charles River Breeding Laboratories) maintained on a 12-h light/dark schedule. A reference electrode was placed 11 mm posterior to bregma, and a miniature connector was affixed to the skull. Implanted rats were allowed 2 weeks of recovery from the surgery before use. EEG (sampling rate 200 Hz) was recorded from previously habituated rats inside sound-attenuating chambers. Before experiments began, a flexible cable was attached to the implanted miniature connector that allowed the rats unrestricted movement during the recording sessions. Standard EEG amplifiers (Grass Instrument Division, Astro-Med, West Warwick, RI) and a computer-based system (Stellate Systems, Montreal, Canada) were used to acquire and analyze data. The average EEG amplitude in microvolts was determined by using fast Fourier transform analysis and broken down into an analysis of the 1- to 4-Hz slow-wave band activity. Dose-response effects on EEG were determined for intraperitoneal administration of ABT-288 and compared with saline vehicle. The treatments were administered in a random order on different days with one treatment per day and 3 days between each treatment. On one of these treatment days, the rat would receive a vehicle control treatment. This within-subjects design allowed each rat to serve as its own control. EEG recordings were conducted during the light cycle and began within 5 min after injection, and recording sessions lasted for 120 min. A total of eight rats was used in these studies.

Cardiovascular Parameters

hERG Binding and Function. HEK-293 cells, stably transfected with the hERG channel, were obtained from Dr. C. W. Janu-ary (Cardiology Division, University of Wisconsin, Madison, WI). For [3H]dofetilide competition binding to hERG channels, membrane preparations were incubated with [3H]dofetilide in the presence or absence of increasing concentrations of ABT-288 for 45 min at 37°C in a final volume of 0.2 ml of Tris buffer as described previously (Diaz et al., 2004). Isolated whole-cell patch-clamp electrophysiology experiments to determine IC50 values for functional inhibition of hERG channel activity were performed as described previously (Diaz et al., 2004).

Anesthetized Dog Model. Male beagle dogs (Marshall BioRe- sources, North Rose, NY, weighing 9.4 to 11.5 kg, were anesthetized with pentobarbital (35.0 mg/kg i.v.) and immediately placed on a
constant intravenous infusion of pentobarbital (6.0 mg/kg/h). Once anesthetized, the dogs were intubated with a cuffed endotracheal tube and ventilated with room air by means of a mechanical respiration pump (Harvard Apparatus Inc., Holliston, MA). Expiratory CO₂ was monitored with an end-tidal CO₂ monitor (Criticare Systems, Inc., Waukesha, WI) and maintained at 4 to 5% CO₂. Electrocardiogram limb leads were attached to the animals, and a lead II ECG was recorded. A Swan-Ganz catheter (5.5 F; Hospira, Inc., Lake Forest, IL) was advanced into the pulmonary artery via the right jugular vein for measurement of cardiac output by using a cardiac output computer (Oximetrix 3; Abbott Laboratories). Central venous and pulmonary artery pressures were measured through the proximal and distal ports of the catheter, respectively. A dual tip micro-manrometer catheter (model SPC-770, 7F; Millar Instruments Inc., Houston, TX) was advanced into the left ventricle of the heart via the right carotid artery for the measurement of left ventricular and aortic blood pressure. Polyethylene catheters were inserted into the right femoral vein and artery for infusion of test agents and collection of blood samples, respectively. Systemic vascular resistance was calculated as [(mean arterial pressure – mean central venous pressure)/cardiac output]. Pulmonary vascular resistance was calculated as [(pulmonary arterial pressure – central venous pressure)/cardiac output]. Body temperature was monitored throughout the experiment.

The primary hemodynamic variables were computed by using commercial software and a signal processing workstation (Ponemah; Gould Instrument Systems, Inc., Cleveland, OH). The electronic lead II ECG record was assessed for changes in QT interval (QTc, corrected for heart rate using Fridericia’s and Van de Water’s formulae) and the PR interval via the Ponemah system with manual over-reads conducted at 15-min intervals (Polakowski et al., 2009). ABT-288 was dissolved in vehicle (polyethylene glycol 400; Sigma) and administered by intravenous infusion (0.02 ml/kg/min) in two sets of experiments at 15-min intervals using microcapillary tubes. Whole blood samples were collected into standard blood tubes containing heparin, and post-treatment time point, two-sample, two-sided t tests. In addition, for each parameter at each treatment group, time point, and treatment group by time point interaction. Because the readings at the 10 time points (repeated measures) for each animal were correlated, a heterogeneous autoregression covariance matrix was used to account for the correlation of repeated measurements. The change in each hemodynamic parameter in antagonist-treated animals versus vehicle was also evaluated at each time point when the ANOVA model indicated a significant treatment group by time point interaction (p < 0.05). All statistical tests and comparisons were assessed at a 0.05 level of significance.

Pharmacokinetic Analysis

ABT-288 was selectively removed from the plasma or brain homogenate by using protein precipitation with acetonitrile at neutral pH. The samples were vortexed vigorously followed by centrifugation. The supernatant was transferred and evaporated to dryness with a gentle stream of nitrogen over low heat (−35°C). The samples were reconstituted by vortexing with mobile phase. ABT-288 and internal standard were separated from c-extracted contaminants on a 5 mm x 3 mm 3-μ Luna CN column with an acetonitrile/0.1% trifluoroacetic acid (30:70, by volume) mobile phase at a flow rate of 0.4 ml/min with a 25-μl injection. ABT-288 was quantified by using multiple reaction monitoring detection, m/z 373.3->343.2 using a turbo ionspray source on a PerkinElmerSciex Instruments (Boston, MA) mass spectrometer. The limits for quantification were approximately 1 ng/ml for plasma and 10 ng/g for brain samples. For more sensitive analysis of plasma and brain samples obtained from the H3 receptor occupancy and behavioral studies, a 5 mm x 3 mm 5-μ Aquasil C18 (Thermo Fisher Scientific, Waltham, MA) column was used with a 10-μl injection volume. All other parameters were the same as described previously (Fox et al., 2005). The limits for quantification were approximately 0.08 ng/ml for plasma and 0.25 ng/g for brain samples under these conditions.

Results

Histamine Receptor Binding

The H3R binding affinities for ABT-288, ABT-239, and ciproxifan were determined for the full-length recombinant rat or human H3Rs as well as for H3Rs present in rat, mouse, and human brain cortical membranes (Table 1). All three compounds exhibited high affinity for the recombinant rat H3R and rodent brain cortical membrane H3Rs with Kᵢ values less than 10 nM. ABT-288 was 4- to 8-fold more potent at human recombinant (Kᵢ = 1.9 nM) and brain cortical (0.64 nM) H3Rs than at the corresponding rat receptors in contrast to the more rat-selective H3R antagonist ciproxifan (Table 1). ABT-288 recognized a single high-affinity H3R binding site bound by the agonist radioligand [3H]N-α-methylhistamine.
as demonstrated by displacement curve Hill slopes that approached unity in each of the membrane preparations tested. ABT-288 exhibited no binding at concentrations up to 10 μM to human H₁, H₂, and H₄ receptors providing a more than 5000-fold H₃R selectivity versus other histaminergic receptors, whereas the imidazole ciproxifan was 20-fold selective for the human H₃R compared with the human H₄R (Table 1). In addition, a Cerep (Celle l’Evescault, France) binding screen of 85 other G protein-coupled receptor and ion channel targets revealed good selectivity for ABT-288 with more than 50% displacement of specific binding seen only at the 5-HT₁B (52%) and sigma (85%; Kᵢ = 760 nM) receptors, Na⁺ channel (51%), and 5-HT transporter (58%).

**Functional Antagonism and Inverse Agonism at Recombinant H₃Rs**

ABT-288 demonstrated competitive H₃R antagonism in Schild experiments, shifting the concentration response curves for (R)-α-MeHA-mediated stimulation of [³⁵S]GTPγS binding by human (Fig. 2A) and rat H₃Rs in a parallel, dextral manner with Schild slopes approaching unity and respective pA₂ values of 8.7 and 8.2 (Table 2). In these studies, whereas the basal [³⁵S]GTPγS binding was decreased up to 50% in a concentration-dependent manner by the inverse agonist activity of ABT-288 described below, the maximal agonist response to (R)-α-MeHA in the presence of ABT-288 was still achieved, indicating the competitive antagonism properties of this compound. The pA₂ values compared favorably to those for ABT-239 at both receptors as was seen in the binding assays, whereas ciproxifan exhibited lower antagonist potency at human H₃Rs. ABT-288 is also an inverse agonist, reversing basal [³⁵S]GTPγS binding mediated by human and rat H₃Rs in a concentration-dependent manner with respective EC₅₀ values of 3.78 and 7.2 nM, equipotent with ABT-239 at both receptors and more potent than ciproxifan at the rat H₃R (Table 2).

**Effects of ABT-288 in In Vitro Models of Neurotransmitter Release**

ABT-288 exhibited competitive antagonism by shifting the concentration response curves for (R)-α-MeHA-mediated reversal of EFS contractions of guinea pig ileal segments with a pA₂ value of 9.1, comparable with the potencies exhibited by both ABT-239 and ciproxifan (Table 2). In another model of neurotransmitter release, the activation of presynaptic H₃Rs inhibited the release of [³⁵S]histamine from rat brain synaptosomes caused by potassium-stimulated depolarization. This histamine-mediated reversal of [³⁵S]histamine release from rat synaptosomes was inhibited by ABT-288 in a concentration-dependent manner with a Kᵢ value of 3.2 nM (Fig. 2B), a somewhat greater antagonist potency in this model than that shown by ABT-239 (Table 2).

**Effects of ABT-288 on In Vivo Neurotransmitter Release**

ABT-288 dose-dependently increased microdialysate acetylcholine (Fig. 3, top) and dopamine (Fig. 3, middle) levels in the medial prefrontal cortex. Acetylcholine levels peaked at 40 to 60 min after dosing with an elevation in acetylcholine levels that lasted for up to 2 h seen at the higher doses. A similar profile for acetylcholine release was seen in the hippocampus although the amplitude of the increase in acetylcholine levels was smaller (data not shown). Higher doses of ABT-288 (3.0 and 10.0 mg/kg) were required to increase dopamine levels that also peaked at approximately 40 min after dosing with a sustained elevation seen for up to 2 h. One-way ANOVA for the area under the curve from time 0 to 120 min revealed a significant treatment effect for microdialysate acetylcholine (F₅,₄₀ = 6.41; p < 0.0002) and dopamine (F₅,₄₀ = 18.84; p < 0.0001) levels in the medial prefrontal cortex. Subsequent Dunnett’s pairwise comparisons revealed a significant increase of microdialysate acetylcholine (p < 0.05 at 0.3, 1.0, and 3.0 mg/kg) and dopamine (p < 0.01 at 3.0 and 10 mg/kg) levels compared with vehicle-treated animals in the medial prefrontal cortex after administration of ABT-288. It is noteworthy that dopamine levels were not increased.
Effects of ABT-288 in Rodent Models of Cognition

Five-Trial Inhibitory Avoidance in SHR Pups. ABT-288 treatment 30 min before training produced a significant improved performance compared with vehicle-treated rats (Fig. 4). Plasma samples collected from a separate set of pups revealed efficacious plasma concentrations of 0.1 to 2.0 ng/ml. Plasma levels of 1 ng/ml (at 0.01 mg/kg) were considered an indicator of preclinical cognitive efficacy to establish the safety index for this compound. To assess efficacy with repeated dosing, SHR pups were dosed for 5 days with the maximally efficacious dose of ABT-288 (0.01 mg/kg). After 5 days of repeated dosing, the efficacy of ABT-288 was maintained with observed mean crossover latencies of 309 ± 38 s (± S.E.M.) compared with acute treatment (342 ± 42 s) and vehicle control (175 ± 46 s), suggesting that the development of tolerance is unlikely. In addition, no diminution of the 0.01 mg/kg response was seen when animals were repeatedly dosed at the higher efficacious dose of 0.03 mg/kg for 4 days, then acutely tested on day 5 with the lower effective dose of 0.01 mg/kg (data not shown).

Social Recognition. Overall differences in the ratios of the ABT-288 treatment groups compared with vehicle were evaluated by applying a one-way ANOVA (F9,83 = 4.574; p < 0.05) with significance assessed by a subsequent Dunnett’s post hoc analysis. ABT-288 produced significant increases in recall performance (expressed as investigation ratio) ratio in the social recognition assay at doses of 0.03 and 0.1 mg/kg i.p., equivalent to that seen with ABT-239 (Fig. 5). Lower (0.01 mg/kg) and higher (0.3 mg/kg) doses of ABT-288 did not improve performance. To assess efficacy with repeated dosing in this model, rats were dosed for 5 days with 0.03 mg/kg ABT-288. There was no significant difference between repeatedly dosed animals and acutely dosed animals with observed investigation ratios of 0.71 ± 0.06 (mean ± S.E.M.) for the repeatedly dosed treatment and acute treatment (0.62 ± 0.06) compared with vehicle control (0.91 ± 0.06). ABT-288 did not exhibit any nonspecific effects in this task, as evidenced by a lack of an effect on the investigation of a novel juvenile (data not shown), indicating the effects in this model were not caused by general changes in investigational behavior.

Scopolamine Induced Deficit in Rat Two-Platform Discrimination Water Maze. Vehicle-treated rats normally learned the task over 5 days of testing, whereas rats treated with scopolamine (0.3 mg/kg i.p.) exhibited impaired discrimination learning in this assay as demonstrated by a consistent number of errors (contacts with the incorrect, floating platform) across test days (Fig. 6). Overall differences in errors were evaluated by applying a repeated-measures ANOVA, indicating an overall effect for day (F5,44 = 6.446; p < 0.0001), treatment (F5,44 = 6.268; p = 0.0002) and a significant day × treatment interaction (F20,44 = 1.753; p = 0.03). For each day, significance was assessed by ANOVA with subsequent post hoc analysis using Bonferroni’s method. ABT-288, administered each day of testing, significantly attenuated the scopolamine deficit at doses of 0.1 and 1 mg/kg (Fig. 6). This response is comparable with the positive control group dosed with ABT-239 (1 mg/kg), which also significantly attenuated the scopolamine deficit.

Methamphetamine-Induced Hyperactivity. Clinically effective antipsychotic agents can block the long-lasting hyperactivity induced by methamphetamine in rodents. ABT-288 attenuated methamphetamine-induced hyperactivity in a dose-related manner, reaching significance after the administration of 1.0 mg/kg (data collapsed over 90 min after methamphetamine administration; Fig. 7). Antipsychotics that are efficacious in this assay, such as risperidone, significantly attenuated methamphetamine-induced hyperactivity.

EEG Slow-Wave Activity

ABT-288 at doses of 0.01 and 0.1 mg/kg i.p. had no significant effect on slow-wave EEG activity for up to 2 h after administration (Fig. 8). At the highest dose tested (1.0 mg/kg), ABT-288 significantly decreased the amplitude of the 1- to 4-Hz slow wave (p < 0.01; Neuman-Keuls multiple comparison test) (Fig. 8). Corresponding plasma levels of ABT-288 at the two lower doses were 0.9 and 11.2 ng/ml, whereas the plasma level at 1 mg/kg corresponded to 165 ng/ml. In this model, the histamine H3 antagonist diphenhydramine increased slow-wave activity (Fig. 8), consistent with its sedative effects in humans.

Receptor Occupancy

ABT-288 inhibited cortical [3H]A-349821 H₃R occupancy as shown in Fig. 9. At the dose of [3H]A-349821 used, cortical...
levels of [3H]A-349821 were approximately 2-fold greater in the cerebral cortex than cerebellum. In rats pretreated with maximal doses of ABT-288, [3H]A-349821 levels in cortex and cerebellum were indistinguishable, indicating that ABT-288 completely inhibited [3H]A-349821 H3R occupancy. ABT-288 occupied cortical H3 Rs in a dose-dependent manner with an ED50 of 0.1 mg/kg, and at the dose of 1.0 mg/kg it produced more than 90% occupancy. ABT-288 plasma levels were determined in these studies, and an EC50 value of 3.17 ± 0.7 ng/ml was calculated from a plasma level-occupancy plot (Fig. 9).

The H3R occupancy was 0.15 fmol/mg tissue, which was equivalent to approximately 1.5% of the total cortical H3 Rs. The levels of [3H]dofetilide were approximately 2-fold greater in the cerebral cortex than cerebellum. In rats pretreated with maximal doses of ABT-288, H3 Rs were indistinguishable in cortex and cerebellum, indicating that ABT-288 completely inhibited [3H]A-349821 H3R occupancy. ABT-288 occupied cortical H3 Rs in a dose-dependent manner with an ED50 of 0.1 mg/kg, and at the dose of 1.0 mg/kg it produced more than 90% occupancy. ABT-288 plasma levels were determined in these studies, and an EC50 value of 3.17 ± 0.7 ng/ml was calculated from a plasma level-occupancy plot (Fig. 9).

Cardiovascular Effects
ABT-288 exhibited low affinity for [3H]dofetilide binding to the hERG channel with an IC50 value of 8830 nM. Blockade of hERG current by ABT-288 in a stably transfected cell line in vitro was characterized with an IC50 value of 3760 ng/ml, more than 3000-fold the preclinical efficacious level (1 ng/ml). These results contrast with those for ABT-239, which blocked [3H]dofetilide binding and the hERG current more potently (Kd = 250 nM and IC50 = 56 ng/ml, respectively).

Detailed cardiovascular studies were conducted in anesthetized dogs where peak blood concentrations of 570 ± 40, 1870 ± 120, and 6300 ± 330 ng/ml for ABT-288 were achieved at the end of each infusion period corresponding to 570- to 6300-fold above the preclinical efficacious level (1 ng/ml). At the highest concentration tested, ABT-288 produced no physiologically relevant effects on any hemodynamic parameter measured including mean arterial pressure (Fig. 10, top) as well as systolic arterial pressure, diastolic arterial pressure, heart rate, contractility, cardiac output, pulmonary arterial pressure, pulmonary vascular resistance, and systemic vascular resistance, central venous pressure, left ventricular end-diastolic pressure, or hematocrit. At all
doses tested and at the highest blood concentration tested (6300 ± 330 ng/ml) and relative to vehicle controls, ABT-288 produced no statistically significant changes in the QT interval corrected for heart rate (Fig. 10, bottom).

**Pharmacokinetic Properties of ABT-288**

The pharmacokinetics of ABT-288 were evaluated in Sprague-Dawley rats and CD-1 mice. ABT-288 exhibited blood clearance (CLb) values of 1.3 l/h · kg in mouse and 2.7 l/h · kg in the rat. ABT-288 exhibited high volumes of distribution, with \( V_{ss} \) values of 1.7 l/kg in mouse and 5.3 l/kg in rat. In vitro plasma protein binding of ABT-288 was 86 and 88%, respectively, in mouse and rat. The elimination half-lives for ABT-288 were 1.0 and 1.3 h in mouse and rat, respectively. Rats demonstrated the highest clearance values and correspondingly the lowest bioavailability at approximately 37% after a 1 mg/kg dose in the rat. Mice exhibited higher bioavailability at 66%.

**Discussion**

Our findings show that ABT-288 is an \( H_3 \)R competitive antagonist that potently and selectively binds rat and human \( H_3 \)Rs, exhibiting slightly higher affinity toward the human receptor. The high degree of selectivity of ABT-288 is illustrated by its more than 5000-fold greater affinity for the human histamine \( H_3 \)R versus other histamine receptor subtypes and its good selectivity against other G protein-coupled receptor and ion channel targets. Functionally, ABT-288 is a competitive antagonist, inhibiting agonist-stimulated \( H_3 \)R GTP\(_y\)S binding by both rat and human receptors and reversing \( H_3 \)R agonist-induced inhibition of neurotransmitter release in vitro native \( H_3 \)R models. Schild analysis of the inhibition of (\( R \))-\( \alpha \)-MeHA-induced GTP\(_y\)S binding mediated by \( H_3 \)Rs clearly demonstrates the competitive antagonist properties of ABT-288, revealing \( pA_2 \) values that approximate the binding affinities. Although ABT-239 binding affinities were somewhat greater than ABT-288, the antagonist potencies of ABT-288 and ABT-239 were similar. Like almost all \( H_3 \)R antagonists described to date, ABT-288 is also an \( H_3 \)R inverse agonist, potently reversing basal human and rat
ABT-288 enhanced the in vitro release of histamine and acetylcholine, two neurotransmitters important for cognition and vigilance (Ebenshade et al., 2008; Haas et al., 2008; Passani et al., 2009), by potently and competitively reversing H₃R agonist-mediated inhibition of their release in the rat brain synaptosome and EFS guinea pig ileal segment assays, respectively. This work was extended in vivo, where ABT-288 significantly enhanced acetylcholine and dopamine levels at equivalent doses in the rat prefrontal cortex. ABT-288 was most potent and efficacious in enhancing acetylcholine release in prefrontal cortex in comparison with hippocampus, where higher doses were required and the magnitude of the effect was lower, and in comparison with histamine and dopamine in the prefrontal cortex where higher doses were required to enhance release. It is noteworthy that GSK189254 enhanced acetylcholine and dopamine levels at equivalent doses in the rat interior cingulate cortex (Medhurst et al., 2007), perhaps a brain region-specific event. In addition, ABT-288 increased dopamine release only in the prefrontal cortex and not striatum. Although ABT-288 did not significantly increase histamine release in the prefrontal cortex, there was a trend toward enhanced release, consistent with the brain region-specific enhancement of histamine release by the H₃ antagonist GSK189254 (Giannoni et al., 2010). These findings suggest that the effects of H₃ antagonists on the release of neurotransmitter release probably are not caused by general brain activation but instead are probably more regionally and functionally discrete, dependent on histaminergic innervation and localization of H₃Rs.

Preclinical evidence suggests that histamine H₃R blockade can decrease impulsivity, improve attention, and enhance learning and memory. Much of the research in these areas has focused on cognition assays that measure the different learning and memory domains thought to be most affected in CNS diseases. ABT-288 was efficacious in three different cognitive tests designed to assess attention, short-term social memory, and spatial cognition. As is often seen with H₃ antagonists, the potency and efficacy of ABT-288 varied depending on the cognitive domain measured. ABT-288 produced a dose-dependent improvement of acquisition in a five-trial inhibitory avoidance task in SHR pups previously linked to attention/impulsivity deficits (Fox et al., 2002). Full efficacy was achieved at 0.001 mg/kg and maintained over a broad dose range up to 0.03 mg/kg with corresponding plasma exposure levels of 0.1 to 2.0 ng/ml. It is noteworthy that these exposure levels correspond to an H₃R occupancy (EC₅₀ = 3.17 ng/ml) of 10 to 40%, indicating that only a small proportion of H₃Rs needed to be blocked to achieve efficacy in this model. Although slightly less potent in vitro than ABT-239 at the rat H₃R, ABT-288 is 100-fold more potent on a dosage basis than ABT-239 in this model, which is reflective of the higher volume of distribution and higher plasma protein binding of ABT-239. It has been reported for GSK189254 and other GlaxoSmithKline compounds that more than 60% H₃R occupancy is required for efficacy, but it must be recognized that these agents were tested in different behavioral models (passive avoidance and novel object recognition).

ABT-288 was less potent and exhibited a narrower range of efficacious doses in a model of short-term social memory in adult rats than in the five-trial inhibitory avoidance model, with full efficacy achieved at 0.03 to 0.1 mg/kg i.p. Another aspect of cognition that is impaired in patients with AD and schizophrenia is working memory that can be measured with a spatial navigation test in the water maze. ABT-288 reversed scopolamine-induced deficits in this task at 0.1 and 1.0 mg/kg i.p., similar to the doses required for efficacy in the social recognition assay. It is noteworthy that efficacy across the cognitive models is first seen at lower doses (0.001–0.1 mg/kg) of ABT-288 than the dose (1.0 mg/kg) that causes a decrease in the amplitude of 1- to 4-Hz slow waves. This suggests that improvements in cognitive performance occurs at lower levels of H₃R occupancy than is required for wakefulness, in agreement with an earlier report showing that a high degree of receptor occupancy was required to increased wakefulness by H₃R antagonists when sleep parameters were measured (Le et al., 2008). Additional studies seem warranted to determine whether differential neurotransmitter release, different sites or signaling events, or other reasons account for the differences in doses of H₃R antagonists required for cognitive effects and those that cause EEG activation.

Acetylcholine release in the frontal cortex was the most robust and sensitive (0.3–3 mg/kg) of the three neurotrans-
mitters measured, but dopamine levels were also increased at higher doses in the prefrontal cortex, perhaps implicating these neurotransmitters in the cognitive effects of ABT-288. Although the doses required for stimulating release of the neurotransmitters were higher than those required for cognitive effects, probably reflecting the limitations in sensitivity (spatial, temporal resolution) of the microdialysis approach, it is likely that more discrete changes in local levels of these neurotransmitters in general occur after administration of ABT-288. On the other hand, all microdialysis studies reported here have been performed under so-called resting conditions: no behavioral/cognitive challenges or environmental stimuli (Pepeu and Giovannini 2004) that may result in limited sensitivity of neurotransmitter release toward H₃ blockade by ABT-288. Social memory is impaired in Alzheimer’s disease and schizophrenia (Pinkham et al., 2003), and both acetylcholine (Winslow and Camacho, 1995) and histamine (Prast et al., 1996) have been implicated as being involved in the social recognition assay. Cholinergic neurotransmission in the hippocampus is integral to spatial memory, and ABT-288 was able to overcome the deficit produced by scopolamine in the water maze task. It is possible that the positive effects of ABT-288 observed across the cognitive domains examined in this study are mediated at least in part by acetylcholine with contributions from histaminergic and dopaminergic systems depending on the doses and brain regions involved in the processes.

The effect of ABT-288 in a model related to schizophrenia was also assessed. Current antipsychotics are believed to work through the blockade of dopamine hyperactivity in mesolimbic and mesocortical regions, which is hypothesized to underlie the positive symptoms in schizophrenia. Positive symptoms of schizophrenia are thought to be modeled in the methamphetamine-induced psychomotor hyperactivity rodent test, where antipsychotic drugs have been shown to be efficacious in blocking methamphetamine-induced hyperlocomotion. In these studies, ABT-288 (1.0 mg/kg) blocked the psychostimulant effects of methamphetamine. The efficacious dose of ABT-288 in this model was higher than that required for efficacy in the cognitive models, an effect also seen with ABT-239 (Fox et al., 2005). It is noteworthy that GSK207040 did not reverse hyperlocomotion induced by methamphetamine (Southam et al., 2009), and pitolisant was only partially effective (Ligneau et al., 2007a).

ABT-288 exhibits good drug-like attributes including favorable pharmacokinetic properties in mouse and rat characterized by moderate to high plasma clearance values and moderate to high volumes of distribution. The plasma elimination half-life was short in rodents (<1.5 h), but still more than adequate for testing in rodent cognition models. The oral bioavailability values ranged from 37% in rat to 67% in mouse. The brain-to-plasma ratios were greater than 1.5:1 in both mouse and rat, indicating ABT-288 readily partitioned into the brain. In addition, ABT-288 exhibited no appreciable effects in vitro hERG models or in vivo canine cardiovascular studies at dose multiples of up to 6300-fold over the behaviorally efficacious level in rodents, a margin superior to that previously seen with ABT-239.

ABT-288 is a highly selective H₃R competitive antagonist with high potency at both rat and human H₃Rs. ABT-288 modulates neurotransmitter release in vitro, enhances the release of acetylcholine and dopamine in vivo, and exhibits preclinical efficacy in rodent behavioral tests across multiple cognitive domains affected in disorders of cognition such as AD, attention deficit hyperactivity disorder, and cognitive deficits of schizophrenia. ABT-288 exhibits a safe preclinical profile in rodent and dog studies and has advanced to the clinical stage to determine its pharmacokinetics and tolerability in healthy volunteers. ABT-288 is currently in phase 2 trials to evaluate its therapeutic benefit for the treatment of cognitive deficits in Alzheimer’s disease and schizophrenia.

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