In Vivo Pharmacological Characterization of a Novel Selective α7 Neuronal Nicotinic Acetylcholine Receptor Agonist ABT-107: Preclinical Considerations in Alzheimer’s Disease


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ABSTRACT

We previously reported that α7 nicotinic acetylcholine receptor (nAChR) agonist 5-(6-[(3R,4S)-2,2-dimethyl-5-(6-phenyl-pyridazin-3-yl)-5-aza-2-azoniabicyclo[2.2.1]heptanes; BSA, bovine serum albumin; EEG, electroencephalography; CNS, central nervous system; IHC, immunohistochemistry; p-tau, tau phosphorylation; PBS, phosphate-buffered saline; ABC, avidin-biotin complex; IR, immunoreactivity; CI, confidence interval; ANOVA, analysis of variance; Cmax, maximum concentration; IA, inhibitory avoidance; AChEI, acetylcholinesterase inhibitor; Cmax, steady-state concentration; pERK, phosphorylated extracellular-regulated kinase; pCREB, phosphorylated cAMP response element-binding protein.

Introduction

The role of acetylcholine (ACh) and cholinergic transmission in cognition has generated much interest and effort in neuroscience drug discovery research (Bartus, 2000). Although preclinical studies have indicated the involvement of both muscarinic and nicotinic acetylcholine receptors (nAChR), in the past 5 years there has been significant progress supporting a distinct role of the α7 nAChR subtype in mediating cognitive function under both physiological and pathological conditions (Mudo et al., 2007; Leiser et al., 2009). The γ7 nAChR approach in the treatment of AD may have potential utility for symptomatic alleviation and slowing of disease progression in the treatment AD, and expand the understanding of the potential therapeutic viability associated with the α7 nAChR approach in the treatment of AD.

ABBREVIATIONS: ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; A-582941, 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c]pyrrole; ERK, extracellular signal-regulated kinase; CREB, cAMP response element-binding protein; AD, Alzheimer’s disease; APP, amyloid precursor protein; NFT, neurofibrillary tangle; GSK3β, glycogen synthase kinase-3; pS9, phospho-Ser9; Tg, transgenic; AChE, acetylcholine esterase; PK-PD, pharmacokinetic-pharmacodynamic; ABT-107, 5-(6-[[3R]-1-azabicyclo[2.2.2]oct-3-yl]oxy]pyridazin-3-yl)-1H-indole (ABT-107) was evaluated in behavior-}
Abundantly expressed in the frontal cortex and hippocampus, anatomical substrates of memory formation, the α7 nAChR is a homopentameric ligand-gated ion channel that is distinct from the α4β2 subtype counterpart in showing relatively higher permeability for calcium (Ca2+). For this reason, α7 nAChRs have been described as having metabotropic-like properties resulting from the activation of Ca2+-dependent second messenger cellular signaling events critical to cognitive processes (Berg and Conroy, 2002; Buckingham et al., 2009).

Consistent with α7 nAChR-mediated procognitive signaling, we previously showed that the selective α7 agonist 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c]pyrrole (A-582941) produced broad-spectrum efficacy in animal models at doses that enhance signaling pathways associated with synaptic plasticity and cognition, specifically extracellular signal-regulated kinase (ERK) and cAMP response element-binding protein (CREB) phosphorylation, supporting the potential utility of α7 nAChR agonism in the treatment of cognitive disorders, in particular Alzheimer’s disease (AD) (Bitner et al., 2007). AD is the most prevalent neurodegenerative disease today and is pathologically defined by two distinct biochemical abnormalities: 1) aberrant amyloid precursor protein (APP) leading to production of extracellular Aβ plaques; and 2) hyperphosphorylation of the microtubule-associated protein tau leading to the formation of intracellular neurofibrillary tangles (NFTs) (Pallàs and Camins, 2007). AD is the most prevalent neurodegenerative disease (Bitner et al., 2009). Specifically, A-582941 increased expression of phospho-Ser9 (pS9)-GSK3β, a regulatory means of GSK3β inhibition, in the cortex and hippocampus of normal and transgenic (Tg) AD mice but not in α7 nAChR knockout mice. Moreover, this α7 agonist reduced tau hyperphosphorylation in a mouse tauopathy Tg model.

Therapeutic agents used to treat AD today, such as acetylcholine esterase (AChE) inhibitors and glutamate-NMDA receptor antagonists, only provide modest symptomatic relief. Therefore, a major challenge in developing improved AD pharmacotherapies is identifying disease-modifying mechanisms, in addition to symptomatic alleviation (Giacobini and Becker, 2007). Results observed with A-582941 led us to speculate that α7 nAChR agonism may provide both symptomatic and disease-modifying efficacy in treatment of AD. However, a number of uncertainties will need to be addressed in the clinical development of an α7 nAChR agonist for the treatment of AD that may include drug-drug interactions in patients already receiving other AD therapeutics, dosing constraints defined by pharmacokinetic-pharmacodynamic (PK-PD) limitations, and adverse events, in particular, nicotine-like abuse liability. In the present study, we describe the use of another α7 agonist, 5-[(6-(3R)-1-azabicyclo[2.2.2]oct-3-yl)pyridazin-3-yl]-1H-indole (ABT-107) (Fig. 1A), to not only validate the procognitive and biochemical properties previously shown with A-582941 but also, more importantly, to address the potential issues that are likely to be encountered in the clinical development of an α7 nAChR agonist for the treatment of AD. Although continuing to support the pharmacological utility suggestive of symptomatic and disease-modifying efficacy, results presented with ABT-107 provide further understanding of the potential therapeutic viability associated with an α7 nAChR approach in the treatment of AD.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI) weighing 275 to 400 g and male CD1 (Charles River Breeding Laboratories) and female tau P301L(+/−) × APP(+/−)(TAPP) (Taconic Farms, Germantown, NY) mice weighing 20 to 30 g were used. Animals were group-housed in an American Association for Accreditation of Laboratory Animal Care–approved facility at Abbott Laboratories (Abbott Park, IL) in a temperature-regulated environment with lights on between 7:00 AM and 8:00 PM.
All the experimental procedures involving animals were conducted under protocols approved by Abbott’s Institutional Animal Care and Use Committee. In delayed matching to sample (DMTS) studies, young adult rhesus monkeys (Macaca mulatta) well trained (>100 individual sessions) in the DMTS task were used. The monkeys were maintained on a 12-h light/dark cycle and were tested each weekday between 9:00 AM and 2:00 PM. Room temperature and humidity were maintained at 72.0 ± 1.0°C and 52.0 ± 2.0%, respectively.

DMTS task procedures were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with American Association for Accreditation of Laboratory Animal Care guidelines.

Chemicals. ABT-107 (Di, 2007) and donepezil were synthesized at Abbott Laboratories; nicotine hydrogen tartrate, d-amphetamine, and methylphenidate were obtained from Sigma-Aldrich (St. Louis, MO). Compounds were prepared in water.

Blood Plasma and Brain Concentrations. For the determination of plasma and brain concentrations of the parent compound, naive rats or mice were dosed with the compounds as indicated and sacrificed at various time points postdosing. In monkey, plasma concentrations were assessed after intramuscular administration of ABT107. For analytical determination of ABT-107 plasma concentrations, blood was collected into heparinized tubes and then centrifuged, and the separated plasma was frozen at −20°C until analysis. For the determination of brain concentrations, animals were decapitated at the various time points, and the brains were immediately removed and rapidly freed from blood vessels as much as possible. The tissues were immediately frozen at −20°C and then centrifuged, and the separated plasma was stored at −20°C. For analysis, compounds were extracted from the samples via liquid-liquid extraction and were quantified by liquid chromatography/mass spectroscopy.

α7 nAChR-Binding Density in Rat Brain. Both a selective α7 nAChR antagonist [3H]methyllycaconitine (MLA) and agonist [3H]nicotine ([18,4S]-2,2-dimethyl-5-(6-phenylpyridazin-3-yl)-5-aza-2-azoniabicyclo[2.2.1]heptane (A-585539)) radioligand assay were used to determine α7 nAChR-binding densities as described previously (Anderson et al., 2008). Briefly, membrane-enriched fractions were prepared fresh from sacrificed male Sprague-Dawley rats, four saline-treated and four treated with ABT-107 (Charles River Laboratories, Inc., Wilmington, MA), 3 to 5 months old. Hippocampus and frontal cortex from each rat were homogenized for 10 s in BSS (balanced salt solution)-Tris buffer (120 mM NaCl, 5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, and 50 mM Tris-Cl, pH 7.4, 4°C) using a Kinematica (Littau-Lucerne, Switzerland) Polytron homogenizer. The tissue suspensions were centrifuged at 40,000 g for 15 min at 4°C. The resulting pellets were again homogenized and centrifuged in ice-cold H2O, and the pellets were frozen at −80°C until the day of the assay. Pellets were thawed, homogenized in BSS-Tris buffer, pH 7.4, and diluted with buffer to approximately 100 (hippocampus) or 150 (frontal cortex) μg protein/ aliquot. Binding conditions for the α7 nAChR antagonist [3H]MLA and agonist [3H]nicotine were as described in detail elsewhere (Anderson et al., 2008). Membrane aliquots, 7 nM [3H]MLA (25 Ci/mmol) and 0.1% bovine serum albumin (BSA) in BSS-Tris buffer, pH 7.4, were incubated in quadruplicate in a final volume of 500 μl for 60 min at 22°C. Nonspecific binding was determined in the presence of 30 μM MLA. Bound radioactivity was isolated by vacuum filtration onto glass fiber filter plates presoaked with 2% BSA (Millipore Corporation, Billerica, MA) using a 96-well filtration apparatus (PerkinElmer Life and Analytical Sciences, Waltham, MA) and washed with 2 ml of ice-cold BSS-Tris buffer. Forty microliters per well of scintillant were added to each well, and radioactivity was determined using scintillation counting in a Top-Count (PerkinElmer Life and Analytical Sciences). Binding conditions for the α7 nAChR agonist [3H]nicotine were as follows: 1.0°C and 52.0°C.

In Vivo Characterization of nAChR α7 Agonist ABT-107

Behavioral Methodology. DMTS procedure. Studies were conducted in young male rhesus monkeys that were initially trained in the DMTS procedure (Buccafusco et al., 2002). Animals were tested simultaneously in their home cages using a computer-automated training and testing system. Daily sessions consisted of 96 trials. Each test unit included three touch-sensitive screen (15-inch Accu-Touch LCD Panelmount TouchMonitor; Elo TouchSystems, Menlo Park, CA)/pellet dispenser units (MED Associates, St. Albans, VT) mounted in a light-weight aluminum chassis that could be attached to the home cage. A trial began by the presentation of a 5.7 (height) × 8.8 (width)-cm colored rectangle located in the upper center of the screen. Monkeys were trained to touch the illuminated sample (red, blue, or yellow) area to initiate a trial. This action also extinguished the sample during a computer-specified delay interval. After the delay interval, two choice colored rectangles, but not the sample, were illuminated. One of the two choices matched the sample, whereas the other (incorrect) choice was presented as one of the two remaining colors. Correct trials (matches) were rewarded by the delivery of a reinforcement food pellet (intertrial interval = 5 s). The various combinations of stimulus color were arranged so that each appeared an equal number of times as a sample; each color appeared an equal number of times as choices; and each color appeared an equal number of times in combination with each other color. Likewise, when two colors appeared in combination, each color was counterbalanced between left and right sides of the screen in a nonpredictable pattern. Finally, all the stimulus counterbalancing procedures were matched to the length of delay interval. For each subject, baseline accuracy was normalized by adjusting the length of delay intervals to provide: zero delay (85–100% correct), short delay (75–84% correct), medium delay (65–74% correct), and long delay (55–64% correct). Baseline data were obtained after the administration of drug vehicle (sterile saline).

Social recognition. The experimental room was set up with clean cages (52 × 28 × 20 cm) with metal wire lids, one for each adult rat to be tested on that day. Small cages (20 × 22 × 20 cm) were also set up below each large cage, one for each juvenile to be used that day. New cages and lids were used daily. Adult and juvenile animals were brought from the animal room and placed in the social recognition room. Animals were allowed to acclimate to the room for 90 to 120 min. After the acclimation period, adult rats were tail-marked in numerical order and weighed. Juvenile rats were numbered such that no two juveniles from the same cage were next to each other for purposes of third trial novelty. In numerical order in the smaller square cages below the large cages. Adult rats were placed alone in their respective test cages 30 min before the first interaction period (Trial 1). At the time of test, a juvenile of corresponding number was placed in the test cage with the adult for a period of 5 min, and the investigation time was recorded. Investigation included sniffing, grooming, holding, and close following of the juvenile by the adult. Adult rats were separated and placed back into their home cage. The juvenile rats were placed back in their respective smaller cages. Trials 2 and 3 occurred 2 h after Trial 1. Again animals were moved to their test cage 30 min before test. For Trial 2, the same juvenile used in Trial 1 was placed back into the test cage with the adult for 5 min, and investigation time was recorded. Immediately after Trial 2, the two juveniles were switched between the two cages under observation and used as novel juveniles for the opposite adult. The investigation time was recorded for the third 5-min period (Trial 3). Data were recorded in seconds. Recognition ratios of time spent investigating the same juvenile in Trial 2 divided by time spent investigating the juvenile in Trial 1 (familiar) and time spent investigating the novel juvenile in Trial 3.
divided by time spent investigating the juvenile in Trial 1 (unfamiliar) were calculated.

For subchronic administration, ABT-107 and/or donepezil was administered via subcutaneous osmotic minipumps. Minipumps were surgically implanted under isoflurane anesthesia 6 to 10 days before start of testing. Animals were weighed and anesthetized using 2% isoflurane inhalant anesthesia. The rats were injected with 2.5 mg/kg s.c. flunixin meglumine for analgesia. The body hair was clipped from the back of the neck between the scapulae, and the skin was surgically prepared with povidone iodine scrubbing. A lateral incision (3 cm) was made through the skin at the base of the neck, and a prefilled minipump (model 2ML2; Alzet, Cupertino, CA; 5 µl/h, 14 days) was implanted with the flow moderator facing away from the incision. The incision was closed with wound clips, and the rat was allowed to recover from anesthesia before returning it to its home cage. Surgical rats were maintained in group housing after recovery.

24-h recall two-trial inhibitory avoidance. Inhibitory avoidance was tested in two-compartment chambers from San Diego Instruments (San Diego, CA) modified to require manual opening of the guillotine door. The floor consisted of stainless steel rods. A foot shock was delivered from a Coulbourn Instruments (Allentown, PA) model E13-12 scrambled shocker. After a 1-h habituation period in the experimental room, mice were administered the compound (intrapertoneally) 30 min before the training session. For the training session, mice were placed one at a time into the light (safe) compartment, during which time the retractable door was closed. After 30 s, the door was opened (lifted gently). Measurement of the training latency commenced at this point. If a mouse did not cross within 60 s, the animal was excluded from the experiment. After the animal crossed into the dark chamber, the door was lowered, and inescapable foot shock (0.12–0.13 mA, 1 s duration) was presented. The mouse was immediately removed from the chamber and returned to the home cage. Twenty-four hours later the mouse was tested using methods identical to those on the training day but without shock. If the mouse did not enter the dark chamber after 180 s the trial was terminated. The latency to enter (all four paws on the grids in the dark side) the dark chamber was recorded and used as the dependent variable measured for assessing memory retention.

Nicotine-induced behavioral sensitization. To assess the ability of ABT-107 to induce locomotor sensitization was determined using an assay where nicotine has previously been shown to be effective (Stolerman et al., 1995). Rats were administered ABT-107 (1.0 µmol/kg s.c.), nicotine (2.5 µmol/kg s.c.), or vehicle (normal saline) daily for 10 consecutive days; all the groups received vehicle on day 11. Locomotor activity (total distance traveled in centimeters) was assessed for 1 h immediately after compound administration in 1 of 16 acrylic open-field environments (42 cm long × 42 cm breadth × 40 cm high; Piper Plastics, Libertyville, IL) situated inside Versamaz/ Digiscan activity monitors (AccuScan Instruments, Inc., Columbus, OH). Each monitor was equipped with 32 horizontal and 16 vertical infrared sensors (AccuScan Instruments, Inc.) and located in a dimmed room.

To further compare the effects of ABT-107 with the stimulant effects of nicotine, a cross-sensitization study was conducted in which the effects of ABT-107 (0.1 and 10.0 µmol/kg s.c.) were assessed in nicotine-experienced rats. To do this, a similar locomotor sensitization study was conducted where rats were repeatedly administered either nicotine (2.5 µmol/kg s.c.) or normal saline (1 ml/kg s.c.), a summary of the dosing scheme described in Table 1. Specifically, the dosing regimen during the first treatment week consisted of rats receiving nicotine (or saline) daily for 4 consecutive days, followed by 3 consecutive days of no injections. During the 2nd treatment week, nicotine (or saline) was only administered on day 8, with no injections on days 9 through 14. During the 3rd treatment week, nicotine (or saline) was administered on days 15 and 16. On day 17, the nicotine group received saline (along with the saline group) to assess any conditioned effects, returning to nicotine on day 18, and followed by no injections in either group on days 19 through 21. During the 4th treatment week, nicotine (or saline) was administered on days 22 and 23. On day 24, ABT-107 was assessed for nicotine cross-sensitization where two thirds of the nicotine group received ABT-107 at a dose of either 0.1 or 10.0 µmol/kg, whereas the remaining one third of the historic nicotine group received nicotine. Similarly, two thirds of the saline group received ABT-107 at a dose of either 0.1 or 10.0 µmol/kg, whereas the remaining one third of the historic saline group received saline. Locomotor activity (distance traveled in centimeters) was measured for 1 h immediately after each daily injection.

Spontaneous locomotor activity. Mice were acclimated to the test room for 30 min. After injections of the test compound, rats were placed into chambers (40 × 40 × 40 cm) for activity measures in an open field. Measures were automatically taken by the Versamaz system (AccuScan Instruments, Inc.), which records such things as horizontal activity, total distance, vertical activity, rearing, and so on. Activity was monitored in 5-min time bins, and data were analyzed by repeated measures and summed for overall analysis.

Electroencephalography assessment. Electroencephalography (EEG) recordings were obtained from adult rats as a measure of cortical neuronal activity. Under pentobarbital anesthesia (50 mg/kg, i.p.), stainless steel EEG recording electrodes were implanted over the frontal (anterior-posterior 3.0, lateral 2.0) and parietal cortices (anterior-posterior −3.0, lateral 4.0) such that the electrode tips were in contact with, but not penetrating, the dura. A reference electrode was placed over the cerebellum 11 mm posterior to bregma. A miniature electrical connector was affixed to the skull with dental cement, and the animals were allowed to recover for 2 weeks before experiments.

EEG slow waves are large amplitude synchronous electrical potentials present during periods of somnolence or low vigilance. Reduction of the amplitude is consistent with heightened brain activation and arousal, i.e., central nervous system (CNS) stimulation. These slow waves have a periodicity of 1 to 4 Hz and are blocked by stimulant compounds, including nicotine and like amphetamine. Decreased amplitude of the 1 to 4 Hz is representative of greater stimulant-like activity. ABT-107 (0.1, 1.0, and 10.0 µmol/kg i.p.), methylphenidate (1.3, 4.3, and 12.9 µmol/kg i.p.), or amphetamine (0.7, 4.3, 7.4, and 22.2 µmol/kg i.p.) was administered immediately before EEG assessment.

In vivo microdialysis. Rats (male Sprague-Dawley; 350–380 g b.wt.) were anesthetized with 60 mg/kg i.p. pentobarbital (Narcoren; Rhone-Merieux, Toulouse, France), mounted in a Kopf Instruments

<table>
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<tr>
<th>TABLE 1</th>
<th>Nicotine (2.5 µmol/kg) and ABT-107 (0.1 and 10.0 µmol/kg) cross-sensitization injection regimen</th>
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<tr>
<td>Injection Day</td>
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<td>Group I</td>
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(Tujunga, CA) stereotactic frame, and implanted with one microdialysis guide cannula (CMA/12; Axel Semrau GmbH, Sprockhövel, Germany) into the medial prefrontal cortex (AP = 2.5; ML = 0.6; DV = −0.2). Guide cannula was secured with dental cement (Technovit 2060; Kulzer GmbH, Dorma, Germany) and four anchor screws into the skull. Next, a CMA/12 microdialysis probe (3-mm membrane length; outer diameter, 0.5 mm) 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 CaCl$_2$, containing 1 μM neostigmine) for approximately 1 h (CMA/102 microdialysis pump, 1.5 μl/min).

Approximately 60 min after surgery, each animal was transferred into a freely moving animal system (CMA/120; Axel Semrau GmbH), consisting of a plastic bowl, wire attachment, counterbalance arm, and swivel assembly connecting inlet/outlet of the probe with the perfusion pump. After 24 h, the probe was perfused for at least 1 h before microdialysate fractions were collected every 30 min. Four fractions before and six fractions after drug administration were analyzed for microdialysate levels of ACh by high-performance liquid chromatography with electrochemical detection. ABT-107 (1, 3 μmol/kg i.p.) or saline was administered daily for 3 days to the rats during the 3-h microdialysis collection period, during which time the animals were tethered but awake and freely moving as described.

For analytical assessment of ACh, a microdialysate fraction (10 μl) was injected onto a reversed-phase column (MF-8908 ACh SepStik Kit; BAS Bioanalytical Systems, West Lafayette, IN; microparticle column, 530 × 1.0 mm, 10-μm particle size coupled to immobilized enzyme reactor; 50 × 1.0 mm, 10-μm particle size, containing acetylcholinesterase and choline oxidase) using a refrigerated autosampler (CMA/200; Axel Semrau GmbH). The mobile phase consisted of 50 mM Na$_2$HPO$_4$, pH 8.5, and 5 ml/l biocide (Kathon; Bioanalytical Systems Inc, West Lafayette, IN). Flow rate was set to 0.13 ml/min (CMA/250 pump; Axel Semrau GmbH), and the sample run time was less than 15 min. ACh and choline were assessed via an electrochemical 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 (ACh, choline) containing 1 pmol/10 μl injection.

**In Vivo Phosphorylation Signaling Studies and Immuno- histochemical Assays.** A series of studies were conducted in either male CD1 or female TAPP mice consisting of either acute administration or 2-week continuous infusion of ABT-107. To examine the acute effects on ERK1/2, CREB, Ser9 GSK3β, and tau phosphorylation (p-tau), male CD1 mice were administered varying doses of ABT-107 (0.01, 0.1, and 1.0 μmol/kg i.p.). Fifteen minutes after ABT-107 (or vehicle) administration, all the mice were deeply anesthetized (CO$_2$) and perfused through the aorta with normal saline (5 ml/kg, 0.9% NaCl, 4.0 mM KCl, and 2.4 mM CaCl$_2$, containing 10 μl/ml biocide). After perfusion, brains were postfixed in 10% formalin and swivel assembly connecting inlet/outlet of the probe with the perfusion pump. After 24 h, the probe was perfused for at least 1 h before microdialysate fractions were collected every 30 min. Four fractions before and six fractions after drug administration were analyzed for microdialysate levels of ACh by high-performance liquid chromatography with electrochemical detection. ABT-107 (1, 3 μmol/kg i.p.) or saline was administered daily for 3 days to the rats during the 3-h microdialysis collection period, during which time the animals were tethered but awake and freely moving as described.

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After perfusion, brains were removed and postfixed in 30% sucrose–phosphate-buffered saline (PBS) overnight and subsequently cut frozen on a cryostat (40-μm coronal sections) and collected as free-floating sections in PBS. Sections containing cortex were then immunostained using a three-step avidin-biotin complex (ABC)-peroxidase technique beginning with 30 min incubation with blocking serum. Sections were next incubated with primary antibodies—anti-phospho-ERK1/2 (mouse monoclonal IgG 1:500; Cell Signaling Technology Inc., Danvers, MA), anti-phospho-CREB (rabbit monoclonal IgG 1:1000; Cell Signaling Technology Inc.), or anti-phospho-S9-GSK3β (rabbit polyclonal IgG 1:500; Biosource, Camarillo, CA) antibody—overnight at room temperature, washed with PBS, and incubated for 1 h with biotinylated secondary anti-rabbit (1:200). Finally, sections were washed in PBS, incubated with ABC reagent (Vectastain Elite; Vector Laboratories, Burlingame, CA), and then developed in a peroxidase substrate solution (diaminobenzidine, 0.625 mg/ml). All the incubations were done at room temperature.

**IHC p-tau.** After perfusion, brains were postfixed in 10% formalin for 6 to 12 h, then processed and embedded in paraffin, and subsequently cut on a microtome (0.3-μm coronal sections). Sections containing the hippocampus were mounted and dried on aminosilane-coated slides and subsequently deparaffinized through a xylene and graded alcohol series. A three-step ABC-peroxidase technique was used to examine p-tau immunoreactivity where sections were first incubated for 20 min in blocking serum (2.0% horse serum) followed by 90-min incubation with anti-phospho-tau (AT8, mouse monoclonal IgG, 1:200; Pierce Endogen, Rockford, IL). After primary antibody incubation, sections were washed (three times for 5 min) in PBS incubated in a biotinylated secondary antibody solution (10 μg/ml, horse anti-mouse IgG; Vector Laboratories) for 60 min, washed in PBS, incubated with ABC reagent (Vectastain Elite; Vector Laboratories), and then developed in a peroxidase substrate solution (diaminobenzidine, 0.625 mg/ml) and counterstained with hematoxylin.

All the incubations were done at room temperature.

**Image analysis and IHC quantification.** To minimize experimental variability, tissue processing, immunoreagent incubations, and chromatographic development were always conducted concurrently. In this regard, all the tissues examined from a given experiment were exposed to the same experimental conditions, and samples were processed by the same investigator. For IHC processes, a prerequisite for quantifying changes in immunoreactivity, after IHC processing, four to six serial sections from each animal were cover-slipped and photographed with a light microscope (DMRB; Leica Microsystems, Inc., Deerfield, IL). Sections were chosen based on optimal immunoreactivity (IR) and anatomical similarity for immunocounting, in which the experimenter was blind to treatment conditions. Phospho-ERK1/2, -CREB, and -S9 GSK3β IR in the cingulate cortex was quantified using an image analysis system (Quantimet 500; Leica Microsystems, Inc.) that determined relative intensity of peroxidase substrate-positive stained neurons from digitized photomicrographs according to a pixel gray level empirically determined before analysis. For studies involving p-tau IR in the spinal motoneurons or hippocampal mossy fibers, a mean gray level was determined from immunopositive motoneurons using an image analysis system (Quantimet 500; Leica Microsystems, Inc.) and converted to an optical density value using an optical density standard curve generated with a densitometry step tablet (Edmund Optics, Inc., Barrington, NJ). Whereas within-experiment IHC parameters were constant, IHC conditions affecting staining intensity can fluctuate between experiments; therefore, changes in IR were expressed as percentage of control.

**Data Analysis.** Data are presented as mean ± S.E.M. with 95% confidence intervals (CIs). Significant differences between group means were assessed by one-way analysis of variance (ANOVA) followed by either Dunnett’s or Fisher’s protected least-squares difference post hoc analyses. For monkey DMTS, data were analyzed with a within-subject design by use of a multiafactorial ANOVA with repeated measures (JMP statistical software package; SAS Institute, Cary, NC). For mouse inhibitory avoidance, nonparametric analysis using a Mann-Whitney t test was used. Microdialysis data (area under curve, 0–180 min) were assessed for significance using one-way ANOVA followed by Dunn’s pairwise comparison post hoc test. A p value <0.05 was considered statistically significant.

**Results**

**Efficacy in Models of Cognition.** The in vivo efficacy profile of ABT-107 was examined across a battery of in vivo models representing various domains of memory processing, including short-term memory (recognition memory and vi-
sual working memory), long-term memory (memory consolidation and recall), and attention. ABT-107 exhibits good bioavailability in mouse (orally, 51.1%; intraperitoneally, >100%), rat (orally, 81.2%; intraperitoneally, >100.0%), and monkey (orally, 40.6%; intramuscularly, >100%), and good CNS penetration in rodents with a brain/plasma ratio of ~1.

Short-term memory. To determine the effects on short-term visual working memory, ABT-107 was evaluated in the DMTS task in young adult rhesus monkeys. Under vehicle control conditions, animals show a decline in DMTS accuracy approaching chance levels with increasing delays (55–65% correct at the long delay intervals). ABT-107 (10, 20, 40, and 80 nmol/kg i.m.) administration 30 min before testing produced significant task improvement at the long, but not immediate, short, or medium delay trials (Fig. 1B). At the long delay, a significant increase in DMTS performance ranged between 10 and 20% at the 20, 40, and 80 nmol/kg doses (Fig. 1C). The plasma levels of ABT-107 were assessed after intramuscular administration of 100 nmol/kg dose in a separate group of animals. Extrapolation of these values from a 100 nmol/kg dose (maximum concentration \( C_{\text{max}} \), 17.5 ng/ml) to the doses tested in monkeys yielded plasma levels ranging from 1.8 to 14.4 ng/ml. The efficacy noted with the 20 nmol/kg dose corresponds to plasma levels of ~3.5 ng/ml. These results show the ability of ABT-107 to enhance cognitive performance in this model.

Efficacy of ABT-107 was also assessed in rats using the social recognition model, a test of short-term recognition memory. Adult rats, administered ABT-107 (0.001, 0.01, and 0.1 μmol/kg i.p.) immediately after initial juvenile exposure (Trial 1), displayed a significant decrease in the duration of subsequent juvenile interaction (Trial 2) 2 h later, consistent with short-term memory improvement (Fig. 1D). Whereas ABT-107 decreased the duration of investigation of the familiar juvenile (ratio of Trial 2/Trial 1), the compound had no effect on the investigation duration when a novel juvenile was subsequently introduced, showing that the reduced investigation of the familiar juvenile after ABT-107 reflects improved memory rather than nonspecific effects on exploratory behaviors (data not shown). The efficacious dose range spanned 0.001 to 0.1 μmol/kg (although lacking a clear dose response), which corresponds to plasma levels ranging from 0.034 to 3.4 ng/ml. In addition, continuous infusion of ABT-107 that maintained a plasma steady-state concentration of 0.2 ng/ml for 7 days also significantly improved memory in this model, suggesting a lack of tolerance to the cognition-enhancing effects of the compound (Fig. 1E). Moreover, no change in α7 receptor-binding density occurred in the hippocampus or in the frontal cortex from these rats, indicating no agonist-induced receptor up-regulation after continuous exposure to efficacious plasma levels (Table 2).

Long-term memory consolidation. To determine the effects of α7 nAChR agonism on long-term memory consolidation, ABT-107 was evaluated in a mouse two-trial inhibitory avoidance (IA) test. In this model, pretraining administration of compound resulting in increased crossover latency during retention testing 24 h later is consistent with enhanced long-term memory consolidation. ABT-107 administered 30 min before training increased memory retention at doses of 0.03 and 0.1 μmol/kg. A lack of effect at the 0.3-μmol/kg dose suggested an inverted U relationship (Fig. 1F). The plasma \( C_{\text{max}} \) at maximal efficacious dose (0.1 μmol/kg) was 2.6 ng/ml. At the time of testing 24 h later, the plasma level of ABT-107 was undetectable.

PK-PD Profile. To examine the time course relationship of plasma levels associated with procognitive efficacy, studies were conducted by varying intervals between drug administration and cognitive assessment in both the monkey DMTS and mouse IA models. In separate animals, concentrations of ABT-107 were determined at the same time intervals.

As noted earlier (Fig. 1B), ABT-107 improved monkey/DMTS performance at long delay intervals when administered 30 min before the test session. To assess the duration of action in this model, an efficacious dose of ABT-107 (30 nmol/kg) was administered, and DMTS testing was carried out 0.5, 2, or 6 h later. As depicted in Fig. 2A, a significant increase in DMTS accuracy was observed at all the time points. The largest improvement in DMTS accuracy was observed at 2 h, despite substantial reduction in plasma concentration of ABT-107 at this time point compared with the peak levels. Moreover, at the 6-h interval, significant effects were still observed, although the plasma concentration was decreased 150-fold from the \( C_{\text{max}} \) (5.4 ng/ml) of the 30-nmol/kg efficacious dose. These data suggest that efficacy in this model is not temporally congruent with drug levels in plasma and that continuous drug levels may not be required for sustaining efficacy. However, this assumes that time course of plasma and brain levels are comparable, which is indeed the case in the mouse vide infra.

In the mouse inhibitory avoidance test, ABT-107 was administered at the maximal efficacious dose of 0.1 μmol/kg at varying time intervals (0.5, 1, 3, and 6 h) before training. When assessed 24 h later, a significant improvement in memory consolidation (as assessed by the increase in crossover latency) was observed at all the time points, although both plasma and brain levels of ABT-107 declined to almost undetectable levels at the 6-h time point (Fig. 2A). These results, along with results from the monkey DMTS model, suggest that continuous presence of ABT-107 may not be necessary for sustaining memory effects in these preclinical models.

Combination Studies with Donepezil. Given the widespread use of acetylcholinesterase inhibitors (AChEIs), clinical evaluation of novel mechanisms in AD will necessitate recruiting patients already receiving an AChEI such as donepezil. Accordingly, studies were conducted to examine whether ABT-107 could interfere with the efficacy of donepezil. In the rat social recognition model of short-term memory, ABT-107 or donepezil was continuously infused (subcutaneously) alone or in combination and tested 8 to 9 days later (Fig. 3). Continuous infusion of ABT-107 (0.5 μmol/kg/day; steady-state concentration \( C_{\text{ss}} \) = 2–3 ng/ml) significantly improved memory in the social recognition model as revealed by a significant decrease in the Trial 2/Trial 1 recognition ratio, also indicating a lack of tolerance to the cognition-enhancing effects of the compound. Infusion of donepezil alone (1 and 3 mg/kg/day) showed a trend toward improvement of recognition memory that did not achieve statistical significance. The plasma \( C_{\text{ss}} \) of

<table>
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<tr>
<th>TABLE 2</th>
<th>α7 binding density (fmol/mg protein) in rat hippocampus (HPC) and frontal cortex (FCX) after 1-week ABT-107 infusion (plasma ( C_{\text{ss}} ) = 0.2 ng/ml)</th>
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<td>([\text{H}]\text{MLA})</td>
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<tr>
<td>HPC</td>
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were made to identify potential adverse effects that may be associated with α7 nAChR agonism. In particular, testing was conducted to evaluate ABT-107 for potential nicotine-like abuse liability and psychomotor stimulant properties.

**Nicotine-induced behavioral sensitization.** Repeated injections of psychomotor stimulants, including nicotine, can produce behavioral sensitization, as evidenced by an augmented locomotor response to a subsequent injection of the drug (Clarke and Kumar, 1983). To determine whether ABT-107 would induce behavioral sensitization similar to nicotine, a study was conducted assessing the locomotor response evoked by daily repeated injections of ABT-107 or nicotine in rats. Assessment of locomotor activity (total distance traveled) for 1 h was made immediately after drug administration (Fig. 4A). Daily injections of ABT-107 (0.1 μmol/kg s.c.) for 10 days did not evoke a significant increase in locomotor activity compared with saline-treated controls, both groups exhibiting low levels of total distance traveled throughout the 10-day regimen. In contrast, daily injections of nicotine (2.5 μmol/kg s.c.) induced locomotor sensitization, i.e., subsequent increased locomotor activity, as expected, that was observed as early as day 3. Administration of nicotine on day 11 to animals treated with ABT-107 did not lead to an augmented response, further supporting a lack of locomotor sensitization-like activity.

A second study was conducted to determine whether ABT-107 would cross-sensitize to nicotine after induction of locomotor sensitization. Similar to the first study, rats dosed daily (days 1, 2, 3, 4, 8, 15, 16, 18, 22, and 23) with nicotine (2.5 μmol/kg s.c.) exhibited a progressive increase in locomotor activity that reached asymptote by day 15, consistent with locomotor sensitization (Fig. 4B). Nicotinetreated rats did not display an augmented locomotor response when administered saline on day 17 but returned to the sensitized state (hyperlocomotion) when subsequently administered nicotine the following day. On day 24 of the treatment regimen, the administration of ABT-107 (0.1 and 10.0 μmol/kg s.c.) in the nicotine-treated (or vehicle) groups failed to produce an augmented response, indicating that ABT-107 administered up to a dose (10 μmol/kg) 100 to 10,000 times greater than that required for efficacy in the rat social recognition test of short-term memory. Both compounds were delivered by continuous minipump infusion assessed on days 9 and 10. In the absence of donepezil, ABT-107 infusion (open circles) improved short-term memory as indicated by a reduction in the Trial 2/Trial 1 ratio compared with vehicle-infused controls (open squares). Infusion of donepezil (DPZ) alone (1 and 3 mg/kg/day; closed squares) showed a trend toward improvement of recognition memory that did not achieve statistical significance. ABT-107 in combination with donepezil (closed circles) improved recognition memory similar to ABT-107 alone. All the data are represented as mean ± S.E.M.; *p < 0.05 versus vehicle infusion controls; n = 8/ treatment.
ABT-107 was also evaluated for effects on spontaneous motor activity in mice at 10, 30, and 100 µmol/kg i.p. (Fig. 5A). No significant reduction in locomotor activity was noted up to 30 µmol/kg, a dose that is 300-fold over the maximal efficacious dose (0.1 µmol/kg) in the mouse IA test. A significant reduction (~43%) in locomotor activity was noted at 100 µmol/kg. 1000 times the maximally efficacious ABT-107 dose required for enhancing memory consolidation.

**Effects on EEG in rats.** Slow-wave EEG is present during periods of somnolence from drowsiness to very deep non-REM sleep. Slow waves can be indicative of a state of the brain in which processes associated with attention or vigilance are functioning minimally. The 1- to 4-Hz band, as well as its association with slow-wave activity, allows for characterization of general stimulant or sedative actions of CNS active compounds. Unlike the stimulants amphetamine and methylphenidate that differentially decreased slow-wave EEG in rats, ABT-107 administered acutely at 0.1, 1, and 10 µmol/kg i.p. did not alter slow-wave activity (Fig. 5B). The lack of EEG effect up to 10 µmol/kg, a dose 100 and 10,000 times greater than the maximal and minimal efficacious dose in rat social recognition, respectively, suggests that the procognitive effects of ABT-107 are not associated with CNS stimulatory effects as measured by EEG.

**Neurochemical and Biochemical Mechanistic Assessment.** Both neurochemical and biochemical assessment of ABT-107 were carried out in the present studies. Because cholinergic transmission is recognized as a key neurochemical pathway in cognitive processing, microdialysis studies were conducted to examine the effects of ABT-107 on ACh release in rat. In addition, ABT-107 was examined for its effects on ERK1/2 and CREB phosphorylation-activation as a biochemical index of procognition, as well as S9-GSK3β and p-tau as a biochemical index of disease-modifying effects relevant to AD. As we have previously shown, IHC methodology was chosen as a more germane procedure for examination of α7 nAChR-induced signaling involving the use of phospho-specific antibodies (Bitner et al., 2007, 2009).

**ABT-107 enhances ACh release from prefrontal cortex in freely moving rats.** ACh plays a critical role in cognition, as degeneration/dysfunction of cholinergic neurons is closely associated with cognitive deficits of AD (Coyle et al., 1983); it may be expected that α7 agonists like ABT-107 would be expected to release ACh, in addition to other neurotransmitters. The effects of ABT-107 on ACh release from freely rats were examined in microdialysis studies in the medial prefrontal cortex of freely moving rats. ABT-107 was administered once daily over 3 consecutive days. As shown in Fig. 6A, the acute administration at day 1 of ABT-107 (3
ABT-107 activates ERK1/2 and CREB phosphorylation in mouse cingulate. To examine potential biochemical mechanisms involved in mediating proognitive activity, the effects of ABT-107 on signaling pathways known to be involved in cognitive function, specifically phosphorylation of mitogen-activated protein kinase ERK1/2 and its downstream effector CREB, were examined in normal (CD1) mice. Administration of ABT-107 (0.01–1 μmol/kg i.p., 15 min before sacrifice) produced a dose-dependent increase in ERK1/2 (Fig. 7A) and CREB (Fig. 7B) phosphorylation in the cingulate cortex as determined immunohistochemically using a phospho-specific antibody against the phosphorylated, i.e., activated form of ERK1/2 and CREB. In contrast to more diffuse intracellular phosphorylated ERK (pERK) distribution, phosphorylated CREB (pCREB) immunoreactivity was limited primarily to the nucleus, where CREB is constitutively expressed, as described previously (Bitner et al., 2007). Similar to pERK, the dose-dependent increase in CREB phosphorylation was observed at behaviorally efficacious doses. As a negative control, omission of the primary pERK1/2 antibody resulted in a complete loss of immunostaining (data not shown). The significant increases in ERK1/2 phosphorylation observed at the 0.1 and 1.0 μmol/kg doses parallel the behavioral efficacy measured in the inhibitory avoidance model (see Fig. 1E).

ABT-107 increases S\(^{\text{\*}}\)-GSK3\(^{\beta}\) and decreases p-tau in mouse cortex and hippocampus. Studies were conducted to address the biochemical pathways involved in ABT-107-mediated signaling relevant to AD pathology. The ability of α7 nACh agonism to increase phosphorylation of the inhibitory regulating amino acid residue Ser9 on GSK3\(^{\beta}\), a biochemical index of GSK3\(^{\beta}\) inhibition, was examined in normal (CD1) mice. Specifically, ABT-107 (0.01, 0.1, and 1.0 mg/kg i.p.) produced a dose-dependent increase in Ser9 phosphorylation in the cingulate cortex 15 min after acute administration in mice (Fig. 7C). In the same animals, ABT-107 treatment dose-dependently reduced basal p-tau in hippocampal CA3 mossy fibers, as immunohistochemically assessed with the

![Fig. 6](http://example.com/figure6.png)

**Fig. 6.** Effect of 3 days of daily injections of ABT-107 (1.0 and 3.0 μmol/kg i.p.) on ACh levels in the medial prefrontal cortex of freely moving rats (A). Time course: data are mean ± S.E.M. percentage change of the average of two preapplication basal ACh levels. Basal levels were found to be 4.65 ± 0.638 pmol/30 μl fraction. ABT-107 was administered once daily over 3 consecutive days (3 μmol/kg i.p., n = 5). B, data are mean ± S.E.M. area under the curve (AUC in arbitrary units, data collapsed across 0–180 min after administration of vehicle or ABT-107; 1 and 3 μmol/kg i.p.). One-way ANOVA revealed a significant treatment effect for ACh concentration in the prefrontal cortex \(F(3,20) = 10.39; p < 0.0011\). Subsequent Dunnett's multiple comparison revealed a significant increase for microdialysate ACh level compared with vehicle-treated rats \(*, p < 0.05\) ABT-107 (1 and 3 μmol/kg i.p.).

μmol/kg i.p.) did not significantly affect microdialysate ACh levels in the medial prefrontal cortex. However, ABT-107 induced a significant, dose-dependent increase in ACh release by day 3 of repeated administration (Fig. 6B). It should be noted that the doses examined were higher than behaviorally active doses in rats, specifically the social recognition memory test. However, it may be speculated that only subtle localized changes in synaptic ACh are required for efficacy that occur at concentrations below the sensitivity of in vivo microdialysis using 20-min collection periods, as used here. Alternatively, higher ABT-107 doses may be required to evoke ACh release in naive rats not engaged in stimulated, i.e., cognitive-related behavior.

![Fig. 7](http://example.com/figure7.png)

**Fig. 7.** Acute effects of ABT-107 on biochemical signaling in mice. Acute administration of ABT-107 (0.01, 0.1, and 1.0 μmol/kg i.p.) induced changes in biochemical signaling in normal (CD1) mice that included increased cortical phosphorylation of ERK1/2 (A), CREB (B), S\(^{\text{\*}}\)-GSK3\(^{\beta}\) (C), and decreased hippocampal (CA3 mossy fibers) p-tau (D). All the data are represented as mean ± S.E.M.; *, \(p < 0.05\) versus vehicle-treated controls; n = 6/treatment.
ABT-107 infusion attenuates tau hyperphosphorylation in AD transgenic APP-tau mice. To further confirm effects on p-tau under conditions more relevant to the pathology of AD, the effects of ABT-107 were examined in a double transgenic AD mouse line. TAPP mice have previously been reported to exhibit hyperphosphorylated tau in motoneurons of the ventral horn spinal cord, beginning at 10 months of age (Lewis et al., 2001). TAPP female mice (12–13 months old) continuously infused subcutaneously with ABT-107 (5.5 mg/kg/day) showed significantly decreased p-tau immunoreactivity in ventral horn spinal cord motoneurons compared with saline-infused TAPP mice, such that p-tau levels in TAPP mice treated with ABT-107 approached p-tau levels observed in wild-type controls (Fig. 8). The mean steady-state plasma concentration of ABT-107 in the TAPP-infused mice was 15 ng/ml. In comparison, the Cmax of the acute 1-μmol/kg dose of ABT-107 that increased pS9-GSK3β and decreased basal p-tau expression was ~32 ng/ml.

Discussion

Although AD is the most prevalent neurodegenerative disease today, affecting approximately 25 million patients worldwide (Kelley and Petersen, 2007), current treatments are limited and essentially only provide modest symptomatic relief. We have previously reported that α7 nAChR agonism may represent a novel AD therapeutic approach offering both symptomatic and disease-modifying efficacy (Bitner et al., 2006, 2007, 2009; Hu et al., 2008). Reported here, studies were conducted to address questions of PK-PD discordance, interactions with AChEIs, and issues of abuse liability that may be associated with the use of an α7 nAChR agonist in the treatment of AD. Using the novel α7 agonist ABT-107, which displays excellent biochemical and drug-like properties (Malysz et al., 2010), efficacy was observed across different cognitive domains and experimental species that was maintained even as plasma and brain levels decreased. ABT-107 efficacy was maintained when coadministered with donepezil, supporting adjunctive utility with AChEIs. Furthermore, ABT-107 did not exhibit nicotinic-like psychomotor stimulant properties. Finally, ABT-107 exhibited neurochemical and biochemical properties consistent with its procognitive activity, suggesting therapeutic potential in AD symptomatic alleviation, as well as in the attenuation of disease progression.

An early and predominant cognitive deficit observed in the AD patient is short-term memory loss (Germano and Kinsella, 2005). ABT-107 improved performance in both the monkey DMTS and rat social recognition tests, cognitive measures representing domains of working and short-term recognition memory, respectively. In the monkey DMTS, ABT-107 only produced significant enhancement at the long delay, a period that in the absence of drug treatment yields close to chance level performance. However, this may not be unexpected given the greater demand on short-term memory likely associated with the long delay as opposed to the short and intermediate delay periods that produce more accurate performance, and thus may be less sensitive to α7 agonism-mediated effects. In contrast to ABT-107, the AChEI donepezil shows only marginal efficacy in the monkey DMTS (Buccafusco and Terry, 2004). In rat social recognition, similar efficacy was observed with both acute administration and continuous 1-week subcutaneous infusion of ABT-107, the latter supporting a lack of tachyphylaxis on subchronic dosing. The efficacious levels of ABT-107 across various cognition models ranged from 0.2 to 20 ng/ml (0.63–6.3 nM), levels that were significantly lower than concentrations that evoke substantial current activation in vitro and more in line with α7 nAChR-binding Kᵩ value (Malysz et al., 2010). For example, acute Cmax and Css ABT-107 concentrations for sustaining procognitive efficacy were 0.3 ng/ml (0.94 nM) and 0.2 ng/ml (0.63 nM), respectively, 300 to 500 times lower than the EC5₀ (~340 nM) measured from rat α7 nAChR currents in oocytes. In mouse IA, a model of memory consolidation, ABT-107 efficacy was observed with doses producing brain Cmax levels up to approximately 2 ng/g (6 nM), still below in vitro concentrations evoking substantial current activation. These findings suggest that minimal receptor activation is indeed sufficient for triggering procognitive effects and, as discussed below, support the view that the behavioral efficacy associated with α7 agonism may be more related to a metabolotropic than inotropic function (Berg and Conroy, 2002; Bitner et al., 2006, 2007).

Time course studies were conducted in the monkey DMTS and mouse inhibitory avoidance tests to investigate the PK-PD relationship associated with ABT-107 efficacy. In both models, procognitive efficacy was maintained at extended time intervals between ABT-107 administration and behavioral testing/training despite substantial reductions in compound levels, as determined by measuring ABT-107 plasma and/or brain levels in satellite animals. As shown in Fig. 2A, maximal DMTS performance was observed at 2 h, an interval in which monkeys exhibited a 95% reduction in plasma levels compared with the 0.5-h Tmax plasma concentration. Furthermore, DMTS performance remained significant compared with nondrug control conditions at 6 h when
ABT-107 plasma levels were only 0.1% of $T_{\text{max}}$. Similarly, mice trained in two-trial inhibitory avoidance 6 h after ABT-107 administration showed a significant increase in memory consolidation, yet satellite mice at 6 h did not have detectable ABT-107 plasma levels and brain levels only 8% of the 0.5-h $T_{\text{max}}$. Collectively, these findings indicate discordance between the PK-PD properties of ABT-107 that may represent a key mechanistic feature of the procognitive efficacy associated with $\alpha 7$ nAChR agonism. In this regard, nicotine continues to enhance working memory in rats long after discontinuation, with effects persisting for at least 4 weeks after termination of drug (Levin et al., 1992). Like nicotine, ABT-107 appears to offer prolonged efficacy independent of receptor occupancy that may be associated with activation of signaling pathways leading to lasting secondary functional changes linked to synaptic plasticity.

Indeed, activation of biochemical pathways provides a potential mechanism for the prolonged efficacy of $\alpha 7$ nAChR agonists such as ABT-107. As previously reported from our laboratory and elsewhere, the higher calcium permeability associated with $\alpha 7$ nAChR agonist stimulation activates calcium-dependent signal transduction pathways implicated in cognition (Berg and Conroy, 2002; Bitner et al., 2006, 2007). In particular, the mitogen-activated protein kinase/ERK pathway regulates a diverse array of cellular functions, such as cell growth, differentiation and survival, that may underlie the synaptic plasticity required for cognitive processing and memory formation (Adams and Sweatt, 2002; Giovannini, 2006). As a primary effector-substrate of the ERK pathway, phosphorylation-activation of the transcription factor CREB can induce gene expression of proteins responsible for the synaptic plasticity implicated in cognitive function that includes brain-derived neurotrophic factor, a neurotrophic factor required for neurite outgrowth (Ring et al., 2006). Reduced pCREB and brain-derived neurotrophic factor expression have been observed in AD transgenic mice and AD patients (Connor et al., 1997; Yamamoto-Sasaki et al., 1999; Gong et al., 2004). Procognitive doses of ABT-107 increased both ERK1/2 and CREB phosphorylation in mouse (CD1) cingulate cortex, an anatomical site recognized for processing short-term memory, particularly working memory (Dalley et al., 2004). In addition, ABT-107 administered to rats daily over 3 days was shown to progressively enhance ACh release in the medial prefrontal cortex, consistent with $\alpha 7$ nAChR-mediated cellular signaling activation and subsequent augmented neurotransmission.

Studies were also conducted to examine the procognitive effects of ABT-107 in combination with donepezil in the rat social recognition model. Although only providing temporary symptomatic efficacy, AChEIs such as donepezil represent the mainstay therapeutic approach currently used in the treatment of AD (Seltzer, 2005). The development of a novel AD therapeutic may likely encounter clinical testing involving the concurrent use with an AChEI, and thus will necessitate the need for examining potential pharmacodynamic interactions. In particular, the pharmacological action of a cholinergic agonist may be altered when administered in combination with an AChEI as a result of the latter leading to increased cholinergic tone because of reduced ACh degradation. Our studies show that coinfusion of ABT-107 with donepezil at clinically relevant plasma steady-state concentrations (11–17 ng/ml) (Rogers et al., 1998) improved rat recognition memory similarly to ABT-107 infusion alone. Therefore, we conclude from these results that donepezil did not interfere with ABT-107 efficacy in this paradigm.

The reinforcing properties of nicotine are well recognized as underlying the addiction associated with tobacco use (Bennowitz, 2009). Nicotine’s reinforcing properties have been examined using a variety of behavioral, pharmacological antagonism, and genetic knockout approaches aimed at elucidating the nAChR subtype involved in nicotine reward (Picciotto et al., 1998). These studies generally indicate a greater importance of the $\alpha 4\beta 2$ subtype in mediating the reinforcing properties of nicotine, although it has been suggested $\alpha 7$ nAChRs may have a regulatory role for long-term maintenance of nicotine consumption (Levin et al., 2009). However, in a battery of studies comparing ABT-107 with nicotine we found no evidence of nicotine-like abuse liability. Specifically, daily repeated administration of ABT-107 in rats produced no signs of behavioral sensitization, a measure of progressive locomotor response augmentation to repeated administration of psychomotor stimulants that includes amphetamine, cocaine, methylphenidate, and nicotine. Furthermore, ABT-107 at doses 100 to 10,000 times greater than the dose range required for procognitive activity did not cross-sensitize to nicotine when given to rats maintained on daily nicotine. ABT-107 also presented no signs of CNS stimulatory effects at doses 100 to 1000 times greater than doses producing procognition, as measured by locomotor activity and EEG activation. Collectively, our studies show no evidence that ABT-107 displayed activity suggestive of nicotine-like abuse liability. However, as $\alpha 7$ agonists continue to advance toward clinical development, more complex assessment of abuse liability testing such as self-administration may be required to provide further confirmation.

In addition to cellular signaling linked to cognition, the effects of ABT-107 on biochemical pathways relevant to AD pathology were examined. Acute administration of ABT-107 in CD1 mice increased cortical phosphorylation of the Ser9 inhibitory residue of GSK-3$\beta$. Acute administration of ABT-107 also decreased basal p-tau in the hippocampus of normal mice. Moreover, 2 weeks of continuous ABT-107 infusion reversed tau hyperphosphorylation in the spinal cord of TAPP (tau $\times$ APP) Tg-AD mice (Lewis et al., 2001). These findings confirmed earlier results showing that $\alpha 7$ nAChR agonism leads to increased S9-GSK3$\beta$ in cingulate cortex and hippocampus and to decreased p-tau in normal and Tg-AD mice, the latter including both spinal cord and hippocampus (Bitner et al., 2009). Based on these findings, we speculate that $\alpha 7$ nAChR agonists may function as indirect inhibitors of GSK3$\beta$ via increased Ser-9 phosphorylation, possibly through signaling involving the phosphoinositide-3 kinase/Akt pathway, resulting in reduced phosphorylation of the GSK3$\beta$ substrate tau. Activation of Akt, a Ser-Thr kinase associated with cell survival and neuroprotection (Chong et al., 2005), leads to S9-GSK3$\beta$ phosphorylation and can be initiated by a number of upstream signaling pathways that include cell surface proteins like the insulin receptor (Bondy and Cheng, 2004). Neuroprotection observed with nicotine in vitro has been shown to involve $\alpha 7$ nAChR stimulation and subsequent activation of phosphoinositide-3 kinase/Akt cascade through signaling involving the tyrosine kinase Janus kinase 2 (Shaw et al., 2002; Marrero et al., 2004).
In summary, results presented continue to support the therapeutic utility of α7 nAChR agonism in the treatment of cognitive-neurodegenerative disorders, in particular AD, as evidenced by the broad-spectrum procognitive activity correlating with the activation of biochemical signaling known to be involved in memory (e.g., pCREB) and neuroprotection (e.g., pS9^GSK3β). Moreover, in addressing the potential issues that may be encountered with the clinical development of a novel AD therapeutic, ABT-107 exhibited a preclinical efficacy profile that included 1) PD-PK discordance consistent with prolonged biochemical signaling germane to synaptic plasticity and enhanced cognition; 2) maintenance of efficacy with concurrent use of an AChEI; and 3) lack of nicotine-like abuse liability and CNS stimulatory activity. Together, our findings show that targeting α7 nAChRs may have potential utility for symptomatic alleviation and slowing of disease progression as a novel therapeutic in the treatment of AD.

Acknowledgments

We wish to recognize the long-standing and productive collaboration that existed with coauthor Dr. Jerry Buccafusco of Medical College of Georgia and Abbott Neuroscience Discovery that sadly came to an end on March 6, 2010 with his untimely death. In addition to being a key collaborator, Jerry was a good friend and will be greatly missed.

References


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