An Allosteric Modulator of the α7 Nicotinic Acetylcholine Receptor Possessing Cognition-Enhancing Properties in Vivo

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ABSTRACT

Augmentation of nicotinic α7 receptor function is considered to be a potential therapeutic strategy aimed at ameliorating cognitive and mnemonic dysfunction in relation to debilitating pathological conditions, such as Alzheimer’s disease and schizophrenia. In the present report, a novel positive allosteric modulator of the α7 nicotinic acetylcholine receptor (nAChR), 1-(5-chloro-2-hydroxy-phenyl)-3-(2-chloro-5-trifluoromethyl-phenyl)-urea (NS1738), is described. NS1738 was unable to displace or affect radioligand binding to the agonist binding site of nicotinic receptors, and it was devoid of effect when applied alone in electrophysiological paradigms. However, when applied in the presence of acetylcholine (ACh), NS1738 produced a marked increase in the current flowing through α7 nAChRs, as determined in both oocyte electrophysiology and patch-clamp recordings from mammalian cells. NS1738 acted by increasing the peak amplitude of ACh-evoked currents at all concentrations; thus, it increased the maximal efficacy of ACh. Oocyte experiments indicated an increase in ACh potency as well. NS1738 had only marginal effects on the desensitization kinetics of α7 nAChRs, as determined from patch-clamp studies of both transfected cells and cultured hippocampal neurons. NS1738 was modestly brain-penetrant, and it was demonstrated to counteract a (−)-scopolamine-induced deficit in acquisition of a water-maze learning task in rats. Moreover, NS1738 improved performance in the rat social recognition test to the same extent as (−)-nicotine, demonstrating that NS1738 is capable of producing cognitive enhancement in vivo. These data support the notion that α7 nAChR allosteric modulation may constitute a novel pharmacological principle for the treatment of cognitive dysfunction.

In clinical conditions where it is desirable to augment the function of a particular receptor type, positive allosteric modulation of the receptor in question is frequently considered preferable to classical (orthosteric) agonism. This primarily relies on the fact that actions of positive allosteric modulators are use-dependent, because only receptors activated by the endogenous ligand (agonist) are subject to modulation. Therefore, the temporospatial characteristics of endogenous receptor activation are preserved, and the function of the modulator can be considered as increasing the gain of individual receptor activation events. Agonists, in contrast, tonically activate all receptors. This will lead to a nonphysiological pattern of receptor activation, and it is also well known that prolonged agonist exposure will lead to receptor desensitization and that it affects receptor expression patterns (for review, see Quick and Lester, 2002).

The cysteine-loop-containing family of ligand-gated ion channels encompasses anion-prefering receptor channels for γ-aminobutyric acid (GABA_A and GABA_C receptors) and glycine as well as cation-prefering receptor channels activated by 5-hydroxytryptamine (5-hydroxytryptamine_3 receptors) and acetylcholine (nicotinic receptors; nAChRs). These ion channels are assembled from five structurally similar subunits.
An Allosteric Modulator of the \( \alpha 7 \) nAChR

units, either as homomeric or heteromeric protein complexes (for review, see Lindstrom, 2000). Within this family of ion channels, positive allosteric modulators have been known for decades: Several clinically used anesthetic, sedative, and anxiolytic drugs exert their effects through positive allosteric modulation of \( \text{GABA}_A \) receptors.

The discovery of positive allosteric modulators acting on the homomeric \( \alpha 7 \) nAChR is of more recent date. The anhelminic agent ivermectin (Krause et al., 1998), 5-hydroxyindole (Zwart et al., 2002), and serum albumin from various species (Conroy et al., 2003) have been shown to enhance \( \alpha 7 \) nAChR-mediated currents using in vitro electrophysiological methods. Moreover, two distinct peptides have also been found to enhance \( \alpha 7 \) function, namely, a peptide fragment from the C terminus of acetylcholinesterase (Greenfield et al., 2004) and the polypeptide SLURP-1, which is structurally related to lynx1 (see above) but highly selective for \( \alpha 7 \) (Chimenti et al., 2003). However, due to their physicochemical properties, none of these agents are useful for exploring the pharmacological actions of \( \alpha 7 \)-selective modulators in vivo. However, the recent discovery of two small-molecule \( \alpha 7 \) nAChR modulators (PNU-120596 and compound 6), capable of penetrating the blood-brain-barrier, was reported in Hurst et al. (2005) and Ng et al. (2007).

The \( \alpha 7 \) nAChR is abundantly expressed in the brain, and its functional significance is not only attributable to its electrophysiological properties (i.e., modulation of neuronal excitability and neurotransmitter release) but also to its high Ca\(^{2+} \) permeability and association with biochemical signaling pathways (for review, see Dajas-Bailador and Wonnacott, 2004). Nicotine is known to enhance cognitive and attentional function in both laboratory animals (Levin and Simon, 1998) and in humans (Rezvani and Levin, 2001), indicative of an important role for nAChRs in these processes. Moreover, sensory gating (a neurophysiological phenomenon thought to be important for processing of sensory information) and performance in models of cognition and memory is known to be impaired in rodents with decreased expression/pharmacological blockade of \( \alpha 7 \) nAChRs (Stevens et al., 1996; Felix and Levin, 1997). Consistent with this finding, expression of the \( \alpha 7 \) protein is dramatically reduced in the brains of patients suffering from Alzheimer’s disease (Burghaus et al., 2000) and schizophrenia (Freedman et al., 1995). The latter phenomenon has been correlated with polymorphisms in the promoter region of the \( \alpha 7 \) gene in schizophrenic patients; such polymorphisms have been shown to result in decreased gene transcription (Freedman et al., 1997; Leonard et al., 2002). These and other observations have attracted considerable attention to the \( \alpha 7 \) nAChR as a drug target in recent years, the working hypothesis being that drugs capable of augmenting \( \alpha 7 \) nAChR function can ameliorate the cognitive and mnemonic deficits of demented and/or schizophrenic patients. Approaching this hypothesis, we present here the pharmacological profile of the novel \( \alpha 7 \)-selective positive allosteric modulator 1-(5-chloro-2-hydroxy-phenyl)-3-(2-chloro-5-trifluoromethyl-phenyl)-urea (NS1738).

Materials and Methods

Materials

NS1738 was synthesized at NeuroSearch A/S (Ballerup, Denmark). \( \alpha \)-Bungarotoxin (\( \alpha \)-BgTx), acetylcholine chloride, and \((-\))-scopolamine HBr were purchased from Sigma-Aldrich (Brøndby, Denmark); methylycysteine (MLA) was purchased from Tocris Cookson Inc. (Bristol, UK); and all other salts and chemicals were of analytical grade or higher. \([\text{3H}]\alpha\)-BgTx (46 Ci/mmol) was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK).

The GH4C1 and TE671 cell lines were purchased from the American Type Culture Collection (Manassas, VA). Wistar rats and NMRI mice were purchased from Taconic Farms ( Ry, Denmark).

Methods

Cell Culture and Transfection (GH4C1 and TE671). Cells of the rat pituitary carcinoma cell line GH4C1 were propagated in tissue culture flasks kept at 37°C in a humidified atmosphere containing 5% \( \text{CO}_2 \). The growth medium consisted of Ham’s F-10 medium, supplemented with 15% horse serum and 2.5% fetal bovine serum (Invitrogen, Tåstrup, Denmark). Twenty-four hours before transfection, cells were plated into 35-mm Petri dishes containing 10 to 12 round glass coverslips (3.5 mm diameter) that had previously been coated with poly-d-lysine. The GH4C1 cells were transfected with a mixture of cDNAs encoding the human \( \alpha 7 \) nAChR subunit (0.8 \( \mu \)g/dish) and green fluorescent protein (0.2 \( \mu \)g/dish) to identify transfected cells. Transfection was performed using the Lipofectamine (Invitrogen) method, according to the manufacturer’s instructions. After transfection, the GH4C1 cells were incubated in growth medium (see above) supplemented with 50 mM KCl, because elevated K\(^+ \) has been shown to facilitate expression of \( \alpha 7 \) nAChR in this cell line (Quik et al., 1986). In the present study, treatment with elevated K\(^+ \) was also found to elicit a profound increase in peak current amplitude following transient transfection (data not shown).

Cells of the human medulloblastoma cell line TE671 were propagated in tissue culture flasks kept at 37°C in a humidified atmosphere containing 5% \( \text{CO}_2 \). The growth medium consisted of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Two to 3 h before patch-clamp recording, the cells were plated into 35-mm Petri dishes containing 10 to 12 round glass coverslips (3.5 mm diameter) that had been coated previously with poly-d-lysine.

Cloning of nAChR Subunits. The human nAChR \( \alpha 7 \) subunit was cloned using human brain mRNA as template for PCR. In brief, human hippocampus poly(A\(^+ \)) mRNA (Clontech, Mountain View, CA) was reverse-transcribed using oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (GE Healthcare). One hundred nanograms of first-strand cDNA then was subjected to PCR using Expand HF polymerase (Roche Diagnostics, Mannheim, Germany) and a gene-specific primer set spanning the full-length cDNA sequence. Amplified products were polished with PFu polymerase (Stratagene, La Jolla, CA), purified on a QiAquick column (QIAogen, Valencia, CA), and cloned into the cloning vector pCRScript (Stratagene).

The human nAChRs \( \alpha 3, \alpha 4, \beta 2, \) and \( \beta 4 \) subunits were cloned using RNA from the human cell line IMR32 (American Type Culture Collection) as template for PCR. In brief, mRNA was isolated from IMR32 using the Oligotex Direct mRNA kit (QIAGEN). Then, 25 to 100 ng of mRNA was subjected to a reverse transcription-PCR reaction using the RobuS\(T \) kit (Finzymes, Espoo, Finland) and gene-specific primers in a PCR round using the Touchdown technique. PCR products were purified using the QIAquick PCR purification kit (QIAGEN), and 1/50th of the product from the first PCR round was used in a second PCR round using Expand HF polymerase (Roche Diagnostics) and nested gene-specific primers. Amplified products were purified on QIAquick columns (QIAGEN) and cloned into pSws (custom-made cloning vector).

Maxprep DNA (QIAGEN) for all the subunits was prepared from positive clones, and two to four individual clones were sequenced bidirectionally to identify error-free clones. cRNA was prepared from cloning vectors containing the respective subunit cDNAs using the T7 mMessage mMachine T7 Transcription kit (Ambion, Austin, TX).

For expression experiments in GH4C1, the \( \alpha 7 \) cDNA was subcloned into the pNSSH vector (custom-designed vector), and the
DNA for green fluorescent protein (Clontech) was subcloned into the pNS1z vector (custom-designed vector). Both of these vectors use the cytomegalovirus promoter to drive expression of the inserted cDNA.

Hippocampal Neurons. Hippocampi of neonatal pups were dissected under 10x magnification, placed in HIB (129 mM NaCl, 5 mM KCl, 25 mM HEPES, 9.1 mM glucose, and 0.04 mM phenol red, pH 7.4), and then they were roughly dissociated mechanically by forceps. Next, the tissue was dissociated by mild trypsinization (0.2% trypsin and 40 μg/ml DNase) at 37°C for 15 min. Trypsinization was ended by addition of trypsin inhibitor (7.5 mg/20 ml of HIB) and 0.3 mM MgSO4. The tissue was rapidly centrifuged, resuspended in Dulbecco’s modified Eagle’s media containing 23 mM glucose, 10% horse serum, 7 μM p-aminobenzoate, 0.5 mM glucose, 100 μM insulin, 0.1% penicillin, and 19.1 mM KCl; and then disaggregated by mechanical trituration by the use of a steel needle and a disposable syringe. This step was repeated, and the cells were plated at a final density of 0.5 × 10^6 cells/ml on 10 μg/ml poly-L-lysine-coated plates and prepared for electrophysiology. The neurons were maintained in culture for 14 days at 37°C (5% CO2, 95% O2) with addition of antimitic agent (5 μM cytosine arabinoside) from day 3 to day 4 to limit glial proliferation.

Saturation Binding Analysis. Preparations were performed at 0–4°C unless otherwise indicated. Cerebral cortices and hippocampi from male Wistar rats (Taconic Farms), weighing 150 to 250 g, were homogenized for 10 s in 15 ml of 20 mM HEPES buffer containing 118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO4, and 2.5 mM CaCl2, pH 7.5, by using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmingtom, NC). The tissue suspension was centrifuged at 27,000 × g for 10 min. The supernatant was discarded, and the pellet was washed twice by centrifugation at 27,000 × g for 10 min in 20 ml of fresh buffer, and the final pellet was resuspended in fresh buffer containing 0.01% bovine serum albumin (35 mg/ml original tissue). The reaction mixture consisted of 500 μl of homogenate, 25 μl of [3H]α-BTX, and 25 μl of buffer or (–)nicotine (1 mM to define nonspecific binding), Saturation curves were obtained for the binding of 0.05 to 10 nM [3H]α-BTX in the absence and presence of 10 μM NS1738. The binding at each concentration was determined using three samples for total and nonspecific binding, respectively. After an incubation time of 2 h at 37°C, the reaction was terminated by the addition of 5 ml of ice-cold HEPES buffer containing 0.05% polyethyleneimine, and the solution was poured directly onto Whatman GF/C glass fiber filters (pre-soaked in 0.1% polyethyleneimine for at least 0.5 h; Whatman, Maidstone, England) under suction, and they were immediately washed with 2 × 5 ml of ice-cold buffer. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting. Protein concentration was measured by the method of Lowry et al. (1951).

Two-Electrode Voltage Clamp on Xenopus laevis Oocytes. The X. laevis oocytes were prepared for electrophysiological experiments as described previously (Briggs et al., 1995; Briggs and McKenna, 1998). In brief, three to four lobes from ovaries of female adult X. laevis (Blades Biological Ltd., Cowden, Kent, UK) were removed, manually defolliculated, and treated with collagenase type 1A (2 mg/ml; Sigma-Aldrich) prepared in low-Ca2+- Barth’s solution (90 mM NaCl, 1.0 mM KCl, 0.66 mM NaNO3, 2.4 mM NaHCO3, 10 mM HEPES, 2.5 mM sodium pyruvate, 0.82 mM MgCl2, and 0.5% (v/v) penicillin-streptomycin solution (Sigma-Aldrich), pH 7.55) for 1.5 to 2 h at ±18°C under constant agitation to obtain isolated oocytes. The oocytes were injected with ~20 to 25 ng of human α7 nAChR cRNA, kept at 18°C in a humidified incubator in modified Barth’s solution (90 mM NaCl, 1.0 mM KCl, 0.66 mM NaNO3, 2.4 mM NaHCO3, 10 mM HEPES, 2.5 mM sodium pyruvate, 0.74 mM CaCl2, 0.82 mM MgCl2, and 0.5% (v/v) penicillin-streptomycin solution, pH 7.55), and used 2 to 7 days after injection.

Responses were measured by two-electrode voltage-clamp techniques using Parallel Oocytes Electrophysiology Test station (POETs) (Trumbull et al., 2003). During recordings the oocytes were bathed in Ba2+-OR2 solution (90 mM NaCl, 2.5 mM KCl, 2.5 mM BaCl2, 1.0 mM MgCl2, 5.0 mM HEPES, and 0.005 mM atropine, pH 7.4), and held at ~60 mV at room temperature (~20°C). ACh was applied to the recording chamber for 1 s at 6 ml/s. NS1738 compound was applied for a minimum of 60 s before ACh application, allowing for sufficient preincubation. ACh application after preincubation with NS1738 was always done in the presence of NS1738.

The oocyte electrophysiology experiments involving α3β4 and α3β2 nAChRs were conducted under similar conditions. Oocytes were injected with cRNA (as a 1:1 mixture, giving ~25 ng in total) encoding human α3 and β4 subunits or human α4 and β2 subunits, respectively. Experiments were conducted using a custom-built system in which solutions were applied to the oocytes via a glass capillary (placed at the vicinity of the cell). Solutions were delivered via a Gilson 233 XL autosampler (Gilson, Inc., Middleton, WI), and the flow (2.5 ml/min) was created by the use of syringe pumps (Gilson 402; Gilson, Inc.). The composition of saline solutions was as described above. Two-electrode voltage-clamp was achieved using a GeneClamp 500 amplifier in combination with a Digidata 1322A interface (both Molecular Devices, Sunnyvale, CA).

Patch-Clamp Electrophysiology. Cells used for patch-clamp experiments were grown on poly-L-lysine-coated glass coverslips as described above. Coverslips were placed in a diamond-shaped polycarbonate recording chamber (Warner Instruments, Hamden, CT) fixed at the stage of an inverted microscope (Olympus, Ballerup, Denmark). The cells were then superfused with extracellular buffer (140 mM NaCl, 4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, and 10 mM HEPES, pH adjusted to 7.4 using NaOH) throughout the experiment. Micropipettes were made from borosilicate capillary tubes by means of a horizontal micropipette puller (Zeit Z Instrumente, Munich, Germany), and they were filled with intracellular buffer (120 mM potassium glutonate, 6 mM KCl, 5 mM NaCl, 2 mM MgCl2, 10 mM HEPES, 0.5 mM EGTA, 2 mM ATP, and 0.2 mM GTP (ATP + GTP were added to the pipette buffer immediately before use), pH was adjusted to 7.4 using KOH. Pipette resistances were in the range of 2 to 5 MΩ under these conditions. Cells were subjected to voltage clamp in the whole-cell configuration using an EPC-9 patch clamp amplifier (HEKA, Lambrecht/PFalz, Germany), operated via a Macintosh G4 computer (Apple, Cupertino, CA). The holding potential was ~60 mV in all experiments presented. Data were sampled at 20 kHz, and they were low-pass filtered at 6.7 kHz and only accepted for analysis if the series resistance was <~10 MΩ. Series resistance was compensated by 80%. Agonists and drugs were delivered via an ultrafast solution application system. A micropipette was fabricated using a specialized glass capillary tube, bifurcated by a thin glass septum (a so-called θ-tube; WPI, Stevenage, Hertfordshire, UK). The tip of this micropipette was then cut using a sapphire knife to attain a tip opening of appropriate size (~0.05–0.1 mm diameter). Solutions were applied through the two halves of the θ-tube via Teflon tubing. Typically, solution flowing in one half would be extracellular solution (without drugs used for preincubation), and the other half would contain ACh dissolved in extracellular solution. The tip of the θ-tube was positioned in the immediate vicinity of the voltage-clamped cell under visual guidance. The lateral position of the θ-tube was controlled via a piezo-ceramic device (Burleigh Instruments, Fishers, NY), allowing the environment of the cell to be switched from extracellular buffer to ACh-containing buffer with an extremely high time resolution. Exposing the tip of a pipette electrode to a solution of reduced ionic strength using the piezo-electric device allowed the environment of the cell to be switched from extracellular buffer to ACh-containing buffer with an extremely high time resolution. Exposing the tip of a pipette electrode to a solution of reduced ionic strength using the piezo-electric device indicated that the 10 to 90% settling time of the liquid junction potential shift was 200 to 400 ms (data not shown), providing an upper limit for the time resolution of solution exchange around a voltage-clamped cell. Agonist pulses used for stimulation of α7 nAChR lasted 200 ms, whereas cells expressing other nAChR subtype were stimulated with pulses lasting 1 s. In all experiments, the stimulation frequency was 1 agonist pulse/30 s, to ensure full recovery of the nAChRs from agonist-induced desensitization between pulses.
Pharmacokinetics. Concentration of NS1738 in rat plasma and brain were measured using high-performance liquid chromatography in combination with a triple-quadrupole mass spectrometry (LC-MS/MS). This system is highly specific to the analyte NS1738, and it monitors fragmentation of parent molecule to a prominent daughter ion by a process termed multiple reaction monitoring. By this means, it is possible to measure concentration of NS1738 down to 1 ng/ml in plasma (approximately 3 nM) and 10 ng/g in brain (30 nM).

Plasma samples were prepared by protein precipitation with acetonitrile containing an internal standard, centrifugation, and analysis of the supernatant by LC-MS/MS. Quantification was performed by preparing control (blank) plasma samples spiked with known concentrations of NS1738 and by extraction in the same manner as study samples. Brain samples were prepared by extreme agitation with zirconium oxide pellets in acetonitrile/water (80:20, v/v) containing internal standard in a “bead-beater” (Biospec, Bartlesville, UK) to achieve efficient homogenization and extraction. The homogenates were subsequently centrifuged, and the supernatant was analyzed by LC-MS/MS. Quantification was performed in a similar manner to the plasma samples, except brains from control rats were spiked at known concentration with NS1738 (dissolved in dimethyl sulfoxide), and they were subjected to the same extraction procedures used for study samples.

Morris’ Water-Maze Model. Scopolamine HBr (Sigma-Aldrich) was dissolved in 0.9% saline and administered s.c. at 1 ml/kg. NS1738 was dissolved in 10% Tween 80 and administered i.p. at 1 ml/kg. Predosing time was 30 min for scopolamine and 15 min for NS1738, with these drugs being administered before the first acquisition trial on each day during the acquisition phase of the experiment and before the first reversal trial. The rats were drug-free in the probe trial on test day 5.

Male Wistar rats (Taconic Farms) with free access to laboratory chow and tap water were used. The rats were kept at room temperature with a 12/12-h light/dark cycle in Scantainers (Scanbur, Karlslund, Denmark). The water maze consisted of a black metal pool (160 cm in diameter × 50 cm in height) filled with tap water at 23°C (40 cm in depth) divided into four quadrants. In the center of the northeast quadrant (30 cm from the wall), a removable escape platform (10 × 10 cm) was placed 1 cm below the water level.

The location of the pool in the room and the location of the platform in the pool were the same for all of the acquisition trials, maintaining constant distal spatial cues throughout all swim sessions. Acquisition training consisted of four trials per day (intertrial interval = 15 s). The rat was placed in the water facing the wall of the pool at a predetermined, selected quadrant (north, south, east, or west). For subsequent trials, the rat was released in each of the other three quadrants. For each trial, the rat swam in the pool until climbing onto the platform or for 60 s. If the rat did not find the platform within the 60 s, it was taken to the platform. The rat remained on the platform for 15 s between all trials, and after four trials it was removed from the pool, dried, and returned to its home cage. Thirty-two rats were trained per day, and they were divided into four dosing groups (i.e., eight animals per dosing group). The training continued for 4 days, with four trials per day. All experiments were performed according to the guidelines of the Danish Committee for Experiments on Animals.

Social Recognition. The rat social recognition test measures short-term memory on the basis of olfactory cues. Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI) were group-housed and acclimated to the animal facility for at least 1 week before testing. Animals were tested in the light phase of a 12:12-h light/dark schedule (lights on at 6:00 AM), and they were allowed free access to food and water, except for during test period. All experimental procedures were conducted under protocols approved by Abbott Laboratories Institutional Animal Care and Use Committee.

Adult (2–4 months; 400–450 g) and juvenile (50–60 g) animals were allowed to acclimate to the test room for 90 to 120 min before starting. After acclimation, adult rats were placed alone in their respective test cages. After a brief habituation period (30 min), they were allowed to interact for 5 min with a juvenile rat (trial, T1). During the interactive trial, the adult exhibits investigative behaviors that include close following, grooming, and/or sniffing of the juvenile for as much as 40 to 50% of the trial duration. The time of the investigative interaction was recorded in seconds. The juvenile rat was then removed, and the adult rats were immediately administered varying doses of NS1738 (prepared in 5% ethanol/95% hydroxypropyl-B-cyclodextrin (34% solution); 2.0 ml/kg i.p.) or nicotine, and then they were returned to their home cage. A second 5-min interactive trial (T2) was conducted 120 min later in the same test cage, and investigative behavior of the adult rat was again monitored and the time was recorded. Recognition ratios of time spent investigating the familiar juvenile in T2 divided by time spent investigating the juvenile in T1 were calculated. If recognition memory was lost over the 120-min interval between trials, the investigative behavior would be similar for the two trials. However, if memory were retained, the ratio (T2:T1) would be lower (<1). Decreased T2:T1 ratio is considered as an index of improved short-term recognition memory. Significant differences between group means were assessed by a one-way analysis of variance. Post hoc analysis was done using Dunnett’s multiple comparison, and P < 0.05 was considered statistically significant.

Data Analysis and Statistics. In two-electrode voltage-clamp studies, responses were quantified by measuring peak current amplitude. For concentration-response curves, ion current amplitudes were measured, and they were normalized to the amplitude of control responses as indicated for each experiment. Data were analyzed by using the POET software Omine (Trumbull et al., 2003) or Clampfit (Molecular Devices) and GraphPad Prism (GraphPad Software Inc., San Diego, CA).

For patch-clamp experiments, measured peak current amplitudes were typically normalized with respect to the peak current amplitude evoked by a saturating concentration of ACh, as detailed in the figures and accompanying legends. Normalized peak current amplitudes were graphically depicted and fitted to the empirical Hill equation (see figure legends for details) using GraphPad Prism 3.0 software (GraphPad Software Inc.). Curve fitting of current traces to a monoexponentially decaying function to obtain estimates of desensitization time constants (see figure legends for details) was performed using IgorPro 4.0 software (Wavemetrics, Lake Oswego, OR). Student’s t test was performed using GraphPad Prism 3.0 software (GraphPad Software Inc.), and one- and two-way ANOVAs were performed using SigmaStat software (Systat Software, Inc., Point Richmond, CA).

Results

NS1738 (Fig. 1A) was initially identified among thousands of compounds screened for allosteric modulation of the α7 nAChR, using POETs (Trumbull et al., 2003). In this screening paradigm, X. laevis oocytes expressing the human α7 nAChR were subjected to ACh stimulation (EC_{50} = 100 μM) with or without preincubation with test compounds (at 10 μM), and compounds that increased peak current amplitude above a predefined level were selected for further evaluation. Several diaryl urea compounds were identified as highly efficacious modulators of α7 nAChR function, and among these, NS1738 (Fig. 1A) was selected for detailed characterization.

Effects on Radioligand Binding. To assess the ability of NS1738 to interact with the orthosteric (agonist) binding site of the α7 nAChR, the ability of the compound to affect bind-
ing of [3H]α-BgTx was investigated. Using rat brain membrane, NS1738 was found unable to displace [3H]α-BgTx at concentrations up to 100 µM (data not shown). Likewise, no displacement of [3H]methyllycaconitine binding to rat brain membranes was observed until at least 100 µM (data not shown). NS1738 was also without effect on binding of [3H]cytisine or [3H]epibatidine to rat brain membrane and [3H]α-BgTx on membranes from the TE671/RD cell line (expressing the neuromuscular nAChR; data not shown), indicating that NS1738 does not affect binding to heteromeric or neuromuscular nicotinic receptors.

We next investigated whether NS1738 could alter the affinity of the α7 nAChR for [3H]α-BgTx. Again, using rat brain membrane, a saturation binding experiment was performed. The data conformed nicely with binding of the radioligand to a single site both in the presence and absence of 10 µM NS1738 (Fig. 1B). However, the presence of NS1738 did not affect either the $K_d$ or the $B_{\text{max}}$ value for [3H]α-BgTx (Fig. 1B, inset).

**Potency and Efficacy: Xenopus Oocyte Electrophysiology.** One-minute preincubation of oocytes expressing human α7 nAChR with NS1738, followed by test application of ACh at a half-maximal concentration (100 µM), led to an ~2 to 3-fold increase in peak current amplitude compared with the control condition (Fig. 2A). Plotting peak current amplitude against the logarithm of the NS1738 concentration used for preincubation revealed a sigmoidal concentration-response relationship that was well fit by the Hill equation [$EC_{50} = 3.4$ µM; $E_{\text{max}} = 322\%$; $n_H = 2.0$ ($n = 7$)] (Fig. 2B). Under similar experimental conditions, NS1738 showed comparable efficacy and potency at the rat α7 nAChR ($EC_{50} = 3.9$ µM; $E_{\text{max}} = 490\%$) (data not shown).

To resolve the action of the compound more clearly, concentration-response curves for ACh were constructed in the presence and absence of 10 µM NS1738. These curves are depicted in Fig. 2C, and they demonstrate that in addition to increasing ACh potency by an order of magnitude [ACh: $EC_{50} = 139$ µM ($n = 4$); ACh + NS1738: $EC_{50} = 15$ µM ($n = 6$)], NS1738 also produced an ~2-fold increase in the maximal efficacy of ACh [ACh: $E_{\text{max}} = 109\%$ ($n = 4$); ACh + NS1738: $E_{\text{max}} = 184\%$ ($n = 6$)]. Moreover, the concentration-response curve became more steep in the presence of NS1738 (ACh: $n_H$...
fected GH4C1 cells preincubated with 10 μM H9262 and H9251. Previous report on rat activation by choline, and antagonism by MLA and kinetics of desensitization, current-voltage relation, current ACh-evoked currents measured under these conditions (e.g., (Virginio et al., 2002).

Amplitude, compared with control levels (Fig. 3A). The concentration of ACh more efficiently activates the human α7 nAChR in the presence of the modulator.

**Potency and Efficacy: Patch-Clamp Electrophysiology.** Whole-cell patch-clamp recordings were performed on GH4C1 cells transiently transfected with cDNA encoding human α7, using an ultrafast solution application system. Basic pharmacological and biophysical characteristics of ACh-evoked currents measured under these conditions (e.g., kinetics of desensitization, current-voltage relation, current activation by choline, and antagonism by MLA and α-Bgt) were in excellent agreement with the properties reported for native α7 nAChRs (data not shown), consistent with a previous report on rat α7 expressed in the GH4C1 cell line (Virginio et al., 2002).

As shown in Fig. 3A, application of 10 μM NS1738 alone did not elicit any measurable current (n = 5), whereas application of a saturating ACh concentration (1 mM) to α7-transfected GH4C1 cells preincubated with 10 μM NS1738 resulted in an approximately 10-fold increase of peak current amplitude, compared with control levels (Fig. 3A). The concentration-response relationship for NS1738-mediated current potentiation was well fit by the Hill equation, giving rise to a rather steep sigmoidal curve [EC50 = 1.6 μM; Emax = 1170%; nH = 3.2; ACh: EC50 = 2.2). The tendency of NS1738 to increase the slope of the sigmoidal curve noted in the oocyte experiments was also evident here (ACh: nH = 3.4; ACh + NS1738: nH = 3.2).

Because pharmacological and biophysical properties of nAChRs may differ between native and heterologously expressed receptors (Lewis et al., 1997), the ability of NS1738 to modulate ACh-induced currents in primary cultures of rat hippocampal neurons was assessed. Expression and composition of nAChRs in this neuronal preparation have been characterized extensively, and it has been estimated that...
80% of these neurons almost exclusively express α7 nAChRs (Alkondon and Albuquerque, 1993).

Whole-cell patch-clamp recording from cultured rat hippocampal neurons at 12 to 14 days in vitro revealed rapidly desensitizing currents conforming to the type IA characteristics typical of α7-mediated currents in these neurons (Alkondon and Albuquerque, 1993) upon application of ACh (Figs. 4 and 5B). Thus, τ_{desensitization} = 14 ± 3 ms (n = 8) (Fig. 5B), determined using 1 mM ACh applications, which is in good agreement with the average desensitization time constant of 27 ms reported previously for type IA currents evoked by 3 mM ACh (Alkondon and Albuquerque, 1993). Moreover, preincubation of hippocampal neurons with 100 nM α-bungarotoxin for 2 min abolished ACh-evoked currents.
(n = 4) (Fig. 4A), an effect that was not reversed by washout of the toxin. By analogy, preincubation of the hippocampal neurons with 10 nM MLA, a concentration at which this toxin is highly selective for /H9251 7 nAChRs, also led to disappearance of ACh-induced current, and this blockade was fully reversible upon a few minutes of washing (Fig. 4B). Together, these findings indicate that the ACh-evoked currents measured in cultured hippocampal neurons were exclusively mediated by /H9251 7 nAChRs. Preincubation of hippocampal neurons with 10 μM NS1738 increased peak amplitude of ACh-evoked currents to 297 ± 32% of control current (at 1 mM ACh; mean ± S.E.M.; n = 8) (Fig. 4C), demonstrating the ability of NS1738 to modulate native /H9251 7 nAChRs.

**Effect on Desensitization.** Inspection of the current traces presented in Fig. 3A (GH4C1 cells) and Fig. 4C (hippocampal neurons) indicates that NS1738 does not pro-
foundly alter the rapid kinetics of α7 nAChR desensitization. Because these recordings were done using ultrafast solution application (complete solution exchange occurring on a submillisecond time scale; see Materials and Methods), the time resolution of these experiments should be of sufficient quality for allowing quantification of desensitization kinetics. Therefore, time constants of desensitization (τdesensitization) were determined from monoeXponentially decaying fits of the decaying phase of current traces recorded, respectively, in the absence and presence of 10 μM NS1738. Because the potency of ACh was essentially identical under these two conditions (see above), applications of ACh at 100 μM (EC50) were used. As shown in Fig. 5A, inspection of scaled current traces suggests only slight effects of NS1738 on desensitization, and comparison of τdesensitization values reveals that although NS1738 did significantly increase τdesensitization, this effect was modest [ACh: τdesensitization = 30 ± 4 ms (n = 5); ACh + NS1738: τdesensitization = 53 ± 7 ms (n = 5); P = 0.014, paired t test] (Fig. 5A). By analogy, the desensitization time constant of 1 mM ACh-induced currents in cultured hippocampal neurons (see above) was found to be τdesensitization = 14 ± 3 ms (n = 8) under control conditions and τdesensitization = 21 ± 2 ms (n = 8) in the presence of 10 μM NS1738 (P = 0.025, paired t test) (Fig. 5B), suggesting only a minor effect on desensitization of native α7 nAChRs.

Selectivity Profile. We also investigated whether allosteric modulation by NS1738 was specific for the α7 nAChRs or whether it would extend to other nAChR subtypes. Using a fluorometric imaging plate reader, we found that Ca2+ responses evoked by subsaturating concentrations of (-)-nicotine in human embryonic kidney 293 cells expressing α4β2 or α3β4 nAChRs were not potentiated by NS1738. In contrast, at high concentrations, a modest 20% (IC50 > 30 μM; n = 4) and 58% (IC50 > 30 μM; n = 4) inhibition at α4β2 and α3β4 nAChRs, respectively, was observed (data not shown).

In an attempt to corroborate these findings, Xenopus oocytes expressing α4β2 or α3β4 nAChRs were stimulated with subsaturating concentrations of acetylcholine in the absence or presence of varying concentrations of NS1738. As shown in Fig. 6A, ACh-induced (α4β2, 1 μM; α3β4, 100 μM) currents were not increased in amplitude by NS1738; in fact, they were inhibited at high concentrations. NS1738 was tested at concentrations up to 30 μM, at which concentration currents mediated by α4β2 and α3β4 nAChRs were inhibited by 24 ± 4% (n = 3) and 58 ± 3% (n = 3), respectively, in good agreement with the findings of the fluorometric imaging
plate reader experiments. Assuming complete inhibition of both α4β2- and α3β4-mediated currents at saturation, half-maximal concentrations (IC50 values) for this inhibiting effect of NS1738 were extrapolated to be 89 μM (nH = 1.0) for α4β2 and 27 μM (nH = 1.0) for α3β4 nAChR. Compared with the potency of NS1738 with respect to positive modulation of α7 nAChRs (EC50 = 3.4 μM in oocyte experiments; see above), this gives approximately 8- and 26-fold selectivity for potentiation of α7 versus inhibition of α3β4 and α4β2, respectively. Indeed, comparing the effect of a saturating concentration of NS1738, 10 μM, with respect to positive modulation of α7 in Xenopus oocytes, GH4C1 cells or hippocampal neurons (current traces of Figs. 2, 3, and 4) with the inhibiting effect at α3β4 and α4β2 nAChRs at the same concentration (Fig. 6A, inset), indicates that the inhibiting effect at heteromeric receptors is only marginal. Thus, NS1738 seems to selectively potentiate α7 nAChR effects, while not substantially interfering with the effects at heteromeric α4* and α3* receptors.

We also investigated whether NS1738 acts as a modulator of α1-containing nicotinic receptors by means of patch-clamp recordings from the human clonal cell line TE671/RD, which...
is known to express the fetal (α1)βγδ form of the neuromuscular nicotinic receptor (Somnir, 1994). As expected (Sine, 1988), inward currents evoked by 1 mM ACh in TE671/RD cells were abolished by preincubation with 100 nM α-BgtTx (data not shown). However, as presented in Fig. 6C, preincubation of cells with 10 μM NS1738 did not affect the amplitude of ACh-evoked currents [I<sub>NS1738</sub> = 95 ± 5% of I<sub>control</sub>] (n = 4). Likewise, NS1738 also did not potentiate human 5-hydroxytryptamine<sub>3A</sub> currents at least until 30 μM (data not shown).

**Absorption, Distribution, Metabolism, and Excretion Properties.** To estimate the ability of NS1738 to permeate the blood-brain barrier, rats were administered 10 mg/kg NS1738 intraperitoneally. Peak brain concentrations were measured approximately 30 min after injection, and they amounted to ~80 ng/ml (~200 nM) at this dose (Table 1). The ratio between the amount of compound entering the brain and that in plasma was AUC<sub>brain/AUC_plasma</sub> = 0.50. The half-life in plasma was estimated to 42 min, but it could not be measured reliably in the brain. Incubation of NS1738 with isolated liver microsomes in vitro indicated that approximately 60 and 75% of the compound was metabolized via the cytochrome P450 system in mouse and rat, respectively, within 1 h (Table 1). Preliminary analyses of mass spectra suggested that NS1738 was primarily metabolized in microsomes by either sulfation or glucuronidation of the hydroxyl group (Fig. 1A; data not shown).

**Behavioral Effects.** Having demonstrated modest brain penetration of NS1738, the behavioral pharmacology of this compound was investigated using various animal models. As an index of general sedative or stimulant properties, NS1738 was tested for effects on locomotor function by measuring the novelty-induced exploratory activity in mice at various time points (0–3 h) after injection with vehicle or 10, 30, or 100 mg/kg i.p. NS1738. The compound did not affect locomotor function at 10 and 30 mg/kg, whereas a slight but statistically significant (P < 0.05; one-way ANOVA) increase in activity was noted at 100 mg/kg (data not shown).

To assess the effect of NS1738 on cognitive/mnemonic function, the compound was first tested in the rat Morris’ water maze, a model of spatial long-term memory; (−)-scopolamine at 0.1 mg/kg i.p. was used to impair performance of the animals. Acquisition learning was determined as the latency to find the submerged platform over four consecutive days, using four trials each day, as detailed under Materials and Methods. Acquisition of the task was impaired by (−)-scopolamine, as shown by the significantly increased latencies to locate the submerged platform through days 2 to 4 in the scopolamine/vehicle group, relative to the vehicle/vehicle group (P < 0.05; one-way ANOVA) (Fig. 7), despite identical latencies on day 1. The latter observation suggests that the ability of the rats to identify and climb the platform was unaffected by NS1738. However, NS1738 at 30 mg/kg improved acquisition learning to near control levels, and latencies in the scopolamine/NS1738 group were significantly smaller compared with latencies in the scopolamine/vehicle group throughout trials 2 to 4 (P < 0.05; one-way ANOVA).

None of the treatment groups displayed alterations in swimming speed (data not shown), indicating that latency is an adequate measure of the ability of the rats to find the platform.

Acquisition of the task was followed by a probe trial (the platform is removed and the swimming time spent in the quadrant previously containing it is measured) and a reversal trial (the platform is returned to the water maze but in a new location; the latency to find the platform is measured) on day 5. Although there were tendencies toward alleviation of the (−)-scopolamine-induced deficits by NS1738 in both probe and reversal trials, this did not amount to statistical significance at either dose (data not shown).

The efficacy for NS1738 was also assessed in the rat social recognition test, a model of short-term memory. Adult rats administered NS1738 at 10 and 30 mg/kg i.p. immediately following the initial exposure to a juvenile rat (T1) displayed significant decreases in the investigative duration of a subsequent exposure to the same juvenile (T2) 2 h later [T2/T1 ratio of 0.69 ± 0.13 and 0.61 ± 0.07, respectively (mean ± S.E.M.; n = 9). In contrast, vehicle-treated rats exhibited a T2/T1 ratio of ~1.0, indicating loss of memory. (−)-Nicotine

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg i.p.)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (min)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>AUC (ng · min/ml)</th>
<th>Brain-Plasma Ratio</th>
<th>Microsomal Stability (% Left at 1 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>10</td>
<td>30</td>
<td>42</td>
<td>172</td>
<td>13,381</td>
<td>0.50</td>
<td>mouse:41 rat:25</td>
</tr>
</tbody>
</table>
(0.1 mg/kg free base) used as positive control in this study displayed a significantly lower ratio (0.62 ± 0.06; n = 9), comparable with that observed with NS1738.

Discussion

Taken together, the oocyte and patch-clamp electrophysiology data presented demonstrate that NS1738 augments ACh-induced α7 receptor activity, with potency in the low micromolar range and with excellent selectivity against other nAChR subtypes. It is noteworthy that augmentation was observed regardless of the concentration of ACh used for activating the α7 nAChR, including saturating concentrations. From the ACh concentration-response relationships recorded in the presence of NS1738, it is evident that the maximal efficacy of ACh was robustly enhanced (by ~2-fold in the oocyte experiments and by ~6-fold in the patch-clamp experiments). Analysis of the oocyte experiments suggested that NS1738 also produced a leftward shift of the ACh concentration-response relationship, i.e., made ACh ~10-fold more potent at the receptor, whereas this effect was not observed in the patch-clamp experiments on transfected GH4C1 cells (compare Figs. 1 and 2). Although this discrepancy may be accounted for by the different experimental paradigms used, it is also conceivable that this observation is explained by inherent differences in α7 nAChR pharmacology between the two expression systems. Indeed, differences in the single-channel properties of α3β4 nAChRs have been reported comparing receptors expressed in oocytes and mouse L929 cells (Lewis et al., 1997).

Interestingly, the rapid desensitization kinetics of the α7 nAChR was affected only marginally by NS1738, as determined from patch-clamp recordings on transfected GH4C1 cells. The time constant of desensitization was slightly less than doubled by NS1738 when measured under these conditions. This finding was corroborated by the observation of a similar increase in the value of the time constant in hippocampal neurons. Although the increase in the value of the time constant did reach statistical significance in both cases, the absolute magnitude of the increase was quite modest, and it did not substantially affect the rapidly desensitizing waveform of the α7 nAChR current traces (Fig. 5).

Together, these findings are consistent with the idea that NS1738 modulates the activity of the α7 nAChR by facilitating the energetic coupling between agonist binding and ion channel gating, thereby increasing the receptor open probability at all ACh concentrations. However, it is also clear that this enhanced efficiency of the binding-gating coupling is dissociated from the energetics of the desensitization process, which proceeds with nearly unaltered kinetics.

The mechanistic profile of NS1738 is qualitatively reminiscent of various agents reported previously to produce allosteric α7 nAChR modulation in vitro. Thus, 5-hydroxyindole, ivermectin, serum albumin, the C-terminal fragment of ACh esterase and the peptide modulator SLURP-1 all increase the potency and/or efficacy of ACh. Furthermore, these α7 nAChR modulators display no, or at most marginal, effects on desensitization kinetics (Krause et al., 1998; Zwart et al., 2002; Chimienti et al., 2003; Conroy et al., 2003; Greenfield et al., 2004). To date, three α7 nAChR modulators have been demonstrated to be bioavailable to some extent and to be efficacious in in vivo pharmacological models, namely, PNU-120596 (Hurst et al., 2005), compound 6 (Ng et al., 2007), and NS1738 (present report). These compounds display some degree of chemical similarity, and yet their in vitro pharmacological profiles differ substantially. Although all three compounds have been found to increase the potency and/or maximal efficacy of ACh to varying degrees, PNU-120596 was reported to produce profound inhibition of the rapid desensitization of the α7 nAChR. Compound 6 and NS1738, in contrast, are similar in the sense that they hardly affect desensitization kinetics at all; therefore, it seems that NS1738 and compound 6 belong to a novel class of α7 nAChR modulators sharing a similar mode of action.

Extrapolating these mechanistic differences to a more physiological scenario, it seems likely that the dramatic effect of PNU-120596 on desensitization could distort the temporal characteristics of normal α7-mediated neurotransmission, i.e., by prolonging excitatory synaptic events. Moreover, considering the high calcium permeability of the α7 nAChR, it is easily conceivable that prolonged activation of PNU-120596-modulated receptors could have excitotoxic consequences. Indeed, PNU-120596 was reported to be neurotoxic in an in vitro model (Ng et al., 2007). NS1738 and compound 6, in contrast, exert their main effect through enhancement of peak current amplitudes. At the synaptic level, this would probably translate into increased α7-mediated EPSCs, giving larger postsynaptic depolarizations/Ca2+ signals, while completely preserving the temporal characteristics of normal neurotransmission. In addition, compound 6 was found not to be neurotoxic (Ng et al., 2007), consistent with the notion that the type of modulation produced by NS1738 and compound 6 does not lead to excessive calcium influx.

The pharmacokinetic data obtained after intraperitoneal administration of NS1738 revealed modest systemic absorption and brain penetration of NS1738. Peak concentrations were measured in plasma and brain ca. 30 min after administration, in accordance with the time frame during which the pharmacology experiments were conducted. After administration of 10 mg/kg i.p., this peak brain concentration was in the order of 200 to 300 nM (Table 1), and after 30 mg/kg, the peak brain concentration would approach 1 μM, assuming linear pharmacokinetics. Extrapolating from the in vitro potency of NS1738 (Figs. 2B and 3B), such brain concentrations would be within a range expected to confer α7 nAChR modulation in vivo.

Consistent with this, 10 and 30 mg/kg NS1738 improved performance to the same extent as (−)-nicotine in the social recognition test, a model of short-term memory, and it reversed the cognitive impairment induced by (−)-scopolamine of acquisition of a water-maze learning task (at 30 mg/kg), a model of long-term spatial memory (in the format used in the present study). The social recognition test determines the ability of an adult rat to recall a previous encounter with a juvenile rat, considering the time spent investigating the juvenile rat as a parameter being inversely proportional with memory. In the present protocol, a 2-h delay between trials was sufficient to make the adult rat completely forget the encounter (ratio of ~1 in vehicle controls; Fig. 8), whereas both (−)-nicotine and NS1738 reduced this ratio (to approximately 0.6), reflecting a strengthening of short-term memory. Interestingly, in this experiment, NS1738 was administered immediately after the first trial, implying an effect on memory consolidation.
In the Morris’ water-maze test, the ability (latency) of a rat to find the submerged platform is measured over four consecutive days. Using spatial cues (i.e., laboratory surroundings), the rat gradually learns the location of the platform, thus reducing swimming latency. However, drug-naïve animals learn this task extremely efficiently (Fig. 7, vehicle controls), and it is virtually impossible to improve acquisition using pharmacological means (our unpublished observations). In the present experiments, (−)-scopolamine was therefore used to induce a state of cognitive impairment (Patel and Tariot, 1991), and this treatment dramatically reduced the rate of task learning. Coadministration of 30 mg/kg NS1738 reversed the effect of (−)-scopolamine to near control levels, whereas the effect of the 10-mg/kg dose was intermediate, albeit nonsignificant. In these experiments, NS1738 was administered before each test session, suggesting an effect on either memory acquisition and/or consolidation.

These observations are in agreement with recent reports highlighting the involvement of α7 nAChRs for memory formation in these particular models. Thus, two different agonists selective for the α7 nAChR, AR/R-17779 and N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide, have been demonstrated to enhance performance in the rat social recognition model (Van Kampen et al., 2004; Boess et al., 2007). Moreover, N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide was shown to improve performance of aged rats in a water-maze acquisition paradigm (Boess et al., 2007), and interestingly, in vivo administration of antipsychotic olanzapine targeting α7 was recently shown to impair acquisition of a water-maze task with a concomitant reduction in α7 nAChR expression (Curzon et al., 2006). Collectively, these data indicate that stimulation of α7 nAChR function facilitates, whereas reducing α7-mediated neurotransmission inhibits, memory formation.

Generally, there is a growing body of evidence demonstrating that agonists of the α7 nAChR facilitate cognitive function in a variety of species ranging from rodents to humans (Arendash et al., 1995; Briggs et al., 1997; Freedman et al., 1997; Kitagawa et al., 2003; Hajós et al., 2005; Olincy et al., 2006; Wishka et al., 2006; Pichat et al., 2007). Moreover, several α7 nAChR agonists alleviate auditory gating deficits in animal models (Stevens et al., 1998; Hajós et al., 2005; Wishka et al., 2006) and in humans (Olincy et al., 2006). Recently, evidence that allosteric modulators of the α7 nAChR display efficacy in similar models of cognitive function and auditory gating has become available. The recently published compound 6 found to improve working memory, as assessed in the eight-arm radial maze, and both compound 6 and the desensitization inhibitor PNU-120596 have been shown to improve deficient auditory gating in rodent models (Hurst et al., 2005; Ng et al., 2007).

Collectively, the data presented in this report show NS1738 to be an efficacious and highly selective modulator of the α7 nAChR, capable of producing cognitive enhancement in vivo. These findings testify to the utility of allosteric modulation of the α7 nAChR as a novel therapeutic principle for treating cognitive dysfunction associated with various forms of dementia and/or schizophrenia.

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