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RESEARCH PAPER

Role of channel activation in cognitive enhancement mediated by α7 nicotinic acetylcholine receptors

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Background and purpose: Several agonists of the α7 nicotinic acetylcholine receptor (nAChR) have been developed for treatment of cognitive deficits. However, agonist efficacy *in vivo* is difficult to reconcile with rapid α7 nAChR desensitization *in vitro*; and furthermore, the correlation between *in vitro* receptor efficacy and *in vivo* behavioural efficacy is not well delineated. The possibility that agonists of this receptor actually function *in vivo* as inhibitors via desensitization has not been finally resolved.
Experimental approach: Two structurally related α7 nAChR agonists were characterized and used to assess the degree of efficacy required in a behavioural paradigm.
Key results: NS6784 activated human and rat α7 nAChR with EC_{50}s of 0.72 and 0.88 μM, and apparent efficacies of 77 and 97% respectively. NS6740, in contrast, displayed little efficacy at α7 nAChR (<2% in oocytes, ≤8% in GH4C1 cells), although its agonist-like properties were revealed by adding a positive allosteric modulator of α7 nAChRs or using the slowly desensitizing α7V274T receptor. In mouse inhibitory avoidance (IA) memory retention, NS6784 enhanced performance as did the 60% partial agonist A-582941. In contrast, NS6740 did not enhance performance, but blocked effects of A-582941.
Conclusions and implications: Collectively, these findings suggest that a degree of α7 nAChR agonist efficacy is required for behavioural effects in the IA paradigm, and that such behavioural efficacy is not due to α7 nAChR desensitization. Also, a partial agonist of very low efficacy for this receptor could be used as an inhibitor, in the absence of α7 nAChR antagonists with favourable CNS penetration.
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Keywords: nicotinic acetylcholine receptor; ligand-gated ion channels; desensitization; functional systems; learning and memory; biopharmaceuticals

Abbreviations: c.i., confidence interval; IA, inhibitory avoidance; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; PAM, positive allosteric modulator

Introduction

Recently, several groups have developed agonists selective for the α7 nicotinic acetylcholine receptor (nAChR) that enhance performance in pre-clinical models of cognitive function (Kem *et al.*, 2004; Wishka *et al.*, 2006; Bitner *et al.*, 2007; Boess *et al.*, 2007; Pichat *et al.*, 2007; Romanelli and Gualtieri, 2007; Briggs *et al.*, 2008; Tietje *et al.*, 2008). However, it is somewhat difficult to reconcile *in vivo* efficacy of systemically administered α7 nAChR agonist with the rapid (milliseconds to seconds) desensitization of α7 nAChR observed *in vitro*. A variety of *in vitro* studies, including Ca^{2+} and protein kinase signalling as well as electrophysiological recording, found that α7 nAChR agonists must be applied rapidly in order to elicit a maximal response. With slow application *in vitro*, over a period of tens of seconds, desensitization severely curtails signal recognition unless a positive allosteric modulator of the α7 nAChR is included to inhibit desensitization (Hurst *et al.*, 2005; Dickinson *et al.*, 2007; Grønlien *et al.*, 2007). Nevertheless, agonists are effective *in vivo*, as measured by electrophysiology, signalling and behavioural assays following systemic administration, which would deliver the agonist to the CNS over several minutes (Olincy *et al.*, 2006; Bitner *et al.*, 2007; Tietje *et al.*, 2008).

One hypothesis is that α7 nAChR agonists may function *in vivo* as inhibitors by desensitizing the receptor rather than as agonists *per se*. This is difficult to test with α7 nAChR antagonists because of problems with selectivity and

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CNS penetration (see Discussion). In another approach to testing this hypothesis, we selected two high-affinity α7 nAChR ligands with markedly different abilities to activate α7 nAChR currents – NS6784 (2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-5-phenyl-1,3,4-oxadiazole; also known as A-803401) and NS6740 (1,4-diazabicyclo[3.2.2]nonan-4-yl(5-(3-(trifluoromethyl)phenyl)furan-2-yl)methanone; also known as A-793394). Both compounds are members of the diazabicyclononane chemical series and have nanomolar α7 nAChR binding affinities.

In this study, NS6740 was a weak (<10%) partial agonist that predominantly inhibited α7 nAChR, while NS6784 was a full agonist at rat recombinant α7 nAChR. In an avoidance learning behaviour paradigm, the effects of these compounds correlated with their *in vitro* characterization. NS6784 enhanced avoidance learning, while NS6740 did not enhance avoidance learning but inhibited the effect of a more efficacious α7 nAChR agonist. These results argue against the concept that enhancement of cognitive function may be due to α7 nAChR inhibition, and demonstrate that some level of α7 agonist efficacy, as defined *in vitro*, is required for *in vivo* efficacy. Further studies using selective α7 nAChR ligands other than classic antagonists, including weak partial agonists and positive allosteric modulators, will help to identify the signalling mechanisms critical for α7 nAChR function *in vivo*.

Methods

Receptor binding

Binding to rat brain membrane α7 nAChR and α4β2 nAChR was measured by [^{3}H]A-585539 ([^{3}H]-(*S*,*S*)-2,2-dimethyl-5-(6-phenyl-pyridazin-3-yl)-5-aza-2-azonia-bicyclo[2.2.1]heptane iodide) and by [^{3}H]cytisine displacement, respectively, using standard techniques (Anderson *et al*., 2008). Briefly, rat brain membrane pellets were thawed at 4°C, washed and resuspended with a Polytron (Cardinal Health, Dublin, OH, USA) (setting 7) in 30 volumes of balanced salt solution (BSS)–Tris buffer (120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 50 mM Tris–Cl, pH 7.4, 4°C). Bound radioactivity was collected by vacuum filtration onto Millipore MultiScreen harvest plates FB (Millipore Corporation, Billerica, MA, USA) pre-soaked with 0.3% polyethyleneimine using a Packard cell harvester (PerkinElmer, Waltham, MA, USA). The filters were then rapidly rinsed with 2 mL of ice-cold BSS. PerkinElmer MicroScint-20 scintillation cocktail (40 μL) was added to each well, and bound radioactivity was determined using a PerkinElmer TopCount instrument. Non-specific binding was defined using 10 μM A-585539 (α7) or 10 μM nicotine (α4β2). K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation, where $K_i = IC_{50}/(1 + [\text{ligand}]/K_D)$.

Electrophysiological measurements

Functional evaluation at human and rat α7 wild-type nAChR and human α7V274T mutagenized nAChR was performed using recombinant receptors expressed in *Xenopus laevis* oocytes and in GH4C1 cells.

Oocyte responses were measured at room temperature using two-electrode voltage clamp (–60 mV) in the presence of 0.5 μM atropine to block endogenous muscarinic receptors. Assays were conducted as previously described (Briggs *et al*., 1995; Trumbull *et al*., 2003; Grønlien *et al*., 2007). Responses were measured as the peak (maximal) inward current relative to the baseline holding current, and were normalized to the maximal response to ACh (10 mM for wild type or 10 μM for α7V274T) determined in each oocyte before and after application of test compound. In some experiments, the α7 nAChR positive allosteric modulator, PNU-120596 (10 μM), was added before and during exposure to NS6740 (Grønlien *et al*., 2007) in order to observe the effects of the low-efficacy partial agonist.

Additionally, NS6740 was evaluated using human α7 nAChR transfected into GH4C1 cells and recorded by standard whole-cell patch clamp technique as previously described (Timmermann *et al*., 2007).

Cell line Ca^{2+} signalling in FLIPR

To determine nAChR functional selectivity, effects on non-α7 nAChR were measured by FLIPR using the Ca^{2+}-sensitive fluorescent probe Fluo-4 and human neuroblastoma IMR-32 or HEK293 cell lines expressing recombinant human α4* and α3* nAChR as previously described (Grønlien *et al*., 2007). Responses were normalized to the response to 100 μM (-)-nicotine determined in the same 96-well plate.

Cognitive performance – inhibitory avoidance (IA) assay

For behavioural evaluation, the IA procedure was used with male CD-1 mice (30–40 g) acclimatized to their housing for at least 14 days after receipt. Testing was carried out in a dimly lit room (~20 lux) following habituation (≥90 min).

Single compound assays. Each mouse was injected i.p. (10 mL·kg^{-1}) with compound or vehicle control (0.9% saline), and 30 min later placed into the illuminated (~25 lux) side of a two-chamber light/dark shuttle box. After 30 s, the door separating the light and dark compartments was raised, and the time taken (latency) to enter the darkened compartment was measured. Training consisted of one trial in which the mouse received mild footshock (0.12 mA, 1 s) through the floor upon crossing to the darkened side (encased in black foam/plastic), and was then gently removed from the apparatus and returned to the home cage. Testing was conducted 24 h later in the same apparatus, except the compound was not administered and footshock was not delivered. Memory retention was indexed by increased latency to enter the darkened compartment with all four paws on the grids. Data were analysed by one-way analysis of variance followed by Dunnett's *post hoc* test, if differences were found.

Two compound assays. To determine the ability of NS6740 to inhibit the response to A-582941, NS6740 (0.1–10 μmol·kg^{-1}) was administered 10 min prior to A-582941 (1 μmol·kg^{-1}), and training commenced 30 min following A-582941. Otherwise, methods were the same as for the single compound assay.

Results

Receptor binding

The diazabicyclononanes NS6740 (A-793394) and NS6784 (A-803401; Figure 1A) were synthesized as prospective α7

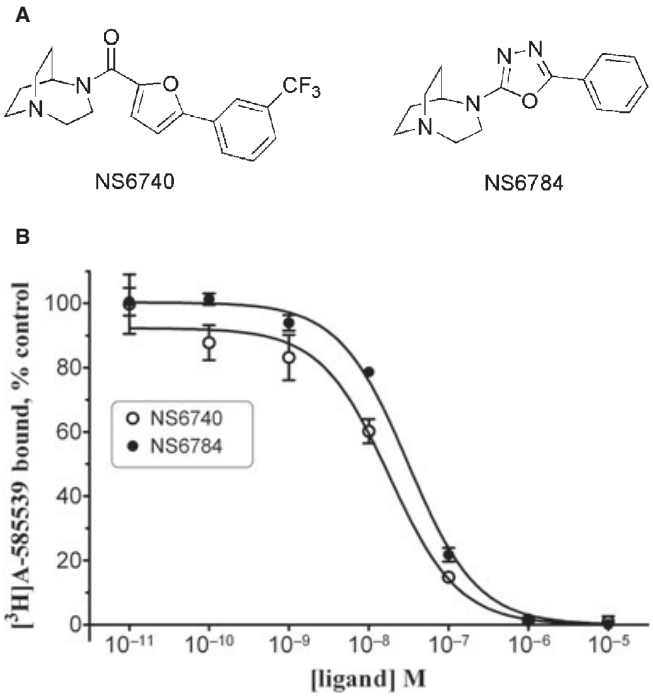

Figure 1 NS6740 and NS6784 are structurally similar α7 nicotinic acetylcholine receptor (nAChR) ligands. (A) Structures of the diazabicyclononane compounds NS6740 (A-793394) and NS6784 (A-803401). (B) Binding affinities at rat brain α7 nAChR, as measured by displacement of the α7-selective agonist [^{3}H]A-585539. Data are shown as mean ± SEM from three experiments. Affinities of the compounds for α7 nAChR were similar, with K_i values of 1.1 nM for NS6740 and 3.3 nM for NS6784.

nAChR agonists. Both exhibited high-affinity binding to α7 nAChR sites in rat brain membranes, displacing the α7-selective agonist [^{3}H]A-585539 with K_i values of 1.1 nM [0.95–1.3 nM, 95% confidence interval (c.i.), n = 3] and 3.3 nM (3.0–3.7 nM c.i., n = 3), respectively (Figure 1B). Both compounds exhibited weak binding affinities to rat brain α4β2 nAChR with [^{3}H]cytisine displacement K_i values of ≥8000 nM for NS6740 (n = 4) and 2300 nM (1720–3070 nM c.i., n = 7) for NS6784.

Receptor function

NS6784 exhibited clear agonist efficacy at human and rat α7 nAChR expressed in oocytes, with EC_{50} values of 0.72 μM (0.53–0.99 μM c.i., n = 6) and 0.88 μM (0.51–1.5 μM c.i., n = 3), respectively (Figure 2). Concentration–response curves plateaued at 77 ± 3% (mean ± SEM) at human α7 and 97 ± 6% at rat α7 nAChR, relative to the maximal ACh response. In contrast, the response to NS6740 was <2% of the response to ACh at both human and rat α7 nAChR. For both compounds, <25% activation of human α4β2, α3β4 and IMR-32 native nAChR was detected in FLIPR at concentrations up to 100 μM (data not shown; n = 4 quadruplicate determinations).

The observation that NS6740 had low efficacy prompted us to further examine the nature of its interactions at α7 nAChRs by whole-cell patch clamp and two-electrode voltage clamp studies. As shown in Figure 3A, patch clamp recording from GH4C1 cells transiently transfected with human α7 nAChR revealed a small α7-like response to NS6740. The response was reversibly blocked by the α7-selective antagonist 10 nM methyllycaconitine (MLA; 10 nM, n = 5), consistent with α7 nAChR activation. Nevertheless, the response to NS6740 at micromolar concentrations averaged <10% of the maximal response to ACh (Figure 3B). Pre-exposure to nanomolar concentrations of NS6740, on the other hand, was able to block the response to ACh applied as agonist (Figure 3C). The IC_{50} value for NS6740 was 2.7 nM (1.9–3.9 nM c.i., n = 5–7), demonstrating interaction of NS6740 with human α7 nAChR in this assay at concentrations similar to those at which NS6740 was found to bind α7 nAChR in rat brain membranes.

In the oocyte expression system, addition of the α7 nAChR positive allosteric modulator PNU-120596 to the bathing media revealed functional interaction of NS6740 with human α7 nAChR. Agonist efficacy of NS6740 was apparent in the presence of PNU-120596 (Figure 4A), similar to the ability of PNU-120596 to convert the partial agonist GTS-21 to full efficacy (Grønlien *et al*., 2007). The EC_{50} value for NS6740 was 63 nM (46–87 nM c.i., n = 5) in combination with PNU-120596. Furthermore, the slowly desensitizing α7V274T mutagenized nAChR was fully activated by NS6740 with an EC_{50} value of 27 nM (23–33 nM c.i., n = 4) in the absence of modulator (Figure 4B). In parallel experiments, the EC_{50} value for ACh at α7V274T was 1200 nM (950–1500 nM c.i., n = 2), similar to results from an earlier study (Briggs *et al*., 1999).

All together, the data showed that NS6740 bound to wild-type α7 nAChR, had very low (≤10%) efficacy to activate channel opening, inhibited the response of α7 nAChR to a full agonist and appeared as an agonist when a positive allosteric modulator or a mutagenized receptor was used to slow desensitization. This profile of NS6740 was consistent with those of other α7 nAChR partial agonists (Briggs and McKenna, 1998; Briggs *et al*., 1999; Grønlien *et al*., 2007).

Cognitive function

With the IA paradigm in mice, the full agonist NS6784 (97% efficacy at rat α7 nAChR) was efficacious in improving memory consolidation and recall, but not more effective than the 60% partial agonist A-582941 (Figure 5, top; see also Tietje *et al*., 2008). In contrast, the weaker partial agonist NS6740 failed to improve IA performance, compared to nicotine used as a positive control in the same experiment (Figure 5, middle). However, NS6740 did block the effect of A-582941 in the IA paradigm (Figure 5, bottom).

Discussion

In lead optimization studies directed towards novel α7 nAChR agonists, the diazabicyclononane NS6784 was found to be an efficacious α7 nAChR agonist, while the analog NS6740 showed a strikingly distinct *in vitro* profile. NS6740 bound rat brain α7 nAChR with high affinity, yet exhibited little (<10%) activation of either human or rat recombinant α7 nAChR current, as measured electrophysiologically in oocyte and GH4C1 recombinant receptor expression systems.

Behavioural studies of α7 nAChR-mediated effects are complicated by two pharmacological issues. One is that α7

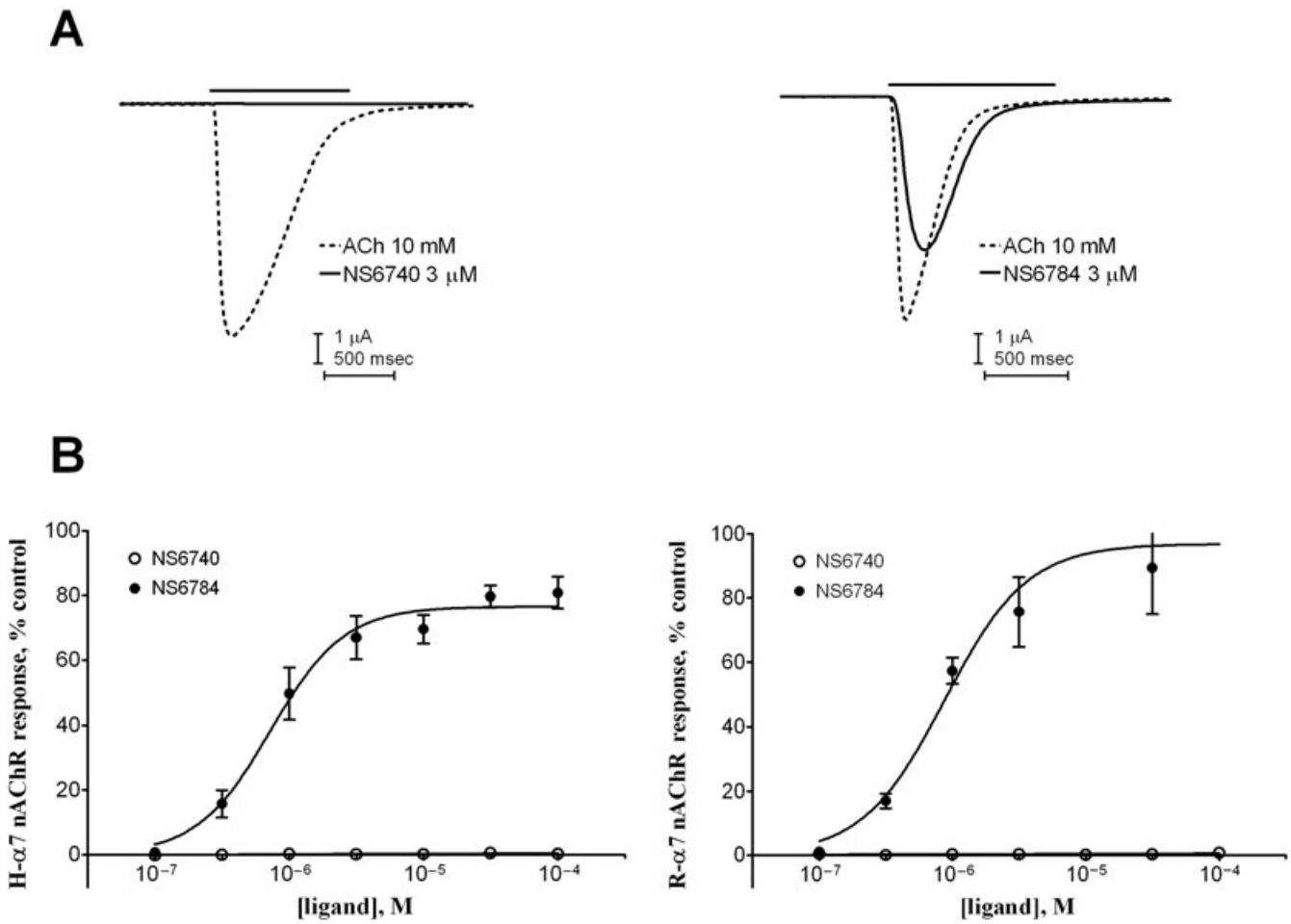

Figure 2 NS6784 was an agonist, while NS6740 showed little efficacy at α7 nicotinic acetylcholine receptor (nAChR) expressed in oocytes. (A) Examples of responses from human α7 nAChR expressed in oocytes. Left: NS6740 (3 μM) had little effect compared to the response to ACh (10 mM) recorded in the same oocyte. Right: NS6784 (3 μM) elicited a clear response with peak amplitude about 70% as large as the maximal response to ACh (10 mM) in the same oocyte. Lines above the traces mark the duration of compound application. Scale bars represent 1 μA and 500 ms. (B) Concentration–response curves for NS6740 and NS6784 from six oocytes expressing human α7 nAChR (left graph) and three oocytes expressing rat α7 nAChR (right graph). Responses were normalized to control responses to 10 mM ACh in each oocyte. Data are shown as mean ± SEM, and curves represent the Hill equation fit to the data. EC_{50} values for NS6784 were 0.72 μM at human α7 nAChR, and 0.88 μM at rat α7 nAChR. NS6740 had little effect up to 100 μM.

nAChR are known to desensitize very rapidly – in <1 s (Galzi *et al.*, 1992; Gotti *et al.*, 1994; Edelstein and Changeux, 1996; Elliott *et al.*, 1996; Fenster *et al.*, 1997; Alkondon *et al.*, 1998; Briggs *et al.*, 1999; Sudweeks and Yakel, 2000). *In vitro* approaches including calcium signalling, kinase signalling and electrophysiological measures detect little or no α7 nAChR agonist response unless care is taken to apply agonist very rapidly, or a positive allosteric modulator is included to inhibit desensitization (Dickinson *et al.*, 2007; Dunlop *et al.*, 2007; Briggs *et al.*, 2008). Yet, α7 nAChR agonists are able to elicit behavioural effects despite the fact that systemically administered compounds clearly do not equilibrate with the CNS within the time thought to be necessary from *in vitro* studies. With equally slow administration *in vitro*, the predominant effect of an α7 nAChR agonist would be inhibition, likely through desensitization in the case of a full agonist, or a combination of desensitization and competitive inhibition in the case of a partial agonist (Briggs and McKenna, 1998; Briggs *et al.*, 1999). This leads to an alternative hypothesis, that the behavioural effects are due to inhibition of α7 nAChRs, not their activation *per se*. Consistent with this is the observation that α7 partial agonists such as A-582941 appear to be fully efficacious in at least some behavioural models (Bitner *et al.*, 2007; Tietje *et al.*, 2008), as partial agonists are able to fully inhibit α7 nAChR (Briggs and McKenna, 1998). Our present results, however, would argue against this hypothesis.

To test the hypothesis, we utilized NS6740, rather than a classic antagonist, as an α7 nAChR inhibitor because there is no specific antagonist for α7 nAChR with high CNS penetration. The most selective α7 antagonist is α-bungarotoxin, but this is a peptide with little or no CNS penetration, and its potent irreversible block of muscle nicotinic receptors clearly prevents its use in behavioural studies, except following i.c.v. administration. MG-624 and derivatives have been described as α7-selective antagonists, but selectivity among mammalian nAChR is lower than among chick nAChR (Maggi *et al.*, 1999; Gotti *et al.*, 2000), and the compounds' quaternary nitrogen

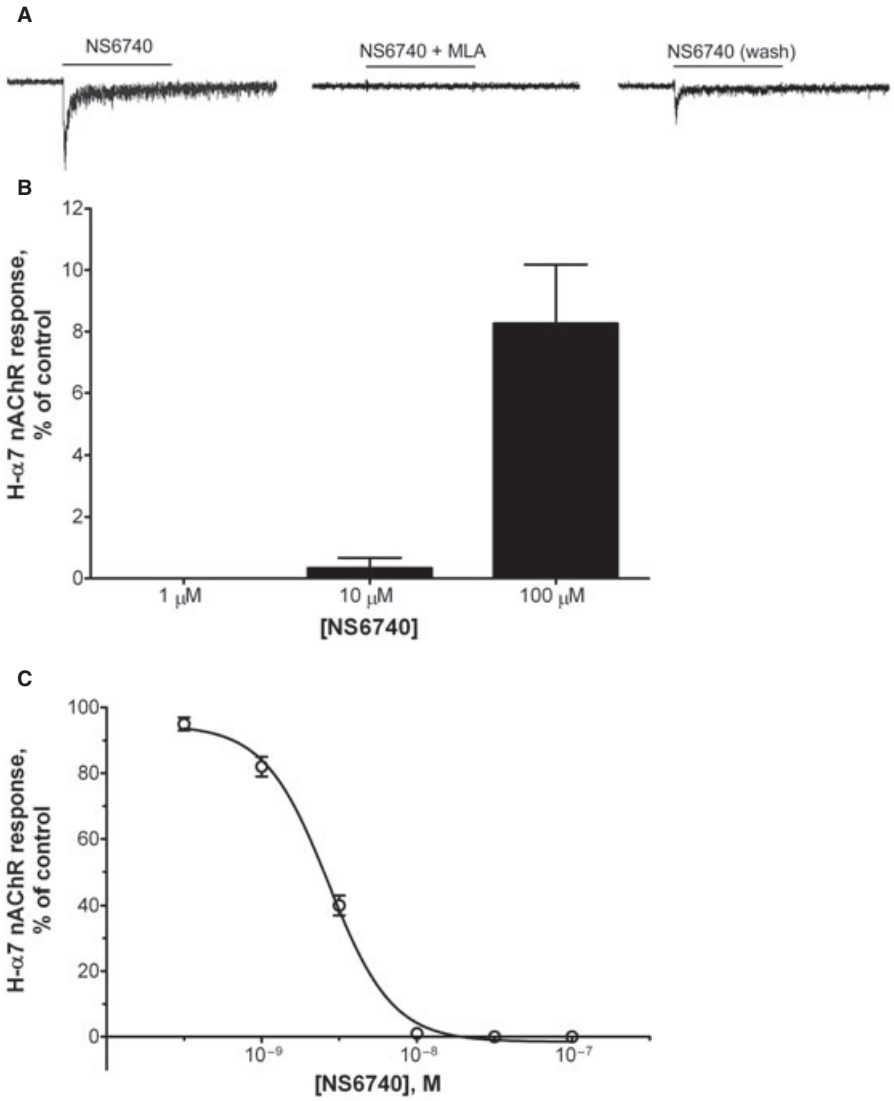

Figure 3 NS6740 displayed weak agonist activity at human α7 nicotinic acetylcholine receptor (nAChR) expressed in GH4C1 cells. (A) An example of response of human α7 nAChR to 100 μM NS6740 (left) that was blocked by 10 nM MLA (middle). Inhibition by MLA was partially reversed following a 5 min wash (right). Lines above the traces mark duration of compound application. (B) Responses to 100 μM NS6740 were detected in 13 of 15 cells, and averaged 8.3 ± 1.9% of the response to 10 mM ACh. Lower concentrations of NS6740 had little effect, with a detectable (2%) response to 10 μM NS6740 in only one of six cells, and no detectable response to 1 μM NS6740 in six cells. (C) Interaction of NS6740 with human α7 nAChR expressed in GH4C1 cells was demonstrated by its ability to inhibit the response to 10 mM ACh. Data are shown as mean ± SEM (n = 5–7), and the curve represents the fitted Hill equation (IC_{50} = 2.7 nM).

seems likely to limit CNS penetration. MLA is the most widely used α7 nAChR antagonist. MLA does cross the blood–brain barrier, albeit poorly. A dose of 6 μmol·kg^{-1} i.p. produced an estimated 50–100 nM MLA in brain (Turek *et al.*, 1995), and chronic nicotine has been observed to further reduce CNS penetration of MLA (Lockman *et al.*, 2005). Furthermore, MLA is selective but not specific. It inhibits α7 and rat α9 nAChR with IC_{50} values around 1 nM (Alkondon *et al.*, 1992; Briggs and McKenna, 1996; Verbitsky *et al.*, 2000), α6* ~50–300 nM (Evans *et al.*, 2003; Salminen *et al.*, 2004), α4β2 ~100–1000 nM (Buisson *et al.*, 1996; Salminen *et al.*, 2004; Briggs *et al.*, 2006) and other nAChR > 1000 nM. Thus, when used under conditions where the concentrations are not known or well controlled, it is difficult to be certain that all effects of MLA are due to α7 nAChR block alone.

NS6740 bound to rat brain α7 nAChR with a K_i of 1.1 nM, elicited ≤10% agonist response (up to 100 μM) at recombinant α7 nAChR, and inhibited the α7 nAChR response to ACh with an IC_{50} value of 2.7 nM. Thus, NS6740 was a weak partial agonist. It produced a small, MLA-sensitive, α7 nAChR current response in transfected GH4C1 cells and exhibited clear α7 nAChR activation only under conditions where the apparent rate of desensitization was slowed – in the presence of the positive allosteric modulator PNU-120596 and at the mutagenized α7V274T nAChR. These are characteristics of partial agonists, not of classic antagonists such as *d*-tubocurarine, mecamylamine and MLA which remain antagonists in the presence of PNU-120596 (Hurst *et al.*, 2005; Dickinson *et al.*, 2007) and at α7V274T (Briggs *et al.*, 1999). In previous studies, we showed that bath application of low

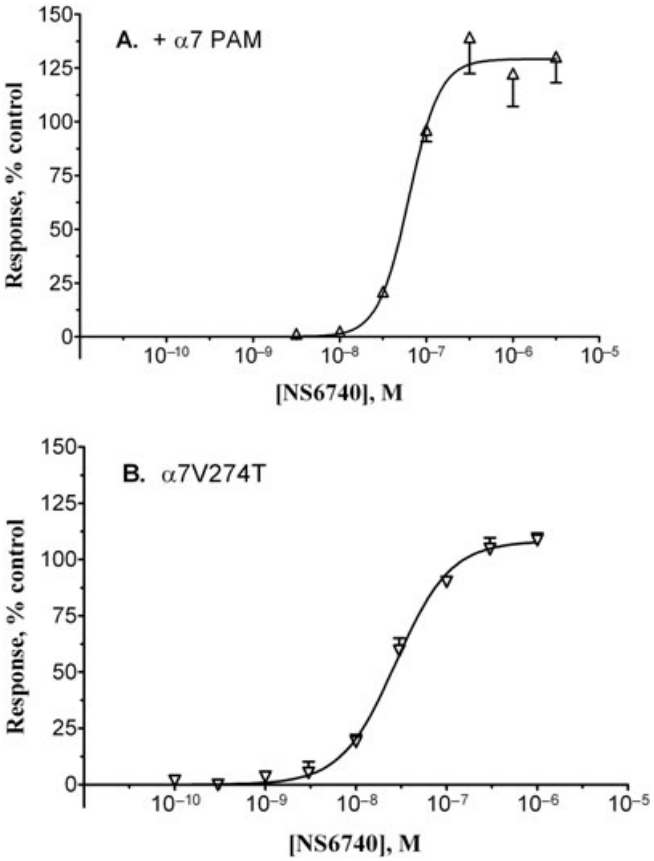

Figure 4 Agonist activity of NS6740 revealed by α7 nicotinic acetylcholine receptor (nAChR) modulation and receptor modification. (A) At wild-type α7 nAChR, NS6740 elicited an agonist-like response in the presence of the positive allosteric modulator, PNU-120596 (10 μM). (B) Additionally, NS6740 acted as an agonist at the mutagenized α7V274T nAChR in the absence of the modulator. Responses were normalized to 10 mM ACh control responses for wild-type α7, and 10 μM ACh for α7V274T in each oocyte. Data are shown as mean ± SEM ($n = 5$ wild-type α7 with modulator; $n = 4$ with α7V274T). Curves represent the fitted Hill equation (EC_{50} values 63 nM for α7 with modulator, 27 nM for α7V274T).

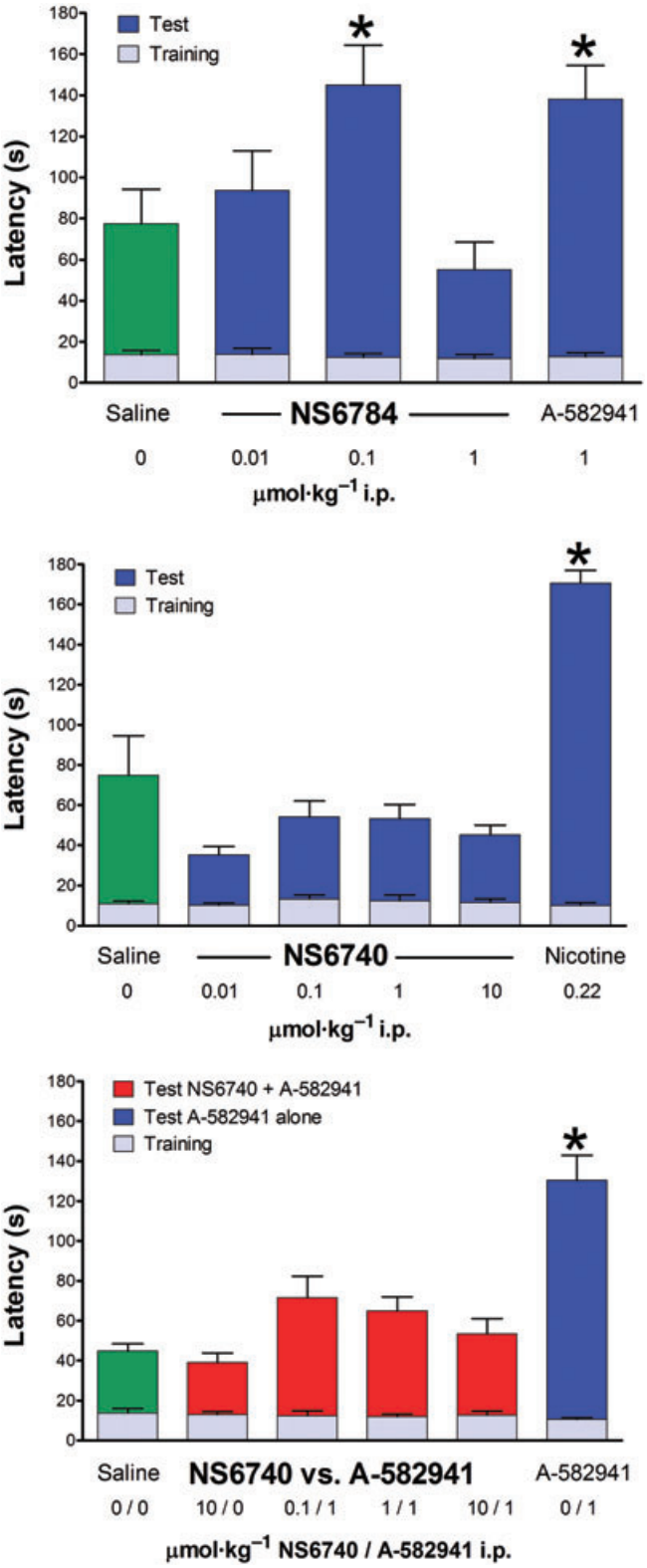

Figure 5 NS6740 inhibited responses to agonists of the α7 nicotinic acetylcholine receptor (nAChR) in the inhibitory avoidance cognitive performance model. During training, the time taken (latency) to enter the dark compartment was <20 s in all groups, as shown by the light grey bars. After training, the latency increased to 40–80 s in control saline-treated mice. Top panel: avoidance learning was enhanced by α7 nAChR agonists NS6784 (0.01–1 μmol·kg^{-1}) and A-582941 (1 μmol·kg^{-1}). Middle panel: NS6740 (0.01–10 μmol·kg^{-1}) did not enhance avoidance learning, while the positive control nicotine (0.22 μmol·kg^{-1}) did enhance learning in the same set of experiments. Bottom panel: in another group of mice, the ability of A-582941 (1 μmol·kg^{-1}) to enhance learning was blocked by prior treatment with NS6740 (0.1–10 μmol·kg^{-1}). Data are shown as mean ± SEM latency to enter the dark compartment with 10–12 mice per group. *Significantly ($P < 0.05$) different from saline control.

concentrations of partial or full agonists, while producing little detectable response, could nevertheless inhibit α7 nAChR sensitivity to subsequent application of a higher concentration of full agonist (Briggs and McKenna, 1998). The mechanism of inhibition is thought to include a slow accumulation of receptors in a desensitized state, a process that could generate little instantaneous response amplitude but eventually affect the whole α7 nAChR population, in the continued presence of agonist. Analogously, NS6740 may inhibit α7 nAChR through desensitization while generating

little instantaneous current. Additionally, as a partial agonist, NS6740 could inhibit the α7 nAChR competitively by occupying the ligand binding site but failing to activate the receptor. Both competition and desensitization may pertain to NS6740. The proportion of inhibition by each mechanism under physiological conditions has not been defined, and is likely to vary with time and concentration of ligands. Nevertheless, the predominant effect of NS6740, at wild-type α7 nAChR in the absence of a positive allosteric modulator of α7 nAChRs was inhibition of these receptors.

Thus, if the behavioural effect of α7 nAChR agonists and partial agonists were due to α7 nAChR inhibition, we would expect NS6740 as well as NS6784 to produce the behavioural effect. This was not the case. NS6740 did not enhance performance in the IA paradigm. In contrast, the α7 nAChR full agonist NS6784 (97% efficacy at rat α7 nAChR *in vitro*) and the α7 nAChR partial agonist A-582941 (60% efficacy at rat α7 nAChR *in vitro*) (Bitner *et al*., 2007; Tietje *et al*., 2008) did enhance performance in the IA paradigm. Further, NS6740 blocked the ability of A-582941 to enhance cognitive function in the same model. Thus, it would appear that in this model of cognitive performance, the behavioural response to α7 nAChR agonists was due to receptor activation, despite the known ability of α7 nAChR to undergo rapid desensitization and the relatively slow rate of ligand equilibration in brain following systemic administration.

Low concentrations of agonist can produce a sustained current at α7 nAChRs (Sullivan *et al*., 1997; Briggs and McKenna, 1998). Such a sustained current is likely to result from the ability of individual receptors to cycle through various states, including resting (channel closed), activated (agonist bound, channel open) and desensitized (agonist bound, channel closed). In the continued presence of agonist, at any given moment, there can be a small but finite pool of activated channels, even while other channels are in resting or desensitized states. This can produce a sustained α7 nAChR current that has a relatively small magnitude and may be difficult to detect *in vitro*. However, in neurons, a small current may suffice to depolarize the cell, potentially reducing Mg^{2+} block of NMDA channels or altering neuron firing through effects on voltage-sensitive channels. Further, it should be noted that the α7 nAChR itself is highly permeant to Ca^{2+} (Sands *et al*., 1993; Séguéla *et al*., 1993) and is often found not in synapses but in extrasynaptic regions (Jones and Wonnacott, 2004; Coggan *et al*., 2005; Guo *et al*., 2005; Wonnacott *et al*., 2006) and even in asynaptic and non-neuronal cells (Arredondo *et al*., 2002; Bruggmann *et al*., 2002; Jones *et al*., 2006; Li and Wang, 2006; Fujii *et al*., 2007; Egleton *et al*., 2008). While the α7 nAChR electrical current may be small, the effects of a small influx of Ca^{2+} could be magnified by co-localization of channels with Ca^{2+}-sensitive effectors, and by activation of kinase signalling cascades. Our previous studies have demonstrated that selective α7 nAChR agonists improve performance across various domains of cognition in pre-clinical models and that these effects correlate with activation of MAPK and CREB phosphorylation pathways in hippocampus and cingulate cortex (Bitner *et al*., 2007). Thus, perhaps the α7 nAChR should be thought of less as a means of triggering action potentials and more as a ligand-gated Ca^{2+} channel, associated with key biochemical signalling processes.

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Conflict of interest

All authors were, at the time of the experiments, employees of Abbott or NeuroSearch.

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