

The characterization of a novel rigid nicotine analog with $\alpha 7$ -selective nAChR agonist activity and modulation of agonist properties by boron inclusion

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Abstract—The $\alpha 7$ nAChR subtype is of particular interest as a potential therapeutic target since it has been implicated as a mediator of both cognitive and neuroprotective activity. The rigid nicotine analog ACME and the *N*-cyanoborane conjugate ACME-B are selective partial agonists of rat $\alpha 7$ receptors expressed in *Xenopus* oocytes, with no significant activation of either $\alpha 3\beta 4$ or $\alpha 4\beta 2$ receptors. ACME-B is both more potent and efficacious than ACME. The efficacies of ACME-B and ACME are approximately 26% and 10% of the efficacy of ACh, respectively. Similar *N*-conjugation of *S*(–)nicotine with cyanoborane decreased efficacy for $\alpha 3\beta 4$ and $\alpha 4\beta 2$ receptors, as well as for $\alpha 7$ nAChR. Structural comparison of ACME with the benzylidene anabaseines, another class of previously identified $\alpha 7$ -selective agonists, suggests that they share a similar structural motif that may be applicable to other $\alpha 7$ -selective agonists.

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1. Introduction

There is a remarkable diversity of nicotinic acetylcholine receptor (nAChR) subtypes in the vertebrate nervous system, and an even greater diversity in the chemical structures which have been identified as nicotinic agonists. One goal for dealing with this diversity of both the ligands and receptor subtypes is to define general principles that may have predictive value for anticipating the selectivity, potency, and efficacy of new drug candidates. One approach of proven value is to study simple defined receptor subtypes in heterologous expression systems such as the *Xenopus* oocyte.¹ Nine genes have been identified that code for the α subunits of neuronal nAChRs ($\alpha 2$ – $\alpha 10$) along with three genes coding for β subunits ($\beta 2$ – $\beta 4$), and the potential diversity that could arise from the pentameric assemblies of these subunits is enormous.² Nonetheless, simple pairwise combinations or single subunits can serve as useful models for

the major subtypes of the brain and autonomic nervous system. For example, nAChRs of the brain are broadly categorized into two main subtypes. One subtype binds nicotine and other agonists with high affinity upon desensitization, and these receptors are largely made up of $\alpha 4$ and $\beta 2$ subunits.^{3,4} The other main subtype, of roughly equal abundance, binds α -bungarotoxin with high affinity and is associated with homomeric assemblies of $\alpha 7$ subunits.^{5,6} These two receptor subtypes have complementary and largely non-overlapping patterns of distribution, serve different functions, and have been identified as potential therapeutic targets for different indications. The $\alpha 7$ subtype is of particular interest as a therapeutic target in association with schizophrenia⁷ and Alzheimer's disease.⁸ Receptors containing $\alpha 3$ and $\beta 4$ subunits are commonly used as a model for the nAChRs of the autonomic nervous system,⁹ and, to avoid unwanted side effects, drugs for CNS indications should have low activity for receptors formed with these subunits.

In this paper, we study three receptor subtypes, $\alpha 7$, $\alpha 4\beta 2$, and $\alpha 3\beta 4$, and we report that ACME (*cis*-1-methyl-

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2,3,3a,4,5,9b,-hexahydro-1*H*-pyrrolo[3,2-*h*]isoquinoline), a rigid analog of nicotine, is a selective partial agonist for $\alpha 7$ -type receptors. The partial agonist activity of an *N*-cyanoborane-conjugated form of ACME at $\alpha 7$ nAChRs was increased compared to the parent compound, while, interestingly, *N*-cyanoborane conjugation to *S*(-)-nicotine resulted in a decrease in agonist activity at this receptor subtype. Structural comparison of ACME with other $\alpha 7$ -selective agonists suggests a common structural motif, which may be exploited for the further development of new $\alpha 7$ -selective agonists.

2. Methods

2.1. Preparation of RNA

The nAChR clones were obtained from Dr. Jim Boulter (UCLA). After linearization and purification of cloned cDNAs, RNA transcripts were prepared in vitro using the appropriate mMessage mMachine kit from Ambion Inc. (Austin, TX).

2.2. Expression in *Xenopus* oocytes

Mature (>9 cm) female *Xenopus laevis* African toads (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Prior to surgery, frogs were anesthetized by placing the animal in a 1.5 g/L solution of MS222 (3-aminobenzoic acid ethyl ester) for 30 min. Oocytes were removed from an incision made in the abdomen.

To remove the follicular cell layer, harvested oocytes were treated with 1.25 mg/ml collagenase from Worthington Biochemical Corporation (Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 1 mM KCl, 2.38 mM NaHCO₃, 0.82 mM MgSO₄, 15 mM HEPES (pH 7.6), and 0.1 mg/ml gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nl (5–20 ng) each of the appropriate subunit cRNAs. Recordings were made 5–15 days after injection.

2.3. Chemicals

ACME, BCME (*cis*-1-methyl-2,3,3a,4,5,9b,-hexahydro-1*H*-pyrrolo[2,3-*f*]quinoline), ACME-B, *S*(-)-nicotine, and *S*(-)-nicotine-B (see Fig. 1 for structures) were synthesized at the University of Kentucky utilizing previously reported methods.¹⁰ All other chemicals used in electrophysiology studies were obtained from Sigma Chemical (St. Louis, MO). Fresh acetylcholine stock solutions were made up daily in Ringer's solution and diluted.

2.4. Electrophysiology

Experiments were conducted using OpusXpress 6000A (Axon Instruments, Union City CA), or manual oocyte two-electrode voltage-clamp systems as previously reported.¹¹ OpusXpress is an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel. Cells were automatically per-

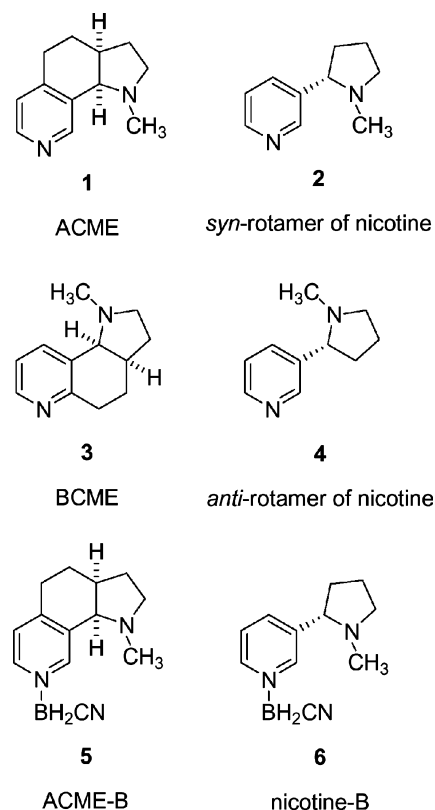


Figure 1. Structure of rigid nicotine analogs.

fused with bath solution, and agonist solutions were delivered from a 96-well plate. Both the voltage and current electrodes were filled with 3 M KCl. The agonist solutions were applied via disposable tips, which eliminated any possibility of cross-contamination. Drug applications alternated between acetylcholine (ACh) controls and experimental applications. Flow rates were set at 2 ml/min for experiments with $\alpha 7$ receptors and 4 ml/min for other subtypes. Cells were voltage-clamped at a holding potential of -60 mV. Data were collected at 50 Hz and filtered at 20 Hz. ACh applications were 12 s in duration followed by 181 s washout periods with $\alpha 7$ receptors and 8 s with 241 s wash periods for other subtypes. For manual oocyte recordings, Warner Instruments (Hamden, CT) OC-725 C oocyte amplifiers were used, and data were acquired with a minidigi or digidata 1200A with pClamp9 software (Axon Instruments). Sampling rates were between 10 and 20 Hz and the data were filtered at 6 Hz. Cells were voltage clamped at a holding potential of -50 mV.

2.5. Experimental protocols and data analysis

Each oocyte received two initial control applications of ACh, then an experimental drug application, and then a follow-up control application of ACh. The control ACh concentrations for $\alpha 3\beta 4$, $\alpha 4\beta 2$, and $\alpha 7$ receptors were 100, 10, and 300 μM , respectively. In other experiments (not shown¹²), these concentrations were determined to be the EC₁₅, EC₂₂, and EC₁₀₀, respectively. These standard control concentrations¹³ were found to give robust reproducible responses for the indicated subtypes with-

out significant desensitization or rundown following repeated applications at the specified interval.

Responses to experimental drug applications were calculated relative to the preceding ACh control responses to normalize the data, compensating for the varying levels of channel expression among the oocytes. Drug responses were initially normalized to the ACh control response values and then adjusted to reflect the experimental drug responses relative to the ACh maximums. Responses for $\alpha 7$ receptors were calculated as net charge.¹⁴ For subtypes other than $\alpha 7$, responses were calculated from the peak current amplitudes. Means and standard errors (SEM) were calculated from the normalized responses of at least four oocytes for each experimental concentration. The application of some experimental drugs caused the subsequent ACh control responses to be reduced, suggesting some form of residual inhibition (or prolonged desensitization). To measure the residual inhibition, this subsequent control response was compared to the pre-application control ACh response.

For concentration–response relations, data derived from net-charge analyses were plotted using Kaleidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated from the Hill equation:

$$\text{Response} = \frac{I_{\max}[\text{agonist}]^n}{[\text{agonist}]^n + (\text{EC}_{50})^n},$$

where I_{\max} denotes the maximal response for a particular agonist/subunit combination, and n represents the Hill coefficient. I_{\max} , n , and the EC_{50} were all unconstrained for the fitting procedures. Negative Hill slopes were applied for the calculation of IC_{50} values.

3. Results

3.1. ACME and ACME-B

ACME (Fig. 1, structure 1) is a conformationally restrained analog of the *syn*-rotamer of nicotine (Fig. 1, structure 2), while BCME (Fig. 1, structure 3) is a conformationally restrained analog of the *anti*-rotamer of nicotine (Fig. 1, structure 4). ACME and its *N*-cyanoborane conjugate ACME-B (Fig. 1, structure 5) were tested on oocytes expressing rat $\alpha 7$, $\alpha 4\beta 2$, or $\alpha 3\beta 4$ nAChR. As shown in Figure 2A, both ACME and ACME-B produced significant activation of $\alpha 7$, but there was essentially no significant receptor activation of $\alpha 4\beta 2$ or $\alpha 3\beta 4$ receptors. The alternative conformationally restrained *anti*-rotameric nicotine analog, BCME (*cis*-1-methyl-2,3,3a,4,5,9b,-hexahydro-1*H*-pyrrolo[2,3-*f*] quinoline, Fig. 1, structure 3) had no agonist activity at any nAChR subtype studied (data not shown). Likewise the boron-conjugated analog of BCME, BCME-B (not shown), had no agonist activity (data not shown).

The efficacy and potency of ACME-B at $\alpha 7$ nAChRs were roughly twice that of ACME (see Table 1). After the application of ACME and ACME-B at concentrations above 30 μM , subsequent ACh control responses were not fully recovered after a 5 min wash (not shown),

indicating some residual inhibition or prolonged desensitization. This effect was most pronounced with ACME-B.

3.2. Nicotine and nicotine-B

N-Cyanoboration of (*S*)-nicotine has previously been reported¹⁰ to form *S*(–)nicotine-B (Fig. 1, structure 6) and resulted in a loss of binding affinity to $\alpha 4\beta 2$ -type but not $\alpha 7$ -type receptors in rat brain membranes when compared with (*S*)-nicotine. To determine whether *N*-cyanoborane conjugation enhanced the efficacy of (*S*)-nicotine as it did for ACME, we tested *S*(–)nicotine and *S*(–)nicotine-B on the same receptor subtypes expressed in *Xenopus* oocytes. As shown in Figure 2B, the *N*-cyanoborane-conjugated nicotine produced significantly less activation of all the nAChR subtypes tested. As measured by the amplitude of ACh controls following a 5 min wash, *S*(–)nicotine-B also produced less receptor desensitization of both $\alpha 7$ and $\alpha 4\beta 2$ than did *S*(–)nicotine (data not shown).

4. Discussion

We report three principle findings: first, that the conformationally restrained nicotine analog ACME is an $\alpha 7$ -selective partial agonist; second, that the *N*-conjugation of ACME with a cyanoborane moiety increases its efficacy and potency for activation of $\alpha 7$; and third, that in contrast to the effects of *N*-cyanoborane conjugation of ACME, similar *N*-conjugation of *S*(–)nicotine has the effect of decreasing efficacy for $\alpha 7$ as well as for other nAChR subtypes.

Bicyclic nAChR agonist molecules such as nicotine and anabaseine have rotational freedom about the C–C bond joining the two azaheterocyclic rings in these molecules. Both *ab initio* and molecular dynamics studies of the nicotine molecule have identified two low energy conformations, in which the pyridyl ring is rotated such that the pyridyl nitrogen atom can either be close to, or far apart from, the pyrrolidinyl nitrogen; these conformational forms are referred to as '*syn*' and '*anti*' rotomers, respectively.^{15,16} These two nicotine rotomers were found to be isoenergetic with each other in the gas-phase^{15,16}; however, in solution a slight (<1 kcal/mol energy difference) preference for the *syn* rotomer was observed.¹⁶ In the present work, molecular mechanics (MM2, Chem3D Ultra) or semi-empirical calculations¹⁷ suggest that anabaseine may behave similarly, although direct comparison with the previous work on the nicotine molecule must be made cautiously, since the steric features of the anabaseine and nicotine molecules differ. In this respect, the *syn* rotomer of anabaseine is only 0.3 kcal/mol preferred over the *anti* conformer with the PM3 parameter set (Fig. 3A). These results indicate that either conformer in solution is readily available for binding to nAChRs. It has not been previously established if one of these low energy conformers preferentially binds to the $\alpha 7$ nAChR. Our use of conformationally restrained or rigid molecules that mimic the *syn* and *anti* conformers of nicotine strongly suggests that it is the

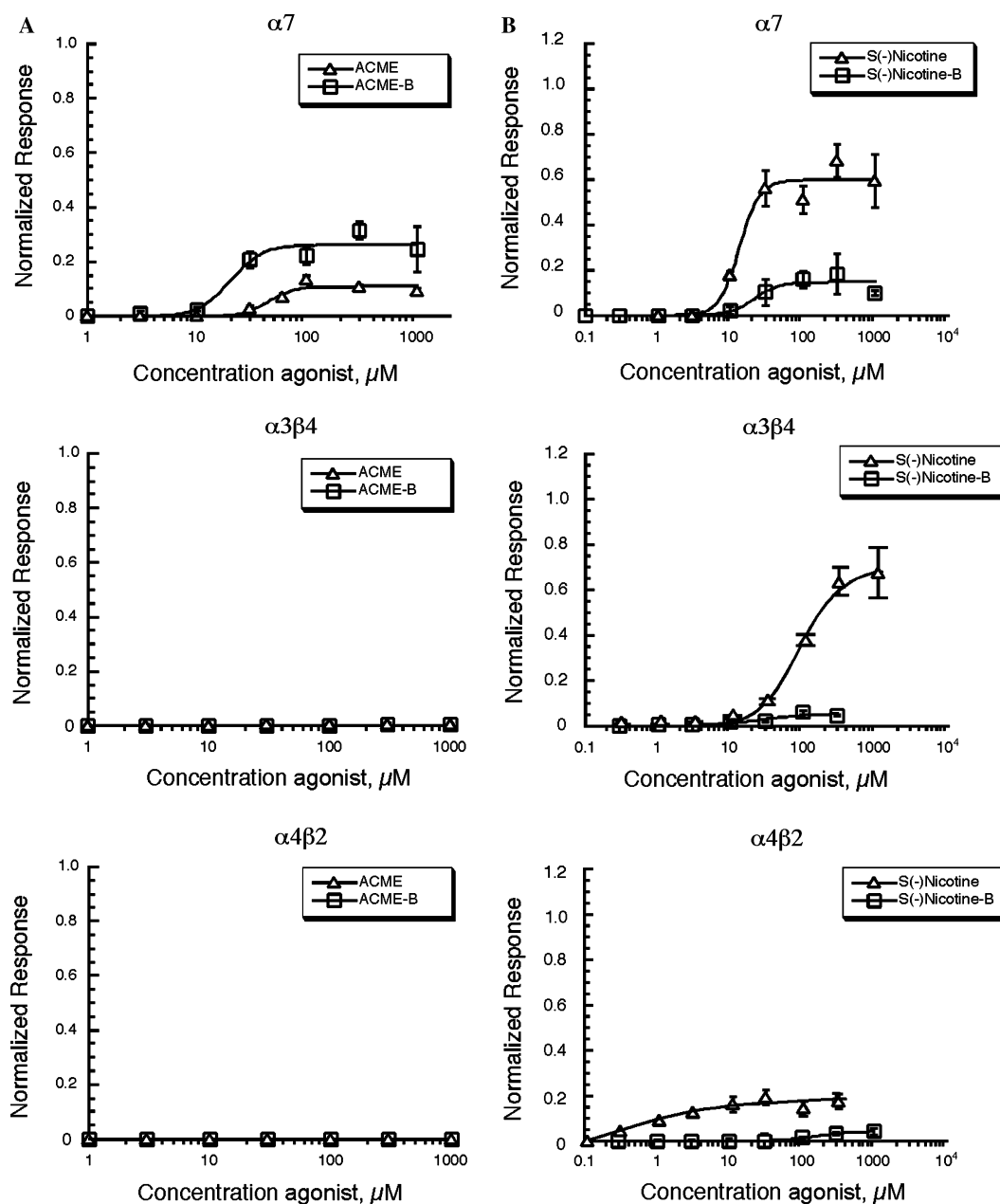


Figure 2. (A) Concentration–response curves for $\alpha 7$, $\alpha 3\beta 4$, and $\alpha 4\beta 2$ nAChR subunits expressed in *Xenopus* oocytes to either ACME or ACME-B. Responses are normalized to internal ACh controls for each cell and then expressed relative to maximal ACh-evoked responses determined in separate experiments. Each point is the mean \pm SEM for at least four separate cells. (B) Concentration–response curves for $\alpha 7$, $\alpha 3\beta 4$, and $\alpha 4\beta 2$ nAChR subunits expressed in *Xenopus* oocytes to either S(-)-nicotine or S(-)-nicotine-B. Responses are normalized to internal ACh controls for each cell and then expressed relative to maximal ACh-evoked responses determined in separate experiments. Each point is the mean \pm SEM for at least four separate cells.

syn conformer that is preferentially recognized by nAChR, since the *anti*-rotamer analog of nicotine, BCME, had no agonist activity at the $\alpha 7$ nAChR or at any other nAChR subtype studied.

There is a long history of research into the nature of the nAChR pharmacophore (for a recent review see Ref. 18), most of which has focused on muscle-type receptors or binding to the high-affinity desensitized form of brain-type (i.e., $\alpha 4\beta 2$) receptors. One early model was that developed in 1970 by Beers and Reich,¹⁹ who suggested that nicotinic ligands require a charged nitrogen

(onium) group and a hydrogen-bond acceptor feature that interacts with a receptor-based hydrogen bond donor site. Refinements of this basic model have been suggested by numerous groups.¹⁸ However, in regard to the activation of neuronal nAChRs, we have shown that the minimal structure required is nothing more than the cationic quaternary ammonium ion alone, as in tetramethylammonium ion (TMA).²⁰ Elaborations of this minimal structure may produce changes in potency and/or selectivity, as in the case of choline, which becomes selective for the $\alpha 7$ nAChR, but is 10-fold less potent than either TMA or ACh. A number of lines of

Table 1.

	ACME		ACME-B	
	I_{\max}^a	$EC_{50} (\mu M)^b$	I_{\max}	$EC_{50}(\mu M)$
$\alpha 7$	$0.11 \pm .01$	44 ± 9	$0.26 \pm .02$	20 ± 5
$\alpha 3\beta 4$	NA ^b	NA	NA	NA
$\alpha 4\beta 2$	NA	NA	NA	NA
	<i>S</i> (–)nicotine		<i>S</i> (–)nicotine-B	
	I_{\max}	$EC_{50}(\mu M)$	I_{\max}	$EC_{50}(\mu M)$
$\alpha 7$	$0.60 \pm .04$	13 ± 3	$0.15 \pm .01$	21 ± 7
$\alpha 3\beta 4$	$0.69 \pm .14$	86 ± 4.4	$0.06 \pm .02$	28 ± 19
$\alpha 4\beta 2$	$0.17 \pm .01$	$1 \pm .5$	$0.05 \pm .01$	150 ± 7

NA, insufficient response to obtain a value.

^a I_{\max} expressed relative to the maximum ACh evoked responses.

^b From curve fits in figures.

research suggest that $\alpha 7$ receptors tolerate extensive elaboration on the basic activation pharmacophore, within certain structural requirements. For example, anabaseine is a nonselective nicotinic agonist, while benzylidene-conjugated anabaseines are selective for $\alpha 7$ nAChR.²¹ A comparison of the $\alpha 7$ -selective nAChR agonists shown in Figure 3B suggests that in the ACME molecule the carbon bridge between the pyridyl and pyrrolidine rings may extend into a large pocket in the $\alpha 7$ nAChR binding site. It is known that the $\alpha 7$ nAChR binding site accepts agonists containing bulky aromatic moieties, for example, the benzylidene anabaseine series of compounds and the tropisetron molecule, which contains a heteroaromatic indole moiety.^{13,22} Thus, overlay of 3-benzylidene anabaseine with ACME reveals that

the bridged methylene moiety adjacent to the piperidinyll ring in ACME overlaps with the sp^2 benzylidene carbon of benzylidene anabaseine (Fig. 3B). We hypothesize that the $\alpha 7$ selective agonist activity of ACME may result from this specific interaction. Alternatively, the $\alpha 7$ binding site may accept the relatively rigid *syn*-rotameric conformation of ACME, while non- $\alpha 7$ nAChR ligands may interact with other nAChR subtypes in alternative rotameric conformations that are precluded in the ACME molecule.

It is interesting that the *N*-cyanoborane conjugate, ACME-B, is both more potent and efficacious than the parent compound, ACME, particularly since a similar *N*-cyanoborane conjugation of *S*(–)nicotine (nicotine-B) decreased efficacy for $\alpha 3\beta 4$ and $\alpha 4\beta 2$ receptors as well as for $\alpha 7$ nAChRs. *N*-Cyanoborane conjugation of *S*(–)nicotine has previously been reported to result in a large reduction in binding affinity for $\alpha 4\beta 2$ receptors in brain membrane preparations and relatively little change in affinity for putative $\alpha 7$ receptors.¹⁰ This is consistent with our current studies, and the effect of nicotine-B on EC_{50} values for $\alpha 7$ and $\alpha 4\beta 2$ nAChRs. Note that although *S*(–)nicotine-B has lower efficacy for $\alpha 4\beta 2$ or $\alpha 3\beta 4$ nAChRs than for $\alpha 7$ nAChRs, *S*(–)nicotine-B is not a selective agonist.

While ACME has comparable low affinity for $\alpha 4\beta 2$ and $\alpha 7$ binding sites in brain membranes (Table 2), it has agonist activity for $\alpha 7$ receptors but not $\alpha 4\beta 2$ receptors expressed in oocytes. However, comparing the ACME profile for binding and activity to that of nicotine, the

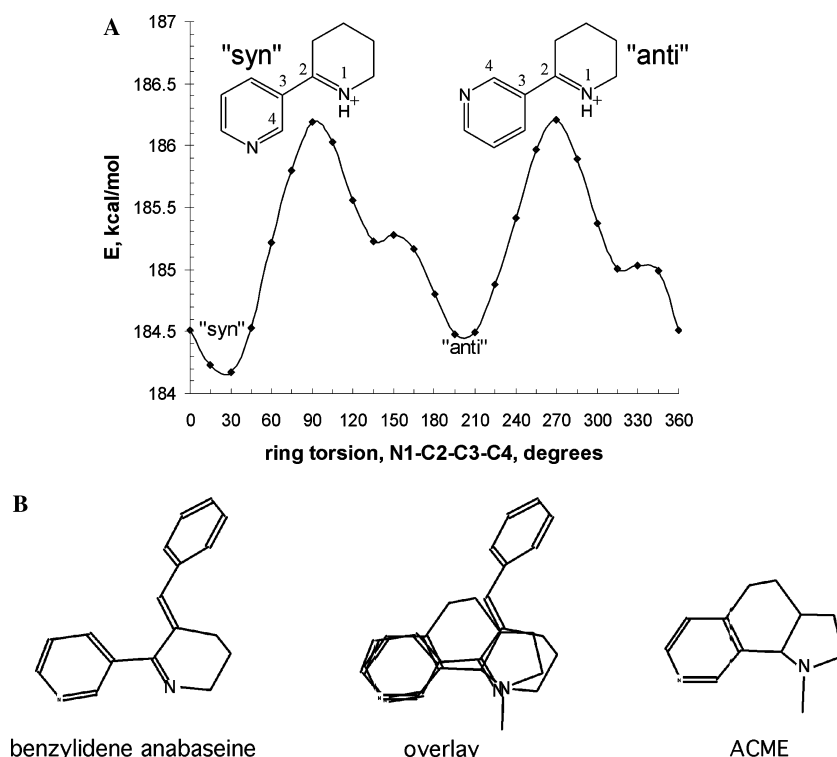


Figure 3. (A) Gas-phase rotamer energy profile for anabaseine. Calculations utilized the semi-empirical PM3 parameter set, and were performed by holding the torsion angle fixed while optimizing all other coordinates to tight convergence. (B) Comparison of the $\alpha 7$ -selective agonists benzylidene anabaseine and ACME, showing the correspondence of hydrophobic structural elements in the overlay.

Table 2. K_i values for nicotine, ACME, BCME and their cyanoborate analogs for the displacement of either radiolabeled nicotine or MLA from rat brain membranes^a

	K_i values (μ M)	
	[³ H]Nicotine	[³ H]MLA
Nicotine	0.001	0.77
ACME	0.40	0.59
BCME	12.2	>100
NIC-B	0.041	15.2
ACME-B	0.60	2.4
BCME-B	>100	>100

These values are taken to reflect the binding affinity of these compounds for putative $\alpha 4\beta 2$ or $\alpha 7$ -type receptors, respectively.

^a Data take from Xu et al.¹⁰

loss of high affinity for nicotine binding would seem to be predictive for loss of $\alpha 4\beta 2$ function. Consistent with this, the cyanoborate form of nicotine shows both decreased binding affinity and decreased agonist activity compared to nicotine. In contrast to the relatively predictive relationships between binding and function for $\alpha 4\beta 2$ receptors, there is relatively poor correlation between MLA (i.e., putative $\alpha 7$) binding and $\alpha 7$ function. ACME-B is more potent than ACME for $\alpha 7$ receptor activation but has lower affinity for the brain binding sites. Likewise, *S*(–)nicotine-B is essentially equipotent as *S*(–)nicotine for activating $\alpha 7$ although it shows 20-fold lower affinity for the MLA binding sites in rat brain membranes. In the case of the BCME compounds though, there are parallel and complete losses of both binding affinity and agonist activity for $\alpha 7$.

The effects of the *N*-cyanoborane conjugation may be either intramolecular, changing the properties of the pharmacophore, or intermolecular, affecting interactions between the agonist and the amino acids in the agonist binding site of the receptor. Alternatively, both kinds of effects may be important. If intermolecular effects do occur, then perhaps the rotational freedom in *S*(–)nicotine-B molecule permits a deleterious intermolecular interaction that is excluded in the more rigid ACME-B molecule.

Ligand-gated ion channels such as nAChRs are marvelously complex molecular machines that encompass the features of drug selectivity and the translation of agonist binding to the conformational changes detected as channel activation and receptor desensitization. Selectivity, potency, and efficacy are all key criteria for therapeutic targeting of $\alpha 7$ or any other CNS receptor. The data we report here provide additional guidelines for future drug design, which ultimately may have translational value for emerging therapies for such diverse indications as Alzheimer's disease, schizophrenia, and inflammation. While our analysis of the rigid rotamer analogs of nicotine is informative regarding the basis through which drugs can selectively activate $\alpha 7$ nAChR, it is unclear if ACME and ACME-B themselves will be good lead compounds for further drug development since both of these compounds produced significant residual inhibition (or prolonged desensitization). Similar mixed agonist/antagonist properties have

been reported for select benzylidene and cinnamylidene anabaseines, including the most well-studied analog, GTS-21.^{23,24} Interestingly, while in vitro studies of cinnamylidene compounds suggested that analogs which produced significant residual inhibition were less effective as cytoprotective agents than similar compounds with less antagonist or desensitizing effects, agents which produced residual inhibition were equally effective as nondesensitizing compounds at producing in vivo behavioral effects.²⁴

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References and notes

- Boulter, J.; Connolly, J.; Deneris, E.; Goldman, D.; Heinemann, S.; Patrick, J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7763.
- Clementi, F.; Fornasari, D.; Gotti, C. *Eur. J. Pharmacol.* **2000**, *393*, 3.
- Whiting, P. J.; Lindstrom, J. M. *J. Neurosci.* **1988**, *8*, 3395.
- Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe, B. B.; Kellar, K. J. *Mol. Pharmacol.* **1992**, *41*, 31.
- Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A. *J. Neurosci.* **1985**, *5*, 1307.
- Seguela, P.; Wadiche, J.; Dinely-Miller, K.; Dani, J. A.; Patrick, J. W. *J. Neurosci.* **1993**, *13*, 596.
- Adler, L. E.; Olincy, A.; Waldo, M.; Harris, J. G.; Griffith, J.; Stevens, K.; Flach, K.; Nagamoto, H.; Bickford, P.; Leonard, S.; Freedman, R. *Schizophr. Bull.* **1998**, *24*, 189.
- Levin, E. D. *J. Neurobiol.* **2002**, *53*, 633.
- Costa, V.; Nistri, A.; Cavalli, A.; Carloni, P. *Br. J. Pharmacol.* **2003**, *140*, 921.
- Xu, R.; Dwoskin, L. P.; Grinevich, V. P.; Deaciuc, G.; Crooks, P. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1245.
- Stokes, C.; Papke, J. K. P.; McCormack, T.; Kem, W. R.; Horenstein, N. A.; Papke, R. L. *Mol. Pharmacol.* **2004**, *66*, 14.
- Papke, R. L.; Webster, J. C.; Lippiello, P. M.; Bencherif, M.; Francis, M. M. *J. Neurochem.* **2000**, *75*, 204.
- Papke, R. L.; Schiff, H. C.; Jack, B. A.; Horenstein, N. A. *Neurosci. Lett.* **2005**, *378*, 140.
- Papke, R. L.; Papke, J. K. P. *Br. J. Pharmacol.* **2002**, *137*, 49.
- Wu, Y.; Schmitt, J. D.; Car, R. *J. Chem. Phys.* **2004**, *121*, 1193.
- Elmore, D. E.; Dougherty, D. A. *J. Org. Chem.* **2000**, *65*, 742.
- Frisch, M. J. et al. Gaussian Revision A.7, Gaussian, Inc., Pittsburgh, PA, **1998**.
- Glennon, R. A.; Dukat, M.; Liao, L. *Curr. Top. Med. Chem.* **2004**, *4*, 631.
- Beers, W. H.; Reich, E. *Nature* **1970**, *228*, 917.
- Papke, R. L.; Bencherif, M.; Lippiello, P. *Neurosci. Lett.* **1996**, *213*, 201.

21. Papke, R. L.; Meyer, E. M.; Lavieri, S.; Bollampally, S.; Papke, T.; Horenstein, B.; Papke, J. K. P. *J. Neuropharmacol.* **2004**, *46*, 1023.
22. Papke, R. L.; Rose, G.; Papke, J. K. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1849.
23. Meyer, E.; Kuryatov, A.; Gerzanich, V.; Lindstrom, J.; Papke, R. L. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 918.
24. Meyer, E. M.; Tay, E. T.; Zoltewicz, J. A.; Papke, R. L.; Meyers, C.; King, M.; Fiebre, C. M. D. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 1026.