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Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors

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Running Title: Varenicline actions at neuronal nicotinic receptors

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Nonstandard abbreviations: ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; Var, varenicline.

Abstract

Varenicline, a new nicotinic ligand based on the structure of cytosine, has recently been approved by the U.S. Food and Drug Administration for use as a smoking cessation aid. Varenicline has been shown to be a partial agonist of $\alpha 4\beta 2$ receptors and in equilibrium binding assays varenicline is highly selective for the $\alpha 4\beta 2$ receptor. In this study, we have examined the functional activity of varenicline at a variety of rat neuronal nicotinic receptors expressed in *Xenopus* oocytes and assayed under two electrode voltage clamp. We also find that varenicline is a potent, partial agonist at $\alpha 4\beta 2$ receptors, with an EC_{50} of $2.3 \pm 0.3 \mu M$ and an efficacy (relative to acetylcholine) of $13.4 \pm 0.4\%$. Varenicline has lower potency and higher efficacy at $\alpha 3\beta 4$ receptors with an EC_{50} of $55 \pm 8 \mu M$ and an efficacy of $75 \pm 6\%$. Varenicline also appears to be a weak partial agonist at $\alpha 3\beta 2$ and $\alpha 6$ -containing receptors, with an efficacy $<10\%$. Remarkably, varenicline is a potent, full agonist at $\alpha 7$ receptors with an EC_{50} of $18 \pm 6 \mu M$ and an efficacy of $93 \pm 7\%$ (relative to acetylcholine). Thus, while varenicline is a partial agonist at some heteromeric neuronal nicotinic receptors, it is a full agonist at the homomeric $\alpha 7$ receptor. Some combination of these actions may be involved in the mechanism of varenicline as a smoking cessation aid.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels composed of α and β subunits, which assemble to form pentamers with a variety of pharmacological and biophysical properties (Corringer et al., 2000). These receptors are widely distributed in the CNS and have been proposed as potential therapeutic targets for a variety of conditions and disorders, such as Alzheimer's disease, anxiety, depression, drug addiction, epilepsy, pain, Parkinson's disease, schizophrenia and Tourette's syndrome (Jensen et al., 2005). The development of subtype selective ligands is an essential part of attempts to make progress in these areas. Consequently, much effort is currently directed toward synthesizing and screening analogs of various nicotinic compounds (recently reviewed in Jensen et al., 2005). The $\alpha 4 \beta 2$ nAChR (and more complex $\alpha 4 \beta 2$ containing receptors) is a target of particular interest for the development of smoking cessation therapies, due to the location of this receptor on presynaptic terminals in the striatum and the role of this receptor in modulating dopamine release (Salminen et al., 2004).

Cytisine is a plant alkaloid with a relatively rigid conformation. In equilibrium binding assays, cytisine is selective for the $\alpha 4 \beta 2$ subunit combination, as compared to other important nAChR subtypes such as $\alpha 3 \beta 4$ and $\alpha 7$ (Parker et al., 1998; Stauderman et al., 1998; Chavez-Noriega et al., 2000; Carbonnelle et al., 2003; Slater et al., 2003; Xiao et al., 2004). In functional assays, cytisine shows greater potency at $\alpha 4 \beta 2$ receptors than at many other subunit combinations. Cytisine also displays a wide variation in efficacy at various subunit combinations. While cytisine is a high efficacy agonist at $\alpha 7$ receptors and at various $\beta 4$ containing receptors such as $\alpha 3 \beta 4$, cytisine is a low efficacy partial agonist at $\alpha 4 \beta 2$ and other $\beta 2$ containing receptors (Luetje and Patrick, 1991; Papke and Heinemann, 1994; Chavez-Noriega et al., 1997; Stauderman et al., 1998; Chavez-Noriega et al., 2000; Papke and Porter Papke, 2002; Carbonnelle et al., 2003; Slater et al., 2003). Manipulation of the cytisine structure results in changes in the efficacy at various neuronal nAChRs. For example, bromination or iodination of the 3-position in the pyridone ring of cytisine preserves the full efficacy at $\alpha 7$ receptors while increasing the efficacy at $\alpha 4 \beta 2$ receptors (Slater et al., 2003), while the addition of side groups at the

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basic nitrogen of cytosine can reduce or eliminate efficacy for various receptors (Carbonnelle et al., 2003).

Varenicline is a recently developed nicotinic ligand that has recently been approved by the U.S. Food and Drug Administration for use as a smoking cessation therapy (Coe et al., 2005c; Coe et al., 2005b; Coe et al., 2005a; Obach et al., 2006). The structure of varenicline (Figure 1) is loosely based on that of cytosine and it was the partial agonist activity of cytosine that was one of the initial characteristics that lead to the development of varenicline (Coe et al., 2005c; Coe et al., 2005b; Coe et al., 2005a). In equilibrium binding assays, varenicline is selective for the $\alpha 4\beta 2$ receptor as compared to $\alpha 3\beta 4$, $\alpha 7$ and muscle-like nAChRs, while in a functional assay, varenicline is a partial agonist at $\alpha 4\beta 2$ receptors (Coe et al., 2005c). Here we examine the functional potency and efficacy of varenicline at several neuronal nAChRs subunit combinations. We find that varenicline displays only moderate differences in functional potency, but shows wide variation in efficacy at these receptors.

Materials and Methods

Materials. *Xenopus laevis* frogs were purchased from Nasco (Ft. Atkinson, WI). Care and use of *Xenopus* frogs in this study were approved by the University of Miami Animal Research Committee and meet the guidelines of the National Institutes of Health. RNA transcription kits were from Ambion (Austin, TX). All other chemicals were from Sigma-Aldrich (St. Louis, MO).

Varenicline. 6,7,8,9-tetrahydro-6,10-methano-6*H*-pyrazino[2,3-*h*][3]benzazepine (varenicline) was synthesized as the dihydrochloride salt using reported methods (Brooks et al., 2004; Coe and Brooks, 2002). We thank Dr. Jotham Coe for helpful suggestions. The structure of varenicline is shown in Figure 1.

Expression of Neuronal nAChRs in *Xenopus* Oocytes. Mature *Xenopus laevis* frogs were anesthetized by submersion in 0.1% 3-aminobenzoic acid ethyl ester. Oocytes were surgically removed, and follicle cells were removed by treatment with collagenase B for 2 hr at room temperature. cRNA encoding the rat $\alpha 3$, $\alpha 4$, $\alpha 6/\alpha 3$ (see below), $\alpha 7$, $\beta 2$, $\beta 3$ and $\beta 4$ neuronal nAChR subunits was synthesized using mMessage mMachine kits (Ambion). Oocytes were injected with 10-40 ng of cRNA in 13-50 nl of water and incubated at 19°C in modified Barth's saline (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.3 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 100 ug/ml gentamicin, 15 mM HEPES, pH 7.6) for 2 to 6 days. For heteromeric nAChRs, cRNA transcripts encoding each subunit were injected into oocytes at a molar ratio of 1:1.

Neuronal nAChRs containing the $\alpha 6$ subunit are narrowly distributed, but functionally important, in the mammalian CNS (Salminen et al., 2004). Unfortunately, receptors containing this subunit are difficult to functionally express. However a successful strategy has been to use a chimeric subunit consisting of the N-terminal extracellular domain of $\alpha 6$ joined to the transmembrane and intracellular domains of $\alpha 3$ (Kuryatov et al., 2000; McIntosh et al., 2004; Azam et al., 2005). This chimeric $\alpha 6/\alpha 3$

subunit was kindly provided by Dr. Michael McIntosh (University of Utah). As reported previously (McIntosh et al., 2004), we found that while functional expression of the $\alpha 6/\alpha 3$ chimera with the $\beta 2$ subunit was very weak, useful levels of functional expression could be achieved with the inclusion of the $\beta 3$ subunit. Indeed, $\alpha 6\beta 2\beta 3$ is a functional nAChR subtype in the mammalian CNS (Salminen et al., 2004).

Electrophysiological methods. Agonist-induced current responses were measured 2-6 days after cRNA injection using two-electrode voltage clamp in an automated parallel electrophysiology system (OpusExpress 6000A, Molecular Devices). Micropipettes were filled with 3M KCl and had resistances of 0.2-2.0 M Ω . Current responses were recorded at a holding potential of -40 mV to minimize the contribution of calcium activated chloride channels. For all receptors except $\alpha 7$, current responses were filtered (4-pole, Bessel, low pass) at 20 Hz (-3db) and sampled at 100 Hz. For $\alpha 7$ receptors, current responses were filtered (4-pole, Bessel, low pass) at 100 Hz (-3db) and sampled at 500 Hz. Current responses were captured and stored using *OpusXpress 1.1* software (Molecular Devices). Initial analysis was done using *Clampfit 9.1* software (Molecular Devices). Oocytes were perfused at room temperature (20-25°C) with Ringer's saline (in mM: 115 NaCl, 1.8 CaCl₂, 2.5 KCl, 0.0001 atropine, 10 HEPES, pH7.2). For all receptors except $\alpha 7$, acetylcholine and varenicline were applied for 20 sec at a flow rate of 4 ml/min, with 3 min washes between applications. For $\alpha 7$ receptors, acetylcholine and varenicline were applied for 5 sec at a flow rate of 4 ml/min, with 3 min washes between applications. The acetylcholine concentration, applied prior to each varenicline application, was 20 μ M for $\alpha 4\beta 2$ (EC₃₀), 110 μ M for $\alpha 3\beta 4$ (EC₂₀), 10 μ M for $\alpha 3\beta 2$ (EC₂₀), 30 μ M for $\alpha 6/\alpha 3\beta 2\beta 3$ (~EC₅₀) and 300 μ M for $\alpha 7$ (EC₅₀). Each current response to varenicline was normalized to the preceding acetylcholine-induced response. Dose response data were fit to the equation: $I = I_{\max} / [1 + (EC_{50}/X)^n]$, where I is the current response at agonist concentration (X), I_{\max} is the maximum current, EC₅₀ is the ligand concentration producing the half-maximal current response, and n is the Hill

coefficient. Dose response data for inhibition curves were fit to the equation: $I = I_{\max} / [1 + (IC_{50}/X)^n]$, where I is the current response at agonist concentration (X), I_{\max} is the maximum current, IC_{50} is the ligand concentration producing half-maximal inhibition of the current response, and n is the Hill coefficient.

Results and Discussion

We first examined the effect of varenicline on rat $\alpha 4\beta 2$ receptors expressed in *Xenopus* oocytes. Application of 100 μ M varenicline yielded a peak current response that was smaller in amplitude than the response evoked by 20 μ M ACh (the EC_{30} for ACh), suggesting that varenicline might have a low efficacy at this receptor (Figure 2B). The response to this high concentration of varenicline desensitized rapidly, as compared to the response to a low concentration of varenicline (1 μ M, Figure 2A). The small deflection seen when the varenicline is washed away suggests that in addition to the desensitizing effect, varenicline may cause a small amount of channel block. When the $\alpha 4\beta 2$ receptor was challenged with a range of varenicline concentrations and the current responses normalized to the maximal response to ACh (see Methods), we found that varenicline was a potent ($EC_{50} = 2.3 \pm 0.3$ μ M) partial agonist with an efficacy $13.3 \pm 0.4\%$ that of ACh (Figure 2C). These results are similar to what has been previously reported for human $\alpha 4\beta 2$ ($EC_{50} = 2.3$ μ M, efficacy = 24% relative to nicotine, Coe et al., 2005c). When a range of varenicline concentrations were co-applied with ACh (40 μ M, the EC_{60}), we find that varenicline can antagonize the ACh response with an IC_{50} of 0.2 ± 0.03 μ M (Figure 2D), consistent with varenicline being a potent partial agonist of $\alpha 4\beta 2$ receptors.

Next we examined the action of varenicline on other neuronal nAChR subtypes. Application of 200 μ M varenicline to an oocyte expressing $\alpha 3\beta 4$ receptors yielded a peak current response with an amplitude substantially larger than the response evoked by 110 μ M ACh (the EC_{20} for ACh), suggesting that varenicline might have a high efficacy at this receptor (Figure 3B). The response to this high

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concentration of varenicline desensitized rapidly, as compared to the response to a lower concentration of varenicline (30 μ M, Figure 3A). The small deflection seen when the varenicline is washed away suggests that in addition to the desensitizing effect, varenicline may cause a small amount of channel block. When the $\alpha 3\beta 4$ receptor was challenged with a range of varenicline concentrations and the current responses normalized to the maximal response to ACh (see Methods), we found that varenicline had an efficacy of $75 \pm 6\%$ at these receptors (Figure 3C). Varenicline was 24-fold less potent at $\alpha 3\beta 4$ receptors ($EC_{50} = 55 \pm 8 \mu$ M) than at $\alpha 4\beta 2$ receptors.

We also examined the efficacy of varenicline at $\alpha 3\beta 2$ receptors and $\alpha 6$ -containing receptors (as represented by an $\alpha 6/\alpha 3$ chimera coexpressed with the $\beta 2$ and $\beta 3$ subunits, see Methods). When 100 μ M varenicline was applied to oocytes expressing $\alpha 3\beta 2$ receptors, current responses were $3.7 \pm 0.4 \%$ of the maximal ACh response. This was a saturating response, as 1mM varenicline did not yield larger current responses (data not shown). Thus, varenicline is a low efficacy partial agonist at $\alpha 3\beta 2$ receptors, with an $EC_{50} < 100 \mu$ M. Varenicline also displayed low efficacy on the $\alpha 6/\alpha 3\beta 2\beta 3$ receptor, with 100 μ M varenicline yielding responses that were $8.8 \pm 1.4\%$ of the maximal response to ACh. Again, this appears to be a saturating response because the response to 10 μ M varenicline was similar (data not shown).

While varenicline proved to be a partial agonist at many of the neuronal nAChR subtypes that we tested, the effect of varenicline was remarkably different at $\alpha 7$ containing receptors. When 100 μ M varenicline was applied to $\alpha 7$ expressing oocytes, peak current responses were substantially larger than the responses to 300 μ M ACh (the EC_{50}), suggesting that varenicline has high efficacy at these receptors (Figure 4A). This was borne out when we applied a range of varenicline concentrations to $\alpha 7$ expressing oocytes. Varenicline was a potent full agonist, activating $\alpha 7$ receptors with an EC_{50} of $18 \pm 6 \mu$ M and an efficacy that was $93 \pm 7\%$ of the maximal response to ACh (Figure 4A, B). The rapid desensitization kinetics of the $\alpha 7$ receptor at all agonist concentrations has raised concerns about the use peak current measurements to study the $\alpha 7$ receptor and an alternate method, net charge analysis,

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has been proposed (Papke and Porter Papke, 2002). When using net charge analysis, the EC_{50} for ACh is greatly reduced and 300 μ M ACh becomes a saturating concentration (Papke and Porter Papke, 2002). When we examine the actions of varenicline on $\alpha 7$ receptors using net charge analysis, we again find that varenicline is a potent, high efficacy agonist, with an EC_{50} of $2.3 \pm 0.2 \mu$ M and an efficacy of $84 \pm 2\%$.

We find relatively modest differences in the functional potency of varenicline at several neuronal nAChRs. The EC_{50} for varenicline activation of $\alpha 4\beta 2$ is $2.3 \pm 0.3 \mu$ M, while varenicline was 8-fold and 24-fold less potent at $\alpha 7$ and $\alpha 3\beta 4$ receptors, respectively. These results contrast with the high degree of selectivity reported for varenicline at $\alpha 4\beta 2$ as compared to $\alpha 7$ and $\alpha 3\beta 4$ receptors (Coe et al., 2005c). However, in this earlier study it is the equilibrium binding affinity of varenicline that is being compared. In this context, varenicline is highly selective for $\alpha 4\beta 2$, displaying an affinity that is 4000-fold and >5000-fold greater than the affinities for the $\alpha 3\beta 4$ and $\alpha 7$ subtypes, respectively. The binding affinity derived from this type of assay is primarily the affinity of the ligand for the desensitized state(s) of the receptor. Unfortunately, large differences in affinity observed in equilibrium binding assays are generally not observed in functional assays and the rank order of affinities do not necessarily correlate with the rank orders of functional potency or efficacy (Avalos et al., 2002; Jensen et al., 2005). Thus, we find that the functional potency of varenicline at $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ receptors varies by 24-fold or less. More interesting is the variation in efficacy, which ranges from low (<15% for $\alpha 4\beta 2$, $\alpha 3\beta 2$ and $\alpha 6$ -containing receptors), to high (93% and 75% for $\alpha 7$ and $\alpha 3\beta 4$ receptors, respectively). This variation in efficacy is not surprising considering that the initial inspiration for the development of varenicline was cytisine (Coe et al., 2005c), an agonist that displays low efficacy at $\alpha 4\beta 2$ receptors and high efficacy at $\alpha 3\beta 4$ and $\alpha 7$ receptors (Luetje and Patrick, 1991; Papke and Heinemann, 1994; Chavez-Noriega et al., 1997; Stauderman et al., 1998; Chavez-Noriega et al., 2000; Papke and Porter Papke, 2002; Carbonnelle et al., 2003; Slater et al., 2003). When compared to cytisine, varenicline has a similarly

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low efficacy at $\alpha 4\beta 2$ and other $\beta 2$ containing receptors, and a similarly high efficacy at $\alpha 3\beta 4$ and $\alpha 7$ receptors.

We have characterized the pharmacological properties of varenicline using rat neuronal nAChRs. Thus, it is important to consider how our results may relate to the actions of varenicline at human receptors. As discussed above, varenicline displays similar potency and efficacy values at both rat and human $\alpha 4\beta 2$ receptors (Table 1; Coe et al., 2005c). In addition, cytisine displays similar potencies and efficacies on both rat and human $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ receptors (Chavez-Noriega et al., 1997; Papke and Porter Papke, 2002; Carbonnelle et al., 2003). Thus, the actions of varenicline on rat neuronal nAChRs that we describe here are likely to be relevant to the actions of varenicline on human receptors.

All of the actions of varenicline should be taken into account when considering a potential mechanism for smoking cessation. Varenicline is a potent partial agonist at $\alpha 4\beta 2$ receptors, a less potent high efficacy agonist at $\alpha 3\beta 4$ receptors, a partial agonist at $\alpha 3\beta 2$ and $\alpha 6$ -containing receptors and a potent full agonist at $\alpha 7$ receptors. The involvement of presynaptic $\alpha 4$ -containing and $\alpha 6$ -containing receptors in regulating dopamine release in the striatum (Salminen et al., 2004), makes the partial agonism of varenicline at these receptors an attractive potential mechanism (Coe et al., 2005c). However, the potent high efficacy activation of $\alpha 7$ receptors may also be important. In addition, because therapeutic use of varenicline involves chronic exposure, it may be that it is the greater affinity of varenicline for the $\alpha 4\beta 2$ receptor in the desensitized state(s) that is important. That is, varenicline may have a selectively greater ability to maintain $\alpha 4\beta 2$ receptors in a desensitized state. While it is possible that one of these actions is critical, it is also possible that that some combination of these actions might work in concert to achieve a desirable smoking cessation outcome. Regardless of the exact mechanism of action, it is ultimately the results of clinical trials that determine the usefulness of varenicline as a smoking cessation therapeutic. It should also be noted that the potent, high efficacy

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agonism of the $\alpha 7$ receptor suggests that varenicline may have utility as a treatment of disorders such as schizophrenia and Alzheimer's disease (Jensen et al., 2005).

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References

Avalos M, Parker MJ, Maddox FN, Carroll FI and Luetje CW (2002) Effects of pyridine ring substitutions on affinity, efficacy, and subtype selectivity of neuronal nicotinic receptor agonist epibatidine. *J Pharmacol Exp Ther* **302**:1246-1252.

Azam L, Dowell C, Watkins M, Stitzel JA, Olivera BM and McIntosh JM (2005) Alpha-conotoxin Bula, a novel peptide from *Conus bullatus*, distinguishes among neuronal nicotinic acetylcholine receptors. *J Biol Chem* **280**:80-87.

Brooks PR, Caron S, Coe JW, Ng KK, Singer RA, Vazquez E, Vetelino MG, Watson Jr HH, Whritenour DC and Wirtz MC (2004) Synthesis of 2,3,4,5-Tetrahydro-1,5-methano-1H-3-benzazepine via Oxidative Cleavage and Reductive Amination Strategies. *Synthesis* **11**: 1755-1758.

Carbonnelle E, Sparatore F, Canu-Boido C, Salvagno C, Baldani-Guerra B, Terstappen G, Zwart R, Vijverberg H, Clementi F and Gotti C (2003) Nitrogen substitution modifies the activity of cytosine on neuronal nicotinic receptor subtypes. *Eur J Pharmacol* **471**:85-96.

Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ and Johnson EC (1997) Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors $\alpha 2\beta 2$, $\alpha 2\beta 4$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 4\beta 4$ and $\alpha 7$ expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* **280**:346-356.

Chavez-Noriega LE, Gillespie A, Stauderman KA, Crona JH, Claeps BO, Elliott KJ, Reid RT, Rao TS, Velicelebi G, Harpold MM, Johnson EC and Corey-Naeve J (2000) Characterization of the recombinant

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human neuronal nicotinic acetylcholine receptors $\alpha 3\beta 2$ and $\alpha 4\beta 2$ stably expressed in HEK293 cells. *Neuropharmacology* **39**:2543-2560.

Coe, JW and Brooks, PRP, Aryl Fused Azapolycyclic Compounds, US Patent 6,410,550 B1, date of patent June 25, 2002.

Coe JW, Vetelino MG, Bashore CG, Wirtz MC, Brooks PR, Arnold EP, Lebel LA, Fox CB, Sands SB, Davis TI, Schulz DW, Rollema H, Tingley FD 3rd and O'Neill BT (2005a) In pursuit of $\alpha 4\beta 2$ nicotinic receptor partial agonists for smoking cessation: carbon analogs of (-)-cytisine. *Bioorg Med Chem Lett* **15**:2974-2979.

Coe JW, Brooks PR, Wirtz MC, Bashore CG, Bianco KE, Vetelino MG, Arnold EP, Lebel LA, Fox CB, Tingley FD 3rd, Schulz DW, Davis TI, Sands SB, Mansbach RS, Rollema H and O'Neill BT (2005b) 3,5-Bicyclic aryl piperidines: a novel class of $\alpha 4\beta 2$ neuronal nicotinic receptor partial agonists for smoking cessation. *Bioorg Med Chem Lett* **15**:4889-4897.

Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollema H, Lu Y, Mansbach RS, Chambers LK, Rovetti CC, Schulz DW, Tingley FD 3rd and O'Neill BT (2005c) Varenicline: an $\alpha 4\beta 2$ nicotinic receptor partial agonist for smoking cessation. *J Med Chem* **48**:3474-3477.

Corringer PJ, Le Novere N and Changeux JP (2000) Nicotinic receptors at the amino acid level. *Annual Review of Pharmacology and Toxicology* **40**:431-458.

MOL #25130

Jensen AA, Frolund B, Liljefors T and Krogsgaard-Larsen P (2005) Neuronal nicotinic acetylcholine receptors: Structural revelations, target identifications, and therapeutic inspirations. *J Med Chem* **48**:4705-4745.

Kuryatov A, Olale F, Cooper J, Choi C and Lindstrom J (2000) Human $\alpha 6$ AChR subtypes: subunit composition, assembly and pharmacological responses. *Neuropharmacology* **39**:2570-2590.

Luetje CW and Patrick J (1991) Both α - and β -subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. *J Neurosci* **11**:837-845.

McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ and Whiteaker P (2004) Analogs of alpha-conotoxin MII are selective for alpha6-containing nicotinic acetylcholine receptors. *Molec Pharmacol* **65**:944-952.

Obach RS, Reed-Hagen AE, Krueger SS, Obach BJ, O'Connell TN, Zandi KS, Miller S and Coe JW (2006) Metabolism and disposition of varenicline, a selective alpha4beta2 acetylcholine receptor partial agonist, in vivo and in vitro. *Drug Metab Dispos* **34**:121-130.

Papke RL and Heinemann SF (1994) Partial agonist properties of cytisine on neuronal nicotinic receptors containing the b2 subunit. *Molec Pharmacol* **45**:142-149.

Papke RL and Porter Papke JK (2002) Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. *Br J Pharmacol* **137**:49-61.

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Parker MJ, Beck A and Luetje CW (1998) Neuronal nicotinic receptor $\beta 2$ and $\beta 4$ subunits confer large differences in agonist binding affinity. *Molec Pharmacol* **54**:1132-1139.

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC and Grady SR (2004) Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* **65**:1526-1535.

Slater YE, Houlihan LM, Maskell PD, Exley R, Bermudez I, Lukas RJ, Valdivia AC and Cassels BK (2003) Halogenated cytosine derivatives as agonists at human neuronal nicotinic acetylcholine receptor subtypes. *Neuropharmacology* **44**:503-515.

Stauderman KA, Mahaffy LS, Akong M, Velicelebi G, Chavez-Noriega LE, Crona JH, Johnson EC, Elliott KJ, Gillespie A, Reid RT, Adams P, Harpold MM and Corey-Naeve J (1998) Characterization of human recombinant neuronal nicotinic acetylcholine receptor subunit combinations $\alpha 2\beta 4$, $\alpha 3\beta 4$ and $\alpha 4\beta 4$ stably expressed in HEK293 cells. *J Pharmacol Exp Ther* **284**:777-789.

Xiao Y, Baydyuk M, Wang HP, Davis HE and Kellar KJ (2004) Pharmacology of the agonist binding sites of rat neuronal nicotinic receptor subtypes expressed in HEK 293 cells. *Bioorg Med Chem Lett* **14**:1845-1848.

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Footnotes

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Figure Legends

Figure 1. Structure of varenicline.

Figure 2. Action of varenicline at $\alpha 4\beta 2$ receptors. **A)** Current responses of an oocyte expressing $\alpha 4\beta 2$ receptors to 20 μ M ACh and 1 μ M varenicline. Scale: 200 nA, 20 sec. **B)** Current responses of an oocyte expressing $\alpha 4\beta 2$ receptors to 20 μ M ACh and 100 μ M varenicline. Scale: 200 nA, 20 sec. **C)** Responses of $\alpha 4\beta 2$ expressing oocytes to a range of varenicline concentrations, normalized to the maximal ACh response. Values are the mean \pm SEM, n = 5. **D)** Varenicline inhibition of ACh responses. Responses of oocytes to 40 μ M ACh (EC_{60}) combined with various concentrations of varenicline are normalized to the response to ACh alone. Values are the mean \pm SEM, n = 4-5.

Figure 3. Action of varenicline at $\alpha 3\beta 4$ receptors. **A)** Current responses of an oocyte expressing $\alpha 3\beta 4$ receptors to 110 μ M ACh and 30 μ M varenicline. Scale: 0.5 μ A, 20 sec. **B)** Current responses of an oocyte expressing $\alpha 3\beta 4$ receptors to 110 μ M ACh and 200 μ M varenicline. Scale: 0.5 μ A, 20 sec. **C)** Responses of $\alpha 3\beta 4$ expressing oocytes to a range of varenicline concentrations, normalized to the maximal ACh response. Values are the mean \pm SEM, n = 4-10.

Figure 4. Action of varenicline at $\alpha 7$ receptors. **A)** Current responses of an oocyte expressing $\alpha 7$ receptors to 300 μ M ACh and various concentrations of varenicline (in μ M). Scale: 1 μ A, 5 sec. **B)** Responses of $\alpha 7$ expressing oocytes to a range of varenicline concentrations, normalized to the maximal ACh response. Values are the mean \pm SEM, n = 5-11.

Table 1. Functional potency and efficacy values for varenicline at rat neuronal nAChRs. EC_{50} values were determined from the fit data in Figures 2C, 3C and 4B. Efficacy values are presented as the percent of maximum ACh response (see Methods). Efficacy values for $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ are taken from the fit data in Figures 2C, 3C and 4B. Efficacy values for $\alpha 3\beta 2$ and $\alpha 6/\alpha 3\beta 2\beta 3$ are derived from a single concentration of varenicline (100 μ M). All values are the mean \pm SEM. nd, not determined.

Receptor	EC_{50} (μ M)	n_H	Efficacy (% of Max ACh)
$\alpha 4\beta 2$	2.3 ± 0.3	1.1 ± 0.1	13.4 ± 0.4
$\alpha 3\beta 4$	55 ± 8	2.0 ± 0.5	75 ± 6
$\alpha 3\beta 2$	nd	nd	3.7 ± 0.4
$\alpha 6/\alpha 3\beta 2\beta 3$	nd	nd	8.8 ± 1.4
$\alpha 7$	18 ± 6	1.0 ± 0.3	93 ± 7

Figure 1

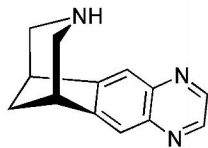


Figure 2

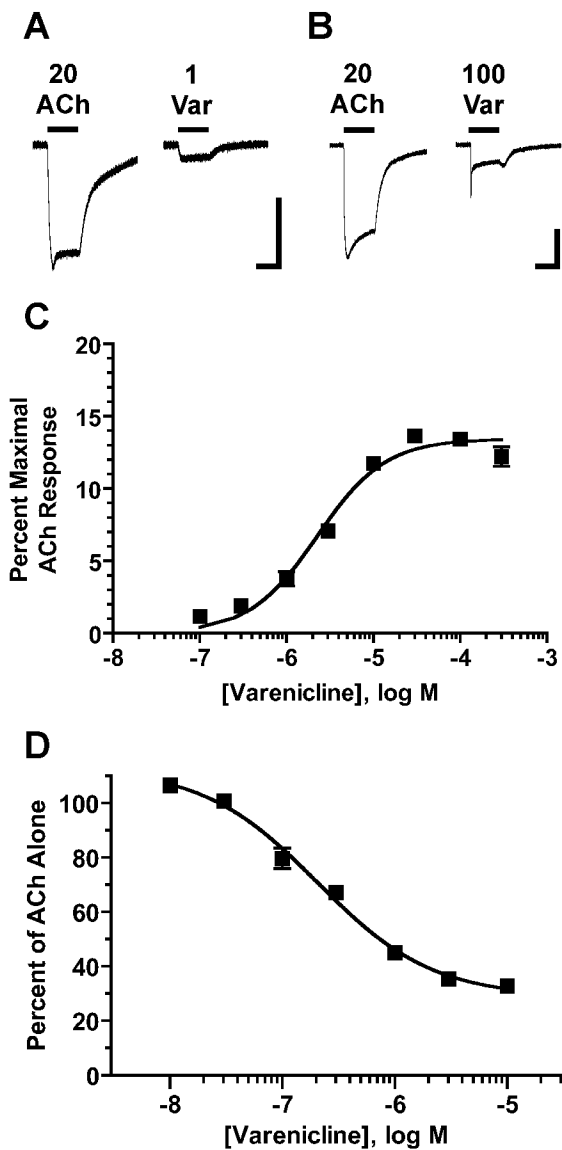


Figure 3

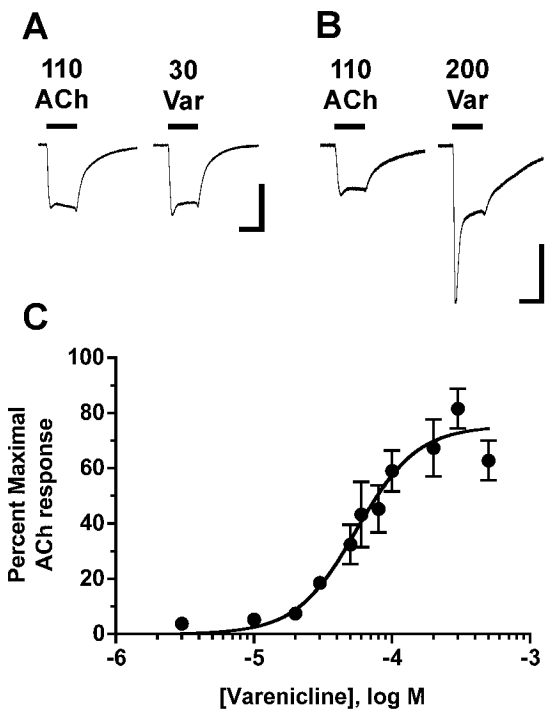


Figure 4

