Pharmacological profile of the \(\alpha_4\beta_2\) nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid

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Received 24 August 2006; received in revised form 24 October 2006; accepted 25 October 2006

Abstract

The preclinical pharmacology of the \(\alpha_4\beta_2\) nicotinic acetylcholine receptor (nAChR) partial agonist varenicline, a novel smoking cessation agent is described. Varenicline binds with subnanomolar affinity only to \(\alpha_4\beta_2\) nAChRs and in vitro functional patch clamp studies in HEK cells expressing nAChRs show that varenicline is a partial agonist with 45% of nicotine's maximal efficacy at \(\alpha_4\beta_2\) nAChRs. In neurochemical models varenicline has significantly lower (40–60%) efficacy than nicotine in stimulating [3H]-dopamine release from rat brain slices in vitro and in increasing dopamine release from rat nucleus accumbens in vivo, while it is more potent than nicotine. In addition, when combined with nicotine, varenicline effectively attenuates the nicotine-induced dopamine release to the level of the effect of varenicline alone, consistent with partial agonism. Finally, varenicline reduces nicotine self-administration in rats and supports lower self-administration break points than nicotine. These data suggest that varenicline can reproduce to some extent the subjective effects of smoking by partially activating \(\alpha_4\beta_2\) nAChRs, while preventing full activation of these receptors by nicotine. Based on these findings, varenicline was advanced into clinical development and recently shown to be an effective and safe aid for smoking cessation treatment.

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Keywords: \(\alpha_4\beta_2\) nAChR; Smoking cessation; Nicotine; Partial agonist; Varenicline; Dopamine; Self-administration

1. Introduction

Nicotine is a bioactive substance in tobacco that serves as a positive reinforcer in humans and animals (Henningfield and Goldberg, 1983; Corrigall and Coen, 1989; Shoaib et al., 1997). Because nicotine produces physical dependence and is readily available in the form of cigarettes, most smokers repeatedly fail to quit despite the serious health consequences of tobacco-related illnesses such as cancer, respiratory and cardiovascular diseases (Balfour, 2004; U.S. Department of Health and Human Services, 2004; Doll et al., 2004). Currently available pharmacotherapies, including nicotine replacement therapy and the antidepressant bupropion, are only modestly effective, approximately doubling the quit rate compared with placebo (Silagy et al., 2006; Hughes et al., 2006), illustrating the pressing need for more effective smoking cessation pharmacotherapies (Cryan et al., 2003).

An alternative approach to discovering a more efficacious treatment was founded on the knowledge that high affinity \(\alpha_4\beta_2\) nAChRs in the mesolimbic dopamine system mediate the reinforcing effects of nicotine (Pontieri et al., 1996;...
Corrigall, et al., 1994). Activation of \( \alpha 4\beta 2 \) nAChRs in the ventral tegmental area triggers downstream events such as increased mesolimbic dopamine release, which transmits salient reward and aversive signals to higher cortical centers. Repeated use of nicotine causes recurrent and transitory increases in dopamine release, which facilitate the association and learning that ultimately lead to dependence (Laviolette and Van der Kooy, 2004; Dani and Harris, 2005; Everitt and Robbins, 2005). Convincing evidence for the crucial role of mesolimbic \( \alpha 4\beta 2 \) nAChRs came from studies in which elimination of either the \( \alpha 4 \) or \( \beta 2 \) subunit attenuated the pharmacological and behavioral effects of nicotine (Picciotto et al., 1998; Marubio et al., 2003). Additionally, targeted expression of \( \beta 2 \) subunits in the ventral tegmental area of \( \beta 2 \) knock-out mice reinstated nicotine-seeking behavior and nicotine-induced dopamine release (Maskos et al., 2005). These findings strongly support the hypothesis that targeting \( \alpha 4\beta 2 \) nAChRs with a partial agonist would provide improved efficacy, since the agonist and antagonist properties of a partial agonist would both relieve craving and withdrawal symptoms in smokers who try to quit, and reduce or eliminate the reinforcing aspects of tobacco (Rose and Levin, 1991; Cohen et al., 2003).

We recently described a screening strategy leading to the discovery of partial \( \alpha 4\beta 2 \) nAChR agonists and the identification of varenicline, a compound with the desired properties (Coe et al., 2005a,b), which was recently shown to be an efficacious smoking cessation agent (Gonzales et al., 2006; Jorenby et al., 2006; Tonstad et al., 2006). This report describes the key in vitro and in vivo pharmacological properties of varenicline.

2. Materials

2.1. Drugs and chemicals

Varenicline (6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino[2,3-b][3]benzazepine) was synthesized according to Coe et al. (2005a,b); \((-\) nicotine bitartrate, ketamine/xylazine, mecamylamine, \((-\)epibatidine and all analytical grade chemicals were purchased from Sigma (St. Louis, MO), \([\text{3H}]\)-nicotine, \([\text{3H}]\)-dopamine, \([\text{125I}]\)-alpha-bungarotoxin and Solvable from PerkinElmer electroplax membranes from Receptor Biology Inc., Beltsville, MD.

2.2. Animals

Sprague–Dawley and Long–Evans rats were obtained from Charles River Labs (Wilmington, MA or Kingston, NY) and kept in a temperature and light-controlled (lights on 07:00–19:00 h) animal facility. For dopamine turnover and microdialysis studies male Sprague–Dawley rats had free access to food and water and weights ranged from 200–300 g to 280–320 g, respectively, at the time of the experiments. For behavioral studies male Sprague–Dawley rats (drug discrimination) or male Long–Evans rats (nicotine self-administration), were held at approximately 400 g and 350 g, respectively, by post-session supplemental feeding (Purina Rat Chow). All animal experiments were conducted in accordance with the “Principles of Laboratory Animal Care” from the National Institutes of Health and approved by the Institutional Animal Care and Use Protocol Committee. Protocols and equipment were in agreement with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care.

3. Methods

3.1. In vitro binding to nAChRs receptors

In vitro binding affinities of varenicline to different nAChRs were determined essentially as described previously (Pabreza et al., 1991; Arneric et al., 1994), using \([\text{3H}]\)-nicotine (for \( \alpha 3\beta 4 \) in HEK293 cells and in cortical human or rat tissue), \([\text{3H}]\)-epibatidine (for \( \alpha 3\beta 4 \) in HEK293 cells), and \([\text{125I}]\)-alpha-bungarotoxin (for \( \alpha 7 \) in IMR32 cells and \( \alpha 1\beta 2\gamma 0 \) in Torpedo electroplax membranes) as radioligands. \( K_i \) values were calculated according to \( K_i = IC_{50}/(1 + [\text{3H-ligand}] / K_d) \) and expressed as \( pK_i \pm SEM \) (\( n = 4 \)).

3.2. In vitro patch clamp electrophysiology

Agonist-evoked whole-cell currents were recorded from HEK293 cells stably transfected with human \( \alpha 4\beta 2 \) or \( \alpha 3\beta 4 \) nAChRs, and from TE671 cells, which endogenously express human muscle-type nAChRs (\( \alpha 1\beta 1\gamma 0 \)). Cells were continuously superfused at room temperature with a solution containing 140 nM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM HEPES, 10 mM glucose (pH 7.4 with NaOH). Patch pipettes were filled with an internal pipette solution containing 126 mM Cs\(_2\)SO\(_4\), 10 mM CsCl, 4 mM NaCl, 1 mM MgCl\(_2\), 0.5 mM CaCl\(_2\), 5 mM EGTA, 10 mM HEPES, 3 mM ATP-Mg, 0.3 mM GTP-Na, 4 mM Phosphocreatin (pH 7.2 with CsOH) and resistances ranged from 3 to 6 MΩ. Analog signals were acquired with an Axopatch 200B amplifier, filtered at 1/5 the sampling frequency, digitized, stored, and measured using pCLAMP software (Molecular Devices) and data were expressed as mean ± SEM.

3.2.1. Intrinsic efficacy

Cells were voltage-clamped at −60 mV and varying concentrations of nicotine or varenicline were applied for 3 s once every 45 s, using a multi-barrel fast perfusion exchange system (Warner Instrument, Hamden, CT). Each concentration was applied at least three times to ensure that the peak current was stable. The currents evoked by varenicline were normalized to the response evoked by a single control concentration of nicotine applied to the same cell (10 μM for \( \alpha 4\beta 2 \) nAChRs, 10 μM for \( \alpha 3\beta 4 \) nAChRs and 100 μM for \( \alpha 1\beta 1\gamma 0 \) nAChRs). The normalized data were fit by a simple logistic function and expressed as the mean percent of the maximum nicotine-evoked response.

3.2.2. Acute inhibition

Various varenicline concentrations were applied for 5 s in the middle of a 15 s challenge with 10 μM nicotine. Co-application of varenicline started 5 s after initiation of the nicotine challenge and inhibition was measured as the reduction in current at the end of the varenicline co-application, compared to the current measured in the absence of varenicline.
3.2.3. Steady-state inhibition

Nicotine (10 μM) was applied for 3 s once every 30 s before and during a continuous bath application of low concentrations of varenicline. For every concentration of varenicline, the amplitude of the nicotine-evoked current was monitored until it reached a new steady-state level. This process was repeated at a series of concentrations for varenicline to produce a steady-state inhibition curve.

3.3. In vitro [3H]-dopamine release in rat striatal slices

Striatal tissue slices (300 μm) from Sprague–Dawley rats were placed in oxygenated Krebs buffer (134 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 1 mM MgSO₄, 10 mM glucose, 1.3 mM CaCl₂, 250 mM NaHCO₃, pH 7.4) and washed twice with Krebs buffer. After addition of 0.1 μM [3H]-dopamine, slices were incubated at 37 °C for 15 min with periodic oxygenation. The buffer was removed, 40 μl slices were placed in 300 μl chambers in a Brandel 12-channel superfusion machine and superfused at 0.5 ml/min with Krebs buffer for 30 min at 37 °C, after which 4 min fractions were continuously collected and counted. After superfusion with Krebs buffer for 16 min, nicotine (10 μM), varenicline (0.03–100 μM), or varenicline (100 μM) plus either 10 μM nicotine or mecamylamine, were perfused for 4 min, followed by superfusion with Krebs buffer for 60 min. Slices were removed, digested overnight in scintillation vials with Solvable and counted. The fractional release of [3H]-dopamine was calculated by dividing the DPMs in each fraction by the sum of [3H]-dopamine remaining in the slices and [3H]-dopamine released in all following fractions. The drug-evoked [3H]-dopamine release, calculated by subtracting baseline release from each sample, was expressed as the percentage of the total [3H]-DA release stimulated by 10 μM nicotine ± SEM (n = 4 for each condition). Statistical differences were analyzed by One-Way ANOVA.

3.4. In vivo dopamine turnover

Varenicline was administered with either vehicle (2 ml/kg saline) or nicotine to Sprague–Dawley rats at 1 h (s.c.) or 2 h (p.o.) prior to rapid removal of nucleus accumbens tissue. Accumbents tissue samples were homogenized in cold 0.1 N perchloric acid and after 10 min centrifugation at 14,000 rpm, concentrations of dopamine and the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in 10 μl of the supernatant. The analytes were quantified electrochemically at 650 mV (Coulochem II ESA, Chelmsford, MA) after separation over a C18 Beckman Ultrasphere 5 μ ODS 4.6 mm × 25 cm column with a mobile phase consisting of 20 mM NaH₂PO₄, 9% methanol, 0.2 mM octanesulfonic acid, 0.2 mM EDTA, pH 3.8, at 1 ml/min (LC-10AD pump, Shimadzu, Columbia, MD). Dopamine turnover was calculated as the ratio ([DOPAC] + [HVA])/[DA] and expressed as percent of controls (vehicle-treated animals). Statistical significance was analyzed by One-Way ANOVA using Dunnett’s post hoc test.

3.5. In vivo dopamine microdialysis

Effects on extracellular dopamine, DOPAC and HVA concentrations were determined by standard microdialysis procedures. Briefly, probes with a 2 mm microdialysis tip were implanted into the nucleus accumbens (AP +1.7, ML +1.5, DV −6.2) of Sprague–Dawley rats under ketamine/xylazine anesthesia. The next day the probe was connected to a microperfusion pump (CMA/102, CMA/Microdialysis, Chelmsford, MA, USA) and perfused at 2 μl/min with artificial cerebral spinal fluid (NaCl 147 mM, CaCl₂ 1.3 mM, KCl 2.7 mM, MgCl₂ 1.0 mM). The probe outlet was directly connected to a 30 μl HPLC sample loop and samples were collected on-line and automatically injected on a C18 3 μ Hypersil BDS 150 × 3 mm column. The mobile phase (75 mM NaAc pH 4.3, 8% MeOH, 0.1 mM EDTA, 2.3 mM heptanesulfonic acid) was delivered at a flow rate of 0.4 ml/min (Shimadzu LC-10AD pump) and dopamine, DOPAC and HVA were detected amperometrically at 650 mV (DECADE detector, Antec, Leiden, The Netherlands). After baseline levels of dopamine stabilized, effects of varenicline (p.o.), nicotine (s.c.) and varenicline with 0.32 mg/kg s.c. nicotine (given 1 h after varenicline) were monitored and expressed as the percentage of baseline (i.e. average of last five pre-drug baseline levels) ± SEM (n = 3–6). For dose–response curves, the maximal effect of each dose was calculated as the mean ± SEM of three sequential samples around the tₘₐₓ (i.e. 54–108 min after p.o. varenicline and 27–81 min after s.c. nicotine). Statistical significance was analyzed by One-Way ANOVA using Student–Newman–Keul’s post hoc test.

3.6. In vivo behavioral models

Nicotine self-administration and drug discrimination studies were essentially performed as previously described (Mansbach et al., 2000), in standard operant test chambers equipped with a house light, two response levers and a food dispenser (BRS/LVE Beltsville, MD). For i.v. experiments, rats were tethered to an infusion pump that delivered a 35 μl infusion over a 1.2 s period. Data and delivery of stimuli were recorded with commercially available interfacing equipment and software (Med Associates, Georgia, VT). All self-administration and VI30 control experiments, whether pretreatment or substitution studies, were designed as within-subjects experiments. Each animal received all doses of a treatment, in random order and only one treatment was given per test day, while test days were separated by control testing until stability criteria were met. Control baselines were calculated by taking the mean of the three previous control sessions, which all had to be within 20% of the mean for the animal to be considered stable. Data were analyzed using One-Way or Two-Way ANOVA with repeated measures, with Bonferroni/Dunnnett, Fisher’s PLSD, or paired-t tests as post hoc comparisons.

3.6.1. Nicotine self-administration on a fixed ratio schedule

Long–Evans rats were trained to lever press under a fixed ratio (FR) schedule of food reinforcement, requiring one lever
press to obtain food (FR1 schedule), then maintained under a 30 s variable interval schedule until steady response rates were observed. Rats were then implanted with jugular catheters under ketamine/xylazine anesthesia and 4–7 days later reintroduced to the testing apparatus for self-administration training. The beginning of the session was signaled by illumination of a house light and drug availability was signaled by illumination of three jeweled lamps located above the right lever. A reinforced lever press extinguished the lamps for a 10 s timeout period, during which responses on the lever did not count toward the next reinforcer. Nicotine (30 μg/kg/infusion) was used to maintain responding and the FR schedule was increased from FR1 to FR5. Responding on the right lever was reinforced; responses on the left lever were recorded but had no programmed consequences, i.e., no significant responding took place on this lever and therefore no data are reported. The effects of varenicline pretreatment on nicotine self-administration were tested when the self-administration rate was stable. To ensure that a decrease in responding for nicotine was not due to an inability to lever press, a separate group of rats was trained under a schedule of food-maintained responding, initially on a FR1 schedule of food delivery, then to a variable interval of 30 s (VI30) schedule with 30 min sessions. Rats were administered nicotine (0.6 mg/kg s.c.) after each session, to approximate the daily nicotine exposure of the self-administration rats. Data were expressed as percent of baseline, the number of infusions or response rates (VI30 rats) taken under test conditions were divided by the mean number of the previous three baseline sessions.

3.6.2. Nicotine self-administration on a progressive ratio schedule

Under the progressive ratio schedule the response requirement was elevated after each successive infusion according to a log 0.2 progression, starting with FR1. If the next FR value was not completed within 45 min of the previous infusion and 3 h total session time had not yet elapsed, then the final completed infusion was defined as the break point (i.e., highest ordinal infusion attained during the 3 h session) and the session terminated. As in the FR procedure, a 10 s timeout followed each infusion. Varenicline substitution studies, which started after stable behavior was established, were conducted by substituting varenicline for nicotine for 4 days. The first day data were excluded from the analysis and results were expressed as the mean break point of the last 3 days of substitution. The most efficient progression of rat use was to start in VI30 (control), move to self-administration under FR5 and then go into self-administration under progressive ratio. In this progression, rats can start on a nicotine FR1 and progressed through FR5, usually in one session and were stable on FR5 within 3–5 days. It was therefore possible, but not necessary, that an individual animal may have participated in all of the studies described.

3.6.3. Drug discrimination

Sprague–Dawley rats were trained to discriminate nicotine from saline with a standard 2-lever drug discrimination procedure, pairing each of the levers with either nicotine (0.4 mg/kg) or saline injections (s.c., 5 min prior to 20 min session), with lever assignments randomized across animals. Food pellets were delivered according to a FR30 schedule and responses on the incorrect lever reset the FR response requirement on the correct lever. Tests were conducted by administering nicotine or varenicline prior to the session where responses on either lever were reinforced with food pellet delivery. Full substitution for nicotine was considered to have occurred when the mean percentage of nicotine-lever responding was 80% or greater. Lever-selection results for individual subjects were included in group averages only if the response rate for that subject was 0.05 responses/s or greater. Mean lever-selection results were plotted only if at least half of the subjects met this rate criterion. Points on the varenicline dose–response curve were preceded by test days on which 0.4 mg/kg nicotine or saline were administered. These data served as a reference for the degree of stimulus control exerted by nicotine and saline under testing conditions. The highest dose of varenicline (1 mg/kg) was also dosed in combination with 0.56 mg/kg mecamylamine.

4. Results

4.1. In vitro binding affinity at nAChRs

As reported previously (Coe et al., 2005b) varenicline has much higher affinity for α4β2 nAChRs than for other nAChR subtypes, with $K_i$ values for rat brain, human cortex and human cloned α4β2 nAChRs from 0.11 to 0.17 nM (Table 1). Varenicline has approximately 20-fold higher affinity for human α4β2 nAChRs than nicotine ($K_i = 2.1$ nM) and binds with low affinity to non-nicotinic neurotransmitter receptors, modulatory binding sites, ion channels, transporters and enzyme sites ($K_i > 350$ nM).

4.2. In vitro functional activity at nAChRs

The intrinsic efficacy of varenicline was determined by normalizing currents evoked by varenicline (0.1–1000 μM) to the current evoked by a single nicotine concentration, as illustrated in Fig. 1 for α4β2 nAChRs. Nicotine-evoked concentration-dependent inward currents from all receptor

<table>
<thead>
<tr>
<th>Receptor (species, tissue/cell line)</th>
<th>$K_i$ ± SEM</th>
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<tbody>
<tr>
<td>α4β2 (rat, brain)</td>
<td>9.76 ± 0.05</td>
</tr>
<tr>
<td>α4β2 (human, cortex)</td>
<td>9.81 ± 0.10</td>
</tr>
<tr>
<td>α4β2 (human, HEK293a)</td>
<td>9.95 ± 0.07</td>
</tr>
<tr>
<td>α3β4 (human, HEK293a)</td>
<td>7.08 ± 0.11</td>
</tr>
<tr>
<td>α2 (human, IMR32)</td>
<td>6.21 ± 0.13</td>
</tr>
<tr>
<td>α1β1γδ (Torpedo)</td>
<td>5.47 ± 0.17</td>
</tr>
</tbody>
</table>

a Human embryonic kidney cell membranes.
b Immortalized neuroblastoma cell membranes.
c Torpedo electroplax cell membranes.
populations with EC_{50} values of 5.2 μM for α4β2, 13 μM for α3β4 and 92 μM for α1β1γδ nAChRs (Fig. 2). The intrinsic agonist efficacy of varenicline at α4β2 nAChRs was found to be 45% relative to nicotine with an EC_{50} = 3.1 μM (Figs. 1a and 2a), in agreement with previous data in Xenopus laevis oocytes (Coe et al., 2005b). Acute application of varenicline during a continuous challenge with 10 μM nicotine caused a concentration-dependent, but partial, inhibition of the nicotine-evoked currents by 14.1 ± 4.7%, 39.6 ± 5.4% and 45.8 ± 9.7% with 0.1, 1 and 10 μM varenicline, respectively (Fig. 1b). Varenicline was considerably more potent at inhibiting α4β2 nAChRs under steady-state conditions (IC_{50} of 6 nM (n = 4, see Section 3). Varenicline’s intrinsic efficacy at α3β4 nAChRs was 63% (EC_{50} = 1.1 μM, Fig. 2b), and it evoked no response from α1β1γδ nAChRs at concentrations up to 100 μM (Fig. 2c).

4.3. In vitro and in vivo effects on the dopaminergic system

In vitro, varenicline produced concentration-dependent increases in [^{3}H]-dopamine release in rat striatal slices that were significantly lower than the increase induced by 10 μM nicotine (F_{5,25} = 95.06, p < 0.001) and reached a maximal effect at 1 μM that was 51% of the maximal nicotine effect (Fig. 3a). When combined with nicotine, 10 μM varenicline reduced the nicotine-evoked [^{3}H]-dopamine release by 53% to a level that was not significantly different from the maximal effect of varenicline alone (F_{2,9} = 0.88, p > 0.45). Finally, mécamylamine, a noncompetitive nAChR antagonist, completely blocked the varenicline-evoked [^{3}H]-dopamine release (Fig. 3a).

Results from in vivo studies confirm and extend preliminary data on varenicline’s effects on mesolimbic dopamine (Coe et al., 2005b). Time courses for maximally effective doses of nicotine (0.32 mg/kg s.c.) and varenicline (0.1 mg/kg p.o.) indicate that varenicline-induced increases in the extracellular levels of dopamine as well as of its metabolites DOPAC and HVA are significantly smaller than nicotine-induced increases at approximately 0.5–1 h after drug administration (Fig. 3b). Dose–response studies (Fig. 4a, b) showed significant treatment effects on dopamine turnover and dopamine release for nicotine (F_{6,24} = 12.74, p < 0.001; F_{1,22} = 21.91, p < 0.001) and varenicline (F_{6,47} = 9.68, p < 0.001; F_{7,26} = 6.45, p < 0.001), with the maximal effects of varenicline being only 40–60% of the maximal nicotine response. When escalating doses of varenicline were co-administered with a maximally effective nicotine dose (1 mg/kg in turnover, 0.32 mg/kg

![Fig. 2](image-url)
in release), the increases in turnover and release (F$_{1,36} = 14.46, p < 0.001$; F$_{3,15} = 13.96, p < 0.001$) were significantly reduced by 1 mg/kg varenicline compared with the maximal nicotine response (p < 0.05), approximately to the level of varenicline’s maximal effect. ED$_{50}$ values for nicotine and varenicline were 0.2 mg/kg s.c. and 0.06 mg/kg p.o. in turnover and 0.2 mg/kg s.c. and 0.03 mg/kg p.o. in release, respectively. Varenicline doses of 3.2 mg/kg and higher had no significant effect on dopamine release, resulting in an inverted U-shaped dose–response curve (Fig. 4b).

4.4. Nicotine self-administration (fixed ratio schedule)

Fig. 5a shows that pretreatment with s.c. or p.o. varenicline in rats that self-administer nicotine (30 μg/kg/inf) under a FR5 schedule, significantly reduced nicotine intake to a response level similar to that under saline substitution (F$_{3,15} = 15.6, p < 0.001$). This reduction was specific to nicotine intake, because food-maintained control responding on a VI30 schedule was not decreased in a separate group of nicotine-experienced rats pretreated with varenicline. The response rate in control animals was actually significantly increased (F$_{3,15} = 6.0, p < 0.01$), but was not associated with increased locomotor activity or food intake.

4.5. Nicotine self-administration and varenicline substitution (progressive ratio)

Progressive ratio schedules require an animal to fulfill an increasingly higher FR requirement for each successive infusion and can be considered an index of the drug’s reinforcing efficacy, defined by the maximum FR value (“break point”) that supports responding. Fig. 5b shows that nicotine supported significantly greater break points than saline under a progressive ratio schedule over a wide dose range of 30–100 μg/kg/inf (F$_{5,20} = 13.8, p < 0.001$). In contrast, varenicline treatment

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Fig. 3. (a) Effects of nicotine, varenicline, nicotine + varenicline and mecamylamine + varenicline, on in vitro [3H]-dopamine release from rat striatal slices. Data are expressed as percent of [3H]-dopamine release evoked by 10 μM nicotine ± SEM (n = 4). **p < 0.01 vs 10 μM nicotine (open bar); *p < 0.01 vs varenicline. (b) Time courses for maximally effective doses of nicotine (0.32 mg/kg s.c. open squares) and varenicline (0.1 mg/kg p.o., closed circles) on in vivo extracellular levels of the metabolites (DOPAC and HVA) and dopamine (DA) in rat nucleus accumbens, expressed as mean percent of baseline ± SEM (n = 3–5). *p < 0.05, **p < 0.01 varenicline vs nicotine.

Fig. 4. Dose–response curves for the effects of vehicle (open circle), varenicline (filled circles), nicotine (open squares) and varenicline co-administered with 1 or 0.32 mg/kg s.c. nicotine (triangles) on (a) dopamine turnover and (b) dopamine release in rat nucleus accumbens. Dopamine turnover data are expressed as mean percentage of controls ± SEM (n = 10). Microdialysis data are expressed as mean percentage of baseline ± SEM (n = 3–5); *p < 0.05, **p < 0.01 vs vehicle; *p < 0.05 vs 1 mg/kg (turnover) or 0.32 mg/kg (release) nicotine.
Varenicline-dependent manner with complete substitution at 1 mg/kg. The effect of 1 mg/kg s.c. varenicline was blocked by 0.56 mg/kg s.c. mecamylamine (Y-axis denotes the mean break point ± SEM (n = 5–8), expressed both as the number of infusions obtained and the corresponding FR value. Open bars represent nicotine, filled bars varenicline infusions; *p < 0.05, **p < 0.01 vs saline (One-Way repeated measures ANOVA with Bonferroni/Dunnett’s test). (b) Varenicline substitution under a progressive ratio schedule. The Y-axis denotes the mean break point ± SEM (n = 5–8). Data represent percentage of baseline responding %nicotine lever responding + SEM (vehicle), % of baseline + SEM (% nicotine lever responding + SEM).)

Fig. 6. Effects of varenicline in the rat drug discrimination model. (a) Varenicline (filled circles) substitutes for 0.4 mg/kg s.c. nicotine (open square) in a dose-dependent manner with complete substitution at 1 mg/kg. The effect of 1 mg/kg s.c. varenicline was blocked by 0.56 mg/kg s.c. mecamylamine (triangle). Data represent the percent responses ± SEM (n = 6–8) on the lever associated with nicotine. **p < 0.01, *p < 0.05 vs nicotine. (b) Varenicline doses of 0.01–1 mg/kg s.c. do not affect response rate. Data represent responses per second ± SEM (n = 8).

In rats trained to reliably discriminate the reference substance nicotine, varenicline significantly and dose-dependently increased response rate (F_{5,20} = 12.14, p < 0.001), supporting self-administration only at the 56 μg/kg/infusion dose (F_{5,20} = 3.53, p < 0.05), with a corresponding break point that was significantly lower than the maximum break point supported by nicotine.

4.6. Drug discrimination

In rats trained to reliably discriminate the reference substance nicotine, varenicline significantly and dose-dependently increased response rate (F_{5,20} = 14.1, p < 0.001), supporting self-administration only at the 56 μg/kg/infusion dose (F_{5,20} = 3.53, p < 0.05), with a corresponding break point that was significantly lower than the maximum break point supported by nicotine.

was associated with significantly fewer infusions than nicotine treatment (F_{5,20} = 14.1, p < 0.001), supporting self-administration only at the 56 μg/kg/infusion dose (F_{5,20} = 3.53, p < 0.05), with a corresponding break point that was significantly lower than the maximum break point supported by nicotine.

5. Discussion

This report describes the neuropharmacological profile of the recently approved pharmacotherapeutic aid for smoking cessation, varenicline (Coe et al., 2005b), a partial agonist at α4β2 nAChRs that play a pivotal role in nicotine addiction. An α4β2 nAChR partial agonist may facilitate the extinction of behaviors associated with nicotine dependence by partially stimulating nAChRs in the absence of nicotine, while preventing nicotine from triggering a full response. Such an agent should thus provide relief from craving and withdrawal and concomitantly dampen the reinforcing effects of smoking, offering benefits over currently available smoking cessation agents. The recent demonstration of the clinical efficacy of
varenicline (Gonzales et al., 2006; Jorenby et al., 2006) supports the validity of this approach to smoking cessation. Here we describe the preclinical assessment of varenicline’s partial agonist profile and efficacy in animal models that supported its full development.

Additional in vitro receptor binding studies confirmed the previously reported (Coe et al., 2005b) high affinity and selectivity of varenicline for z4ß2 nAChRs (Table 1). Functional patch clamp studies in mammalian cell lines expressing human z4ß2 receptors showed that varenicline’s intrinsic activity is 45% of that of nicotine, with an EC50 of 3.1 μM (Figs. 1 and 2). These results are in good agreement with our preliminary results (Coe et al., 2005b) and recent data (Mihalak et al., 2006; Xiao et al., 2006) obtained in oocytes. It should be noted that the large difference between functional potency (EC50 = 3.1 μM) and binding affinity (Kd = 0.15 nM) is a known feature of human z4ß2 nAChRs, thought to reflect higher affinity binding to desensitized states than to closed or open states of the nAChRs (Eaton et al., 2003). Consistent with this idea, prolonged exposure to sub-activating concentrations of varenicline, which could stabilize a desensitized state of the receptor (Fenster et al., 1997; Paradiso and Steinbach, 2003; Xiao et al., 2006), potently (IC50 = 6 nM) inhibited the functional activity of z4ß2 nAChRs.

Given the importance of the mesolimbic dopaminergic system in nicotine dependence, we compared the effects of varenicline and nicotine in three different measures of dopaminergic activity, which all showed an agonist—antagonist profile consistent with observations in the patch clamp studies. Varenicline has 53% agonist efficacy and blocks nicotine’s effect on [3H]-dopamine release in vitro (Fig. 3a), while its agonist efficacy in vivo was 45% and 63% in dopamine turnover and dopamine microdialysis, respectively (Fig. 4), confirming preliminary data (Coe et al., 2005b). The reasonable agreement between the intrinsic efficacies of varenicline in turnover (changes in metabolite levels) and in microdialysis (changes in transmitter release) is consistent with the parallel changes in metabolites and dopamine that nicotine and varenicline produce (Fig. 3b). These data also show that the effects of varenicline on dopamine release have a slower onset and longer duration of action than nicotine. Interestingly, the dose—response curve for varenicline’s effect on dopamine release has an inverted-U shape, possibly a consequence of partial agonist effects at z4ß2 nAChRs on GABA-ergic and/or glutamatergic neurons that modulate dopamine release in the accumbens and are thought to play an important role in nicotine dependence (Laviolette and Van der Kooy, 2004; Markou, 2005).

Varenicline acts in vivo as an antagonist in the presence of nicotine and the present dopamine studies show that varenicline effectively blocks the nicotine-induced dopamine increase in a dose-dependent manner (Fig. 4). Taken together, all data consistently show that varenicline is a potent partial agonist with comparable intrinsic efficacies (40—60%) across several in vitro and in vivo models and that it reduces nicotine-induced effects by 40—55%, close to the maximum response level of varenicline alone (Table 2). This dual action will create a ceiling effect on dopamine release and ‘stabilize’ the dopaminergic status of the mesolimbic pathway, both in the presence and the absence of nicotine from tobacco.

![](https://example.com/table2.png)

**Table 2.** Relative agonist (varenicline alone) and antagonist (varenicline with nicotine in indicated concentrations/doses) efficacy of varenicline compared with nicotine (=100%), as determined in the in vitro and in vivo functional models used in this study.

Behavioral animal models provided evidence that varenicline modifies the dependence-related behavioral effects of nicotine. In the nicotine self-administration procedure, a model with good construct and face validity for the reinforcing effects of tobacco (Rose and Corrigan, 1997; Shoaib et al., 1997), varenicline pretreatment significantly decreased nicotine self-administration by up to 50%, at doses that do not compromise the animals’ ability to respond (Fig. 5a). The maximal varenicline effect is comparable to the effect of saline substitution for nicotine, which also causes a 50% reduction in responding, suggesting that self-administration is partly being maintained by secondary reinforcements associated with the self-administration session, such as signaling lights and pump noise. There is a growing appreciation for the complex nature of the reinforcing effects of nicotine, with important roles for primary and secondary reinforcements (Chaudhri et al., 2006). We found, in agreement with other reports (Mathieu-Kia et al., 2002) that it takes 6—20 sessions of saline substitution for nicotine to completely extinguish the lever-pressing behavior. This implies that the 50% reduction by varenicline is the maximal effect that can be expected in an acute situation and that repeated varenicline treatment could completely extinguish lever pressing. Translating this to the chronic relapsing condition of smoking, it seems reasonable to assume that it takes time to allow the extinction of all secondary and enhanced reinforcing effects imparted by non-pharmacological stimuli in their environments and that varenicline treatment might give smokers that time.

As expected for a partial agonist, varenicline is self-administered in rats trained to self-administer nicotine (Fig. 5b). The progressive ratio experiments show, however, that nicotine is a more efficacious reinforcer than varenicline, consistent with the idea that a partial agonist should be less reinforcing and consequently have a significantly lower abuse potential than nicotine. Finally, in the drug discrimination paradigm varenicline substitutes for a nicotine cue, an effect that is blocked by mecamylamine, indicating that varenicline can reproduce the subjective effects of nicotine and that this effect is mediated via nAChRs (Fig. 6a). Comparable full substitution
by partial agonists has been described for other classes of drugs, such as benzodiazepines and cannabinoids (Ator and Griffiths, 1999; De Vry and Jentzsch, 2003).

In summary, varenicline displays the desired profile of a potent and selective α4β2 nAChR partial agonist that can effectively reduce nicotine self-administration. These properties, together with varenicline’s straightforward dispositional profile (Obach et al., 2006), prompted the full clinical development of varenicline. In humans, varenicline is well absorbed, has linear pharmacokinetics and an elimination half-life of 24 h, low plasma protein binding and is almost completely renally excreted as unchanged varenicline (Faessel et al., 2006). The efficacy and safety of varenicline were demonstrated in several smoking cessation trials (Nides et al., 2006; Gonzales et al., 2006; Jorenby et al., 2006; Oncken et al., 2006; Tonstad et al., 2006) and varenicline, as the prescription drug Champix (Chantix in the USA), has been approved as an effective treatment for smoking cessation.

Acknowledgement

This work was performed at Pfizer Global Research and Development, Groton, CT 06340, USA.

References


