Original Investigation

Varenicline Blocks $\beta 2^*$ -nAChR–Mediated Response and Activates $\beta 4^*$ -nAChR–Mediated Responses in Mice In Vivo

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Abstract

Introduction: The smoking cessation aid, varenicline, has higher affinity for the $\alpha 4\beta 2^*$ -subunit of the nicotinic acetylcholine receptor ($\alpha 4\beta 2^*$ -nAChR) than for other subtypes of nAChRs by in vitro assays. The mechanism of action of acute varenicline was studied in vivo to determine (a) subtype activation associated with physiological effects and (b) dose relationship as an antagonist of nicotine.

Methods: Acute doses of saline, nicotine, and varenicline were given to mice, and locomotor depression and hypothermia were measured. Subunit null mutant mice as well as selective antagonists were used to study mode of action of varenicline as an agonist. Varenicline as an antagonist of nicotine was also investigated.

Results: Varenicline evokes locomotor depression and hypothermia at higher doses than necessary for nicotine. Null mutation of the $\alpha 7$ - or $\beta 2$ -nAChR subunit did not decrease the effectiveness of varenicline; however, null mutation of the $\beta 4$ subunit significantly decreased the magnitude of the varenicline effect. Effects of the highest dose studied were blocked by mecamylamine (general nAChR antagonist) and partially antagonized by hexamethonium (largely peripheral nAChR antagonist). No significant block was seen with ondansetron antagonist of 5-hydroxytryptamine 3 receptor. Using a dose of nicotine selective for $\beta 2^*$ -nAChR subtype effects with these tests, dose-dependent antagonism by varenicline was seen. Effective inhibitory doses were determined and appear to be in a range consistent with binding affinity or desensitization of $\beta 2^*$ -nAChRs.

Conclusions: Varenicline acts as a functional antagonist of $\beta 2^*$ -nAChRs, blocking certain effects of nicotine. At higher doses, varenicline is an agonist of $\beta 4^*$ -nAChRs producing physiological changes in mice.

Introduction

Varenicline, a smoking cessation aid, is marketed as a selective partial agonist for the $\alpha 4\beta 2$ ($\alpha 4\beta 2^*$)-nicotinic acetylcholine

receptor (nAChR). Data on the affinity, potency, and efficacy of varenicline at various nAChR subtypes measured in vitro indicate varenicline has considerably higher affinity for the $\alpha 4\beta 2^*$ -nAChR than for other subtypes of nAChRs in humans, rats, and mice (Anderson et al., 2008; Carroll et al., 2008; Coe et al., 2005; Grady et al., 2010; Rollema, Faessel, & Williams, 2009; Rollema et al., 2007; Smith et al., 2007). The activation potency is also selective for the $\alpha 4\beta 2^*$ -nAChR subtype (Grady et al., 2010; Kuryatov, Berrettini, & Lindstrom, 2011; Mihalak, Carroll, & Luetje, 2006; Papke, Wecker, & Stitzel, 2010; Rollema et al., 2007). Varenicline is a partial agonist at both $\alpha 4\beta 2^*$ - and $\alpha 6\beta 2^*$ -nAChR subtypes and a full agonist at both the $\alpha 7$ - and $\alpha 3\beta 4^*$ -subtypes (Grady et al., 2010; Kuryatov et al., 2011; Mihalak et al., 2006; Papke et al., 2010; Rollema et al., 2007).

Despite the in vitro data indicating that varenicline has a higher affinity than nicotine, in animal models, a higher dose of varenicline is needed to produce an effect equivalent to nicotine for some behaviors (Carroll et al., 2008; Cunningham & McMahon, 2011; Turner, Castellano, & Blendy, 2010). In addition, a few reports have indicated that varenicline can block certain behavioral effects of nicotine (Raybuck et al., 2008; Zaniowska, McCreary, Stefanski, Przegalinski, & Filip, 2008). It is unclear whether these observations are the result of the partial agonist properties of varenicline or combinations of activity and/or desensitization at various nAChR subtypes. With widespread use in humans for smoking cessation, a number of side effects of varenicline have been reported (Ebbert, Wyatt, Hays, Klee, & Hurt, 2010). More knowledge on the relative effects of nicotine and varenicline at the various subtypes of nAChR may be helpful in designing new compounds with fewer side effects.

As a first step, we evaluated potential nicotine-like pharmacological effects of varenicline in a mouse model. We used both wild-type (WT) C57BL/6 mice as well as nAChR subunit-null mutant mice on the C57BL/6 background to assess the ability of varenicline to evoke locomotor depression and hypothermia, two effects of nicotine commonly studied in mice. In addition, we have used antagonists to block certain receptors in order to ascertain which subtypes of nAChR are mediating varenicline-induced responses. To assess only $\beta 2^*$ -nAChR-mediated effects, we used a lower dose of nicotine (0.5 mg/kg intraperitoneal [ip])

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that selectively elicits locomotor depression and hypothermia mediated by $\beta 2^*$ -nAChRs (Tritto et al., 2004) and investigated whether a prior dose of varenicline blocked these nicotine-mediated effects. Our results show that, at low doses (below ~ 0.1 mg/kg), varenicline acts as a functional antagonist of the $\beta 2^*$ -nAChR, while at higher doses (1.0 mg/kg and above), it acts as an agonist at $\beta 4^*$ -nAChRs possibly at peripheral locations.

Methods

Mice

C57BL/6 mice and subunit null mutant mice were bred and housed at the Institute for Behavioral Genetics, University of Colorado (Boulder, CO). All animal care and experimental procedures were in accordance with National Institutes of Health (NIH) guidelines and approved by the Animal Care and Utilization Committee of the University of Colorado. The subunit null mutant mice ($\beta 2$, Picciotto et al., 1995; $\beta 4$, Xu et al., 1999; and $\alpha 7$, Orr-Urtreger et al., 1997) have been maintained via heterozygous matings and backcrossed onto the C57BL/6 background at this facility for a minimum of 10 generations. Genotypes were determined by polymerase chain reaction from tail clippings (Salminen et al., 2004).

Mice had free access to food and water. A 12-hr light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) was maintained, and room temperature was 22 ± 2 °C. Mice of both sexes and between 60 and 150 days were tested, and weights at time of testing were between 17 and 32 g.

Drugs

(–)-Nicotine (freebase), hexamethonium dihydrochloride, and ondansetron hydrochloride dihydrate were products of Sigma Chemical Co. (St Louis, MO). Mecamylamine hydrochloride was a gift from Merck, Sharp and Dohme Research Lab (Rahway, NJ). Varenicline tartrate was synthesized and donated by Targacept, Inc. (Winston-Salem, NC). All doses were calculated as freebase.

Behavioral Tests

Testing equipment and procedures used have been previously described (Collins, Evans, Miner, & Marks, 1986; Marks, Romm, Bealer, & Collins, 1985; McCallum, Collins, Paylor, & Marks, 2006; Tritto et al., 2004). Briefly, a symmetrical red acrylic Y-maze with three enclosed arms, each 26 cm long, 6.1 cm wide, and 10.2 cm high, was used. Crosses and rears were monitored by infrared beam breaks. A circular open-field arena (60-inch diameter) with bright-field illumination (4,500 lumens) was used, with activity monitored by infrared beam breaks set at 15 cm intervals. A Thermalert rectal probe for mice was lubricated with peanut oil. All injections were ip in isotonic saline (0.01 ml/g of body weight), and drugs were calculated as the free-base form. Mice were allowed to acclimate to the behavioral test room for at least 1 hr prior to testing. Y-maze activity (crosses and rears) was evaluated for 3 min starting 3 min after the final injection, followed by open field (distance traveled in 5 min) starting at 6 min after the final injection, and followed by rectal temperature measurement at 15 min after the final injection.

Protocol 1

Mice (C57BL/6 or various subunit null mutant mice and littermate WT controls) were injected ip and tested as described above.

Protocol 2

Mice were given saline, hexamethonium, mecamylamine, or ondansetron ip and placed in an empty cage with bedding. After 10 min, a second injection of either saline or varenicline was administered and temperature measured 15 min after the second injection (Collins et al., 1986; Zambrano, Marks, Cassels, & Maccioni, 2009). Mecamylamine, a general nAChR antagonist that is permeable to the blood-brain barrier, was administered at a dose (1 mg/kg; 0.8 mg/kg freebase) previously shown to block hypothermia mediated by nAChRs (Collins et al., 1986; Zambrano et al., 2009). Hexamethonium, an antagonist of ganglionic-type nAChR that does not cross the blood-brain barrier, was given at a dose (10 mg/kg; 7.8 mg/kg freebase) previously shown to be effective in blocking nAChR-mediated hypothermia (Zambrano et al., 2009). Ondansetron, an antagonist of the antagonist of 5-hydroxytryptamine 3 receptor (5 HT3) receptor (a receptor that has similarities to nAChRs and used to block nausea in humans, one of the reported side effects of varenicline), was given at a dose (2 mg/kg; 1.6 mg/kg freebase) previously shown to reduce sleep apnea in susceptible mice (Real, Seif, Adrien, & Escourrou, 2009).

Protocol 3

Mice were given an injection of low-dose varenicline or saline and placed in an empty cage with bedding. After 10 min, mice received a second injection of either saline or 0.5 mg/kg nicotine, and the behavioral testing procedure followed as above.

Data Analysis

Data were analyzed by one- or two-way analysis of variance (ANOVA) as indicated using Graph Pad Prism 5.0 (Graph Pad Software, La Jolla, CA). Dunnett's multiple comparison post-hoc test was used to compare null mutant mice to WT as well as antagonist treatment to saline. To determine the dose for 50% maximum effect (ED_{50}) and the dose for 50% inhibition (ID_{50}), data were fit to modified Hill equations using SigmaPlot 5.0 DOS (Tritto et al., 2004). For varenicline-induced activity measures:

$$R_{\text{dose}} = R_{\text{sal}} / [1 + (\text{Dose}/ED_{50})^n],$$

where R_{dose} is the response measured following a given dose of varenicline, R_{sal} is the average response following saline injection, ED_{50} is the dose giving 50% maximal effect, and n is the Hill coefficient. For varenicline-induced hypothermia:

$$R_{\text{dose}} = td / [1 + (\text{Dose}/ED_{50})^n] + (R_{\text{sal}} - td),$$

where td = maximal temperature decrease. For varenicline inhibition of an effect of nicotine:

$$R_{\text{dose}} = R_{\text{max}} \times \{1 - [1 + (\text{Dose}/ID_{50})^n]^{-1}\} + R_0,$$

where R_{dose} is the response following a given dose of varenicline prior to 0.5 mg/kg nicotine, R_{max} is the effect of nicotine after saline (i.e., maximum effect of 0.5 mg/kg nicotine with no prior varenicline), ID_{50} is the dose of varenicline giving 50% maximal effect, R_0 is the residual response not affected by 0.5 mg/kg nicotine, and n is the Hill coefficient.

Results

The effects of acute administration of varenicline to WT and $\alpha 7$ -, $\beta 2$ -, and $\beta 4$ -null mutant mice are shown in Figure 1 with statistical analyses summarized in the figure legend. Like nicotine

Figure 1. Effects of acute varenicline in nicotinic acetylcholine receptor (nAChR) subunit-null mutant mice. Mice were injected intraperitoneally (ip) with saline or varenicline (mg/kg as freebase) and tested by Protocol 1. For each condition, 4–14 mice were tested for each genotype at each dose. Analysis by two-way analysis of variance (ANOVA) for genotype and dose indicated significant effects as follows: for Y-maze crosses, main effects of genotype, $F(3, 126) = 7.2$, $p < .001$, power = 0.97; dose $F(3, 126) = 73.5$, $p < .001$, power = 1.00; and interaction, $F(9, 126) = 4.8$, $p < .001$, power = 0.99; for Y-maze rears, main effects of genotype, $F(3, 126) = 3.2$, $p < .05$, power = 0.55; dose, $F(3, 126) = 76.2$, $p < .001$, power = 1.00; and interaction, $F(9, 126) = 5.4$, $p < .001$, power = 1.00; for distance traveled, main effects of genotype, $F(3, 125) = 4.8$, $p < .01$, power = 0.82; dose, $F(3, 125) = 44.3$, $p < .001$, power = 1.00; and interaction, $F(9, 125) = 3.0$, $p < .01$, power = 0.83; and for temperature, main effects of genotype, $F(3, 126) = 20.3$, $p < .001$, power = 1.00; dose, $F(3, 126) = 116.3$, $p < .001$, power = 1.00; and interaction, $F(9, 126) = 8.0$, $p < .001$, power = 1.00. These data were also analyzed by one-way ANOVA for effects of genotype at each dose and analyzed by Dunnett's post-hoc test for difference from wild type (WT). Significant differences are indicated by $**p < .01$ and $***p < .001$. ED₅₀ values were calculated as described in Methods section.

(for nicotine effects, see McCallum et al., 2006; Tritto et al., 2004), acute administration of varenicline elicits dose-dependent decreases in locomotor measures (Y-maze crosses and rears and distance traveled in the open field) and body temperature. Full pharmacological effects were observed over a relatively narrow dose range yielding steep dose–response curves. No measurable changes were observed for a dose of 0.6 mg/kg ip (2.8 μ mol/kg) varenicline, while a dose of 2.4 mg/kg (11.4 μ mol/kg) elicited maximal locomotor depression and a 4 °C decrease in body temperature in WT mice. The maximal response is similar to that observed following a dose of 1 mg/kg (6.2 μ mol/kg) nicotine ip. The ED₅₀ values for varenicline in WT mice, which are summarized in Figure 1, are similar for each of the tests and range from 0.80 ± 0.02 mg/kg for Y-maze crosses to 1.35 ± 0.24 mg/kg for body temperature. The effects of varenicline administration were measured on WT mice as well as $\alpha 7^-$, $\beta 2^-$, and $\beta 4^-$ null mutant mice for each of the

responses (Figure 1). Statistical analyses are summarized in the legend to Figure 1. Comparison of the differences in ED₅₀ values for each test reveals the source of the interaction term. Unlike with nicotine (Tritto et al., 2004), deletion of the $\beta 2$ subunit did not decrease the effect of varenicline and even showed a significant increase from WT for the hypothermia measure. As was seen for nicotine, deletion of the $\alpha 7$ subunit showed no decrease from WT. However, deletion of the $\beta 4$ subunit did significantly decrease the effect of varenicline as shown by decreased effectiveness of varenicline and increases in ED₅₀ values for each of the four tests (Figure 1). Therefore, it appears that these measures of effects of varenicline are mediated largely through activation of $\beta 4^*$ -nAChRs. In contrast, the effects of nicotine are via activation of $\beta 2^*$ -nAChRs at doses below 0.5 mg/kg and via activation of a mixture of $\beta 2^*$ -nAChRs and $\beta 4^*$ -nAChRs at higher doses (McCallum et al., 2006; Tritto et al., 2004).

In order to further characterize potential sites of action of varenicline in vivo, various antagonists, at doses known to be effective in mice (see Methods section), were given ip before saline or varenicline administration to WT-, $\beta 2$ -, $\beta 4$ -, or $\alpha 7$ -null mutant mice. Results are presented in Figure 2. Statistical analyses are summarized in the legend to Figure 2. No significant effects of antagonists were detected when administered prior to a saline injection in either WT- or $\beta 2$ -null mutant mice (Figure 2A). As seen in Figure 1, varenicline has a diminished effect in $\beta 4$ -null mutant mice (Figure 2B). By two-way ANOVA, varenicline effects differ from WT only in the $\beta 4$ -null mutant genotype, having less effect. However, in all genotypes, the broad-spectrum, central nervous system-permeable nAChR antagonist mecamylamine blocked varenicline-induced hypothermia confirming varenicline's action as an agonist at nAChRs (Figure 2B). The ganglionic antagonist, hexamethonium, that does not easily cross the blood-brain barrier also significantly decreased varenicline-induced hypothermia, indicating that part of the hypothermia induced

by varenicline is peripherally mediated. Note that hexamethonium was effective in the $\beta 4$ -null mutants indicative of activation of $\alpha 3\beta 2^*$ -nAChR remaining in the periphery in these mice. The 5-HT₃ antagonist, ondansetron, was without significant effect in this test. Activation of the 5-HT₃ receptor has been shown to result in hypothermia in mice (Naumenko, Kondaurova, & Popova, 2009); however, varenicline does not appear to activate this receptor in mice (Lummis, Thompson, Bencherif, & Lester, 2011). The results presented above indicate that the in vivo locomotor depression and hypothermic effects elicited by varenicline in this study are at least partially mediated by $\beta 4^*$ -nAChR (Figure 1) and that a fraction of the hypothermia resulting from varenicline administration may be mediated by peripheral nAChR (Figure 2). Based on an affinity comparison, varenicline is selective for the $\alpha 4\beta 2^*$ -nAChR and should be somewhat more potent than nicotine at this subtype. However, varenicline is a partial, low-efficacy agonist at the $\alpha 4\beta 2^*$ -nAChR (Grady et al., 2010; Kuryatov et al., 2011; Mihalak et al., 2006; Papke et al.,

Figure 2. Response to varenicline after antagonists in wild-type (WT) and null mutant mice. Mice were injected ip as indicated; protocol 2 was followed. The first intraperitoneal (ip) injection was saline or an antagonist calculated as freebase (Hex = hexamethonium, 7.8 mg/kg; Ond = ondansetron, 1.6 mg/kg; Mec = mecamylamine, 0.8 mg/kg). A second injection of saline (A) or varenicline (2.3 mg/kg freebase; B) was given 10 min later. For each condition, 3–10 mice were tested. Analysis by one-way analysis of variance (ANOVA) indicated no effect of antagonists alone for either WT mice or $\beta 2$ -null mutant mice. By two-way ANOVA (for antagonist and dose of varenicline), significant effects were seen for genotype, $F(3, 78) = 17.4$, $p < .001$, power = 1.00, and antagonist treatment, $F(8, 78) = 18.9$, $p < .001$, power = 1.00. The $\beta 4$ genotype differs from all others, $p < .001$, while the other null mutants did not differ from WT. The data were also analyzed by one-way ANOVA for antagonist effects within each genotype using Dunnett's post-hoc test for difference from saline. Significant differences are indicated by * $p < .05$, ** $p < .01$, *** $p < .001$.

2010; Rollema et al., 2007). Being a partial agonist, perhaps, varenicline does not elicit enough receptor activation to elicit locomotor depression or hypothermia mediated by the $\alpha 4\beta 2^*$ -nAChR subtype. Consequently, it could be acting primarily as a competitive antagonist or desensitizer of these receptors. Therefore, to evaluate the hypothesis that varenicline could inhibit $\alpha 4\beta 2$ -nAChR responses, we administered low doses of varenicline prior to a 0.5 mg/kg dose of nicotine that elicits effects of locomotor depression and hypothermia mediated primarily by the $\beta 2^*$ -nAChR (McCallum et al., 2006; Tritto et al., 2004).

Results shown in Figure 3 demonstrate that varenicline inhibits the effects of a 0.5 mg/kg dose of nicotine. ID_{50} values for varenicline inhibition of nicotine at 0.5 mg/kg ip (3.1 μ mol/kg) were similar for inhibition of nicotine-elicited decreases in Y-maze crosses and rears and body temperature (0.004–0.007 mg/kg or 0.02–0.03 μ mol/kg), while the ID_{50} for blockade of nicotine-induced decreases in open-field activity was somewhat higher (0.031 mg/kg or 0.14 μ mol/kg). On a mole for mole basis, these varenicline ID_{50} values are considerably lower than the nicotine ED_{50} values (Tritto et al., 2004) needed to produce effects on Y-maze rears, distance traveled in the open-field, Y-maze crosses, and hypothermia (18-, 25-, 74-, and 110-fold, respectively). It appears that varenicline at low doses acts as a functional antagonist at $\beta 2^*$ -nAChRs and at higher doses as an agonist at $\beta 4^*$ -nAChRs, while nicotine can affect these measures as an agonist at both of these subtypes.

Discussion

By *in vivo* tests, measuring the well-known locomotor-depressant and hypothermia-inducing effects of nicotinic agonists, varenicline appears to have two different actions. Varenicline, *in vivo*, acts effectively as an antagonist at $\beta 2^*$ -nAChRs and as an agonist at $\beta 4^*$ -nAChRs.

Acute administration of relatively high doses of varenicline elicits locomotor depression and hypothermia. Nicotine elicits these same effects by activation of either $\beta 2^*$ -nAChRs or $\beta 4^*$ -nAChRs (McCallum et al., 2006; Tritto et al., 2004), but not by activation of $\alpha 7$ -nAChRs (Tritto et al., 2004). The lack of effect of deletion of the $\alpha 7$ subunit indicates the same is true for varenicline (Figure 1). Nicotine effects mediated by activation of $\beta 2^*$ -nAChRs are seen with lower doses of nicotine than those mediated by activation of $\beta 4^*$ -nAChRs, a result predicted by their relative *in vitro* concentration for 50% effect (EC_{50}) (Tritto et al., 2004). In contrast to the reduced potency of nicotine determined in $\beta 2$ -null mutant mice (1.4- to 4.0-fold shift to higher ED_{50} values; Tritto et al., 2004), deletion of the $\beta 2$ subunit did not decrease the effectiveness of varenicline (Figure 1). In fact, an increase in effectiveness was seen for hypothermia in the $\beta 2$ -null mutant mice at the highest varenicline dose (Figure 1). Thus, varenicline appears to elicit its direct effects on locomotor depression and hypothermia largely via activation of $\beta 4^*$ -nAChRs, as demonstrated by decreases in the effect of

Figure 3. Effect of low-dose varenicline on nicotine-induced behaviors. C57Bl/6 mice were injected intraperitoneally (ip) with the indicated dose of varenicline (calculated as freebase), followed by an injection of saline or nicotine (0.5 mg/kg freebase) ip 10 min later followed by testing (see Protocol 3). For each condition, 4–8 mice were tested. ID_{50} values were calculated as described in Methods section for varenicline to block the effect of nicotine.

varenicline in $\beta 4$ -null mutant mice (2.0- to 3.7-fold shift to higher ED_{50} values; Figure 1). Furthermore, blockade by hexamethonium indicates that the locomotor-depressant and hypothermic effects of varenicline are elicited by activating, in part, peripheral, probably ganglionic, receptors (Figure 2). This result is also predicted by in vitro assays showing that varenicline is a full agonist at $\beta 4^*$ -nAChRs (Grady et al., 2010; Mihalak et al., 2006; Rollema et al., 2010). Varenicline is also a full agonist at the $\alpha 7^*$ -nAChRs (Grady et al., 2010; Mihalak et al., 2006; Papke et al., 2010; Rollema et al., 2007, 2010). However, as seen for nicotine, this receptor subtype does not decrease these measures; no loss of effectiveness of varenicline was seen in $\alpha 7$ -null mutant mice (Figure 1).

By in vitro assays, both nicotine and varenicline are more potent at activating $\beta 2^*$ - than $\beta 4^*$ -nAChRs (Grady et al., 2010; Mihalak et al., 2006; Rollema et al., 2007, 2010). With a molar comparison, varenicline is less potent than nicotine at producing in vivo locomotor depression and hypothermia in WT mice with ED_{50} values of 3.9–5.8 μ mol/kg for varenicline (Figure 1) versus 0.6–3.2 μ mol/kg for nicotine (Tritto et al., 2004). Nicotine sufficiently activates both $\beta 2^*$ - and $\beta 4^*$ -nAChRs, while varenicline is producing these effects solely by activation of $\beta 4^*$ -nAChRs. The data presented here support the idea that varenicline does not activate $\beta 2^*$ -nAChRs sufficiently to produce measurable effects on the tests administered but does produce effects by activation of $\beta 4^*$ -nAChRs. These data are, therefore, consistent with previous in vitro data on affinity, potency, and efficacy of these drugs at various subtypes of nAChRs (Grady et al., 2010; Mihalak et al., 2006; Rollema et al., 2007, 2010).

In vitro experiments have also demonstrated that exposure of $\alpha 4\beta 2$ -nAChR to low concentrations of varenicline effectively blocks subsequent activation by ACh by either competing for the agonist site and/or desensitizing the receptor (Mihalak et al., 2006; Rollema et al., 2010). For mouse $\alpha 4\beta 2^*$ -nAChRs, there is a factor of ~100-fold in the concentrations required to bind versus activate (Marks, Robinson, & Collins, 1996). A similar factor appears to be true for varenicline (Grady et al., 2010; Mihalak et al., 2006; Rollema et al., 2007). Thus, in vivo, functional blockade of the effects of nicotine on $\beta 2^*$ -nAChR by varenicline is consistent with in vitro data. The functional blockade is achieved following administration of relatively low doses of the drug (ID_{50} values of 0.004–0.030 mg/kg [0.02–0.14 μ mol/kg] depending on the test; Figure 3).

The actions of varenicline at low doses, as an antagonist of $\beta 2^*$ -nAChR, and at higher doses, as an agonist of $\beta 4^*$ -nAChR, appear consistent with previous investigations with rodents in other laboratories. Tests where an effect of 0.6 mg/kg nicotine was seen, but that showed no effect of doses up to 1 mg/kg varenicline, include distance traveled in an elevated zero maze and homecage activity (Turner et al., 2010). Varenicline had little effect on tail flick or hot plate tests for antinociception at doses up to 10 mg/kg subcutaneous (sc) in mice, and the ED_{50} value for inducing hypothermia was 2.8 mg/kg (Carroll et al., 2008). These authors also reported that varenicline was very potent in antagonizing the effects of nicotine (2.5 mg/kg sc) in the tail flick (dose for 50% antagonism [AD_{50}] of 0.0002 mg/kg) and hot plate tests (AD_{50} 0.47 mg/kg); however, varenicline did not inhibit induction of hypothermia by a dose of nicotine that activates both $\beta 2^*$ - and $\beta 4^*$ -nAChRs (2.5 mg/kg). No direct effect of varenicline up to 1.0 mg/kg was found in a fear-conditioning paradigm, although at 0.1 mg/kg varenicline was able to prevent nicotine withdrawal effects

in fear conditioning (Raybuck et al., 2008). In rats, acute nicotine (0.4 mg/kg sc) has a stimulatory effect on locomotor activity measured as distance traveled and varenicline at 0.3 mg/kg (0.18 mg/kg as freebase) had a smaller but significant effect on this measure also (Zaniewska et al., 2008). Perhaps, varenicline does produce enough activation via nAChRs for this stimulatory effect, which is not readily measured in mice. In this study, the effect of nicotine could be partially blocked by 0.1 mg/kg varenicline (0.06 mg/kg as freebase), again likely indicating an antagonist effect at $\beta 2^*$ -nAChR. In a test of the ability of nicotine and varenicline to decrease responding for food in C57Bl/6 mice, nicotine (ED_{50} 0.83 mg/kg ip) was more potent than varenicline (ED_{50} 2.71 mg/kg ip; Cunningham & McMahon, 2011). While mecamylamine was able to block the effects of both drugs in this test, dihydro- β -erythroidine, selective for $\alpha 4\beta 2^*$ -nAChRs, only blocked nicotine. This is consistent with agonism at $\alpha 4\beta 2$ -nAChRs by nicotine and agonism at $\beta 4^*$ -nAChRs by varenicline.

In two more complex tests for anxiety, the marble-burying test and the NIH test for latency to feed in a novel environment, varenicline had effects at 0.1 mg/kg, a lower dose than for nicotine at 0.3 mg/kg (Turner et al., 2010). It has not been demonstrated whether these tests depend on activation or blockade of nAChRs. However, these data, indicating that varenicline is more potent than nicotine, would support the hypothesis that it is the blocking effect that may mediate changes in these behaviors.

Pharmacokinetics influences the concentrations of both drugs in the brain. Nicotine reaches the brain very quickly, likely somewhat faster than varenicline in mice (Reperant et al., 2010) where, after ip injection, peak effect of nicotine was seen at 15 min and varenicline not until 30 min. Nicotine is also cleared more quickly with a half-life of 6–7 min (Matta et al., 2007; Petersen, Norris, & Thompson, 1984) versus 1.4 hr for varenicline (Obach et al., 2006). It has been shown that rats and mice given the same dose of varenicline orally had similar peak concentrations even though the elimination of half-lives differ by species (1.4 and 4 hr, respectively). Varenicline is not metabolized extensively in mice; 90% is excreted unchanged (Obach et al., 2006), while nicotine is extensively metabolized (Matta et al., 2007). These pharmacokinetic factors will collectively affect brain concentrations; therefore, brain concentrations for these acute experiments are estimates.

Therapeutic doses of varenicline in humans are 0.5–2.0 mg/day or ~0.01 to 0.03 mg/kg/day. Plasma varenicline levels effective for decreasing smoking are 7–10 ng/ml or ~30 to 50 nM (Faessel et al., 2006), indicating that varenicline likely acts via binding to $\beta 2^*$ -nAChRs. These pharmacologically effective concentrations would be insufficient to produce much agonist activity, even at the $\alpha 4\beta 2^*$ -nAChR subtype (reported EC_{50} values for human $\alpha 4\beta 2^*$ -nAChR range from 0.1 to 1.4 μ M with efficacy values of 13%–22% of nicotine; Papke et al., 2010; Rollema et al., 2010). It appears likely that almost all $\beta 2^*$ -nAChRs need to be occupied by varenicline to decrease smoking. Alternatively, these receptors could be desensitized by varenicline. Estimates for desensitization by varenicline for various forms of $\alpha 4\beta 2^*$ -nAChR by expression in *Xenopus* oocytes are in the low nanomolar range, similar to binding K_i values (Papke, Trocme-Thibierge, Guendisch, Al Rubaiy, & Bloom, 2011; Papke et al., 2010; Rollema et al., 2010; Xiao et al., 2006). Therefore, the block by low doses of varenicline could be either by competition with nicotine for $\beta 2^*$ -nAChR or by desensitization of $\beta 2^*$ -nAChR.

Whether side effects of varenicline in humans are caused by binding to $\alpha 4\beta 2^*$ -nAChRs or binding to (or activation of) other subtypes of nAChR, by interaction with other receptors, or by secondary effects such as histamine release (Rollema et al., 2009) or corticosterone release (Pauly, Grun, & Collins, 1990) is beyond the scope of this report. However, it appears that in order to be effective, concentrations of varenicline in humans need to be high enough to completely block or desensitize $\alpha 4\beta 2^*$ -nAChRs (~30 to 50 nM), about 100 times the binding K_i value. At doses that achieve 70 nM plasma levels, all subjects have nausea; however, this may be via interaction with receptors in the gut where concentrations could be higher (Rollema et al., 2009). Partial activation of $\alpha 6\beta 2^-$, $\alpha 7^-$, or $\beta 4$ -nAChR or nonnicotinic receptors (such as 5-HT₃; Lummis et al., 2011) may be possible at therapeutic doses leading to some side effects such as nausea. Alternatively, side effects, especially those involving complex behavior or physiology (e.g., vivid dreams or neuropsychiatric symptoms), may be inherent with this total block or desensitization of $\alpha 4\beta 2^*$ -nAChRs (Mineur & Picciotto, 2010).

Summary

In mice, a dose of 0.5 mg/kg ip, nicotine acts as an agonist predominately at $\beta 2^*$ -nAChRs to promote locomotor depression and hypothermia. At higher doses, nicotine can also act at $\beta 4^*$ -nAChRs causing similar physiological effects. Varenicline, however, does not affect locomotor activity or body temperature at doses in the range where it activates $\beta 2^*$ -nAChRs in vitro. Varenicline, a partial agonist at $\beta 2^*$ -nAChRs, appears to have insufficient agonist activity at these receptors to affect these physiological measures. At higher doses, varenicline does decrease locomotor activity and body temperature via activation of $\beta 4^*$ -nAChRs, many of which are in the periphery. Varenicline acts in the brain at low doses as an antagonist by its ability to block the effects of the natural agonist, ACh, or of a $\beta 2^*$ -selective dose of nicotine.

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Declaration of Interests

None declared.

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