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# Varenicline Is a Potent Partial Agonist at α6β2* Nicotinic Acetylcholine Receptors in Rat and Monkey Striatum

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**ABSTRACT**

Extensive evidence indicates that varenicline reduces nicotine craving and withdrawal symptoms by modulating dopaminergic function at α4β2* nicotinic acetylcholine receptors (nAChRs) (the asterisk indicates the possible presence of other nicotinic subunits in the receptor complex). More recent data suggest that α6β2* nAChRs also regulate dopamine release and mediate nicotine reinforcement. The present experiments were therefore done to test the effect of varenicline on α6β2* nAChRs and their function, because its interaction with this subtype is currently unclear. Receptor competition studies showed that varenicline inhibited α6β2* nAChR binding ($K_i$ = 0.12 nM) as potently as α4β2* nAChR binding ($K_i$ = 0.14 nM) in rat striatal sections and with ~20-fold greater affinity than nicotine. Functionally, varenicline was more potent in stimulating α6β2* versus α4β2* nAChR-mediated [$^3$H]dopamine release from rat striatal synaptosomes with $EC_{50}$ values of 0.007 and 0.086 μM, respectively. However, it acted as a partial agonist on α6β2* and α4β2* nAChR-mediated [$^3$H]dopamine release with maximal efficacies of 49 and 24%, respectively, compared with nicotine. We also evaluated varenicline's action in striatum of monkeys, a useful animal model for comparison with humans. Varenicline again potently inhibited monkey striatal α6β2* ($K_i$ = 0.13 nM) and α4β2* ($K_i$ = 0.19 nM) nAChRs in competition studies. Functionally, it potently stimulated both α6β2* ($EC_{50}$ = 0.014 μM) and α4β2* ($EC_{50}$ = 0.029 μM) nAChR-mediated [$^3$H]dopamine release from monkey striatal synaptosomes, again acting as a partial agonist relative to nicotine at both subtypes. These data suggest that the ability of varenicline to interact at α6β2* nAChRs may contribute to its efficacy as a smoking cessation aid.

## Introduction

Tobacco use leads to major health problems worldwide and is a leading cause of preventable deaths. Smoking decreases life expectancy because of tobacco-related cancer, cardiovascular disease, and pulmonary disease and leads to increased susceptibility to numerous other adverse conditions. Despite the availability of smoking cessation therapies, most smokers fail to quit because of the addictive nature of tobacco (Hatsukami et al., 2008; Dwoskin and Bardo, 2009; Benowitz, 2010; De Biasi and Dani, 2011; Raupach and van Schayck, 2011). Although tobacco contains numerous chemicals, the presence of nicotine is thought to underlie smoking addiction. Nicotine seems to exert its rewarding effects by acting at nicotinic acetylcholine receptors (nAChRs) and increasing neurotransmission in the mesolimbic and nigrostriatal dopaminergic pathways (Dwoskin and Bardo, 2009; Wise, 2009; Benowitz, 2010; De Biasi and Dani, 2011).

The primary nAChRs in CNS dopaminergic systems that mediate the addictive effects of nicotine are the α4β2* and α6β2* subtypes (the asterisk indicates the possible presence of other subunits in the receptor complex) (Barik and Wonnacott, 2009; Changeux, 2010; Mao and McGehee, 2010; De Biasi and Dani, 2011). Evidence for this idea stemmed from studies showing that mice lacking the α4, α6, and/or β2 nAChR subunits fail to self-administer nicotine, whereas re-expression of these subunits in the ventral tegmental area of knockout mice restored nicotine self-administration (Picciotto et al., 1998; Marubio et al., 2003; Maskos et al., 2005; Pons et al., 2008; Jackson et al., 2009; Gotti et al., 2010). In addition, infusion and systemic administration of drugs that selectively inhibit α4β2* and/or α6β2* nAChRs blocked behaviors linked to nicotine reward and reinforcement (Corrigall et al., 1992, 1994; Jackson et al., 2009; Brunzell et al., 2010; Wooters et al., 2011).

Because the α4β2* and α6β2* nAChRs play an important role in addictive behaviors, these receptors represent important therapeutic targets for smoking cessation therapies. In-

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**ABBREVIATIONS:** nAChR, nicotinic acetylcholine receptor; CNS, central nervous system; α-CtxMII, α-conotoxin MII; BSA, bovine serum albumin.

deed, numerous studies have shown that varenicline, a Food and Drug Administration-approved drug widely used to help people quit smoking, potently acts as a partial agonist at α4β2* nAChRs (Coe et al., 2005; Gonzales et al., 2006; Jorenby et al., 2006; Rollema et al., 2007). This action at α4β2* nAChRs is postulated to block the reinforcing effects of nicotine while also relieving craving and withdrawal symptoms (Biala et al., 2010; O'Connor et al., 2010; De Biasi and Dani, 2011; George et al., 2011). However, smoking relapse rates remain high even with varenicline treatment (McNeil et al., 2010; Raupach and van Schayck, 2011). In addition, varenicline is not without side effects, including nausea and serious psychiatric problems (Jorenby et al., 2006; Moore et al., 2011; Williams et al., 2011).

Thus there is an urgent need to develop better smoking cessation drugs. To achieve this, it is important to understand the receptors with which varenicline interacts in the brain. Initial studies suggested that the primary impact of varenicline was on α4β2* nAChRs; however, subsequent work showed that varenicline had a significant effect at α7 nAChRs, α3β4* nAChRs, and 5-hydroxytryptamine type 3 receptors (Coe et al., 2005; Mihalak et al., 2006; Grady et al., 2010; Ween et al., 2010; Chatterjee et al., 2011; Lummis et al., 2011). Although α6β2* nAChRs play an important role in addiction, the effect of varenicline on α6β2* nAChR-mediated function in CNS dopaminergic systems is currently unclear (Anderson et al., 2009; Grady et al., 2010). The goal of the present study was to investigate the potential of varenicline to interact with α6β2* nAChRs and modulate dopaminergic function. The results show that varenicline is a potent partial agonist at α6β2* nAChRs in striatum of both rats and monkeys.

## Materials and Methods

**Animals.** Male Sprague-Dawley rats (200–250 g) were purchased from Charles River Laboratories (Hollister, CA). Upon arrival, they were housed in a temperature-controlled room with a 12-h light/dark cycle with free access to food and water. The animals were killed several days later by decapitation.

Squirrel monkeys (*Saimiri sciureus*) (600–1050 g) were purchased from World Wide Primates (Miami, FL). Animals were housed separately in a temperature-controlled room with a 12-h light/dark cycle. Food (consisting of monkey chow, fruits, and vegetables) was given twice daily, and water was provided ad libitum. The monkeys initially underwent a 1-month state-mandated quarantine. Monkeys were then euthanized according to the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. They were injected with 1.5 ml of euthanasia solution intraperitoneally (390 mg of sodium pentobarbital and 50 mg of phenytoin sodium/ml) followed by 1.5 ml/kg of the same solution administered intravenously.

All procedures were performed according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and approved by the Institutional Animal Care and Use Committee at SRI International.

**Tissue Preparation.** Rat brains were quickly removed and bisected coronally at a midstriatal level. The striatum from the anterior portion of the brain was dissected and used to measure [$^3$H]dopamine release. The posterior brain was frozen in isopentane on dry ice and stored at −80°C. Eight-micrometer sections were cut at −20°C by using a cryostat (Leica Microsystems Inc., Bannockburn, IL), thaw-mounted onto poly-L-lysine-coated slides, dried, and stored at −80°C.

Monkey brains were rapidly removed and rinsed in cold phosphate-buffered saline. They were placed in a squirrel monkey brain mold and cut into 2-mm-thick blocks by using stainless-steel blades. The slice at level A14.0-15.0 was bisected along the midline. The striatum from one half of the brain slice was dissected for the measurement of synaptosomal [$^3$H]dopamine release. The other half was immediately frozen in isopentane on dry ice and stored at −80°C. These blocks were later used for preparation of 10-μm-thick sections at −20°C by using a cryostat.

**[$^{125}$I]α-Conotoxin MII Autoradiography.** Striatal α6β2* nAChRs were determined by using [$^{125}$I]α-CtxMII (specific activity, 2200 Ci/mmol) binding as described previously (Quik et al., 2001, 2003). Striatal sections were preincubated for 15 min at room temperature in binding buffer [144 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$ 1 mM $MgSO_4$, 20 mM HEPES, and 0.1% bovine serum albumin (BSA), pH 7.5] plus 1 mM phenylmethylsulfonyl fluoride in the absence or presence of the indicated concentrations of varenicline (Sigma-Aldrich, St. Louis, MO) or nicotine (Sigma-Aldrich). This was followed by 1-h incubation at room temperature in binding buffer also containing 0.5% BSA, 5 mM EDTA, 5 mM EGTA, and 10 μg/ml each of aprotinin, leupeptin and pepstatin A, plus 0.5 nM [$^{125}$I]α-CtxMII, also with or without competing ligands. Nonspecific binding was determined by using nicotine (100 μM). Binding was terminated by washing the slides for 10 min at room temperature in binding buffer, 10 min in ice-cold binding buffer, and twice for 10 min in 0.1×buffer at 0°C, with two final 10-s washes in ice-cold deionized water. The striatal sections were dried and exposed to Kodak MR film (Eastman Kodak Co., Rochester, NY) for several days with $^3$H-microscale standards (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK).

**[$^{125}$I]Epibatidine Autoradiography.** Striatal α4β2* nAChRs were assessed by using [$^{125}$I]epibatidine (specific activity, 2200 Ci/mmol; GE Healthcare) (Quik et al., 2003; Huang et al., 2011) in the presence of $10^{-7}$ M α-CtxMII. Slides were preincubated in buffer pH 7.0 containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1.0 mM $MgCl_2$ in the absence or presence of the indicated concentrations of varenicline or nicotine. They were then incubated in the same buffer containing 0.015 nM [$^{125}$I]epibatidine for 40 min with or without competing ligands. Nonspecific binding was assessed in the presence of nicotine (100 μM). The sections were then washed, dried, and exposed to Kodak MR film for several days together with $^3$H-microscale standards.

**[$^3$H]Dopamine Release.** Synaptosomal [$^3$H]dopamine release was done as described previously (McCallum et al., 2005). Striatal tissue (~15 mg) was homogenized in 2 ml of cold homogenization buffer (0.32 M sucrose and 5 mM HEPES, pH 7.5) and centrifuged at 12,000*g* for 20 min. The pellets were resuspended in 0.8 ml of uptake buffer (128 mM NaCl, 2.4 mM KCl, 3.2 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 25 mM HEPES, pH 7.5, 10 mM glucose, 1 mM ascorbic acid, and 0.01 mM pargyline), followed by incubation for 10 min at 37°C. [$^3$H]dopamine (4 μCi, final dopamine concentration of 100 nM; PerkinElmer Life and Analytical Sciences, Waltham, MA) was added, and the synaptosomes were incubated for 5 min. An 80-μl aliquot was then pipetted onto 5-mm-diameter A/E glass-fiber filters (Gelman Instrument Co., Ann Arbor, MI) and perfused with uptake buffer also containing 0.1% BSA and 10 μM nomifensine at a rate of 1 ml/min for 10 min before fraction collection. Several baseline fractions were then collected, followed by stimulation with either nicotine or varenicline at the indicated concentrations for 18 s. Some filters were pre-exposed to α-CtxMII (50 nM) for 3 min before agonist stimulation. Twelve fractions (18 s each) were collected per sample, including basal release before and after stimulation. Radioactivity was counted with a liquid scintillation counter.

**Quantitation of the Autoradiographic Images and Data Analysis.** The ImageQuant program from GE Healthcare was used to determine optical density values. A calibration curve of radioactivity (nCi/mg tissue) versus optical density was generated by using $^3$H-microscale standards, which allowed for conversion to femtomole

per milligram of tissue. The $^{3}H$ standards were calibrated for $^{125}I$ autoradiography as described previously (Artymyshyn et al., 1990). The sample optical density readings were within the linear range of the standards. Nonspecific binding was subtracted from total tissue binding to determine specific binding. Competition analyses were done by using Prism (GraphPad Software, Inc., San Diego, CA).

[$^{3}H$]dopamine release was determined as described previously (McCallum et al., 2005), with release calculated as counts over basal release from samples immediately before and after stimulation. Release units were corrected for the wet weight of each tissue sample. Stimulated release was then normalized to baseline to yield units of release as a fraction of baseline. α4β2* and α6β2* nAChR-mediated release components were distinguished by using α-CtxMII, with release remaining in the presence of the toxin designated as that occurring via α4β2* nAChRs. The α6β2* receptor-mediated component was assessed by subtracting α4β2* nAChR-mediated release from total release. $R_{max}$ and $EC_{50}$ values for dose-response curves were calculated by nonlinear regression equations using Prism.

## Results

**Varenicline Potently Interacts at Both α6β2* and α4β2* nAChRs in Rat Striatum.** Receptor autoradiography was done to investigate the effect of varenicline on nAChR binding. Competition analyses of [$^{125}I$]α-CtxMII binding at varying concentrations of varenicline ($10^{-12}$ to $10^{-7}$ M) showed that varenicline potently inhibited α6β2* nAChR binding with a $K_i$ value of 0.12 ± 0.02 nM (Fig. 1; Table 1). Varenicline also potently inhibited [$^{125}I$]epibatidine binding in the presence of α-CtxMII (α-CtxMII-resistant [$^{125}I$]epibatidine binding), which was used as a measure of α4β2* nAChRs. [$^{125}I$]epibatidine competition studies yielded a $K_i$ value of 0.14 ± 0.01 nM, which was very similar to that for varenicline at α6β2* nAChRs (Fig. 1; Table 1). These data indicate that varenicline interacts equally well with α6β2* and α4β2* nAChRs in rat striatum.

Nicotine competition studies of [$^{125}I$]α-CtxMII (α6β2* nAChRs) and α-CtxMII-resistant [$^{125}I$]epibatidine (α4β2* nAChRs) binding yielded $K_i$ values of 1.68 ± 0.15 and 3.77 ± 0.76 nM, respectively (Fig. 1; Table 1). Thus varenicline is approximately 20 times more potent than nicotine at α6β2* and α4β2* nAChRs in rat striatum.

**Varenicline Acts as a Potent Partial Agonist at α6β2* nAChRs to Stimulate [$^{3}H$]Dopamine Release from Rat Striatal Synaptosomes.** As an approach to evaluating functional effects of varenicline, we measured [$^{3}H$]dopamine release from rat striatal synaptosomes. The results (Fig. 2) show that total varenicline-evoked [$^{3}H$]dopamine release was ~60% lower than that evoked by nicotine, in agreement with previous studies (Rollema et al., 2007; Anderson et al., 2009). To determine varenicline's impact on release mediated by either α4β2* and α6β2* nAChRs [$^{3}H$]dopamine release was measured in the presence of α-CtxMII. The results show that varenicline-stimulated release was less than that induced by nicotine at both nAChR subtypes (Fig. 2; Table 2). Varenicline-evoked α6β2* nAChR-mediated [$^{3}H$]dopamine release was 49% of that evoked by nicotine, whereas release mediated via α4β2* nAChRs was 24% of nicotine-stimulated release (Fig. 2; Table 2).

Although varenicline was less efficacious than nicotine, it was a more potent agonist at both α6β2* and α4β2* nAChRs compared with nicotine. The $EC_{50}$ values for varenicline-stimulated α6β2* and α4β2* nAChR-mediated release were 0.007 and 0.086 μM, respectively (Table 3), compared with 0.19 and 5.42 μM, respectively, for nicotine.

A point of note is that both varenicline and nicotine interacted somewhat more potently at α6β2* than at α4β2* nAChRs. The ratios of the $EC_{50}$ values for α6β2*/α4β2* were 0.083 and 0.036 for varenicline and nicotine, respectively. Thus varenicline may be a more potent partial agonist at α6β2* than α4β2* nAChRs in rat striatum.

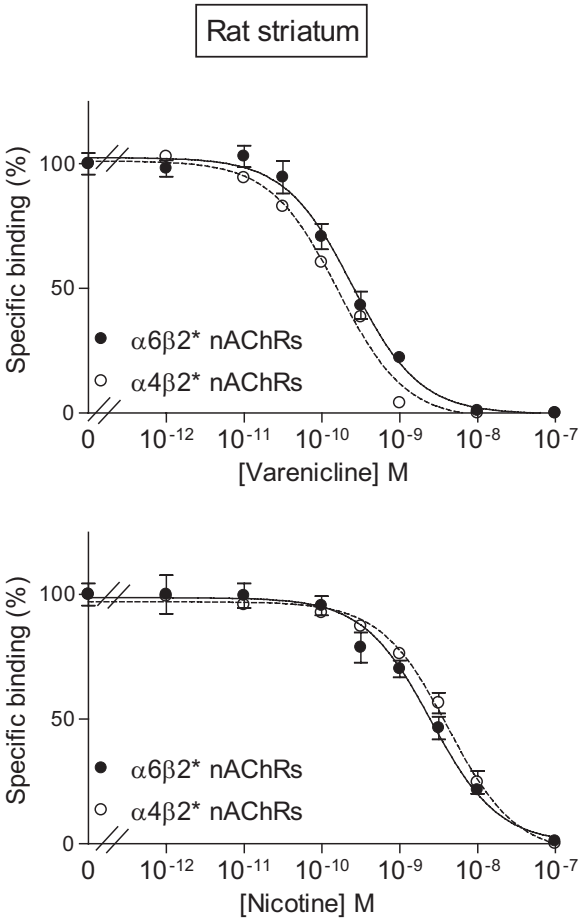


**Fig. 1.** Varenicline potently interacts at both α6β2* and α4β2* nAChRs in rat striatum. [$^{125}I$]α-CtxMII autoradiography was used to determine the effect of varenicline on α6β2* nAChRs, while [$^{125}I$]epibatidine binding in the presence of α-CtxMII was done to evaluate effects on α4β2* nAChRs (top panel). The bottom panel depicts the effect of nicotine on rat striatal α6β2* and α4β2* nAChRs for comparison. Values represent the mean ± S.E.M. of 8 rats.

TABLE 1

$K_i$ values for varenicline at α6β2* and α4β2* nAChRs in rat and monkey striatum

[$^{125}I$]α-CtxMII competition studies were done in rat and monkey striatum to measure α6β2* nAChRs, whereas [$^{125}I$]epibatidine binding in the presence of α-CtxMII was used to detect α4β2* nAChRs. Results show that varenicline potently interacts with both α6β2* and α4β2* nAChRs. Data with nicotine are provided for comparison. The values represent the mean ± S.E.M. of eight rats or three monkeys.

| Drug | Animal | $K_i$ | | Ratio of $K_i$ Values α6β2*/α4β2* |
|---|---|---|---|---|
| | | α6β2* nAChRs | α4β2* nAChRs | |
| | | *nM* | | |
| Varenicline | Rat | 0.12 ± 0.02 | 0.14 ± 0.01 | 0.86 |
| | Monkey | 0.13 ± 0.01 | 0.19 ± 0.11 | 0.68 |
| Nicotine | Rat | 1.68 ± 0.15 | 3.77 ± 0.76 | 0.46 |
| | Monkey | 0.61 ± 0.09 | 1.43 ± 0.24 | 0.43 |

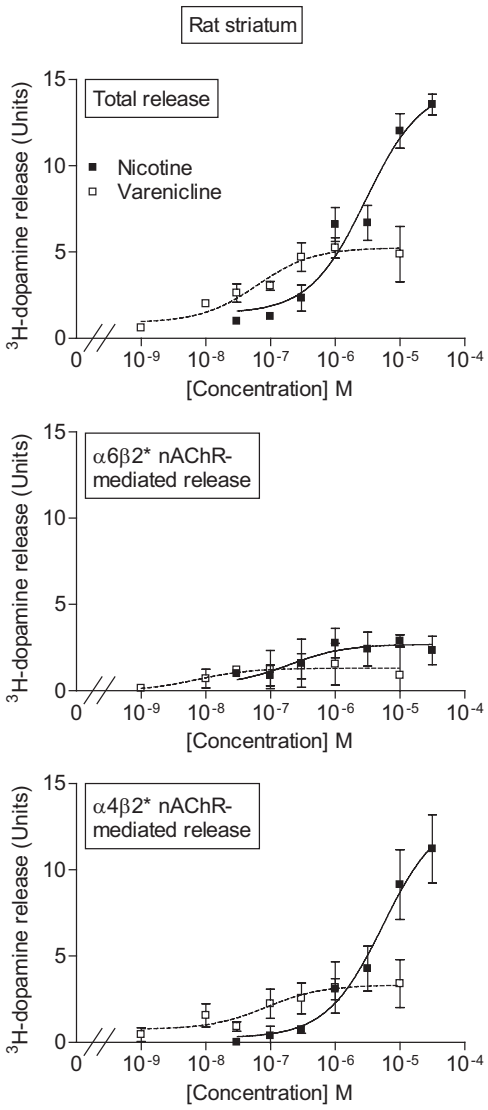


**Fig. 2.** Varenicline acts as a partial agonist at rat striatal α6β2* nAChRs. Top, the dose-response curve of varenicline and nicotine on total synaptosomal [$^3$H]dopamine release from rat striatum. Middle, α6β2* nAChR-mediated release was defined as the difference in release in the absence and presence of 50 nM α-CtxMII. Bottom, α4β2* nAChR-mediated release was designated as that occurring in the presence of α-CtxMII. Both α6β2* and α4β2* varenicline-mediated maximal [$^3$H]dopamine release were lower than that evoked by nicotine, although varenicline was more potent than nicotine. The values represent the mean ± S.E.M. of four to eight rats.

**Varenicline Potently Competes for Both α6β2* and α4β2* nAChRs in Monkey Striatum.** We next examined the effect of varenicline at α6β2* and α4β2* nAChRs in monkey striatum. Competition binding studies with [$^{125}$I]α-CtxMII showed that varenicline ($10^{-12}$ to $10^{-7}$ M) potently inhibited binding to α6β2* nAChRs in monkey striatal sections with a $K_i$ of 0.13 ± 0.01 nM (Fig. 3; Table 1). The monkey striatum is a relatively large structure that can readily be subdivided into distinct regions, including the medial and lateral caudate and ventral and dorsal putamen. Because previous work had shown that these areas were differentially affected by treatments (Quik et al., 2001; McCallum et al., 2006), we assessed the effect of varenicline in the striatal subregions. The $K_i$ values of varenicline at α6β2* nAChRs were as follows ($n$ = 3): medial caudate, 0.11 ± 0.01 nM; lateral caudate, 0.13 ± 0.01 nM; ventral putamen, 0.14 ± 0.01 nM; and dorsal putamen, 0.11 ± 0.01 nM. Because the $K_i$ values for varenicline at α6β2* nAChRs were similar in the different areas, only the competition curve for the total striatum is provided in Fig. 3.

The $K_i$ of varenicline at striatal α4β2* nAChRs was determined by measuring [$^{125}$I]epibatidine binding in the presence of α-CtxMII. The value for the total striatum was 0.19 ± 0.11 nM (Fig. 3; Table 1). This was very similar to that for the individual subregions, which were as follows ($n$ = 3): medial caudate, 0.16 ± 0.10 nM; lateral caudate, 0.18 ± 0.10 nM; ventral putamen, 0.18 ± 0.10 nM; and dorsal putamen, 0.20 ± 0.12 nM. Thus, varenicline acts with a similar potency at α4β2* and α6β2* nAChRs in monkey striatum.

As for the rat, nicotine had a lower affinity than varenicline at both α6β2* and α4β2* nAChRs in monkey striatum. [$^{125}$I]α-CtxMII and α-CtxMII-resistant [$^{125}$I]epibatidine competition binding experiments in monkey striatum yielded $K_i$ values of 0.61 ± 0.09 and 1.43 ± 0.24 nM, respectively (Fig. 3; Table 1). There was no difference in the $K_i$ values for either α6β2* or α4β2* nAChRs in the different striatal subregions (data not shown). Thus, varenicline is approximately six times more potent than nicotine at α6β2* and α4β2* nAChRs in competition binding studies in monkey striatum.

**Varenicline Is a Partial Agonist at Monkey Striatal α6β2* nAChRs.** Varenicline-evoked [$^3$H]dopamine release was next determined from monkey striatal synaptosomes to investigate its functional characteristics in striatum of a model that may more closely resemble humans. Total varenicline-evoked [$^3$H]dopamine release in monkey striatum was ~80% lower than that evoked by nicotine (Fig. 4). This was caused by a decline in both varenicline-evoked α6β2* and

TABLE 2

$R_{max}$ values for varenicline-evoked [$^3$H]dopamine release from rat and monkey striatum

Rat and monkey striatal synaptosomes were exposed to nicotine or varenicline to evoke [$^3$H]dopamine release. Release was done in the absence and presence of α-CtxMII to identify the component mediated by α6β2* and α4β2* nAChRs. Release units were normalized to the wet weight of each tissue sample as detailed under *Materials and Methods*. Varenicline acts as a partial agonist at α6β2* and α4β2* nAChRs in both rat and monkey striatum. The values represent the mean of four to eight rats or three monkeys. The numbers in parentheses are the 95% confidence intervals.

| | Animal | Maximal Dopamine Release, $R_{max}$ | | | |
|---|---|---|---|---|---|
| | | Nicotine | | Varenicline | |
| | | Units | % Control | Units | % Control |
| α6β2* nAChR- mediated | Rat | 2.69 (2.10–3.28) | 100 | 1.31 (0.90–1.71) | 49 |
| | Monkey | 2.45 (2.09–2.81) | 100 | 0.32 (0.21–0.42) | 13 |
| α4β2* nAChR-mediated | Rat | 13.26 (10.80–15.72) | 100 | 3.23 (2.63–4.01) | 24 |
| | Monkey | 1.04 (0.74–1.33) | 100 | 0.44 (0.33–0.54) | 42 |

TABLE 3

$EC_{50}$ values for varenicline-evoked [$^3$H]dopamine release in rat and monkey striatum

Rat and monkey striatal synaptosomes were exposed to varenicline or nicotine to evoke [$^3$H]dopamine release. Release was done in the absence and presence of α-CtxMII to identify the component mediated by α6β2* and α4β2* nAChRs. The data show that varenicline stimulated release somewhat more potently via α6β2* compared with α4β2* nAChRs from rat and monkey striatal synaptosomes. The values represent the mean of four to eight rats or three monkeys. The numbers in parentheses are the 95% confidence intervals.

| Drug | Animal | Dopamine Release, $EC_{50}$ | | Ratio of $EC_{50}$ Values α6β2*/α4β2* |
|---|---|---|---|---|
| | | α6β2* nAChRs | α4β2* nAChRs | |
| | | *μM* | | |
| Varenicline | Rat | 0.007 (0.001–0.15) | 0.086 (0.023–0.31) | 0.083 |
| | Monkey | 0.014 (0.001–0.40) | 0.029 (0.002–0.39) | 0.49 |
| Nicotine | Rat | 0.19 (0.012–3.23) | 5.42 (2.71–10.84) | 0.036 |
| | Monkey | 0.68 (0.28–1.65) | 1.21 (0.22–6.51) | 0.56 |

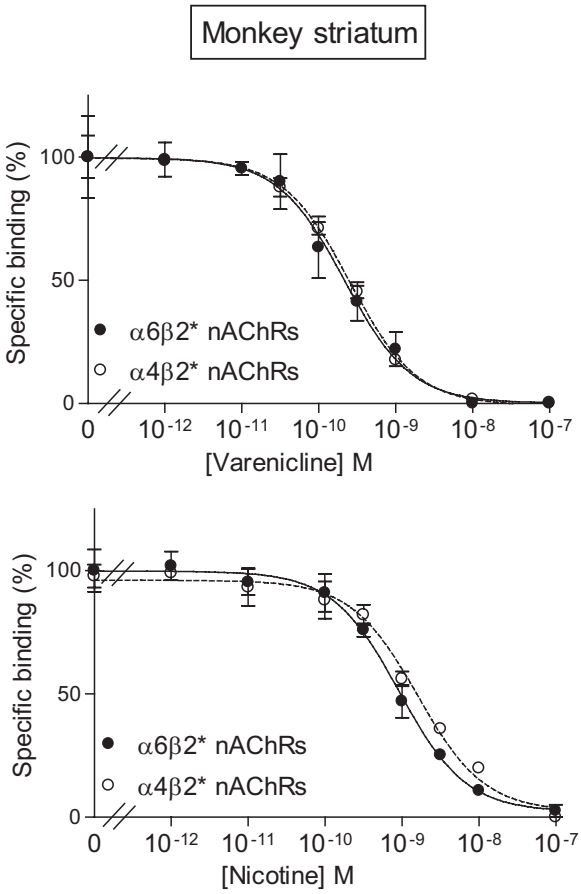


**Fig. 3.** Varenicline potently competes for both α6β2* and α4β2* nAChRs in monkey striatum. [$^{125}$I]α-CtxMII autoradiography was used to test the effect of varenicline on α6β2* nAChRs, while [$^{125}$I]epibatidine binding in the presence of α-CtxMII was conducted to determine the effect on α4β2* nAChRs (top panel). The effects of nicotine on α6β2* and α4β2* nAChRs are shown in the bottom panel for comparison. The values represent the mean ± S.E.M. of 3 monkeys.

α4β2* nAChR-mediated release, which were 13 and 42%, respectively, of that induced by nicotine (Fig. 4; Table 2).

Although varenicline again elicited less [$^3$H]dopamine release compared with nicotine, it was a more potent agonist at both α6β2* and α4β2* nAChRs (Fig. 4, middle and bottom). Varenicline's $EC_{50}$ value for α6β2* nAChR-mediated release was 0.014 μM, and for α4β2* nAChR-mediated release it was 0.029 μM (Table 3). These values were both lower than the $EC_{50}$ values for nicotine-evoked release, which were 0.68 μM for α6β2* nAChRs and 1.21 μM for α4β2* nAChRs. Again, both varenicline and nicotine interacted somewhat more potently at α6β2* than at α4β2* nAChRs, with ratios of the $EC_{50}$ values for α6β2*/α4β2* being 0.49 and 0.56 for varenicline and nicotine, respectively. Thus varenicline also seems to be a potent partial agonist at α6β2* nAChRs in monkey striatum.

## Discussion

Numerous studies indicate that varenicline potently and selectively interacts at α4β2* nAChRs (Mihalak et al., 2006; Rollema et al., 2007; Reperant et al., 2010; Brandon et al., 2011; Maloku et al., 2011; Dutra et al., 2012; Lotfipour et al., 2012; Shim et al., 2012). In fact, it has been suggested that varenicline exerts its beneficial effects as a smoking cessation aid by targeting this nAChR population. Varenicline's ability to interact more potently at α4β2* nAChRs than nicotine, but yet only act as a partial agonist in stimulating dopamine release, has been postulated to underlie its potential to reduce nicotine craving and withdrawal symptoms (Coe et al., 2005; Gonzales et al., 2006; Mihalak et al., 2006; Rollema et al., 2007; Reperant et al., 2010). However, accumulating studies now indicate that α4β2* nAChRs are not the only ones involved in addiction and withdrawal, but α6β2* nAChRs also play a role (Picciotto et al., 1998; Marubio et al., 2003; Maskos et al., 2005; Pons et al., 2008; Jackson et al., 2009; Gotti et al., 2010). Indeed, α6β2* nAChRs have been shown to have a critical and possibly dominant effect on dopaminergic neurotransmission in the mesolimbic and nigrostriatal dopaminergic system (Meyer and McIntosh, 2006; Exley et al., 2008; Perez et al., 2008, 2011; Brody et al., 2009). Moreover, their function is modified by nicotine exposure (Perez et al., 2008, 2011). These observations underscore the importance of determining whether varenicline also interacts with α6β2* nAChRs to more fully understand its mechanism of action as a smoking cessation aid.

Thus far, a limited number of studies have investigated the ability of varenicline to act at α6β2* nAChRs in the rodent dopaminergic system with conflicting results. The first of these evaluated effects of varenicline on [$^3$H]dopamine release from rat striatal slices (Anderson et al., 2009). These investigators showed that there was no shift in the $EC_{50}$ value of the α-CtxMII-sensitive release component in the presence of varenicline, a finding that suggested that varenicline did not affect α6β2* nAChR dopamine release. By contrast, a more recent report showed that varenicline potently interacted at α6β2* nAChRs in [$^{125}$I]α-CtxMII receptor competition studies ($K_i$ = 5.5 nM) using mouse membranes and was also a potent partial agonist ($EC_{50}$ = 77 nM) in eliciting α6β2* nAChR-mediated [$^3$H]dopamine release from striatal

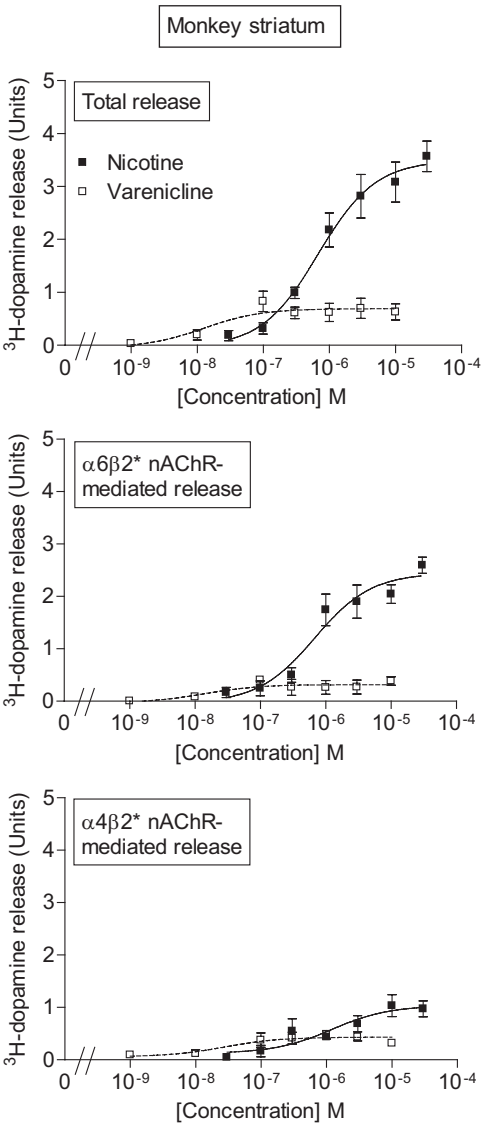


**Fig. 4.** Varenicline is a partial agonist at monkey striatal α6β2* nAChRs. Top, the dose-response curve of varenicline and nicotine on total [$^3$H]dopamine release from monkey striatum. Middle and bottom, α4β2* nAChR-mediated release (bottom) was designated as that occurring in the presence of 50 nM α-CtxMII, with α6β2* nAChR-mediated release defined as the difference in release in the absence and presence of α-CtxMII (middle). Both maximal α6β2* and α4β2* varenicline-stimulated [$^3$H]dopamine release were less than that evoked by nicotine, although varenicline seemed to act more potently at both subtypes than nicotine. The values represent the mean ± S.E.M. of three monkeys.

synaptosomes (Grady et al., 2010). Another series of studies in oocytes also showed that varenicline activated expressed α6β2β3* nAChR concatamers at nanomolar concentrations (75–178 nM) (Kuryatov and Lindstrom, 2011).

In view of these divergent findings, we initiated the current study and investigated whether varenicline acts at α6β2* nAChRs by using two different animal models. Our studies in rats showed that varenicline potently binds to α6β2* nAChRs with ~20-fold higher affinity than nicotine. It is noteworthy that the binding affinity of varenicline for α6β2* nAChRs was very similar to that of α4β2* nAChRs, in agreement with Grady et al. (2010). In addition, our data show that varenicline evoked striatal α6β2* nAChR-mediated [$^3$H]dopamine release, and its potency at the α6β2* nAChR was greater than at the α4β2* subtype. Varenicline also had a higher affinity than nicotine at both the α6β2* and α4β2* nAChRs. However, varenicline-evoked total [$^3$H]dopamine release was only one-third of the total nicotine-evoked release, supporting the idea that varenicline acts as a partial agonist. Work with α-CtxMII to delineate the contribution of the α6β2* and α4β2* nAChRs to varenicline-evoked release showed that varenicline was a partial agonist at both subtypes, in agreement with others (Grady et al., 2010).

We also did experiments to evaluate varenicline's ability to act at nAChRs in striatum of nonhuman primates, which offers the advantage that it more closely resembles humans with respect to genetics, anatomy, and physiology. The results are the first to show that varenicline interacts with high affinity at striatal α6β2* nAChRs and α4β2* nAChRs in receptor competition studies. Moreover, varenicline's $K_i$ values at both subtypes is several times greater than that observed for nicotine, as in the rat. The results also show that varenicline stimulated [$^3$H]dopamine release from monkey striatal synaptosomes more potently than nicotine at the α6β2* and α4β2* nAChR subtypes. On the other hand, it was much less efficacious in stimulating [$^3$H]dopamine release compared with nicotine at both nAChR subtypes. Thus, the results in monkeys corroborate those in the rat with respect to varenicline's ability to interact at α6β2* nAChRs.

The current results show that in acute experiments varenicline has a higher potency but is less efficacious than nicotine at α6β2* nAChRs in both monkey and rat striatum. Thus the mode of interaction of varenicline at α6β2* nAChRs is similar to that at α4β2* nAChRs, as shown here and reported by others (Grady et al., 2010). These data suggest that the antismoking effects of varenicline may be caused by an interaction at both β2* nAChR populations. Because α6β2* nAChRs exhibit a more restricted localization in the CNS than α4β2* nAChRs, drugs targeting α6β2* nAChRs may be more selective for smoking cessation, with fewer side effects compared with varenicline, which activates multiple subtypes.

With respect to a long-term mechanism of action, chronic varenicline exposure may subsequently lead to nAChR desensitization (Ortiz et al., 2012). This idea is consistent with an extensive literature suggesting that nicotine and other nAChR agonists induce their behavioral effects via this mechanism (Picciotto et al., 2008; Buccafusco et al., 2009; Bordia et al., 2010; Mineur and Picciotto, 2010; Grady et al., 2012). Desensitization would result in a further reduction in dopamine release, with varenicline acting like a virtual antagonist. Such a mechanism of action to explain the effects of varenicline on smoking cessation is consistent with the result of extensive studies showing that β2, α4, or α6 nAChRs knockout mice fail to self-administer nicotine (Picciotto et al., 1998; Marubio et al., 2003; Maskos et al., 2005; Pons et al., 2008). With respect to the CNS site of action, the present results show that varenicline potently interacts at α6β2* and α4β2* nAChRs in striatum, a region implicated in the rewarding and addictive effects of nicotine (Wise, 2009; Changeux, 2010). Another critical site of action would be the mesolimbic dopamine system, which is a major contributor to reward function and addiction, as well as the habenulo-interpeduncular pathway, which has been implicated in the addictive effects of smoking (Dani and Bertrand, 2007; De Biasi and Dani, 2011).

In summary, the present data demonstrate that varenicline binds with high affinity to α6β2* nAChRs in rat and monkey striatum. They also show that varenicline is a potent partial agonist in stimulating α6β2* nAChR-mediated dopamine release from striatal synaptosomes in both animal models. Because the striatum is implicated in motivational effects of nicotine (Wise, 2009; Changeux, 2010), these observations may suggest that varenicline's action at α6β2* nAChRs contributes to its efficacy as a smoking cessation aid.

### Authorship Contributions

*Participated in research design:* Bordia and Quik.
*Conducted experiments:* Bordia, Hrachova, and Chin.
*Contributed new reagents or analytic tools:* McIntosh.
*Performed data analysis:* Bordia, Hrachova, Chin, and Quik.
*Wrote or contributed to the writing of the manuscript:* Bordia, Hrachova, Chin, McIntosh, and Quik.

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